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ARTICLE

The Role of Prohexadione-Calcium in Mediating the Alleviation of Shading Stress in *Hesperis sibirica*

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ABSTRACT: Shading stress significantly compromises the ornamental quality and ecological adaptability of *Hesperis sibirica*. To investigate the potential mitigating effects of plant growth regulators, this study systematically analyzed the regulatory mechanisms of foliar-applied prohexadione-calcium (Pro-Ca) at different concentrations (0, 100, 300, 600, and 900 mg·L^{-1}) under both full sunlight and heavy shading (70% light reduction, retaining 30% of full sunlight intensity) conditions. Through comprehensive evaluation of plant morphological traits, chlorophyll content, photosynthetic parameters, and chlorophyll fluorescence characteristics, combined with structural observations using scanning electron microscopy, light microscopy, and transmission electron microscopy, 600 mg·L^{-1} Pro-Ca was identified as the optimal concentration for alleviating shading stress. This treatment significantly reduced plant height compared to the shading control, while also increasing floret number (50 d). During the observation period, the chlorophyll a/b ratio showed a notable improvement, accompanied by significant enhancements in the net photosynthetic rate (P_n) and maximum photochemical efficiency (F_v/F_m). Structural analysis revealed that this concentration promoted leaf thickening, stomatal optimization, and chloroplast ultrastructure repair. The comprehensive evaluation results confirmed that 600 mg·L^{-1} Pro-Ca provided the comparatively comprehensive regulatory effect under shading conditions.

KEYWORDS: Chlorophyll fluorescence; membership function analysis; photosynthetic characteristics; shade adaptation; ultrastructure

1 Introduction

Hesperis sibirica (syn. *Hesperis oreophila*) is a wild ornamental herb native to northern China and adjacent Eurasian regions, belonging to the *Brassicaceae* family. It has proved irreplaceable for its application in indigenous landscape design due to its distinctive inflorescences, vibrant flower coloration, and prolonged blooming period [1]. Existing field investigations of *Hesperis sibirica* mainly involve population morphological traits and natural reproductive performance [2], with a critical neglect of its adaptation to shading stress, which severely restricts its evidence-based application in shade-adapted environment.

In urban landscapes with high canopy density, heavy shading (70% light reduction) represents a major environmental constraint limiting the application of *H. sibirica*. Under such conditions, plants typically exhibit classic shade avoidance syndrome, including stem elongation, lodging, and leaf yellowing, resulting in substantial deterioration of their ornamental value [3–5]. Consequently, developing effective physiological interventions to mitigate shading stress has emerged as a pivotal challenge for promoting its application in shade-prone landscaping.

Plant growth regulators serve as effective tools for enhancing plant adaptability in landscape environments. Among them, prohexadione-calcium (Pro-Ca), an inhibitor of gibberellin (GA) biosynthesis, has demonstrated remarkable efficacy in modulating plant architecture (e.g., reducing plant height and increasing branch number in *Chrysanthemum* [6]) and enhancing stress resistance [7,8]. However, systematic studies on the regulatory role of Pro-Ca in the shade adaptation of *H. sibirica* remain scarce, particularly regarding the integrated physiological and structural mechanisms underlying its action. This study has shown that: Pro-Ca can alleviate the excessive shading phenotype induced by heavy shading, and significantly enhance plant shade tolerance by optimizing plant morphology, root development, and photosynthetic structure. The mechanism underlying its mitigation of shading stress may be closely associated with key physiological processes such as protection of photosynthetic pigments and maintenance of leaf and chloroplast structural integrity. This hypothesis finds indirect support from studies on other hormones; for instance, cytokinin (CTK) and salicylic acid (SA) have been shown to enhance abiotic stress tolerance in plants by increasing chlorophyll content and optimizing physiological status, respectively [9–11].

Based on the above framework, the present study focuses on the comprehensive regulatory effects of Pro-Ca on the growth phenotype, photosynthetic performance, and leaf microstructure of *H. sibirica* under shading conditions, aiming to systematically elucidate the physiological mechanisms through which it enhances shade tolerance. The findings are expected not only to fill critical theoretical gaps in the shade physiology of this species but also to provide key technical support for the precise cultivation and sustainable landscape development of wild ornamental plants in shaded urban habitats.

2 Materials and Methods

2.1 Geographical Location of the Study Area

This study utilized wild *H. sibirica* grown from seeds collected from the natural population as the experimental material. The plant material originated from the Wuling Mountain and Saihanba National Nature Reserve in Chengde city, Hebei province, possessing significant value for introduction, cultivation, and research. We successfully introduced it to the Experimental Station No. 3 of Hebei Agricultural University in 2020. After seedling cultivation in an environmentally controlled intelligent greenhouse in September 2023, the plants were transplanted to the station's open nursery and subjected to routine management of irrigation, fertilization, and pest control to ensure normal growth. The experimental station is located in a temperate continental monsoon climate zone. The specific meteorological parameters are as follows: an annual average temperature of 12.7°C, annual sunshine duration of 2560 h, annual precipitation of 575.4 mm, annual evaporation of 1760 mm, and an average annual frost-free period of 200 days. The climatic characteristics of this region provide a typical environment for studying plant adaptability. The plants used in this experiment were sown and germinated in September 2023, and experimental treatments began in April 2024. By the start of the experiment, seeds had been germinating for approximately seven months, the plants had not yet undergone flower bud differentiation, and vegetative growth was healthy.

2.2 Experimental Design and Hormone Treatment

This study employed a two-factor randomized complete block design to investigate the combined effects of light intensity and exogenous hormones on the growth of *H. sibirica*. After seedling cultivation in an environmentally controlled intelligent greenhouse, the plants were transplanted to the station's open nursery and grown under natural conditions with routine irrigation, fertilization, and pest management. Two light intensity treatments were applied as required by the experimental design: (1) Full sunlight control: Plants were grown under full sunlight, which served as the baseline (100% light) for calculating shading percentages. The PPFD in this condition was maintained at 1200–1500 μmol m^{-2} s^{-1} during midday. (2) 70% heavy shading treatment: Achieved using black shading nets (officially deployed on 24 March 2024), resulting in an interior PPFD of 350–450 μmol m^{-2} s^{-1}, which was measured to be approximately 30% of the concurrent full sunlight PPFD. In order to counteract the effects of cloud cover and solar angle on PPFD and ensure a constant shading rate of 70%, the LI-190R quantum sensor (LI-COR Biosciences, USA) was used for PPFD synchronous measurement in shaded and full-sun areas every noon (12:00–14:00) throughout the entire experimental period. One week after the shading treatment commenced (April 1), foliar spraying of Pro-Ca was initiated. Hormone concentrations were set at five gradients: 0 (control, sprayed with an equal amount of distilled water), 100, 300, 600, and 900 mg·L^{-1} (detailed codes for each treatment combination are listed in Table 1). The gradient was designed based on our pre-screening trials and literature. Lower levels (100–300 mg·L^{-1}) typically induce weak growth regulation, while higher levels (600–900 mg·L^{-1}) help test stress mitigation effects and dose dependence [11]. This range captures effects from beneficial to inhibitory, identifies optimal and threshold concentrations, and remains practical for real-world applications. The evenly spaced gradient also aids dose–response analysis and supports robust statistical evaluation. To maintain effective hormone levels within the plants, a second spray at the same concentration was applied on April 15. Both applications were conducted in the evening under clear and windless conditions, continuing until the leaf surfaces were uniformly moist without dripping. For all physiological and biochemical measurements, fully expanded and healthy leaves were collected from the third node from the apex of each plant to minimize variation caused by leaf age and position. Seven days after the second foliar application of Pro Ca (April 22), leaf samples were collected for unified biochemical analysis, paraffin sectioning, and observation of chloroplast ultrastructure for one-time comparative testing. At the same time, dynamic morphological indicators (plant height, leaves, roots, and small flower traits) and gas exchange photosynthetic parameters were continuously measured every 10 days from 0 to 50 days after initial spraying. Photosynthetic measurements were conducted on May 13th, and microscopic structure observation and anatomical material sampling were arranged as independent post sampling events on May 15th.

Table 1: Experimental treatments involving Pro-Ca application at different concentrations under two light conditions.

Full Sunlight Treatments	Pro-Ca Concentration (mg·L^{-1})	Heavy Shading Treatments	Pro-Ca Concentration (mg·L^{-1})
qCK	0	zT0	0
qT1	100	zT1	100
qT3	300	zT3	300
qT6	600	zT6	600
qT9	900	zT9	900

2.3 Morphological Trait Measurements

Starting from April 10th to June 1st, 2024, a morphological feature analysis will be conducted every 10 days on the plant height, leaves, and root traits of the *H. sibirica*. The counting of small flowers starts from the 10 d and records the data of small flowers on the 10 d, 20 d, 30 d, 40 d, and 50 d in sequence. All samples were collected 7 days after the second spraying. Plant height was measured with a tape measure from the substrate surface to the top of the canopy and recorded in centimeters (cm); leaf length and width were measured with a ruler by randomly selecting three leaves per plant, recorded in centimeters (cm); at the full bloom stage, flower and leaf color parameters were quantitatively analyzed using a CR-400 color difference meter, flower counting was performed using automated image processing techniques; after flowering, root morphological data including root length, root surface area, root volume, and average diameter were obtained using an MRS-9600TFU2L root scanner combined with the Wansen LA-S analysis system, recorded in meters (m). All measurements were performed with three biological replicates and three technical replicates.

2.4 Chlorophyll and Photosynthetic Characteristics Measurements

Chlorophyll content and photosynthetic parameters were determined using leaf samples collected 7 days after the second Pro-Ca application. The ethanol extraction method was adopted for chlorophyll determination [12]. For chlorophyll fluorescence analysis during the peak flowering period, leaves were dark-adapted for 20 min and then measured with a Pocket PEA fluorimeter (Hansatech Instruments Ltd., UK) to obtain basic parameters including F_o, F_m, and F_v, from which derived parameters F_v/F_o and F_v/F_m were calculated. Photosynthetic parameters were measured on 13 May 2024 (peak flowering stage) between 07:30 and 16:30 at 2-h intervals using a CIRAS-3 portable photosynthesis system (PP Systems, USA). For subsequent analysis, the peak photosynthetic data obtained during the midday period (11:30–13:30) were used, including net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) [13]. Three biological replicates were established for each treatment, and all measurements were conducted under controlled environmental conditions (25 ± 1°C temperature, 60 ± 5% relative humidity, and 1000 μmol·m^{-2}·s^{-1} photosynthetically active radiation).

2.5 Observation of Paraffin Sections

On 15 May 2024 (08:00–09:00), mature leaf midrib tissues of *H. sibirica* were collected and fixed in Bouin's solution under vacuum overnight. The specimens subsequently underwent graded ethanol dehydration (70% to 100%), with immersion at each concentration for 0.5–1 h. Tissue clearing was performed through two xylene treatments (0.5–1 h each) to completely remove ethanol. Paraffin infiltration was conducted for 4–8 h to replace xylene with molten paraffin, followed by embedding in paraffin blocks. Serial sections (5–10 μm thickness) were obtained using a rotary microtome (Leica RM2235) and mounted on albumin-glycerol coated slides. After xylene-mediated dewaxing and ethanol rehydration, sections were stained following standard protocols. Coverslips were applied using synthetic resin mounting medium (Entellan, Merck) for microscopic examination.

2.6 Microscopic Observation of Leaf Epidermis

On 15 May 2024 (08:00–09:00), fresh leaf tissue samples (1 mm^2) adjacent to midribs of functional *H. sibirica* leaves were collected and immediately fixed in 2.5% glutaraldehyde solution. The samples were then rinsed three times (15 min each) with 0.1 M phosphate buffer (pH 7.0), followed by post-fixation in 1% osmium tetroxide for 1–2 h and subsequent buffer washes (3 × 15 min) [14].

2.7 Ultrastructural Observation of Chloroplasts

On 15 May 2024 (08:00–09:00), fresh leaf tissue samples (1 mm^2) adjacent to the midrib of mature *H. sibirica* leaves were collected and immediately fixed in 2.5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.0) for 4 h at 4°C, followed by post-fixation in 1% osmium tetroxide for 2 h. After three 15-min washes in 0.1 M phosphate buffer (pH 7.0), samples were dehydrated through a graded acetone series (30%, 50%, 70%, 80%, 90%, and 95%, 20 min per step) and infiltrated with Spurr's epoxy resin [15]. Ultrathin sections (70–90 nm) were cut using a Leica UC7 ultramicrotome, mounted on 200-mesh copper grids, and stained with 2% uranyl acetate (15 min) followed by Reynolds' lead citrate (5 min) for contrast enhancement. Samples were examined under a Hitachi HT-7800 transmission electron microscope (Hitachi High-Technologies, Japan) operating at 80 kV, and high-resolution digital images were captured using an AMT XR-16 CCD camera (Advanced Microscopy Techniques, USA).

2.8 Statistical Analysis

The two factor factorial design and two-way ANOVA used in this experiment followed the mature statistical scheme proposed [16]. All datasets were processed using SPSS 18.0 through a two factor randomized complete block design and two-way analysis of variance, where light intensity and Pro Ca concentration were designated as two fixed factors and blocks were treated as random factors. We studied the main effects and interactions of light intensity and Pro Ca concentration at a significance level of $\alpha = 0.05$ (light × Pro Ca). If the interaction between light and Pro Ca reaches statistical significance ($p < 0.05$), a simple effects analysis will be conducted and combined with Duncan's multiple range test to compare the differences in Pro Ca concentration under full sunlight and severe shading conditions. Comprehensive physiological measurements are used to systematically elucidate the interaction between light regimes and plant growth regulators under shading stress. All charts were generated using Microsoft Excel 2016 and R 3.5.1.

2.9 Membership Function Analysis

This study employed membership function analysis to comprehensively evaluate the effects of different Pro-Ca concentrations on various parameters of *H. sibirica*. The measured indicators were transformed into dimensionless membership values (range: 0–1) using standardized functions. The optimal Pro-Ca concentration was determined by calculating the mean membership value across all parameters, with higher values indicating superior overall performance.

3 Results

3.1 Morphological Characteristics

3.1.1 Effects on Plant Height

Under heavy shading conditions, *H. sibirica* displayed a significant increase in plant height relative to plants grown under full sunlight. In full sunlight environments, applications of Pro-Ca at concentrations of 300, 600, and 900 $mg{\cdot}L^{-1}$ resulted in significant reductions in plant height ($p < 0.05$), with the most marked suppression observed under the 600 $mg{\cdot}L^{-1}$ treatment. Throughout the observation timeline (10, 20, 30, 40, and 50 days), the 600 $mg{\cdot}L^{-1}$ Pro-Ca treatment led to height reductions of 39.03%, 16.69%, 11.39%, 11.29%, and 13.91%, respectively. A similar dwarfing effect was observed under heavy shading, where Pro-Ca treatments at 300, 600, and 900 $mg{\cdot}L^{-1}$ also induced sustained suppression of plant height ($p < 0.05$), with the 600 $mg{\cdot}L^{-1}$ treatment again exhibiting the strongest inhibitory effect. Compared to the shaded control group, the 600 $mg{\cdot}L^{-1}$ Pro-Ca treatment under heavy shading reduced plant height by 32.19%, 27.65%,

23.48%, 15.94%, and 22.02% at the corresponding time points (Table 2). Consistent with the significant Light × Pro-Ca interaction (Table A1), the same concentration produced a more pronounced effect under heavy shading.

Table 2: The effect of Pro-Ca on the plant height of *H. sibirica* (cm).

Treatment	Days after Initial Pro-Ca Application					
	0 d	10 d	20 d	30 d	40 d	50 d
qCK	17.33 ± 0.58^{b}	48.67 ± 2.08^{c}	72.33 ± 2.52^{bcd}	93.67 ± 3.51^{bc}	100.33 ± 4.73^{cd}	115.00 ± 5.00^{bc}
qT1	18.67 ± 1.53^{b}	47.33 ± 2.52^{bc}	69.33 ± 2.31^{c}	88.67 ± 2.08^{bc}	98.00 ± 6.00^{cd}	113.33 ± 8.50^{b}
qT3	18.00 ± 2.00^{b}	37.67 ± 1.53^{de}	61.67 ± 3.21^{d}	86.33 ± 2.08^{cd}	92.00 ± 2.65^{de}	110.33 ± 5.51^{bc}
qT6	19.00 ± 1.00^{b}	29.67 ± 2.52^{f}	60.33 ± 2.52^{d}	83.00 ± 4.00^{cd}	89.66 ± 1.53^{ef}	99.00 ± 1.00^{d}
qT9	17.33 ± 3.21^{b}	36.33 ± 4.04^{de}	63.00 ± 4.58^{d}	80.09 ± 4.58^{bc}	91.67 ± 2.52^{de}	104.00 ± 1.00^{cd}
zT0	25.00 ± 1.00^{a}	55.67 ± 2.52^{a}	80.33 ± 2.08^{a}	100.02 ± 4.36^{a}	110.67 ± 1.53^{a}	126.33 ± 5.69^{a}
zT1	25.67 ± 2.08^{a}	50.33 ± 7.37^{ab}	76.33 ± 3.21^{ab}	93.67 ± 1.53^{b}	104.67 ± 2.52^{b}	119.00 ± 6.00^{ab}
zT3	25.00 ± 0.00^{a}	47.00 ± 4.58^{bc}	71.67 ± 3.51^{bc}	81.67 ± 3.79^{d}	93.33 ± 3.51^{de}	114.00 ± 1.00^{b}
zT6	26.33 ± 1.53^{a}	33.00 ± 2.00^{ef}	52.33 ± 3.21^{e}	71.67 ± 4.73^{e}	84.33 ± 4.51^{f}	89.67 ± 2.08^{e}
zT9	26.33 ± 1.53^{a}	41.33 ± 3.51^{cd}	60.33 ± 2.52^{d}	81.00 ± 1.73^{d}	92.67 ± 2.52^{de}	101.33 ± 8.08^{cd}

Note: Data in the table were expressed as mean ± SD, the LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$) in the same column.

3.1.2 Effects on Leaf Blades

Under full sunlight conditions, heavy shading significantly reduced leaf length, width, and area in *H. sibirica* ($p < 0.05$). The experimental results showed that low concentrations of Pro-Ca (100, 300 mg·L^{-1}) had a weaker promoting effect on leaf morphology, while high concentrations of 900 mg·L $^{-1}$ had a weaker effect compared to 600 mg·L^{-1} Pro Ca application. However, 600 mg·L^{-1} produced the strongest leaf improvement effect under severe shading. For leaf length, the 600 mg·L^{-1} Pro-Ca treatment under shading exhibited the most prominent effect, increasing values by 16.12% to 45.05% from 10 d to 50 d after treatment, whereas the promoting effects of both 600 and 900 mg·L^{-1} Pro-Ca under full sunlight were comparatively limited (Table 3). In terms of leaf width, the shaded 600 mg·L^{-1} Pro-Ca treatment demonstrated the greatest efficacy, resulting in increases of 37.35% to 51.58% between 20 d and 50 d, which significantly exceeded the control. The 600 mg·L^{-1} Pro-Ca treatment under full sunlight also induced moderate promotion (Table 4). Regarding leaf area, the most substantial enhancement was observed under the shaded 600 mg·L^{-1} Pro-Ca treatment, which increased leaf area by 98.52% to 116.98% from 20 d to 50 d, markedly outperforming the same concentration applied under full sunlight (Tables 5 and A1).

Table 3: The effect of Pro-Ca on the leaf length of *H. sibirica* (cm).

Treatment	Days after Initial Pro-Ca Application					
	0 d	10 d	20 d	30 d	40 d	50 d
qCK	11.22 ± 0.33^{a}	12.13 ± 0.54^{a}	13.28 ± 0.37^{bc}	14.63 ± 0.71^{bcd}	16.25 ± 0.17^{cde}	16.90 ± 0.36^{cd}
qT1	11.30 ± 0.18^{a}	11.75 ± 0.23^{ab}	12.55 ± 0.61^{bc}	14.25 ± 0.44^{cd}	16.58 ± 0.20^{bcde}	17.67 ± 0.99^{bcd}
qT3	11.10 ± 0.85^{a}	11.97 ± 0.54^{a}	12.73 ± 0.65^{bc}	15.16 ± 0.21^{bc}	17.47 ± 1.24^{bcd}	18.43 ± 1.21^{bc}
qT6	11.24 ± 0.17^{a}	12.87 ± 0.40^{a}	14.37 ± 0.77^{ab}	16.47 ± 1.10^{ab}	18.79 ± 0.58^{ab}	20.05 ± 0.35^{ab}
qT9	11.15 ± 0.58^{a}	12.40 ± 0.69^{a}	13.07 ± 0.35^{bc}	15.44 ± 1.00^{bc}	17.61 ± 0.54^{bc}	18.07 ± 1.16^{bc}

Table 3: *Cont.*

Treatment	Days after Initial Pro-Ca Application					
	0 d	10 d	20 d	30 d	40 d	50 d
zT0	9.32 ± 0.52^{b}	10.36 ± 0.58^{bc}	11.47 ± 0.51^{c}	12.83 ± 0.64^{d}	14.43 ± 0.12^{e}	15.10 ± 0.66^{d}
zT1	9.28 ± 0.63^{b}	9.77 ± 0.69^{c}	13.01 ± 0.83^{bc}	14.68 ± 0.76^{bcd}	15.16 ± 0.30^{de}	16.83 ± 0.29^{cd}
zT3	9.20 ± 1.14^{b}	10.02 ± 0.04^{c}	13.33 ± 1.23^{bc}	15.24 ± 0.62^{bc}	16.5 ± 0.46^{bcde}	17.68 ± 0.15^{bcd}
zT6	9.19 ± 0.60^{b}	12.03 ± 0.07^{a}	15.97 ± 0.51^{a}	18.38 ± 0.52^{a}	20.93 ± 2.10^{a}	21.77 ± 2.11^{a}
zT9	9.28 ± 0.19^{b}	10.22 ± 0.69^{c}	13.89 ± 0.12^{b}	16.55 ± 0.67^{ab}	18.17 ± 0.32^{bc}	19.17 ± 0.76^{abc}

Note: Data in the table were expressed as mean ± SD, the LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$) in the same column.

Table 4: The effect of Pro-Ca on the leaf width of *H. sibirica* (cm).

Treatment	Days after Initial Pro-Ca Application					
	0 d	10 d	20 d	30 d	40 d	50 d
qCK	2.62 ± 0.29^{a}	3.25 ± 0.15^{a}	3.91 ± 0.22^{ab}	4.76 ± 0.44^{cd}	7.27 ± 0.27^{cde}	7.30 ± 0.45^{cde}
qT1	2.67 ± 0.15^{a}	2.97 ± 0.30^{ab}	3.38 ± 0.4^{bcde}	5.08 ± 0.23^{bcd}	6.67 ± 0.26^{efg}	7.22 ± 0.39^{cde}
qT3	2.5 ± 0.06^{ab}	3.15 ± 0.37^{a}	3.60 ± 0.10^{bcd}	5.29 ± 0.23^{bc}	6.93 ± 0.38^{def}	7.63 ± 0.39^{bcd}
qT6	2.44 ± 0.22^{abc}	2.88 ± 0.12^{abc}	4.12 ± 0.18^{ab}	5.72 ± 0.12^{ab}	8.13 ± 0.22^{ab}	8.38 ± 0.10^{ab}
qT9	2.69 ± 0.24^{a}	2.00 ± 0.26^{c}	3.62 ± 0.37^{bc}	5.18 ± 0.16^{bc}	7.50 ± 0.30^{bcd}	7.81 ± 0.31^{bc}
zT0	1.98 ± 0.2^{bcd}	2.43 ± 0.33^{abc}	3.21 ± 0.44^{bcde}	4.11 ± 0.13^{e}	6.01 ± 0.12^{g}	6.48 ± 0.39^{e}
zT1	1.83 ± 0.04^{d}	2.02 ± 0.28^{c}	2.44 ± 0.40^{e}	4.48 ± 0.22^{de}	6.42 ± 0.43^{fg}	6.91 ± 0.09^{de}
zT3	1.92 ± 0.16^{cd}	2.20 ± 0.63^{bc}	2.67 ± 0.22^{de}	4.81 ± 0.17^{cd}	7.38 ± 0.33^{bcde}	7.67 ± 0.15^{bcd}
zT6	1.73 ± 0.12^{d}	2.74 ± 0.21^{abc}	4.67 ± 0.33^{a}	6.23 ± 0.23^{a}	8.67 ± 0.15^{a}	8.90 ± 0.10^{a}
zT9	1.96 ± 0.33^{bcd}	2.31 ± 0.32^{abc}	2.90 ± 0.40^{cde}	5.39 ± 0.11^{bc}	7.82 ± 0.08^{bc}	8.37 ± 0.24^{ab}

Note: Data in the table were expressed as mean ± SD, the LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$) in the same column.

Table 5: The effect of Pro-Ca on the leaf area of *H. sibirica* (cm).

Treatment	Days after Initial Pro-Ca Application					
	0 d	10 d	20 d	30 d	40 d	50 d
qCK	20.64 ± 2.87^{a}	27.57 ± 1.22^{a}	36.33 ± 1.59^{bc}	48.82 ± 5.80^{c}	82.63 ± 2.5^{de}	86.42 ± 6.12^{de}
qT1	21.08 ± 0.91^{a}	24.45 ± 2.47^{abc}	29.77 ± 4.98^{cde}	50.59 ± 1.12^{c}	77.41 ± 3.42^{ef}	89.40 ± 9.47^{d}
qT3	19.39 ± 1.38^{a}	26.49 ± 4.03^{a}	32.08 ± 1.7^{bcd}	56.16 ± 2.5^{bc}	84.55 ± 3.08^{de}	98.28 ± 1.8^{cd}
qT6	19.22 ± 1.67^{a}	25.90 ± 1.04^{ab}	41.41 ± 3.12^{b}	65.98 ± 5.55^{b}	106.83 ± 0.88^{b}	117.7 ± 3.45^{ab}
qT9	21.06 ± 2.48^{a}	17.40 ± 3.20^{bcde}	33.16 ± 3.55^{bcd}	55.96 ± 3.26^{bc}	92.41 ± 4.11^{cd}	98.85 ± 8.9^{bcd}
zT0	12.94 ± 1.39^{b}	17.71 ± 3.19^{bcde}	25.68 ± 2.63^{de}	36.97 ± 2.68^{d}	60.72 ± 0.75^{g}	68.33 ± 1.70^{e}
zT1	11.92 ± 0.99^{b}	13.85 ± 2.57^{e}	22.14 ± 3.05^{e}	46.00 ± 0.25^{cd}	68.12 ± 4.21^{fg}	81.43 ± 2.28^{de}
zT3	12.30 ± 0.99^{b}	15.42 ± 4.49^{de}	24.94 ± 3.58^{de}	51.29 ± 2.34^{c}	85.17 ± 1.68^{de}	94.91 ± 2.44^{cd}
zT6	11.11 ± 0.03^{b}	23.09 ± 1.67^{abcd}	52.25 ± 4.81^{a}	80.22 ± 4.5^{a}	126.94 ± 12.25^{a}	135.65 ± 13.78^{a}
zT9	12.74 ± 2.37^{b}	16.61 ± 3.50^{cde}	28.17 ± 3.65^{cde}	62.46 ± 3.6^{b}	99.41 ± 2.49^{bc}	112.30 ± 1.83^{bc}

Note: Data in the table were expressed as mean ± SD, different letters indicated significant differences ($p < 0.05$).

3.1.3 Effects on Leaf Color

Heavy shading significantly influenced leaf color parameters in *H. sibirica* ($p < 0.05$). Compared to full sunlight conditions, shaded leaves exhibited significantly higher lightness (L* value), lower a* values, and higher b* values. Pro-Ca application exerted marked regulatory effects on these color attributes: it significantly reduced L* values, with the most substantial reduction observed under 600 mg·L^{-1} Pro-Ca in full sunlight (8.74%) and an even greater reduction under the same concentration in heavy shading (13.91%). Notably, the 600 mg·L^{-1} Pro-Ca treatment under shading decreased L* values by 9.80% relative to the full sunlight control, demonstrating enhanced efficacy in shaded environments (Fig. 1A). For a* values, Pro-Ca treatment induced a significant increase, with 600 mg·L^{-1} Pro-Ca under full sunlight raising a* by 14.42%, while the same concentration under shading resulted in a more pronounced increase of 28.77% (Fig. 1B). In the case of b* values, Pro-Ca application led to a significant decrease, with the strongest effect again observed under 600 mg·L^{-1} Pro-Ca in both full sunlight (28.56% reduction) and heavy shading (34.32% reduction). All Pro-Ca treatments under shading significantly lowered b* values compared to the shaded control (Fig. 1C). These findings indicate that Pro-Ca effectively modulates leaf color parameters across light regimes, with particularly strong regulatory outcomes under shaded conditions. Consistent with the significant Light × Pro-Ca interaction (Table A1), the 600 mg·L^{-1} Pro-Ca treatment consistently performed best in enhancing leaf color traits under heavy shading.

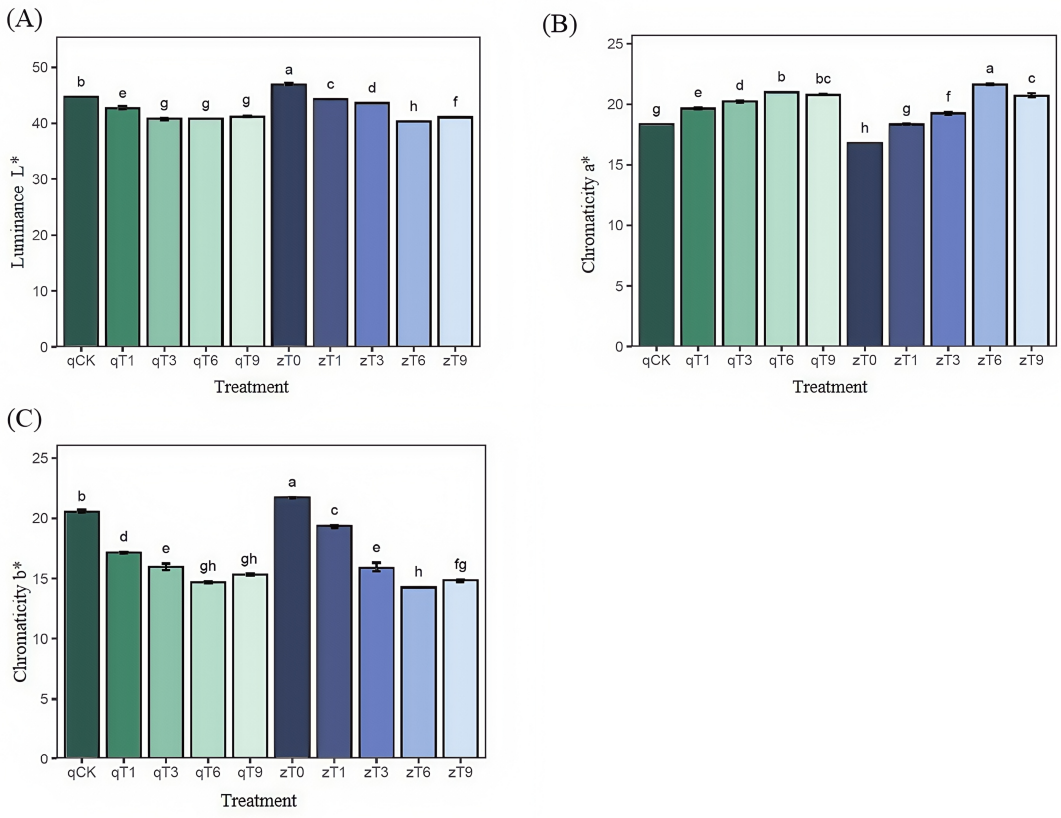

Figure 1: The effect of Pro-Ca on the leaf color of *H. sibirica*. (**A**) Lightness (L*); (**B**) Red-green chromaticity index (a*); (**C**) Yellow-blue chromaticity index (b*). Treatment codes: qCK (full sunlight control), zT0 (shading control), qT1 (100 mg·L^{-1}, full sun), qT3 (300 mg·L^{-1}, full sun), qT6 (600 mg·L^{-1}, full sun), qT9 (900 mg·L^{-1}, full sun), zT1 (100 mg·L^{-1}, shading), zT3 (300 mg·L^{-1}, shading), zT6 (600 mg·L^{-1}, shading), zT9 (900 mg·L^{-1}, shading). The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

3.1.4 Effects on Floret Number

Based on the final observation data (50 d), heavy shading significantly reduced the floret number in *H. sibirica* compared to full sunlight conditions ($p < 0.05$). At this time point, the effect of Pro-Ca treatment on floret development exhibited distinct light-dependent responses: under shaded conditions, the 600 mg·L^{-1} treatment significantly increased the floret number by 57.07% compared to the shaded control, while the 900 mg·L^{-1} treatment also achieved a 43.46% increase ($p < 0.05$). In contrast, under full sunlight conditions, only the 600 mg·L^{-1} Pro-Ca treatment showed a significant effect compared to the full sunlight control, increasing the floret number by 12.13% ($p < 0.05$) (Table 6). Collectively, these results indicate that Pro-Ca exerts a light-environment-dependent regulation on floret number, with a particularly strong flowering-promoting effect under shaded conditions. Among all treatments evaluated, the 600 mg·L^{-1} Pro-Ca application under heavy shading achieved optimal results, followed by the 900 mg·L^{-1} treatment under the same light regime. These findings offer valuable insights for improving flowering performance in *H. sibirica* cultivated under light-limiting environments.

Table 6: The effect of Pro-Ca on the floret number of *H. sibirica* (no.).

Treatment	Days after Initial Pro-Ca Application					
	0 d	10 d	20 d	30 d	40 d	50 d
qCK	0.00 ± 0.00^{a}	11.25 ± 2.22^{a}	18.75 ± 1.26^{a}	32.50 ± 3.32^{ab}	51.50 ± 2.65^{ab}	59.75 ± 1.26^{c}
qT1	0.00 ± 0.00^{a}	10.25 ± 1.26^{abc}	17.25 ± 3.1^{ab}	28.25 ± 2.75^{bc}	40.50 ± 2.52^{cd}	48.75 ± 3.30^{de}
qT3	0.00 ± 0.00^{a}	11.25 ± 0.50^{a}	13.25 ± 2.63^{bc}	23.25 ± 1.50^{cd}	35.00 ± 2.16^{de}	53.50 ± 3.70^{cd}
qT6	0.00 ± 0.00^{a}	11 ± 0.82^{ab}	16.50 ± 1.29^{ab}	29.5 ± 3.32^{bc}	48.25 ± 1.71^{b}	67.00 ± 3.16^{b}
qT9	0.00 ± 0.00^{a}	10.5 ± 0.58^{abc}	16.25 ± 2.5^{ab}	27.25 ± 1.26^{bc}	41.50 ± 3.42^{c}	59.50 ± 1.29^{c}
zT0	0.00 ± 0.00^{a}	6.5 ± 1.29^{d}	9.50 ± 2.89^{c}	19.00 ± 2.16^{d}	33.00 ± 2.16^{e}	47.75 ± 2.36^{de}
zT1	0.00 ± 0.00^{a}	6.75 ± 1.89^{d}	8.50 ± 2.38^{c}	18.00 ± 1.63^{d}	36.75 ± 2.75^{cde}	42.75 ± 2.06^{e}
zT3	0.00 ± 0.00^{a}	7.50 ± 1.29^{cd}	10.50 ± 1.91^{c}	24.5 ± 2.08^{bcd}	40.25 ± 1.89^{cd}	50.50 ± 3.51^{d}
zT6	0.00 ± 0.00^{a}	8.00 ± 0.82^{bcd}	13.5 ± 1.73^{abc}	37.75 ± 6.18^{a}	56.50 ± 1.29^{a}	75.00 ± 3.74^{a}
zT9	0.00 ± 0.00^{a}	7.00 ± 0.82^{d}	12.75 ± 2.06^{bc}	31.50 ± 5.32^{ab}	50.25 ± 1.71^{b}	68.5 ± 2.08^{ab}

Note: Data in the table were expressed as mean ± SD, different letters indicated significant differences ($p < 0.05$).

3.1.5 Effects on Petal Color

Under heavy shading conditions, flower color lightness (L*) was significantly reduced compared to full sunlight exposure ($p < 0.05$). Pro-Ca application consistently lowered L* values, with the most substantial reductions observed in the 600 mg·L^{-1} treatment under full sunlight (13.33%) and the same concentration under shading (6.08%). Notably, all shading treatments resulted in greater L* reduction relative to the full sunlight control, with the maximum decrease (15.21%) recorded for the 600 mg·L^{-1} Pro-Ca treatment under shading (Fig. 2A,E), which was supported by the significant Light × Pro-Ca interaction (Table A1). Flower redness-greenness (a*) was significantly lower under shaded conditions than under full sunlight ($p < 0.05$). Pro-Ca treatment effectively enhanced a* values, with the 600 mg·L^{-1} treatment showing the greatest improvement in full sunlight (36.72%) and a markedly stronger effect under shading (98.89%). The 300, 600, and 900 mg·L^{-1} Pro-Ca treatments under shading all significantly increased a* values compared to the full sunlight control (Fig. 2B,E). Shading significantly increased yellowness-blueness (b*) relative to full sunlight ($p < 0.05$). In full sunlight, only the lowest Pro-Ca concentration significantly raised b* (31.81%), whereas under shading, all Pro-Ca treatments reduced b* values. The maximum reduction (43.68%) was achieved

with the 600 mg·L^{-1} Pro-Ca treatment under shading, and the 300, 600, and 900 mg·L^{-1} treatments under shading all showed significant decreases compared to the full sunlight control (Fig. 2C,E). Flower chroma (C*) was significantly lower under shading than under full sunlight ($p < 0.05$). Pro-Ca treatment increased C* values, with the strongest enhancement observed for the 600 mg·L^{-1} treatment in full sunlight (15.63%) and under shading (36.59%). Both the 600 and 900 mg·L^{-1} Pro-Ca treatments under shading significantly elevated C* relative to the full sunlight control (Fig. 2D,E).

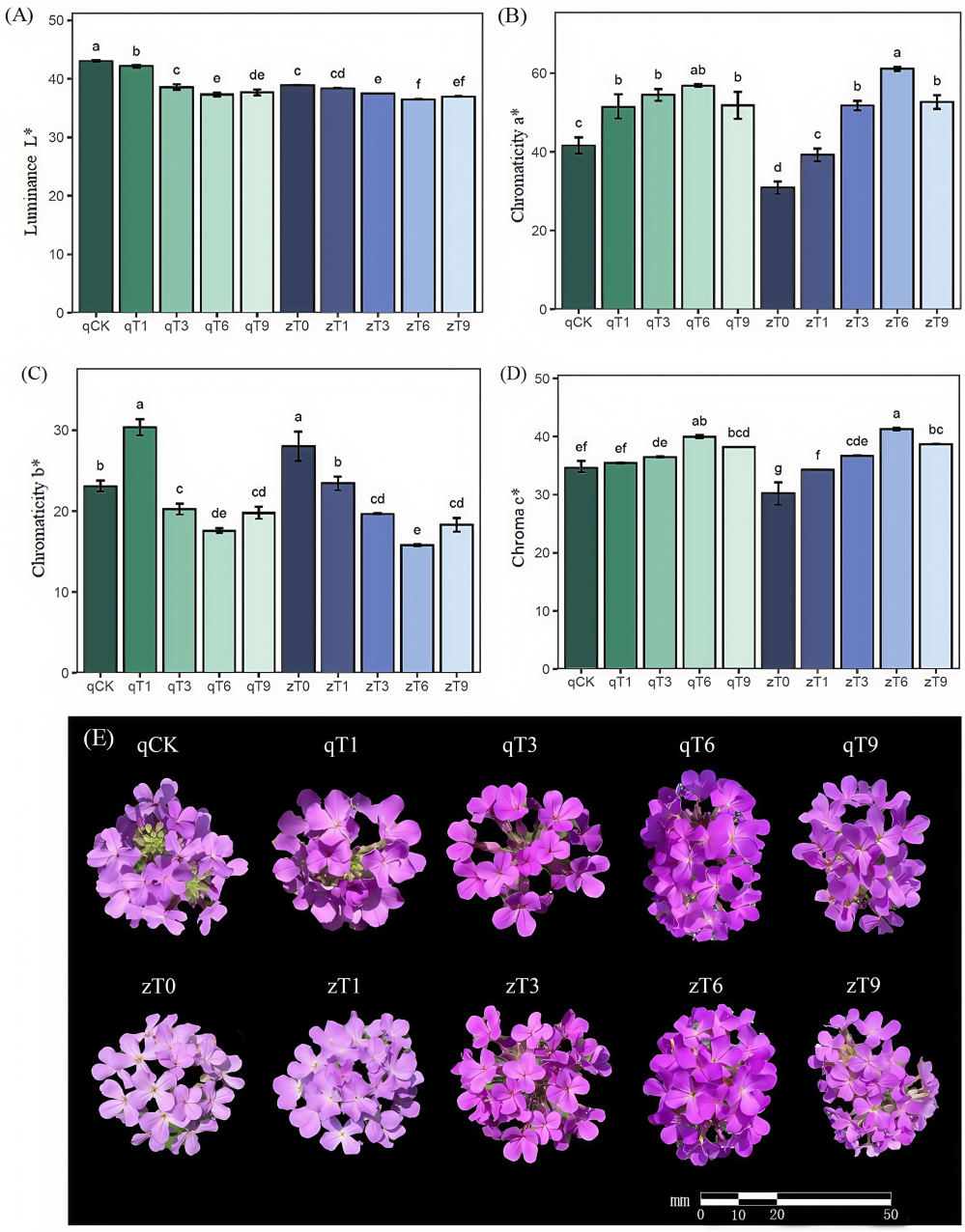

Figure 2: The effect of Pro-Ca on the petal color of *H. sibirica*. (**A**) Lightness (L*); (**B**) Red-green chromaticity index (a*); (**C**) Yellow-blue chromaticity index (b*); (**D**) Chroma (C*); (**E**) Flower phenotypes under different treatments (Scale bar: 50 mm). The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

3.1.6 Effects on Root System

Heavy shading significantly suppressed root growth in *H. sibirica*, resulting in markedly reduced root length compared to plants grown under full sunlight ($p < 0.05$). Pro-Ca application significantly promoted root development, though its efficacy was strongly influenced by light conditions. Under full sunlight, the 600 mg·L^{-1} Pro-Ca treatment increased root length by 67.48%, consistent with the significant Light × Pro-Ca interaction (Table A1), the same concentration produced a more pronounced effect under heavy shading, achieving a 169.09% increase. All shading treatments resulted in significantly greater root length relative to the full sunlight control, with the 600 mg·L^{-1} Pro-Ca treatment under heavy shading exhibiting a 74.43% enhancement (Fig. 3A). In terms of root diameter, the 600 mg·L^{-1} and 900 mg·L^{-1} Pro-Ca treatments under heavy shading increased diameter by 36.01% and 24.46%, respectively, compared to the shading control. All Pro-Ca treatments under shading significantly thickened roots, with the 600 mg·L^{-1} treatment showing an 83.88% increase. Both the 600 and 900 mg·L^{-1} treatments under shading also yielded significantly larger diameters than the full sunlight control (Fig. 3B). For root surface area, all full sunlight Pro-Ca treatments significantly exceeded the full sunlight control, with the 600 mg·L^{-1} treatment increasing area by 14.47%. Under shading, all treatments significantly outperformed the shading control, with the 600 mg·L^{-1} Pro-Ca treatment under heavy shading increasing root area by 63.38%. Moreover, all shading treatments produced greater root surface area than the full sunlight control (Fig. 3C,E). A notable light-dependent response was observed in root volume: while Pro-Ca did not significantly affect root volume under full sunlight, all shading treatments showed significant increases, with the 600 mg·L^{-1} treatment reaching a 122.08% enhancement. Both the 600 and 900 mg·L^{-1} Pro-Ca treatments under shading also significantly surpassed the full sunlight control in root volume (Fig. 3D). These findings demonstrate that Pro-Ca application can effectively alleviate shading-induced inhibition of root development, with the 600 mg·L^{-1} Pro-Ca treatment under heavy shading exhibiting the most substantial restorative effects in shaded environments.

3.2 Photosynthetic Characteristics

3.2.1 Effects on Chlorophyll Content

Under heavy shading conditions, the contents of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (a+b), and the Chla/Chlb ratio in *H. sibirica* were significantly reduced compared to full sunlight conditions ($p < 0.05$). Pro-Ca application significantly increased these chlorophyll-related parameters under both light regimes, with more substantial enhancements observed under shaded environments. Among the treatments, 600 mg·L^{-1} Pro-Ca under shading demonstrated the most pronounced effects, exhibiting a concentration-dependent response characterized by an initial increase followed by a decline at higher concentrations. Temporal analysis revealed that most parameters generally followed a pattern of initial elevation followed by gradual decrease, except for Chlb, which showed a continuous increasing trend. Specifically, while the 600 mg·L^{-1} Pro-Ca treatment showed superior performance under full sunlight conditions, the same concentration under shading provided the most significant improvements in chlorophyll parameters under low light stress (Table A1), with the 900 mg·L^{-1} Pro-Ca treatment under shading also performing well in terms of the Chla/Chlb ratio (Fig. 4A–D).

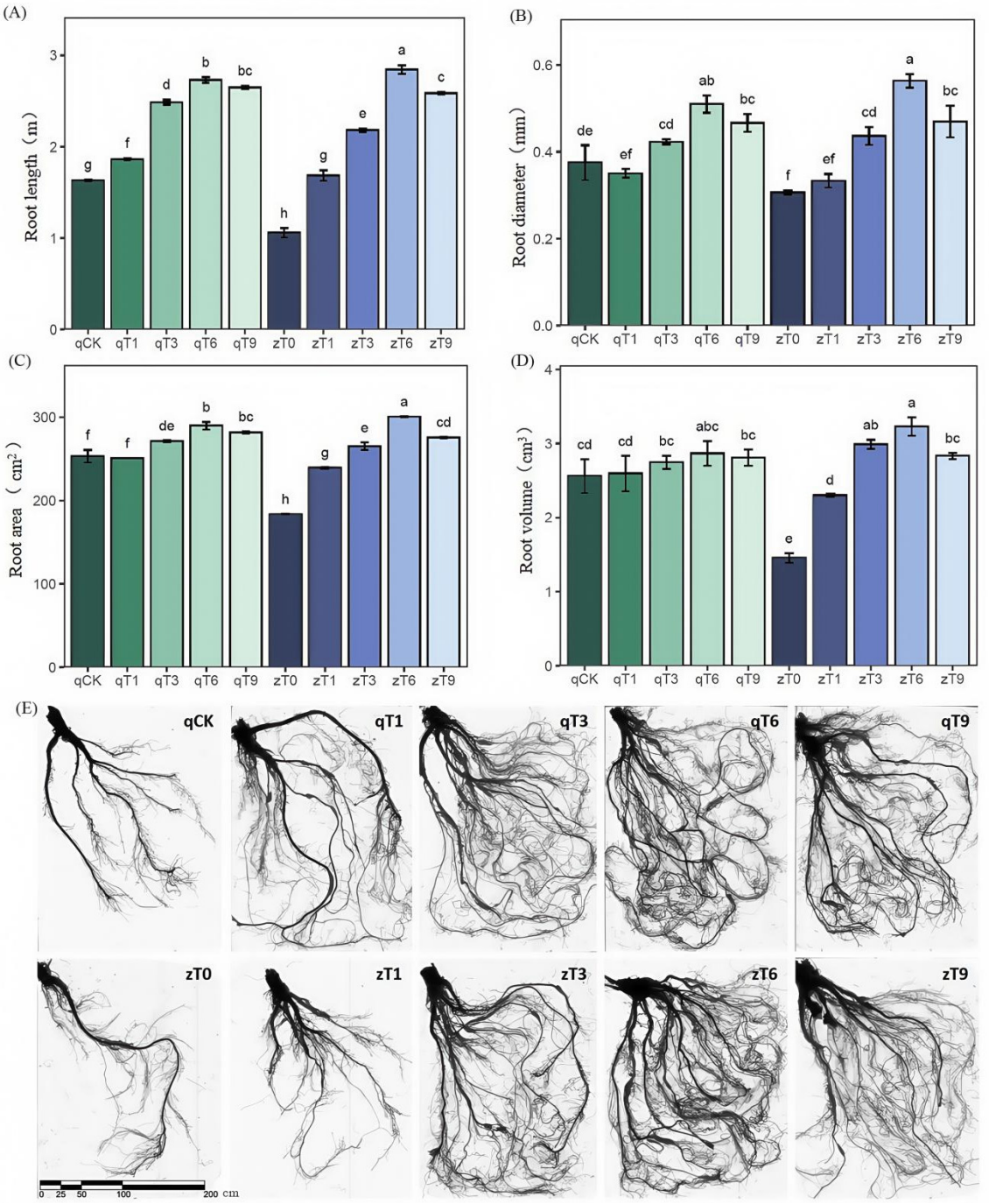

Figure 3: The effect of Pro-Ca on the root system of *H. sibirica*. (**A**) Root length; (**B**) root average diameter; (**C**) root surface area; (**D**) root volume; (**E**) comprehensive root growth comparison. Different lowercase letters above bars denote significant differences at $p < 0.05$ according to LSD test. Treatment codes: qCK (full sunlight control), zT0 (shading control), qT1 (100 mg·L^{-1}, full sun), qT3 (300 mg·L^{-1}, full sun), qT6 (600 mg·L^{-1}, full sun), qT9 (900 mg·L^{-1}, full sun), zT1 (100 mg·L^{-1}, shading), zT3 (300 mg·L^{-1}, shading), zT6 (600 mg·L^{-1}, shading), zT9 (900 mg·L^{-1}, shading). The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

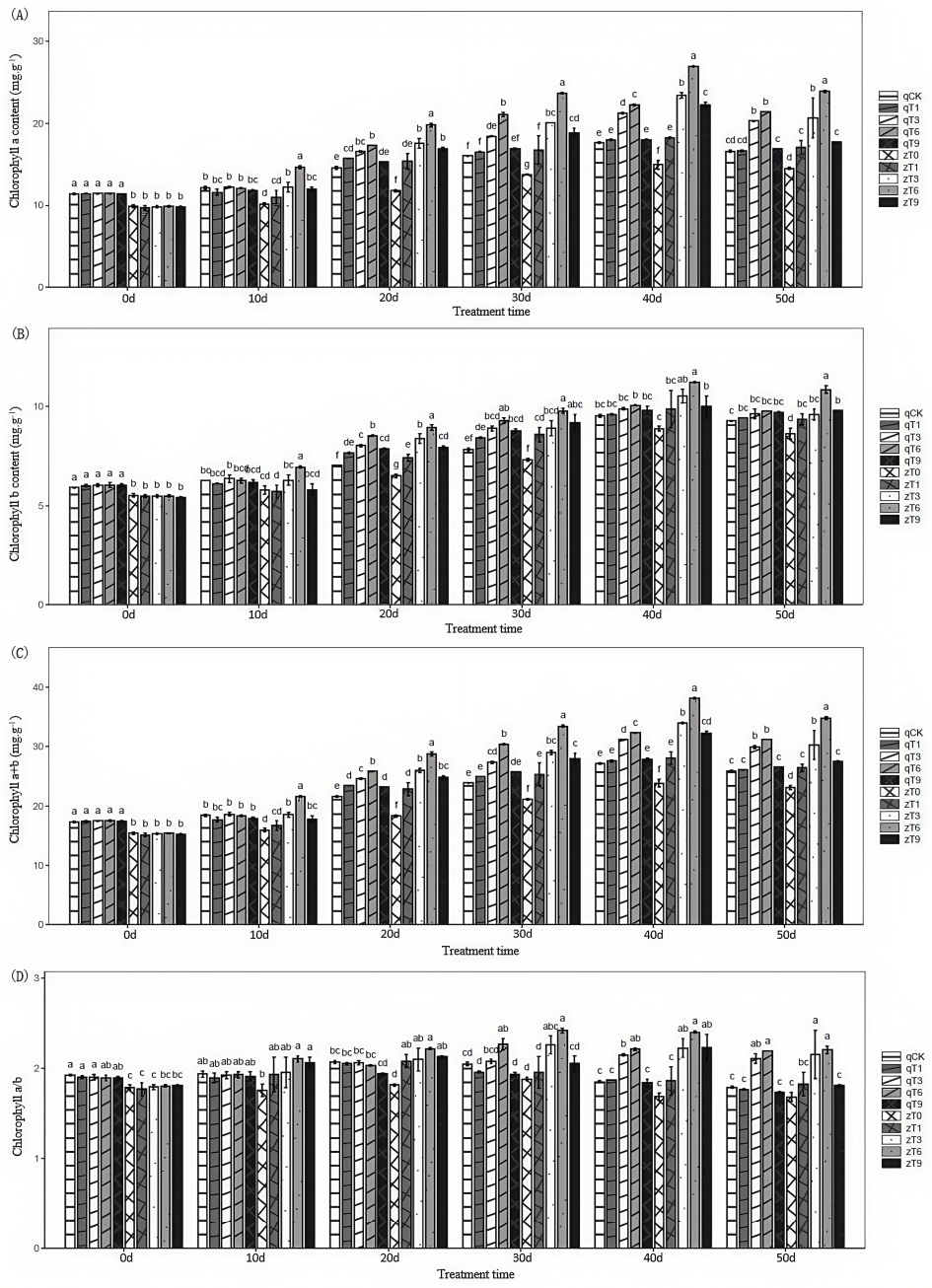

Figure 4: The effect of Pro-Ca on chlorophyll content of *H. sibirica.* (**A**) Chlorophyll a content (Chl a); (**B**) Chlorophyll b content (Chl b); (**C**) Total chlorophyll content (Chl a+b); (**D**) Chlorophyll a/b ratio (Chl a/b). The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

3.2.2 Effects on Photosynthetic Parameters

For photosynthetic parameters, the shaded control exhibited significantly elevated intercellular CO_2 concentration (C_i) but reduced stomatal conductance (G_s), transpiration rate (T_r), and net photosynthetic rate (P_n) relative to the full sunlight control ($p < 0.05$). Pro-Ca application effectively lowered C_i while enhancing G_s, T_r, and P_n under both light regimes, with more substantial improvements observed under shaded conditions—particularly with the 600 mg·L^{-1} Pro-Ca treatment under heavy shading. The responses

followed distinct patterns: C_i decreased initially then increased with rising Pro-Ca concentration, whereas G_s, T_r, and P_n increased initially before declining. Temporal dynamics revealed an initial decline followed by a rise in C_i, an initial increase then decrease in G_s and P_n, and a progressive increase in T_r. Under full sunlight, the 600 mg·L^{-1} Pro-Ca treatment provided relatively better improvement in gas exchange parameters (Fig. 5A–D). These findings indicate that Pro-Ca treatment can effectively alleviate the negative impact of shading stress on the photosynthetic performance of *H. sibirica*.

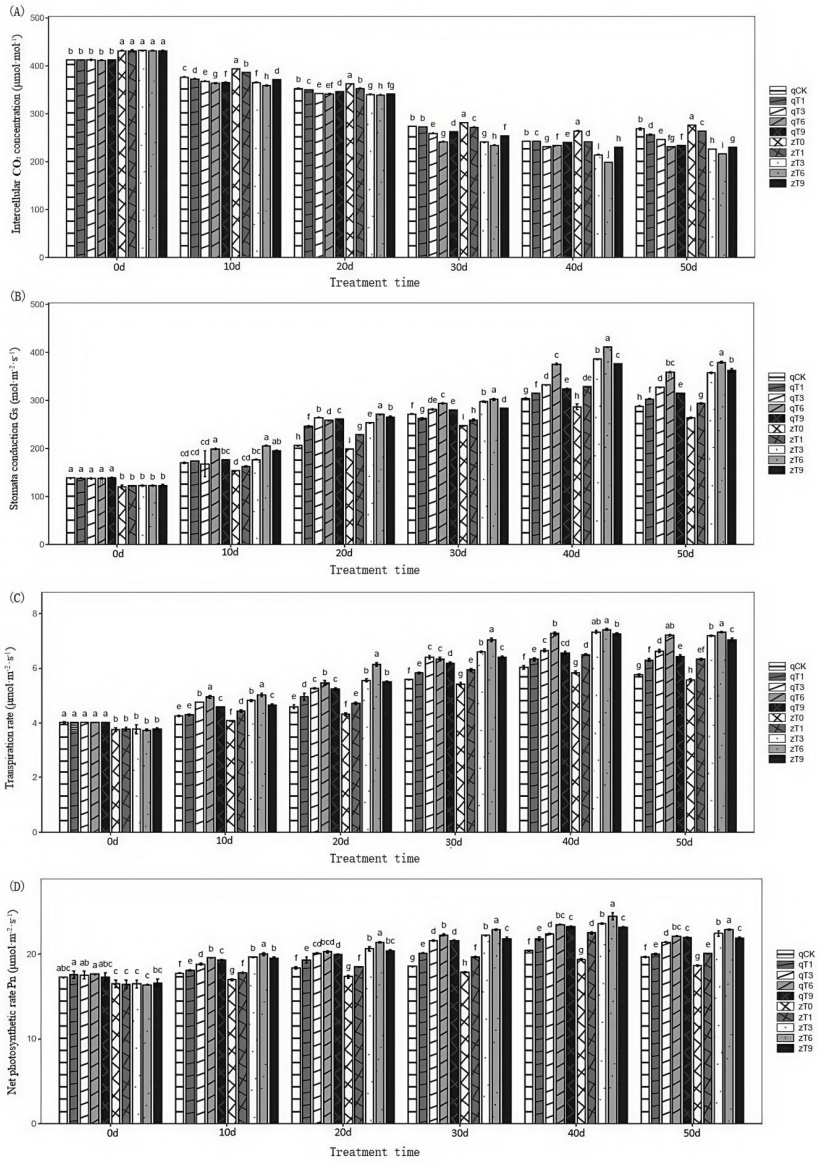

Figure 5: The photosynthetic parameters of *H. sibirica*. (**A**) Intercellular CO_2 concentration (C_i); (**B**) Stomatal conductance (G_s); (**C**) Transpiration rate (T_r); (**D**) Net photosynthetic rate (P_n). The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

3.3 Chlorophyll Fluorescence Characteristics

The shaded control group showed higher initial fluorescence (F_o) relative to the full sunlight control, whereas maximum fluorescence (F_m), variable fluorescence (F_v), the F_v/F_o ratio, and the maximum photo-

chemical efficiency (F_v/F_m) were all significantly lower ($p < 0.05$). Pro-Ca application substantially improved chlorophyll fluorescence characteristics: it reduced F_o while increasing F_m, F_v, and F_v/F_o, with the most pronounced effects observed under the 600 mg·L^{-1} Pro-Ca treatment in heavy shading. This treatment resulted in the greatest reduction in F_o and the most significant enhancements in F_m, F_v, and F_v/F_o. Under full sunlight, the 600 mg·L^{-1} Pro-Ca treatment also induced considerable improvements. In contrast, the effect of Pro-Ca on the F_v/F_m ratio was relatively limited, with only marginal increases detected in specific treatments such as 600 mg·L^{-1} Pro-Ca under heavy shading (Fig. 6). These findings demonstrate that Pro-Ca treatment effectively optimizes the chlorophyll fluorescence characteristics of *H. sibirica*, with particularly prominent effects under shaded conditions.

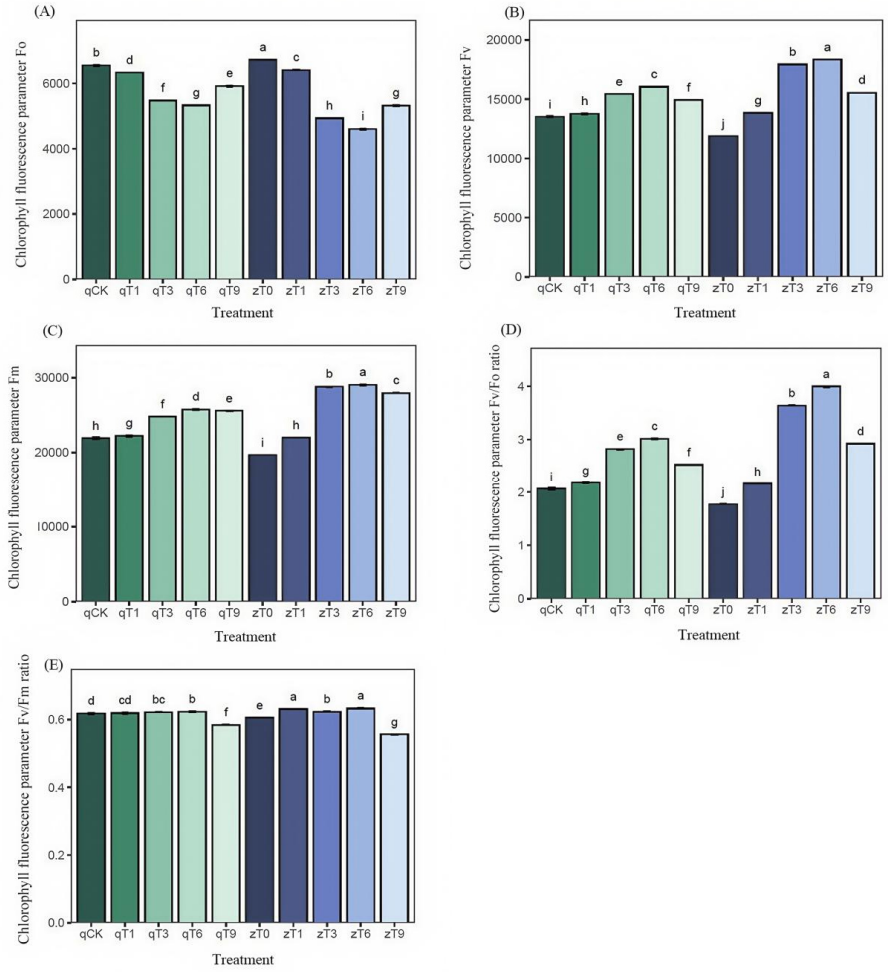

Figure 6: The effect of Pro-Ca on fluorescence parameters of *H. sibirica*. (**A**) F_o: minimal fluorescence yield; (**B**) F_v: variable fluorescence yield; (**C**) F_m: maximum fluorescence yield; (**D**) F_v/F_o ratio; (**E**) F_v/F_m ratio. The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

3.4 Comprehensive Analysis via Membership Function

Based on comprehensive experimental results of growth characteristics, photosynthetic properties, and chlorophyll fluorescence parameters, this study employed membership function analysis to evaluate different treatment groups. This analysis revealed the following performance ranking: zT6 > qT6 > zT9 > zT3 > qT9 > qT3 > qT1 > qCK > zT1 > zT0 (Table 7), with the 600 mg·L^{-1} Pro-Ca under heavy shading

demonstrating optimal performance. The results indicate that Pro-Ca treatment effectively promotes both growth and photosynthetic performance in *H. sibirica* under different light conditions. The most significant comprehensive effects were observed with 600 mg·L^{-1} Pro-Ca application under heavy shading, followed by the same concentration under full sunlight. These findings provide an favorable plant growth regulator application protocol for *H. sibirica* cultivation under shading stress conditions.

Table 7: Comprehensive ranking based on membership function analysis.

Index	Treatment									
	qCK	**qT1**	**qT3**	**qT6**	**qT9**	**zT0**	**zT1**	**zT3**	**zT6**	**zT9**
Plant height	0.68	0.61	0.35	0.46	0.55	1.00	0.60	0.24	0.00	0.22
Leaf length	0.28	0.33	0.47	0.67	0.49	0.00	0.11	0.32	1.00	0.57
Leaf width	0.47	0.25	0.35	0.80	0.56	0.00	0.15	0.52	1.00	0.68
Leaf area	0.33	0.25	0.36	0.70	0.48	0.00	0.11	0.37	1.00	0.58
Leaf color L*	0.67	0.35	0.08	0.07	0.13	1.00	0.60	0.50	0.00	0.11
Leaf color a*	0.85	0.91	0.94	0.97	0.96	0.78	0.85	0.89	1.00	0.96
Leaf color b*	0.84	0.39	0.23	0.05	0.14	1.00	0.69	0.21	0.00	0.08
Floret number	0.53	0.19	0.33	0.75	0.52	0.16	0.00	0.24	1.00	0.80
Petal color L*	1.00	0.87	0.32	0.12	0.18	0.36	0.28	0.15	0.00	0.07
Petal color a*	0.35	0.68	0.78	0.86	0.69	0.00	0.28	0.69	1.00	0.72
Petal color b*	0.50	1.00	0.31	0.12	0.27	0.84	0.53	0.27	0.00	0.17
Petal color c*	0.39	0.47	0.57	0.88	0.72	0.00	0.37	0.58	1.00	0.76
Root length	0.32	0.45	0.80	0.94	0.89	0.00	0.35	0.63	1.00	0.86
Root diameter	0.27	0.17	0.46	0.79	0.62	0.00	0.10	0.51	1.00	0.64
Root area	0.60	0.57	0.75	0.91	0.84	0.00	0.48	0.70	1.00	0.79
Root volume	0.62	0.64	0.73	0.80	0.76	0.00	0.48	0.86	1.00	0.78
Chla	0.22	0.25	0.53	0.61	0.26	0.00	0.27	0.71	1.00	0.61
Chlb	0.27	0.31	0.43	0.51	0.40	0.00	0.42	0.71	1.00	0.49
Chla+b	0.23	0.26	0.51	0.59	0.28	0.00	0.30	0.71	1.00	0.59
Chla/b	0.23	0.26	0.65	0.74	0.21	0.00	0.24	0.75	1.00	0.76
C_i	0.67	0.66	0.50	0.54	0.63	1.00	0.65	0.24	0.00	0.48
G_s	0.14	0.23	0.37	0.71	0.30	0.00	0.34	0.80	1.00	0.72
T_r	0.12	0.31	0.51	0.91	0.46	0.00	0.42	0.94	1.00	0.89
P_n	0.21	0.48	0.59	0.81	0.75	0.00	0.61	0.84	1.00	0.75
F_o	0.92	0.82	0.41	0.34	0.62	1.00	0.85	0.16	0.00	0.34
F_v	0.25	0.29	0.55	0.64	0.47	0.00	0.30	0.93	1.00	0.57
F_m	0.24	0.27	0.55	0.65	0.63	0.00	0.25	0.98	1.00	0.89
F_v/F_o	0.23	0.28	0.43	0.36	0.34	0.00	0.28	0.64	0.00	0.42
F_v/F_m	0.13	0.18	0.47	0.56	0.34	0.00	0.18	0.84	1.00	0.52
Value	0.42	0.43	0.49	0.63	0.51	0.24	0.37	0.59	0.77	0.59
Rank	8	7	6	2	5	10	9	4	1	3

3.5 Leaf Anatomic Structural Responses

Based on the comprehensive analysis result of membership function, this study further compared the effects of 600 mg·L^{-1} Pro-Ca on leaf structure of *H. sibirica* under full sunlight and heavy shading conditions, aiming to investigate the mechanism in alleviating shading stress.

3.5.1 Effects on Leaf Microscopic Structure

Scanning electron microscopy analysis revealed distinct leaf anatomical characteristics under different light regimes. Leaves developed under full sunlight displayed intact architecture with turgid epidermal cells exhibiting well-defined boundaries, accompanied by tightly arranged palisade and well-differentiated spongy mesophyll tissues. In contrast, heavy shading maintained general leaf integrity but induced epidermal cell shrinkage with obscured boundaries, reduced palisade tissue density, and underdeveloped spongy mesophyll organization (Fig. 7a–h). Treatment with 600 mg·L^{-1} Pro-Ca under both light conditions induced significant structural improvements: epidermal cells regained turgidity (particularly evident under shading), stomatal development was enhanced, and total leaf thickness—including the upper and lower epidermis, palisade, and spongy layers—increased substantially (Fig. 7i–p). The 600 mg·L^{-1} Pro-Ca treatment under heavy shading produced more pronounced effects than the same concentration under full sunlight. Specifically, leaf thickness under full sunlight with 600 mg·L^{-1} Pro-Ca reached 1.99 times that of the full sunlight control, while under heavy shading, it increased to 2.35-fold relative to the shaded control. Venation analysis further showed that Pro-Ca promoted xylem cell proliferation and collenchyma cell expansion under full sunlight, while significantly increasing xylem cell numbers under shading (Fig. 8). These results demonstrate that Pro-Ca application effectively enhances the development of all leaf tissue layers, increases overall leaf thickness, and generates more substantial improvements under light-limiting conditions.

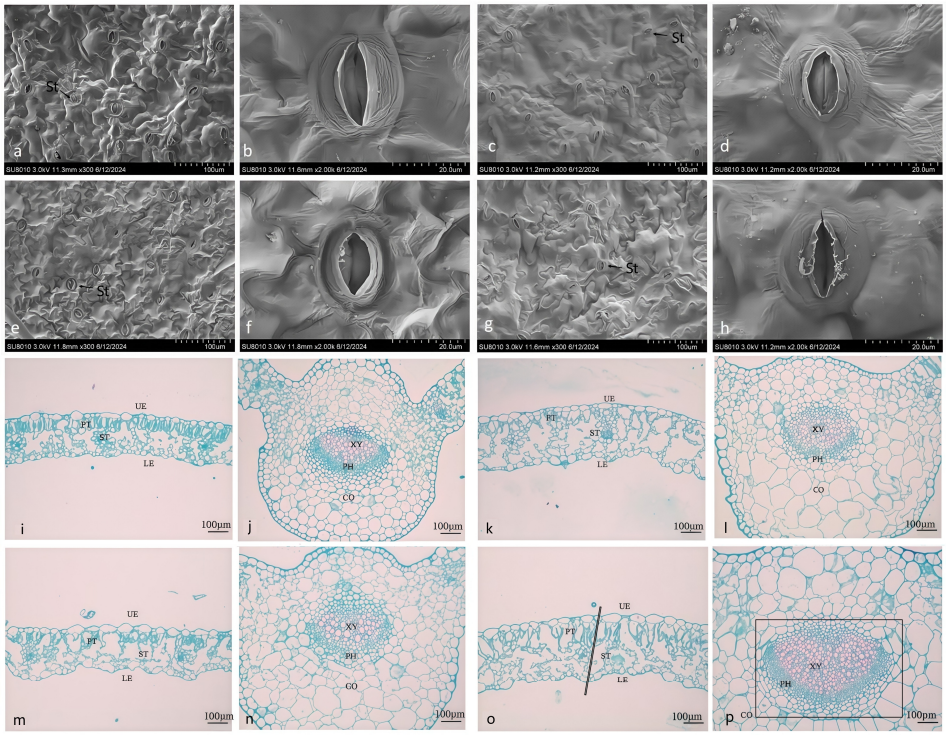

Figure 7: Pro-Ca alleviates shade-induced leaf structural impairment. (**a**,**b**,**i**,**j**). Control in full light; (**c**,**d**,**k**,**l**). Pro-Ca-tuned treatment in full light; (**e**,**f**,**m**,**n**). Control in heavy shade; (**g**,**h**,**o**,**p**). Pro-Ca-tuned treatment in heavy shade.

Scanning electron micrographs of leaf transverse sections show that heavy shading caused epidermal cell shrinkage and disordered mesophyll arrangement, while 600 mg·L^{-1} Pro-Ca treatment restored epidermal cell turgor, enhanced stomatal development, and significantly increased leaf thickness under both full sunlight and heavy shading conditions, with more pronounced improvements under shading.

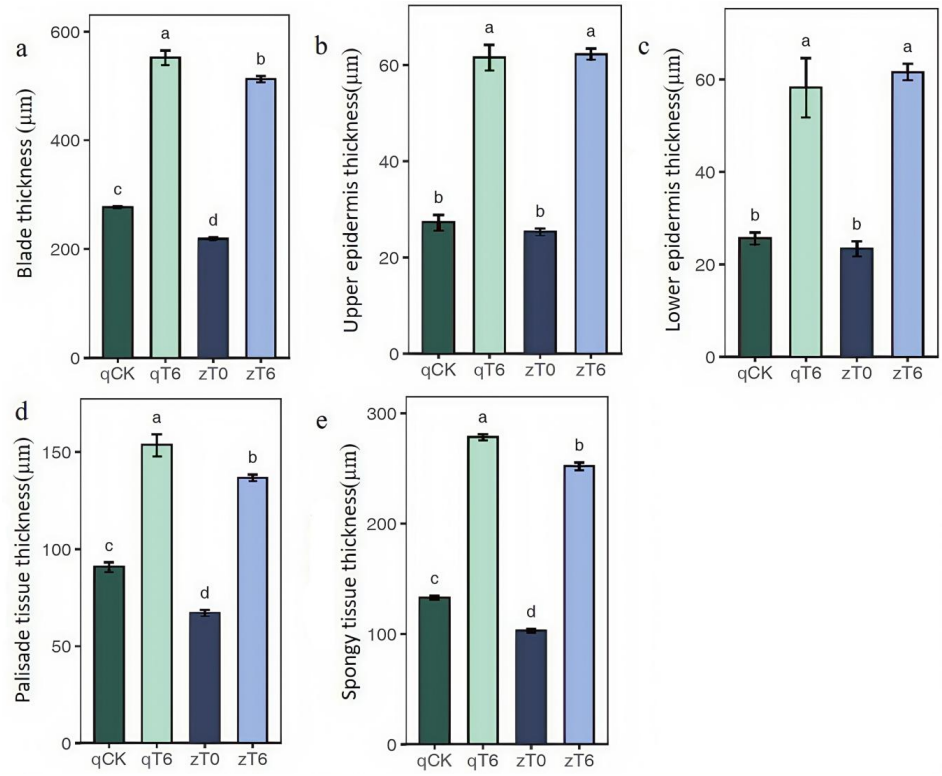

Figure 8: Pro-Ca promotes venation development under different light regimes. Treatment codes combine light regime with Pro-Ca concentration (qCK: Full sunlight control group; zT0: Shading control group; qT6: 600 mg·L^{-1} treatment under full sunlight conditions; zT6: 600 mg·L^{-1} treatment under heavy shading conditions). (**a**) Blade thickness; (**b**) Upper epidermis thickness; (**c**) Lower epidermis thickness; (**d**) Palisade tissue thickness; (**e**) Spongy tissue thickness. The LSD test was employed for mean comparison, different letters indicated significant differences ($p < 0.05$).

Venation anatomy analysis reveals that Pro-Ca treatment stimulated xylem cell proliferation and collenchyma cell enlargement in full sunlight, and significantly increased xylem cell number under heavy shading, demonstrating its role in reinforcing vascular structure to support improved leaf function.

3.5.2 Effects on Leaf Ultrastructure

Transmission electron microscopy examination demonstrated that mesophyll cells of *H. sibirica* treated with 600 mg·L^{-1} Pro-Ca maintained structural integrity under both full sunlight and heavy shading conditions, with spindle-shaped chloroplasts densely arranged along the inner cell periphery (Fig. 9). Pro-Ca application enhanced chloroplast development, though distinct light-dependent responses were observed. In full sunlight, leaves treated with 600 mg·L^{-1} Pro-Ca exhibited relatively fewer chloroplasts but contained larger and more numerous starch grains and osmiophilic globules, along with increased stromal lamellae and reduced grana stacking. Under heavy shading, however, the same Pro-Ca treatment showed more abundant intact chloroplasts under visual observation, promoted tighter stacking and higher frequency of

grana lamellae, enriched stromal lamellae, and enlarged starch grain size. Both treatments led to greater accumulation of osmiophilic globules compared to their respective controls, with those in the shading control displaying darker staining (Table A1). These findings indicate that Pro-Ca promotes chloroplast development under varying light regimes by stimulating starch grain production, enhancing grana and stromal lamellae formation, and increasing starch grain dimensions, while exerting light-specific modulatory effects: it primarily enhances osmiophilic globule accumulation under full sunlight while substantially increasing chloroplast proliferation under shading stress.

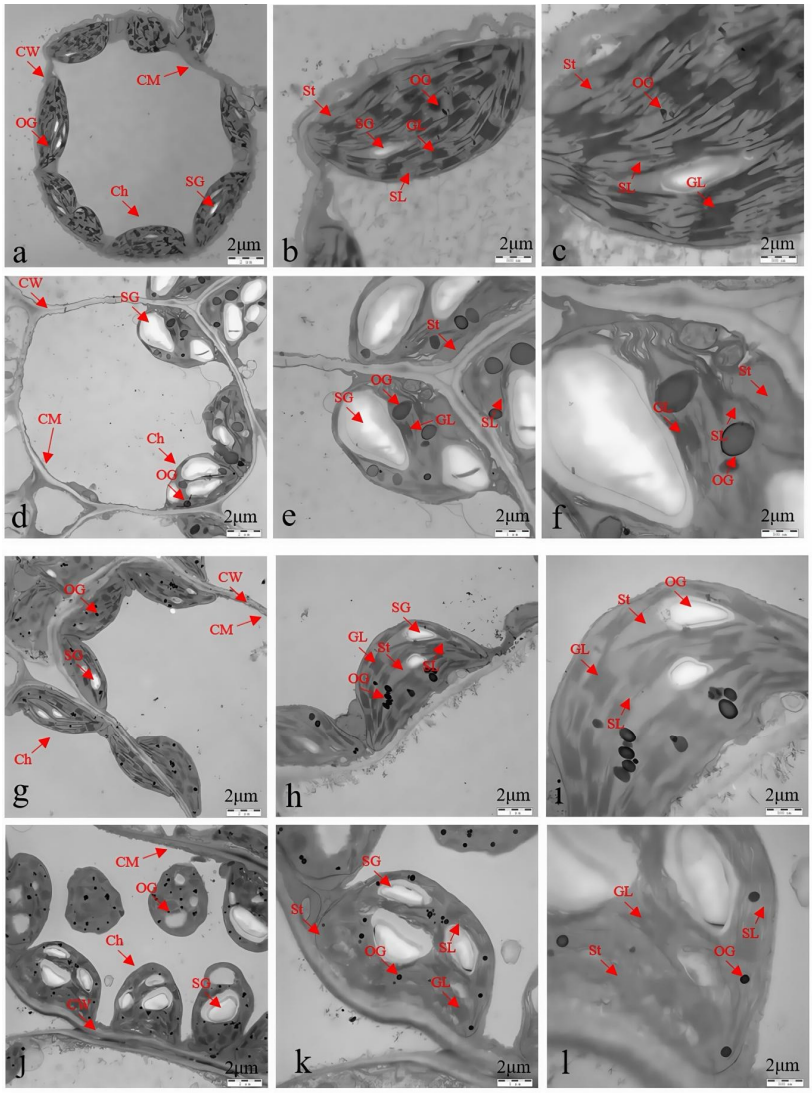

Figure 9: Pro-Ca induces light-dependent optimization of chloroplast ultrastructure under different light regimes. (**a**–**c**) control under full light; (**d**–**f**) Pro-Ca-tuned treatment under full light; (**g**–**i**) control under heavy shade; (**j**–**l**) Pro-Ca-tuned treatment under heavy shade. CM: cell membrane; CW: cell wall; Ch: chloroplasts; GL: grana lamellae, SL: stromal lamellae; OG: osmiophilic granules; SG: starch granules; St: chloroplast stroma.

Transmission electron micrographs show that 600 mg·L^{-1} Pro-Ca treatment maintained intact mesophyll cell structure with chloroplasts arranged along cell walls in both light environments. Under full sunlight, Pro-Ca promoted starch grain and osmiophilic globule accumulation alongside increased stromal

lamellae. Under heavy shading, it significantly increased chloroplast number, enhanced grana stacking tightness, and enlarged starch grains, indicating light-specific strategies for improving photosynthetic efficiency under stress.

4 Discussion

4.1 Pro-Ca-Mediated Modulation of Plant Architecture

Plants show an adaptive response under shaded conditions: elongated stems to escape from shade or capture more light. Excessive elongation leads to thin, lodging-prone stems that have compromised their survival and ornament value [17]. Pro-Ca decreased plant height in *H. sibirica* plants grown under both full sunlight and heavy shade conditions. Consistent with the dwarfing effect of Pro-Ca observed in rice (Oryza sativa) [18], this gibberellin biosynthesis inhibitor shortens basal internodes, increase stem diameter and wall thickness, optimize plant type structure, and enhance lodging resistance, reducing the decline of photosynthetic function and ensure efficient carbon assimilation [19]. In addition to regulating stem length, Pro-Ca also promotes stem growth by enhancing lignification and increasing mechanical resistance, as evidenced by research on *O. sativa* [18] and *Setaria italica* [20]. A series of coordinated mechanisms link the reduction in *GA* gene expression with stem thickening. This involves a complex interplay of hormonal cross-talk with auxins and brassinosteroids [21] and transcriptional regulation that redirects growth towards structural development [22], as well as a shift in carbon allocation for more reliance on structural carbohydrates such as cellulose [23]. Overall, these systems indicate that Pro-Ca plays a multi-functional regulatory role in plant morphology under stress conditions. This corresponding transformation alleviates the plant's adaptive strategy from shade avoidance to shade tolerance, reallocating resources for stem reinforcement. In a shaded environment, it helps reduce carbon loss by improving the plant's adaptive capacity for survival and enhancing its ornamental features. Therefore, Pro-Ca functions to regulate plant adaptation and promote the emergence of small and dense plants in shady spots, a morphology that is advantageous for long-term persistence.

4.2 Pro-Ca-Mediated Enhancement of Root System Development

Apart from the aboveground architectural modifications discussed above, Pro-Ca also substantially influences underground root system growth. This directly affects the plant's ability to tolerate stress. Based on the above research, Pro-Ca increased the root weight, surface area, volume and diameter of *H. sibirica* in both strong light environments and darkened conditions. Shading stress reduces photosynthetic products and restricts root development through a carbon limitation mechanism. Pro-Ca promotes a more robust root system with longer and wider roots. This allows plants under low-light conditions to more efficiently acquire nutrients like nitrogen and phosphorus, compensating for limited leaf growth. Simultaneously, Pro-Ca also alters the shade-induced prioritization of stem elongation. On the contrary, Pro-Ca allocates more carbon to establish a balanced stem to root ratio, thereby avoiding energy consumption [24]. This finding is consistent with reports on the alleviation of waterlogging stress in *Ipomoea batatas* by 6-benzyladenine [25] and the enhancement of salt tolerance in *Populus euphratica* by paclobutrazol [26]. Based on this model, the application of Pro-Ca also makes roots more tolerant of shade stress.

4.3 Pro-Ca-Mediated Improvement of Floral Characteristics

Building upon the root and architectural modifications, Pro-Ca also enhances reproductive growth in the shade, where photosynthetic limitation often inhibits flowering. Pro-Ca increased flower number and intensified flower colour in *H. sibirica* under heavy shade, although this effect was weakened under

direct light. Previous studies confirmed that Pro-Ca can facilitate floral formation in ornamental species such as *Chrysanthemum morifolium* [27] and *Fragaria* × *ananassa* [28], but has no such effect on *Paeonia lactiflora* [29]. The synergistic improvement in photosynthetic efficiency and root growth ensures the continuous provision of carbon and energy resources for floral organ development. Regarding the intensification of flower colour, Pro-Ca reduces shade damage to anthocyanins and carotenoids [29].

4.4 Pro-Ca-Mediated Improvement of Photosynthetic Physiology and Structural Basis

As the main organs for light interception and photosynthetic assimilation, leaves undergo adjustments to perform essential functions in the shade via multiple regulatory pathways. At the pigment level, Pro-Ca enhanced the chlorophyll a/b ratio and total chlorophyll content. These changes help mitigate photodamage [30], maintain photosystem integrity [31], and promote efficient utilization of high light [21]. In terms of leaf architecture, Pro-Ca increased leaf thickness, improved the packing structure of palisade and spongy tissues, induced stomata formation, and promoted xylem growth, thereby enhancing gas exchange and nutrient absorption [32]. The optimization of sponge tissue and palisade tissue morphology increased the leaf light receiving area, while the improvement of stomatal traits increased the supply of CO_2, both of which contributed to a significant increase in net photosynthetic rate under heavy shading conditions. It also optimized chloroplast ultrastructure by tightening grana assembly and increasing stroma lamella formation. The intact structure of the basal layer and the enrichment of starch granules improve the efficiency of light energy capture and stabilize the photosynthetic mechanism, which is the key to the increase of F_v/F_m under shading conditions. It can effectively reduce light energy loss and sustain photosynthetic metabolism. These modifications enhance light-harvesting capacity and bolster protection against oxidative damage through the antioxidant system [31,33]. Consequently, carbon assimilation is no longer the primary limiting factor. In summary, the changes in the microstructure of leaves and chloroplasts enhance gas exchange and photochemical activity, forming a complete mechanism of action for calcium cyclamate to promote the transformation of *H. sibirica* from shade avoidance to shade tolerance. Pro-Ca can stimulate photosynthetic activity and provide the raw materials needed to overcome shade-induced limitations, an effect similar to the cytokinin-mediated stimulation of chlorophyll synthesis [34–36].

4.5 Comprehensive Regulatory Effects and Practical Implications of Pro-Ca

Using membership function analysis to examine the systemic regulation of *H. sibirica* by Pro-Ca, the optimal concentration was determined to be 600 $mg{\cdot}L^{-1}$ under deep shade conditions [33,37,38]. This concentration-dependent response reflects differences in absorption, metabolism, and the activation thresholds for various pathways [19]. In practice, Pro-Ca can improve the shade tolerance of ornamental plants by enhancing multiple factors, including stem strength, root system maturation, leaf photosynthetic capacity, and reproductive activity. For example, by helping plants retain vibrant color and compact form even under strong sun, Pro-Ca ensures they remain aesthetically pleasing during periods of low light [27]. This makes it a valuable tool for cultivation in partially shaded environments.

5 Conclusions

This study employed *H. sibirica* as experimental material and investigated its responses under different light conditions combined with Pro-Ca treatments. A comprehensive analysis was conducted on growth characteristics, chlorophyll-related parameters, and photosynthetic performance. The results demonstrated that under severe shade conditions, foliar application of 600 $mg{\cdot}L^{-1}$ Pro-Ca most effectively promoted growth regulation in *H. sibirica*. The underlying mechanism involves optimized resource allocation, protection of

photosynthetic structure and function, and coordinated "shade avoidance" and "shade tolerance" adaptation strategies, ultimately maintaining or even enhancing plant vigor and ornamental value in shaded environments. This research not only provides theoretical guidance for the cultivation of *H. sibirica*, but also offers practical insights for improving nursery management and landscape design in shaded conditions, while advancing our understanding of the multifaceted roles of plant growth regulators in mitigating abiotic stress. Subsequently, a more detailed gradient test will be conducted within a narrower range of 300–900 $mg{\cdot}L^{-1}$ to further determine the precise optimal dosage, thereby providing guidance for more refined applications.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
CTK	Cytokinins
CAT	Catalase
Chla	Chlorophyll a
Chlb	Chlorophyll b
Ci	CO_2 concentration
Fm	Maximum fluorescence
Fo	Minimum fluorescence
Fv	Variable fluorescence
Gs	Stomatal conductance
LSD	Least significant difference
MDA	Malondialdehyde
Pro-Ca	Prohexadione-calcium
PGRs	Plant growth regulators
POD	Peroxidase
Pn	Net photosynthetic rate
SOD	Superoxide dismutase
SA	Salicylic acid
Tr	Transpiration rate

Appendix A

Table A1: Summary of two-way ANOVA results for all measured morphological, photosynthetic and chlorophyll fluorescence parameters of *H. sibirica*.

Measured Parameters	Light (F, *p*)	Pro-Ca Concentration (F, *p*)	Light × Pro-Ca Interaction (F, *p*)
Plant height	42.35, $p < 0.001^{**}$	38.75, $p = 0.02^{*}$	15.18, $p < 0.001^{**}$
Leaf length	64.86, $p < 0.001^{**}$	2.83, $p = 0.042^{*}$	19.00, $p < 0.001^{**}$
Leaf width	43.08, $p < 0.001^{**}$	5.31, $p = 0.004^{**}$	16.69, $p < 0.001^{**}$
Leaf area	38.25, $p < 0.001^{**}$	10.67, $p < 0.001^{**}$	12.29, $p < 0.001^{**}$
Leaf color L*	0.63, $p = 0.043^{*}$	1.17, $p = 0.035^{*}$	0.087, $p = 0.048^{*}$
Leaf color a*	1.41, $p = 0.24$	1.98, $p = 0.013^{*}$	0.033, $p = 0.029^{*}$
Leaf color b*	2.07, $p = 0.165$	3.01, $p = 0.043^{*}$	0.068, $p = 0.049^{*}$
Floret number	35.73, $p < 0.001^{**}$	5.05, $p = 0.006^{**}$	10.33, $p < 0.001^{**}$
Petal color L*	0.11, $p = 0.073$	0.43, $p = 0.048^{*}$	0.57, $p = 0.068$
Petal color a*	0.57, $p = 0.45$	0.88, $p = 0.043^{*}$	0.42, $p = 0.047^{*}$
Petal color b*	1.61, $p = 0.021^{*}$	1.40, $p = 0.026^{*}$	0.43, $p = 0.047^{*}$
Petal color c*	0.83, $p = 0.37$	1.01, $p = 0.042^{*}$	0.40, $p = 0.051$
Root length	28.64, $p < 0.001^{**}$	8.36, $p < 0.001^{**}$	10.32, $p < 0.001^{**}$
Root diameter	25.02, $p < 0.001^{**}$	7.44, $p < 0.001^{**}$	8.48, $p = 0.003^{**}$
Root area	30.93, $p = 0.01^{*}$	9.11, $p < 0.001^{**}$	11.43, $p < 0.001^{**}$
Root volume	27.45, $p = 0.048^{*}$	6.59, $p < 0.001^{**}$	9.10, $p < 0.001^{**}$
Chla	12.02, $p = 0.017^{**}$	16.46, $p = 0.039^{**}$	8.34, $p < 0.001^{**}$
Chlb	18.30, $p = 0.01^{*}$	7.77, $p = 0.055$	7.39, $p < 0.001^{**}$
Chla+b	20.18, $p = 0.015^{*}$	11.76, $p = 0.017^{*}$	7.34, $p = 0.042^{**}$
Chla/b	15.01, $p = 0.007^{**}$	9.63, $p < 0.001^{**}$	6.67, $p < 0.001^{**}$
C_i	1.00, $p = 0.99$	12.51, $p < 0.001^{**}$	0.00, $p < 0.001^{**}$
G_s	25.25, $p = 0.49^{*}$	22.63, $p < 0.001^{**}$	18.007, $p < 0.001^{**}$
T_r	28.73, $p < 0.001^{**}$	34.25, $p < 0.001^{**}$	20.08, $p < 0.001^{**}$
P_n	32.17, $p < 0.001^{**}$	67.75, $p < 0.001^{**}$	25.15, $p < 0.001^{**}$
F_o	1.008, $p = 0.032^{*}$	0.28, $p = 0.018^{*}$	0.003, $p = 0.048^{*}$
F_v	22.07, $p < 0.001^{**}$	10.27, $p < 0.001^{**}$	15.06, $p < 0.001^{**}$
F_m	18.09, $p < 0.001^{**}$	8.27, $p < 0.001^{**}$	12.06, $p < 0.001^{**}$
F_v/F_o	15.00, $p < 0.001^{**}$	7.075, $p < 0.001^{**}$	10.00, $p < 0.001^{**}$
F_v/F_m	12.00, $p < 0.001^{**}$	6.016, $p < 0.001^{**}$	8.00, $p < 0.001^{**}$

Note: $^{*}p < 0.05$, $^{**}p < 0.01$, indicating significant differences.

References

1. Cheng K, Lu Z, Wang Y, Wu J. *Hesperis oreophila* f. bicolor, a new form of *Hesperis* from Hebei. Hebei J For Orchard Res. 2009;24(1):53–4. (In Chinese). [CrossRef].
2. Li HF, Dong MY, Li YH. Investigation and analysis on the habitat of wild *Hesperis oreophila* in Hebei province. For Ecol Sci. 2024;39(4):422–31. (In Chinese). [CrossRef].

3. Dong MY, Wu M, Tong GJ, Wang JX, Chen XN, Zhao XY, et al. Studies on flowering characteristics and breeding system of *Hesperis Oreophila*. Acta Hortic Sin. 2023;50(3):607–19. (In Chinese). [CrossRef].
4. Wang G, Ryu KH, Dinneny A, Carlson J, Goodstein D, Lee J, et al. Diversification of gene expression across extremophytes and stress-sensitive species in the Brassicaceae. bioRxiv. 2024. [CrossRef].
5. Nguyen NH, Sng BJR, Yeo HC, Jang IC. Comparative phenotypic and transcriptomic analyses unravel conserved and distinct mechanisms underlying shade avoidance syndrome in Brassicaceae vegetables. BMC Genom. 2021;22(1):760. [CrossRef].
6. Kim YH, Khan AL, Hamayun M, Kim JT, Lee JH, Hwang IC, et al. Effects of prohexadione calcium on growth and gibberellins contents of *Chrysanthemum morifolium* R. cv Monalisa White. Sci Hortic. 2010;123(3):423–7. [CrossRef].
7. Lal M, Mir MM, Iqbal U, Kumar A. Influence of prohexadione-calcium and paclobutrazol on growth, yield and mineral content of pear cv. Clapp's favourite. Int J Chem Stud. 2020;8(1):256–9. [CrossRef].
8. Wan YL. Effects of CPPU and prohexadione calcium on shoot growth and flower bud formation in Malus domestica [dissertation]. Taian, China: Shandong Agricultural University; 2024. [CrossRef].
9. Sun YY, Xie H, Ai XM, Meng CD, Wan M. Physiological effects of exogenous auxin on regulating the leaf growth and delaying senescence of water lily. J Southwest For Univ Nat Sci. 2025;45(3):56–64. (In Chinese).
10. Meng Y, Han CY, Nie G, Wang YF. Effects of exogenous cytokinin on the physiological characteristics of perennial ryegrass in the recovery period under flooding stress. Heilongjiang Anim Sci Vet Med. 2023;(13):103–10. (In Chinese). [CrossRef].
11. Wang M, Cao S, Peng J. Effects of gibberellin, salicylic acid and ethephon on flowering and physiological characteristics of *Hydrangea paniculata*. Acta Agric Univ Jiangxiensis. 2023;45(3):605–18.
12. Li HS. Principles and techniques of plant physiological and biochemical experiments. 2nd ed. Beijing, China: Higher Education Press; 2000.
13. Wei Y, Liang M, Ling Y. Effects of bio-fertilizer on maize growth and yield. Trop Agric Eng. 2025;49(2):125–8. (In Chinese).
14. Li J. Mechanism of brassinosteroid on growth and quality of chinese chives [dissertation]. Lanzhou, China: Gansu Agricultural University; 2025. [CrossRef].
15. Xu XL, Cai O, Zhao WQ, Lü Z, Yao WJ, Li L, et al. Changes in cytology and photosynthetic pigments during the formation of colored leaves of *Sasaella* glabra'Albostriata'. Sci Silvae Sin. 2023;59(8):40–51. (In Chinese). [CrossRef].
16. Zhang Y, Yuan Y, Xu S, Li Z, Cui Z, Zhan L, et al. Shading-induced canopy cooling alleviates waterlogging damage during flowering by disrupting heat synergism in field-grown cotton. Field Crops Res. 2025;334:110166. [CrossRef].
17. Franklin KA. Shade avoidance. New Phytol. 2008;179(4):930–44. [CrossRef].
18. Wei M, Jin H, Zhang S. Evaluation of 5% prohexadione-calcium effervescent tablets on *Oryza sativa* growth regulation and lodging prevention. Pestic Sci Adm. 2011;32(9):55–8. (In Chinese).
19. Rademacher W. GROWTH RETARDANTS: effects on gibberellin biosynthesis and other metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol. 2000;51:501–31. [CrossRef].
20. Zhang YX. Effects of prohexadione calcium on plant height and lodging resistance in *Setaria italica* [dissertation]. Taigu, China: Shanxi Agricultural University; 2022. [CrossRef].
21. Rademacher W. Prohexadione-Ca in fruit trees: modes of action of a multifunctional bioregulator. HortScience. 2004;39(4):851D. [CrossRef].
22. Hedden P, Phillips AL. Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci. 2000;5(12):523–30. [CrossRef].
23. Boerjan W, Ralph J, Baucher M. Lignin biosynthesis. Annu Rev Plant Biol. 2003;54:519–46. [CrossRef].
24. Li SS. Effects of prohexadione calcium and CPPU on vegetative growth and flower bud formation in Malus domestica [dissertation]. Taian, China: Shandong Agricultural University; 2020. [CrossRef].
25. Tan C, Zhang H, Yuan J, Zhang YC, Zhu GP, Wang JD, et al. Mitigation effect of different plant growth regulators on waterlogging of sweet potato at branching-tubering stage. Jiangsu Agric Sci. 2022;50(23):81–9. (In Chinese). [CrossRef].

26. Zhang M. Alleviating effects of three plant growth regulators on salt stress in *Populus euphratica* [dissertation]. Alar, China: Tarim University; 2022. [CrossRef].
27. Qu HY, Wang J, Zhu LX. Effects of prohexadione calcium on endogenous hormones and plant architecture in *Chrysanthemum morifolium*. J Shandong For Sci Technol. 2022;52(5):48–53. (In Chinese).
28. Cheng Y. Preliminary study on regulation of prohexadione calcium on growth, development, yield and quality of *Fragaria* × *ananassa* [dissertation]. Yangzhou, China: Yangzhou University; 2022. [CrossRef].
29. Tang YH. Physiological mechanisms of prohexadione calcium and daminozide on flower color of Paeonia lactiflora [dissertation]. Yangzhou, China: Yangzhou University; 2018.
30. Yu ML, Huang L, Zheng DF, Feng NJ, Mou BM, Liu ML. Effects of exogenous prohexadione calcium on growth and physiological characteristics of Glycine max seedlings under saline-alkali stress. Chin J Ecol. 2022;41(4):683–92. (In Chinese). [CrossRef].
31. Shao C, Jiao H, Chen J, Zhang C, Liu J, Chen J, et al. Carbon and nitrogen metabolism are jointly regulated during shading in roots and leaves of *Camellia Sinensis*. Front Plant Sci. 2022;13:894840. [CrossRef].
32. Meng XQ, Zou ZF, Qu CH, Wang SL, Miao YG. Regulatory effects of different plant growth regulators on shoot growth and leaf development in Malus domestica. J Zhejiang Agric Sci. 2023;64(6):1515–8. (In Chinese). [CrossRef].
33. Lin XY, Wang N, Chen T, Song YL, Lu YD, Dong ZX. Effects of three plant growth regulators on shade tolerance of *Zoysia japonica*. Acta Prataculturae Sin. 2025;34(3):224–32. (In Chinese). [CrossRef].
34. Pan MJ, Yin YQ, Shen FK, Luo BX, Tang XL, He HH, et al. Effects of prohexadione calcium on physiological index of tobacco seedlings during cold stress. Southwest China J Agric Sci. 2016;29(2):288–93. [CrossRef].
35. Stoyanova-Koleva D, Stefanova M, Zhiponova M, Kapchina-Toteva V. Effect of N^6-benzyladenine and indole-3-butyric acid on photosynthetic apparatus of *Orthosiphon stamineus* plants grown *in vitro*. Biol Plant. 2012;56(4):607–12. [CrossRef].
36. Zhao JX, Zheng DF, Feng NJ, Liu B. Effects of plant growth regulators on microstructure and photosynthetic characteristics in stems and petioles of Glycine max. Xinjiang Agric Sci. 2008;(5):814–9.
37. Liu BW. Regulatory effects of paclobutrazol on shade tolerance in *Festuca arundinacea* [dissertation]. Yangling, China: Northwest A&F University; 2023. [CrossRef].
38. Zhou Q, Guo ZQ, Xu C, Xu HH, Zhang XS, Yang AY, et al. Effects of plant growth regulators on grafted bitter gourd seedlings growth. China Veg. 2024;(5):115–22. (In Chinese). [CrossRef].