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Study on the growth characteristics of *Pinus yunnanensis* seedlings based on hormonal regulation and chlorophyll metabolism

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Pinus yunnanensis **is an important coniferous species for both ecological and economic purposes in Southwest China. However, its seedlings grow slowly and show low productivity, with notable trait variability under the same cultivation conditions. These characteristics limit its extensive use in reforestation and forest management. Photosynthesis is the foundational process for plant growth and biomass accumulation. Chlorophyll, as the essential pigment in photosynthesis, directly impacts the synthesis of organic materials and the increase in biomass. In this study, the seedlings of *P. yunnanensis* were classified into three grades (I, II, and III) using the mean value ± 1/2 standard deviation method (H ± 1/2σ). Various growth traits, such as the number of branches, plant height, stem thickness, needle length, root characteristics, and biomass, were measured at multiple time points. The content of photosynthetic pigments was determined using spectrophotometry, endogenous hormone levels were analyzed by LC-MS/MS, and the expression of genes related to chlorophyll metabolism was detected through qRT-PCR. Pearson correlation analysis was employed to quantify the associations between hormone content, gene expression, and the seedlings' morphological characteristics. The results indicate that Grade I significantly outperform Grades II and III in terms of above-ground growth metrics. During the rapid growth period in March, Grade I seedlings had the highest chlorophyll content, potentially enhancing photosynthetic efficiency and increasing biomass allocation. Hormonal analysis revealed that compared to other grades, Grade I seedlings exhibited higher levels of ABA, JAs, and ACC, while Auxins and SAs were lower; there was no significant difference in CTK content between Grade I and Grade II, both of which were lower than in Grade III. qRT-PCR analysis confirmed that the expression of genes related to chlorophyll synthesis was significantly upregulated in Grade I seedlings. In contrast, the expression of genes associated with chlorophyll degradation was more active in Grade III seedlings. Correlation analysis further emphasized the strong link between hormone levels, gene expression, and growth traits. This study delves into the intrinsic mechanisms of morphological differentiation in *P. yunnanensis* seedlings by examining the synergistic effects of chlorophyll metabolism and endogenous hormone regulation. These findings can provide valuable references for cultivating high-quality seedlings, optimizing seed source selection, and improving afforestation techniques.**

Keywords *Pinus yunnanensis*, Chlorophyll metabolism, Endogenous phytohormones, Seedling differentiation

Chlorophyll is the core pigment essential for the normal process of photosynthesis. It is primarily found in green plants, algae, and photosynthetic bacteria. It is located within the thylakoid membranes of chloroplasts, where it absorbs, transfers, and converts light energy to facilitate carbon assimilation[1,2]. The content of chlorophyll (total chlorophyll, chlorophyll a, chlorophyll b) and accessory pigments such as carotenoids in leaves not only reflects the photosynthetic capacity and resource acquisition efficiency of plants but is also commonly used as a crucial indicator of plant growth condition[3]. Currently, the biosynthetic pathway of chlorophyll from L-glutamyl-tRNA

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to chlorophyll a and then to chlorophyll b is relatively well understood. It involves up to 15 enzyme-catalyzed reactions and 27 enzyme-encoding genes. The *HEMA1* gene encodes glutamyl-tRNA reductase, a key rate-limiting enzyme in the early stages of chlorophyll synthesis, while the *GSA* gene regulates the production of 5-aminolevulinic acid (ALA), directly determining the subsequent synthesis rate[4,5]. The *CAO* gene controls the critical reaction converting chlorophyll a to chlorophyll b, thereby regulating the ratio of chlorophyll a to b[6]. During the degradation phase, the functionality of genes such as *CLH* (chlorophyllase)[7], *PPH* (pheophytinase)[8], *PAO* (pheophorbide a oxygenase)[9], and *RCCR* (red chlorophyll catabolite reductase)[10] is crucial for chlorophyll breakdown. Deficiencies in these genes can lead to the accumulation of toxic intermediates, adversely affecting cell health and plant growth[8,9,11]. Conversely, the *SGR* (Stay-green) gene is considered a crucial 'switch' for regulating chlorophyll degradation. Overexpression of SGR accelerates leaf senescence and reduces photosynthetic efficiency[12,13].

In addition to genetic factors, plant hormones play a crucial role in regulating chlorophyll synthesis and degradation, photosynthetic efficiency, and seedling growth and development[14–17]. Existing research indicates that auxins (IAA) and cytokinins (CTK) influence chloroplast development, leaf structure, and photosynthetic efficiency. Variations in their levels or ratios often result in differences in photosynthetic characteristics and growth potential[18–20]. For example, the accumulation of carotenoids during the ripening of tomato fruits is regulated by the balance between auxins and ethylene[21]. Abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) are more extensively involved in plant perception and response to stress signals, indirectly affecting chlorophyll degradation and the aging process[22–24].

P. yunnanensis, a light-loving, deep-rooted coniferous tree species, is widely distributed in the southwestern region of China. Due to its high adaptability to poor soils, it has become one of the main species for afforestation and timber production in arid mountainous areas[25,26]. However, its seedlings exhibit slow growth and low productivity and demonstrate significant phenotypic differentiation under the same environmental conditions. These factors limit its survival rate in afforestation and the quality of forest stands[27]. Chlorophyll is the key pigment in photosynthesis, and its content directly influences photosynthetic efficiency and the nutritional status of plants, thereby determining plant biomass and productivity[28]. Currently, research on the differentiation of *P. yunnanensis* seedlings primarily focuses on analyzing phenotypic variation[29] and allometric growth among different provenances[30] and the molecular mechanisms of endogenous hormone regulation of axillary bud differentiation[31]. Research on the impact of chlorophyll metabolism and endogenous hormone regulation on growth has primarily focused on herbaceous and broadleaf species, such as tobacco[32], cotton[33], Arabidopsis[8], and Populus[34]. Currently, there is a lack of systematic studies to elucidate the intrinsic relationships between endogenous hormones, chlorophyll-related gene expression, and seedling growth in *P. yunnanensis*. Therefore, this study categorized *P. yunnanensis* seedlings into three grades (I, II, and III) based on differences in morphological characteristics, using the method of mean ± 1/2 standard deviation. This study combines analyses of seedling phenotypic traits, photosynthetic pigment content, endogenous hormone levels, and chlorophyll metabolism-related gene expression to investigate the intrinsic mechanisms of morphological differentiation and clarify the relationships among genes, hormones, and phenotypes. The goal is to provide a reference for enhancing the quality of *P. yunnanensis* seedlings and optimizing forest management strategies.

Materials and methods

Research location and experimental species

The seeds used in this experiment were sourced from the Dali National *P. yunnanensis* Breeding Base in China and were sown in the nursery of Southwest Forestry University (Kunming, N 25°04'00", E 102°45'41"). In January 2022, after measuring the ground diameter and height of 1,200 *P. yunnanensis* seedlings, the seedlings were classified into three grades based on national or local nursery quality standards: Grade $\mathrm{I} \geq \mathrm{H} + 1/2\sigma$, $\mathrm{H} - 1/2\sigma \leq$ Grade $\mathrm{II} < \mathrm{H} + 1/2\sigma$, Grade $\mathrm{III} < \mathrm{H} - 1/2\sigma$. Here, H represents the average height of the seedlings, and σ is the standard deviation of this height population (Fig. 1). After classification, the seedlings were further cultivated under uniform garden conditions. The area experiences long sunshine hours and a short frost period, with an elevation of 1,945 m. It features a subtropical semi-humid plateau monsoon climate, with an average annual temperature of 14.7 °C, average annual precipitation of 883.8 mm, and an average relative humidity of 68.2%.

Measurement of seedling growth indices

In 2022, based on the seasonal growth characteristics of *P. yunnanensis* and the climatic conditions of the study area, the growth indices of seedlings from three grades were measured in January, March, May, August, and December[35]. A ruler (accurate to 0.1 cm) measured plant height and needle length. A caliper (accurate to 0.01 mm) measured the ground diameter. The number of nodes on all seedlings was counted and recorded. The root systems were scanned using an Epson root scanner to obtain images, which were then analyzed using WinRHIZO software[36].

Measurement of photosynthetic pigment content in seedlings

Fresh tender needles from three grades of *P. yunnanensis* were collected, and according to the method described by Porra et al.'s[37], 0.5 g of the sample were weighed and then ground with a small quantity of quartz sand and calcium carbonate powder. After grinding, 2 mL of 80% acetone was added and the grinding continued until the mixture became a uniform slurry. An additional 10 mL of 80% acetone was added, and the mixture was left at room temperature in the dark until it turned white. The entire mixture was then transferred to a 25 mL brown volumetric flask and made up to volume with 80% acetone. The absorbance of the supernatant was measured at 470, 645, and 663 nm using a UV spectrophotometer (Shimadzu UV-16 A, Shimadzu Corporation, Kyoto,

Fig. 1. Seedlings of *P. yunnanensis* at different grades. I: Grade $\text{I} \geq H + 1/2\sigma$; II: $H - 1/2\sigma \leq \text{Grade II} < H + 1/2\sigma$; III: $\text{Grade III} < H - 1/2\sigma$.

Japan), with acetone serving as the blank control. The content of photosynthetic pigments was computed using the formulas provided by Lichtenthaler[38] and Bao[39]:

$$\text{Chlorophyll a (mg/g)} = (12.72 \times A663 - 2.59 \times A645) \times V/(1000 \times W)$$

$$\text{Chlorophyll b (mg/g)} = (22.88 \times A645 - 4.67 \times A663) \times V/(1000 \times W)$$

$$\text{Total chlorophyll (mg/g)} = (20.29 \times A645 + 80.05 \times A663) \times V/(1000 \times W)$$

$$\text{Carotenoid (mg/g)} = (1000 \times A470 - 3.27 \times \text{Chlorophyll a} - 104 \times \text{Chlorophyll b})/229$$

In these formulas, A470、 A645 and A663 represent the absorbance at the respective wavelengths. (V) is the extract's total volume (mL), and (W) is the fresh weight of the needles used for extraction (g).

Biomass measurement

At each time interval, 15 seedlings were randomly collected from different grades. The roots, stems, and leaves were separately measured for fresh weight using an electronic balance with a precision of 0.0001 g. After 30 min of drying at 110 °C in an oven, the samples were dried again at 80 °C until their mass remained constant. The biomass of each component was recorded, and the distribution of biomass among the components was calculated.

Plant hormone analysis

The content of plant hormones was measured using LC-MS/MS. During the rapid growth phase in March, concentrations of various cytokinins, auxins, jasmonates, salicylates, abscisic acid (ABA), and ethylene (ACC) were determined in the shoot tips of *P. yunnanensis* seedlings of different grades, each tested in triplicate. The cytokinins measured included isopentenyl adenine (IP), dihydrozeatin (DZ), isopentenyl adenosine (IPA), trans-zeatin (TZ), and trans-zeatin riboside (TZR). The auxins included indole-3-acetic acid (IAA), methyl indole-3-acetate (Me-IAA), and indole-3-carbaldehyde (ICAld). Jasmonates consisted of jasmonic acid (JA) and JA-Ile (N-jasmonoyl-L-isoleucine). Salicylates measured were salicylic acid (SA) and its glycosylated form, salicylic acid glucoside (SAG). Additionally, abscisic acid (ABA), and ethylene (ACC) were quantified. The hormone extraction and LC-MS/MS method are as follows: after freezing the shoot tips in liquid nitrogen and grinding into powder, 1 mL of methanol and a mixed internal standard reserve solution were added. The mixture was vortexed for 30 s and ultrasonicated for 10 min, then transferred to a metal bath to oscillate for 4 h[40]. After centrifugation for 10 min (12000 rpm, 4 °C) and filtration through a 0.22 μm filter, the supernatant was analyzed. Chemical analysis was performed using the ExionLC high-performance liquid chromatography system (AB Sciex, USA); data was collected using an AB SCIEX 6500 + Qtrap mass spectrometer (AB Sciex, USA) in the multiple reaction monitoring (MRM) model. Raw data was captured using Analyst 1.7.2 software, and results were qualitatively and quantitatively analyzed using SCIEX OS V2.0.1.48692 software.

Real-time fluorescent quantitative qPCR analysis

The expression of genes linked to chlorophyll metabolism in the tips of the shoots is investigated using qRT-PCR. Total RNA is extracted from the shoot tips using a polysaccharide polyphenol plant total RNA extraction kit, and cDNA is synthesized using a reverse transcription kit, which then serves as the template for real-time fluorescent quantitative PCR. This cDNA is diluted tenfold and stored at -20 °C for subsequent use. Primers for the genes are designed using Primer 5.0 software (Supplementary Table S1), with the *TUBA1* gene acting as the internal reference control. Using Taq Pro Universal SYBR qPCR Master Mix, real-time fluorescence quantitative expression analysis was carried out. The reaction system totaled 20 μL, including 1 μL of cDNA template, 0.4 μL each of F and R primers, 10 μL of SYBR Mix, and 8.2 μL of sterile water. The relative expression levels of the genes were calculated using the $2^{-\Delta\Delta Ct}$ method. Variance analysis was conducted using SPSS 24 software.

Correlation statistical analysis

To elucidate the relationships between endogenous hormone levels, chlorophyll metabolism gene expression, and seedling growth traits, this study employed https://www.omicshare.com to create a network heatmap, including the use of SPSS 24 to perform Pearson correlation analysis on gene expression and seedling growth traits to assess the linear relationships between them. Additionally, the Mantel test assessed the overall correlation between endogenous hormone levels, chlorophyll metabolism gene expression, and seedling growth traits. The Mantel test calculates the correlation coefficient (r) based on different matrices, with values ranging from – 1 to 1. An $r > 0$ indicates a positive correlation, with higher values denoting stronger relationships; an $r < 0$ indicates a negative correlation, with larger absolute values signifying more significant negative relationships; when $r \approx 0$, it suggests no apparent linear correlation between the matrices. The test evaluates statistical significance using a permutation test, typically considering $p < 0.05$ as the threshold for significance.

Results analysis

Analysis of phenotypic traits of *P. yunnanensis* seedlings of different grades

Distinct differences in above-ground traits are evident among different grades of *P. yunnanensis* seedlings (Fig. 2). Grade I seedlings exhibit significantly greater plant height (Fig. 2B), ground diameter (Fig. 2C), and average number of branches (Fig. 2A) compared to Grades II and III. In terms of needle length, significant differences between Grade I and Grade III seedlings are only evident in January and December (Fig. 2D), with no significant differences observed during other periods. Overall, both plant height and average number of seedlings exhibit a 'slow-fast-slow' growth rhythm, accelerating from March, peaking around August, and then decelerating (Fig. 2A and B). In contrast, the growth in ground diameter and needle length displays a certain lag (Fig. 2C and D). Despite some fluctuations across different months, Grade I seedlings consistently maintained a significantly superior above-ground growth performance throughout the various monthly stages. Analysis of the data on the below-ground parts (Table 1) reveals that the differences are relatively limited. In January and May, the average root diameters of Grade I and Grade II seedlings were similar, and both were significantly greater than those of Grade III. By August, Grade I is markedly higher than both Grade II and Grade III, with no significant differences between the latter two. Total root volume only shows significant differences in August, with Grade I being the highest. Apart from the aforementioned time points, the root growth of seedlings across different grades does not exhibit clear gradational characteristics in most months.

Analysis of photosynthetic pigment content in seedlings of different grades

In March, the content of photosynthetic pigments generally reaches a peak, followed by a gradual decline, with levels dropping to their lowest by December (Fig. 3). From March to August, the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids exhibit a trend of 'Grade I > Grade II > Grade III.' In contrast, December shows a trend of 'Grade I < Grade II < Grade III' (Fig. 3A, B, C and D). Additionally, the chlorophyll a/b ratio in Grade I seedlings remains consistently lower than in Grade II from January to August and is also significantly lower than in Grade III during March and May (Fig. 3E). Figure 3F shows that in January, the carotenoid/chlorophyll ratio in Grade I seedlings was significantly higher than in Grade III, with no significant difference from Grade II. There are no significant differences in this ratio among the grades in March and December. However, in May and August, the ratio for Grade II is significantly higher than for Grade I; in August, there is no significant difference compared to Grade III. Grade I seedlings have higher photosynthetic pigment levels during most of the growing season (January to August), while these indices are generally lower in December. In contrast, Grade II typically maintains intermediate levels of photosynthetic pigments, whereas Grade III initially has lower levels than the other two grades, with some indices only increasing later in the season.

Biomass investment and allocation in seedlings of different grades

Significant differences are observed in the stem, leaves, above-ground parts, non-photosynthetic organs, and overall biomass of seedlings across different grades in January and December (Table 2). No significant differences are observed in March; in May, only stem biomass shows significant variation; by August, differences emerge in the leaves, above-ground parts, and overall plant biomass. Overall, the biomass investment across different ages shows that Grade I seedlings invest the most, while Grade III invest the least.

Considering the biomass distribution among different organs (Fig. 4), significant differences in the allocation ratio of the stem are observed only in January, May, and December between Grade I and Grades II and III (Fig. 4A, C and E). Between photosynthetic (leaf) and non-photosynthetic organs, the allocation ratios in January and December are generally similar (Fig. 4A and E). During the rapid growth period in March, except for Grade II, the other grades exhibit a higher allocation ratio in photosynthetic organs (Fig. 4B). Regarding the allocation structure between above-ground and below-ground parts, seedlings exhibit relatively balanced distribution in

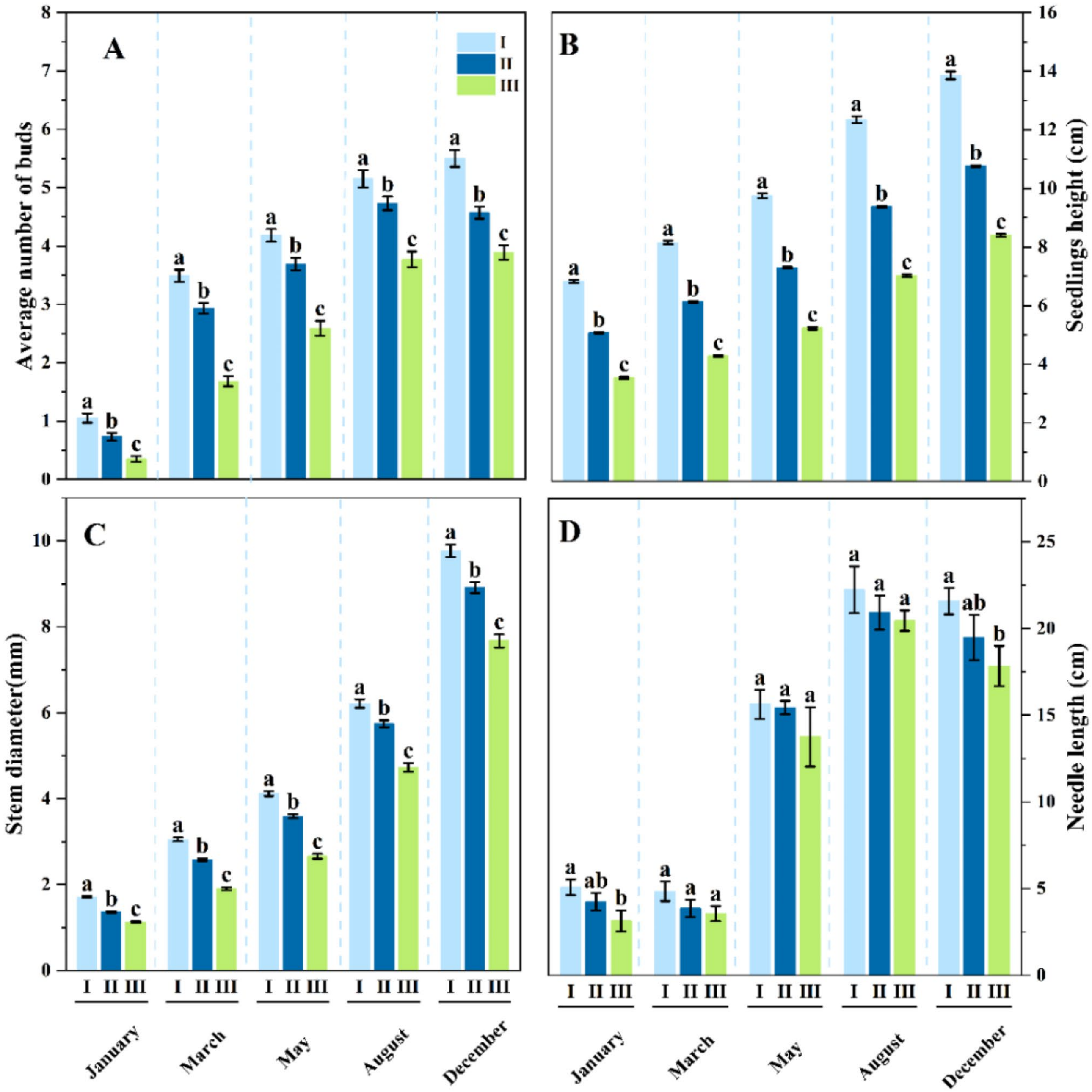

Fig. 2. Changes in growth indicators of *P. yunnanensis* seedlings of different grades. (**A**) average number of buds, (**B**) seedling height, (**C**) stem diameter, and (**D**) needle length. The figure displays the average value ± standard data. Distinct letters signify notable discrepancies among various grades of *P. yunnanensis* ($p < 0.05$).

the early stages. In contrast, the middle and late stages show a greater tendency towards above-ground parts. In summary, the biomass investment and distribution patterns of seedlings at different ages vary by grade and growth stage. Grade I focuses more on increasing biomass investment and enhancing above-ground growth.

Hormone analysis

Significant differences exist among different grades of seedlings in terms of various endogenous hormone contents and ratios (Fig. 5). Compared to Grade II, Grade I seedlings exhibit 1.46 times higher levels of ABA and 1.39 times higher levels of ACC (Fig. 5E and F), while the contents of Auxins and SAs are respectively 1.10 times and 3.39 times lower (Fig. 5B and D).Compared to Grade III, Grade I seedlings show a 7.15-fold increase in JAs, a 1.48-fold increase in ABA, and a 1.16-fold increase in ACC (Fig. 5C, E, F), while their levels of CTKs, Auxins, and SAs are significantly lower (Fig. 5A, B, D).In comparisons between Grade II and Grade III, Grade II exhibits JAs and SAs levels that are 7.07 times and 1.30 times higher, respectively (Fig. 5C and D), while Grade III has 1.09 times higher CTKs and 1.20 times higher ACC levels (Fig. 5A, F).

Months	Grade	Main root length (cm)	Total root length (cm)	Average diameter (mm)	Surface area (cm)	Total volume (cm^3)
January	I	196.589 ± 6.83a	351.190 ± 24.93a	0.733 ± 0.266a	16.253 ± 0.777a	0.927 ± 0.764a
	II	216.634 ± 92.52a	290.893 ± 91.00a	0.545 ± 0.545a	15.179 ± 2.254a	0.528 ± 0.282a
	III	158.956 ± 63.68a	268.274 ± 82.02a	0.515 ± 0.515a	14.722 ± 1.889a	0.336 ± 0.156a
March	I	298.038 ± 290.722a	275.855 ± 79.790a	0.750 ± 0.037a	15.463 ± 1.558a	1.408 ± 1.546a
	II	266.717 ± 94.480a	313.009 ± 51.538a	0.718 ± 0.109ab	16.510 ± 1.023a	1.176 ± 0.650a
	III	230.469 ± 65.777a	261.215 ± 77.639a	0.631 ± 0.068b	15.382 ± 3.825a	0.751 ± 0.307a
May	I	582.935 ± 312.993a	385.572 ± 47.393a	0.763 ± 0.063a	17.688 ± 0.30la	2.519 ± 0.937a
	II	577.465 ± 102.538a	411.133 ± 16.855a	0.687 ± 0.36lab	17.841 ± 0.075a	2.109 ± 0.763a
	III	652.910 ± 353.620a	413.502 ± 11.277a	0.647 ± 0.086b	17.708 ± 0.22la	2.046 ± 0.958a
August	I	1024.125 ± 279.23la	455.023 ± 5.856a	1.104 ± 0.074a	18.856 ± 0.136a	9.750 ± 2.483a
	II	970.160 ± 331.052a	446.397 ± 10.356a	0.936 ± 0.070b	18.775 ± 0.324a	6.541 ± 1.809b
	III	890.371 ± 241.092a	439.480 ± 18.74la	0.913 ± 0.075b	18.582 ± 0.0391a	5.815 ± 1.788b
December	I	1284.767 ± 902.933a	260.204 ± 95.35la	0.624 ± 0.133a	15.659 ± 5.84la	2.656 ± 1.013a
	II	854.411 ± 346.92la	211.317 ± 3.795a	0.534 ± 0.056a	12.820 ± 0.276a	1.822 ± 0.434a
	III	947.469 ± 413.748a	202.629 ± 8.218a	0.539 ± 0.127a	12.592 ± 0.18la	2.251 ± 1.622a

Table 1. Root system growth of *P. yunnanensis* seedlings of different classes.

In terms of hormone ratios, seedlings of different grades also exhibit clear differentiation (Fig. 5G–L). Grade I seedlings exhibit the highest JAs/SAs ratio (Fig. 5K) and show significant differences in most ratios compared to Grades II and III; Grade III displays higher CTKs/ABA and CTKs/JAs ratios (Fig. 5I and L).Grade I seedlings exhibit the highest JAs/SAs ratio (Fig. 5K) and show significant differences in most ratios compared to Grades II and III; Grade III displays higher CTKs/ABA and CTKs/JAs ratios (Fig. 5I and L).

qRT-PCR gene expression analysis

Different grades of *P. yunnanensis* exhibit diversity in the expression of genes related to chlorophyll metabolism (Fig. 6). Compared to Grade II, Grade I exhibits significantly upregulated expression levels of *GSA*, *CHLM*, and *CHLG*, while Grade II shows relatively increased expression of genes such as *HEMA1*, *CLH*, and *SGR*. In the comparison between Grade I and Grade III, only the expression levels of *CHLH* and *PPH* show no significant changes, while most other genes exhibit significant differences. Between Grade II and Grade III, statistical differences are observed in the expression of most genes. *HEMA1*, *GSA*, *CHLH*, and *CRD1* are higher in Grade II, while the expression levels of *CLH*, *PAO*, *RCCR*, and *SGR* are significantly upregulated in Grade III.

Analysis of the correlation between endogenous hormones, chlorophyll metabolism, and seedling indices

Pearson correlation analysis indicates that genes related to chlorophyll synthesis are significantly positively correlated with most seedling traits (Fig. 7). Among them, the *CHLG* and *CHLM* genes show a robust positive correlation with RB ($r = 1$, $p < 0.01$). *CHLM* is also significantly positively correlated with NPB ($r = 0.998$, $p < 0.01$) and *CHLG* with total root volume ($r = 0.999$, $p < 0.01$). Additionally, the *CAO* gene is significantly positively correlated with SD and ANB ($r = 0.999$, $p < 0.05$; $r = 0.998$, $p < 0.05$), and the *GSA* gene also shows significant positive correlations with NI and SD ($r = 0.998$, $p < 0.05$). Conversely, genes associated with chlorophyll degradation generally exhibit negative correlations with seedling traits. *RCCR* and *PAO* show a significant negative correlation with Total Chl ($r = -1$, $p < 0.05$), and *CLH* is negatively correlated with NI ($r = -0.999$, $p < 0.05$).

Further Mantel test results indicate significant associations between ABA, JAs, Auxins, CTKs, gene expression, and seedling indicators. ABA exhibits a strong positive correlation with primary root length (PRL); JAs show a significant positive correlation with Total Chl and are most notably negatively correlated with *RCCR*. Auxins are positively correlated with *PPH* expressions and negatively correlated with PRL and *CHLG*. Meanwhile, CTKs show the most significant negative correlation with Chl c and Chl a. Other hormones, such as SAs and ACC, also exhibit positive or negative correlations with several genes or traits, though these are not statistically significant. Overall, there is a close relationship between hormone levels, gene expression, and seedling growth traits. Hormones such as ABA, JAs, Auxins, and CTKs may play a key role in regulating the expression of chlorophyll-related genes and influencing seedling phenotypes.

Discussion

As an important ecological and economic tree species in Southwest China, *P. yunnanensis* exhibits slow growth and distinct trait differentiation during the seedling stage, limiting its potential for afforestation and utilization[27]. This study found that Grade I reach peak chlorophyll content during the rapid growth period (March), coupled with a lower carotenoid/chlorophyll ratio, indicating higher photosynthetic efficiency and thus outstanding performance in above-ground growth metrics. Correspondingly, the expression levels of key synthesis genes such as *GSA*, *CHLM*, and *CHLG* in Grade I are significantly higher than in other grades, potentially enhancing chlorophyll synthesis by boosting the production of critical intermediates such as 5-aminolevulinic acid (ALA) and magnesium protoporphyrin IX monomethyl ester[41,42]. Higher levels of gene expression may

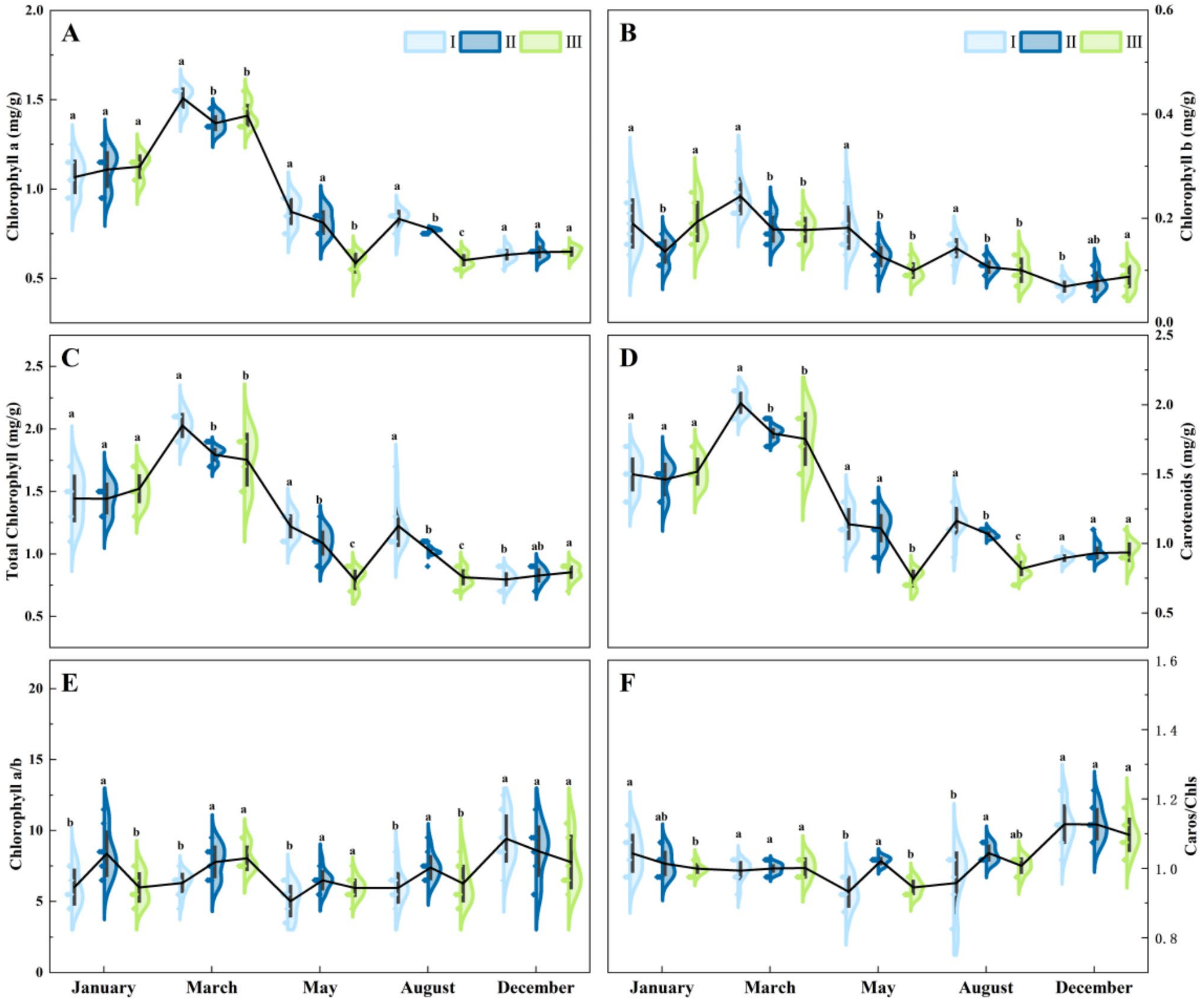

Fig. 3. Variations in the photosynthetic pigment content of different grades of *P. yunnanensis* seedlings. (**A**) Chlorophyll a content, (**B**) Chlorophyll b content, (**C**) Total chlorophyll, (**D**) Carotenoid content, (**E**) Chlorophyll a/b, and (**F**) Carotenoid/Chlorophyll. Each dot represents the actual data point for each sample, with black lines connecting the average values of each grade. Different lowercase letters indicate significant differences between grades at the same time point ($p < 0.05$).

enhance chlorophyll synthesis capacity and photosynthetic efficiency, thereby promoting seedling growth and development. Grade I surpasses other grades in traits such as plant height, ground diameter, and branch number. In contrast, the high expression of degradation genes such as *CLH*, *PAO*, *RCCR*, and *SGR* in Grade III accelerates chlorophyll degradation, reducing photosynthetic capacity and consequently limiting growth[43,44]. This indicates that differences at the genetic level are directly reflected in the seedlings' photosynthetic performance and above-ground morphological indices.

In environments with limited resources, plants optimize their survival and reproductive capacity by adjusting biomass allocation. The above-ground parts of *P. yunnanensis* seedlings, such as stems and leaves, which serve as the principal organs for energy acquisition through photosynthesis, exhibit a significant growth advantage in competing for light resources[45,46]. Conversely, the root system, primarily responsible for the absorption of water and nutrients, often shows less significant growth variations. However, the growth strategies of the primary and lateral roots vary across different growth stages[47]. Under the cold conditions and low soil temperatures of January, the growth of the primary root is inhibited, while some lateral roots may maintain their earlier growth state, causing the primary root length to be shorter than the total root length initially. However, as temperatures rise, the growth of *P. yunnanensis* seedlings accelerates, with the primary root rapidly extending to access deeper water sources and nutrients. In contrast, the growth rate of lateral and other roots is relatively slower, leading to the primary root length gradually surpassing the total root length. In this study, Grade I responded more quickly during the rapid growth period in March, exhibiting earlier primary root growth than other grades. The primary root growth in other grades typically accelerated significantly from May. By December, as plants enter a dormant

Months	Grade	Biomass (g) Root	Stem	Leave	Aboveground	Non-photosynthetic organs	Individual
January	I	0.12 ± 0.04a	0.06 ± 0.02a	0.20 ± 0.05a	0.26 ± 0.07a	0.18 ± 0.02a	0.38 ± 0.08a
	II	0.12 ± 0.46a	0.04 ± 0.01b	0.16 ± 0.06ab	0.20 ± 0.07ab	0.16 ± 0.02ab	0.32 ± 0.11ab
	III	0.08 ± 0.32a	0.03 ± 0.01b	0.11 ± 0.36b	0.13 ± 0.04b	0.11 ± 0.02b	0.21 ± 0.07b
March	I	0.19 ± 0.13a	0.09 ± 0.03a	0.23 ± 0.11a	0.33 ± 0.14a	0.28 ± 0.07a	0.52 ± 0.26a
	II	0.14 ± 0.05a	0.08 ± 0.03a	0.20 ± 0.08a	0.28 ± 0.09a	0.22 ± 0.03a	0.42 ± 0.13a
	III	0.11 ± 0.37a	0.08 ± 0.04a	0.15 ± 0.07a	0.23 ± 0.11a	0.19 ± 0.03a	0.34 ± 0.14a
May	I	0.51 ± 0.18a	0.46 ± 0.13a	1.41 ± 0.27a	1.87 ± 0.33a	0.97 ± 0.31a	2.37 ± 0.47a
	II	0.38 ± 0.08a	0.33 ± 0.08b	1.24 ± 0.28a	1.56 ± 0.35a	0.70 ± 0.15a	1.94 ± 0.42a
	III	0.38 ± 0.16a	0.27 ± 0.05b	1.14 ± 0.36a	1.41 ± 0.39a	0.65 ± 0.19a	1.79 ± 0.53a
August	I	1.23 ± 0.29a	1.08 ± 0.23a	3.73 ± 0.67a	4.81 ± 0.86a	2.31 ± 0.21a	6.04 ± 1.04a
	II	1.15 ± 0.40a	0.97 ± 0.16a	3.55 ± 0.44ab	4.52 ± 0.44ab	2.12 ± 0.23a	5.67 ± 0.41ab
	III	0.91 ± 0.23a	0.83 ± 0.24a	2.89 ± 0.60b	3.72 ± 0.73b	1.74 ± 0.10a	4.63 ± 0.80b
December	I	4.50 ± 1.75a	4.27 ± 1.22a	8.58 ± 1.51a	12.85 ± 2.59a	8.77 ± 2.41a	17.35 ± 3.83a
	II	2.94 ± 0.41a	2.40 ± 0.88b	5.57 ± 2.38b	7.97 ± 3.24b	5.34 ± 1.26b	10.91 ± 3.62b
	III	2.94 ± 1.77a	1.82 ± 0.56b	4.50 ± 0.79b	6.38 ± 1.30b	4.83 ± 2.31b	9.32 ± 3.01b

Table 2. Biomass investment analyses of *P. yunnanensis* seedlings in different classes.

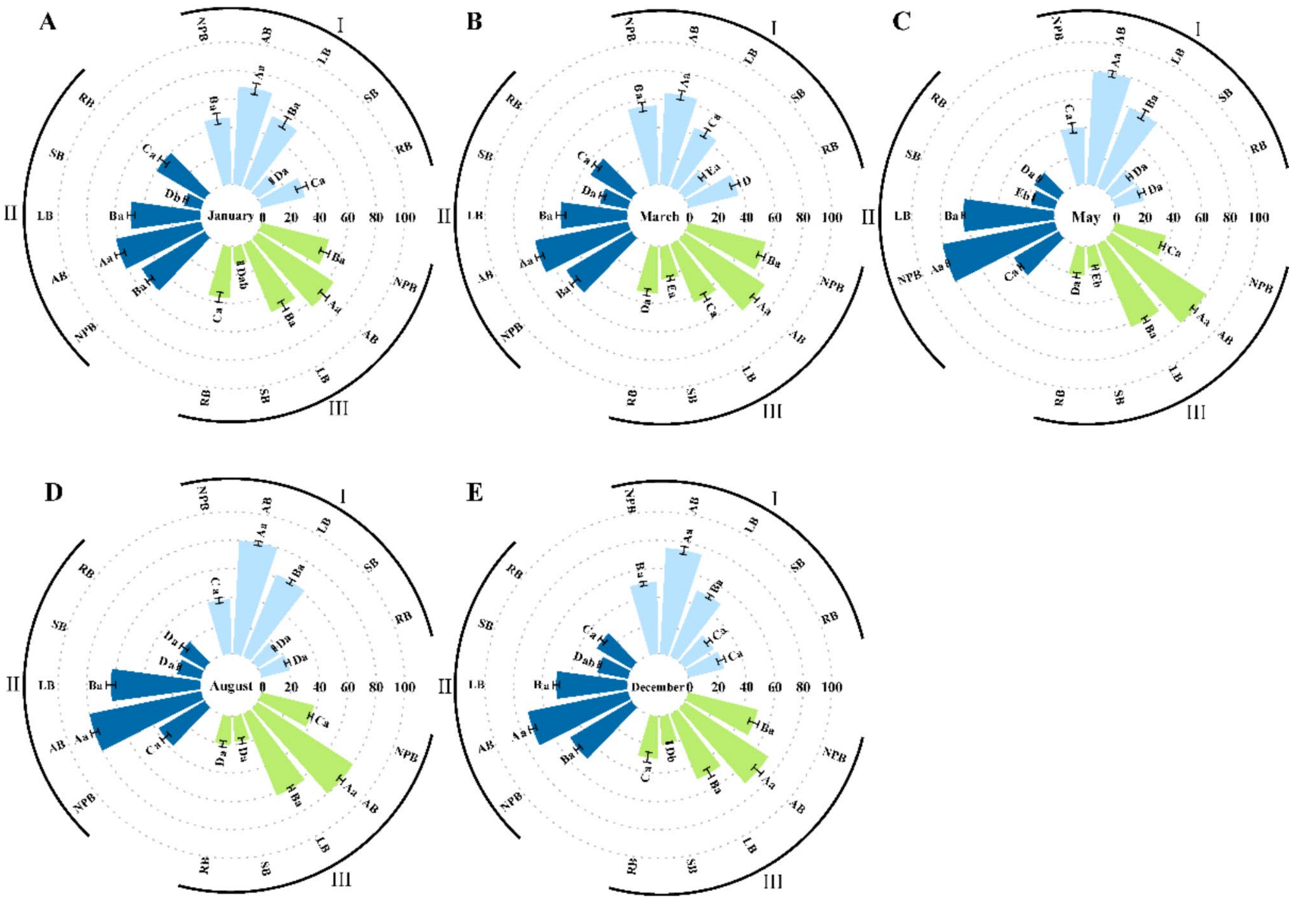

Fig. 4. Biomass allocation in different grades of *P. yunnanensis* seedlings. RB represents root biomass; SB represents stem biomass; LB represents leaf biomass; AB represents above-ground biomass; NPB represents non-photosynthetic biomass. Note: Different lowercase letters indicate significant differences between grades within the same organ ($p < 0.05$), while different uppercase letters highlight significant variations between organs within the same grade ($p < 0.05$).

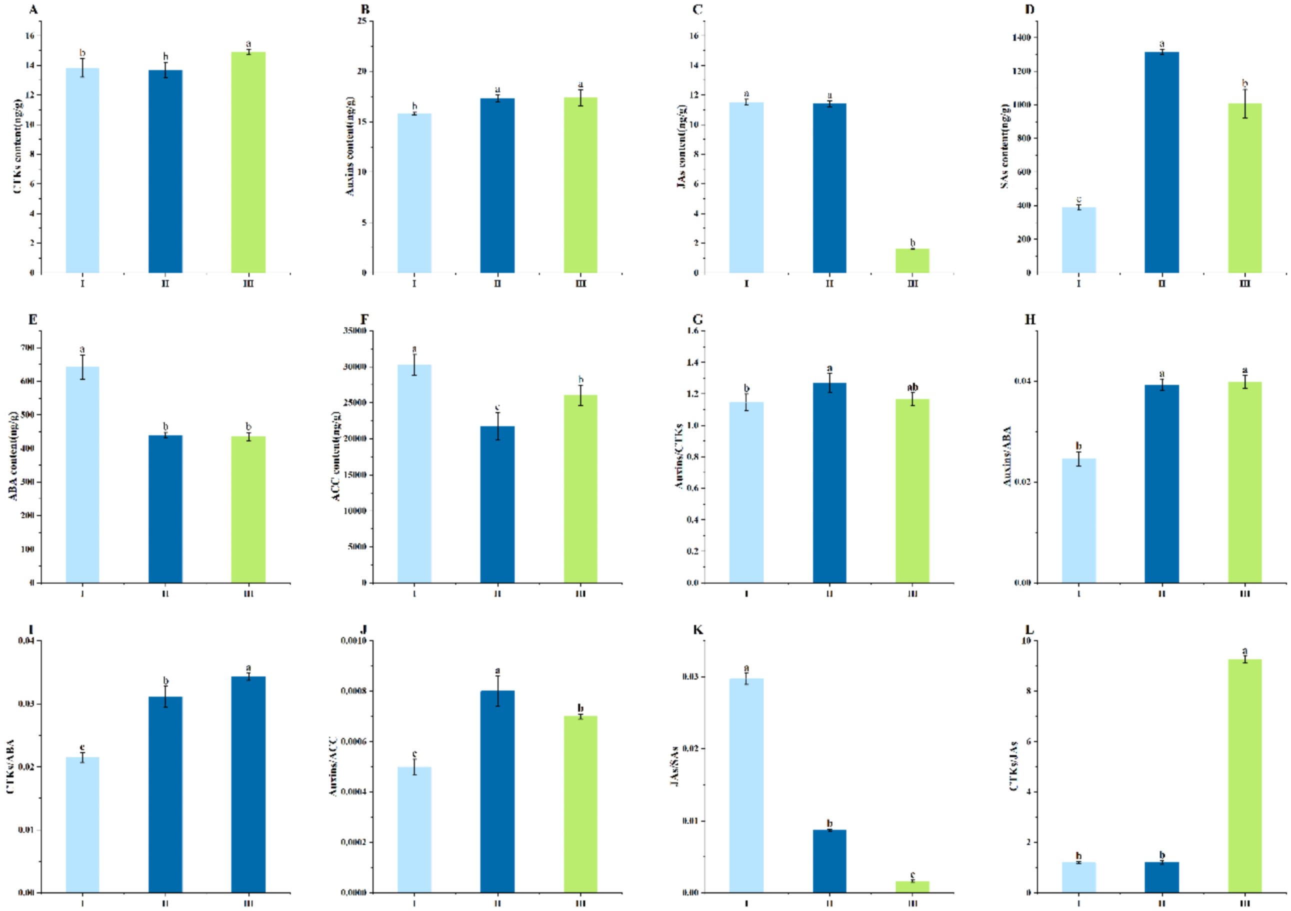

Fig. 5. Plant hormone content and ratio of *P. yunnanensis* seedlings of different grades. (**A**) Cytokinin content, (**B**) Auxins content, (**C**) Jasmonic acid content, (**D**) Salicylic acid content, (**E**) Abscisic acid content, (**F**) Ethylene content, (**G**) Auxins/Cytokinin, (**H**) Auxins/Abscisic acid, (**I**) Cytokinin/Abscisic acid, (**J**) Auxins/Ethylene, (**K**) Jasmonic acid/Salicylic acid, (**L**) Cytokinin/Jasmonic acid. The figure displays the average value ± standard data. Distinct letters signify notable discrepancies among various grades of *P. yunnanensis* ($p < 0.05$).

phase, most of the delicate and transient roots diminish or even disappear due to reduced water, temperature, and carbon allocation, resulting in a sharp decline in total root volume[48].

Endogenous hormones play a key role in regulating the growth process of seedlings[49,50]. This study shows that hormone levels of ABA, JAs, and ACC are significantly elevated in Grade I, potentially enhancing stress tolerance and photoprotection to sustain rapid growth[51–54]. Conversely, Grades II and III, which have higher levels of Auxins and SAs, may have advantages in root growth and pathogen defense, but this appears to inhibit above-ground growth to some extent[18,55,56]. From a genetic perspective, Mantel tests and correlation analyses demonstrate significant associations between these hormones and chlorophyll metabolism-related genes. For instance, JAs are positively correlated with *CHLM* and *CHLG*, as well as with total chlorophyll content, suggesting that JAs may promote chlorophyll accumulation by upregulating the expression of chlorophyll synthesis genes[57–59]. Furthermore, high levels of Auxins are positively correlated with specific degradation genes, suggesting that Auxins may have a negative regulatory effect on photosynthetic pigment metabolism[60]. Excessive levels of CTKs are significantly negatively correlated with Chl Cc and Chl a, potentially exerting adverse effects[61]. This indicates that hormones and gene expression form a synergistic or antagonistic network in seedling photosynthesis and growth development. These insights contribute to a deeper understanding of the role of plant hormones in natural and managed forest ecosystems, providing a scientific basis for future forestry management and breeding.

Beyond individual hormones, complex synergistic and antagonistic relationships exist among various hormones, which are crucial for regulating seedlings' growth and defense responses[51,62–64]. This study observed that an increase in JAs/ACC is often accompanied by a decrease in SAs and a rise in ABA levels, supporting the synergistic role of JAs with ACC and ABA in plant defense[64]. The defense pathway mediated by SAs is antagonistic to the ET/JAs pathway, reflecting how plants rely on different hormonal pathways to regulate their defense strategies when faced with various pathogen attacks[63]. In sugarcane, a high Auxins/CTKs ratio promotes root growth, while a low ratio favors above-ground growth, highlighting the role of hormonal balance in plant morphogenesis[65]. Similarly, the JAs/SAs ratio is closely linked to plant defense responses; a high JAs/SAs ratio

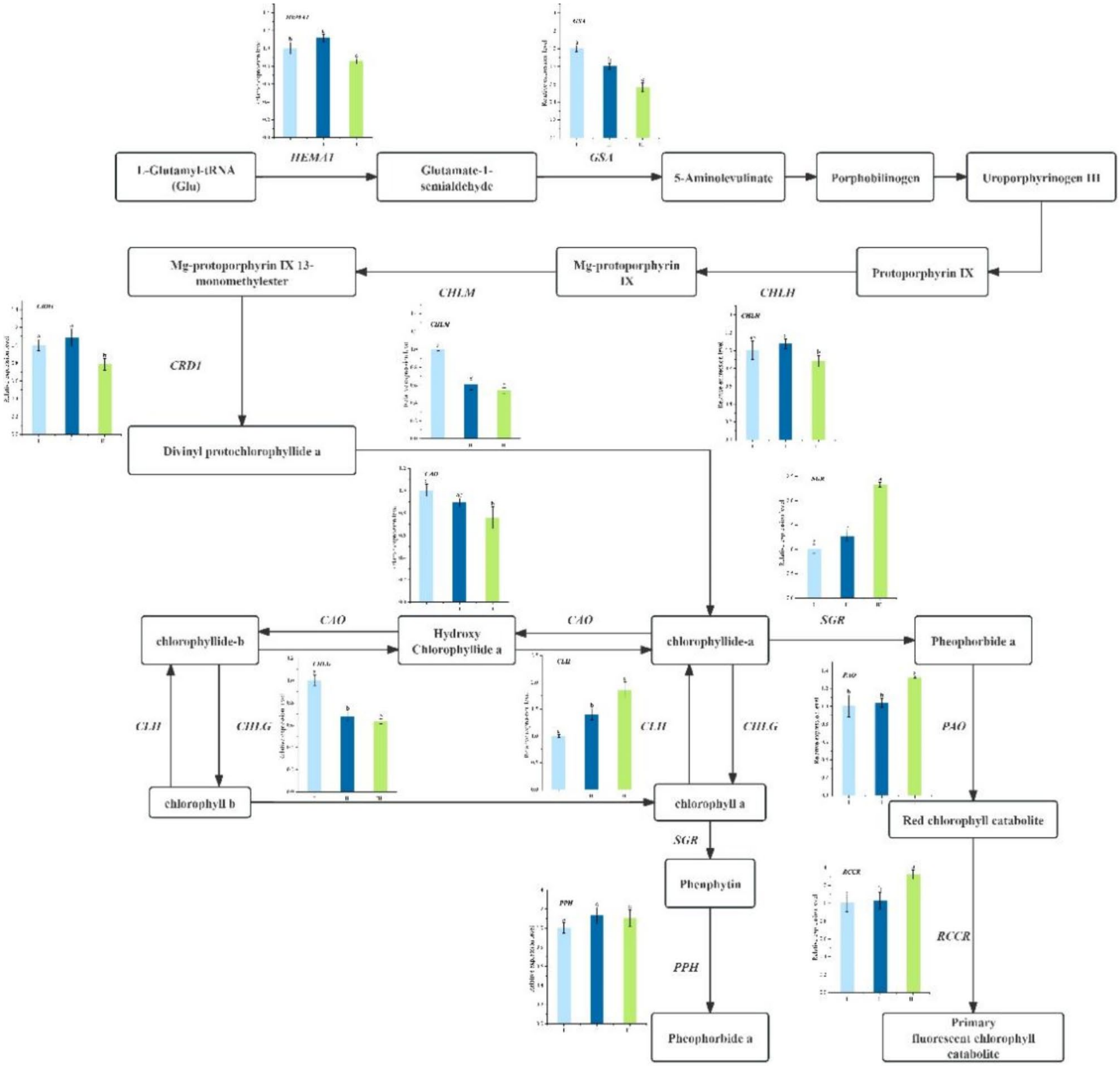

Fig. 6. Metabolic pathways of chlorophyll-related gene expression in different grades of *P. yunnanensis* seedlings. Chlorophyll synthesis-related genes: *HEMA1*, *GSA*, *CHLH*, *CHLM*, *CRD1*, *CHLG*, *CAO*; Chlorophyll degradation-related genes: *CLH*, *PPH*, *PAO*, *RCCR*, *SGR*. The graph displays data as mean values ± standard deviation. Different letters indicate significant differences between the grades of *P. yunnanensis* ($p < 0.05$).

enhances resistance to insect pests[66], while a low ratio favors defense against pathogens[67]. The dynamic balance of these hormones finely controls photosynthetic efficiency, nutrient allocation, and overall growth by regulating transcription levels and activating specific signaling pathways[68]. Adjusting these hormonal ratios through genetic improvements or management practices can enhance plant growth performance and yield, although the specific mechanisms still require further investigation.

Conclusion

This study focuses on different grades of *P. yunnanensis* seedlings, exploring variations in their growth characteristics, photosynthetic pigment content, endogenous hormone levels, and related gene expression. It reveals the synergistic regulatory mechanisms between chlorophyll metabolism and endogenous hormones during the growth process of seedlings. Grade I exhibits high expression of chlorophyll synthesis genes and optimal endogenous hormone levels, facilitating efficient photosynthesis and superior growth performance. Conversely, in lower-grade seedlings, high expression of chlorophyll degradation genes accelerates chlorophyll breakdown, limiting their photosynthetic capacity. Variations in hormone levels, particularly changes in ABA, JAs, Auxins, and CTKs, significantly influence the growth strategies and adaptability of seedlings. For

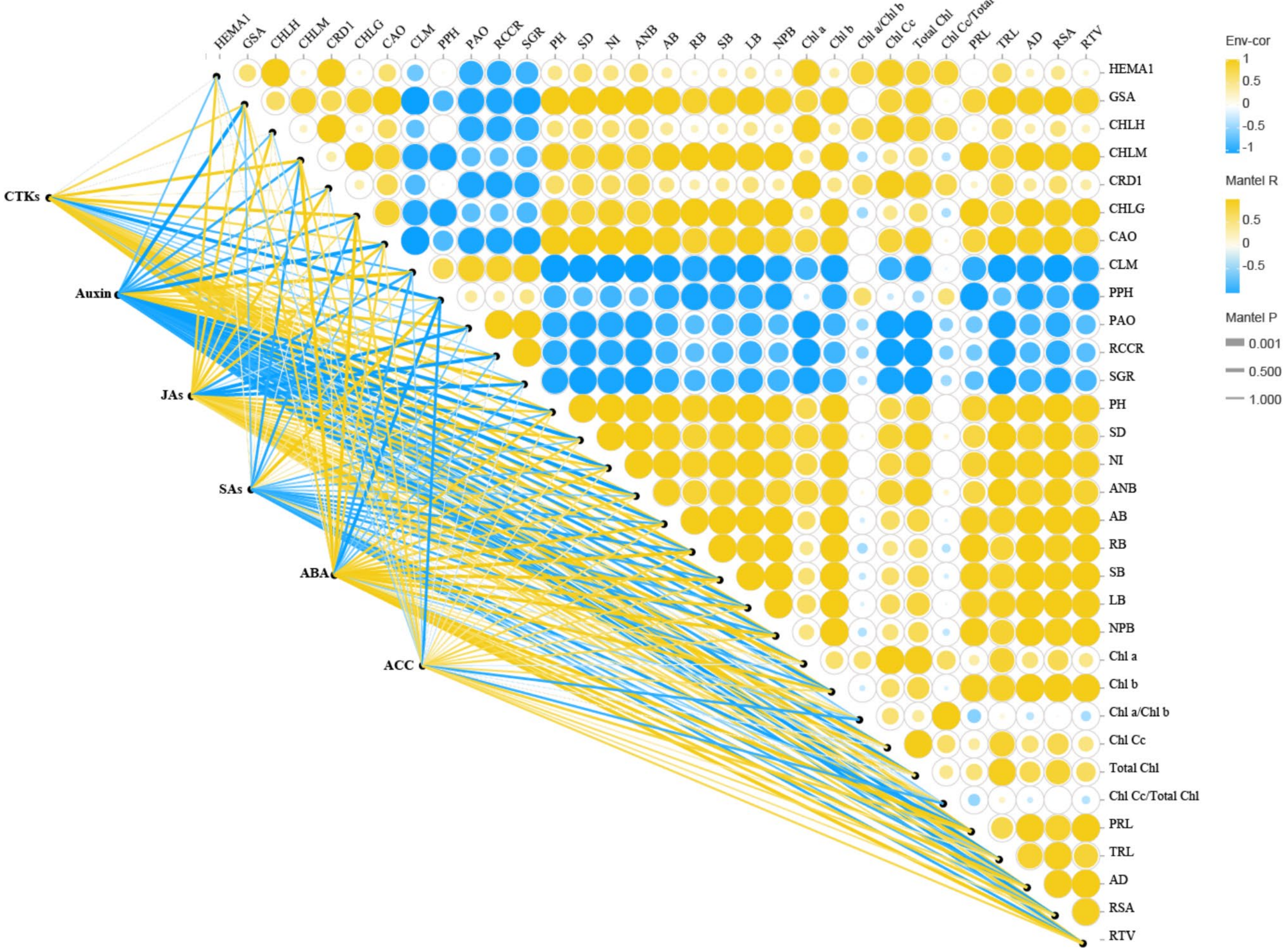

Fig. 7. Network heatmap of the correlations between hormones, chlorophyll metabolism, and seedling growth traits. The color of the lines indicates Mantel R, representing the strength of correlation, while the thickness of the lines denotes Mantel P, which is used to signify the level of significance. *PRL* primary root length, *TRL* total root length, *AD* average root diameter, *RSA* root surface area, *RTV* total root volume.

afforestation and forest management, the regulatory network of genes, hormones, and phenotypes reveals critical factors influencing early seedling growth. Future strategies could involve precisely regulating key gene expression and optimizing endogenous hormone levels to promote ideal trait expression in seedlings, enhance their environmental adaptability, and thereby improve the productivity and ecological benefits of managed forests.

Data availability

All data generated or analyzed during this study are included in the published article, and the gene sequences involved are from the biological project PRJNA1065384.

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Author contributions

Ruilian Li conceptualized the study, processed the data, designed the experiment and wrote the original draft. Qibo Wang processed the data and analyzed results. Yulan Xu collected data, provided resources and edited the manuscript. Nianhui Cai analyzed results. Lin Chen and Sunling Li edited the manuscript. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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