HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

THE ROLE OF EXOGENOUS MELATONIN IN ENHANCING DROUGHT AND SALINITY TOLERANCE IN RANUNCULUS ASIATICUS

DOI: 10.54598/006570

Doctoral (PhD) Dissertation

Eman Abdelhakim Taha Mohamed Eisa

2025

Budapest, Hungary

Name: **Eman Abdelhakim Taha Mohamed Eisa**

Department: Department of Floriculture and Dendrology

Head: **Péter Honfi**

Associated Professor, PhD

Hungarian University of Agriculture and Life Sciences

Institute of Landscape Architecture, Urban Planning and Garden Art

Supervisor(s): **Péter Honfi**

Associated Professor, PhD

Tillyné Mándy Andrea

Associated Professor, CSc

Institute of Landscape Architecture, Urban Planning and Garden Art

.... ………………………………………..

Approval of the Head of Doctoral School Approval of the Supervisor(s)

1. INTRODUCTION

Horticultural plants are increasingly subjected to various abiotic stresses, which significantly hinder growth and productivity (Ahmad et al., 2024). These environmental challenges such as extreme temperatures, heavy metals, drought, and salinity pose severe threats to agricultural sustainability (Altaf et al., 2023). It is estimated that approximately 90% of arable land is susceptible to these stressors (Dos Reis et al., 2012). As climate change intensifies these conditions, understanding plant responses and adaptation mechanisms is crucial for maintaining yield and quality. Abiotic stress disrupts plant physiology, destabilizes ecosystems, and compromises food security.

Among these stressors, drought and salinity are particularly concerning due to their detrimental effects on plant metabolism (Tombesi et al., 2018). Drought stress results from insufficient water availability, leading to impaired photosynthesis, nutrient uptake, and overall plant health (Bidabadi et al., 2020). Prolonged drought conditions can cause reductions in relative water content, loss of turgor, cellular redox imbalances, and oxidative damage due to excessive reactive oxygen species (ROS) accumulation (Sadak et al., 2020). Similarly, salinity stress disrupts water uptake and ion homeostasis, causing osmotic stress and ion toxicity that impair plant metabolic processes (Trușcă et al., 2023). The increasing prevalence of drought and salinity due to climate variability underscores the need for effective mitigation strategies (Besser et al., 2021).

Ranunculus asiaticus L., commonly known as the buttercup, is a perennial geophyte cultivated primarily for its ornamental value (Karlsson, 2003). Native to the Mediterranean and Asia Minor, it has gained prominence due to the development of numerous hybrids (Beruto et al., 2018). However, *R. asiaticus* is highly sensitive to saline soils and drought conditions, making its cultivation challenging in arid regions (Caser et al., 2019). These stressors negatively affect floral quality, stem length, and overall production, particularly in water-scarce regions where floriculture competes with agricultural and urban water demands (Grieve, 2011). To ensure the sustainability of the floral industry, it is essential to identify stress-resistant floricultural varieties.

Melatonin (N-acetyl-5-methoxytryptamine) has emerged as a critical signaling molecule in plants, functioning similarly to phytohormones in regulating growth, development, and stress adaptation (Arabia et al., 2023). Acting as a potent antioxidant, melatonin mitigates oxidative stress by scavenging ROS and enhancing the antioxidant defense system (Pardo-Hernández et al., 2021).

Its application has been shown to improve plant resilience against drought and salinity by optimizing photosynthetic efficiency and root development (Wang et al., 2024).

This study aims to evaluate the role of melatonin in enhancing the drought and salinity tolerance of *R. asiaticus*. By examining physiological and biochemical responses, the research seeks to elucidate melatonin's potential as a biostimulant for mitigating abiotic stress in ornamental plants, contributing to sustainable horticultural practices.

This Work aims:

1. Investigates the role of exogenous melatonin in enhancing *Ranunculus asiaticus* tolerance to drought and salinity stress.
2. Evaluates the negative effects of drought and salinity on plant growth and physiology.
3. Examines the impact of melatonin at different concentrations (50, 100, and 200 µM) in mitigating stress effects.
4. Analyzes physiological responses, including chlorophyll content, relative water content, and oxidative stress markers.
5. Assesses antioxidant defense mechanisms, focusing on peroxidase (POD) activity.
6. Identifies key drought and salinity tolerance indicators, such as POD and proline content.
7. Determines the optimal melatonin concentration for improving plant growth and stress tolerance.

2. MATERIALS AND METHODS

A pot experiment was conducted in the greenhouse of the Floriculture and Dendrology Department, Hungarian University of Agriculture and Life Sciences (MATE), Budapest. Ranunculus asiaticus seedlings (30 days old) were transplanted into 9×9×10 cm pots. The greenhouse maintained a temperature range of 20–15 °C and 60% relative humidity.

Drought Stress Experiment: The experiment involved 160 plants, divided into well-watered and drought-stressed groups. Four melatonin concentrations (0, 50, 100, 200 µM) were applied. A completely randomized design (RCD) was used, with irrigation treatments based on soil moisture levels: 50–120 mbar (well-watered) and 180–200 mbar (drought-stressed). Soil moisture was monitored using a tensiometer.

Salinity Stress Experiment: A total of 135 plants were assigned to three salinity levels: control (0 dS/m), moderate (4.5 dS/m), and high (5.5 dS/m).The randomized complete design (RCD) was used, with melatonin treatments applied at all salinity levels. Salinity was induced using NaCl and $CaCl_2$ solutions, with electrical conductivity monitored by an EC meter.

Melatonin Application: Melatonin solutions (0, 50, 100, 200 µM) were prepared using ethanol and Milli-Q water. Foliar spraying was performed at 45, 60, 75, and 90 days post-planting. Tap water was used as a control for both drought and salinity stress.

Morphological Characteristics: Plants (10 individual plants per treatment) were sampled two weeks after the fourth foliar application to estimate the growth parameters, such as the plant height (cm), (measured from the medium surface to the shoot apex using a meter rod), the number of leaves, (counted manually), fresh and dry weights (shoots and leaves in g). The total leaf area (Area Meter 350, ADC Bioscietific Ltd., Hoddesdon, UK) and the number of flower buds that appeared were also recorded for analysis.

P**hysiological and Biochemical Assesments**: The five uppermost young fully expanded leaf samples were instantly frozen in liquid nitrogen and kept at −80 °C pending inspection. The photosynthetic pigments were measured for the acetone (80%) extract samples of each treatment and the absorbances (644, 663, and 480 nm) were recorded in a UV-VIS Spectrophotometer (Genesys 10S, Waltham, MA, USA). The carotenoid content was computed following the method of (Arnon, 1949), and the relative water content (RWC) was determined using the methods described by Turk and Erdal (2015) and the formula RWC = (FW-DW)/(TW-DW) × 100.

The leaf's proline content (PC) was determined by the methods of (Ábrahám et al., 2010) at 520 nm. A standard curve was constructed to calculate the proline content in µmol g^{-1} leaf FW. The extent of electrolyte leakage (EL) was assessed via the methods of Turk and Erdal (2015) . The peroxidase enzyme activity (POD) was also determined spectrophotometrically (470 nm) using the standard guaiacol method (He et al., 2014). The sodium chloride (NaCl) content of the leaves was determined with a flame photometer according to Mohr's titration method, as described by (Korkmaz, 2017) and is expressed as grams per 100 g dry weight (g 100 g^{-1} DW). Stress Tolerance Indices (Sbei et al., 2014); Drought Tolerance Index (DTI) and Salt Tolerance Index (STI) were calculated based on growth and physiological parameters.

Statistical Analysis

A Two-Way MANOVA was conducted, followed by UNIANOVA with Bonferroni's correction for dependent variables, using the software IBM SPSS27.

3. RESULTS

3.1. Morphological traits of *R. asiaticus* under stressful conditions.

3.1.1. Effect of Melatonin on Morphological Traits Under Drought Stress

Drought stress significantly reduced morphological parameters, but melatonin (MT) application improved plant growth. Under well-irrigated conditions, foliar MT treatments (50, 100, and 200 µM) enhanced shoot length, leaf number, leaf area, and biomass compared to untreated plants (W0MT).

Drought-stressed, untreated plants (D0MT) exhibited severe reductions: shoot length (13.97 cm), leaf number (4.60), leaf area (18.15 cm^2), fresh weight (11.92 g), and dry weight (1.47 g). However, MT application mitigated these effects. At 50 µM MT, minor improvements were observed, while higher concentrations (100 and 200 µM) significantly increased plant growth. At 200 µM MT, shoot length (17.09 cm), leaf number (5.93), leaf area (29.56 cm^2), fresh weight (17.01 g), and dry weight (2.01 g) showed the highest values, demonstrating a dose-dependent enhancement.

3.1.2. Effect of Melatonin on Morphological Traits Under Salinity Stress.

Salinity stress significantly reduced the growth of buttercup plants. Compared to control (non-stressed) plants, those subjected to EC 4.5 (0 MT) and EC 5.5 (0 MT) exhibited severe reductions: Shoot length decreased by 31.78% and 35.64%, respectively. Leaf number declined by 32.23% and 34.03%. Total leaf area shrank by 58.34% and 60.98%. Shoot fresh weight (FW) reduced by 29.32% and 36.61%. Shoot dry weight (DW) dropped by 42.15% and 46.08%.

Melatonin (MT) foliar application at 50, 100, and 200 µM significantly improved all vegetative traits under salinity stress. 200 µM MT provided the greatest enhancement, increasing: Shoot length by 23.37% (EC 4.5) and 30.04% (EC 5.5). Leaf number by 28.32% and 21.14%. Total leaf area by 58.01% and 58.79%. Shoot FW by 29.92% and 42.33%. Shoot DW by 45.20% and 41.82%, respectively.

3.1.3. Effect of Exogenous Melatonin on Flower Bud Emergence Under Drought Stress.

Drought stress significantly accelerated flower bud emergence in buttercup plants. Control plants (D0MT) formed flower buds 29 days earlier than well-watered plants. Melatonin (MT) application at 50, 100, and 200 µM moderated this early flowering response:

D50 MT: Flower buds emerged 23 days earlier. D100 MT: 19 days earlier. D200 MT: 21 days earlier. These results indicate that while drought stress hastens flower bud formation, melatonin treatment mitigates this effect, with 100 µM MT showing the greatest delay.

3.1.4. Effect of Salinity Stress and Melatonin on Flower Bud Emergence

Salinity stress (EC 4.5 and 5.5 dS m^{-1}) significantly accelerated flower bud emergence, with flowering occurring 29 and 36 days earlier, respectively, than in control plants. Melatonin (MT) treatment effectively delayed this premature flowering: S1 (EC 4.5 dS m^{-1}): Flowering was postponed by 22, 24, and 15 days with 50, 100, and 200 µM MT, respectively. S2 (EC 5.5 dS m^{-1}): Flowering was delayed by 17, 8, and 10 days with 50, 100, and 200 µM MT, respectively. These results indicate that while salinity stress hastens flowering, melatonin application mitigates this effect, with 100 µM MT showing the most significant delay under moderate salinity conditions.

3.2. The physiological and biochemical traits of *R. asiaticus* under stressful conditions.

3.2.1. Effect of Drought Stress and Melatonin on Photosynthetic Pigments

Drought stress (180–200 mbar) significantly reduced photosynthetic pigments in *R. asiaticus*, with chlorophyll and carotenoid content decreasing by 68.29% and 51.72%, respectively, in non-MT-treated plants compared to well-watered controls.

Melatonin (MT) application mitigated these losses in a concentration-dependent manner. Foliar spraying with 200 µM MT resulted in the highest recovery, increasing chlorophyll by 75% and carotenoids by 50% compared to untreated drought-stressed plants. These findings highlight MT's protective role in maintaining pigment stability under drought stress.

3.2.2. Effect of Salinity Stress and Melatonin on Photosynthetic Pigments

Salinity stress (EC 4.5 and 5.5) significantly reduced chlorophyll (45.12%–55.49%) and carotenoid (17.24%–44.82%) content in *R. asiaticus* plants without melatonin (MT) treatment.

Melatonin application mitigated these reductions in a dose-dependent manner. At 200 µM MT, chlorophyll and carotenoids increased by 68.89% and 83.56%, respectively, compared to untreated salt-stressed plants. These results confirm MT's protective role in maintaining photosynthetic efficiency and reducing ion toxicity under salinity stress.

3.2.3. Effect of Melatonin on Relative Water Content (RWC) and Proline Accumulation Under Drought Stress

Drought stress significantly reduced RWC in *R. asiaticus*, but melatonin (MT) treatment alleviated this decline. Untreated drought-stressed plants (D0MT) showed a 7.1% decrease in RWC, whereas 200 µM MT application improved RWC by 6.4% compared to untreated stressed plants.

Drought stress also induced proline accumulation, a key osmoprotectant. MT further enhanced proline levels in a dose-dependent manner: 50, 100, and 200 µM MT increased proline content by 17.8%, 28.6%, and 32.1%, respectively, relative to untreated stressed plants. These results indicate that MT improves water retention and enhances osmoprotection, helping plants mitigate drought-induced stress.

3.2.4. Effect of Melatonin on Relative Water Content (RWC) and Proline Accumulation Under Salinity Stress

Salinity stress significantly reduced RWC, with the greatest decline (28.3%) observed at EC 5.5. Melatonin (MT) treatment partially restored RWC, with 200 µM MT providing the most protection, though RWC remained below control levels.

Salinity also triggered a 50% increase in proline content, a key stress-related osmolyte. MT application (50–200 µM) further enhanced proline levels, supporting cellular osmotic balance and stress tolerance. These findings indicate that MT mitigates salinity-induced stress, improving water retention and osmoprotection.

3.2.5. Effect of Melatonin on Sodium Ion (Na^+) Accumulation Under Salinity Stress

Salinity stress significantly increased Na^+ accumulation in *R. asiaticus* leaves, with rises of 280% (EC 4.5) and 311% (EC 5.5) compared to control plants. However, melatonin (MT) treatment mitigated Na^+ buildup, with 200 µM MT reducing Na^+ levels by 50.4% (EC 4.5) and 20.5% (EC 5.5). These results highlight MT's potential to enhance salt tolerance by limiting Na^+ uptake and improving ionic homeostasis.

3.2.6. Melatonin Modulates Peroxidase Activity (POD) and Reduces Electrolyte Leakage (EL) Under Drought Stress

Drought stress significantly increased peroxidase (POD) activity and electrolyte leakage (EL) in *R. asiaticus*. However, melatonin (MT) treatment enhanced POD activity and reduced EL, improving membrane stability.

POD Activity: Drought stress alone increased POD by 64.6% (D0MT vs. W0MT). MT application (200 µM) further boosted POD by 58.3% under drought stress.

Electrolyte Leakage (EL): Drought stress increased EL, indicating membrane damage. MT (200 µM) reduced EL by 14.3%, suggesting ROS mitigation and improved stress tolerance. These results confirm that MT enhances antioxidant defense and protects cell membranes under drought conditions, with 200 µM MT showing the most significant benefits.

3.2.7. Melatonin Modulates Peroxidase Activity (POD) and Reduces Electrolyte Leakage (EL) Under Salinity Stress

Salinity stress significantly impacted peroxidase (POD) activity and electrolyte leakage (EL) in *R. asiaticus*. The application of melatonin (MT) enhanced POD activity and mitigated EL, offering protective effects under saline stress.

POD Activity: Salinity stress (S1 and S2) increased POD by 61.9% and 82.2%, respectively.

MT treatment (200 µM) further boosted POD by 48.8% (S1) and 46.7% (S2), showing a concentration-dependent enhancement in antioxidant activity.

Electrolyte Leakage (EL): Salinity stress increased EL by 64.01% and 67.74%.

MT (200 µM) reduced EL by 28.93% (S1) and 20.57% (S2), suggesting significant membrane protection under saline stress. These results demonstrate that MT application enhances antioxidant defense and improves membrane integrity, providing salinity tolerance by reducing oxidative damage and membrane leakage.

3.3. Stress response index

3.3.1. Drought Tolerance Index (DTI) in *R. asiaticus* under Drought Stress

The Drought Tolerance Index (DTI), calculated based on various plant traits under drought stress and well-irrigated conditions, revealed the following insights:

Most responsive traits to drought stress: POD activity had the highest DTI of 282.35%, indicating its significant role in drought tolerance. Proline content followed with a DTI of 207.40%, highlighting its importance in protecting cells under drought stress. Electrolyte leakage (EL) showed a DTI of 120.49%, reflecting its relevance in maintaining membrane integrity during stress.

Less sensitive traits to drought stress: Carotenoids, shoot dry weight, leaf area, and total chlorophyll exhibited DTI values below 100%, with chlorophyll (32%) and leaf area (37.84%) being the most sensitive to drought conditions.

Overall, POD activity and proline accumulation were identified as key indicators of drought tolerance, while chlorophyll and leaf area were more affected by drought stress.

3.3.2. Salt Tolerance Index (STI) in *R. asiaticus* under Salinity Stress

The Salt Tolerance Index (STI), based on comparisons between non-melatonin-treated plants under unstressed conditions and those subjected to S2 salinity levels (EC 5.5 dS m^{-1}), revealed the following:

Most sensitive traits to salinity stress: Leaf Na^+ content exhibited the highest STI of 437.9%, indicating its strong sensitivity to salt stress. POD activity and electrolyte leakage (EL) followed with values of 321.3% and 309.9%, respectively, highlighting their significant role in stress response. Proline content ranked third with an STI of 202.5%, contributing to osmotic regulation under saline conditions.

Less responsive traits to salinity stress: Total chlorophyll showed the lowest STI value of 44.4%, suggesting it was the least responsive to salinity stress. Other traits exhibited STI values above 50%, indicating moderate sensitivity to salinity.

This analysis highlights Na^+ accumulation, POD activity, and electrolyte leakage as key indicators of salt tolerance, while chlorophyll content was the least affected by salinity.

4. DISCUSSIONS

Water scarcity and salinity are significant environmental stressors that adversely affect plant growth, particularly in arid and semi-arid regions (Liu et al., 2015). These stresses induce physiological, anatomical, and morphological changes that hinder plant development. Drought stress, for example, suppresses growth as an adaptive strategy to minimize transpiration. This leads to reduced cell elongation, turgor pressure, and overall growth (Alam et al., 2014). As water availability declines, leaf area reduces, which further limits photosynthesis and exacerbates water loss (Naeem et al., 2018). Similarly, salinity stress disrupts osmotic balance, leading to ionic imbalances and nutrient deficiencies, ultimately inhibiting growth at EC levels of 4.5 and 5.5 dS m^{-1}. The sensitivity of *R. asiaticus* to salinity stress underscores the need for effective management strategies to mitigate growth inhibition (Valdez-Aguilar et al., 2009; Poorter et al., 2010).

Melatonin (MT) is an effective regulator of plant growth and stress tolerance (Imran et al., 2021). Even in trace amounts, MT modulates physiological functions, improving plant resilience under both drought and salinity stress (Zhang et al., 2015). Under drought stress, MT (100 µM and 200 µM) enhances shoot length, leaf number, and biomass, mitigating damage caused by water deficit. The most significant improvement was observed at 200 µM MT, aligning with previous findings in soybean, lupin, and tomato (Imran et al., 2021; Altaf et al., 2023). MT also alleviates salinity-induced growth suppression, with 200 µM being the most effective concentration, as shown in studies on cotton (Bajwa et al., 2014). By activating antioxidant enzymes and modulating gene expression, MT reduces oxidative damage caused by ROS and stabilizes cellular integrity under stress (Zhang et al., 2015; Li et al., 2018).

Negative Effects of Stress: The detrimental effects of both drought and salinity stress are evident in several key physiological and biochemical processes. Drought stress significantly reduces chlorophyll and carotenoid content, impairing light absorption and photosynthetic efficiency, which limits plant growth and biomass production (Pandey et al., 2012). Similarly, salinity stress leads to excessive Na^+ accumulation, causing ionic toxicity, which disrupts cell membrane stability and water uptake (Hand et al., 2017). These stresses also reduce relative water content (RWC), an indicator of water availability and plant hydration status, further exacerbating dehydration and metabolic disturbances (Keyvan, 2010). Under both stress conditions, proline accumulation is observed as a response to osmotic adjustments, but excessive proline accumulation can also indicate stress-related cellular damage (Sheikhalipour et al., 2022).

In terms of growth, drought stress reduces the size of leaves and stems, leading to stunted growth and poor biomass accumulation (Naeem et al., 2018). Salinity stress similarly limits growth parameters like shoot fresh weight, leaf count, and root length, which affects overall plant development. This growth suppression can severely impact reproductive potential and overall survival, especially under prolonged or severe stress conditions.

Protective Role of Melatonin (MT): Despite the adverse effects of drought and salinity stress, MT application significantly improved plant growth and stress tolerance. MT mitigates the negative impact of drought by enhancing chlorophyll and carotenoid content, improving photosynthetic efficiency, and stabilizing cell membranes (Gohari et al., 2023). Under drought stress, MT-treated plants exhibited higher pigment retention, with the most effective concentration being 200 µM. This concentration also promoted increased biomass, shoot length, and leaf number, underscoring the protective role of MT in mitigating drought-induced damage.

Similarly, under salinity stress, MT application reduced Na^+ accumulation in the leaves, particularly at 200 µM, and improved overall plant water status, as evidenced by higher RWC values. MT likely improves ion homeostasis by upregulating ion transporter genes such as NHX1 and AKT1, which help plants compartmentalize excess ions in vacuoles, mitigating ion toxicity (Wei et al., 2022). This action helps maintain cellular integrity and ion balance, thus improving salt tolerance.

MT also enhances proline accumulation under both drought and salinity stress, promoting osmotic adjustment and improving water retention (Sheikhalipour et al., 2022). The most significant proline accumulation was observed at 200 µM MT, suggesting that MT plays a crucial role in regulating osmotic balance under stress conditions. Additionally, MT improved peroxidase (POD) activity, which is vital for scavenging ROS and reducing oxidative damage, further enhancing plant stress tolerance (Zhang et al., 2015; Altaf et al., 2022). MT's role in stabilizing membranes, as indicated by reduced electrolyte leakage (EL) and enhanced antioxidant activity, supports the overall resilience of plants under drought and salinity stress (Imran et al., 2021; Saneoka et al., 2004).

Finally, the use of DTI% and STI% indices revealed that traits like POD activity, proline levels, and EL can serve as effective markers for drought and salinity stress tolerance in *R. asiaticus*. The high DTI% and STI% values for these traits indicate that they are key indicators of plant resilience under stress conditions and offer valuable insights for future breeding programs focused on stress tolerance (Rafi et al., 2019; Roshdy et al., 2021).

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion:

This study demonstrates the beneficial effects of exogenous melatonin (MT) treatment on the tolerance of *R. asiaticus* to both drought and salinity stress. Under drought conditions, MT application improved the plants' biochemical profiles, significantly enhancing the performance of the antioxidant defense system. This was evidenced by the activation of peroxidase (POD), reduced electrolyte leakage (EL), and decreased ROS generation. Additionally, MT treatment bolstered osmotic regulation by promoting osmolyte accumulation and maintaining water status, which collectively mitigated drought-related damage. The increase in carotenoid content further contributed to non-enzymatic antioxidant activity, helping to sustain total chlorophyll levels and thereby enhancing vegetative growth parameters.

In parallel, under saline stress conditions (EC 4.5 and EC 5.5), exogenous MT treatment also led to improved relative water content (RWC), increased photosynthetic pigments, and reduced sodium accumulation in leaves, thereby reinforcing growth and vegetative parameters in a dose-dependent manner. The results indicated that MT enhanced osmotic regulation capacity, similarly through increased proline accumulation and improved antioxidant defense mechanisms, as evidenced by POD activation and decreased EL. Notably, under the more severe salinity level (EC 5.5), sodium content in leaves, along with enzyme activity, EL, and leaf proline levels, emerged as critical indicators of salinity stress response.

Overall, the optimal dose of 200 µM MT was effective in enhancing the morpho-physiological characteristics and stress tolerance of *R. asiaticus* under both drought and salinity conditions. These findings suggest that the application of MT could be a promising strategy for improving the resilience of *R. asiaticus* in challenging environmental conditions, warranting further investigation with varying MT concentrations for optimal stress tolerance.

5.2. Recommendations:

Based on the findings of this study, several recommendations can be proposed for future research and practical applications:

1. Field Trials and Validation: Conduct extensive field studies to validate the effectiveness of melatonin under natural drought and salinity conditions. This will ensure the applicability of the results to real-world agricultural and horticultural systems.

2. Optimal Application Protocols: Further investigations are needed to establish precise application protocols, including timing, frequency, and delivery methods of melatonin treatments for achieving maximum stress tolerance in *Ranunculus asiaticus* and other related plant species.

3. Molecular Mechanisms: Future studies should explore the molecular pathways and signaling networks through which melatonin modulates stress responses, particularly focusing on its interactions with plant hormones and transcription factors.

4. Vegetative Health and Floral Productivity: Future research should build upon these findings to investigate how vegetative health translates into floral quality and productivity. Understanding post-stress recovery and reproductive performance under melatonin treatment would provide additional insights into its role in flowering regulation, particularly in stress-adapted plants.

4. Broader Crop Applications: Extend research on melatonin's stress-alleviating effects to other economically important horticultural and ornamental crops, aiming to identify cross-species benefits and limitations.

5. Integration with Sustainable Practices: Explore combining melatonin treatments with eco-friendly agricultural practices, such as optimized irrigation systems and soil amendments, to further improve plant resilience while minimizing environmental impact.

6. Commercial Formulations: Development of commercial melatonin-based formulations tailored for stress mitigation in ornamental crops should be considered, emphasizing ease of application and cost-efficiency for growers.

These recommendations provide a pathway for enhancing the sustainable production of *Ranunculus asiaticus* under abiotic stress conditions while contributing to the broader field of stress management in horticulture.

6. NEW SCIENTIFIC RESULTS

1. 1- This study is the first to investigate the physiological responses of *Ranunculus asiaticus* to drought and salinity stress, providing new insights into its adaptation mechanisms.
2. Melatonin significantly improved plant growth under drought and salinity, with 100–200 µM being the most effective concentrations, delayed premature flowering, aligning bud emergence with well-watered plants under drought and salinity stress.
3. Photosynthetic pigment levels (chlorophyll and carotenoids) were preserved with melatonin, maintaining photosynthetic efficiency despite stress.
4. Relative water content (RWC) and proline accumulation increased with melatonin, enhancing water retention and osmotic adjustment.
5. Melatonin application enhanced antioxidant activity (peroxidase), reduced electrolyte leakage, and lowered sodium accumulation, leading to improved membrane stability, better ion balance, and increased overall stress tolerance.
6. This is the first study to comprehensively assess Drought Tolerance Index (DTI%) and Salinity Tolerance Index (STI%) in *R. asiaticus*, offering valuable insights for future research in plant physiology and breeding strategies.

LIST OF PUBLICATIONS

1. **Eisa, E.A**., Honfi, P., Kohut, I., AndreaTilly-Mándy (2025). Advancing Tuberose (*Agave Amica* Medik.) Thiede and Govaerts Cultivation: Insights into Characteristics, Cultivation Practices, and Breeding Strategies. In: Wani, M.A., Al-Khayri, J.M., Jain, S.M. (eds) Breeding of Ornamental Crops: Bulbous Flowers. Advances in Plant Breeding Strategies, vol 5. Springer, Cham. DOI: 10.1007/978-3-031-77900-8_3
2. **Eisa, E. A**., Pasquel Davila, D. S., & Ördögh, M. (2025). Enhancing Acclimatization Conditions for Vriesea splendens 'Fire': A Comparative Analysis of Substrate Effects on Growth and Survival. Plants , 14 (2), 172.
3. El-Mahrouk, ES, Ebrahim, HM, Gaber, MK, Aly, MA, El-Naggar, AA, Honfi, P., Andrea Tilly-Mándy, and **Eisa, EA** (2025). Performance of Growth and Remediation Potency of Jacaranda mimosifolia in Cadmium and Lead Contaminated Soil. BioResources , 20 (1), 1547-1576.
4. El-Mahrouk E-SM, Atef EA-M, Gabr MK, Aly MA, Mohamed AE, Eisa EA and Gururani MA (2024). Response of Salvia officinalis to zinc and silicon nanoparticles and pollen extract as alternates to traditional fertilizers. Front. Plant Sci. 15:1469691. doi: 10.3389/fpls.2024.1469691.
5. El-Mahrouk EM,, Eldawansy SM,, El-Tarawy AM,, Ebrahim HMA., Eisa EA*, Tilly-Mandy A and Honfi P*. (2024) (2024) Evaluation of Growth, Enzymatic Activity, Electrolyte leakage and Phytoremediation Efficiency of Conocarpus erectus under Cadmium and lead Lead Stress. Frontiers, 10.3389/fpls.2024.1466697.
6. Kovács, Z., Portocarrero, L. K., Honfi, P., Kohut, I., **Eisa, E. A.*** & Tilly-Mándy, A. (2024). Enhancing Micropropagation of Adenophora liliifolia: Insights from PGRs, Natural Extracts, and pH Optimization. Plants, 13(13),1735.https://doi.org/10.3390/plants13131735
7. Shala, A. Y., Elmenofy, H. M., **Eisa, E. A**. E.-H., and Al-Khayri, J. M. (2024). Phytochemistry, Antioxidants, Antimicrobial Activities and Edible Coating Application of Aloe vera. In M. Anis & M. N. Khanam (Eds.), In Vitro Propagation and Secondary Metabolite Production (Part 2) (pp. 119–160). Bentham Science Publishers.

8. Honfi, P.; **Eisa, E.A*.;** Tilly-Mándy, A.; Kohut, I.; Ecseri, K.; Mosonyi, I.D.(2023) Salt Tolerance of Limonium gmelinii subsp. hungaricum as a Potential Ornamental Plant for Secondary Salinized Soils. Plants, 12, 1807.https://doi.org/10.3390/plants12091807
9. **Eisa, E.A**.; Honfi, P.; Tilly-Mándy, A.; Mirmazloum, I. Exogenous Melatonin Application Induced Morpho-Physiological and Biochemical Regulations Conferring Salt Tolerance in Ranunculus asiaticus L. Horticulturae 2023, 9, 228. DOI: 10.3390/horticulturae9020228
10. **Eisa, E.A**.; Honfi, P.; Tilly-Mándy, A.; Gururani, M.A. Exogenous Application of Melatonin Alleviates Drought Stress in Ranunculus asiaticus by Improving Its Morphophysiological and Biochemical Attributes. Horticulturae 2023, 9, 262. DOI: 10.3390/horticulturae9020262
11. Eisa, E.A.; Tilly-Mándy, A.; Honfi, P.; Shala, A.Y.; Gururani, M.A. Chrysanthemum: A Comprehensive Review on Recent D evelopments on In Vitro Regeneration. Biology 2022, 11, 1774. DOI: 10.3390/biology11121774

7. REFERENCES

Ábrahám, E., Hourton-Cabassa, C., Erdei, L., and Szabados, L. (2010). Methods for determination of proline in plants. Methods in molecular biology, 639, pp. 317-31. DOI: 10.1007/978-1-60761-702-0_20

DOI: 10.1007/978-1-60761-702-0_20

Ahmad, J., Hayat, F., Khan, U., Ahmed, N., Li, J., Ercisli, S., Iqbal, S., Javed, H.U., Alyas, T., Tu, P. and Chen, J. (2024): Melatonin: A promising approach to enhance abiotic stress tolerance in horticultural plants. South African Journal of Botany, 164, pp. 66–76. DOI: 10.1016/j.sajb.2023.10.045

Alam, M., Nahar, K., Hasanuzzaman, M., and Fujita, M. (2014): Trehalose-induced drought stress tolerance: A comparative study among different Brassica species.Plant Omics,7, pp. 271–283.

Altaf, M.A. , Sharma, N., Singh, J., Samota, M. K., Sankhyan, P., Singh, B., Kumar, A., Naz, S., Lal, M.K., Tiwari, R.K. and Kumar, R. (2023): Mechanistic insights on melatonin-mediated plant growth regulation and hormonal cross-talk process in solanaceous vegetables. Scientia Horticulturae, 308, p. 111570. https://doi.org/10.1016/j.scienta.2022.111570.

Arabia, A., Mu˜noz, P., Pallar´es, N., Munn´e-Bosch, S. (2023) :*Experimental approaches in studying active biomolecules modulating fruit ripening: Melatonin as a case study*, Plant Physiology, 192(3), pp. 1747–1767. https://doi.org/10.1093/plphys/kiad106.

Bajwa, V.S. Shukla, MR., Sherif, SM., Murch, SJ.,and Saxena, PK. (2014) :Role of melatonin in alleviating cold stress in Arabidopsis thaliana. J. Pineal Res., 56, pp. 238–245.

Beruto, M.; Rabaglio, M.; Viglione, S.; van Labeke, M.-C.; Dhooghe, E. (2018): Ranunculus. In Ornamental Crops; Springer: Berlin, Germany, pp. 649–671.

Besser, H., Dhaouadi, L., Hadji, R., Hamed, Y., and Jemmali, H., 2021: Ecologic and economic perspectives for sustainable irrigated agriculture under arid climate conditions: An analysis based on environmental indicators for southern Tunisia. Journal of African Earth Sciences, 177, pp. 104134. DOI: 10.1016/J.JAFREARSCI.2021.104134

Bidabadi, S.S., VanderWeide, J. and Sabbatini, P. (2020): Exogenous melatonin improves glutathione content, redox state and increases essential oil production in two Salvia species under drought stress. Scientific Reports, 10(1), p. 6883. https://doi.org/10.1038/s41598-020-63986-6.

Caser, M. Chitarra, W. D'Angiolillo, F., Perrone, I., Demasi, S., Lovisolo, C., Pistelli, L., Pistelli, L., and Scariot, V. (2019) : Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica* Codd, Industrial crops and products, 129, pp. 85–96.

Dos Reis, S.P., Lima, A.M. and De Souza, C.R.B. (2012): Recent Molecular Advances on Downstream Plant Responses to Abiotic Stress. International Journal of Molecular Sciences, 13(7), pp. 8628–8647. https://doi.org/10.3390/ijms13078628.

Gohari, G., Farhadi, H., Panahirad, S., Zareei, E., Labib, P., Jafari, H., and Fotopoulos, V. (2023): Mitigation of salinity impact in spearmint plants through the application of engineered chitosan-melatonin nanoparticles. International Journal of Biological Macromolecules, 224, pp. 893–907. https://doi.org/10.1016/j.ijbiomac.2022.10.175.

Grieve, C.M. (2011): *Salinity-induced enhancement of horticultural crop quality*, in *:* M. Pessarakli (ed.). Handbook of plant and crop stress. Taylor and Francis, Boca Raton, FL USA., pp. 1173–1194.

Hand, M., Taffouo, V., Nouck, A., Nyemene, K., Tonfack, B., Meguekam, T., and Youmbi, E., 2017. Effects of salt stress on plant growth, nutrient partitioning, chlorophyll content, leaf relative water content, accumulation of osmolytes and antioxidant compounds in pepper (*Capsicum annuum* L.) Cultivars. Notulae Botanicae Horti Agrobotanici Cluj-napoca, 45, pp. 481-490.

DOI: 10.15835/NBHA45210928

Imran, M., Khan, A., Shahzad, R., Khan, M., Bilal, S., Khan, A., Kang, S., and Lee, I. (2021): Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants', *AoB PLANTS*. Edited by O. Wilkins, 13(4), pp. 1–12. https://doi.org/10.1093/aobpla/plab026.

Karlsson, M. (2003) : *Producing ravishing ranunculus*, Greenhouse Prod. News January, P. 44–48.

Keyvan, S. (2010) : The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars.J. Anim. Plant Sci., 8, pp. 1051–1060.

Korkmaz, D. (2017): Precipitation Titration: Determination of Chloride by the Mohr Method. Methods, 4(2), pp. 1–6. http://academic.brooklyn.cuny.edu/esl/gonsalves/tutorials/ Writing_a_Lab_Report/xPrecipitation Titration edited 3.pdf.

Li, J., Zeng, L., Cheng, Y., Lu, G., Fu, G., Ma, H., and Li, C. (2018): Exogenous melatonin alleviates damage from drought stress in *Brassica napus* L.(rapeseed) seedlings. Acta Physiologiae Plantarum, 40(3), pp. 1–11. https://doi.org/10.1007/s11738-017-2601-8.

Liu, N., Jin, Z., Wang, S., Gong, B., Wen, D., Wang, X., and Shi, Q. (2015): Sodic alkaline stress mitigation with exogenous melatonin involves reactive oxygen metabolism and ion homeostasis in tomato. Scientia Horticulturae, 181, pp. 18–25. DOI: 10.1016/j.scienta.2014.10.049

Naeem, M., Naeem, M. S., Ahmad, R., Ahmad, R., Ashraf, M. Y., Ihsan, M. Z., Nawaz, F., Athar, H.U.R., Ashraf, M., Abbas, H.T. and Abdullah, M. (2018): Improving drought tolerance in maize by foliar application of boron: water status, antioxidative defense and photosynthetic capacity. Arch Agron Soil Sci, 64, pp. 626–639.

Pandey, H., Baig, M. and Bhatt, R. (2012): Effect of moisture stress on chlorophyll accumulation and nitrate reductase activity at vegetative and flowering stage in Avena species. Agric Sci Res J., 2, pp. 111–118.

Pardo-Hernández, M., López-Delacalle, M., Martí-Guillén, J., Martínez-Lorente, S., and Rivero, R., (2021): ROS and NO Phytomelatonin-Induced Signaling Mechanisms under Metal Toxicity in Plants: A Review. *Antioxidants,* 10. DOI: 10.3390/antiox10050775

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., and Villar, R.. (2010): Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytologist, 182, pp. 565–588.

Rafi, Z.N., Kazemi, F. and Tehranifar, A. (2019): Morpho-physiological and biochemical responses of four ornamental herbaceous species to water stress. Acta Physiologiae Plantarum, 41(1), pp. 1–13. https://doi.org/10.1007/s11738-018-2797-2.

Roshdy, A.E.-D., Alebidi, A., Almutairi, K., Al-Obeed, R., and Elsabagh, A. (2021): The effect of salicylic acid on the performances of salt stressed strawberry plants, enzymes activity, and salt tolerance index. Agronomy, 11(4), p. 775. https://doi.org/10.3390/agronomy11040775.

Sadak, M.S., Abdalla, A.M., Abd Elhamid, E.M., and Ezzo, M.I. (2020): Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* L. plant under drought stress. Bulletin of the National Research Centre, 44(1), p. 18. https://doi.org/10.1186/s42269-020-0275-

7.

Saneoka, H. Moghaieb, R. E., Premachandra, G. S.,and Fujita, K. (2004): Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in Agrostis palustris Huds. Environmental and Experimental Botany, 52(2), pp. 131–8. doi: 10.1016/j.envexpbot.2004.01.011.

Sbei, H., Shehzad, T., Harrabi, M., and Okuno, K. (2014): Salinity tolerance evaluation of Asian barley accessions (*Hordeum vulgare L.*) at the early vegetative stage. J. Arid Land Studies, 24, pp. 183–186.

Sheikhalipour, M., Mohammadi, S.A., Esmaielpour, B., Zareei, E., Kulak, M., Ali, S., Nouraein, M., Bahrami, M.K., Gohari, G. and Fotopoulos, V. (2022): Exogenous melatonin increases salt tolerance in bitter melon by regulating ionic balance , antioxidant system and secondary metabolism related genes. BMC Plant Biology, 33, p. 363:380. https://doi.org/10.1186/s12870-022-03728-0.

Tombesi, S., Frioni, T., Poni, S., and Palliotti, A. (2018): Effect of water stress "memory" on plant behavior during subsequent drought stress, Environmental and Experimental Botany, 150, pp. 106–114. https://doi.org/10.1016/j.envexpbot.2018.03.009.

Tombesi, S., Frioni, T., Poni, S., and Palliotti, A. (2018): Effect of water stress "memory" on plant behavior during subsequent drought stress, Environmental and Experimental Botany, 150, pp. 106–114. DOI: 10.1016/j.envexpbot.2018.03.009

Trușcă, M., Gâdea, Ș., Vidican, R., Stoian, V., Vâtcă, A., Balint, C., and Vâtcă, S. (2023): Exploring the research challenges and perspectives in ecophysiology of plants affected by salinity stress, Agriculture (Switzerland), 13(3). https://doi.org/10.3390/agriculture13030734.

Turk, H. and Erdal, S. (2015): Melatonin alleviates cold-induced oxidative damage in maize seedlings by up-regulating mineral elements and enhancing antioxidant activity. Journal of Plant Nutrition and Soil Science, 178(3), pp. 433–439.

Valdez-Aguilar, L.A., Grieve, C. M., Poss, J., and Mellano, M. A. (2009): Hypersensitivity of *Ranunculus asiaticus* to Salinity and Alkaline pH in Irrigation Water in Sand Cultures. HortScience, 44(1), pp. 138–144.https://doi.org/10.21273/HORTSCI.44.1.138.

Wang, X. Cao, M., Li, H., Liu, Y., Fan, S., Zhang, N., and Guo, Y. (2024) :Strategies and prospects for melatonin to alleviate abiotic stress in horticultural plants, Horticultural Plant Journal, 10(3), pp. 601–614. DOI: 10.1016/j.hpj.2023.03.011

Wei, J. Liang, J., Liu, D., Liu, Y., Liu, G., and Wei, S. (2022):Melatonin-induced physiology and transcriptome changes in banana seedlings under salt stress conditions, *Frontiers in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.938262.

Zhang, Na., Sun, Q., Zhang, H., Cao, Y., Weeda, S., Ren, S., and Guo, Y. D. (2015): Roles of melatonin in abiotic stress resistance in plants. Journal of Experimental Botany, 66(3), pp. 647–656.https://doi.org/10.1093/jxb/eru336.