BMC Plant Biology

RESEARCH

Open Access

Comparison of the morphological and physiological characteristics of diploid and tetraploid *Luculia pinceana* Hook.

Lele Wang[1†], Yefang Li[2†], Hongling Li[2], Shifeng Li[3], Jie Song[3*] and Wenling Guan[1,2*]

Abstract

Background To explore the physiological and biochemical differences between different ploidy *Luculia pinceana* Hook. varieties and to obtain germplasm resources with excellent horticultural characteristics and strong resistance, we analysed and compared the morphological characteristics, photosynthetic properties, and physiological indices of diploid and tetraploid *L. pinceana*.

Results (1) Tetraploid *L. pinceana* exhibited distinct polyploid characteristics, including a smaller plant, rounder, thicker, and darker green leaves, as well as coarser and longer leaf and stem hairs. Moreover, the flowers of tetraploids are relatively large, and the diameters of the flowers, the lengths of the corolla tubes and the lengths of the pistils are all extremely significantly greater than those of diploids. Compared with those of tetraploids, the leaf width and thickness of the diploid plants increased by 16.38% and 14.71%, respectively, whereas the leaf length, leaf area, and plant height decreased by 21.20%, 3.46%, and 54.86%, respectively. (2) The diurnal variation curve of photosynthesis in *L. pinceana* was unimodal, reaching a maximum value at approximately 10:00. Compared with diploid plants, tetraploid plants presented a significantly greater maximum net photosynthetic rate (P_{max}), light saturation point (LSP), and light compensation point (LCP). (3) Compared with diploids, tetraploid leaves presented significantly greater activities of antioxidant enzymes, including superoxide dismutase (SOD, + 51.61%), peroxidase (POD, + 6226%), and catalase (CAT, + 211.66%). Additionally, the concentrations of osmolytes [soluble sugar (SS, + 80.11%), soluble protein (SP, + 63.49%), and proline (Pro, + 57.40%)] were markedly elevated. Conversely, the malondialdehyde (MDA) content was substantially decreased (-46.16%).

Conclusion These results demonstrate that the tetraploid *L. pinceana* has greater ornamental value, fertility and resistance. It is an excellent breeding material for the germplasm resources of *L. pinceana* and provides a material basis for the cultivation of new varieties of *L. pinceana*.

Keywords *Luculia pinceana* Hook, Tetraploid, Morphological characteristics, Photosynthetic properties, Physiological indices

[†]Lele Wang and Yefang Li contributed equally to this work.

*Correspondence:
Jie Song
songjie2591@126.com
Wenling Guan
13170627151@163.com
Full list of author information is available at the end of the article

Introduction

Luculia pinceana Hook., which belongs to the genus *Luculia* in Rubiaceae, is distinguished by its exceptionally long flowering period, elegant colouration, dense flower clusters, large inflorescences, and strong fragrance [1, 2]. Moreover, all the species within the genus *Luculia* are characterized by their typical distyly. Owing to their high ornamental value, plants of this genus have been introduced and cultivated worldwide [3]. There are approximately five species of *Luculia*, with three species and one variety found in China [4]. The three domestic species of *Luculia* genus are highly ornamental and represent valuable resources due to their floral display and fragrance, making them suitable for garden borders, pot culture, or cut flowers, indicating considerable potential for development [5–7]. Nevertheless, these plant species are prone to frost intolerance and have a high susceptibility to stem rot, which severely constrains industrialized development.

Currently, the development of new varieties of *L. pinceana* is limited primarily to the selection and breeding of natural variants [8, 9]. Polyploid breeding represents a significant strategy for generating new varieties and plays an essential role in plant evolution and speciation [10, 11]. Both natural and artificial polyploidization can significantly increase the vitality of species, and compared with diploids, polyploids exhibit greater physiological adaptability [12]. Polyploidization plays a crucial role in plant breeding and crop improvement. The current trend in polyploid development involves characterizing polyploids in terms of ultrastructures, bioactive substances, photosynthetic capacity, and metabolomics [13–16]. Compared with diploids, polyploids generally exhibit vigorous growth, larger organs, increased nutrient contents, and greater adaptability [17]. In particular, polyploids often present valuable phenotypic traits, such as larger leaves [18], broader and thicker foliage [19], sturdier stems [20], deeper leaf pigmentation [21], as well as more substantial and visually striking flowers [22], which significantly improve the ornamental value of these plants in horticulture.

The photosynthetic capacity of plants is generally correlated with leaf size, leaf thickness, stomatal dimensions, and the composition of photosynthetic pigments. The increase in photosynthesis in tetraploid plants is particularly reflected by photosynthetic parameters such as the net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular CO_2 concentration (Ci), transpiration rate (Tr), maximum net photosynthetic rate (P_{max}), and light saturation point (LSP). Compared with those of diploid varieties, the photosynthetic characteristics of tetraploid barley differ in terms of the Pn, Cond, and Tr. Moreover, the net photosynthetic rate of tetraploids significantly increases under strong light conditions compared with that of diploids [13, 23]. To elucidate the mechanisms underlying the adaptive advantages conferred by polyploidy, it is essential to investigate the physiological traits associated with polyploids. Among these traits, osmotic adjustment plays a crucial role in enabling plants to lower their osmotic potential under abiotic stresses (such as drought, extreme temperatures, salinity and alkalinity) to resist environmental changes [24, 25]. Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) can alleviate oxidative damage to plants under abiotic stress [26–28]. These enzymes are essential for enhancing the cellular capacity to combat reactive oxygen species under stressful conditions [29]. Osmotic adjustment and the antioxidant enzyme protection system are key physiological mechanisms and pathways that plants utilize to respond to stress, effectively preventing oxidative damage and preserving normal cellular functions [30–32].

Previous polyploid induction experiments on *L. pinceana* seeds employed colchicine immersion protocols. While preliminary cytogenetic screening successfully identified tetraploid variants, systematic characterization of their morphological adaptations and physiological modifications remains unaddressed in extant studies [3, 4]. In this study, polyploid *L. pinceana* seedlings were used as the experimental materials. The control group consisted of diploid *L. pinceana*. This study focused on the changes in their morphological characteristics, photosynthetic properties, and physiological indices, with the aim of obtaining germplasm resources with excellent horticultural traits and strong resistance. These findings lay the foundation for the breeding of new varieties of *L. pinceana*.

Materials and Methods

Plant materials

Seeds of *L. pinceana* collected from Motuo, Tibet, were treated with 0.6% (w/v) colchicine solution for 48 h and subsequently sown on MS media. After one month of cultivation in a growth chamber maintained at 25 °C ± 1 °C under a 12-h light/12-h dark photoperiod, the seedlings were transplanted into 32-cell seedling trays filled with a 1:1 (v/v) substrate mixture of peat moss and perlite. Presumed polyploid plants were preliminarily screened based on morphological characteristics, and tetraploid individuals were further confirmed through combined chromosome counting and flow cytometry analysis. Six months later, the tetraploid plants were transferred to plastic pots (15 cm diameter × 17 cm height) containing a 2:1:1 (v/v) mixture of peat moss, coconut coir, and perlite and grown in a greenhouse at 25 ± 3 °C and 60–70% relative humidity with natural sunlight exposure [3, 4]

[*L. pinceana* seeds were collected from Motuo County, Tibet, China (29°07'N, 95°33'E)]. The species identifier was Professor Guan Wenling of Yunnan Agricultural University, and the sample was stored at Yunnan Agricultural University (YAUNo. 20181203).

Measurement of morphological indicators

Following six months of cultivation in plastic pots, 30 diploid and 30 tetraploid *L. pinceana* plants from the same sowing batch, which exhibited uniform growth and the absence of diseases/pests, were selected for subsequent experimental procedures. For the leaf-related parameters, 10 plants were selected at each ploidy level, with three leaves at identical nodal positions (specifically, the 5th to 7th leaves basipetally from the apex) measured on each plant to standardize the developmental stages. The plant height is defined as the natural height from the base of the stem to the apex. The length from the leaf tip to the leaf base is designated the leaf length. The maximum width of the leaf, measured perpendicular to the leaf length, is defined as the width of the leaf. The leaf shape index is the ratio of leaf length to leaf width. The average thickness derived from measurements at the upper, middle, and lower sections of a single leaf is considered its overall thickness.

After two years of greenhouse cultivation, 10 diploid and 10 tetraploid *Luculia pinceana* plants were selected during their flowering phase for floral trait measurements. Pollen grains were collected for microscopic size analysis, and viability was assessed using potassium iodide (IKI) solution staining.Measurement of Photosynthetic Indicators.

In October 2022, three plants of both diploid and tetraploid genotypes with consistent growth were selected. The diurnal variation in photosynthesis and the light response curve of leaf photosynthesis in plants of different ploidy levels were determined using a Li- 6400 portable photosynthesis system (Beijing Ecotek Tech Co., Ltd.). Three biological replicates (individual plants) per ploidy level were analysed. Fully expanded functional leaves at identical developmental stages (node positions 7–9 from the apical meristem) were selected for measurement. Triplicate technical measurements were performed on each of three leaves per plant using nondestructive gas exchange protocols, ensuring methodological precision through measurement replication. These photosynthetic indicators were measured in the greenhouse of the College of Horticulture and Landscape Architecture, Yunnan Agricultural University.

Measurement of the daily variation of photosynthesis

The diurnal variation in photosynthesis was measured using a standard leaf chamber. Measurements were obtained every two hours from 8:00 to 19:00 (the last measurement was conducted at 19:00 due to darkness occurring at 19:30). The variations in temperature and light intensity throughout the day were systematically recorded, along with the net photosynthetic rate (Pn), transpiration rate (Trmmol), intercellular CO_2 concentration (Ci), and stomatal conductance (Cond).

Measurement of the photosynthesis light response curve of leaves

The LI- 6400 light-response automatic measurement program was employed, with the CO_2 concentration maintained at ambient levels (approximately 370–400 $\mu mol \cdot mol^{-1}$). The light intensity gradient was established as follows: 2000, 1800, 1500, 1200, 1000, 800, 600, 400, 200, 100, 50, 20, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The net photosynthesis rate (Pn), transpiration rate (Trmmol), intercellular CO_2 concentration (Ci), and stomatal conductance (Cond) were recorded. The photosynthesis light response curve (Pn-PAR curve) was constructed by plotting photosynthetically active radiation (PAR) on the x-axis and Pn on the y-axis.

Measurement of physiological indicators

One year after the transplantation of the tissue-cultured seedlings, five biological replicates (individual plants) per ploidy level were randomly sampled. Fully expanded functional leaves were surgically excised, immediately fragmented into 5-mm^2 sections using sterile blades, pooled into composite samples, flash-frozen in liquid nitrogen within 30 s of collection, and cryopreserved at − 80 °C until analysis. Three technical replicates per parameter were processed through independent analytical runs to control for batch effects. For this analysis, 0.1 g of fresh leaves from *L. pinceana* was ground in a mortar containing liquid nitrogen, along with 2 ml of phosphate buffer (pH =7.8). The resulting homogenized slurry was then transferred into a centrifuge tube and centrifuged at 4 ℃ for 25 min at 10,000 rpm. The supernatant was then collected and stored in a refrigerator at temperatures ranging from 0 to 4 °C for use as the enzyme mixture for measuring the activities of SOD, POD, and CAT.

The activity of superoxide dismutase (SOD) was determined using the nitroblue tetrazolium (NBT) method in accordance with the approach described by Li Hesheng [33]. To the sample tubes, 0.05 ml of enzyme mixture was added, and 0.05 mL of phosphate buffer was added to the control tubes. Three replicates were established for each sample. Next, 1.5 mL of phosphate buffer, 0.3 mL of 130 mmol/L methionine (Met), 0.3 mL of 750 μmol/L NBT, 0.3 mL of 100 μmol/L EDTA-Na_2, 0.25 mL of distilled water, and 0.3 mL of 20 μmol/L riboflavin were added to all the tubes in succession. All the tubes were illuminated at 4000 lx

for 30 min, and the absorbance at 560 nm (A560) was measured using a microplate reader. The SOD activity ($U \cdot g^{-1}$) was computed via the following formula:

$$\text{SOD activity}\left(U \cdot g^{-1}\right) = [(A_{CK} - A_{560}) \times V_T]/(0.5 \times A_{CK} \times W \times V_S)$$

where: A_{CK} represents the absorbance of the control tube at 560 nm; A_{560} represents the absorbance of the sample tube at 560 nm; V_T represents the total volume of the extracted enzyme mixture (mL); W represents the fresh weight of the sample (g); and V_S represents the volume of the enzyme mixture utilized for the determination (mL).

The peroxidase (POD) activity was determined using the guaiacol method. Fifty-six microlitres of guaiacol was added to 100 mL of phosphate buffer (pH 6.0), and the solution was fully dissolved using a heating stirrer. After cooling to room temperature, 38 μL of 30% hydrogen peroxide was added and thoroughly mixed to obtain the POD reaction mixture. Twenty microlitres of the enzyme mixture and 3 mL of the reaction mixture were added to the test tubes. Each sample was analysed three times, and the absorbance at 470 nm was measured using an enzyme-labelled instrument every minute for a total of three measurements.

$$\text{POD activity}\left[U \cdot (\min \cdot g)^{-1}\right] = (\Delta A_{470} \times V_t)/(W \times V_s \times 0.01 \times t)$$

where: ΔA_{470} represents the change in absorbance at 470 nm of the sample tube within the reaction time; V_t represents the total volume of the extracted enzyme mixture (mL); W represents the fresh weight of the sample (g); V_s represents the volume of the enzyme mixture used for the determination (mL); and t represents the reaction time (min).

A volume of 100 μL of the enzyme mixture was added, and 0.5 mL of 0.1 mol/L H_2O_2 and 2 mL of 0.1 mol/L phosphate buffer (pH 7.8) were added. Absorbance at 240 nm was measured using an enzyme-labelled instrument once every minute for a total of 3 measurements.

$$\text{CAT activity}\left[U \cdot (\min \cdot g)^{-1}\right] = (\Delta A_{240} \times V_t)/(W \times V_s \times 0.1 \times t)$$

where: ΔA_{240} represents the change in absorbance at 240 nm of the sample tube within the reaction time; V_t represents the total volume of the extracted enzyme mixture (mL); W represents the fresh weight of the sample (g); V_s represents the volume of the enzyme mixture used for measurement (mL); and t represents the reaction time (min).

The malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) method. Fresh leaves (0.1 g) were ground to a powder state in a mortar containing liquid nitrogen. Two millilitres of 5% trichloroacetic acid (TCA) was added, and the mixture was ground to form a homogenate, which was then transferred to a 2-mL centrifuge tube. The mixture was subsequently centrifuged at 4 °C and 1000 rpm for 20 min. The supernatant was collected after centrifugation. For 1 mL of the supernatant and 1 mL of 5% TCA as the control, 1 mL of 0.6% TBA was added to each sample. The samples were sealed and heated in boiling water for 15 min. After cooling, the samples were centrifuged again (5000 rpm, 10 min), and the absorbance of the supernatants at 450 nm, 532 nm, and 600 nm was measured using an enzyme marker.

$$\text{The MDA content}\left(\text{mmol} \cdot g^{-1}\right) = [(6.45(A_{532} - A_{600}) - 0.56\, A_{450}) \times V_T \times V]/(W \times V_s)$$

where: A_{450}, A_{532}, and A_{600} represent the absorbances at wavelengths of 450 nm, 532 nm, and 600 nm respectively; W represents the fresh weight of the sample (g); V represents the total volume of the extract (mL); V_S represents the volume of the extract added to the test solution (mL); V_T represents the total volume of the test solution (mL).

The contents of soluble sugar (SS), soluble protein (SP), and proline (Pro) were determined using kits from Suzhou Geruishi Biotechnology Co., Ltd. (Table S1).

To measure the chlorophyll content of leaves from plants with different ploidy levels, a SPAD- 502Plus chlorophyll meter was used. The average value was taken from the upper, middle and lower parts of each leaf. For each plant, three leaves were measured, and ten plants of each ploidy level were measured.

Statistical analysis

Data variance analysis was carried out using SPSS software. The normal distribution of the data was examined,

and the homogeneity of variance was verified. If the data failed to follow a normal distribution or the variance was not homogeneous, nonparametric tests were employed; if the data variance was homogeneous, t tests were adopted for data analysis. Duncan's test was used to detect differences.

Results

Morphological *L. pinceana* Seedlings with Different Ploidy Levels

Compared with diploid seedlings (Fig. 1A and C), tetraploid seedlings are shorter in stature with rounder leaves and a darker leaf colour (Fig. 1D). The leaves of polyploid plants have an increased thickness, presenting a succulent texture both visually and tactilely. Deformation occurs in the leaves of polyploid plants, which are no longer smooth, and some leaves exhibit slight curling and deformation. Moreover, there were villi on the backs of the leaves (Fig. 1B).

The different ploidy levels of *L. pinceana* result in different flower structures. The flowers and pollen grains of tetraploid *L. pinceana* are larger than those of diploid *L. pinceana*, and the pollen viability of tetraploid *L. pinceana* is greater than that of diploid *L. pinceana* (Fig. 2). As shown in Fig. 3, the flower stem length of tetraploids (44.85 ± 2.24 cm) increased by 37% compared with that of diploids (32.68 ± 4.28 cm) (Fig. 3G), the corolla tube length increased by 33% (Fig. 3H), the pistil length increased by 63% (Fig. 3J), the pollen size increased by 32% (Fig. 3K), and the pollen viability increased by 166% (Fig. 3L), all of which were extremely significant differences ($P < 0.001$). However, the increase in ploidy did not significantly change the stamen length of *L. pinceana* (Fig. 3I).

As shown in Fig. 3, extremely significant differences in leaf length, leaf width, leaf shape index, plant height, leaf area, and leaf thickness were detected between the tetraploid seedlings and the diploid control ($P < 0.001$). The leaf width of the tetraploid seedlings (6.18 cm) was 16.38% greater than that of the diploid control (5.31 cm) (Fig. 3C). The leaf thickness of the tetraploid seedlings (0.39 mm) was extremely significantly greater than that of the diploid seedlings (0.34 mm), with an increase of 14.71% (Fig. 3E). The leaf length of the tetraploid seedlings (12.64 cm) was 21.20% shorter than that of the diploid seedlings (16.04 cm) (Fig. 3B). The leaf area of the tetraploid seedlings (53.62 cm^2) was significantly lower than that of the diploid seedlings (55.54 cm^2), with a decrease of 3.46% (Fig. 3D). Similarly, the height of the tetraploid seedlings (19.95 cm) was significantly lower than that of the diploid seedlings (44.20 cm), with a decrease of 54.86% (Fig. 3A). The average leaf shape index of the diploids was 2.581, indicating a long elliptical shape, whereas the average leaf shape index of the tetraploids was 2.038, indicating an elliptical shape (Fig. 3F). Consequently, compared with the diploid, the tetraploid plants were more dwarfed, with rounder and thicker leaves.

Diurnal Variation in *L. pinceana* photosynthesis under different ploidy conditions

In October 2022, a cloudless and sunny day was selected for the measurement of diurnal variation of photosynthesis, which was conducted from 8:00 am to 7:00 pm at 2-h intervals. The light intensity gradually increased in the morning and reached a maximum value of 910.13 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at approximately 12:00 pm (because the determination was conducted in a greenhouse where two layers of shade nets covered the *L. pinceana* plants), after which it gradually decreased (Fig. S1A). Concurrently, as the light intensity increased, the temperature also gradually increased, reaching a maximum temperature of approximately 29 ℃ between 2:00 pm and 4:00 pm before gradually decreasing to 21 ℃

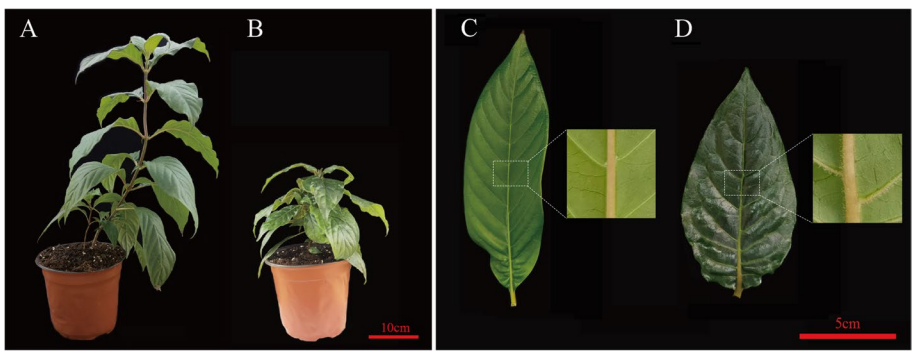

Fig. 1 Morphological comparison of tetraploid and diploid *L. pinceana*. **A** Plant type of the diploid *L. pinceana*; (**B**) plant type of the tetraploid *L. pinceana*; (**C**) leaf characteristics of the diploid *L. pinceana*; (**D**) leaf characteristics of the tetraploid *L. pinceana*. The scale for the plant is 10 cm, and that for the leaf is 5 cm

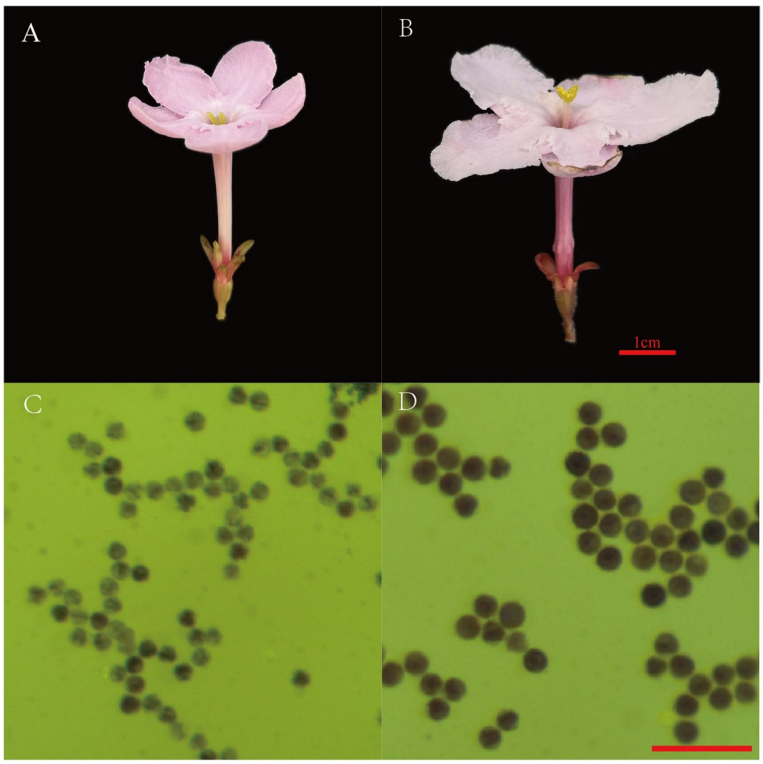

Fig. 2 Morphological comparison of tetraploid and diploid *L. pinceana*. **A** Flower characteristics of the diploid *L. pinceana*; (**B**) flower characteristics of the tetraploid *L. pinceana*; (**C**) pollen viability of the diploid *L. pinceana*; (**D**) pollen viability of the tetraploid *L. pinceana*. The scale for the flower is 1 cm, and that for the pollen is 100 μm

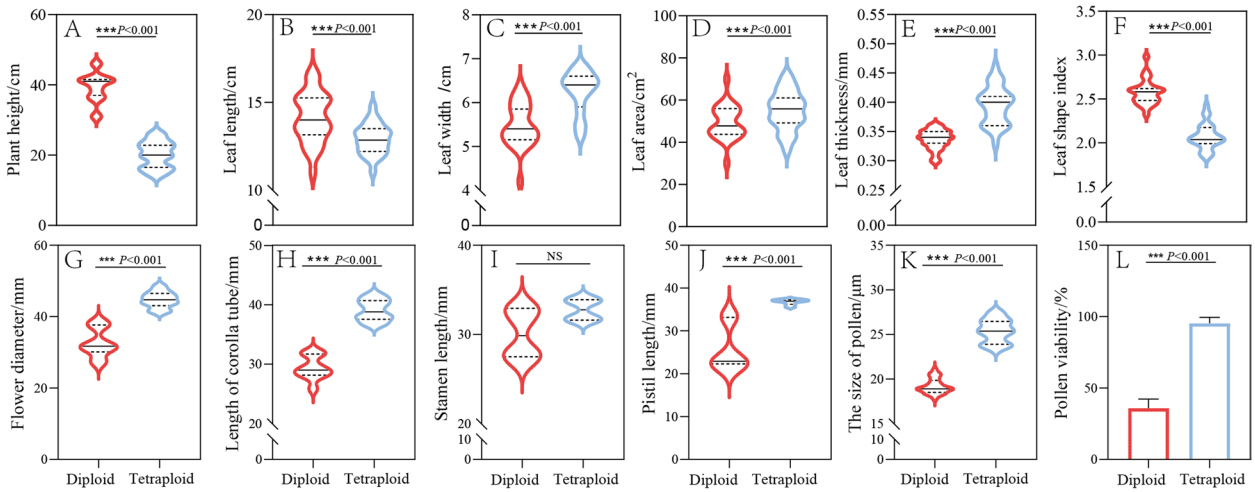

Fig. 3 Morphological variations among different ploidy levels of *L. pinceana*. **A** Plant height difference between diploids and tetraploids of *L. pinceana*; (**B**) leaf length difference between diploids and tetraploids of *L. pinceana*; (**C**) leaf width difference between diploids and tetraploids of *L. pinceana*; (**D**) leaf area difference between diploids and tetraploids of *L. pinceana*; (**E**) leaf thickness difference between diploids and tetraploids of *L. pinceana*; (**F**) leaf shape index difference between diploids and tetraploids of *L. pinceana*; (**G**) flower diameter difference between diploids and tetraploids of *L. pinceana*; (**H**) corolla tube length difference between diploids and tetraploids of *L. pinceana*; (**I**) stamen length difference between diploids and tetraploids of *L. pinceana*; (**J**) pistil length difference between diploids and tetraploids of *L. pinceana*; (**K**) pollen size difference between diploids and tetraploids of *L. pinceana*; (**L**) pollen viability difference between diploids and tetraploids of *L. pinceana*. (* indicates a significant difference between diploids and tetraploids, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

by 7:00 pm (Fig. S1B). Owing to the low air humidity in the greenhouse, an irrigation session was carried out for 1 h before the measurement to control the air humidity within the range of 50% to 60%.

Figure 4 presents the diurnal variations in the net photosynthetic rate (Pn), intercellular carbon dioxide concentration (Ci), stomatal conductance (Cond), and transpiration rate (Trmmol) of *L. pinceana* plants of different ploidy levels under potted experimental conditions. On the day of the experiment, the diurnal variation in Pn for *L. pinceana* of all ploidies exhibited a unimodal pattern, peaking at approximately 10:00 am and then gradually declining. The decline in Pn accelerated aat approximately 16:00. Throughout the day, the Pn values varied among the *L. pinceana* plants of different ploidy levels. At 8:00 am, the difference in the Pn values was not significant. With increasing in temperature and light intensity, the Pn of tetraploids increased more significantly and eventually surpassed that of the diploids. At 10:00 am, when the Pn value reached the maximum value for the day, the Pn value of the tetraploid was 3.57 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, which was greater than that of the diploid (2.39 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). However, at approximately 16:00, the decline in the Pn value of the tetraploid intensified. After 18:00, the Pn value of the tetraploid was lower than that of the diploid. Nevertheless, the tetraploid maintained a greater net photosynthetic rate compared with the diploid for a longer duration throughout the day, and the average net photosynthetic rate of the tetraploid (1.35 μmol$CO_2 \cdot m^{-2} \cdot s^{-1}$) was greater than that of the diploid (0.93 μmol$CO_2 \cdot m^{-2} \cdot s^{-1}$) (Fig. 4A).

The diurnal variation in stomatal conductance (Cond) among the different ploidies of *L. pinceana* did not significantly differ (Fig. 4B), with both presenting a

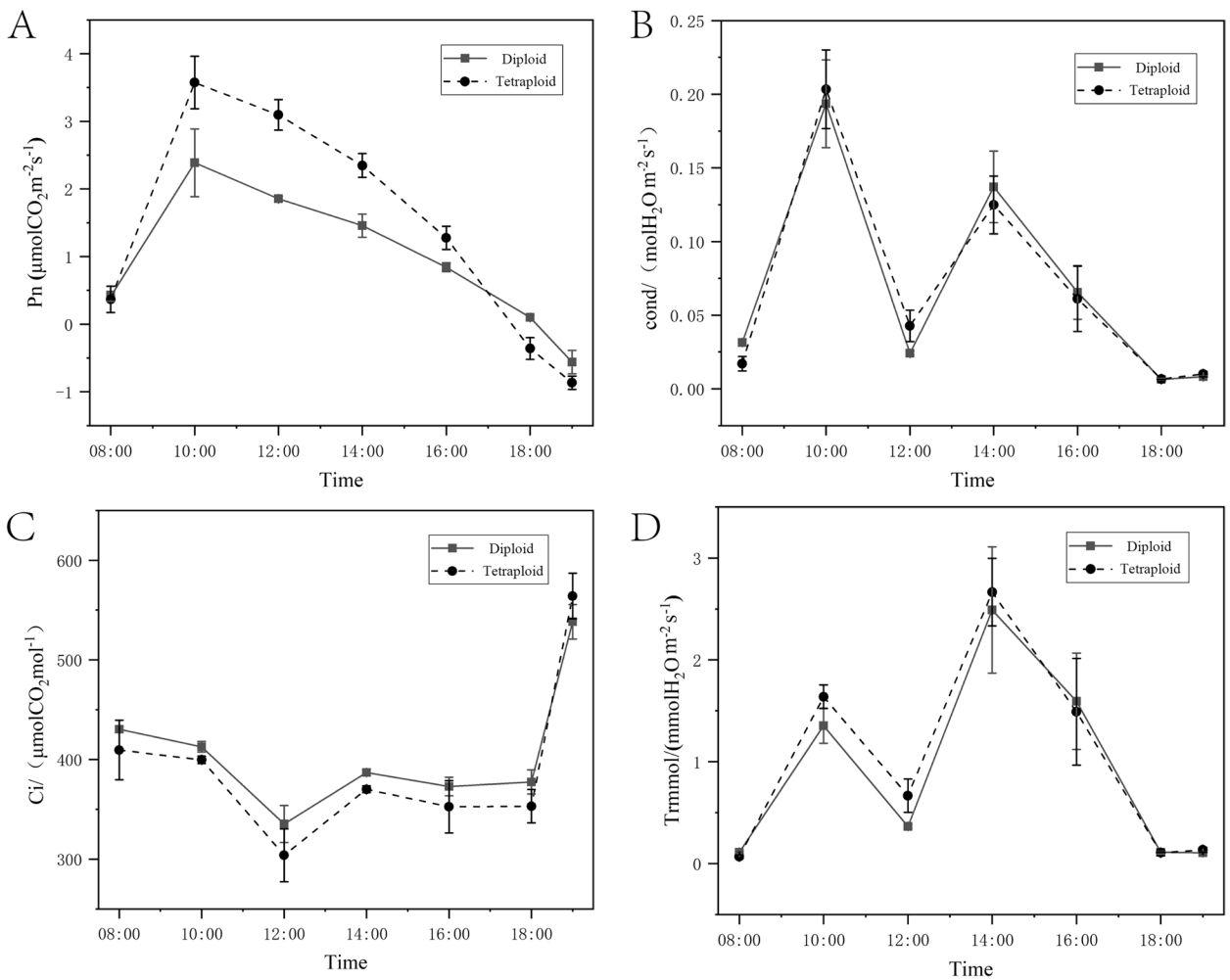

Fig. 4 Diurnal variations in the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate in *L. pinceana* of different ploidies. **A** Diurnal variation in the net photosynthetic rate of *L. pinceana* of different ploidy levels. **B** Diurnal variation in the stomatal conductance of *L. pinceana* of different ploidy levels. **C** Diurnal variation in the intercellular CO_2 concentration of *L. pinceana* of different ploidy levels. **D** Diurnal variation in the transpiration rate of *L. pinceana* of different ploidy levels

bimodal pattern. The first peak was reached at 10:00, when the Cond value of the tetraploid (0.20 mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) was greater than that of the diploid (0.19 mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$). Subsequently, it decreased to the trough at 12:00 before rising again, reaching the second peak at 14:00. At this time, the Cond value of the diploid (0.14 mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) was greater than that of the tetraploid (0.12 mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$). The average Cond value throughout the day was 0.0666 mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$ for the diploids and 0.0665 mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$ for the tetraploids, showing a minimal difference.

The variation trends of the intercellular CO_2 concentration (Ci) among the different ploidies of *L. pinceana* were similar (Fig. 4C). Staring at 8:00, Ci gradually decreased, with a more pronounced decline at 10:00. The lowest value within a day was reached at 12:00, with Ci values of 335.32 μmol $CO_2{\cdot}mol^{-1}$ for the diploids and 303.82 μmol $CO_2{\cdot}mol^{-1}$ for the tetraploids. Subsequently, Ci gradually increased again, with a slight decrease observed between 14:00 and 16:00, followed by a rapid increase at 18:00. The average Ci value within a day was greater for the diploids (407.71 μmol $CO_2{\cdot}mol^{-1}$) than for the tetraploids (393.26 μmol $CO_2{\cdot}mol^{-1}$).

The transpiration rate (Trmmol) variation among the different ploidies of *L. pinceana* also exhibited similar trends (Fig. 4D), with all presenting a bimodal curve. The first peak occurred at 10:00, when the transpiration rate of the tetraploid (1.64 mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) was greater than that of the diploid (1.35 mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$). The second peak occurred at 14:00, with the Trmmol values of the tetraploids being greater than those of the diploids, which were 2.66 and 2.49 mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$, respectively. Within a day, the average Trmmol value of the tetraploids (0.97 mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) was greater than that of the diploids (0.88 mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$).

Correlation analysis of various factors related to the diurnal variation in photosynthesis

Correlation analyses were carried out on the diurnal variation data of photosynthesis in *L. pinceana* of different ploidies, along with data on environmental factors such as temperature and light intensity (Fig. 5). For diploid plants, a highly significant positive correlation was detected between the diurnal variation index of the net photosynthetic rate (Pn) and light intensity ($r = 0.89$, $p < 0.01$), and a significant positive correlation was detected between the stomatal conductance and transpiration rate ($r = 0.77$, $p < 0.05$). In the case of tetraploid plants, the diurnal variation index of the net photosynthetic rate was extremely significantly and positively correlated with light intensity ($r = 0.92$, $p < 0.01$). Additionally, there was a significant positive correlation between stomatal conductance and the net photosynthetic rate ($r = 0.79$, $p < 0.05$), as well as between light intensity and the transpiration rate ($r = 0.76$, $p < 0.05$). Furthermore, a significant positive correlation was observed between stomatal conductance and the transpiration rate ($r = 0.78$, $p < 0.05$).

Light response curves of *L. pinceana* of different ploidy levels

Figure 6 shows the response of the net photosynthetic rate (Pn), transpiration rate (Trmmol), stomatal conductance (Cond), and intercellular CO_2 concentration (Ci) of *L. pinceana* plants of different ploidy levels to various light intensities. When the light intensity was weak (PAR < 400 μmol·m^{-2}·s^{-1}), the net photosynthetic rate

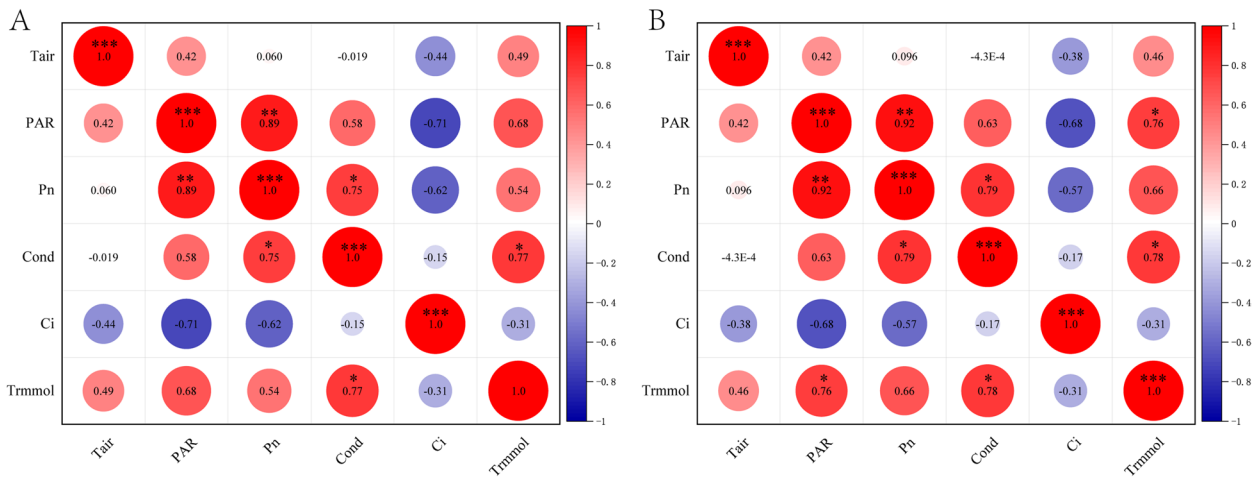

Fig. 5 Analysis of the correlations between various physiological and environmental factors and the diurnal photosynthetic variation in *L. pinceana* of different ploidies. **A** Diploid; (**B**) tetraploid

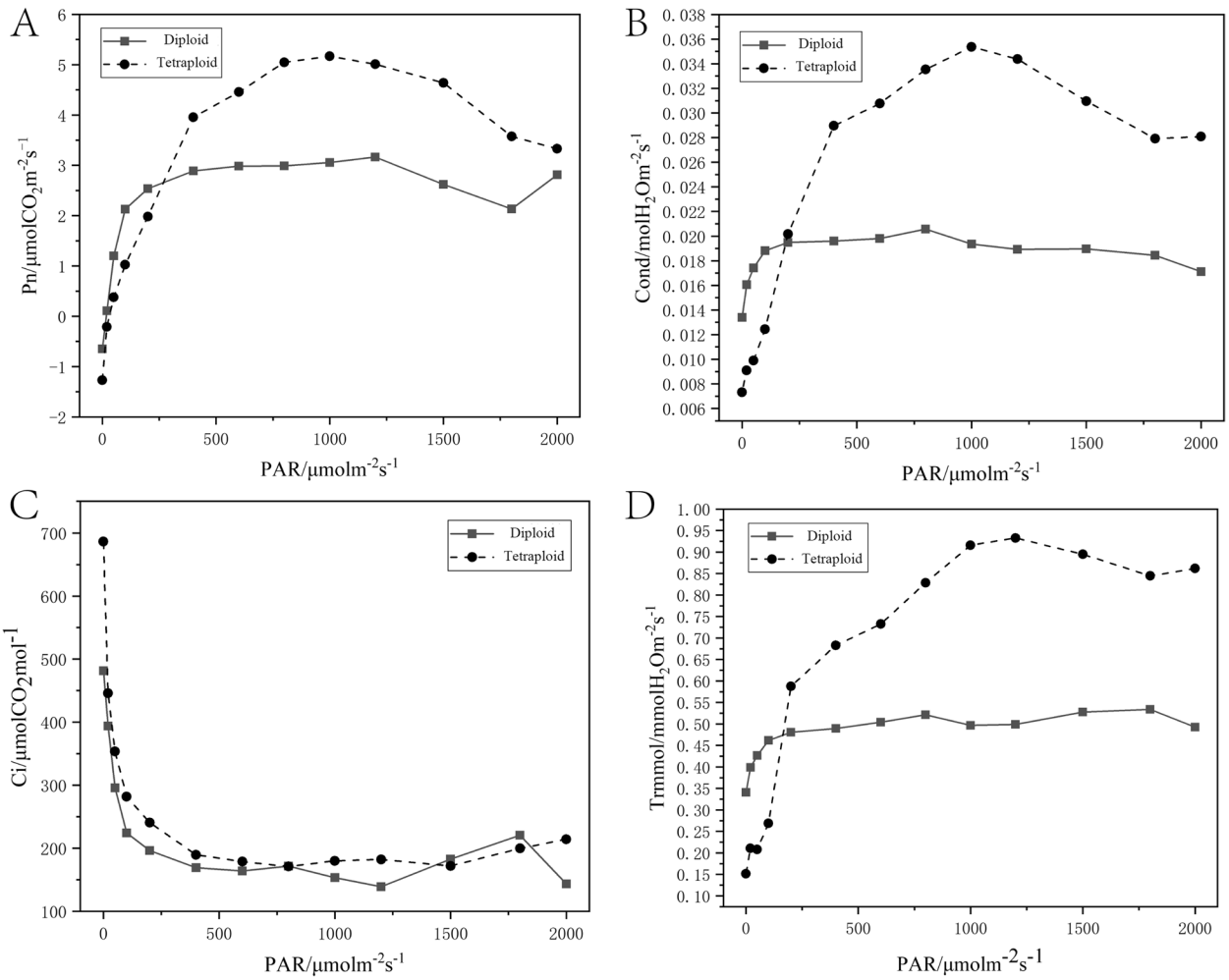

Fig. 6 Parameters of the light response curves of *L. pinceana* plants of different ploidy levels under various light intensities. **A** Variations in the net photosynthetic rates of *L. pinceana* of different ploidy levels under different light intensities. **B** Variations in the stomatal conductance of *L. pinceana* of different ploidy levels under different light intensities. **C** Variations in the intercellular CO_2 concentrations of *L. pinceana* of different ploidy levels under different light intensities. **D** Variations in the transpiration rates of *L. pinceana* of different ploidy levels under different light intensities

increased linearly, albeit with insignificant differences (Fig. 6A). With increasing of light intensity, the increase in Pn decreased and then gradually stabilized when PAR > 500 μmol·m^{-2}·s^{-1}. At this time, the Pn of the tetraploids was approximately 4–5 μmol CO_2·m^{-2}·s^{-1}, which was greater than that of the diploid (2–3 μmol CO_2·m^{-2}·s^{-1}).

When PAR < 200 μmol·m^{-2}·s^{-1}, the stomatal conductance (Cond) of the diploids was greater than that of the tetraploids. As the light intensity increased, the stomatal conductance values of *L. pinceana* of different ploidies initially exhibited linear growth, followed by a stabilization of the growth rate. The growth of the diploids tended to stabilize at a light intensity of approximately 100 μmol·m^{-2}·s^{-1}, whereas the growth rate of the tetraploids slowed at a light intensity of approximately 400 μmol·m^{-2}·s^{-1} (Fig. 6B). The growth rate of tetraploids was greater than that of diploids. When PAR > 400 μmol·m^{-2}·s^{-1}, the Cond of tetraploids (0.28–0.36 mol H2O·m^{-2}·s^{-1}) was greater than that of diploids (0.18–0.20 mol H2O·m^{-2}·s^{-1}).

The intercellular CO_2 concentration (Ci) of *L. pinceana* of different ploidy levels decreased with increasing light intensity, showing the opposite trend as the variation in the Pn. Under weak light intensities of PAR < 200 μmol·m^{-2}·s^{-1}, the Ci of *L. pinceana* plants of different ploidy levels decreased linearly. When PAR > 400 μmol·m^{-2}·s^{-1}, the deceleration gradually decreased, and the Ci ranged from approximately 150 to 220 μmol CO2·mol^{-1}. The Ci values for different ploidies of *L. pinceana* fluctuated with changes in light intensity, but the differences were insignificant (Fig. 6C).

The growth rate of the transpiration rate (Trmmol) in *L. pinceana* of different ploidies was similar to

the variation trends observed in Pn and Cond, which increased with increasing light intensity. Under low light intensity (PAR < 200 μmol·m^{-2}·s^{-1}), the growth rate was extremely rapid and increased linearly (Fig. 6D). When PAR > 200 μmol·m^{-2}·s^{-1}, the growth rate decreased. At this point, the Trmmol of tetraploids (0.48–0.53 mmol H2O·m^{-2}·s^{-1}) was greater than that of diploids (0.68–0.93 mmol H2O·m^{-2}·s^{-1}).

Figure 7 presents the response curves of the net photosynthetic rates of *L. pinceana* plants of different ploidy levels to light intensity. The light saturation point (LSP) for the diploid plants was 220 μmol·m^{-2}·s^{-1}, whereas it was 500 μmol·m^{-2}·s^{-1} for the tetraploid plants, which was 127.27% higher than that of the diploid plants. The LSP reflects the plant's ability to utilize strong light, and a larger LSP indicates greater adaptability and utilization ability for intense light. The LSP of the tetraploid *L. pinceana* was greater than that of the diploid plants, indicating that tetraploid *L. pinceana* has a superior ability to utilize strong light. The light compensation point (LCP) of the diploid *L. pinceana* was 12 μmol·m^{-2}·s^{-1}, and the LCP of the tetraploid was 52 μmol·m^{-2}·s^{-1}, a 333.33% increase from that of the diploid plants. The LCP reflects the ability of plants to utilize weak light, and a lower LCP indicates a greater capacity for utilizing weak light. The LCP of the diploid *L. pinceana* was lower than that of the tetraploid *L. pinceana*, indicating that the diploid *L. pinceana* has greater adaptability and utilization ability for weak light and is more shade tolerant. The maximum net photosynthetic rate (P_{max}) of the diploid *L. pinceana* was 3.48 μmol·m^{-2}·s^{-1}, and that of the tetraploid *L. pinceana* was 5.76 μmol·m^{-2}·s^{-1}. The maximum net photosynthetic rate reflects the photosynthetic capacity of a plant. The P_{max} of tetraploid *L. pinceana* was greater than that of the diploids, suggesting that tetraploids have greater photosynthetic potential.

Physiological characteristics of Tetraploid *L. pinceana*

The activity of antioxidant enzymes in L. pinceana of different ploidy levels

As shown in Fig. 8, there were extremely significant differences in the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) among *L. pinceana* of different ploidies. SOD is one of the most crucial free radical scavengers in organisms and can eliminate superoxide anion radicals to protect cells from harm and maintain metabolic balance. The higher the SOD content is, the greater the tolerance of the plant to oxidative stress. The SOD activity in the tetraploid *L. pinceana* (154.8 U·g^{-1}) was extremely significantly greater than that in the diploid *L. pinceana* (102.1 U·g^{-1}), with an increase of 51.62% ($P < 0.001$) (Fig. 8A). Peroxidase (POD) is localized in peroxisomes and has a protective function in cells. The activity of POD within plants can indicate the strength of their resistance. The POD activity in *L. pinceana* tetraploids (119.5 U·(min·g)$^{-1}$) was significantly greater than that in diploids (1.89 U·(min·g)$^{-1}$) ($P < 0.001$) (Fig. 8B).

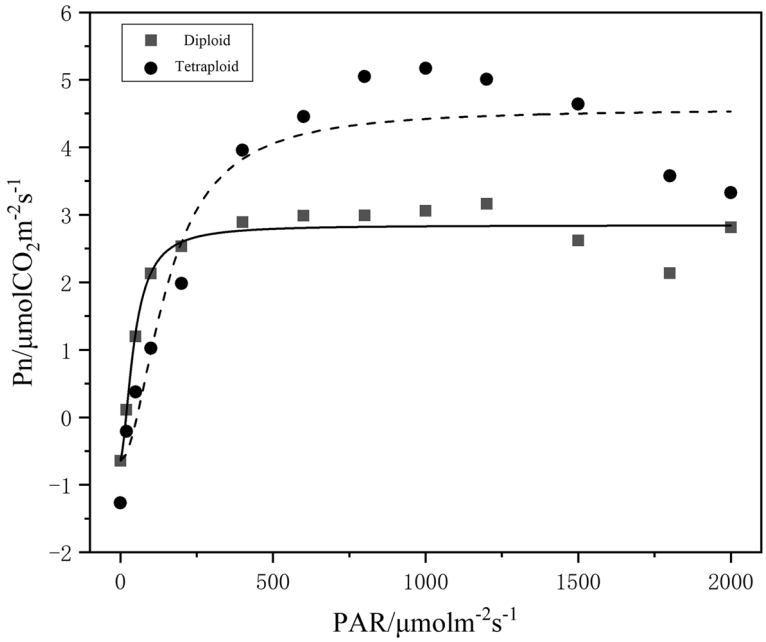

Fig. 7 Fitting curves of the light response of *L. pinceana* to various ploidies under different light intensities

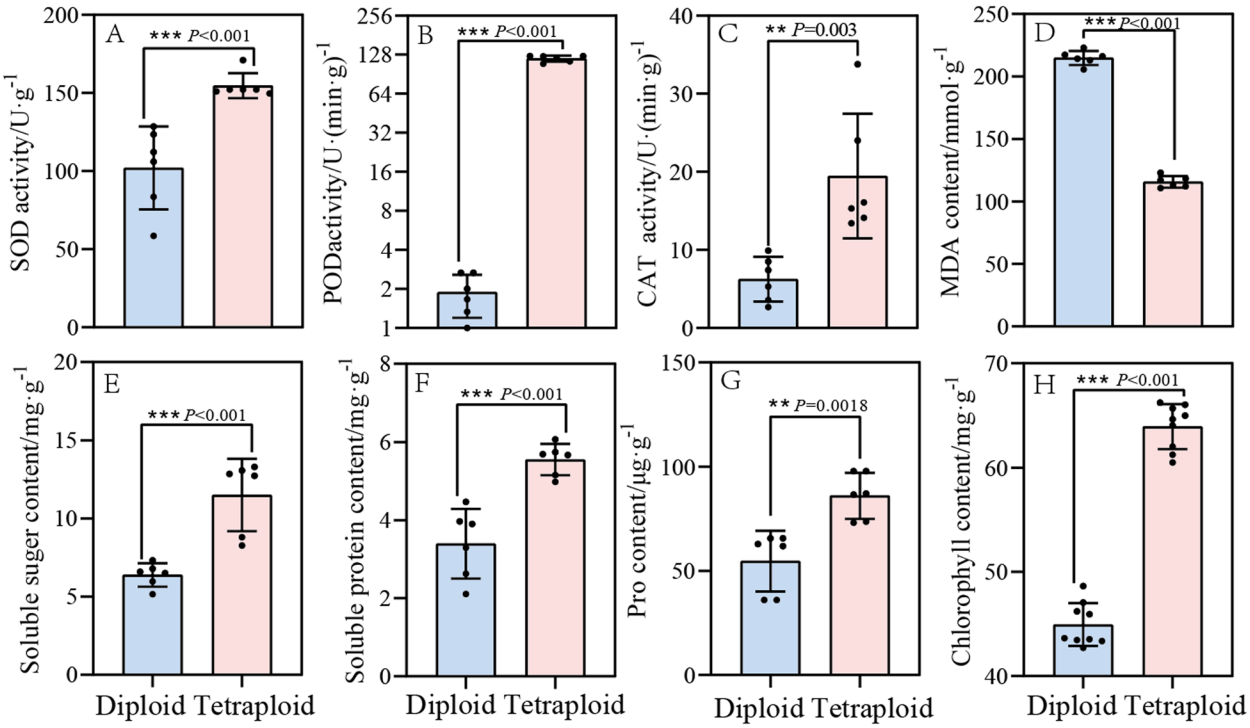

Fig. 8 Physiological index differences of *L. pinceana* with different ploidy levels. **A** The differences in the activity of superoxide dismutase of *L. pinceana* with different ploidies; (**B**) the differences in the activity of peroxidase of *L. pinceana* with different ploidies; (**C**) the differences in the activity of catalase of *L. pinceana* with different ploidies; (**D**) the differences in malondialdehyde (MDA) content of *L. pinceana* with different ploidies; (**E**) the differences in the content of soluble sugar of *L. pinceana* with different ploidies; (**F**) the differences in the content of soluble protein of *L. pinceana* with different ploidies; (**G**) the differences in the content of proline of *L. pinceana* with different ploidies; (**H**) the differences in chlorophyll content of *L. pinceana* with different ploidies; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

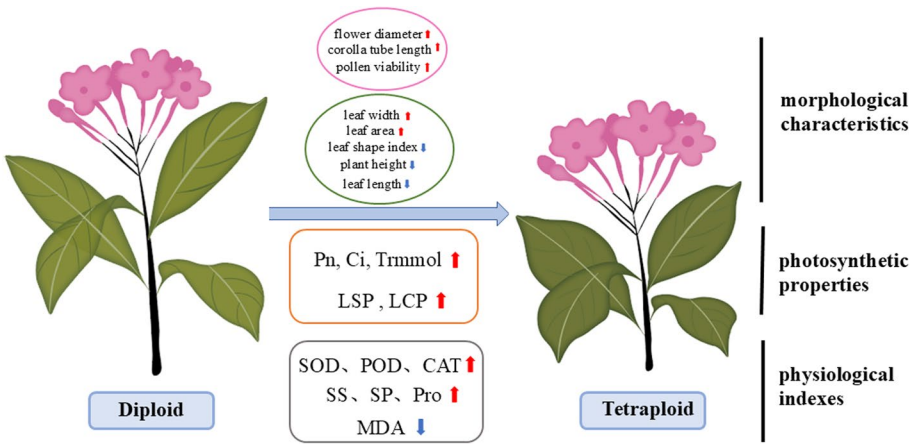

Fig. 9 Conceptual framework summarizing the comparison of the morphological and physiological characteristics of diploid and tetraploid *L. pinceana*

CAT facilitates the decomposition of hydrogen peroxide, thereby protecting cells from the toxic effects of H_2O_2. Varieties with relatively strong resistance presented relatively high CAT activity. The CAT activity in the tetraploid *L. pinceana* (19.46 U·(min·g)$^{-1}$) was significantly greater than that in the diploid variety (6.24 U·(min·g)$^{-1}$) ($P < 0.01$), representing an increase of 211.86% (Fig. 8C). In summary, the activities of antioxidant enzymes were elevated in the tetraploids of *L. pinceana*, which could maintain cellular protection

against damage under adverse conditions and exhibited increased resistance.

The contents of osmotic adjustment substances in *L. pinceana* of different ploidy levels

As illustrated in Fig. 8, highly significant differences were observed in the contents of soluble sugars (SSs), soluble proteins (SPs), and proline (Pro) among *L. pinceana* plants of different ploidies. Soluble sugars serve as crucial energy sources for plants and play a role in osmotic pressure regulation. The SS content in the tetraploids of *L. pinceana* was significantly greater than that in their diploid counterparts ($P < 0.001$), with values of 11.51 $mg{\cdot}g^{-1}$ and 6.39 $mg{\cdot}g^{-1}$, respectively (Fig. 8E). Soluble protein is considered one of the resistance indicators in plants, serving as an osmotic adjustment substance and a nutrient, thus providing cellular protection. The SP content in the tetraploid *L. pinceana* (5.55 $mg{\cdot}g^{-1}$) was significantly greater than that in the diploid varieties (3.40 $mg{\cdot}g^{-1}$) ($P < 0.001$), representing an increase of 63.24% (Fig. 8F). Pro can serve as a protective substance for membranes and enzymes, as well as a scavenger for free radicals. The accumulation of Pro is associated with plant resistance, and a relatively high content of Pro in plants indicates relatively strong resistance. The Pro content in the tetraploid *L. pinceana* (86.13 $\mu g{\cdot}g^{-1}$) was extremely significantly greater than that in the diploid *L. pinceana* (54.72 $\mu g{\cdot}g^{-1}$) ($P < 0.01$), showing an increase of 57.40% (Fig. 8G). In conclusion, the content of osmotic adjustment substances in the tetraploids of *L. pinceana* was greater than that in the diploids, suggesting that the resistance of *L. pinceana* may have been greater and that its ability to adapt to adverse conditions was potentially greater.

Differences in the chlorophyll content and malondialdehyde content of *L. pinceana* plants of different ploidy levels

As depicted in Fig. 8, highly significant differences in both the chlorophyll content and malondialdehyde (MDA) content were detected among *L. pinceana* plants of different ploidy levels. The content of MDA in plant cells is associated with the extent of cell damage, where a higher content of MDA indicates more severe cell damage and is commonly regarded as an indicator of plant resistance. The MDA content in the tetraploid *L. pinceana* (115.7 $mmol{\cdot}g^{-1}$) was significantly lower than that in the diploid *L. pinceana* (214.9 $mmol{\cdot}g^{-1}$) ($P < 0.001$), with a reduction of 46.16% (Fig. 8D). Leaves serve as the primary site for photosynthesis in plants, and the chlorophyll content in leaves is an indicator of a plant's photosynthetic capacity to a certain extent. The chlorophyll content in the tetraploid *L. pinceana* (63.95 $mg{\cdot}g^{-1}$) was significantly greater than that in the diploid *L. pinceana* (44.95 $mg{\cdot}g^{-1}$) ($P < 0.001$), increasing by 42.27% (Fig. 8H). Consequently, the higher chlorophyll content in the tetraploids indicated greater potential for photosynthesis, whereas the lower MDA content indicated that resistance in the tetraploids of *L. pinceana* was greater than that in the diploids.

Discussion

Growth and physiological and biochemical differences in polyploids

Previous studies have indicated that after polyploidization, plants undergo various morphological changes, such as increased height, larger leaves, enlarged cells, accelerated growth rates, and higher nutritional content [34, 35]. Different plants exhibit diverse morphological changes after polyploidization [36]. The morphological characteristics of polyploid plants can be classified into two types: slow-growing and dwarfed [37] and fast-growing and tall [38]. In the present study, the plant height of the tetraploid *L. pinceana* was extremely significantly lower than that of the diploid *L. pinceana*, indicating slower growth than that of the diploid *L. pinceana*. The tetraploid *L. pinceana* is a polyploid that is slow-growing and dwarfed. This is distinct from the manifestations of tetraploid *Morus alba* and *Arabidopsis thaliana* tetraploids [39] but is similar to those of tetraploid *Salix babylonica* [40] and tetraploid *Malus pumila* [41]. Moreover, in this study, the tetraploid *L. pinceana* had larger flowers and pollen grains, as well as greater pollen viability. The tetraploid *L. pinceana* can subsequently be used as the paternal parent for hybridization in the hope of providing a reference for polyploid hybridization breeding and germplasm innovation of *L. pinceana*.

Plants generate a considerable amount of reactive oxygen species under adverse conditions, which can disrupt biological metabolic activities [17]. Antioxidant enzymes such as SOD, POD, and CAT can alleviate oxidative damage to plants under stress, and these enzymes play crucial roles in enhancing the cellular defence against the increase in reactive oxygen species under stressful conditions [42]. The results of this study indicate that the activities of SOD, POD, and CAT in the tetraploid *L. pinceana* were significantly greater than those in the diploid *L. pinceana*, suggesting that the tetraploid *L. pinceana* has a stronger defence ability under adverse stress. Furthermore, the secondary metabolites of the chromosome-doubled plants changed, with significant increases in soluble protein, soluble sugar, and Pro contents [43]. These substances are positively correlated with plant stress resistance, and the accumulation of a greater amount of Pro is regarded as a widespread protective response to environmental stress, indicating that

polyploid plants are more adaptable than conventional plants [44]. Additionally, in this study, the contents of soluble sugars, soluble proteins, and proline in tetraploid *L. pinceana* were significantly greater than those in diploids, suggesting that tetraploid *L. pinceana* has greater resistance. MDA is a decomposition product of plants under adverse conditions, and a higher content indicates more severe damage to plant cells, which can be used as an indicator of plant resistance. Wei et al. [45] reported that the MDA content in the tetraploid *Manihot esculenta* Crantz was significantly lower than that in the diploid *Manihot esculenta*, and there was a significant positive correlation between the mite damage index and the MDA content, suggesting that more MDA accumulates under adverse conditions. The results of this study are consistent with those described above, as the MDA content of tetraploid *L. pinceana* was significantly lower than that of diploids, indicating that tetraploid *L. pinceana* has greater resistance. In conclusion, the differences in physiological indicators between *L. pinceana* of different ploidies indicate that tetraploid *L. pinceana* has greater adaptability and resistance to adverse conditions.

The impact of ploidy changes on the photosynthetic efficiency of *L. pinceana*

As a fundamental mechanism of the carbon/oxygen cycle, photosynthesis plays a crucial role in plant vegetative growth, carbohydrate production, and the conversion of light energy into chemical energy [46]. Polyploids often exhibit unique photosynthetic characteristics that differ from those of diploids because of complex modifications in anatomical features and biochemical processes [47]. In general, compared with diploids, the leaves of polyploids have larger mesophyll cells, more chloroplasts, and higher chlorophyll contents, leading to an increased photosynthetic rate per cell [48]. With increasing ploidy, the photosynthetic rates of species such as *Robinia pseudoacacia* [49], mulberry [50], and *Cucumis melo* [51] also increase. Liao et al. [52] reported that the leaf anatomical structure and chlorophyll content in triploid *Populus* may be linked to an increase in the net photosynthetic rate (Pn), as evidenced by a highly significant positive correlation between the relative chlorophyll content index (CCI) and the Pn. The findings of this study demonstrated that tetraploid *L. pinceana* presented a significantly greater chlorophyll content than its diploid counterpart, and the stomatal content was also greater than that observed in diploids, which may have contributed to the photosynthetic rate of the tetraploid *L. pinceana*.

Under natural conditions, many reports have documented the types of diurnal variation in various plant species. These diurnal variation curves can generally be categorized into three distinct types: unimodal [53], bimodal [54], and irregular [55]. The findings from the diurnal variation in photosynthetic parameters in *L. pinceana* across different ploidies indicated that the net photosynthetic rate (Pn) exhibited a unimodal pattern, peaking at approximately 10:00 before gradually declining. Correlation analyses of the physiological and ecological factors influencing photosynthesis in *L. pinceana* revealed that the Pn was significantly positively correlated with photosynthetically active radiation (PAR) and positively correlated with Cond and Trmmol but negatively correlated with Ci. These results are largely consistent with those reported by Zhu et al. [56]. However, despite the positive correlations between Pn and both Cond and Trmmol, their variations were not parallel, suggesting that the photosynthesis of *L. pinceana* is influenced by a complex interplay of physiological and ecological factors. Furthermore, the reliance on simple correlation analysis may present an incomplete perspective, and the mechanisms underlying factor interactions affecting photosynthesis remain to be elucidated. The relationship between photosynthesis and its associated physiological and ecological factors in *L. pinceana* warrants further investigation. Additionally, the average values for Pn, Ci, and Trmmol in tetraploid *L. pinceana* over a 24-h period were consistently greater than those observed in diploids, indicating that tetraploid individuals accumulate more assimilates throughout the day compared with their diploid counterparts.

To facilitate a more direct and effective comparison of photosynthetic efficiency between diploid and tetraploid *L. pinceana*, this study measured the light response curves for both ploidy levels, thereby obtaining several critical photosynthetic indicators. Parameters such as Pn, Cond, Ci, and Trmmol serve as common indicators that can directly reflect the strength of a plant's photosynthesis efficiency. Wang Tingting et al. [57] assessed the light response curves of *Solanum tuberosum* plants with varying ploidy levels and reported that the net photosynthetic rate in tetraploid potato plants exceeded that in diploids, indicating strong photosynthetic capacity under both high- and low-light conditions. Similarly, Wang Feng et al. [58] examined the photosynthetic characteristics of *Pteroceltis tatarinowii* plants of different ploidy levels and reported that the light energy conversion efficiency of leaves was greater in tetraploid individuals than in their diploid counterparts and that tetraploids presented a superior ability to utilize weak light and produce more assimilates under such conditions. This study revealed that the Pn, Cond, and Trmmol values for tetraploid *L. pinceana* were consistently greater than those for diploids under strong light exposure, and both the light saturation point (LSP) and maximum net photosynthetic efficiency (Pmax) were greater in tetraploids than in diploids,

indicating better adaptability to strong light conditions in tetraploid *L. pinceana*. Conversely, under weak light conditions, there were few differences in the Pn, Cond, Ci, and Trmmol values between the tetraploids and diploids of *L. pinceana*, as well as the light compensation point (LCP) of the tetraploids was greater than that of the diploids, suggesting greater adaptability of the diploid *L. pinceana* to weak light environments. These findings contrast with previous results but further indicate that tetraploid *L. pinceana* possesses increased photosynthetic capacity under intense illumination while potentially exhibiting a preference for brighter environments.

This study investigated the morphological, photosynthetic, and physiological differences between diploid and tetraploid *L. pinceana*. The observed variations in tetraploid plant architecture and physiological characteristics may be influenced by the differential expression of regulatory genes, although the underlying regulatory mechanisms remain unclear. Yao et al. [59] demonstrated that the superior growth vigour and enhanced photosynthetic capacity of triploid *Camellia sinensis* were correlated primarily with genes involved in photosynthesis, cell division, and hormone biosynthesis. Similarly, Zhang et al. [38] identified upregulated developmental genes in tetraploid *Glehnia littoralis* through transcriptomic analysis, which mediated polyploid-specific modifications, including enlarged stomata and thickened leaf tissues. In our study, tetraploid *L. pinceana* exhibited substantial morphological divergence from its diploid counterparts, along with significantly enhanced photosynthetic efficiency and superior physiological performance. Subsequent experiments should focus on elucidating the molecular regulatory mechanisms driving morphological and physiological adaptations in tetraploid *L. pinceana* and identifying key genetic determinants governing phenotypic divergence across ploidy levels.

Compared with diploids, tetraploids exhibit superior phenotypic traits, which may increase their adaptability to novel environments, thereby potentially improving yield and quality in agricultural production. These findings hold significant value for future breeding programs. Notably, tetraploid *L. pinceana* demonstrates exceptional physiological resilience, making it a priority candidate for cultivation in climatically unstable regions. This study contributes to the expanding knowledge of polyploidy and its applications in plant improvement, particularly in horticulture and ornamental species. However, our current analysis provides only preliminary insights into the morphological and physiological divergence between diploid and tetraploid *L. pinceana*. Further investigations are needed to validate the horticultural merits of tetraploid variants under field conditions, elucidate the genetic and epigenetic mechanisms underlying their enhanced stress tolerance, and assess the long-term stability of polyploid traits across generations.

Conclusion

This study focused on research and analysis of the biological characteristics of polyploid *L. pinceana*. With diploid plants as the control, the morphological, photosynthetic and physiological indicators of tetraploid *L. pinceana* were determined. The differences and advantages compared with those of diploids were analysed to provide theoretical guidance for improving the stress resistance, adaptability and ornamental traits of *L. pinceana* varieties, as well as for developing new varieties with enhanced characteristics, laying a foundation for the breeding of new *L. pinceana* varieties. In conclusion, the leaf length, leaf width, leaf area, leaf thickness, leaf shape index and plant height of the tetraploid plants were significantly different from those of the diploid plants. The height of tetraploid *L. pinceana* plants was significantly lower than that of diploid *L. pinceana* plants, which are a type of polyploid with slow growth and short stature. Nevertheless, both the flowers and pollen grains of tetraploids were larger than those of diploids, and the pollen vitality of tetraploids was significantly greater than that of diploids. Consequently, the tetraploid *L. pinceana* has high ornamental value and high fertility and can be utilized as a breeding material for improving the germplasm resources of *L. pinceana* in the future. The daily variations in the Pn, Ci, and Trmmol values of tetraploid *L. pinceana* are greater than those of diploid *L. pinceana* within a day, indicating that tetraploid *L. pinceana* accumulates more assimilates within a day compared with diploid *L. pinceana*. The LSP and LCP of tetraploid *L. pinceana* are greater than those of diploid *L. pinceana*, suggesting that tetraploid *L. pinceana* has better photosynthetic capacity under strong light and can produce more assimilates under light. The activities of SOD, POD and CAT and the contents of SS, SP and Pro in tetraploid *L. pinceana* are significantly greater than those in diploid *L. pinceana*, whereas the content of MDA is significantly lower than that in diploid *L. pinceana*. These findings indicate that tetraploid *L. pinceana* has a stronger defence ability under adverse stress conditions (Fig. 9).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06568-w.

Supplementary Material 1.

Supplementary Material 2.

Authors' contributions
WLL: Conceptualization, Investigation, Methodology, Writing–original draft, Writing–review & editing, Formal analysis. LYF: Methodology, Investigation, Writing–review & editing, Formal analysis. LHL: Investigation, Methodology. LSF: Formal analysis, Writing–review & editing. SJ: Formal analysis, Writing–review & editing, Project administration. GWL: Conceptualization, Resources, Supervision, Writing–review & editing, Funding acquisition.

Statement
The species of *L. pinceana* does not fall within the category of endangered wild plant species, and the collection of materials is in accordance with both Chinese and international guidelines.

Funding
This research was funded by the Key Technologies Research for the Germplasm of Important Woody Flowers in Yunnan Province (Grant No. 202302 AE090018),and by the Scientific Research Fund of Yunnan Education Department "Study on induction of polyploidy in characteristic flower ***Luculia pinceana*** Hook."(2021 J0137).

Data availability
The data generated or analyzed are provided within the manuscript and its additional file. The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]College of Resources and Environment, Yunnan Agricultural University, Kunming, China. [2]College of Landscape and Horticulture, Yunnan Agricultural University, Kunming, China. [3]Flower Research Institute of Yunnan Academy of Agricultural Sciences, National Engineering Research Center for Ornamental Horticulture, Kunming, China.

Received: 18 February 2025 Accepted: 15 April 2025
Published online: 25 April 2025

References

1. Li Y, Wan Y, Sun Z, Li T, Liu X, Ma H, et al. Floral scent chemistry of *Luculia yunnanensis* (Rubiaceae), a species endemic to China with sweetly fragrant flowers. Molecules. 2017;22:1–11.
2. Li Y, Ma H, Wan Y, Li T, Liu X, Sun Z, et al. Volatile Organic Compounds Emissions from *Luculia pinceana* Flower and Its Changes at Different Stages of Flower Development. Molecules. 2016;21:1–10.
3. Wang L, Lu X, Guan W, Li Y. Chemical Mutagenesis and Identification of Polyploid in *Luculia pinciana*. Chinese Agric Bull Sci. 2024;40:38–45.
4. Wang L, Li Y, Lu X, Li S, Song J, Guan W. Polyploid induction of Luculia pinceana Hook by aseptic germination combined with chemical agents. Eur J Hortic Sci. 2024;89:1–9.
5. Kong L, Li F, Du R, Geng H, Li S, Wang J. Effects of different preservatives on cut flower of *Luculia pinceana*: A novel fragrant ornamental species. HortScience. 2021;56:795–802.
6. Gao C, Guo S, Ma C, Yang J, Kang X, Li R. Impact of Climate Change on the Potential Geographical Distribution Patterns of *Luculia pinceana* Hook. f. since the Last Glacial Maximum. Forests. 2024;15(2):253.
7. Ma H, Wan YM, Liu XX, Zhao ZG. 'Hongfu', a new red-flowered *Luculia pinceana* cultivar. Acta Sin Hortic. 2018;45:2.
8. Ma H, Wang Y, Liang N. A study on the breeding system of *Luculia pinciana*. For Res. 2009;22:6.
9. Liu XX, Ma H, Wan YM, Zhao ZG, Li ZH. A new *Luculia pinceana* cultivar'Jinyudian.' Acta Sin Hortic. 2019;46:199–200.
10. van de Peer Y, Ashman TL, Soltis PS, Soltis DE. Polyploidy: an evolutionary and ecological force in stressful times. Plant Cell. 2021;33:11–26.
11. Kang X, Wei H. Breeding polyploid Populus: progress and perspective. For Res. 2022;2:4.
12. Sabooni N, Gharaghani A. Induced polyploidy deeply influences reproductive life cycles, related phytochemical features, and phytohormonal activities in blackberry species. Front Plant Sci. 2022;13:938284.
13. Bharati R, Shmeit YH, Šedivá JH, Cong TTN, Kundu JK, Severová L, et al. Comparative assessment of morphological, cytological, and photosynthetic characteristics of the induced octoploid and its tetraploid counterpart of *Celosia argentea* L. BMC Plant Biol. 2024;24(1):1227.
14. Wang J, Wang R, Luo F, Du W, Hou J, Chen G, et al. Comparative Morphological, Physiological, and Transcriptomic Analyses of Diploid and Tetraploid Wucai (*Brassica campestris* L.). Plants. 2024;13(16):2341.
15. Bharati R, Gupta A, Novy P, Severová L, Šrédl K, Žiarovská J, et al. Synthetic polyploid induction influences morphological, physiological, and photosynthetic characteristics in *Melissa officinalis* L. Front Plant Sci. 2023;14 December:1–15.
16. Sabzehzari M, Hoveidamanesh S, Modarresi M, Mohammadi V. Morphological, anatomical, physiological, and cytological studies in diploid and tetraploid plants of *Plantago psyllium*. Plant Cell Tissue Organ Cult. 2019;139:131–7.
17. Xu J, Jin J, Zhao H, Li K. Drought stress tolerance analysis of *Populus ussuriensis* clones with different ploidies. J For Res. 2019;30:1267–75.
18. Zhou X, Zhao P, Zeng F, Geng X, Zhou J, Sun J. Induction and identification of polyploids in four *Rhododendron* species. Plant Cell Tissue Organ Cult. 2024;158.
19. Pu T, Wang Y, Han W, Li H, Sun P, Suo Y, et al. Physiological Characteristics and Transcriptional Differences of Growth Traits of Persimmon with Different Ploidy. Horticulturae. 2024;10:1–14.
20. Wang L, Zheng P, Ge H, Zhao X, Kou Y, Yang S, et al. Colchicine-Induced Tetraploidy in Protocorms of *Aerides rosea* Lodd. ex Lindl. and Paxton. and Its Identification. Plants. 2024;13(24):3535.
21. Xie N, Zhao Y, Huang M, Chen C, Cao C, Wang J, et al. Polyploid Induction and Identification of *Begonia* × *benariensis*. Horticulturae. 2024;10(1):47.
22. Zhang Y, Qiao Y, Zhang W, Liu X, Gong R, Wang Z, et al. In vitro induction and characterization of *Anthurium andraeanum* 'Pink Champion' tetraploids. Vitr Cell Dev Biol - Plant. 2025:1-9. https://doi.org/10.1007/s11627-024-10476-3.
23. Chen Y, Xu H, He T, Gao R, Guo G, Lu R, et al. Comparative Analysis of Morphology, Photosynthetic Physiology, and Transcriptome Between Diploid and Tetraploid Barley Derived From Microspore Culture. Front Plant Sci. 2021;12:626916.
24. Su X, Yang Z, Zhou C, Geng S, Chen S, Cai N, et al. The Response and Evaluation of Morphology, Physiology, and Biochemistry Traits in Triploid *Passiflora edulis* Sims 'Mantianxing' to Drought Stress. Plants. 2024;13(12):1685.
25. Ye J, Fan Y, Zhang H, Teng W, Teng K, Wu J, et al. Octoploids Show Enhanced Salt Tolerance through Chromosome Doubling in Switchgrass (*Panicum virgatum* L.). Plants. 2024;13(10):1383.
26. Yoo MJ, Hwang Y, Koh YM, Zhu F, Deshpande AS, Bechard T, et al. Physiological and Molecular Modulations to Drought Stress in the *Brassica* Species. Int J Mol Sci. 2024;25(6):3306.
27. Sun L, Ghouri F, Jin J, Zhong M, Huang W, Lu Z, et al. Interspecific Hybridization Enhanced Tolerance to Salinity and Cadmium Stress Through Modifying Biochemical, Physiological, and Resistance Gene Levels, Especially in Polyploid Rice : A Sustainable Way for Stress-Resilient Rice. Rice. 2025;18:19.
28. Ghouri F, Shahid MJ, Zhong M, Zia MA, Alomrani SO, Liu J, et al. Alleviated lead toxicity in rice plant by co-augmented action of genome doubling and TiO2 nanoparticles on gene expression, cytological and physiological changes. Sci Total Environ. 2023;2024(911): 168709.
29. Li X, Zhang L, Wei X, Datta T, Wei F, Xie Z. Polyploidization: A Biological Force That Enhances Stress Resistance. Int J Mol Sci. 2024;25(4):1957.
30. Jiang J, Yang N, Li L, Qin G, Ren K, Wang H, et al. Tetraploidy in *Citrus wilsonii* Enhances Drought Tolerance via Synergistic Regulation of Photosynthesis, Phosphorylation, and Hormonal Changes. Front Plant Sci. 2022;13 April:1–16.

31. Ghouri F, Shahid MJ, Liu J, Lai M, Sun L, Wu J, et al. Polyploidy and zinc oxide nanoparticles alleviated Cd toxicity in rice by modulating oxidative stress and expression levels of sucrose and metal-transporter genes. J Hazard Mater. 2022;2023(448): 130991.
32. Nousheen M, Basit A, Khan MK, Alzayed RM, Alhajouj SA, Alaida MF, et al. Pharmacognistic, proximate and phytochemical analysis of stem of Cistanche tubulosa (Schenck) Hook. F Cell Mol Biol. 2024;70:26–35.
33. Li H. The Principles and Techniques of Plant Physiology and Biochemistry Experiments. Higher Education Press;2000.
34. Zhang Y, Wang B, Qi S, Dong M, Wang Z, Li Y, et al. Ploidy and hybridity effects on leaf size, cell size and related genes expression in triploids, diploids and their parents in *Populus*. Planta. 2019;249:635–46.
35. Lin J, Zhang B, Zou J, Luo Z, Yang H, Zhou P, et al. Induction of tetraploids in Paper Mulberry (Broussonetia papyrifera (L.) L'Hér. ex Vent.) by colchicine. BMC Plant Biol. 2023;23:1–13.
36. Manzoor A, Ahmad T, Bashir MA, Hafiz IA, Silvestri C. Studies on Colchicine Induced Chromosome Ornamental Plants. Plants. 2019;8:1–16.
37. Dong R, Pei Y, Hong L, Nie J, Chen Y, Yan H, et al. Tetraploid induction identification and transcriptome preliminary analysis of black currant (Ribes rubrum L.). Plant Cell Tissue Organ Cult. 2023;155:861–72.
38. Zhang X, Zheng Z, Wang J, Li Y, Gao Y, Li L, et al. In vitro induction of tetraploids and their phenotypic and transcriptome analysis in *Glehnia littoralis*. BMC Plant Biol. 2024;24:1–14.
39. Ni Z, Kim ED, Ha M, Lackey E, Liu J, Zhang Y, et al. Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature. 2009;457:327–31.
40. Dudits D, Török K, Cseri A, Paul K, Nagy AV, Nagy B, et al. Response of organ structure and physiology to autotetraploidization in early development of energy willow *Salix viminalis*. Plant Physiol. 2016;170:1504–23.
41. Ma Y, Xue H, Zhang L, Zhang F, Ou C, Wang F, et al. Involvement of Auxin and Brassinosteroid in Dwarfism of Autotetraploid Apple (Malus × domestica). Sci Rep. 2016;6:1–14.
42. Lin C, Hu W, Qin X, Fei Y, Hu D. Effects of Serendipita indica on the Morphological and Physiological Characteristics of *Agrostis stolonifera* L. Under Drought Stress Agronomy. 2025;15:1–16.
43. Zhou J, Guo F, Fu J, Xiao Y, Wu J. In vitro polyploid induction using colchicine for Zingiber officinale Roscoe cv. 'Fengtou' ginger. Plant Cell Tissue Organ Cult. 2020;142:87–94.
44. Yu Q, Zhang L, Sun D, Hu Y, Li P, Zhang X, et al. Interaction between MAPKs and MKPs in hexaploid chrysanthemum illuminates functional paralogue diversification in polyploids. Hortic Plant J. 2023;9:158–68.
45. Wei W, Huang Z, Chen H, Yang H, Lan X, Liang Z, Li H. Comparison of leaf morphology, physiology and mite-resistant characteristics between diploid and autotetraploid *Manihot esculenta* Crantz. J Nucl Agric Sci. 2022;36:2115–23.
46. Evans JR. Improving photosynthesis. Plant Physiol. 2013;162:1780–93.
47. Coate JE, Schlueter JA, Whaley AM, Doyle JJ. Comparative evolution of photosynthetic genes in response to polyploid and nonpolyploid duplication. Plant Physiol. 2011;155:2081–95.
48. Zhang J, Cheng C, Xiao F, Zhang X, Zhang C, Zhao Y, et al. Effects of ploidy level on leaf morphology, stomata, and anatomical structure of *Hibiscus syriacus* L. BMC Plant Biol. 2024;24:1133.
49. Meng F, Peng M, Pang H, Huang F. Comparison of photosynthesis and leaf ultrastructure on two black locust (*Robinia pseudoacacia* L.). Biochem Syst Ecol. 2014;55:170–5.
50. Wang H, Yu M, Lu C, Wu C, Jing C. Study on Breeding and Photosynthetic Characteristics of New Polyploidy Variety for Leaf and Fruit-Producing Mulberry (*Morus* L). Sci Agric Sin. 2011;44:562–9.
51. Chen Y, Chen J, Li W, Cui L, Chen L. Photosynthetic characteristics and ultrastructure in *Cucumis* allopolyploids witn different ploid levels. Sci Agric Sin. 2010;44:32–6.
52. Liao T, Cheng S, Zhu X, Min Y, Kang X. Effects of triploid status on growth, photosynthesis, and leaf area in *Populus*. Trees - Struct Funct. 2016;30:1137–47.
53. Pan X, Liu S, Li Q, He J, Sun zhaojun, MaO X. Photosynthetic diurnal variations of *Symphytum officinale* in different regions of Ningxia and selection of suitable introduction sites. J Northwest A&F Univ Sci Ed). 2023;51.
54. Zhao P, Zhao X, Zhang Y, Zhai Z, Guan Z, Luo H. Study on Daily Changes in Photosynthesis of Different Ploidy Wheat. Acta Agric Jiangxi. 2018;30:19–23.
55. Liu W, Yan Z, Wang M. Study on the Diurnal Changes of Photosynthetic Rate in Watermelons with Different Chromosome Ploidy. Chinese watermelons and cantaloupes. 2003;2:10–2.
56. Zhu Z. Induction of Polyploid of *Populus davidiana* Dode and *Populus simonii* X *Populus nigra* and analyses of their growths and photosynthetic characteristics. 2010.
57. Tingting W, Guanghai Z, Wenbo C, Huachun G. Comparison of Photosynthetic Characteristics between Diploid Potato and Its Chromosomedoubled Individuals. J Yunnan Agric Univ Sci. 2019;34:371–6.
58. Wang F, Liu Z, Yan L, Sun Z, Cheng T, Yang B, et al. Photosynthetic Characteristics of Different Ploidy Varieties of *Pteroceltis tatarinowii*. Chinese Agric Sci Bull. 2018;34:26–30.
59. Yao X, Qi Y, Chen H, Zhang B, Chen Z, Lu L. Study of *Camellia sinensis* diploid and triploid leaf development mechanism based on transcriptome and leaf characteristics. PLoS One. 2023;18(2)e0275652.

Publisher's Note