

The Effect of Foliar Application of Melatonin on the Growth and Digital Phenotypic Traits of China Rose (*Hibiscus rosa-sinensis* L.) Seedlings Under Drought Stress

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Abstract

This study was conducted in the saran-covered canopy belonging to the Department of Horticulture and Landscape Architecture, College of Agriculture and Marshes, University of Dhi Qar, to evaluate the efficacy of foliar melatonin application in enhancing the drought tolerance of China rose (*Hibiscus rosa-sinensis* L.) seedlings subjected to water stress induced by prolonged irrigation intervals. The experiment was implemented as a two-factor completely randomized block design (RCBD) with three replicates. The first factor comprised three irrigation regimes: daily irrigation (control), irrigation every four days (moderate drought), and weekly irrigation (severe drought). The second factor consisted of three exogenous melatonin concentrations: 0, 50, and 100 mg L⁻¹. Quantitative and qualitative data were statistically analyzed using the Least Significant Difference (L.S.D.) test at a significance threshold of P≤0.05.

The results demonstrated that water deficit induced by expanding irrigation intervals caused a statistically significant decline across all evaluated vegetative and physiological traits. Specifically, plant height, leaf count, total leaf area, stem diameter, and total chlorophyll content systematically decreased with rising drought severity. Conversely, leaf chlorosis and tissue damage percentages escalated significantly under water stress, while the healthy green leaf area percentage dropped sharply. The weekly irrigation treatment recorded the lowest structural and biochemical values; total chlorophyll content dropped by over 46% compared to the daily irrigated control, while chlorosis and cellular damage reached their peak levels.

In contrast, exogenous foliar spray with melatonin achieved significant and substantial ameliorations in all studied parameters, particularly at the maximum concentration of 100 mg L⁻¹. This treatment markedly increased plant height, leaf number, functional leaf area, stem diameter, and chlorophyll synthesis, while successfully minimizing chlorosis and tissue necrosis percentages. Furthermore, the dual interaction between irrigation intervals and melatonin treatments demonstrated that daily irrigation combined with 100 mg L⁻¹ melatonin optimized overall seedling performance. Crucially, melatonin application effectively mitigated the deleterious impacts of water deficit even under the most severe weekly irrigation regime. The study concludes that melatonin exerts a vital protective, signaling, and physiological role in bolstering the drought tolerance of *Hibiscus rosa-sinensis* L. by preserving photosystem integrity, maintaining cellular turgor, improving growth efficiency, and reducing oxidative cellular damage caused by severe water deficit

Keywords: *Hibiscus*, *Melatonin*, *Drought Stress*, *Digital Phenotyping*, *HSV Color Space*.

I. Introduction

The China rose (*Hibiscus rosa-sinensis* L.) represents one of the most prominent ornamental shrubs extensively employed in urban landscaping, public parks, private gardens, and highway greenbelts across



arid and semi-arid zones due to its high aesthetic value and distinct architectural features. Belonging to the Malvaceae family, this evergreen shrub is characterized by a remarkable diversity in floral morphology and coloration, alongside dense canopy development (Trivellini *et al.*, 2023). Beyond their ornamental merits, these shrubs improve urban microclimates by moderating temperatures, reducing gaseous pollutants, and serving as vital bio-indicators of environmental health in urban ecosystems (Mezghani *et al.*, 2026).

Morphologically, *H. rosa-sinensis* is an evergreen shrub attaining heights of 2.5–5 meters and canopy widths of 1.5–3 meters, possessing erect aerial stems, alternate deep-green leaves with serrated margins, and a branched taproot system. Its solitary, elegant flowers emerge from the leaf axils, displaying a prominent, elongated staminal column and pistil extending well beyond the five major petals. Flower color polymorphisms range from red (which is the predominant form in Iraq) to orange, yellow, and white. The species exhibits near-continuous flowering throughout the year and tolerates heavy pruning and topiary shaping. Propagation is conventionally achieved via stem cuttings, air layering, or grafting during the spring. Although *H. rosa-sinensis* exhibits a reasonable capacity for environmental acclimatization, exposure to severe abiotic stresses—particularly drought—drastically reduces its landscape performance, vegetative vigor, and reproductive capacity (Mezghani *et al.*, 2026).

Drought, or water deficit, stands as a premier abiotic constraint restricting plant development. It induces intricate physiological, biochemical, and anatomical disruptions that directly impair vegetative growth and vital metabolic pathways (Lu *et al.*, 2023). Water shortages provoke an immediate drop in intracellular turgor pressure, thereby hindering cell division and cell elongation rates, culminating in plant stunting, diminished leaf production, and restricted lateral branching (Gulia *et al.*, 2025). In urban environments, these adverse effects are frequently exacerbated by concurrent anthropogenic stressors, including heavy metal toxicity and exhaust emissions, which threaten the long-term sustainability of urban flora (Mezghani *et al.*, 2026). A decline in soil moisture levels restricts root water and nutrient absorption, which immediately harms the hydraulic status of plant tissues. Persistent drought causes the loss of cellular turgor pressure, suppressing dry matter accumulation and structural development of stems, branches, and leaves, while reducing floral bud differentiation (Gulia *et al.*, 2025).

Photosynthesis is critically sensitive to water deficits. To prevent excessive transpirational water loss under moisture scarcity, plants trigger stomatal closure, which concomitantly restricts intercellular carbon dioxide (CO₂) diffusion, thereby suppressing the Calvin cycle and carbohydrate synthesis (Li *et al.*, 2024). This suppression leads to a shortage of metabolic energy required for structural development. Concurrently, drought promotes the overproduction of Reactive Oxygen Species (ROS), inducing lipid peroxidation of thylakoid membranes and the subsequent degradation of photosynthetic pigments (chlorophylls), which severely undermines photosystem efficiency (Gul *et al.*, 2022). Furthermore, water stress induces severe hormonal imbalances within the plant; it triggers an abrupt synthesis of abscisic acid (ABA) to drive stomatal closure, while suppressing growth-promoting phytohormones such as auxins and cytokinins, ultimately accelerating premature leaf senescence and abscission (Trivellini *et al.*, 2023).

To counteract these adverse impacts, contemporary botanical research has focused on exogenous physiological elicitors capable of reinforcing plant defense frameworks. Among these, melatonin (N-acetyl-5-methoxytryptamine), an indoleamine compound biosynthesized from tryptophan, has emerged as a potent regulator. Melatonin shares structural and biosynthetic pathways with indole-3-acetic acid (IAA), allowing it to directly modulate plant growth, rooting, and organogenesis (Shaheen *et al.*, 2024). It has demonstrated great efficacy in boosting crop resistance against drought, salinity, and oxidative stress by regulating key metabolic pathways and stimulating the plant's internal defense machinery (Asif *et al.*, 2019). Acting as a first-line, amphiphilic antioxidant, melatonin directly scavenges ROS and transcriptionally upregulates endogenous enzymatic defense systems, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), thereby shielding cellular membranes and chloroplast architectures from oxidative degradation (Shreya *et al.*, 2022). Additionally, exogenous melatonin application regulates stomatal conductance, delays dark-induced leaf senescence, optimizes nutrient assimilation, maintains osmotic

homeostasis, and prevents chlorophyll breakdown under water-limited conditions (Luo *et al.*, 2024; Shaheen *et al.*, 2024).

Given the scarcity of comprehensive studies evaluating the precise physiological responses of *H. rosa-sinensis* to drought and the potential ameliorative role of melatonin, this study was designed to investigate the individual and interactive impacts of varied irrigation frequencies and foliar melatonin doses on the vegetative, physiological, and digital phenotypic attributes of China rose seedlings under simulated water-deficit stress..

II. Materials and Methods

A. Experimental Site and Plant Material

The investigation was conducted within the saran-screen greenhouse facility at the Department of Horticulture and Landscape Architecture, College of Agriculture and Marshes, University of Dhi Qar, during the 2025–2026 agricultural season. The experimental material consisted of uniform, healthy 1.5-year-old China rose (*Hibiscus rosa-sinensis* L.) seedlings evaluated to determine their response to varied irrigation intervals and foliar melatonin treatments.

B. Experimental Treatments and Design

The experiment was implemented as a two-factor completely randomized block design (RCBD) with three replications ($3 \times 3 \times 3 = 27$ experimental units).

Factor 1: Drought Stress (D): Placed in the main plots, comprising three irrigation intervals:

1. D1: Continuous daily irrigation (control).
2. D2: Irrigation every 4 days (moderate drought).
3. D3: Irrigation every 7 days (severe drought).

Factor 2: Melatonin Concentrations (M): Comprising three foliar spray doses:

1. M1: 0 mg L⁻¹ (solvent control).
2. M2: 50 mg L⁻¹.
3. M3: 100 mg L⁻¹.

B.1. Preparation of Melatonin Solutions

To prepare the 50 mg L⁻¹ solution, 0.05 g of pure melatonin powder was completely dissolved in 2–3 mL of absolute ethanol (100%), and the volume was adjusted to 1 L using deionized distilled water under constant magnetic stirring to ensure homogeneity. For the 100 mg L⁻¹ solution, 0.1 g of melatonin powder was similarly dissolved in 2–3 mL of absolute ethanol and diluted to 1 L with distilled water. The 0 mg L⁻¹ control treatment was prepared by adding 2–3 mL of absolute ethanol to 1 L of distilled water without melatonin to eliminate potential solvent artifacts (solvent control). A non-ionic surfactant, Tween 20 (0.05%v/v), was integrated into all formulations to minimize droplet surface tension and maximize foliar wetting efficiency..

B.2. Timeline and Crop Management

- October 1, 2025: Seedlings were carefully transplanted into larger plastic pots filled with a uniform substrate mixture of local soil and peat moss (2:1v/v). Following transplanting, pots were irrigated to field capacity and maintained under greenhouse conditions for 24 hours to stabilize.
- October 2025 – March 2026: Seedlings were arranged randomly according to the RCBD layout and grown under standard cultural practices to achieve full acclimatization and establishment within the growth medium.
- April 1, 2026 – May 1, 2026: The irrigation treatments (D1, D2, D3) were strictly enforced to coincide with rising ambient temperatures, maximizing natural evaporative demand.



- Foliar Spray Schedule: Melatonin treatments were applied via a calibrated manual pressure sprayer until absolute foliar runoff. Sprays were administered at 10-day intervals across three sequential dates:
 - First Application: April 10, 2026
 - Second Application: April 20, 2026
 - Third Application: April 30, 2026
- Irrigation volumes were strictly tailored to replenish moisture levels up to field capacity, and all pots featured basal drainage perforations to prevent waterlogging and salt accumulation.

B.3 Sampling and Tissue Preparation

Destructive and non-destructive measurements were conducted at the termination of the trial on May 1, 2026, capturing the cumulative response to the treatments. Leaf samples intended for biochemical extraction were harvested during early morning hours (06:00–08:00 AM) to preserve cellular turgidity and prevent biochemical degradation. Leaves were gently rinsed with deionized water to eliminate dust and surface residues prior to chlorophyll extraction.

C. Quantitative and Qualitative Traits

1. Plant Height (cm): Plant height was measured linearly from the soil-root crown junction to the apical meristem tip using a calibrated metric tape.

2. Stem Diameter (mm): Stem diameter was recorded utilizing a high-precision digital vernier caliper at a fixed point 2 cm above the soil substrate level to ensure accuracy in tracking radial stem development.

3. Leaf Count (leaves plant⁻¹): The total number of fully expanded, functional leaves per seedling was manually counted. Emergent, unexpanded apical leaves and fully senesced leaves were excluded.

4. Total Leaf Area (cm²): Leaf area was determined via digital image processing using ImageJ software (v1.54) following a modified protocol based on Sadik *et al.* (2011). Two fully expanded, non-damaged leaves were selected per plant. Individual leaves were placed flat on a non-reflective, matte-white background panel alongside a standard metric calibration scale. Images were captured using a high-resolution mobile camera positioned at a fixed perpendicular distance (40 cm) under uniform diffuse illumination. The images were loaded into ImageJ, calibrated to spatial dimensions (pixels mm⁻¹), thresholded, and the total surface area was computed automatically in square centimeters (cm²).

5. Total Chlorophyll Content (mg 100g⁻¹ Fresh Weight): Total chlorophyll extraction and quantification were performed according to the spectrophotometric method of Goodwin (1976). Fresh leaf tissue (0.5 g) was finely homogenized in a porcelain mortar with 10 mL of 80% aqueous acetone. The homogenate was transferred to light-shielded centrifuge tubes wrapped in aluminum foil to prevent photo-oxidation and centrifuged at 5000 rpm for 15 minutes. The supernatant was decanted, and optical densities were recorded at wavelengths of 663 nm (A₆₆₃) and 645 nm (A₆₄₅) using a UV-1700 spectrophotometer. Total chlorophyll concentration was computed via the following empirical formula:

$$\text{Total Chlorophyll (mg 100g}^{-1}\text{)} = \frac{[20.2 \times A_{645} + 8.2 \times A_{663}] \times V}{W \times 1000} \times 100$$

Where:

- A: Optical density at the specified wavelength.
- V: Final volume of the acetone extract (10 mL).
- W: Fresh leaf weight (0.5 g).

6. Digital Phenotyping for Leaf Chlorosis, Necrosis, and Healthy Green Areas (%)

To quantify leaf health non-destructively and eliminate human bias, a digital phenotyping methodology was integrated within ImageJ using Hue-Saturation-Value (HSV) color-space segmentation. Digital images acquired under identical lighting and spatial configurations (Section 4) were processed as follows:



1. Color Space Conversion: RGB images were converted into the 8-bit HSV color space using the Image > Type > HSV Stack function to decouple color information (Hue) from brightness variation.
2. Color Thresholding and Segmentation: The Image > Adjust > Color Threshold menu was used to isolate distinct pixel groups based on predefined Hue angle ranges:
 - **Healthy Green Tissue Area (A_{green}):** Segmented within the Hue range of 60° to 150°.
 - **Chlorotic/Yellow Tissue Area (A_{yellow}):** Segmented within the Hue range of 35° to 59°.
 - **Necrotic/Damaged Tissue Area (A_{damage}):** Segmented within the Hue range of 0° to 34°, alongside low brightness values (Value<50) to capture dark brown desiccated regions.
3. Pixel Area Computation: The Analyze > Measure tool computed the total pixel count for each thresholded phase alongside the absolute total leaf area (A_{total}). The respective tissue percentages were calculated using the following mathematical models:
 - 1- **Healthy Green Area Percentage (%)** = $\left(\frac{A_{green}}{A_{total}}\right) \times 100$
 - 2- **Chlorosis Percentage (%)** = $\left(\frac{A_{yellow}}{A_{total}}\right) \times 100$
 - 3- **Tissue Necrosis/ damage Percentage (%)** = $\left(\frac{A_{damage}}{A_{total}}\right) \times 100$

D. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using GenStat software (Release 11.1, 2008). Treatment means were statistically resolved and separated utilizing the Least Significant Difference (L.S.D.) test at a verification probability level of $\alpha=0.05$ according to Al-Rawi and Khalaf Allah (2000).

III. Results and Discussion

3.1 Plant Height (cm)

Statistical analysis revealed that both primary experimental factors and their binary interactions exerted highly significant impacts on plant height ($P<0.05$). Extending the irrigation intervals induced a severe, linear restriction on seedling elongation (Figure 1A). The grand mean for plant height decreased significantly from 39.569 cm under daily watering (D1) to 28.271 cm under severe weekly drought (D3). Conversely, exogenous melatonin application stimulated stem elongation (Figure 1B); the mean height increased from 31.662 cm in the control group (M1) to 35.233 cm at the maximum concentration of 100 mg L⁻¹ (M3).

The dual interaction analysis highlighted distinct variations (Figure 1C). The combination of daily irrigation and 100 mg L⁻¹ melatonin (D1 + M3) produced the highest plant height (40.980 cm), whereas the severe drought treatment without melatonin (D3 + M1) resulted in the absolute minimum height (26.460 cm). Notably, under severe water stress conditions (D3), foliar spraying with 100 mg L⁻¹ melatonin (D3 + M3) successfully mitigated the growth restriction, raising plant height significantly to 30.130 cm.

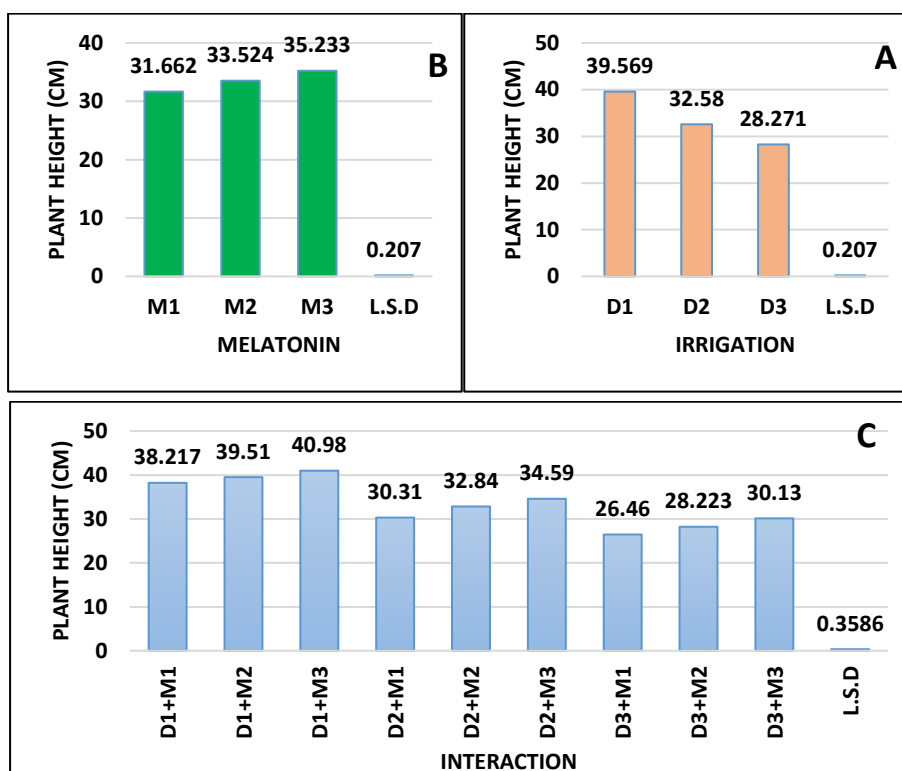


Figure (1) The effect of spraying melatonin on rose seedlings under the influence of drought stress in the trait of Plant Height (cm)

2. Leaf Count (leaves plant⁻¹)

Drought stress and melatonin treatments significantly modified leaf production per seedling ($P \leq 0.05$). Severe water deficit provoked a major decline in leaf retention and emergence (Figure 2A), reducing the mean leaf count from 21.78 leaves plant⁻¹ under daily irrigation (D1) to 10.78 leaves plant⁻¹ under weekly irrigation (D3). Conversely, foliar treatment with melatonin significantly promoted leaf production and minimized abscission (Figure 2B), with the mean leaf count rising from 14.22 leaves (M1) to 18.00 leaves under 100 mg L⁻¹ melatonin (M3).

Regarding interactive effects (Figure 2C), the D1 + M3 combination optimized leaf count at 23.00 leaves plant⁻¹, while the D3 + M1 treatment recorded the lowest value (8.00 leaves plant⁻¹). However, the application of 100 mg L⁻¹ melatonin under severe drought conditions (D3 + M3) restored leaf production to 13.00 leaves plant⁻¹.

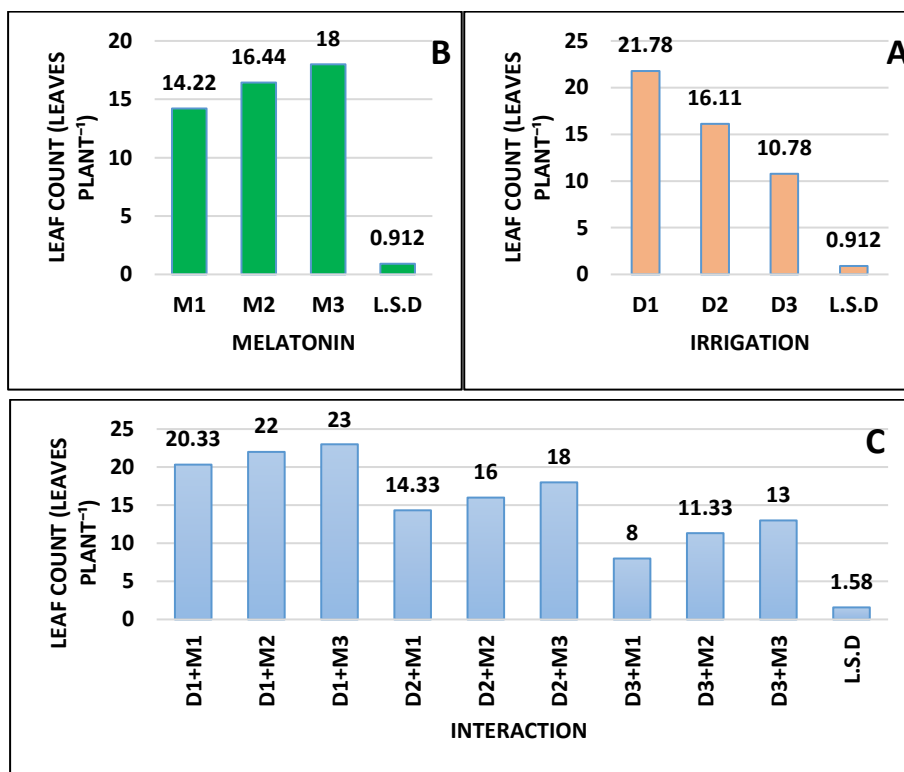


Figure (2) The effect of spraying melatonin on rose seedlings under the influence of drought stress in the trait of number of leaves (plant leaf⁻¹)

3.Total Leaf Area (cm²)

Leaf expansion was strictly regulated by the experimental treatments ($P \leq 0.05$). Data compiled in Table 1 indicate that decreasing irrigation frequency resulted in a significant reduction in total leaf area, which declined from 258.29 cm² (D1) to 133.63 cm² (D3). Melatonin application at 100 mg L⁻¹ (M3) countered this trend, increasing the mean leaf area to 213.96 cm² compared to 172.83 cm² in the untreated control (M1).

The interaction matrix showed that the maximum leaf area was achieved under the D1 + M3 combination (271.36 cm²), while the minimum area occurred under the D3 + M1 treatment (104.19 cm²). Exogenous application of 100 mg L⁻¹ melatonin under weekly drought (D3 + M3) successfully expanded the functional leaf area to 161.64 cm².

Table 1: Effect of melatonin foliar spray on the leaf area (cm²) of China rose seedlings under drought stress.

Irrigation Regimes	Melatonin (mg L ⁻¹)			Irrigation Mean
	0 (mg L ⁻¹)	50 (mg L ⁻¹)	100 (mg L ⁻¹)	
Daily (D1)	246.35	257.15	271.36	258.29
4 Days (D2)	167.94	189.81	208.87	188.87
Weekly (D3)	104.19	135.04	161.64	133.63
Melatonin Mean	172.83	194.00	213.96	
L.S.D. 0.05	Melatonin	Interaction		Irrigation
	0.814	1.409		0.814



4. Stem Diameter (mm):

Stem radial growth was significantly limited by water deficit and enhanced by melatonin treatments ($P \leq 0.05$). According to Table 2, increasing the irrigation interval reduced the mean stem diameter from 5.427 mm under the control regime (D1) to 4.383 mm under weekly stress (D3). Conversely, melatonin applications induced a gradual increase in stem diameter, peaking at 4.996 mm in the M3 treatment compared to 4.670 mm in the M1 control.

The interaction analysis indicated that the D1 + M3 combination yielded the thickest stem diameter (5.540 mm), while the D3 + M1 treatment resulted in the lowest value (4.216 mm). Melatonin supplementation at 100 mg L^{-1} under weekly irrigation stress (D3 + M3) significantly improved radial stem expansion, raising it to 4.550 mm.

Table 2: Effect of melatonin foliar spray on the stem diameter (mm) of China rose seedlings under drought stress.

Irrigation Regimes	Melatonin (mg L^{-1})			Irrigation Mean
	0 (mg L^{-1})	0 (mg L^{-1})	0 (mg L^{-1})	
Daily (D1)	5.30	5.43	5.54	5.42
4 Days (D2)	4.48	4.69	4.90	4.69
Weekly (D3)	4.21	4.38	4.55	4.38
Melatonin Mean	4.6700	4.8367	4.9967	
L.S.D. $_{0.05}$	Melatonin	Interaction		Irrigation
	0.021	0.03		0.021

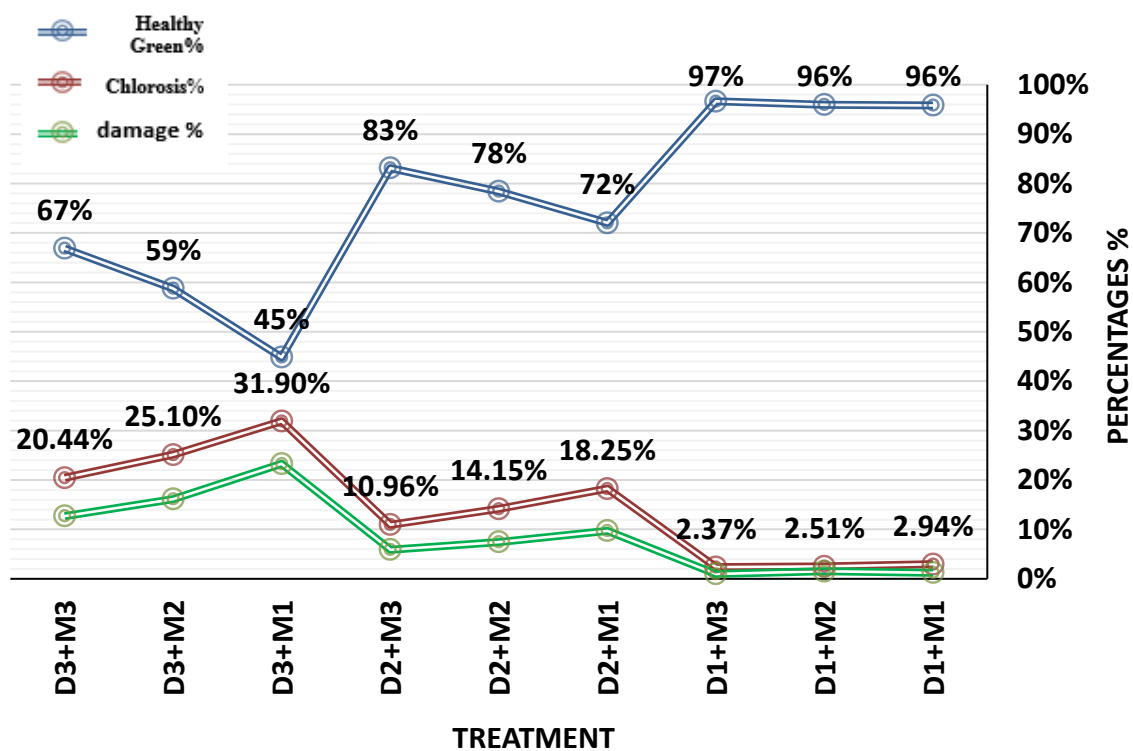
5. Total Chlorophyll Content ($\text{mg } 100\text{g}^{-1}$ Fresh Weight): Total chlorophyll concentrations dropped sharply by over 46.5% under severe weekly drought (D3), falling from a control mean of $91.28 \text{ mg } 100\text{g}^{-1}$ FW to $48.80 \text{ mg } 100\text{g}^{-1}$ FW (Table 3). Melatonin treatments provided significant protection against pigment degradation ($P \leq 0.05$); the grand mean for total chlorophyll increased from $62.45 \text{ mg } 100\text{g}^{-1}$ FW (M1) to $79.43 \text{ mg } 100\text{g}^{-1}$ FW in the 100 mg L^{-1} group (M3). The interaction matrix confirmed that the D1 + M3 combination achieved the maximum chlorophyll concentration ($96.10 \text{ mg } 100\text{g}^{-1}$ FW), while the unremediated severe drought group (D3 + M1) fell to a minimum of $35.73 \text{ mg } 100\text{g}^{-1}$ FW. However, applying 100 mg L^{-1} melatonin to weekly stressed seedlings (D3 + M3) significantly maintained chlorophyll levels at $61.30 \text{ mg } 100\text{g}^{-1}$ FW.

Table 3: Effect of melatonin foliar spray on total leaf chlorophyll $\text{mg } 100\text{g}^{-1}$ FW) of China rose seedlings under drought stress.

Irrigation Regimes	Melatonin (mg L^{-1})			Irrigation Mean
	0 (mg L^{-1})	0 (mg L^{-1})	0 (mg L^{-1})	
Daily (D1)	86.95	90.77	96.10	91.28
4 Days (D2)	64.67	74.65	80.87	73.40
Weekly (D3)	35.73	49.38	61.30	48.80
Melatonin Mean	62.45	71.60	79.43	
L.S.D. $_{0.05}$	Irrigation	Interaction		Melatonin
	3.42	5.92		3.42

6. Digital Phenotypic Percentages: Healthy Green, Chlorosis, and Necrosis (%)

The relative percentages of green, chlorotic, and damaged tissue reflect the physiological balance between cell viability and programmed senescence under environmental stress (Figure 3). Under daily irrigation (D1), seedlings maintained high health indices; the healthy green percentage stabilized between 96% and 97%, chlorosis remaining exceptionally low (2.37%–2.94%), and tissue necrosis dropping below 1.5%. These values indicate stable hydraulic conditions and operational balance in carbon-fixation pathways. Withholding water for four days without melatonin intervention (D2 + M1) significantly reduced the healthy green area to 72%, while increasing the chlorosis index to 18.25% and structural leaf damage to 9.72%. This decline worsened under severe weekly drought lacking melatonin (D3 + M1); the healthy green area dropped to a study minimum of 45%, chlorosis rose to 31.90%, and cellular tissue necrosis expanded substantially to 23.28%. Foliar melatonin application significantly mitigated these symptoms, indicating a powerful protective mechanism. In the moderate drought group, 100 mg L⁻¹ melatonin (D2 + M3) reduced tissue necrosis from 9.72% to 5.91%, limited chlorosis to 10.96%, and restored the healthy green area to 83%. Under severe weekly drought, the 100 mg L⁻¹ treatment (D3 + M3) rescued leaf tissues from severe degradation, maintaining the healthy green canopy area at 67%, restricting chlorosis to 20.44%, and checking tissue necrosis and leaf-margin scorch at 12.70% compared to 23.28% in the untreated group.



Discussion

The systematic reduction in morphological growth metrics (height, leaf number, leaf area, and stem diameter) and biochemical profiles under drought conditions is directly linked to a reduction in water potential within plant cells, causing a subsequent loss of intracellular turgor pressure. Non-optimal turgor pressure acts as a severe biophysical constraint that prevents cell wall expansion and longitudinal stretching, which are required for height and lateral stem growth. Furthermore, water deficit prompts stomatal closure to limit transpirational water loss, which reduces intercellular CO₂ availability, decreases photosynthesis,



and suppresses dry matter accumulation (Farooq *et al.*, 2012). The marked increase in leaf chlorosis and tissue damage under drought stress (Figure 3) can be explained by the bio-chemical degradation of chlorophyll molecules triggered by high chlorophyllase activity and the accumulation of Reactive Oxygen Species (ROS). These free radicals induce severe lipid peroxidation of cellular membranes, causing cellular leakage and desiccation (Sharma *et al.*, 2012). Accelerated leaf abscission and reduced leaf counts serve as an adaptive escape mechanism mediated by endogenous abscisic acid (ABA) accumulation to lower the total transpirational surface area of the plant (Kapoor *et al.*, 2020). Exogenous melatonin application induced substantial recovery across all evaluated growth parameters, most notably preserving total chlorophyll content and increasing the green leaf area percentage. This physiological promotion highlights melatonin's function as an auxin-like growth regulator, which activates plasma membrane, ATPases to enhance cell wall extensibility and stimulate cellular elongation (Arnao and Hernández-Ruiz, 2019). Additionally, melatonin expanded total leaf surface area and prolonged leaf retention by delaying stress-induced senescence. As a primary amphiphilic antioxidant, melatonin directly neutralizes ROS while upregulating the enzymatic activities of SOD, CAT, and POD, thereby protecting thylakoid membranes from oxidative damage under drought (Reiter *et al.*, 2015). This protective shield ensures photosystem operational stability, allowing continuous carbohydrate synthesis and allocation to active sinks. The binary interaction data proved that melatonin treatment under drought stress functions as a highly effective osmoregulator. It triggers the cellular accumulation of compatible osmolytes such as proline and total soluble sugars, allowing cells to maintain a positive turgor pressure under reduced soil moisture conditions (Wang *et al.*, 2013). Melatonin also reduces ion leakage by stabilizing cellular membranes. Moreover, it regulates the gene expression of water-transport proteins (aquaporins), optimizing hydraulic conductivity from root systems to the canopy. This metabolic reprogramming establishes melatonin as a crucial signaling molecule that restructures plant physiological pathways to overcome harsh environmental conditions (Li *et al.*, 2019).

IV. Conclusions

The results demonstrated that water stress caused by increasing irrigation intervals significantly reduced vegetative growth and physiological characteristics of *Hibiscus rosa-sinensis* plants, as evidenced by decreases in plant height, leaf number, leaf area, stem diameter, and chlorophyll content. In contrast, foliar application of melatonin significantly improved all studied traits, with the concentration of 100 mg L⁻¹ showing the greatest effectiveness in enhancing plant growth, increasing chlorophyll content, and reducing drought-induced damage. Furthermore, the interaction between daily irrigation and 100 mg L⁻¹ melatonin produced the highest values for most measured traits, while melatonin effectively mitigated the negative effects of water deficit and helped maintain plant growth and vitality under drought conditions.

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