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Uncovering Physiological Responses of *Idesia polycarpa* 'Yuji' Under Alkali Stress via Transcriptomics

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Abstract

This study aimed to investigate the growth, physiological, and molecular responses of *Idesia polycarpa* 'Yuji' under different intensities of alkali stress, thereby providing a theoretical basis for its wider cultivation and application in saline-alkali areas. Two-year-old *I. polycarpa* 'Yuji' seedlings were used in a pot experiment. A mixed alkali stress solution of $\text{NaHCO}_3:\text{Na}_2\text{CO}_3 = 9:1$ molar ratio was applied at three concentration gradients: J1, $60 \text{ mmol}\cdot\text{L}^{-1}$; J2, $90 \text{ mmol}\cdot\text{L}^{-1}$; and J3,

120 mmol·L⁻¹. Deionized water was used as the control treatment (CK). Our integrated analysis revealed that *I. polycarpa* ‘Yuji’ exhibited heightened sensitivity during the later stages of alkali stress, which progressively inhibited growth, damaged the microstructure of leaves and root tips, and severely impaired photosynthetic function, as evidenced by reduced pigment content and decreased net photosynthetic and transpiration rates. Physiological analysis revealed that as stress intensity and duration increased, soluble sugars (SS), soluble proteins (SP), proline (Pro) accumulated significantly, while malondialdehyde (MDA) content rose continuously, indicating severe oxidative damage to the cell membrane system. Peroxidase (POD) and superoxide dismutase (SOD) activities showing distinct activation patterns. Hormonal analysis revealed that under J1 to J2 stress, abscisic acid (ABA) levels fluctuated, while indole-3-acetic acid (IAA), zeatin riboside (ZR), and gibberellic acid-3 (GA₃) levels increased, whereas under J3 stress they were suppressed. A total of 9,995 differentially expressed genes (DEGs) were identified in the transcriptome sequencing of the control group (CK) and the J3 group. KEGG enrichment analysis revealed that DEGs were significantly enriched in pathways such as photosynthesis-antenna proteins. Transcriptome analysis identified 9 995 DEGs. KEGG enrichment analysis indicated that photosynthesis-antenna proteins, photosynthesis, and the plant MAPK signaling pathway played key roles in the response to alkali stress. In addition, hormone signal transduction-related genes, including *ARR*, *ABF*, *PYR/PYL*, and *AHP*, were significantly DEGs. This study clarified the physiological and molecular mechanisms underlying alkali tolerance in *I. polycarpa* ‘Yuji’ and identified candidate genes and key metabolic pathways associated with alkali stress resistance. These findings provide a theoretical foundation and genetic resources for stress-resistance improvement, efficient utilization of saline-alkali land, and the large-scale cultivation of this tree

species.

Keywords *Idesia polycarpa* ‘Yuji’; alkali stress; morphological response; physiological response; transcriptome sequencing

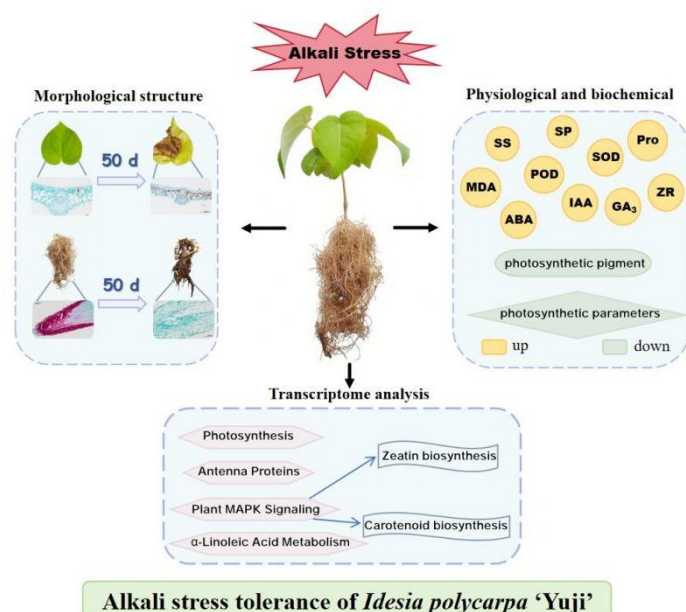
1 Introduction

Soil salinization is one of the principal abiotic stresses affecting global agricultural and forestry production, severely restricting plant growth and ecosystem stability (Sun et al., 2024). The total area of saline-alkali soils worldwide has risen to 950 million hectares, with Asia accounting for the largest share (52.7 million hectares, 68.8 % of the global total) (Shahid et al., 2018). In China, saline-alkali land covers approximately 99.13 million hectares, about 1.03 % of the country’s territory, with over 6.7 million hectares available for agricultural development (Zhang et al., 2025). Cultivating salt-tolerant woody plants on saline-alkali soils can improve soil quality while generating ecological, economic, and social benefits.

Alkali stress in saline-alkali environments adversely affects tree growth and physiological functions. Studies found that plants adapt to alkali conditions by thickening the epidermis and pulp, reducing leaf size, and altering tissue structure (Zhao et al., 2020). High pH in alkali stress disrupts ion balance, impedes photosynthesis and chlorophyll synthesis, damages biomolecules such as DNA, lipids, and proteins, and ultimately leads to stunted growth or plant death (Ge et al., 2022). Qiao et al. (2024) observed a gradual decline in photosynthetic pigment content in three willow species under increasing alkali stress. Wu et al. (2021) reported dynamic changes in antioxidant enzyme activities under alkali stress, where SOD and POD activities initially increased but later declined due to enzyme inactivation or structural damage. Transcription factors play a pivotal role in stress responses by activating or suppressing downstream genes, forming an integrated defense

mechanism. Under alkali stress, wild jujube (*Ziziphus acidojuba*) significantly upregulated calmodulin and calcium channel expression to mitigate oxidative damage (Guo et al., 2017), while oats (*Avena sativa*) regulated Pro accumulation and chlorophyll-related proteins to maintain photosynthetic function (Bai et al., 2021). In-depth studies on these transcriptomic responses enhance our understanding of plant adaptation mechanisms and provide theoretical foundations for improving alkali tolerance.

I. polycarpa, a deciduous tree of the Flacourtiaceae family, is widely distributed in China, Japan, Korea, and Russia (Fang et al., 2023). As an emerging woody oil crop, it holds significant potential for alleviating edible oil shortages and promoting ecological restoration (Qiu et al., 2021). *I. polycarpa* ‘Yuji’ is the only officially certified elite variety in Henan Province, with a lifespan exceeding 20 years, making it an ideal germplasm for alkali tolerance research. Current studies on *I. polycarpa* stress resistance primarily focus on drought and salt tolerance, while alkali tolerance mechanisms remain poorly understood, limiting its cultivation and application. Additionally, Our research group recently elucidated the physiological responses of *I. polycarpa* seedlings to salt stress (Xu et al., 2026). Although both salt and alkali stresses trigger ion toxicity and osmotic injury, alkali stress is coupled with high-pH perturbation, which inflicts unique damage patterns distinct from neutral salt stress. Therefore, this study investigated the morphological, physiological, biochemical, and transcriptomic responses of 2-year-old *I. polycarpa* ‘Yuji’ seedlings under alkali stress (Graphical Abstract). The findings elucidate alkali tolerance mechanisms at multiple levels, providing a basis for breeding alkali-resistant varieties, developing stress mitigation strategies, and supporting large-scale cultivation and industrial development.



Graphical Abstract: Integrated response mechanism of *I. polycarpa* 'Yuji' to alkali stress. Under alkali stress, *I. polycarpa* 'Yuji' exhibits distinct morphological changes in leaves and roots after 50 d of treatment, accompanied by significant alterations in key physiological and biochemical indicators. Transcriptome analysis reveals widespread transcriptional reprogramming, involving four pathways: photosynthesis, antenna proteins, plant MAPK signaling pathway, and α -linolenic acid metabolism. Among them, zein biosynthesis and carotenoid biosynthesis in the MAPK pathway are significantly regulated by gene families, collectively underpinning the species' adaptive tolerance mechanism to alkali stress.

2 Material and methods

2.1 Experimental design

The experiment was conducted from May to June 2023 at the forestry experiment station of Henan Agricultural University, located within a temperate continental monsoon climate zone. The plant material consisted of two-year-old *I. polycarpa* 'Yuji' seedlings. Seeds were mixed with wet sand and sown in March 2022 within a substrate composed of a 1:1 (v/v) mixture of vermiculite and peat soil. Seedlings were grown outdoors under natural conditions. In March 2023, they were

transplanted into plastic pots and acclimatized for 60 d prior to stress induction.

Neutral alkali stress was applied using a solution of NaHCO_3 and Na_2CO_3 mixed at a 9:1 molar ratio. Three concentration gradients were established: 60, 90, and 120 $\text{mmol}\cdot\text{L}^{-1}$, designated as treatments J1, J2, and J3, respectively. The corresponding final substrate pH values of 8.21, 8.50, and 8.74. The control group (CK) was irrigated with deionized water. The experiment comprised four treatment groups (CK, J1, J2, J3), each containing 10 plants. Irrigation was standardized for a 3 d period prior to the initiation of stress treatment.. Alkali treatment was applied via root irrigation every 5 d (250 mL per pot at 17:00), with leachate collected and reapplied. The stress period lasted a total of 50 d.

During the stress period, growth indices (e.g., plant height, ground diameter) were measured every 10 d. Leaf samples were periodically collected and cryopreserved for subsequent physiological and biochemical assays. Microstructural observations of leaves and root tips were performed at the early (10 d), middle (30 d), and late (50 d) stages of stress. On the final day (50 d), leaf samples from the CK and J3 treatment groups were selected for transcriptome sequencing. All experiments and analyses were conducted with three biological replicates. This study aimed to elucidate the integrated response mechanisms of *I. polycarpa* ‘Yuji’ to salt stress.

2.2 Morphological structure determination and growth measurement

Plant morphological changes were observed and recorded at 10 d intervals. On 10 d, 30 d, and 50 d of stress, samples were collected from each treatment. The fourth functional leaf from the apex was selected, washed with distilled water, blotted dry, and sections (1 cm × 1 cm) excised from both sides of the midrib. Primary root tips (1 cm segments) were also collected. All samples were fixed in 50% FAA fixative and stored at room temperature.

Leaf samples underwent a gradient ethanol dehydration and clearing process. Root tips were rinsed with tap water for approximately 12 h to remove fixative, then softened in 12% hydrofluoric acid for 3 d. Longitudinal leaf sections and various root tip regions were observed and imaged using an Eclipse Ci-L microscope (Nikon Corporation, Tokyo, Japan).

From each treatment group, three plants were selected and tagged. Plant height, basal diameter, leaf length, leaf width, number of attached leaves, and number of fallen leaves were measured every 10 d. On 10 d, 30 d, and 50 d, three seedlings per treatment were randomly uprooted. Root systems were scanned using an EPSON PERFECTION V700 Photo scanner (Epson Corporation, Suwa, Nagano, Japan). Root length, surface area, volume, average diameter, and root density were analyzed with the WINRHIZO PRO 2007 system (Regent Instruments Inc., Quebec, Canada).

2.3 Determination of leaf photosynthetic characteristics

The contents of chlorophyll and carotenoids in leaves were determined using an ethanol–acetone extraction method. Briefly, fresh leaf samples were cut into small pieces, and 0.1 g of samples was weighed and placed into a 10 mL centrifuge tube containing 10 mL of a 95% ethanol-acetone mixture (1:1, v/v). The samples were extracted in darkness at room temperature for 36 h, with manual shaking every 8 h to ensure complete extraction. After extraction, the absorbance values of the extracts were measured at 663, 646, and 470 nm using a spectrophotometer. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were calculated according to the following equations:

$$\text{Chlorophyll a (mg} \cdot \text{L}^{-1}) = 12.7A_{663} - 2.69A_{646} \quad (1)$$

$$\text{Chlorophyll b (mg} \cdot \text{L}^{-1}) = 22.9A_{646} - 4.68A_{663} \quad (2)$$

$$\text{Total chlorophyll (mg} \cdot \text{L}^{-1}) = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (3)$$

$$\text{Carotenoids}(\text{mg}\cdot\text{L}^{-1}) = \frac{1000A_{470} - 2.05 \times \text{Chlorophyll a} - 114.8 \times \text{Chlorophyll b}}{245} \quad (4)$$

Leaf gas exchange parameters were measured using an LCpro-SD portable photosynthesis system (ADC BioScientific Ltd., UK). Measurements were conducted between 9:00 and 11:00 a.m. on sunny days. Three seedlings were randomly selected from each treatment, and fully expanded functional leaves from the upper canopy were selected for measurements. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) were recorded. During measurements, natural sunlight was used as the light source, and an open gas exchange system was applied. The leaf chamber temperature was maintained at 25°C , relative humidity was controlled at 60-70%, CO_2 concentration was set at $400 \mu\text{mol}\cdot\text{mol}^{-1}$, and photosynthetic photon flux density (PPFD) was maintained at $800 \mu\text{mol}\cdot\text{m}^{-2} \text{ s}^{-1}$.

2.4 Determination of osmotic adjustment substances

Malondialdehyde (MDA) content was assessed according to Bates et al. (1973): A 0.2 g sample was homogenized in 5% trichloroacetic acid (TCA), centrifuged, and the supernatant reacted with 0.67% thiobarbituric acid (TBA). After incubation in a water bath (30 min), absorbance was read at 450, 532, and 600 nm. Soluble protein (SP) content was determined using the Coomassie Brilliant Blue method (Cheng et al., 2016): The soluble protein (SP) content of leaves was determined using the Coomassie Brilliant Blue G-250 staining method. Briefly, 0.5 g of fresh leaf samples were homogenized with 2 mL phosphate buffer (0.05 mol L^{-1} , pH 7.8), and the homogenate was diluted to a final volume of 5 mL. The extracts were centrifuged at 10,000 rpm for 10 min, and 0.1 mL of the supernatant was collected and mixed with 5 mL Coomassie Brilliant Blue G-250 reagent. After standing for 2 min, the absorbance was measured at 595 nm using a spectrophotometer. The soluble protein content was calculated according to a standard protein calibration curve. Soluble sugar (SS)

content was assayed using the anthrone method (Xu et al., 2021): The soluble sugar (SS) content in leaves was determined using the anthrone colorimetric method. Briefly, 0.5 g of fresh leaf samples were accurately weighed, finely chopped, and homogenized. The samples were extracted with 15 mL distilled water in test tubes, sealed, and incubated in a boiling water bath for 20 min. After cooling, the extracts were filtered into 100 mL volumetric flasks, and the residues were rinsed thoroughly before the extracts were brought to a constant volume. Subsequently, 1 mL of the extract was transferred into a test tube and mixed with 5 mL of anthrone reagent. The mixture was incubated in a boiling water bath for 10 min and then cooled to room temperature. The absorbance was measured at 630 nm using a spectrophotometer. The soluble sugar content was calculated based on a standard glucose calibration curve. Proline (Pro) content was quantified as described by Abubakar et al. (2022): The tissue was extracted in 3% sulfosalicylic acid, heated, and then the supernatant was reacted with the ninhydrin reagent. Absorbance was measured at 520 nm and calculated against a standard curve.

2.5 Determination of antioxidant enzyme activities

Superoxide dismutase (SOD) activity was measured based on the inhibition of nitroblue tetrazolium (NBT) photoreduction (Xu et al., 2018). A 0.2 g sample was homogenized in phosphate buffer (pH 7.0), centrifuged (8 000 rpm, 15 min), and the supernatant incubated with NBT reaction mixture under light (4 000 lx). Absorbance was recorded at 560 nm. Peroxidase (POD) activity was determined using guaiacol as substrate (Zhou and Leul, 1999). The supernatant from a similar extraction was added to a reaction mix containing guaiacol and H₂O₂, and the increase in absorbance at 470 nm was monitored.

2.6 Measurement of endogenous hormone levels

Endogenous hormone contents, indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid-3 (GA₃), and zeatin riboside (ZR), were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Aidisheng Biological Technology Co., Ltd., China), following the manufacturer's instructions.

2.7 Transcriptome sequencing

To ensure the quality of samples for transcriptome sequencing, a NanoDrop 2 000 spectrophotometer was used to determine RNA purity and concentration, and an Agilent 2 100/LabChip GX system was used to accurately assess RNA integrity. After the samples passed quality assessment, eukaryotic mRNA was enriched using Oligo(dT) magnetic beads and fragmented for cDNA library construction. Following end repair, A-tailing, and sequencing-adaptor ligation, appropriately sized fragments were selected using AMPure XP beads, and the cDNA libraries were finally completed by PCR enrichment. Libraries that passed quality control were sequenced on an Illumina NovaSeq 6 000 platform in PE150 mode.

To validate the transcriptome data, six hormone-signaling-related differentially expressed genes (DEGs) were selected for real-time quantitative PCR (qRT-PCR) analysis. Primers were designed using Primer-BLAST and are shown in Table S1. The UBA80 gene was used as the internal reference, and first-strand cDNA was synthesized using PrimeScript™ RT Master Mix. The qPCR reaction system is detailed in Table S2. Thermal cycling conditions were as follows: initial denaturation at 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 10 s; followed by a melt-curve analysis (95°C for 15 s, 60°C for 30 s, 95°C for 15 s). Relative gene expression was calculated using the 2^{-ΔΔCt} method.

2.8 Statistical analysis

Experimental data were organized using Microsoft Excel 2024 (Microsoft Corp., Redmond, WA, USA). A two-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics 29.0 (IBM Corp., Armonk, NY, USA), followed by Tukey's honest significant difference (HSD) test for multiple comparisons. Data are presented as mean $\pm$ standard error (SE). Differences were considered statistically significant at $p < 0.05$. Graphs were generated with Origin 2024 (OriginLab Corporation, Northampton, MA, USA).

For transcriptome data, raw reads were filtered to remove adapters and low-quality sequences, yielding high-quality clean data. Clean reads were aligned to the *I. polycarpa* reference genome. Subsequent analyses included quality assessment, gene expression quantification, differential expression analysis ($|\log_2(\text{fold change})| \geq 2$ and $\text{FDR} < 0.01$), and functional enrichment (GO and KEGG).

3 Results

3.1 Morphological changes of *I. polycarpa* 'Yuji' under alkali stress

External morphology directly reflects plant adaptability to alkali stress. The change in plants (Figure 1A), leaves (Figure 1B), and roots (Figure 1C) of *I. polycarpa* 'Yuji' under alkali stress are shown in Figure 1. CK plants grew normally, whereas alkali-stressed groups exhibited significantly reduced plant height, ground diameter, and leaf growth in (Figure 2) ($p < 0.05$). As the intensity and duration of stress increased, the leaves of the alkali stress treatment group exhibited gradual scorching, curling, or cracking of the leaf margins, an increase in black spots on the leaves, and worsening wilting and leaf drop. As shown in (Table S3), treatments J1 and J3 promoted root length elongation, while other root parameters under J1 exhibited an initial increase followed by decline, and J3 demonstrated continuous reduction across all metrics. In contrast, J2 treatment resulted in

progressive decrease in root volume alongside initial enhancement and subsequent reduction in other root indices. At the late stress stage, J1-treated seedlings showed significant increases of 16.54 % in root length and 7.94 % in average root diameter compared to CK ($p < 0.05$), whereas root surface area, root volume, and root density were prominently reduced by 38.71 %, 31.41 %, and 36.86 % respectively ($p < 0.05$). Both J2 and J3 treatments significantly inhibited all root growth parameters ($p < 0.05$), with the most severe morphological damage observed under severe alkali stress (J3).

Cross-sectional structures of leaves and root tips at different stages under J3 stress are shown in Fig. S1. With increasing stress duration, leaf tissue structure was well developed during the pre-stress stage (CK). No significant change was observed in upper epidermal thickness under J3 stress, whereas the other tissue structures showed increasing trends to varying degrees (Table S4). During the pre-stress stage (CK), root-tip cells were regular in shape, compactly and neatly arranged, and relatively small. As stress duration increased, cell volumes in the root cap, meristematic zone, elongation zone, and maturation zone all tended to increase. Cell arrangement became more compact, intercellular spaces decreased, and the degree of cell damage increased. The volume of cortical parenchyma cells increased significantly, and cell morphology showed varying degrees of deformation.

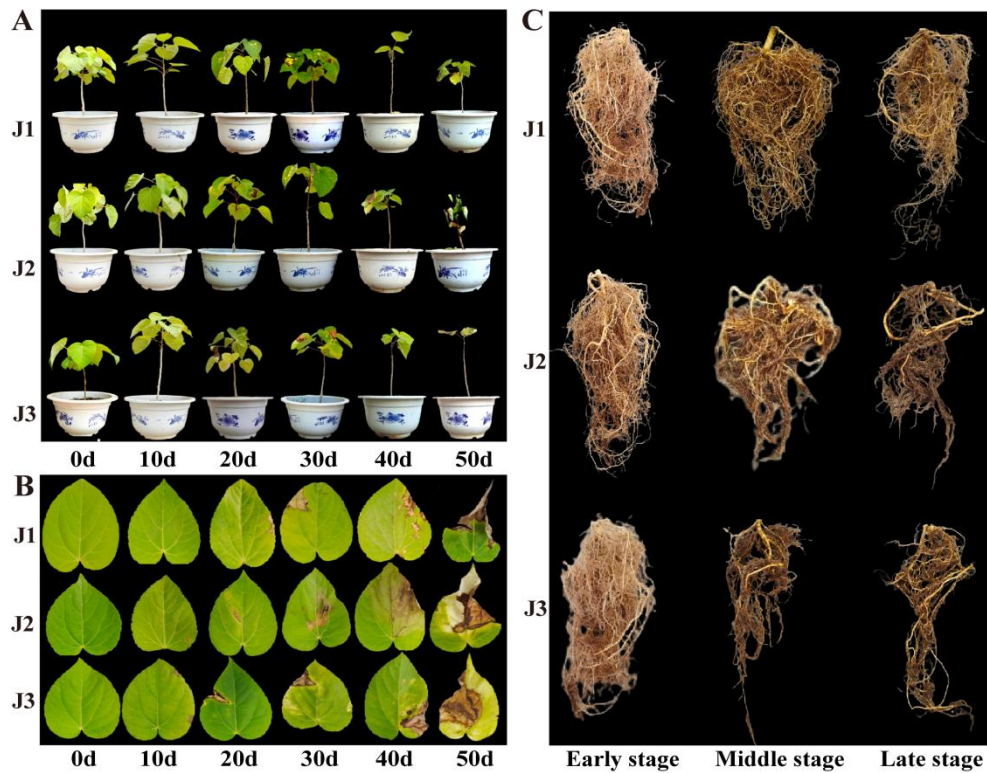


Fig. 1. Plant, leaf, and root morphology under alkali stress of *I. polycarpa* 'Yuji'. In (A and B), each row from left to right corresponds to alkali stress days 0, 10, 20, 30, 40, and 50. In (C), each row from left to right corresponds to the early stage of stress (CK), the middle stage of stress, and the late stage of stress. (A - C) The first three rows from top to bottom are the J1, J2, and J3 treatment groups, respectively.

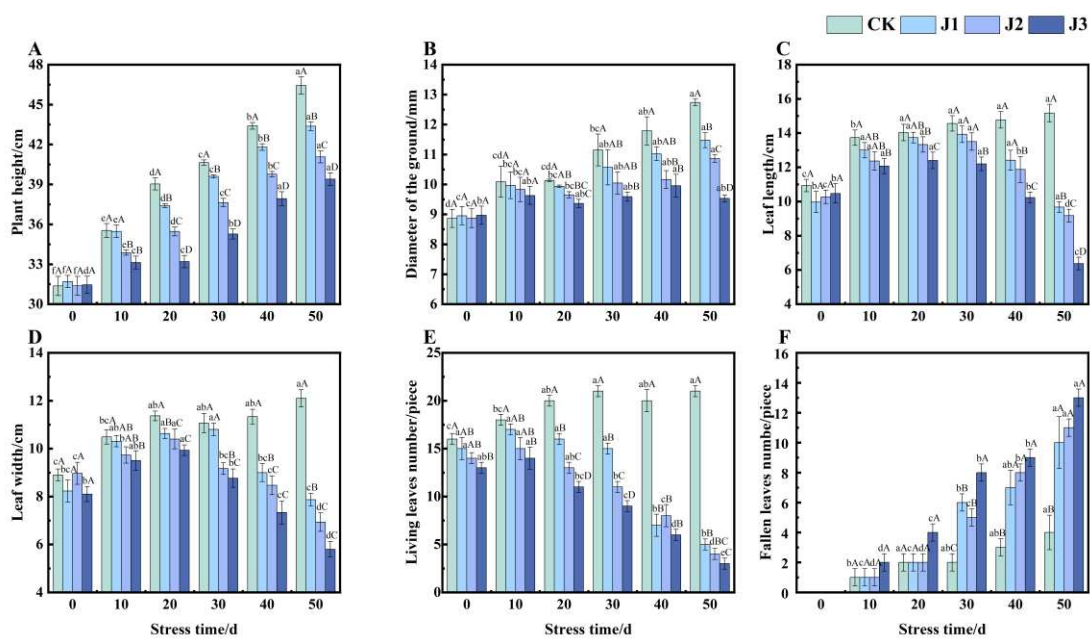


Fig. 2. Effects of alkali stress on morphological changes of *I. polycarpa* ‘Yuji’. Effects of four treatments (CK, J1, J2, J3) on plant height (A), diameter of the ground (B), leaf length (C), leaf width (D), living leaves number (E), and fallen leaves number (F). Capital letters (ABC) indicate significant differences ($p < 0.05$) among different treatments at the same time point, and lowercase letters (abc) indicate significant differences ($p < 0.05$) among different time points for the same treatment, with the mean standard error ($n = 3$).

3.2 Photosynthetic characteristics of *I. polycarpa* ‘Yuji’ under alkali stress

As shown in Fig. 3, chlorophyll a and chlorophyll b contents under J1 stress generally showed mild fluctuations, whereas carotenoid and total chlorophyll contents were significantly reduced by 63.41% and 13.61%, respectively, compared with CK at the same time point ($p < 0.05$). Under J2 and J3 stress, chlorophyll a, chlorophyll b, and total chlorophyll contents continuously declined after 30 d, while carotenoid content also showed a trend from relative stability to decline and was significantly reduced by 67.48% and 71.54%, respectively, compared with CK at the same time point ($p < 0.05$), indicating that J2 and J3 stress had more pronounced effects on photosynthetic pigment synthesis and stability. As shown in Fig. 4, alkali stress generally reduced P_n , Tr , and G_s , with stronger inhibition during the middle and late stages. At 30 d, G_s was 84.00%, 82.00%, and 88.00% lower than the corresponding CK values ($p < 0.05$), and C_i varied with stress intensity and duration. Overall, Tr , C_i , and G_s followed the order $CK > J1 > J2 > J3$, whereas P_n followed $CK > J1 > J3 > J2$. These results indicate that alkali stress weakened the photosynthetic capacity of *I. polycarpa* ‘Yuji’ by reducing photosynthetic pigment contents and restricting gas exchange.

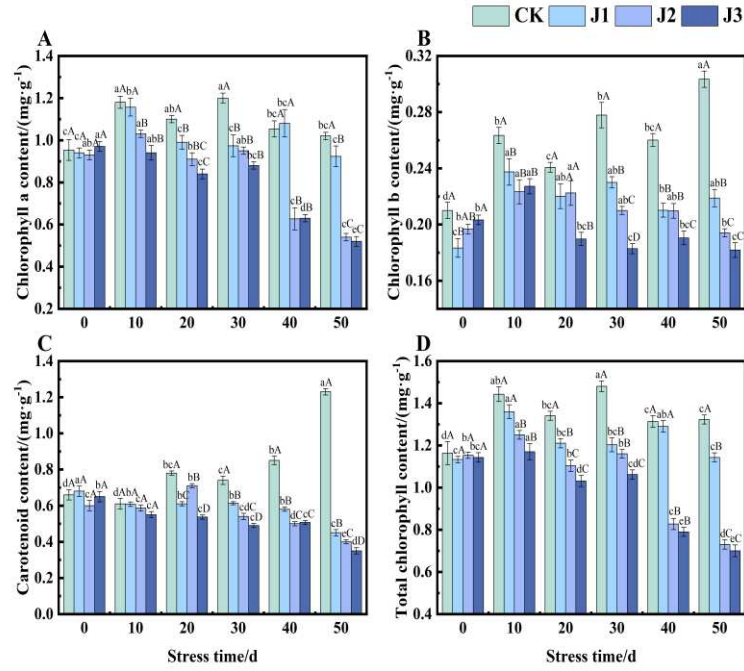


Fig. 3. Effect of Alkali stress on plant height, ground diameter growth and leaf growth of *I. polycarpa* ‘Yuji’. Effects of alkali stress on photosynthetic pigment content of *I. polycarpa* ‘Yuji’. Effects of four treatments (CK, J1, J2, J3) on Chlorophyll a content (A), Chlorophyll b content (B), Carotenoid content (C), and Total chlorophyll content (D) under different stress. Capital letters (ABC) indicate significant differences ($p < 0.05$) among different treatments at the same time point, and lowercase letters (abc) indicate significant differences ($p < 0.05$) among different time points for the same treatment, with the mean standard error ($n = 3$).

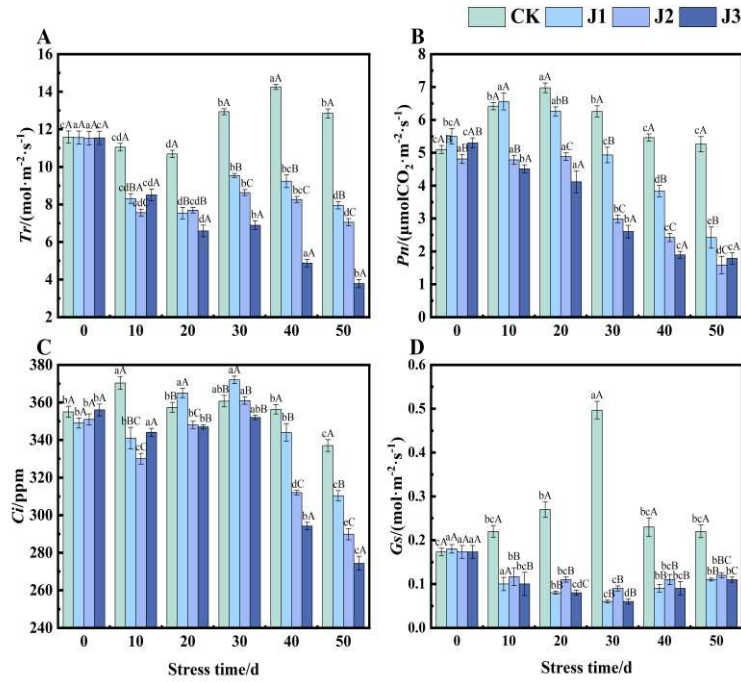


Fig. 4. Effects of alkali stress on photosynthetic parameters of *I. polycarpa* 'Yuji'. Effects of 4 treatments (CK, J1, J2, J3) on Tr (A), Pn (B), Ci (C), and Gs (D) under different stress. Capital letters (ABC) indicate significant differences ($p < 0.05$) among different treatments at the same time point, and lowercase letters (abc) indicate significant differences ($p < 0.05$) among different time points for the same treatment, with the mean standard error ($n = 3$).

3.3 Changes in osmotic adjustment substances and antioxidant enzyme activities of *I. polycarpa* 'Yuji' under alkali stress

As shown in Fig. 5, the SS content in leaves of *I. polycarpa* 'Yuji' under alkali stress generally decreased first and then increased. It increased significantly during the late stage under J1 and J2 stress, whereas under J3 stress it increased significantly at 20 d, indicating an earlier soluble-sugar response under J3 stress (Fig. 5A). SP content generally decreased, then increased, and subsequently decreased again; all stress treatments reached relatively high levels at 40 d, indicating that protein accumulation may be promoted during the middle stage of stress to enhance stress resistance, but declines during the late stage as stress continues (Fig. 5B). Pro content changed relatively slowly

under J1 stress but increased significantly during the late stage under J2 and J3 stress, reaching peaks at 40 d in J2 and J3, indicating that proline mainly contributes to osmotic adjustment during the late stage of stress (Fig. 5C). MDA content peaked at 30 d and then declined, indicating that alkali stress induced a certain degree of membrane lipid peroxidation during the middle stage, while the plants exhibited some capacity for regulation and recovery during the late stage (Fig. 5D).

With respect to antioxidant enzymes, POD activity increased significantly during the late stage under J1 and J3 stress and followed the order J3>J1>CK>J2 at 50 d (Fig. 5E). SOD activity recovered and increased after 40 d and followed the order J3>J2>J1>CK at the end of stress (Fig. 5F). Overall, *I. polycarpa* ‘Yuji’ can alleviate alkali-stress injury by increasing SS, SP, and Pro contents and POD and SOD activities, showing relatively strong adaptability to alkali stress.

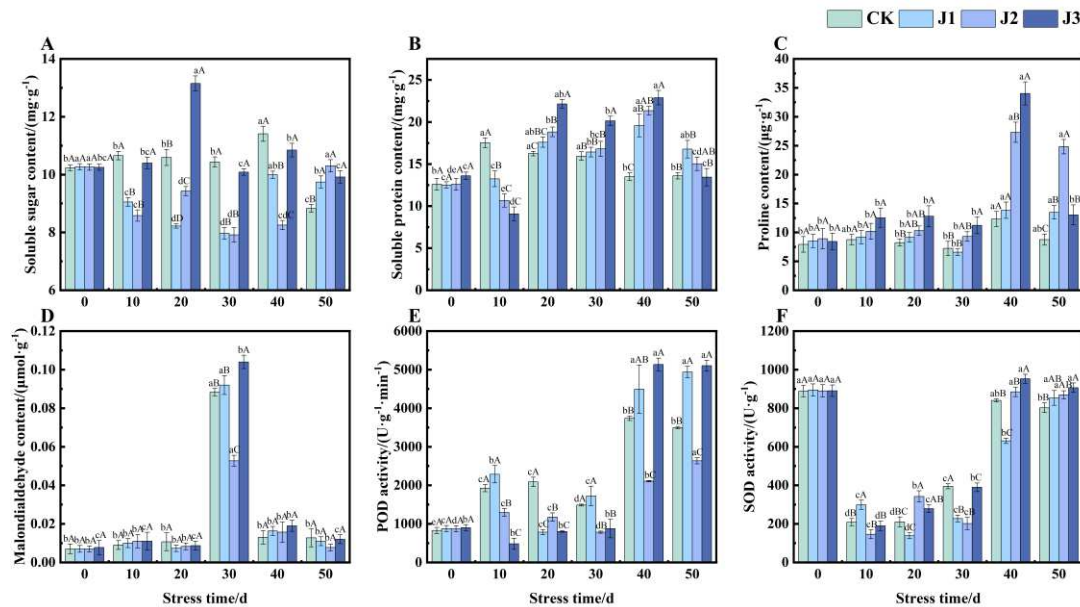


Fig. 5. Effects of alkali stress on osmotic adjustment substances and antioxidant enzyme activities of *I. polycarpa* ‘Yuji’. Effects of four treatments (CK, J1, J2, J3) on SS (A), SP (B), PRO (C), MDA (D), POD (E) and SOD (F) under different stress. Capital letters (ABC) indicate significant differences ($p < 0.05$) among different treatments at the same time point, and lowercase letters (abc) indicate significant differences ($p < 0.05$) among different time points for the same treatment, with the mean standard error ($n = 3$).

3.4 Changes in endogenous hormones of *I. polycarpa* ‘Yuji’ under alkali stress

As shown in Fig. 6, ABA content in leaves of *I. polycarpa* ‘Yuji’ showed a fluctuating downward trend under J1 and J2 stress, but accumulated significantly during the late stage of J3 stress and remained higher than CK from 30 to 50 d, indicating that sustained severe alkali stress can activate ABA-mediated stress-defense responses. IAA content increased significantly during the late stage under J1 and J2 stress, whereas the increase in IAA was limited under J3 stress, indicating that moderate alkali stress may maintain leaf physiological activity by promoting auxin accumulation. ZR content during the late stage under J1 and J2 stress was significantly higher than CK, whereas ZR content under J3 stress decreased markedly during the late stage. Overall, J1 and J2 alkali stress promoted the accumulation of IAA, GA₃, and ZR and maintained growth regulation; J3 stress was characterized by enhanced ABA accumulation together with inhibition of growth-promoting hormone production.

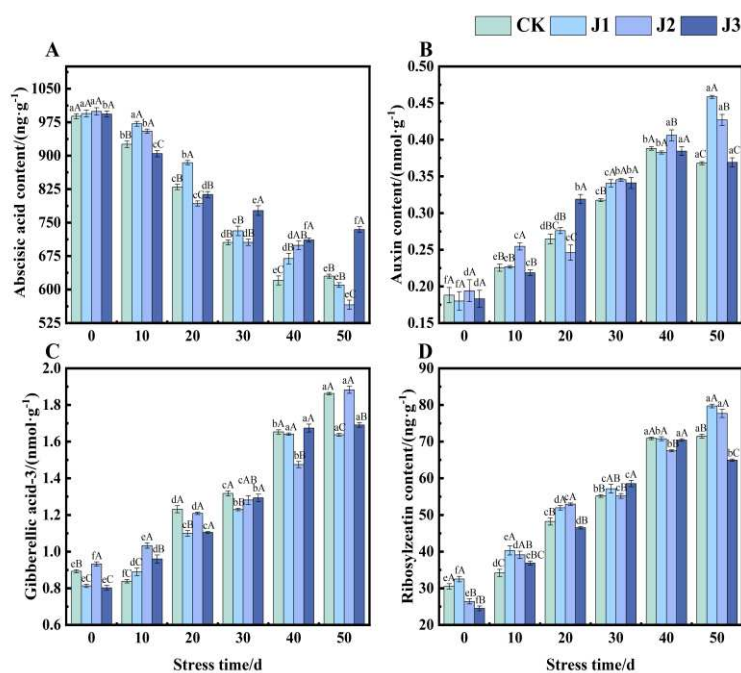


Fig. 6. Effects of alkali stress on endogenous hormones of *I. polycarpa* ‘Yuji’. Effects of 4 treatments (CK, J1, J2, J3) on ABA (A), IAA (B), GA₃ (C) and ZR (D) under different stress. Capital letters (ABC) indicate significant differences ($p < 0.05$) among different treatments at the same time point, and lowercase letters (abc) indicate significant differences ($p < 0.05$) among different time points for the same treatment, with the mean standard error ($n = 3$).

3.5 Transcriptomic analysis of *I. polycarpa* ‘Yuji’ leaves under alkali stress

To reveal the molecular response mechanism of *I. polycarpa* ‘Yuji’ under alkali stress, six seedling leaf samples from the CK and J3 treatment groups after 50 d of stress were selected for eukaryotic reference-based transcriptome (RNA-seq) analysis. The results showed that Clean Data for each sample were not less than 6.36 Gb, Q30 $\geq$ 97.00%, and the alignment rate of Clean Reads to the *I. polycarpa* reference genome was 92.34%-93.49%, indicating that the sequencing data were of high quality and suitable for subsequent analyses. As shown in Fig. 7, a total of 9 995 DEGs were identified in the CK vs J3 comparison, including 5 081 up-regulated genes and 4 914 down-regulating genes, indicating that alkali stress mainly enhanced plant stress resistance through up-regulation of gene expression.

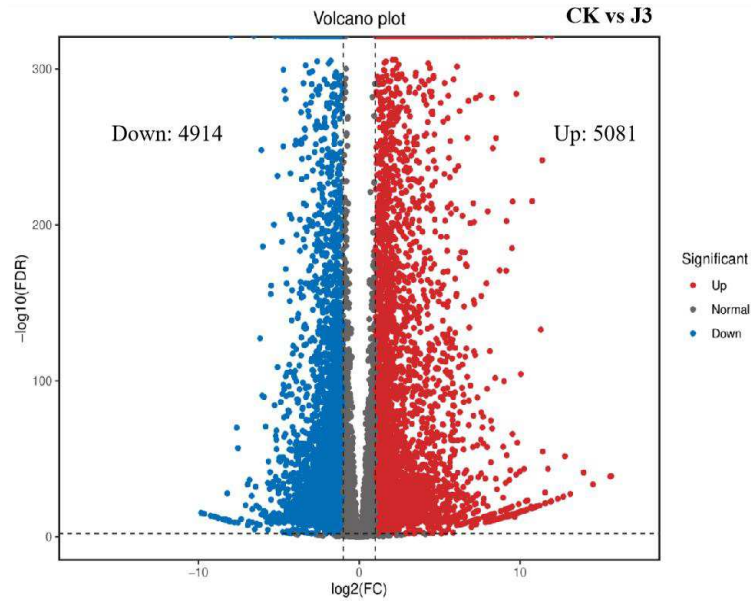


Fig. 7. Differentially expressed volcano picture. The horizontal axis represents the logarithm of the fold change in expression of a certain gene between two samples; the vertical axis represents the negative logarithm of the statistical significance of the gene expression change. Blue indicates down-regulated DEGs, red indicates up-regulated DEGs, and gray indicates non-DEGs.

The GO functional annotation results are shown in Fig. 8. DEGs were mainly distributed among three major categories: biological process, molecular function, and cellular component. Biological process included 22 subcategories, mainly enriched in cellular processes, metabolic processes, biological regulation, localization, and response to stimulus. Molecular function included 15 subcategories, mainly involving binding, catalytic activity, transporter activity, transcription regulator activity, and molecular transducer activity. Cellular component included three subcategories, mainly involving cellular anatomical entities, intracellular structures, and protein-containing complexes. Overall, these results indicate that under J3 stress, *I. polycarpa* ‘Yuji’ maintains cellular functional stability and improves its adaptability to alkali-stress environments by participating in processes such as metabolic regulation, substance transport, and stress response.

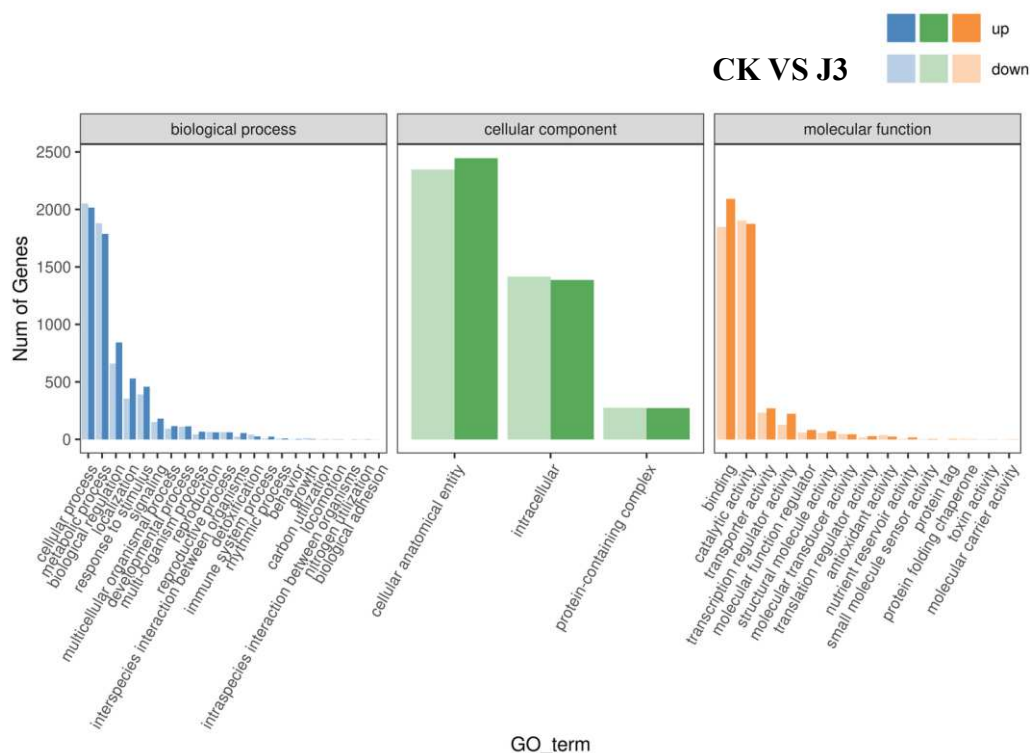


Fig. 8. GO function analysis of DEGs in different comparison group. The horizontal coordinate is the GO classification, the vertical coordinate is the number of genes, and different colors represent the different primary classifications to which they belong.

The KEGG pathway annotation results are shown in Fig. S2. DEGs in the CK vs J3 comparison were significantly enriched in 15 key pathways (Q-value < 0.01) (Fig. S3, Table S6). Photosynthesis-related pathways showed differential regulation: the photosynthesis-antenna proteins pathway was significantly induced and showed up-regulated expression, whereas DEGs in core pathways including photosynthesis, porphyrin and chlorophyll metabolism, and carbon fixation in photosynthetic organisms were predominantly down-regulated. These molecular results corroborate the physiological changes in photosynthetic characteristics described above. In addition, pathways closely related to signal transduction and stress adaptation, including the plant MAPK signaling pathway, α -linolenic acid metabolism, and plant hormone signal transduction, were also significantly regulated.

3.6 Analysis of key DEGs

Further analysis of plant hormone signal-transduction-related pathways showed that 330 DEGs participated in this pathway in the CK vs J3 comparison, among which 61 key genes closely related to hormone regulation, including zeatin biosynthesis and carotenoid biosynthesis, were screened (Table S7). In the zeatin biosynthesis pathway, 39 related DEGs were identified, mainly involving core components such as AHK2/3/4, AHP, ARR-B, and ARR-A. Some ARR-B and ARR-A family genes were significantly up-regulated, indicating that J3 stress regulates cell division and growth by activating the cytokinin-mediated two-component signaling system, thereby participating in adaptive responses to alkali stress. A total of 22 key DEGs were identified in carotenoid biosynthesis and ABA-signaling-related pathways, involving core regulatory factors such as PYR/PYL, PP2C, SNRK2, ABA, and ABF. Notably, some PYR/PYL and ABF genes were significantly up-regulated, suggesting that the ABA signaling module was effectively activated under J3 stress. Through regulation of the PP2C-SnRK2-ABF pathway, this signaling cascade further induces the expression of downstream stress-responsive genes and participates in processes such as stomatal closure, growth-rate regulation, and maintenance of cellular homeostasis, achieving a dynamic balance between maintaining growth and enhancing stress resistance and providing an important molecular basis for adaptation to alkali-stress environments. In addition, the expression trends of the six DEGs validated by qRT-PCR were consistent with the RNA-seq results (Fig. 9), confirming the reliability of the data.

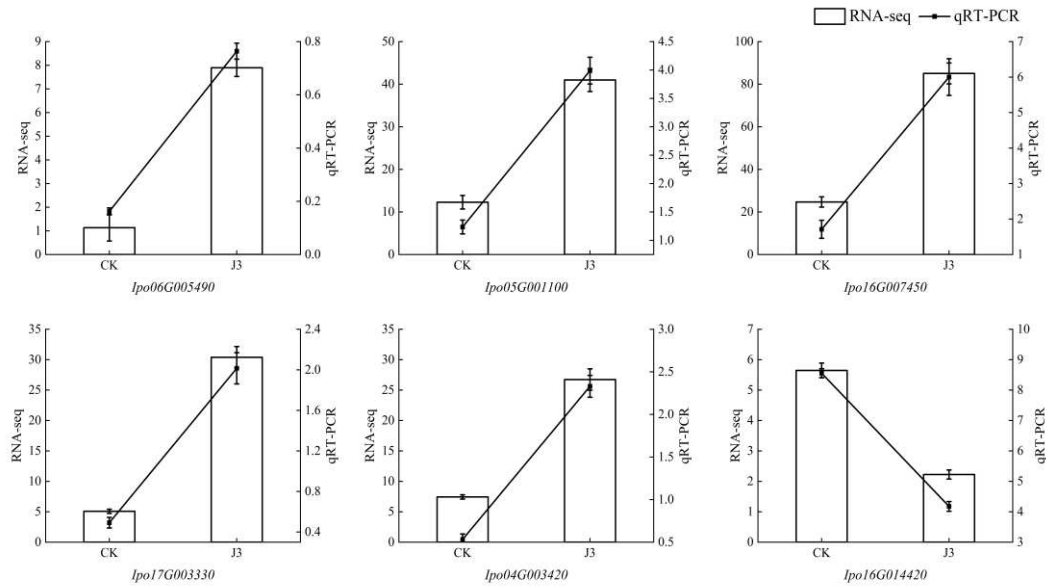


Fig. 9. Quantitative qRT-PCR validation of DEGs of *I. polycarpa* ‘Yuji’

4 Discussion

There exists a close interconnection among plant morphological changes, physiological and biochemical responses, and transcriptomic levels, collectively constituting an integrated response mechanism of plants to environmental stresses. To reduce water evaporation and growth consumption, *I. polycarpa* ‘Yuji’ adapts to alkali environments through morphological growth modifications including reducing leaf length, leaf width, leaf number, plant height, basal diameter increment, root length, root surface area, root volume, root density, and root-shoot ratio, while increasing leaf abscission. These findings are consistent with Aung et al, (2022) study on Peony . Under severe alkali stress, microscopic structures of *I. polycarpa* ‘Yuji’ leaves exhibit varying degrees of changes across different periods: epidermal cells gradually become thicker with increased cutinization to reduce water evaporation and salt penetration; palisade tissue shows increasing cell layers with gradually decreasing cell volume; spongy tissue becomes loosely arranged with enlarging intercellular spaces. These observations align with Yao et al, (2023) research results on *Lycium barbarum*, indicating that *I. polycarpa* ‘Yuji’ copes with alkali stress environments by

adjusting leaf microstructure. Compared with neutral salt stress at equivalent Na⁺ levels (Xu et al., 2026), alkali stress caused more pronounced inhibition of root growth and eliminated the mild hormetic effect on root elongation observed under low salinity. This discrepancy is likely attributable to additional high-pH damage to root tip meristems and reduced rhizosphere nutrient availability under alkaline conditions.

Photosynthesis serves as the fundamental process for energy acquisition and organic compound synthesis in plants, making photosynthetic characteristics crucial for plant growth and development. In this study, the photosynthetic pigment content in *I. polycarpa* ‘Yuji’ leaves showed a continuous decline under severe alkali stress, consistent with the performance of *Populus tomentosa* Carrière in response to alkali stress (Lu et al., 2017). The photosynthetic capacity of *I. polycarpa* ‘Yuji’ was reduced to varying degrees across different alkali stress treatments. During the initial stress phase, stomatal closure limited atmospheric CO₂ absorption, leading to decreased *Pn*. As stress duration prolonged, non-stomatal limitations became the primary factor constraining photosynthetic activity (Ju et al., 2021). These findings align with previous research results by Wang et al, (2020) and Mousavi et al, (2022). Relative to neutral salt stress (Xu et al., 2026), alkali stress induced a steeper decline in photosynthetic pigment content and net photosynthetic rate, suggesting that high pH inflicts additional direct damage to chloroplast ultrastructure beyond ionic osmotic stress.

Osmotic regulators play pivotal roles in plant growth and development. Under alkali stress, plants adapt to environmental changes by increasing osmotic substances such as Pro, SP, and SS to maintain physiological stability and promote healthy growth (Zhao et al., 2020). Our results demonstrated that MDA content gradually decreased under alkali stress, while SP and Pro levels initially increased before declining. SS content increased under mild stress but showed an initial rise

followed by reduction under moderate and severe stress. This pattern mirrors observations in *Cannabis sativa* (Yuan et al., 2024), *Astragalus cicer* (Zhang et al., 2024), and *Machilus faberi* (Mo et al., 2024) under salt stress, suggesting that *I. polycarpa* ‘Yuji’ primarily activates its osmotic regulation mechanism during advanced stages of severe alkali stress. Prolonged stress exposure caused severe damage to cellular structures and disrupted physiological processes, ultimately impairing normal synthesis of SS and Pro. In comparison with salt stress responses (Xu et al., 2026), MDA content accumulated earlier and reached higher levels under alkali stress, indicating that high pH exacerbates membrane lipid peroxidation and oxidative injury on top of ionic toxicity.

Antioxidant enzymes perform critical functions in plant development by protecting against reactive oxygen species (ROS)-induced damage and maintaining normal cellular functions (Petr et al., 2021). In this study, SOD activity in *I. polycarpa* ‘Yuji’ leaves increased under mild alkali stress but exhibited an initial increase followed by decrease under moderate and severe stress. POD activity increased under mild-to-moderate stress but showed the same pattern of initial rise and subsequent decline under severe stress. These results indicate that *I. polycarpa* ‘Yuji’ seedlings possess effective self-adaptation mechanisms and considerable alkali tolerance in mild stress conditions. However, prolonged stress led to ROS accumulation beyond tolerance thresholds, causing significant cellular damage and consequent reductions in POD and SOD activities. These findings corroborate previous salt stress studies by Akbari et al. (2020). When compared with salt stress (Xu et al., 2026), POD activity peaked earlier and declined more sharply under severe alkali stress, while SOD showed a weaker late-stage recovery, indicating a faster exhaustion of the antioxidant defense capacity under high-pH conditions.

Endogenous phytohormones are indispensable regulatory factors in various life activities from

seed germination to organ growth and flowering (Zeng et al., 2024; Ma et al., 2024; Kaur et al., 2021). Research shows that plants accumulate ABA content to protect themselves under stress conditions (Sah et al., 2016). This study found that during early mild alkali stress, *I. polycarpa* ‘Yuji’ resisted stress by reducing ABA while increasing IAA, ZR and GA₃ contents to adjust plant growth. With prolonged stress duration, severe cellular damage and internal physiological dysfunction led to increased ABA content and decreased IAA, ZR and GA₃ contents. The overall hormonal reconfiguration pattern was generally consistent with that observed under salt stress (Xu et al., 2026), but alkali stress triggered a greater magnitude of ABA accumulation and more severe suppression of growth-promoting hormones, reflecting a stronger stress intensity imposed by combined ionic and high-pH challenges.

Plants also respond to alkali stress at transcriptomic levels through altered expression of specific genes to cope with adverse environmental conditions, including up-regulation of stress-responsive genes such as antioxidant enzyme genes, osmotic adjustment substance synthesis genes, and heat shock protein genes (Yan et al., 2020). This study identified 9,995 significantly DEGs in CK vs J3 comparison, indicating that *I. polycarpa* ‘Yuji’ shows greater sensitivity during late severe alkali stress while demonstrating relative alkali tolerance. The most abundant functional annotations in CK vs J3 comparison included binding, catalytic activity, transporter activity, transcription regulator activity, and molecular transducer activity. These patterns resemble *Populus davidiana* × *P. Alba var.pyramidalis* to saline-alkali and drought stress (Yang, 2023). These gene expression changes may increase corresponding protein synthesis, triggering physiological and biochemical responses including photosynthesis adjustment, osmotic regulator (such as Pro and sugars) accumulation, enhanced antioxidant enzyme activity, and hormonal level adjustments to mitigate

stress damage (Li et al., 2022). In this study, alkali stress significantly enriched pathways including photosynthesis-antenna proteins, plant MAPK signaling pathway, porphyrin and chlorophyll metabolism, photosynthesis, and α -linolenic acid metabolism. *I. polycarpa* 'Yuji' may resist alkali stress by regulating these pathways to control expression of genes related to photosynthesis and physiological biochemistry. Research shows that plant MAPK signaling pathways and photosynthesis pathways can defend against saline-alkali stress by reducing photosynthetic rate, decreasing substance consumption, and promoting accumulation of osmotic regulators like SS, SP, Pro and MDA, consistent with this study's findings (Liu et al., 2019; Shrestha et al., 2018; Majeed et al., 2023; Yu et al., 2022). Notably, while core pathways such as plant MAPK signaling and photosynthesis were commonly modulated under both salt and alkali stress (Xu et al., 2026), alkali stress specifically enriched gene sets associated with pH homeostasis, cell wall modification, and mineral element transport, representing unique transcriptional adaptations to high-pH disturbance.

Morphological structure adjustments may alter plant sensitivity to environmental stress, thereby affecting related gene expression levels (Sun et al., 2023). In this study, reduced leaf area and photosynthetic pigments in *I. polycarpa* 'Yuji' decreased photosynthetic conversion efficiency. Under alkali stress, DEGs in leaves significantly enriched photosynthesis pathways and photosynthesis-antenna proteins, which may further influence energy metabolism-related gene expression. Root system modifications not only affect water and nutrient absorption capacity but may also alter rhizosphere environments through root exudates, changing rhizosphere microbial community structures. These changes feedback to plant transcriptomic levels through signal transduction pathways (Haichar and Achouak, 2021; Vissenberg, 2020). Collectively, these cross-level comparisons confirm that salt and alkali stress share approximately 60-70% of core

adaptive pathways in *I. polycarpa* ‘Yuji’, but the high-pH component of alkali stress drives distinct phenotypic and molecular responses that cannot be inferred from salt stress studies alone (Xu et al., 2026).

5 Conclusion

Alkali stress significantly inhibited the growth of *I. polycarpa* ‘Yuji’ seedlings, restricting leaf expansion, stem growth, and root development, and the inhibitory effects became stronger with increasing stress duration and concentration. Under alkali stress, the microstructures of leaf and root-tip cells were damaged to varying degrees, and photosynthetic pigment contents, *Pn*, *Tr*, and *Gs* all decreased, indicating marked inhibition of photosynthetic capacity. Under J1 and J2 alkali stress, *I. polycarpa* ‘Yuji’ alleviated growth inhibition by accumulating osmotic adjustment substances such as SS, SP, and Pro, increasing POD and SOD activities, and increasing IAA, GA₃, and ZR contents; J3 alkali stress intensified membrane lipid peroxidation, promoted ABA accumulation, and inhibited the production of growth-promoting hormones. Transcriptome analysis showed that DEGs were mainly enriched in pathways such as photosynthesis, the plant MAPK signaling pathway, and hormone signal transduction, indicating that *I. polycarpa* ‘Yuji’ adapts to alkali-stress environments through coordinated photosynthetic regulation, antioxidant defense, osmotic adjustment, and reconstruction of hormone signaling. This study provides a basis for breeding alkali-tolerant superior *I. polycarpa* varieties, a reference for alkali-stress regulation technologies, and support for the large-scale cultivation and industrial development of *I. polycarpa*.

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Data availability All the data generated during and/or analyzed during the current study are included in this published article.

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