RESEARCH ARTICLE

Comparative Analysis of Growth and Photosynthetic Characteristics of \((\text{Populus simonii} \times P. \text{nigra}) \times (P. \text{nigra} \times P. \text{simonii})\) Hybrid Clones of Different Ploidides

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Abstract

To evaluate differences among poplar clones of various ploidies, 12 hybrid poplar clones \((\text{Populus simonii} \times P. \text{nigra}) \times (P. \text{nigra} \times P. \text{simonii})\) with different ploidies were used to study phenotypic variation in growth traits and photosynthetic characteristics. Analysis of variance showed remarkable differences for each of the investigated traits among these clones \((P < 0.01)\). Coefficients of phenotypic variation (PCV) ranged from 2.38\% to 56.71\%, and repeatability ranged from 0.656 to 0.987. The \(Pn\) (photosynthetic rate) photosynthetic photon flux density (PPFD) curves of the 12 clones were S-shaped, but the \(Pn\)-ambient CO\(_2\) (Ca) curves were shaped like an inverted “V”. The stomatal conductance (Gs)-PPFD and transpiration rate (Tr)-PPFD curves had an upward tendency; however, with increasing PPFD, the intercellular CO\(_2\) concentration (Ci)-PPFD curves had a downward tendency in all of the clones. The \(Pn\)-PPFD and \(Pn\)-Ca curves followed the pattern of a quadratic equation. The average light saturation point and light compensation point of the triploid clones were the highest and lowest, respectively, among the three types of clones. For \(Pn\)-Ca curves, diploid clones had a higher average CO\(_2\) saturation point and average CO\(_2\) compensation point compared with triploid and tetraploid clones. Correlation analyses indicated that all investigated traits were strongly correlated with each other. In future studies, molecular methods should be used to analyze poplar clones of different ploidies to improve our understanding of the growth and development mechanisms of polyploidy.
Introduction

Poplars (Populus spp.) are some of the most important economic tree species in the temperate regions of the world [1, 2]. With the publication of the P. trichocarpa genome [3], poplar has become a model organism for the study of trees and is the most intensively studied tree genus. In 1959, the Chinese Academy of Forestry (CAF) conducted a large-scale crossing experiment on poplar. After 20 years, hybridized combinations of P. simonii × P. nigra and P. nigra × P. simonii were selected as excellent materials for afforestation in Northern China because of the rapid growth, excellent wood properties, high cold resistance and drought endurance of their offspring [4, 5]. In recent years, many studies have been conducted using these two families as materials, which have mainly focused on growth traits [6, 7], resistance [8], physiology [9], and molecular research [10, 11].

Polyploidy is a ubiquitous phenomenon in higher plants. It is estimated that polyploidy has occurred in 50–70% of flowering species [12, 13], most of which have experienced one or more polyploidization events during their evolution [14]. The increase in chromosomes has resulted in increased gene dosages and cell volumes [15]. Therefore, polyploid plants usually have larger leaves, greater height and diameter, and an increased ability to adapt to their environment [16, 17]. The first natural European aspen polyploid (Populus tremula) was discovered by Nilsson-Ehle [18] in Sweden. Since then, breeders have paid close attention to different ploidy level (diploid, triploid and tetraploid) breeding in forest trees because of the huge growth of forestry. Many natural triploid poplars were found in the Soviet Union, Sweden, Finland and other countries [19–22]. In research on different types of polyploids, triploid poplars are often characterized as having fast growth, large leaves, vigor and low fertility compared with their diploid counterparts [23]. However, fewer comparative studies on poplars with different ploidies, including tetraploids, have been reported [24]. In this study, with the aim of obtaining high growth rate and highly resistant offspring, P. simonii × P. nigra and P. nigra × P. simonii were selected as parents. Colchicine was used in the crossing experiment and many offspring were obtained with different ploidies. Twelve offspring with different ploidies were used as materials in this experiment. Our primary objectives were to explore the variation in growth, photosynthesis and chlorophyll fluorescence traits among hybrid clones with different ploidies, and to provide a theoretical basis for polyploid poplar clone selection.

Materials and Methods

Plant materials

Female Populus simonii × P. nigra and male P. nigra × P. simonii plants (both of the parents were diploids) were selected as parents, and artificially controlled pollination was performed in 2006. During male and female flower development, a 0.5% colchicine solution was injected into flower buds at the beginning of reduction mitosis to obtain reduplicated pollen and metocytes. The crossing experiment was carried with reduplicated pollen and reduplicated metocytes to obtain polar seeds with different ploidies. After sowing, the DNA contents in the leaves were evaluated by flow cytometry using the method of Zhang [25]. Twelve hybrid clones with different ploidies [(Populus simonii × P. nigra) × (P. nigra × P. simonii)] were used for this study, including three tetraploid clones (sn4.1, sn4.2 and sn4.3), five triploid clones (sn3.1, sn3.2, sn3.4, sn3.8 and sn3.9) and four diploid clones (sn, sn2.1, sn2.2, sn2.3). The total DNA contents of each clone were measured and the percentages of the cells that showed varied DNA content were processed by the DPAC software. The results of sn2.1, sn3.8 and sn4.1 are shown in Fig 1. In 2013, 50 hardwood cuttings (15 cm long each) of each clone were collected from 1-year-old seedlings in a greenhouse at the Northeast Forestry University. All cuttings were
moistened and stored in plastic bags at 4°C for about 2 weeks and then planted in 1.5-L plastic pots containing garden soil mixed with peat at a ratio of 3:1 (v/v). The cuttings were grown in artificial climate chambers of Northeast Forestry University under a cycle of 1000 μmol m⁻² s⁻¹ light for 16 h from 8:00 to 24:00 and dark for 8 h. The temperature and humidity were set at 27°C and 60%, respectively.

Growth traits observation

After growth for 3 months, 30 healthy and uniform cuttings of each clone were used as materials to measure tree height (H), basal diameter (BD) and leaf number (LN) of each plant. Nine leaves (4–12) from a shoot tip per cutting were taken to measure the leaf area (LA). Each leaf and stem (the whole stem) was weighed for the leaf fresh weight (LFW) and stem fresh weight (SFW). Leaves and stems were then dried in an oven (90°C) to a constant weight and measured for leaf dry weight (LDW) and stem dry weight (SDW).

Photosynthetic analysis

Nine uniform plants of each clone were selected to measure the photosynthetic parameters. Net photosynthesis rate (Pn), intercellular CO₂ concentration (Ci), stomatal conductance (Gs) and transpiration rate (Tr) were measured from 8:30 a.m. to 11:30 a.m. using a Lico-6400 portable photosynthesis measuring system (Li-Cor Inc., Lincoln, NE, USA) on the third to fifth fully expanded leaf of each plant. The conditions for photosynthetic trait measurements were: leaf temperature 28°C, photosynthetic photon flux density (PPFD) 1400 μmol m⁻² s⁻¹, relative air humidity (RH) 60% and ambient CO₂ concentration (Ca) 400 μmol mol⁻¹.

Light and CO₂ response measurements

Pn, Gs, Ci and Tr responses to PPFD and Ca were also measured. Photosynthesis traits-PPFD curves were produced under a Ca concentration of 400 μmol mol⁻¹, leaf temperature around

Fig 1. DNA content of sn2.1 (a: the main peak at channel 100), sn3.8 (b: the main peak at channel 150) and sn4.1 (c: the main peak at channel 200). The ordinate (count) represents the relative value of the cell population, the abscissa (FL1) represents the passageway value of fluorescence. The abscissa location which peak point corresponding were the ploidy of test sample.

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28°C and relative humidity about 60%. The light intensities varied with 0, 20, 50, 80, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 μmol m$^{-2}$ s$^{-1}$. Response curves were modeled by a quadratic equation (Eq 1) according to Zhao [26, 27]. Light saturation point (LSP) and light compensation point (LCP) were evaluated by fitting data to the model function. Pn-Ca curves were measured under saturating light intensity (1400 μmol m$^{-2}$ s$^{-1}$), and a temperature and humidity around 28°C and 60%. The CO$_2$ concentrations in the leaf chamber were set at 0, 50, 80, 100, 200, 300, 400, 600, 800, 1000, 1200, 1400 and 1600 μmol mol$^{-1}$. Model 1 was used to create response curves and to evaluate the carbon dioxide saturation point (CSP) and carbon dioxide compensation point (CCP).

$$Y = b_0 + b_1X + b_2X^2$$

(1)

Where $Y$ was the Pn value, $X$ was the PPFD (Ca), $b_0$ was constant, and $b_1$ and $b_2$ were coefficients, respectively.

**Chlorophyll fluorescence parameter measurements**

Chlorophyll fluorescence parameters were measured using a pulse amplitude modulation chlorophyll fluorometer MINI-PAM2500 (Walz, Effeltrich, Germany). Minimal fluorescence, $F_0$, was measured in 30-min dark-adapted leaves using weak modulated light of < 0.15 μmol m$^{-2}$ s$^{-1}$. Maximal fluorescence, $F_m$, was measured after a 0.8-s saturating white light pulse (6000 μmol m$^{-2}$ s$^{-1}$) in the same leaves. Maximal variable fluorescence ($F_v = F_m - F_0$) and the photochemical efficiency of PSII ($F_v/F_m$) for dark-adapted leaves were calculated.

**Data analysis**

Statistical analyses were carried out using the Statistical Product and Service Solutions (SPSS 19.0) software. All the growth traits, instantaneous photosynthetic and chlorophyll fluorescence parameters were compared using analysis of variance; the significance of fixed effects was tested with F-tests.

The coefficient of phenotypic variation (PCV) of all the investigated traits was calculated using the following formula [28]:

$$PCV = \frac{SD \times 100}{\bar{X}}$$

(2)

where $\bar{X}$ is the phenotypic mean of the trait and SD is the standard deviation of the trait.

The repeatability ($R$) of all the investigated traits was calculated as follows [29]:

$$R = \frac{\sigma^2}{\sigma^2 + \sigma^2_e}$$

(3)

where $\sigma^2$ is the genetic variance component between clones and $\sigma^2_e$ is the error variance component.

The phenotype correlation $r_A(xy)$ of traits $x$ and $y$ was calculated as follows [30]:

$$r_A(xy) = \frac{\sigma_{a(xy)}}{\sqrt{\sigma^2_{a(x)} \cdot \sigma^2_{a(y)}}}$$

(4)

where $\sigma^2_{a(x)}$ is the clone variance component for the trait $x$, $\sigma^2_{a(y)}$ is the clone variance component for the trait $y$ and $\sigma_{a(xy)}$ is the clone covariance component.
Results

Variation among growth traits

The results of ANOVA for all growth traits are presented in Table 1. There were significant differences among the clones with different ploidies ($P < 0.01$) and clones, except for LFW among clones ($P = 0.014$) based on overall $F$-tests. The PCVs of different growth traits ranged from 12.60% to 43.36%, with the value of BD being the lowest and that of SFW being the highest, respectively. The repeatabilities ($R$) of the different traits are also presented in Table 1. The $R$ values for all growth traits were higher than 0.60. High $R$ and PCV are favorable for excellent clone selection.

Average growth traits for different ploidy poplar clones

The average growth traits of hybrid poplar clones with different ploidies are summarized in Table 2. The overall average tree height and average base diameter were 35.54 cm and 3.49 mm, respectively, for all plants. The average values of all growth traits in the triploids were higher than those in the tetraploid and diploid hybrid clones, and the diploid hybrid clones had the lowest growth traits.

Pn-PPFD curves

The Pn-PPFD curves of different poplar clones are shown in Fig 2. The Pn-PPFD curves of all 12 clones were S-shaped. The Pn values of all clones increased as PPFD increased in low light conditions and reached a plateau when PPFD reached 1200–1400 μmol m$^{-2}$ s$^{-1}$. The triploid hybrid clones showed higher Pn values than the diploid and tetraploid hybrid clones. In particular, sn3.8 had the highest Pn values across all light intensities.

Table 1. ANOVA analyses, PCV and R of different traits among different ploidies and clones.

| Traits | Variation source | SS     | df  | MS     | F      | $P$ value | PCV (%) | R   |
|--------|------------------|--------|-----|--------|--------|-----------|---------|-----|
| H      | Ploidies         | 1058.400 | 2   | 529.200 | 41.422 | 0.000**   | 18.76   | 0.892|
|       | Clones           | 1197.333 | 11  | 108.848 | 9.242  | 0.000**   | 12.60   | 0.690|
| BD     | Ploidies         | 2.896   | 2   | 1.448  | 12.221 | 0.000**   | 12.60   | 0.690|
|        | Clones           | 4.058   | 11  | 0.369  | 3.221  | 0.008**   | 18.86   | 0.822|
| LN     | Ploidies         | 179.706 | 2   | 89.853 | 14.886 | 0.000**   | 12.60   | 0.690|
|        | Clones           | 272.889 | 11  | 24.808 | 5.617  | 0.000**   | 27.89   | 0.807|
| LA     | Ploidies         | 1365.937| 2   | 682.969| 17.530 | 0.000**   | 27.89   | 0.807|
|        | Clones           | 1867.071| 11  | 169.734| 5.192  | 0.000**   | 27.89   | 0.807|
| SFW    | Ploidies         | 77.325  | 2   | 38.663 | 17.593 | 0.000**   | 43.36   | 0.943|
|        | Clones           | 104.961 | 11  | 9.542  | 17.593 | 0.000**   | 42.08   | 0.899|
| SDW    | Ploidies         | 3.054   | 2   | 1.527  | 10.839 | 0.000**   | 42.08   | 0.899|
|        | Clones           | 6.310   | 11  | 0.574  | 9.878  | 0.000**   | 22.73   | 0.656|
| LFW    | Ploidies         | .568    | 2   | .284   | 11.716 | 0.000**   | 22.73   | 0.656|
|        | Clones           | 0.782   | 11  | 0.071  | 2.907  | 0.014*    | 22.73   | 0.656|
| LDW    | Ploidies         | .079    | 2   | .040   | 15.886 | 0.000**   | 22.73   | 0.656|
|        | Clones           | 0.101   | 11  | 0.009  | 3.613  | 0.004**   | 27.01   | 0.723|

**(*) indicated variance is significant at the 0.01(0.05) level

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Gs-PPFD, Ci-PPFD and Tr-PPFD curves

Gs-PPFD, Ci-PPFD and Tr-PPFD curves are shown in Figs 3–5. Gs and Tr values had an upward tendency in all clones as PPFD increased. Clones sn3.8, sn3.2 and sn3.1 had higher Gs values than the other clones at different light intensities: as PPFD increased, these differences became larger. Clones sn2.3, sn2.2 and sn had obviously lower Gs values than the other clones.

By contrast, the Ci-PPFD curves showed a downward tendency, and the Ci values of all clones declined as PPFD increased. This indicated that as Pn values increased, more CO2 was needed for photosynthesis, and the concentration of CO2 used between the cell spaces decreased.

### Table 2. Average growth traits for different hybrid clones of different ploidies.

| Ploidy | clone | H (m)   | BD (mm)  | LN | LA (cm²) | SFW (g) | SDW (g) | LFW (g) | LDW (g) |
|--------|-------|---------|----------|----|----------|---------|---------|---------|---------|
| Tetraploid | 4.1   | 33.00±2.65 | 3.51±0.18 | 15.67±2.08 | 27.44±1.64 | 3.88±0.16 | 0.95±0.18 | 0.78±0.16 | 0.21±0.04 |
|         | 4.2   | 35.00±2.68 | 3.54±0.24 | 16.67±2.08 | 30.00±1.73 | 3.92±0.80 | 0.99±0.14 | 0.88±0.19 | 0.27±0.06 |
|         | 4.3   | 31.00±2.44 | 3.52±0.12 | 15.67±2.08 | 28.11±2.03 | 3.85±0.46 | 0.90±0.19 | 0.79±0.10 | 0.21±0.05 |
|         | average | 33.00±2.87 | 3.52±0.16 | 16.00±1.87 | 28.52±2.61 | 3.88±0.47 | 0.95±0.19 | 0.82±0.14 | 0.23±0.05 |
| Triploid | 3.1   | 39.33±2.08 | 3.77±0.18 | 20.67±2.08 | 37.00±6.09 | 5.46±0.31 | 1.46±0.45 | 0.94±0.12 | 0.34±0.04 |
|         | 3.2   | 41.33±3.15 | 3.81±0.18 | 23.33±2.31 | 39.33±6.37 | 6.52±0.50 | 1.61±0.10 | 1.07±0.20 | 0.31±0.03 |
|         | 3.4   | 38.67±2.03 | 3.55±0.39 | 18.00±2.65 | 31.67±7.64 | 4.71±0.49 | 1.03±0.28 | 0.92±0.20 | 0.33±0.04 |
|         | 3.8   | 45.00±4.58 | 4.17±0.30 | 21.33±2.89 | 47.00±3.67 | 7.56±0.64 | 2.21±0.33 | 1.19±0.11 | 0.29±0.02 |
|         | 3.9   | 39.33±3.21 | 3.62±0.50 | 17.00±1.73 | 36.33±4.73 | 5.01±0.89 | 0.96±0.22 | 0.94±0.10 | 0.25±0.03 |
|         | Average | 40.73±3.83 | 3.78±0.35 | 20.07±3.10 | 38.27±8.27 | 5.85±1.36 | 1.45±0.53 | 1.01±0.17 | 0.31±0.06 |
| Dihplod | 2.1   | 30.33±4.93 | 3.40±0.42 | 15.67±2.31 | 27.67±5.49 | 3.71±0.88 | 0.87±0.19 | 0.75±0.23 | 0.20±0.05 |
|         | 2.2   | 25.67±1.15 | 2.96±0.62 | 14.00±1.80 | 23.67±3.79 | 1.11±0.28 | 0.61±0.02 | 0.74±0.20 | 0.20±0.04 |
|         | 2.3   | 28.33±5.03 | 2.92±0.39 | 14.67±2.08 | 19.78±1.58 | 2.16±0.10 | 0.85±0.09 | 0.64±0.10 | 0.20±0.03 |
|         | 2.4   | 29.00±2.65 | 3.21±0.18 | 16.67±1.53 | 26.56±5.87 | 2.94±0.20 | 0.94±0.26 | 0.78±0.07 | 0.21±0.02 |
|         | average | 28.33±3.70 | 3.13±0.42 | 15.25±1.82 | 24.42±5.00 | 2.48±1.08 | 0.82±0.20 | 0.73±0.15 | 0.20±0.04 |

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Gs-PPFD, Ci-PPFD and Tr-PPFD curves

Gs-PPFD, Ci-PPFD and Tr-PPFD curves are shown in Figs 3–5. Gs and Tr values had an upward tendency in all clones as PPFD increased. Clones sn3.8, sn3.2 and sn3.1 had higher Gs values than the other clones at different light intensities: as PPFD increased, these differences became larger. Clones sn2.3, sn2.2 and sn had obviously lower Gs values than the other clones. By contrast, the Ci-PPFD curves showed a downward tendency, and the Ci values of all clones declined as PPFD increased. This indicated that as Pn values increased, more CO2 was needed for photosynthesis, and the concentration of CO2 used between the cell spaces decreased. In

![Fig 2. Pn-PPFD curves of poplar clones of different ploidies.](https://example.com/fig2.png)

Different ploidy clones are shown by different line types and identification. Triploids are shown by solid lines, diploid and tetraploid are shown as different types of dotted and dashed lines.

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the Ci-PPFD curves, the Ci values of sn2.3, sn2.2 and sn were higher than those of the other clones, while the Ci values of sn3.8, sn3.2 and sn3.1 were much lower.

Pn-PPFD curve model

To understand the change in Pn values at different levels of PPFD, quadratic functions were used to simulate the Pn-PPFD relationship. Two simulation curves for sn3.8 and sn2.3 are presented in Fig 6. The parameters used for the simulation curves are listed in Table 3. Parameter \( R^2 \) was higher than 0.9, which indicated that the model was effective. The parameters used for LSP and point LCP are also presented in Table 3. The average LSP of the triploid poplar clones
exceeded that of the tetraploid and diploid clones. The LCP of the triploid poplar clones was lower than that of the diploid and tetraploid clones, which suggested that the triploid poplar clones had greater adaptability to different light intensities. When the PPFD reached the LCP, different clones expressed Pn differently. Pn varied from $15.90 \mu\text{mol m}^{-2}\text{s}^{-1}$ (sn2.3) to $25.01 \mu\text{mol m}^{-2}\text{s}^{-1}$ (sn3.8) and the average Pn of the triploid clones was higher than that of the tetraploids and diploids.

Pn-Ca curves

The Pn-Ca curves of different poplar clones are shown in Fig 7. The Pn-Ca curves were shaped like an inverted “V”. As Ca increased, Pn increased initially and then decreased. The pattern was the same as the Pn-PPFD curves; triploid clones showed higher values than diploid and tetraploid clones. In particular, sn3.2 and sn3.8 showed clearly higher values at different CO$_2$ concentrations. The Pn-Ca curves were also simulated using quadratic models; the simulated curves for sn3.8 and sn2.3 are shown in Fig 8 and the model parameters are presented in
Table 4. The $R^2$ values of all curve models were higher than 0.9, which indicated that all models were highly efficient. The diploid poplar clones showed higher average CSP and CCP values than the triploids and tetraploids. The average $P_n$ value of the CO$_2$ concentration at the CSP of diploid clones was lower than in other ploidy level poplar clones. Clone sn3.8 still showed a higher $P_n$ at the CSP, but sn had a lower value.
Instantaneous photosynthetic and chlorophyll fluorescence factors

ANOVA analyses of photosynthetic and chlorophyll fluorescence factors among different clones are shown in Table 5. All factors showed significant differences ($P < 0.01$) among clones or ploidies levels. PCV varied from 2.38% (Fv/Fm) to 56.71% (Gs) and repeatability ranged from 0.745 (Ci) to 0.987 (Fv/Fm). The average values for each photosynthetic factor under conditions of leaf temperature 28°C, PPFD 1400 $\mu$mol m$^{-2}$ s$^{-1}$, RH 60% and Ca 400 $\mu$mol mol$^{-1}$ are shown in Table 6. The triploids showed high Pn, Gs and Tr, but low Ci values. As with the photosynthetic factors, the triploid poplar clones had higher Fv/Fm values than the diploid and tetraploid clones.

![Fig 8. Pn-Ca simulated curve of clone sn3.8 (left) and sn2.3 (right). Solid lines are the simulated curves and white circles are the observed data.](image)

Table 4. Coefficients and variation parameters for each Pn-Ca simulated curve.

| Ploidy | Clone | a     | b     | c     | CSP   | CCP   | Pn   | $R^2$  |
|--------|-------|-------|-------|-------|-------|-------|------|--------|
| Tetraploid | sn4.1 | -2.573E-05 | 0.045 | 0.339 | 865.363 | 11.341 | 19.605 | 0.935  |
|         | sn4.2 | -2.484E-05 | 0.047 | -0.415 | 945.794 | 24.341 | 21.807 | 0.975  |
|         | sn4.3 | -2.485E-05 | 0.048 | -1.334 | 958.039 | 18.604 | 21.475 | 0.988  |
|         | Average | 923.066 | 18.096 | 20.962 |       |       |      |        |
| Triploid | sn3.1 | -2.876E-05 | 0.053 | -0.600 | 919.055 | 21.177 | 23.689 | 0.971  |
|         | sn3.2 | -3.031E-05 | 0.057 | -0.591 | 937.974 | 27.083 | 26.079 | 0.967  |
|         | sn3.4 | -2.950E-05 | 0.055 | -0.565 | 927.981 | 24.179 | 24.836 | 0.969  |
|         | sn3.8 | -2.980E-05 | 0.056 | -0.446 | 940.023 | 27.024 | 25.884 | 0.965  |
|         | sn3.9 | -2.912E-05 | 0.053 | -0.623 | 914.070 | 21.667 | 23.709 | 0.973  |
|         | Average | 927.821 | 24.226 | 24.839 |       |       |      |        |
| Diploid | Sn    | -1.166E-05 | 0.028 | -1.555 | 1216.240 | 33.422 | 15.694 | 0.971  |
|         | sn2.1 | -2.030E-05 | 0.038 | -0.530 | 928.700 | 30.049 | 16.979 | 0.980  |
|         | sn2.2 | -1.174E-05 | 0.029 | -1.599 | 1214.183 | 34.145 | 15.705 | 0.971  |
|         | sn2.3 | -1.572E-05 | 0.035 | -0.746 | 1114.362 | 8.706  | 18.775 | 0.991  |
|         | Average | 1118.371 | 26.580 | 16.788 |       |       |      |        |

Parameters a, b and c are coefficients of the quadratic function. $R^2$ is the criterion for evaluating the effectiveness of the model. The unit for CSP and CCP is $\mu$mol mol$^{-1}$ and the unit for Pn is $\mu$mol m$^{-2}$ s$^{-1}$.  

![image](image)

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Correlation coefficients

The correlation coefficients of different traits are shown in Table 7. All pair-wise correlation coefficients were significant ($P < 0.01$). The correlation coefficients among growth traits (H, BD, LN, LA, LFW, SFW, LDW and SDW) ranged from 0.512 to 0.857. There were also significant positive correlations between $P_n$ and $G_s$, $P_n$ and $T_r$, and $G_s$ and $T_r$, but $C_i$ was negatively correlated with the other photosynthetic factors. All growth traits were positively correlated with $P_n$, $G_s$ and $T_r$, and negatively correlated with $C_i$. $F_v/F_m$ was positively correlated with growth traits. As with growth factors, $F_v/F_m$ was positively correlated with $P_n$, $G_s$ and $T_r$, and negatively correlated with $C_i$.

Table 7. ANOVA analyses for $PCV$ and $R$ of clones of different ploidies and clones in photosynthetic and chlorophyll fluorescence traits.

| Traits | Variation source | SS    | df | MS    | $F$   | $P$ value | PCV (%) | $R$ |
|--------|------------------|-------|----|-------|-------|-----------|---------|-----|
| $P_n$  | Ploidies         | 197.450 | 2  | 98.725 | 30.582 | 0.000**   | 16.29   | 0.961|
|        | Clones           | 279.979 | 11 | 25.453 | 25.453 | 0.000**   | 14.60   | 0.987|
| $G_s$  | Ploidies         | 2.472  | 2  | 1.236  | 26.497 | 0.000**   | 10.68   | 0.745|
|        | Clones           | 3.899  | 11 | 0.354  | 76.298 | 0.000**   | 10.29   | 0.745|
| $C_i$  | Ploidies         | 9426.351 | 2  | 4713.176 | 6.113 | 0.006**  | 56.71   | 0.987|
|        | Clones           | 14620.579 | 11 | 1329.176 | 3.922 | 0.002**  | 56.71   | 0.987|
| $T_r$  | Ploidies         | 78.277  | 2  | 39.138 | 33.372 | 0.000**   | 31.77   | 0.963|
|        | Clones           | 108.312 | 11 | 9.847  | 27.266 | 0.000**   | 31.77   | 0.963|
| $F_v/F_m$ | Ploidies       | 0.010  | 2  | 0.005  | 62.893 | 0.000**   | 2.38    | 0.987|
|        | Clones           | 0.013  | 11 | 0.001  | 79.311 | 0.000**   | 2.38    | 0.987|

** indicates that the variance is significant at the 0.01 level.

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Table 6. Average photosynthetic and chlorophyll fluorescence traits of hybrid clones of different ploidies.

| Ploidy | Clone | $P_n$ | $G_s$ | $C_i$ | $T_r$ | $F_v/F_m$ |
|--------|-------|-------|-------|-------|-------|-----------|
| Tetraploid | 4.1  | 15.90 | 0.40  | 311.00 | 5.19  | 0.813     |
|         | 4.2  | 16.30 | 0.63  | 300.00 | 5.41  | 0.815     |
|         | 4.3  | 18.00 | 0.53  | 310.00 | 6.23  | 0.800     |
|         | Average | 16.73 | 0.52  | 307.00 | 5.61  | 0.809     |
| Triploid | 3.1  | 20.33 | 1.21  | 276.00 | 8.63  | 0.828     |
|         | 3.2  | 22.50 | 0.93  | 274.67 | 7.65  | 0.838     |
|         | 3.4  | 18.20 | 0.58  | 293.67 | 5.84  | 0.815     |
|         | 3.8  | 23.80 | 1.21  | 259.67 | 8.63  | 0.846     |
|         | Average | 20.83 | 0.89  | 280.80 | 7.32  | 0.831     |
| Diploid  | 2.1  | 16.80 | 0.37  | 307.00 | 4.82  | 0.792     |
|         | 2.2  | 14.80 | 0.26  | 321.00 | 3.27  | 0.775     |
|         | 2.3  | 14.63 | 0.22  | 324.00 | 3.39  | 0.786     |
|         | 2.4  | 16.57 | 0.30  | 316.00 | 4.13  | 0.798     |
|         | Average | 15.70 | 0.29  | 317.00 | 3.90  | 0.788     |

The units for $P_n$, $G_s$, $C_i$ and $T_r$ are μmol m⁻² s⁻¹, μmol m⁻² s⁻¹, μmol mol⁻¹ and mol m⁻² s⁻¹, respectively.

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Discussion

Polyploidy is an important force in plant evolution [31]. In general, because of the increased heterozygosity and genic and biochemical flexibility provided by the additional alleles, polyploid plants are often larger and have larger cells. Polyploids are considered better able to colonize a wider range of habitats and survive in harsh, unstable environments [23], [31]. The major cytological mechanisms of polyploidy are the union of unreduced gametes, somatic doubling and polyspermy [32]. The union of unreduced gametes used in this research is one of the most important mechanisms for obtaining polyploids [32]. In terms of growth traits at different ploidies, Zhu [24] found that there were significant differences between diploid and tetraploid *P. davidiana* Dode hybrid clones in terms of tree height, basal diameter and chlorophyll content. In 1995, Zhu found that triploid *P. tomentosa* clones grew faster than diploid clones [33]. In this study, triploid poplar clones had clearly higher values for all growth traits compared with diploid and tetraploid poplar clones. Tetraploid poplar clones did not show superiority to diploid poplar clones in growth traits, which agreed with the results of Comai [34] who indicated that because of the ploidy increase, increased numbers of chromosomes led to increased numbers of cells and nuclei, thereby increasing the possibility of producing aneuploidy in meiosis and mitosis, resulting in epigenetic instability.

The most common parameter used to describe the extent of variability in a breeding population is the average PCV. In this study, the average PCVs of the traits ranged from 2.38% (Fv/Fm) to 56.71% (Gs). Except for Fv/Fm, the PCV of all traits exceeded 10%. The repeatability value indicates the reliability with which a genotype can be recognized by its phenotypic expression. In this study, the estimates of repeatability for all traits at the clone mean level ranged from 0.656 (LFW) to 0.987 (Fv/Fm), which is in general agreement with research by Kien [35]. The high PCV and high repeatability indicated that the variation of each trait in poplar hybrid clones with different ploidies is not significantly influenced by environmental factors and that selection and evaluation of these clones will be effective [36].

Light can affect the enzyme activity and stomatal aperture of plant leaves. Without light, there is no photosynthesis. The light compensation point is a critical value; at this point, the amounts of CO2 used in photosynthesis and created by respiration are equal. The light saturation point is a limiting value; over this value, the photosynthetic rate does not increase, and

Table 7. Pair-wise correlation coefficients of different traits.

| Trait | BD    | LN    | LA    | SFW   | SDW   | LFW   | LDW   | Pn    | Gs    | Ci    | Tr    | Fv/Fm |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| H     | 0.657** | 0.692** | 0.786** | 0.840** | 0.657** | 0.726** | 0.638** | 0.755** | 0.756** | -0.489** | 0.748** | 0.858** |
| BD    | 0.535** | 0.567** | 0.681** | 0.512** | 0.618** | 0.584** | 0.640** | 0.673** | 0.460** | 0.674** | 0.748** |
| LN    | 0.690** | 0.716** | 0.678** | 0.536** | 0.655** | 0.705** | 0.727** | 0.727** | -0.518** | 0.663** | 0.758** |
| LA    | 0.769** | 0.725** | 0.717** | 0.632** | 0.794** | 0.710** | 0.575** | 0.739** | 0.820** |
| SFW   | 0.857** | 0.764** | 0.646** | 0.842** | 0.810** | 0.598** | 0.822** | 0.887** |
| SDW   | 0.689** | 0.594** | 0.768** | 0.664** | 0.803** | 0.722** | 0.770** |
| LFW   | 0.586** | 0.658** | 0.626** | 0.557** | 0.607** | 0.739** |
| LDW   | 0.533** | 0.633** | 0.502** | 0.593** | 0.633** |
| Pn    | 0.858** | -0.449** | 0.920** | 0.856** |
| Gs    | 0.469** | 0.938** | 0.839** |
| Ci    | -0.457** | -0.660** |
| Tr    | 0.845** |

**Correlation is significant at the 0.01 level (2-tailed).**

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may decrease [37]. The LSP in plants is important under high light conditions, as the dark reaction follows the light reaction in photosynthesis and then limits the instantaneous photosynthetic rate [38]. Poplars are shade-avoiding species, and our results showed that they had a high photosynthetic rate (15.90–25.01 μmol m⁻² s⁻¹) when the light radiation reached the LSP. The five triploid clones had higher LSPs and lower LCPs, indicating that triploid clones can adapt to extensive light illumination intensities. These results were similar to those of Fu [39] for rice and Qi [16] for poplar. At the LSP, the Pn values of the triploid clones were higher than those of the tetraploid and diploid clones. There were no significant variations between the tetraploid and diploid clones. These variations might reflect differences in leaf structure, composition, transcriptional level, methylation of DNA or nucleolus dominance in clones of different ploidies, which should be explored in future research [16]. As the light illumination intensity increased, Gs and Tr showed the same regular pattern as Pn, indicating that the photosynthetic indices interacted with each other during photosynthesis. These results are in agreement with research carried out on *P. tomentosa* [26] and *Catalpa bungei* [27].

CO₂ is the most important substrate in photosynthesis. Within a certain concentration range, enhanced CO₂ concentration can promote the instantaneous photosynthetic rate [40]. There is strong evidence that plants have already responded to the increase in atmospheric CO₂ concentration [41]. Atmospheric CO₂ concentrations are projected to double from the current concentration of 350 μmol mol⁻¹ to 700 μmol mol⁻¹, which will further stimulate plant growth and ecosystem changes within the next 80 years [37]. CO₂ concentration is the main limiting factor of photosynthesis for plants when the temperature and humidity are moderate and the light radiation is saturated. In this study, as the CO₂ concentration increased, the Pn values of poplar clones with different ploidies initially increased and then decreased. The CSP values of the diploids were obviously higher than those of the tetraploid and triploid poplar clones. However, at the CSP, the Pn values of the triploids were higher than those of the diploids and tetraploids. These results suggested that after 80 years, when the CO₂ concentration is higher than 700 μmol mol⁻¹, diploid poplar may be more preferable than triploid and tetraploid because of its higher CSP.

Chlorophyll fluorescence is a measure of photosynthetic performance and is widely used by plant physiologists and ecophysiologists [42]. The ratio Fv/Fm is the most frequently used parameter in ecophysiological research to determine the maximum photochemical efficiency of PSII [43, 44]. This ratio, typically ranging between 0.75 and 0.85, is proportional to the effectiveness of light energy utilization under standard conditions of CO₂ fixation and to the quantum yield of photochemical processes [45]. In this study, Fv/Fm values ranged from 0.775 to 0.846, which indicated that the plants were not under stress and that the greenhouse conditions were appropriate for poplar growth. However, the Fv/Fm values were higher in the triploid and tetraploid clones than in the diploid clones. Polyploid poplar clones might have higher pre-resistance than diploid poplar clones [46].

Ceulemans [47] and Ma [48] indicated that the Pn and Gs of *Populus* clones were positively correlated with growth traits; Pn and Gs are tightly coupled [49]. In this research, strong positive correlations between growth traits and Pn, Gs, Tr and Fv/Fm were observed, and there were also significant correlations among all photosynthetic parameters, indicating that all traits influence and restrict each other, ultimately controlling plant growth.

**Conclusion**

In conclusion, there were significant variations in growth, photosynthetic and chlorophyll fluorescence (Fv/Fm) traits between different clones with ploidies, and there were also significant correlations between different traits, which suggested that all traits collaborated to promote
plant growth. Triploid clones showed higher growth traits and higher instantaneous photosynthetic and chlorophyll fluorescence factors than diploid and tetraploid clones, which indicated that triploid clones were preferable at the seedling stage. Clone sn3.8 showed high growth traits, Pn and Fv/Fm values, and should be investigated further in future studies. Additional investigations are also needed to determine the transcriptomic, proteomic, metabolomic and structural differences between poplars with different ploidies, to explain the variation in chromosome polyploidy, and to improve the genetic manipulation of poplar.

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Author Contributions

Conceived and designed the experiments: XZ GL CY. Performed the experiments: XZ MZ XB ML. Analyzed the data: YS SL CY JJ. Contributed reagents/materials/analysis tools: CY JJ FW SL YC XB. Wrote the paper: XZ YL. Critical reading of the manuscript: YL.

References

1. Zhao XY, Zheng HQ, Li SW, Yang CP, Jiang J, Liu GF. The rooting of poplar cutting: a review. New Forests. 2013; doi: 10.1007/s11056-013-9389-1
2. Sixto H, Salvia J, Barrio M, Ciria M, Canellas C. Genetic variation and genotype-environment interactions in short rotation Populus plantations in southern Europe. New Forests. 2011; 42:163–177
3. Tuskan GA, Difazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, et al. The genome of black cottonwood, Populus trichocarpa. Science. 2006; 313:1596–1604 PMID:16973872
4. Li XX, Wang JJ, Pang ZW, Zhao XJ, Li YC. Evaluation of growth and management density for Yingchun poplar. Journal of Northeast Forestry University. 1998; 26:24–27
5. Jiang ZH, Fan SH, Feng GX, Zhang Q, Liu GL, Zong YC. Biomass and distribution patterns of Populus×xiahei plantation in sandy land of north China. Scientia Silvae Sinicae. 2007; 43:15–20
6. Fan SH, Liu GL, Zhang Q, Feng CX, Zong YC, Ren HQ. A study on biomass and productivity of Populus×xiahei plantation on study land in north China. Forest Research. 2010; 23:71–76
7. Li ZX, Zhao XY, Yang CJ, Wang GY, Wang FS, Zhang LF, et al. Variation and growth adaptability analysis of transgenic Populus simonii×P. nigra lines carrying TaLEA gene. Journal of Beijing Forestry University. 2013; 35:57–62
8. Wang L, Zhou BR, Wu LL, Lv CY, Qu YJ, Zheng W, et al. Cloning and expression analysis of a ring zinc-finger gene in Populus simonii×P. nigra. Plant Physiology Communications. 2009; 45(12):1160–1166
9. Wang S, Liu MR, Huang HJ, Mu HZ, Li ZX, Liu GF. Selection of cold resistant strains from TaLEA gene transferred Populus simonii×P. nigra. Journal of Northeast Forestry University. 2011; 39:5–7,16
10. Yang CP, Liu GF, Liang HW, Zhang H. Study on the transformation of Populus simonii×P. nigra with salt resistance gene BET-A. Scientia Silvae Sinicae. 2001; 37:34–38
11. Jiang J, Chang YQ, Dong JX, Wang ZY, Liu GF. Study on two insecticidal transgenic genes in Populus simonii×P. nigra. Plant Physiology Communications. 2004; 40:669–672
12. Masterson J. Stomatal size in fossil plants: evidence for polyploid in majority of angiosperms. Science. 1994; 264:421–424 PMID:17836906
13. Wendel JF. Genome evolution in polyploids. Plant Mol Biol. 2000; 42:225–249 PMID:10688139
14. Li A, Hu BQ, Xue ZY, Chen L, Wang WX, Song WQ, et al. DNA methylation in genomes of several annual herbaceous and woody perennial plants of varying ploidy as detected by MSAP. Plant Mol Biol Rep. 2011; 29:784–793 PMID:21419835
15. Kang XY. Advances in researches on polyploid breeding of forest trees. Journal of Beijing Forestry University. 2003; 25:70–74
16. Qi CL, Jin CL, Li KL, Li ZX, Zhao H. Comparison of photosynthetic characteristics and leaf anatomy structure of different ploidy *Populus ussuriensis* Kom. Plant Physiology Journal. 2010; 46:917–922

17. Xing XT, Zhang ZY, Zhang WJ. Variations in wood thermal properties of triploid clones of *Populus tomentosa*. Journal of Beijing Forestry University. 2000; 22: 21–23

18. Nilsson EH. über eine in der nature gefunden gigasform von *Populus tremula*. Hereditas 1936; 21:379–382

19. Sylven N. Annual report on the work of the Association for Forest Tree Breeding during the year. Svensk PappTidn. 1943; 47: 38

20. Sarvas R. Two triploid aspen two triploid Birches. Commun Inst For Fenn. 1958; 49: 25

21. Johnsson H. Cytological studies of diploid and triploid *Populus tremula* and of crosses between them. Hereditas Lund. 1940; 28: 321–352

22. Buijtenen J P, Joranson P N, Einspahr D W. Diploid versus triploid aspen as pulpwood sources with reference to growth, chemical, physical and pulping differences. Tappi. 1958; 41: 170–175

23. Xi XJ, Li D, Xu WT, Guo LQ, Zhang JF, Li BL. 2n egg formation in populous euramericana (Dode) Guignier. Tree Genetics & Genomes.2012; 8: 1237–1245

24. Ewald D, Ulrich K, Naujoks G, Schroder MB. Induction of tetraploid poplar and black locust plants using colchicines: chloroplast number as an early marker for selecting polyploids in vitro. Plant Cell Tiss Organ Cult.2009; 99: 353–357

25. Zhang JE, Guo WW, Deng XX. Relationship between ploidy variation of citrus calli and competence for somatic embryogenesis. Acta Genetica Sinica. 2006; 33: 647–654 PMID: 16875323

26. Zhao XY, Ma KF, Zhang M, Biao JL, Jiao WY, Zhang ZY. Comparative analysis of the photosynthetic characteristics of three-year-old *Populus tomentosa* clones. Forest Research. 2011; 24:370–378

27. Hai PH, Jansson G, Harwood C, Thinh B, Huy H. Genetic variation in growth, stem straightness and branch thickness in clonal treatments of *Acacia auriculiformis* at three contrasting sites in Vietnam. For Ecol Manag.2008; 255:156–167

28. Hansen JK, Roulund H.Genetic parameters for spiral grain, stem form, Pilodyn and growth in 13 years old clones of Sitka Spruce (*Picea sitchensis* (Bong.) Carr.). Silvae Genet. 1996; 46: 107–113

29. Pliura A, Zhang SY, Mackay J, Mackay J, Bousquet J. Genotypic variation in wood density and growth traits of poplar hybrids at four clonal trials. For Ecol Manag. 2007; 238:92–106

30. Maherali H, Walden AE, Husband BC. Genome duplication and the evolution of physiological responses to water stress. New Phytol. 2009; 184: 721–731 doi: 10.1111/j.1469-8137.2009.02997.x PMID: 19703115

31. Ramsey J. Polyploidy and ecological adaptation in wild yarrow. Proc Natl Acad Sci. 2011; 108: 7096–7101 doi: 10.1073/pnas.1016631108 PMID: 21402904

32. Zhu ZT, Lin HB, Kang XY. Studies on allotriploid breeding of *Populus tomentosa* B301 clones. Sci Silvae Sin. 1995; 31:499–505

33. Corami L. The advantages and disadvantages of being polyploid. Nature reviews genetics. 2005; 6: 836–846 PMID: 16304599

34. Kien ND, Jansson G, Harwood C, Almqvist C, Thinh HH. Genetic variation in wood basic density and pilodyn penetration and their relationships with growth, stem straightness and branch size for *Eucalyptus urophylla* S. T. Blake in Northern Vietnam. N Z J For Sci. 2008; 38: 160–175

35. Maniee M, Kahrizi D, Mohammadi R. Genetic variability of some morphophysiological traits in durum wheat (*Triticum turgidum* var. durum). Journal of Applied Sciences. 2009; 9: 1383–1387

36. Zhou YM, Yang CP, Wang SJ, Wu YL, Wang WZ, Han SJ. A study on photosynthetic characteristics of Betula platyphylla. Journal of Forestry Research. 2002; 13: 209–212

37. Surabhi G, Reddy R, Singh S. Photosynthesis, fluorescence shoot biomass and seed weight responses of three cowpea (*Vigna unguiculata* (L.) Walp) cultivars with contrasting sensitivity to UV-B radiation. Environmental and Experimental Botany. 2009; 66:160–171

38. Fu YP, Yan HL, Li LF, Yu YH, Si HM, Hu GC, et al. Photosynthesis—related characteristics of different ploidy rice plants. Chinese J Rice Sci. 1999; 13:157–160

39. Ward JK, Strain BR. Elevated CO2 studies: past, present and future. Tree Physiol. 1999; 19:211–220 PMID: 12651563

40. Dippery JK, Tissue DT, Thomas RB, Strain BR. Effects of low and elevated CO2 and C3 and C4 annuals. I. growth and biomass allocation. Oecologia, 1995; 101:13–20
42. Cowley R, Luckett D. Chlorophyll fluorescence as a method to detect moisture limiting stress in canola. 17th Australian Research Assembly on Brassicas (ARAB). 2011.

43. Zhao XY, Wang JH, Zhang JF, Zhang ZS, Ma JW, et al. Variation analysis on chlorophyll fluorescence and growth traits of Catalpa bungei clones. Journal of Beijing University. 2012; 34: 41–47 doi: 10.1093/jncimonographs/lgs001 PMID: 22623594

44. Krause GH, Weis E. Chlorophyll fluorescence and photosynthesis: The basics. Annual Review of Plant Physiology and Plant Molecular Biology. 1991; 42: 313–349

45. Demming B, Bjorkman O. Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O2 evolution in leaves of higher plants. Planta. 1987; 171:171–184 doi: 10.1007/BF00391092 PMID: 24227324

46. Zheng HQ, Lin SZ, Zhang Q, Lei Y, Hou L, Zhang ZY. Functional identification and regulation of the PtDrl02 gene promoter from triploid white poplar. Plant Cell Rep. 2010; 29: 449–460 doi: 10.1007/s00299-010-0834-8 PMID: 20179934

47. Ceulemans R, Impens U, Steenackers V. Variations in photosynthetic, anatomical, and enzymatic leaf traits and correlations with growth in recently selected Populus hybrids. Can J For Res. 1987; 17:273–283

48. Ma KF, Song YP, Jiang XB, Zhang ZY, Li BL, Zhang DQ. Photosynthetic response to genome methylation affects the growth of Chinese white poplar. Tree Genetics & Genomes. 2012; 8: 1407–1421

49. Bassman JH, Zwier JC. Gas exchange characteristics of Populus trichocarpa, Populus deltoides and Populus trichocarpa x P. deltoides clones. Tree Physiol.1991; 2:145–159 PMID: 14972886