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

Doctoral (PhD) Dissertation

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ABBERVIATIONS

ABR1	APETALA2-like Ethylene-Responsive Element Binding Protein Related 1
ACS1	1-Aminocyclopropane-1-Carboxylate Synthase 1
AKT1	Arabidopsis K+ Transporter 1
AO	Ascorbate Oxidase
AP2/EREBP-HB-WRKY	APETALA2/Ethylene-Responsive Element Binding Protein/ Homeobox/ WRKY Transcription Factor
APX	Ascorbate Peroxidase
AsA	Ascorbate
Asmap1	Avena sativa Mitogen-Activated Protein Kinase 1
ASMT	Acetyl Serotonin Methyltransferase
Aspk11	Avena sativa Protein Kinase 11
ATGs	Autophagy-Related Genes
ATP	Adenosine Triphosphate
BADH	Betaine Aldehyde Dehydrogenase
bHLH36	Basic Helix-Loop-Helix 36
CaMIPS	Citrus sinensis Myo-Inositol-1-Phosphate Synthase
CAMT	Caffeic Acid O-Methyltransferase
CaMYB73	Citrus sinensis Myeloblastosis 73
CAND2/PMTR1	Candidate 2/Phyto-Melatonin Receptor 1
Car	Carotenoids
CaSLAH1	Citrus sinensis Salt Overly Sensitive 1-like Halophyte 1
CAT	Catalase

Chl	Chlorophyll
CK	Cytokinin
COMT	Caffeic Acid O-Methyltransferase
CRISPR-Cas9	Clustered Regularly Interspaced Short Palindromic Repeats-Associated Protein 9
DELLA	Named after a conserved amino acid sequence: **D**-Aspartate, **E**-Glutamate, **L**-Leucine, **L**-Leucine, **A**-Alanine
DOF36	DNA-Binding with One Finger 36
DOF47	DNA-Binding with One Finger 47
DREB2	Dehydration-Responsive Element-Binding Protein 2
DTI	Drought tolerance index
EC	Electrical conductivity
EIL1	Ethylene Insensitive Like 1
EIL3	Ethylene Insensitive Like 3
El	Electrolyte leakage
ELISA	Enzyme-Linked Immunosorbent Assay
ERF2	Ethylene Response Factor 2
ETR4	Ethylene Receptor 4
FLC	Flowering locus C
FT	The flowering locus T
Fv/Fm	Maximum quantum efficiency of photosystem II
G protein	Guanine nucleotide-binding protein
GA	Gibberellic acid
GC-MS	Gas Chromatography-Mass Spectrometry

GPA1	G Protein Alpha Subunit 1
GPX	Glutathione Peroxidase
GR	Glutathione Reductase
GSH	Glutathione
Gα	G Protein Alpha Subunit
Gβ	G Protein Beta Subunit
$H2O2$	Hydrogen Peroxide
HPLC	High-Performance Liquid Chromatography
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
LBD4	Lateral Organ Boundaries Domain 4
MDA	Malondialdehyde
MdCYP707A1	Malus domestica Cytochrome P450 707A1
MdCYP707A2	Malus domestica Cytochrome P450 707A2
MdNCED3	Malus domestica 9-cis-Epoxycarotenoid Dioxygenase 3
MOF1	Modifier of Floral Organ Abscission 1
MT	N-acetyl-5-methoxytryptamine
MYB	Myeloblastosis Transcription Factor
MYB108A	Myeloblastosis 108A Transcription Factor
MYB4	Myeloblastosis 4
NAA	1-Naphthaleneacetic acid
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NHX1	Na+/H+ Exchanger
NO	Nitric Oxide

NR	Nitrate Reductase
OsABI5	Abscisic Acid Insensitive 5 in Oryza sativa
OsSGT1	Suppressor of the G2 Allele of SKP1 in Oryza sativa
P5CS	Pyrroline-5-Carboxylate Synthase
PAO	Pheophorbide A oxygenase
Phyto-MT	Phytomelatonin
PMTR1	Phytomelatonin Receptor 1
POD	Peroxidase
PSII	Photosystem II
R. asiaticus	*Ranunculus asiaticus*
RBCS	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit
REV1	REVOLUTA 1
RIA	Radioimmunoassay
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RWC	Relative Water Content
SIFR	Shelf-Life Fruit Regulator
SlCOMT1	Solanum lycopersicum Caffeic Acid O-Methyltransferase 1
SNAT	Serotonin N-Acetyltransferase
SOD	Superoxide Dismutase
STI	Salt tolerance index
T5H	Tryptophan 5-Hydroxylase
TDC	Tryptophan Decarboxylase

TFs	Transcription Factors
TPH	Tryptophan Hydroxylase
WRKY1	WRKY Transcription Factor 1
WRKY4	WRKY Transcription Factor 4
ZmPMTR1	Zea mays Phyto-Melatonin Receptor 1

1 INTRODUCTION

Horticultural plants are increasingly exposed to various forms of abiotic stress, which can significantly impede their growth and productivity (Ahmad et al., 2024). These stresses, caused by non-living environmental factors such as extreme temperatures, heavy metals, drought, and salinity, pose a serious threat to agricultural sustainability (Ahmed et al., 2021); (Hassan et al., 2022); (Altaf et al., 2023). It is estimated that approximately 90% of arable land is vulnerable to one or more of these stresses (Dos Reis et al., 2012). As climate change exacerbates these conditions, understanding how plants respond to and cope with such stresses becomes crucial for maintaining plant productivity, yield, and quality. Abiotic stress not only disrupts plant physiology but also destabilizes ecosystems, reducing biodiversity and threatening food security.

Among the various abiotic stresses, drought and salinity are particularly concerning (Tombesi et al., 2018); (Porcel et al., 2012). Drought stress occurs when water availability is insufficient for normal plant growth, leading to physiological and biochemical changes that impair photosynthesis, nutrient uptake, and overall plant health (Bidabadi et al., 2020); (Rafi et al., 2019). Severe drought-induced stress can significantly impact plant functions, causing reductions in relative water content, water potential of leaves, turgor loss, cell size reduction, decreased photosynthetic pigments, and disturbances in various metabolic processes (Sadak et al., 2020). Prolonged water deficiency can lead to an imbalance in cellular redox components, where the accumulation of reactive oxygen species (ROS) overwhelms antioxidant defenses, leading to oxidative damage and impaired plant growth (Munné-Bosch et al., 2001).

Salinity stress, on the other hand, results from excessive soluble salts in the soil, leading to osmotic stress and ion toxicity (Shabala and Cuin, 2008); (Pandolfi et al., 2012). This condition disrupts water uptake and nutrient balance, ultimately affecting plant metabolism and growth (Trușcă et al., 2023). Both drought and salinity stress are becoming increasingly prevalent due to climate variability and human activities, making it essential to explore effective mitigation strategies (Smith et al., 2020); (Besser et al., 2021).

Ranunculus asiaticus L., commonly known as the "buttercup," is a perennial geophyte with tuberous roots belonging to the *Ranunculaceae* family. Endemic to the Mediterranean basin and Asia Minor, it is cultivated primarily for its various colorful terminal flowers used in the floral industry (Karlsson, 2003). The species has gained popularity due to the emergence of numerous hybrids (Margherita et al.1996); (Beruto et al., 2018). However, cut flowers like *R. asiaticus* are generally not recommended for cultivation in saline soils or water-recycling systems due to their intolerance to high salt concentrations in irrigation water and soil, as well as their sensitivity to drought conditions (Caser et al., 2019). Water shortages, particularly in arid and semi-arid regions, pose a significant challenge for floral industries, which compete for water with other sectors such as agriculture and urban management. Both drought and salinity can severely impact the quality of blooms, shorten plant stems, and reduce floral crop production (Carter and Grieve, 2008); (Grieve, 2011). Therefore, it is crucial to evaluate more floricultural varieties for their abiotic stress tolerance to ensure sustainable development from both environmental and economic perspectives.

Melatonin, a well-known hormone in animals, has also been identified as a crucial signaling molecule in plants. Known as N-acetyl-5-methoxytryptamine, melatonin is a versatile and multifaceted indolamine derived from tryptophan, with serotonin serving as a critical metabolic intermediate (Ahammed and Li, 2022); (Arabia et al., 2023). Melatonin is a universal molecule with pleiotropic effects, exhibiting a wide range of activities in various plant species (Arnao et al., 2022). Similar to plant hormones, melatonin plays a vital role in regulating plant growth, development, and adaptability to adverse environmental conditions (Chen and Arnao, 2022). It acts as a potent antioxidant, directly scavenging ROS and improving ROS homeostasis by regulating the antioxidant system, thereby effectively mitigating oxidative stress (Pardo-Hernández et al., 2021). Its application has been shown to enhance plant resilience against both drought and salinity stress by regulating physiological and biochemical pathways, including improving photosynthetic efficiency and promoting root development (Wang et al., 2024).

This research focuses on investigating the effects of melatonin on *Ranunculus asiaticus* under conditions of salinity and drought stress. The study aims to elucidate the role of melatonin in alleviating the harmful effects of these stresses, with a focus on physiological and biochemical aspects. By examining how melatonin influences growth parameters and stress tolerance mechanisms in *R. asiaticus*, this study will contribute to a better understanding of its potential as a biostimulant in horticulture, offering valuable insights into sustainable practices for managing abiotic stress in ornamental plant production.

1.1. WORK AIM AND SPECIFIC OBJECTIVES

This Work aims to:

This work aims to investigate the role of exogenous melatonin in enhancing drought and salinity tolerance in *R. asiaticus* by improving its morphophysiological and biochemical attributes under stress conditions.

Specific Objectives:

1. Assess the Impact of Drought and Salinity Stress:
   - To evaluate the negative effects of drought and salinity stress on the growth and physiological parameters of *R. asiaticus*.
2. Evaluate the Role of Melatonin in Drought and Salinity Tolerance:
   - To assess the effectiveness of exogenously applied melatonin (MT) at concentrations of 50, 100, and 200 µM in mitigating the negative effects of drought and salinity stress on *R. asiaticus*.
3. Examine Plant Growth Responses:
   - To study the impact of various concentrations of melatonin (0, 50, 100, and 200 µM) on the morpho- physiological attributes of *R. asiaticus* under normal, saline, and drought conditions.
4. Measure Physiological Responses:
   - To analyze changes in various physiological parameters, such as chlorophyll content, relative water content, and oxidative stress markers, under different drought, salinity, and melatonin treatments.
5. Evaluate Antioxidant Defense Mechanisms:
   - To assess the influence of melatonin on antioxidant defense systems, including peroxidase enzyme activity (POD), under drought and salt stress.
6. Identify Drought and Salinity Tolerance Indicators:
   - To establish key indicators, such as POD and proline contents, that can be used as markers for assessing drought and salinity tolerance in *R. asiaticus*.
7. Determine Optimal Melatonin Concentration:
   - To identify the most effective concentration of melatonin for enhancing growth, drought, and salinity tolerance in *R. asiaticus*.

2 LITERATURE REVIEW

Phyto-melatonin, scientifically known as N-acetyl-5-methoxytryptamine (MT or Mel) (Fig. 1), was first identified in vascular plants in 1995 (Yang et al., 2022). Three research teams from Germany (Sharma et al., 2024), Japan (Hattori et al., 1995), and Czechoslovakia (Kolář et al. 1995) independently identified melatonin (N-acetyl-5-methoxytryptamine), a biological indolamine that was originally discovered in 1954 in the pineal gland of bovine brains (Lerner et al., 1958), where it was noted for its skin-lightening effects in frogs (Arnao et al., 2022). Structurally akin to auxin and indole-3-acetic acid (IAA), this molecule is highly conserved across various species. Historically, melatonin was thought to be exclusive to animals and humans, where it is recognized for its roles in enhancing immunity, delaying the aging process, and regulating circadian rhythms (Arnao and Hernández-Ruiz, 2014b). However, a pivotal finding by Jackson (1967) revealed trace amounts of melatonin in the endosperm of *Hydrangea lily*, expanding the known range of this molecule. The first successful extraction of plant-derived melatonin, achieved by Dubbels et al. in (1995) through gas chromatography/mass spectrometry, led to its identification in multiple crops, including rice (Li et al., 2022), maize (Muhammad et al., 2022), tomato (Xie et al., 2022), cucumber (K.Liu et al., 2022), grape (Ma et al., 2021), apple (Cao et al., 2022), banana (J.Wei et al., 2022) and medicinal herbs (Chen et al., 2003), thus confirming its widespread occurrence within the plant kingdom.

To distinguish plant-derived melatonin from its animal counterpart, Blask (2004) coined the term "phytomelatonin." The concentrations of phytomelatonin in plant tissues vary considerably, ranging from picograms to micrograms per gram, depending on the species and tissue type (Kanwar et al., 2018). Given its structural similarity to auxin, extensive research has been conducted on the functional roles of Phyto-MT, which is primarily recognized for its potent antioxidant properties. Phytomelatonin is critical in regulating (ROS), reactive nitrogen species (RNS), free radicals, and other oxidative agents within plants. Research has demonstrated that phyto-MT acts as a growth regulator, promoting plant growth and enhancing resilience to environmental stresses. Despite its low concentrations, Phyto-MT is vital for various physiological processes, including seed germination (Castañares and Bouzo, 2019), root development (Ji et al., 2022), leaf senescence (K.Wang et al., 2022), photosynthesis (K. Liu et al., 2022), stomatal movement (J.Wei et al., 2018), circadian rhythm regulation (Matthews et al., 2018), floral transition (Zhang et al., 2019), postharvest fruit ripening (Sun et al., 2016), and plant responses to both biotic and abiotic stresses (Song et al., 2023).

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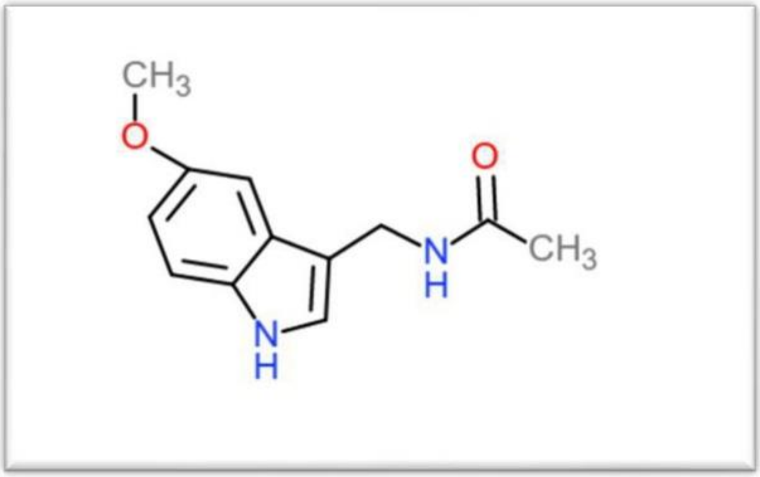

Figure 1 Melatonin structure: N-acetyl-5-methoxytryptamineBiosynthesis and metabolism of phytomelatonin in plants**.**

Melatonin is recognized as a chronoregulator and sleep hormone, functioning in various biological contexts across both animal and plant systems, as highlighted in the literature from 1995 (Socaciu et al., 2020). Melatonin, characterized by its molecular mass of 232.28 g/mol, density of 1.269 g/cm^3, and chemical formula $C_{13}H_{16}N_2O_2$ (Registry No. 73-31-4, CAS Common Chemistry; American Chemical Society), is an endogenous indolamine present in a wide range of organisms throughout evolutionary history. Its synthesis is derived from the amino acid tryptophan, contributing to the molecular architecture of phyto-melatonin. The nomenclature surrounding melatonin often distinguishes between animal-derived or synthetically produced melatonin and phyto-melatonin, which is specifically derived from plant sources. The term "phyto-melatonin" was first introduced in a 2004 study that investigated its potential in liver cancer treatment in rats (Arnao and Hernández-Ruiz, 2018).

Tryptophan is metabolized into melatonin and phyto-MT via established biochemical pathways in both animals and plants (Back et al., 2016). The initial step is catalyzed by the enzyme tryptophan decarboxylase (TDC), which converts tryptophan into either tryptamine or serotonin from 5-hydroxytryptophan. Following this, tryptophan 5-hydroxylase (T5H) facilitates the conversion of tryptophan into 5-hydroxytryptophan and transforms N-acetyl tryptamine into N-acetyl serotonin. The enzyme serotonin N-acetyltransferase (SNAT) is responsible for transferring an acetyl group from acetyl coenzyme A to various biomolecules. This process is succeeded by 5-hydroxyindole O-methyltransferase, which converts N-acetyl serotonin into phyto-melatonin (Moustafa-Farag et al., 2020). Moreover, caffeic acid O-methyltransferase (COMT), an enzyme found in plants, is capable of methylating N-acetyl serotonin as well as other substrates, including caffeic acid and quercetin. In plant systems, ASMT and COMT can also convert serotonin into 5-methoxytryptamine, which contributes to the synthesis of phyto-melatonin through the action of SNAT. Studies on the Sekiguchi mutant rice have indicated an alternative metabolic pathway where N-acetyl tryptamine is produced and then converted into N-acetyl serotonin (Park et al., 2012). Finally, both tryptophan hydroxylase (TPH) and TDC, which are primarily active in mammals but also found in plants, are involved in the conversion of 5-hydroxytryptophan into serotonin. The enzymes involved in the metabolic steps leading to melatonin and its precursors are as follows (Tan et al., 2016):

- **T5H:** Tryptophan 5-hydroxylase
- **TDC:** Tryptophan decarboxylase
- **TPH:** Tryptophan hydroxylase
- **SNAT:** Serotonin N-acetyltransferase
- **ASMT:** Acetyl serotonin methyltransferase
- **COMT:** Caffeic acid O-methyltransferase

These enzymes play crucial roles in the conversion of tryptophan and its derivatives into melatonin and phyto-melatonin in both animal and plant systems. The predominant pathways for melatonin synthesis in plants and animals are depicted using solid and dotted lines, respectively (Fig.2).

The conversion of **tryptophan → tryptamine → serotonin → N-acetyl serotonin → phyto-melatonin** is a crucial pathway for the production of (phyto) melatonin. The enzymes involved in this process, with the exception of tryptophan hydroxylase (TPH), which is predominantly recognized in animals rather than in plants, have been identified and characterized in species such as rice and *Arabidopsis*.

In animals, the primary metabolic pathway is as follows: **tryptophan → 5-hydroxytryptophan → serotonin → N-acetyl serotonin → melatonin.** Various subcellular compartments, including the cytoplasm, endoplasmic reticulum, chloroplasts, and mitochondria, are responsible for generating intermediates of melatonin. In plants, melatonin is frequently hydroxylated, resulting in the formation of 2-, 3-, and 6-hydroxy-melatonin, with 2-hydroxymelatonin being the main metabolite. Consequently, melatonin does not serve as the final product in this metabolic pathway (Byeon and Back, 2014).

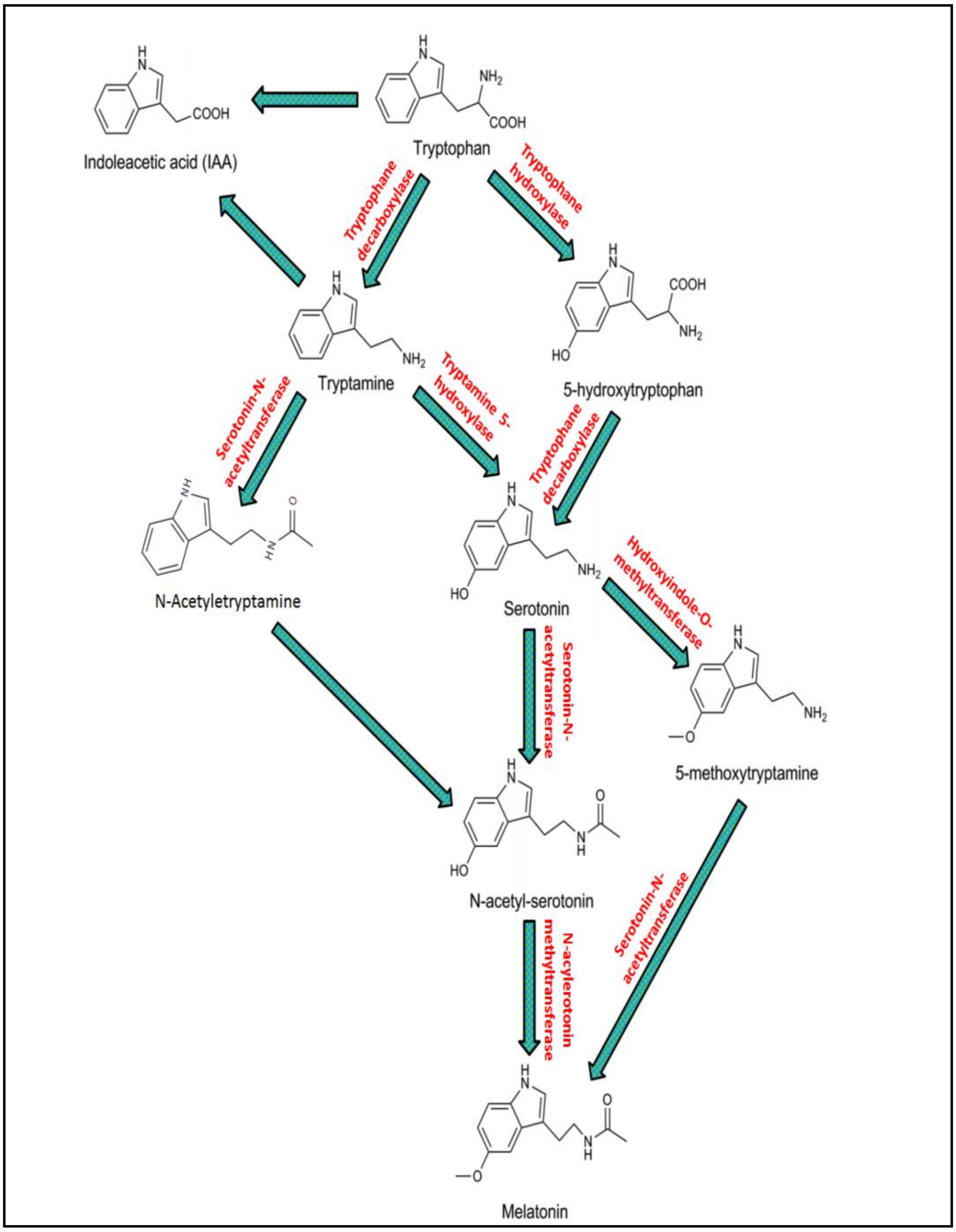

Figure 2 The biosynthesis of melatonin in plants originates from the amino acid tryptophan, involving a series of enzymatic reactions. The key enzymes responsible for each step include tryptophan 5-hydroxylase (T5H), tryptophan decarboxylase (TDC), serotonin N-acetyltransferase (SNAT), and hydroxyindole-O-methyltransferase (HIOMT). Additionally, alternative pathways for these reactions have been identified in specific cases, Modified from Zhang et al. 2014 b.

Melatonin plays a significant role in regulating various aspects of plant growth and development while also enhancing the ability of plants to manage and mitigate diverse stressors (Li et al., 2019 and Qi et al., 2018). Recent studies indicate that calcium ions (Ca^{2+}) may function as a receptor for phytomelatonin in *Arabidopsis thaliana* (J.Wei et al., 2018). This plasma membrane protein exhibits characteristics typical of a receptor and has the capacity to bind melatonin, subsequently interacting with the G protein subunit GPA1. In cells that express Ca^{2+}/PMTR1, melatonin secretion is regulated, which in turn influences the synthesis of Ca^{2+}/PMTR1. Upon the binding of melatonin to Ca^{2+}, the Gα and Gβ subunits dissociate, potentially leading to the stimulation of NADPH oxidase-dependent H_2O_2 production, which promotes potassium (K^+) efflux and Ca^{2+} influx, ultimately resulting in stomatal closure. Notably, this stomatal closure was not observed in Ca^{2+} knockout mutants.

Conversely, findings reported by Lee and Back (2020) challenge this perspective, suggesting that Ca^{2+} is neither localized in the plasma membrane nor involved in the activation of melatonin-triggered defense genes. Moreover, the melatonin signaling pathway appears to operate independently of the downstream Gα and Gβ subunits. Earlier research has also indicated that melatonin treatment does not induce H_2O_2 production in healthy, unstressed plants (Lee and Back, 2017, and Ma et al., 2018), although some studies have produced conflicting results (Chen et al., 2018). It has been proposed that Ca^{2+} may act as a melatonin-binding protein in plants, distinct from conventional melatonin receptors. While some evidence suggests that melatonin is implicated in H_2O_2 production, the mechanisms underlying this relationship remain a topic of debate (Lee and Back, 2017).

2.1. Melatonin: A key regulator of plant growth and physiological processes

Melatonin, a plant hormone, is present in nearly all plant species, with its concentration differing across tissues and in response to light conditions. Initially identified in 1993 in species such as Convolvulaceae ivy morning glory (*Pharbitis nil* L., syn. *Ipomoea nil* L.) and tomato (*Solanum lycopersicum* L.) (Arnao, 2014a), melatonin is particularly abundant in plant families like Rosaceae, Vitaceae, Poaceae, Apiaceae, and Brassicaceae. Certain species are known to have significantly higher endogenous levels of the hormone (Vantassel et al.,1993 and Vantassel et al., 1995). MT is transported via the xylem to various plant tissues, with evidence suggesting its movement within chloroplasts and mitochondria (Arnao and Hernández-Ruiz, 2018).

The variation in melatonin content is influenced by factors such as species, geographic location, growth stage, specific plant organs, and the timing of harvest (Okazaki and Ezura, 2009). This hormone plays a crucial role in regulating plant growth processes (Y.Wei et al., 2018). Various methods have been employed to measure melatonin levels in plants, including radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) with electrochemical, fluorescence, or mass spectrometric detection (Feng et al., 2014). Melatonin production is highly dependent on photoperiod and seasonal shifts, introducing uncertainty in measured values unless circadian rhythms, seasonal factors, and plant age are accounted for (Luboshitzky et al., 1998 and Underwood, 1989).

The stimulatory effect of exogenous phyto-melatonin has been widely investigated in various crop studies, demonstrating its role as a signal molecule that regulates several physiological and biochemical activities (Ye et al., 2020). It enhances plant growth by improving coleoptile length in canary grass, barley, and wheat (Hernández-Ruiz and Arnao, 2005), and boosts seed vigor and quality in maize (Kołodziejczyk et al., 2016). Melatonin treatment in soybean has been shown to increase leaf growth, plant height, and seed production (Wei et al., 2015). It also improves photosynthetic activity, redox balance, root development, and seminal root elongation in crops like barley, wheat, and rice (Park and Back, 2012; Liang et al., 2017; Zuo et al., 2017; Li et al., 2016; Sarropoulou et al., 2012; Li et al., 2015 and Denancé et al., 2013).

Various strategies have been employed to mitigate the detrimental effects of abiotic stress, including the application of exogenous hormones, advancements in genetically modified organisms, the development of stress-tolerant cultivars, and the adoption of modern gene-editing technologies such as CRISPR-Cas9 (Ahmad et al., 2022). Melatonin, an effective anti-stressor hormone, provides both physiological and molecular resistance against a wide range of abiotic stresses by modulating stress signaling pathways (Liang et al., 2018; Posmyk et al., 2008; N. Zhang et al., 2014; Ren et al., 2019 and R. Ahmad et al., 2023). Melatonin's positive impact on photosynthesis and growth-related factors across various crops under different abiotic stresses highlights its potential as a promising application (Meng et al., 2014 and Wang et al., 2016). Earlier studies have shown that the exogenous application of phyto-melatonin can mitigate the negative effects of drought stress in *Moringa oleifera* (Sadak et al., 2020), salt stress in watermelon (Sadak et al., 2020), and cold stress in cucumber (Posmyk et al., 2009). In cucumbers, melatonin enhances seedling growth, nutrient uptake, and nitrogen metabolism, particularly under salt stress (R. Zhang et al., 2017). These improvements in growth and yield are likely due to phyto-melatonin's ability to enhance cell elongation, maintain shoot water content, reduce osmotic stress, and boost plant antioxidant activities (Arnao and Hernández-Ruiz, 2015;

Jan et al., 2023). Recent findings align with those of previous studies, demonstrating that phyto-melatonin treatment effectively mitigates the adverse effects of abiotic stress, leading to improved plant growth and yield (I.Ahmad, et al., 2023; Sharma et al., 2024) with minimal environmental impact.

2.1.1. MT as a biostimulant

Melatonin (MT) plays a crucial role in regulating signaling components, particularly auxin receptors and regulators, which are essential for activating growth processes (Park et al., 2013). It has been observed that MT promotes the formation of both lateral and adventitious roots across different plant species, often in conjunction with auxins such as indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA), which it shares structural and functional similarities (I.Ahmad et al., 2023). Additionally, melatonin interacts with various plant hormones and regulates their effects, including auxin, ethylene, cytokinin (CK), absisic acid (ABA) and gibberellic acid (gibberellins, GA), in a complex network (Zhang et al., 2017), thereby enhancing IAA production, and both compounds (MT and IAA) synergistically promote root morphogenesis (Arnao and Hernández-Ruiz, 2018; Murch et al., 2001). MT demonstrates considerable auxinic activity, exhibiting a growth-promoting efficacy that is 63% greater than that of IAA in *Lupinus albus* (Hernandez-Ruiz et al., 2004) and promoting root regeneration (Arnao and Hernández-Ruiz, 2007). Exogenous MT has been found to enhance root activity in *Brassica juncea* (Chen et al., 2009) and root organogenesis in *Mimosa pudica* (Giridhar and Ravishankar, 2009). In rice, MT regulates root architecture, restricting embryonic root growth and encouraging lateral root growth (Park and Back, 2012). It also improves the root system through its effects on the auxin signaling pathway (Liang et al., 2017). MT has been found to promote GA content in cotton seedlings (Xiao et al., 2019), and induce parthenocarpy in pears (Zeng et al., 2018). In plants subjected to heat stress, CK levels typically degrade over time; however, treatment with exogenous melatonin has been found to stimulate CK biosynthesis, improving the plants' resistance to heat stress through elevated CK levels (J. Zhang et al., 2017).

2.1.2. The Role of Melatonin in Floral Transition

Recent research has identified a novel function for melatonin as a growth regulator in controlling flowering time (Arnao and Hernández-Ruiz, 2020). Early studies demonstrated that disrupting signaling in *Chenopodium rubrum* leaves using electrical currents interfered with flowering, potentially due to alterations in melatonin rhythmicity (Kolář et al., 2003). Notably, *C. rubrum* was the first plant species in which melatonin was found to play a circadian regulatory role (Wolf et al., 2001). Further evidence of melatonin's involvement in floral regulation emerged from transgenic rice plants carrying the *SNAT* gene from sheep, where flowering was significantly repressed (Byeon and Back, 2014). While melatonin does not directly influence the phase of flowering, it modulates key processes involved in the floral transition.

The significance of melatonin in the shift from vegetative to reproductive development was further elucidated by Shi et al. (2016), who proposed a mechanistic model for this regulation. According to their findings, melatonin suppresses floral transition by stabilizing DELLA proteins, thereby disrupting transcription factors associated with flowering. This regulatory function has been corroborated by several studies (Shi et al., 2016; Arnao and Hernández-Ruiz, 2020) (Fig.3.A).

Recent investigations have also highlighted the interplay between melatonin and other signaling molecules in flowering regulation. Zhang et al. (2019) established a direct connection between melatonin and strigolactones carotenoid-derived phytohormones known to influence flowering. Their findings suggest that strigolactones function upstream of melatonin, delaying flowering through the activation of *FLC* when melatonin levels exceed a specific threshold. Additionally, nitric oxide (NO), whose synthesis is induced by melatonin, has been shown to increase DELLA protein levels (Lozano-Juste and León, 2011) and repress flowering in *Arabidopsis* (He et al., 2004). Shi et al. (2016) were the first to report a direct link between melatonin and the floral transition, proposing that NO may contribute to melatonin-mediated stabilization of DELLA proteins, ultimately delaying flowering. However, further research is required to elucidate the precise role of strigolactones in regulating melatonin biosynthesis, as well as the complex crosstalk between NO and melatonin in flowering control.

2.1.3. MT acts as a stress-protective agent

MT may also act as a stress-protective agent, facilitating reproductive success under varying conditions. MT can delay leaf senescence by preserving chlorophyll activity, which is crucial for plant productivity. It slows senescence in barley leaves (Arnao and Hernández-Ruiz, 2009) and increases net photosynthetic rate and chlorophyll content in maize (Ahmad et al., 2021a). In ryegrass, MT aids recovery from heat-induced leaf withering and growth inhibition (J. Zhang et al., 2017). MT also influences carbon fixation during aging and activates genes involved in phytohormone signaling pathways (M. Wang et al., 2019).

2.1.3.1. Anti-senescence

MT acts as an anti-senescence agent by altering redox parameters, such as lowering ROS levels, increasing ascorbate (AsA) and glutathione (GSH) levels, and enhancing antioxidant enzyme activities. MT administration blocked the activation of several autophagy-related genes (ATGs) and the senescence-associated gene Hexokinase-1, which typically occurs during the final stage of leaf senescence (Shi et al., 2015b).

2.1.3.2. Postharvest quality

MT has been shown to significantly influence postharvest produce quality and shelf life (Cao et al., 2018). It mitigates oxidative stress, enhances the production of antioxidant enzymes, and prevents postharvest deterioration (Hu et al., 2018; Cao et al., 2018). Additionally, melatonin regulates signalling pathways involving salicylic acid (SA), jasmonic acid (JA), nitric oxide (NO), and ethylene, which together enhance disease resistance (Zhu and Lee, 2015; and Arnao and Hernández-Ruiz, 2018). By influencing the ethylene biosynthesis pathway, MT improves aroma, color, sugar content, and overall postharvest quality (Sun et al., 2015). As a result, it holds the potential for reducing postharvest losses and extending shelf life. Recent studies have demonstrated that silencing the fruit shelf-life regulator (SIFR) gene can control postharvest ripening and prolong shelf life by inhibiting ethylene production (Zhang et al., 2018). However, further research is required to understand how exogenous MT affects postharvest maturity through the regulation of the SIFR gene (Sharif et al., 2018).

Overall, melatonin is recognized as a biological plant growth regulator, significantly contributing to improved plant productivity (Fig. 3.B).

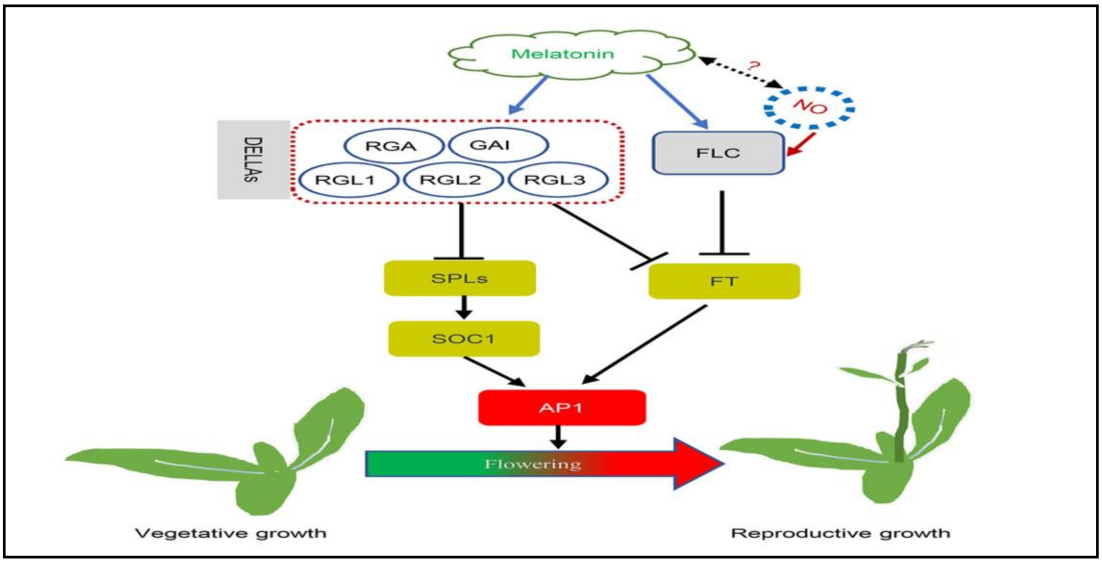

Figure 3 A. Melatonin modulates the expression of DELLAs and FLC, thereby delaying the floral transition in plants. Created by Sun et al., 2021

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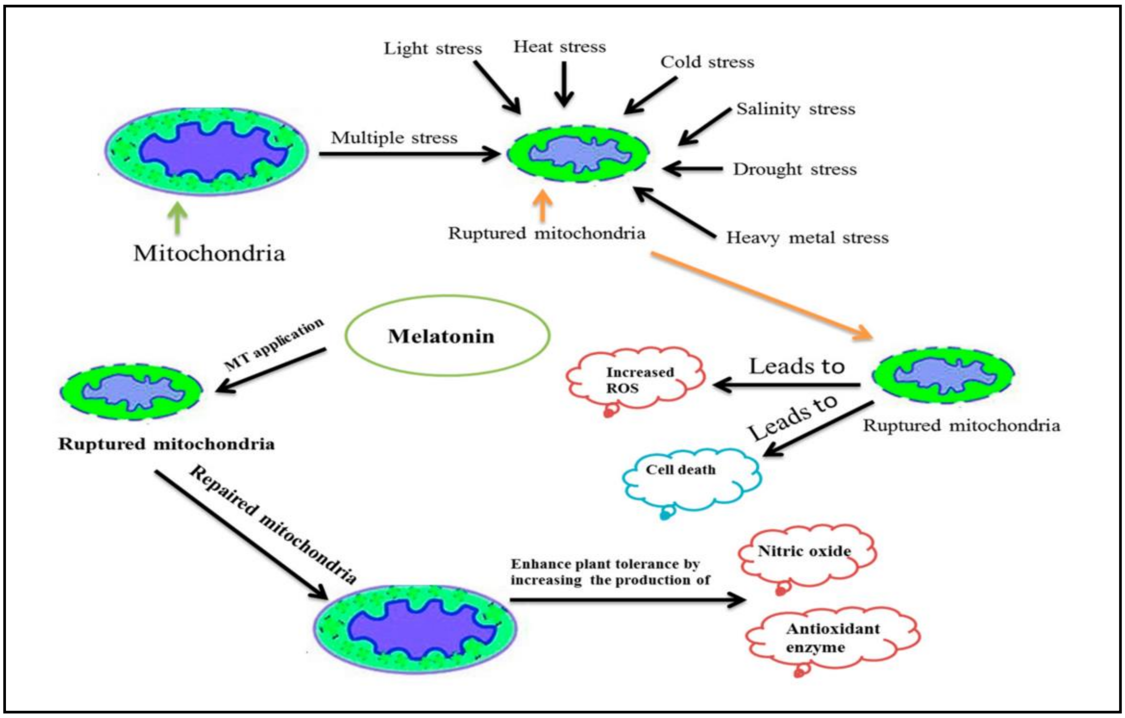


Figure 3. B. Diagrammatic representation of the melatonin defense mechanism pathway. Created by Sharif et al., 2018.

2.2. Melatonin: A crucial mediator in alleviating abiotic stress in plants

Abiotic stress such as (cold temperatures, salinity, heavy metals, waterlogging, heat, and ultraviolet radiation), exacerbated by climate change, disrupts the morpho-physiological activities and yield of plants (Bera et al., 2022). This stress significantly alters the functioning of various physiological processes and overall metabolic health by increasing the formation of ROS, which can lead to lipid and protein damage, and, in severe cases, cell death. To counteract the negative effects of ROS, plants have evolved a range of defense mechanisms, including the production of enzymes such as superoxide dismutase (SOD), catalase (CAT), and POD (Jan et al., 2023; Song et al., 2023; Bartwal et al., 2013). However, prolonged exposure to abiotic stress can overwhelm these protective systems, rendering them ineffective against ROS-induced damage (Bhardwaj et al., 2023).

MT serves as a vital antioxidant in plants, capable of moving across plant tissues due to its lipophilic nature, which allows it to interact with cell membranes and lipid-rich environments (Tan et al., 2013). Moreover, a key component of the sophisticated defense mechanisms plants have evolved to combat the harmful effects of abiotic stresses involves enzymes such as CAT, POD, and SOD. These enzymes play a crucial role in minimizing oxidative damage by reducing the excessive accumulation of ROS (Mittler, 2002). Previous studies have shown that barley and wheat are particularly vulnerable to salt stress, which may be attributed to reduced stomatal conductance or increased accumulation of ROS. This excess ROS triggers oxygen-induced cellular damage in plants, exacerbating the effects of salt stress. Various studies have demonstrated that phyto-MT can enhance antioxidant activity and improve stomatal conductance across a range of crops (S.Khan et al., 2022). Phyto-MT boosts plant antioxidant activity, effectively reducing excess ROS and protecting plants from oxidative damage induced by abiotic stress (Ahmad et al., 2021b). The use of phyto-MT has proven effective in alleviating drought stress and enhancing morpho-physiological functions in crops like citrus and *Phaseolus vulgaris* (Fazal et al., 2018). By bolstering the antioxidant defense system and improving stomatal conductance, phyto-melatonin supports various plants in coping with environmental challenges. However, the exact mechanisms through which it achieves these protective effects under abiotic stress conditions are yet to be fully understood.

2.2.1. Melatonin modulates plant redox status and enhancing plant defense mechanisms under abiotic stress

Redox signalling, especially through ROS-mediated pathways, has lately been acknowledged as a vital mechanism that interacts with hormone-mediated signalling in plants (Xia et al., 2015). The redox idea involves the coordinated equilibrium of several redox pairs in plant cells. Studies demonstrate that unique redox patterns develop at various stages of plant growth, regulated by the synchronised function of ROS-related enzymes, such as SOD, CAT, POD, ascorbate oxidase (AO), glutathione peroxidase (GPX), and glutathione reductase (GR) (Noctor et al., 2012). Abiotic stress is recognised for altering the redox equilibrium in plants (Zinta et al., 2016), wherein minimal quantities of ROS serve as signalling molecules, whereas excessive ROS buildup can induce oxidative stress, hindering growth and destroying macromolecules (Mittler, 2002). Comprehending the impact of stress on redox state is crucial for enhancing plant development and productivity.

In this context, previous studies have demonstrated that MT can mitigate the excessive accumulation of ROS and RNS, thereby enhancing cellular redox status under abiotic stress (Tiwari et al., 2021). This regulation is facilitated through the melatonin-mediated stimulation of redox enzymes, including SOD, POD, CAT, and ascorbate peroxidase (APX) (Debnath et al., 2019). Its effectiveness is notably superior, being five times greater than that of GSH in *Salvia sp*. and fifteen times greater than mannitol (Bidabadi et al., 2020; Naveen Kumar et al., 2020). MT enhances wheat's resistance to low-temperature stress (Kumar et al., 2021; and Wu et al., 2022). Additionally, under high-temperature stress, applying an optimal concentration of melatonin increases the activities of APX and POD by 43.7% and 45.5%, respectively, positively impacting the growth of cherry radish (Jia et al., 2020). In *Prunus persica,* in the presence of 200 µmol·L^{-1} MT, there was an increase in the activities of SOD and POD, accompanied by a decrease in H_2O_2 and ethylene levels, suggesting that melatonin effectively mitigates oxidative stress caused by waterlogging (Jia et al., 2020). Similarly, exogenous MT improved the resistance of pear, *Solanum lycopersicum* and *Clematis sp.* (*C. tientaiensis, C. lanuginosa,* Sen-No-Kaze, and Viva Polonia) to abiotic stresses by enhancing antioxidant enzyme activity and lowering the levels of H_2O_2 and MDA (Liu et al., 2019; Chen et al., 2024).

MT has been shown to alleviate heat stress in tomatoes and soybeans by balancing redox through the modulation of NO and polyamine biosynthesis (Imran et al., 2021). In cotton, phyto-melatonin treatment has been found to regulate the expression of redox-related genes, thus mitigating the adverse effects of salt stress (Y.Zhang et al., 2021). Additionally, MT modulates specific leaf senescence proteins that interact with photosynthesis regulation and macromolecules, influencing redox changes and the plant's response to abiotic stress (Sun et al., 2015). Phyto-MT has been shown to enhance antioxidant activities and mitigate chromium stress by promoting the production of phyto-chelatins and facilitating the compartmentalization of chromium in vacuoles and cell walls (Ayyaz et al., 2022).

Various studies have illustrated that phyto-melatonin not only boosts the antioxidant defense system but also interacts with other resistance pathways, including the regulation of macro-nutrients and gene expression, thereby improving plant resilience under abiotic stress conditions (I.Ahmad et al., 2023; Ahmad et al., 2024; Wang et al., 2024).

2.2.2. MT's influence on plant physiological and biochemical responses to abiotic stress

Melatonin has been shown to mitigate chlorophyll degradation under abiotic stress, enhance leaf photosynthetic rates by boosting Rubisco activity, and promote plant dry matter accumulation (Yang et al., 2018). It also reduces the expression of pheophorbide A oxygenase (PAO), a key gene involved in chlorophyll degradation, while increasing the expression of RBCS, a critical photosynthetic gene, thus improving photosynthetic efficiency under drought conditions (Cherono et al., 2021). Additionally, exogenous foliar application of melatonin has been found to elevate chlorophyll content and the maximum quantum efficiency of photosystem II (Fv/Fm) in *Sorghum bicolor* seedlings, leading to an enhanced rate of photosynthesis (R.Zhang et al., 2021).

Non-enzymatic antioxidant protective substances, such as proline, soluble proteins, and soluble sugars, play a crucial role in plant stress responses. These compounds, acting as primary osmoregulatory agents, can enhance the activity of related enzymes or serve as their substrates.

They also contribute to lowering the osmotic potential of the cell cytoplasm, facilitating water uptake from the external environment, and regulating cellular osmotic pressure to mitigate the effects of waterlogging stress. Consequently, these substances can improve plant survival under such conditions (J.Wang et al., 2019). Moustafa-Farag et al. (2020); Khosraviet al. (2023) have demonstrated that the exogenous application of melatonin increases the concentration of these osmoregulatory substances in various plants, such as kiwifruit and *Capsicum annuum,* thereby alleviating stress-induced damage and enhancing plant resilience. Similarly, Chen et al. (2024) observed that melatonin application enhanced soluble sugar and soluble protein contents in *Clematis* sp. under waterlogging stress, thereby effectively maintaining cellular homeostasis and boosting stress adaptability.

2.2.3. Signaling and Transduction Pathways of Phyto-Melatonin in Response to Abiotic Stress

Transcription factors (TFs) are critical regulators of gene expression and play a vital role in modulating plant responses to various abiotic stresses, one of which is the regulation of stomatal opening and closing, a process influenced by phyto-melatonin signaling (Arnao and Hernández-Ruiz, 2019). In *Arabidopsis thaliana,* the signaling pathway of phyto-MT is mediated by the CAND2/PMTR1 receptor, which governs stomatal movement through G-protein α subunit-regulated hydrogen peroxide (H_2O_2) and calcium ion (Ca^{2+}) signaling pathways (Arnao and Hernández-Ruiz, 2018). This evidence suggests that MT could be considered a potential new candidate for plant hormone classification (Y.Liu et al., 2022).

In a study by Chen et al. (2024), transcriptional sequencing was conducted on wild *Clematis* species with varying degrees of waterlogging tolerance. From this analysis, nine transcription factors were identified as highly correlated with MT and potentially capable of enhancing waterlogging tolerance. Among these, LBD4 and MYB4 showed a significant positive correlation with the antioxidant enzyme system, while bHLH36, DOF36, and WRKY4 exhibited significant negative correlations. Furthermore, photosynthetic capacity was positively associated with DOF36 and WRKY4, but showed significant negative correlations with MYB4, MOF1, DOF47, REV1, and ABR1. These findings suggest a complex regulatory network involving these TFs in the plant’s response to abiotic stress.

Additionally, the study by S. Ahmad et al. (2023), Transcriptomic analysis of *in-vitro* leafless flowering in three *Cymbidium* orchid species identified over 50 differentially expressed genes (DEGs) associated with the tryptophan pathway and melatonin biosynthesis. Key genes in the melatonin pathway included SNAT, CAMT, and TDC. This provides a strong foundation for investigating melatonin as a potential regulator of abiotic stress, contributing to enhanced vegetative growth and flowering in these valuable orchid species.

Applying melatonin exogenously can enhance the expression of genes associated with anthocyanin production in tea leaves subjected to arsenic (AS) stress. This treatment stimulates the biosynthesis and accumulation of anthocyanins, thereby mitigating the adverse effects of AS stress on the photosynthetic system of tea trees (X. Li et al., 2021); (Langaroudi et al., 2023).

Phyto-melatonin affects gene expression in jasmonic acid, salicylic acid, and ethylene signaling pathways (Arnao and Hernández-Ruiz, 2018). It upregulates key ethylene signaling components, such as ERF2, EIL1, EIL3, and receptor genes NR and ETR4 in tomatoes, enhancing ethylene production during fruit ripening (Sun et al., 2015). Additionally, phyto-melatonin treatment increases putrescine and spermidine levels, essential for plant cell growth, in plants like carrots and cucumbers (Zhao et al., 2017).

2.3. Potential role of melatonin in horticultural plants under abiotic stresses

Phyto-melatonin is considered to possess the highest antioxidant capacity among all plant growth regulators and is widely acknowledged for its potent antioxidant properties, positioning it as a highly effective biological stimulant (R.Ahmad et al., 2023). The current study aims to provide a comprehensive overview of the current understanding and potential applications of melatonin in horticultural crops, with a specific focus on the ornamental plant *R. asiaticus*. The goal is to explore how melatonin can be used to enhance the plant's tolerance to drought and salt stresses.

2.3.1. Melatonin's protective role under drought stress conditions

Drought stress poses considerable challenges for horticultural crops, resulting in various detrimental alterations at both molecular and physiological levels, which ultimately compromise growth and productivity (Farooq et al., 2009). Key adverse effects of water stress in plants encompass: Decreased leaf water potential, reduced turgor pressure, inhibited cell expansion, stomatal closure (Farooq et al., 2009). Moreover, drought conditions can profoundly impact a plant's root system architecture, inflict damage on cellular membranes, hinder photosynthetic processes, and disrupt the balance of minerals and nutrients (Tabassum et al., 2021). The ramifications of drought stress on horticultural crops are intricate, affecting everything from molecular mechanisms to broader ecosystem dynamics.

ABA plays a pivotal role in plant drought resistance mechanisms (Hussain et al., 2021). Melatonin, recognized for its role as a biostimulant, has been shown to alleviate oxidative stress caused by water deficits in various species, including *Glycine max* (Ren et al., 2019), *Cucumis* *sativus* (Zhang et al., 2013), and *Vitis vinifera* (Meng et al., 2017). Several studies suggest that phytomelatonin significantly modulates key genes involved in ABA biosynthesis and degradation, such as *MdNCED3*, *MdCYP707A1*, and *MdCYP707A2*, thereby enhancing drought resistance in apple plants (Li et al., 2015). Furthermore, phytomelatonin plays a vital role in regulating the balance between ABA and GA, which is essential for seed germination (H. Li et al., 2022). It also interacts with various hormones and plant growth regulators including zeatin, gibberellin A14, 24-epibrassinolide, and jasmonic acid to bolster plant tolerance to drought stress (Sharma et al., 2020). In studies involving rice seedlings, it was found that mutations in the suppressor of the G2 allele of skp1 (OsSGT1) and abscisic acid insensitive 5 (OsABI5) disrupt the drought tolerance effects mediated by melatonin (Li et al., 2022).

Moreover, Tiwari et al. (2020) highlighted melatonin's ability to safeguard roots and photosynthetic apparatus, enhance antioxidant defenses, and regulate oxidative stress under drought stress. The beneficial effects of melatonin extend to various species, including *Moringa oleifera*, foliar application of melatonin under drought stress positively influenced several growth parameters, such as: dry and fresh weight of shoots and leaves, leaf count, plant height, foliage yield (Sadak et al., 2020). Sharma et al., (2020) emphasized that melatonin application is pivotal in maintaining homeostatic balance and promoting vegetative tissue growth under drought conditions. Additionally, studies conducted by Ding et al., (2018) and Ibrahim et al., (2020) revealed that melatonin supplementation leads to substantial improvements in the photosynthetic machinery of tomato seedlings subjected to drought stress. Similar investigations have highlighted the beneficial effects of melatonin in alleviating drought-induced photosynthetic damage across various crops, including kiwifruit (Xia et al., 2020), fenugreek (Zamani et al., 2020), and grapes (Meng et al., 2014). Further supporting this, research by Karaca and Cekic (2019) demonstrated that melatonin supplementation under drought conditions significantly improved chlorophyll content, antioxidant enzyme activity, and reduced MDA levels in *Solanum lycopersicum* L. Key outcomes associated with melatonin application include the prevention of chloroplast photosynthetic damage (Meng et al., 2014), enhancement of turgor pressure and leaf water content, and stimulation of spongy mesophyll tissue (Meng et al., 2014). Exogenous melatonin has been shown to enhance the activity of tomato roots, mitigate drought-induced damage to PSII reaction centers, reduce toxic substances in cells, and alleviate the negative effects of drought (Liu et al., 2015). The ectopic expression of the melatonin synthesis-related gene SNAT in *Arabidopsis thaliana* increased melatonin production in mitochondria, thereby reducing oxidative stress caused by ROS under drought conditions (Wang et al., 2017).

2.3.1.1. Activation of antioxidant defense systems*:*

Melatonin treatment has been shown to activate the plant's antioxidant defense system, thereby improving the efficiency of reactive oxygen species scavenging. Extensive research has focused on specific mechanisms by which melatonin enhances drought resistance in horticultural plants, notably through the regulation of the ascorbate-glutathione cycle. This cycle plays a crucial role in scavenging hydrogen peroxide (H_2O_2) (Ibrahim et al., 2020). Supporting this, the application of melatonin can enhance drought tolerance in apple varieties (*Malus prunifolia* L.) by boosting antioxidant enzyme activity to mitigate oxidative damage caused by elevated levels of H_2O_2 (N.Liu et al., 2015). Other studies have confirmed that melatonin plays a crucial role in regulating ROS activity and nitrogen metabolic enzymes to maintain nitrogen oxidation homeostasis.

Additionally, melatonin helps regulate the balance of proline, enhancing osmotic protection in plant cells (Antoniou et al., 2017). Antoniou et al. (2017) reported that melatonin treatment in naked oat (*Avena nuda* L.) increased the expression of mitogen-activated protein kinases Asmap1 and Aspk11, as well as drought-related transcription factors WRKY1, DREB2, and MYB, triggering cascade reactions that bolster antioxidant defenses. A Kyoto Encyclopedia of Genes and Genomes enrichment analysis indicated that melatonin promotes the growth of kiwifruit under drought stress mainly by participating in GSH metabolism, AsA, uronic acid metabolism, and carotenoid metabolism (Xia et al., 2020). Moreover, in creeping bentgrass (*Agrostis stolonifera* L.), melatonin was shown to increase the transcription levels of drought-responsive and antioxidant-related genes, suggesting a role in post-transcriptional gene regulation under dehydration stress (Ma et al., 2018). Wang et al. (2012) observed that drought-induced leaf senescence in apple plants, triggered by chlorophyll degradation through polyamine oxidase (PAO) enzyme, could be delayed by melatonin, which reduces the transcript level of the PAO enzyme, thereby postponing water stress-induced leaf senescence. Furthermore, Jafari and Shahsavar (2021) reported that melatonin application boosted the production of secondary metabolites in citrus plants under drought stress, thereby enhancing their drought defense mechanisms. L.-F.Wang et al. (2022) investigated that are overexpressed melatonin receptor ZmPMTR1 in *Arabidopsis thaliana*, demonstrated a strong binding affinity for melatonin, significantly improving cell membrane permeability and reducing the rate of water loss.

2.3.2. Effects of melatonin on plants under salt stress

Salt stress poses a significant abiotic threat to horticultural crops, leading to severe negative consequences for plant growth and productivity (Su et al., 2021). Approximately 45 million hectares, or 19.5% of the world's irrigated land, are affected by salt stress (FAO, 2016). With ongoing climate change, this figure is expected to rise dramatically, potentially affecting 50% of global arable land by 2050 (Machado and Serralheiro, 2017; Abdelrahman et al., 2018). Furthermore, the FAO warns that increasing soil salinity could render 0.3–1.5 million hectares of agricultural land unproductive each year, leading to a yield loss of 20–46 million hectares annually (FAO and ITPS, 2015). This escalating crisis underscores the urgent need for effective salinity management strategies to sustain global food security.

The adverse impacts of increased salinity on plants include oxidative stress, osmotic stress, and nutritional (N, Ca, K, P, Fe, Zn) deficiencies, thereby restricting water absorption from the soil (Van Zelm et al., 2020). Under saline conditions, the overproduction of ROS is often linked to membrane damage, while the accumulation of sodium ions can result in ion toxicity (Munns and Tester, 2008). Moreover, soil salinity significantly hampers phosphorus uptake as phosphate ions tend to precipitate with calcium ions (Bano and Fatima, 2009). Additionally, certain elements, including sodium, chloride, and boron, exert specific toxic effects on plants. Excessive sodium accumulation in the cell wall can rapidly induce osmotic stress, ultimately leading to cell death (Munns, 2002). Furthermore, salt stress disrupts photosynthesis, primarily by reducing leaf area, chlorophyll content, and stomatal conductance, while also impairing photosystem II efficiency to a lesser extent (Netondo et al., 2004). Additionally, salt stress can modify gene expression, influencing mRNA stability and translational regulation, which subsequently affects protein levels (Van Zelm et al., 2020). Plants respond to salinity stress in two primary phases. The first phase, an ion-independent response, occurs within minutes to days and is characterized by stomatal closure and restricted cell expansion, mainly in the shoot, leading to growth inhibition (Rajendran et al., 2009). The second phase unfolds over days or weeks as toxic ions accumulate in plant tissues, disrupting metabolism, inducing premature leaf senescence, and ultimately leading to cell death (Munns and Tester, 2008; Roy et al., 2014).

To counteract these adverse effects, plants employ three primary salinity tolerance mechanisms: osmotic tolerance, ionic tolerance, and tissue tolerance (Roy et al., 2014).

Melatonin's, a multifunctional signaling molecule, plays a crucial role in enhancing these adaptive mechanisms. According to Castañares and Bouzo (2019); and Li et al. (2019), melatonin treatment has been shown to enhance the germination rates of *Limonium bicolor* and *Cucumis melo* L. seeds under conditions of salt stress. This improvement is attributed to the promotion of soluble sugar utilization, the synthesis of new proteins, and an increase in the activities of amylase and α-amylase. Additionally, exogenous melatonin plays a crucial role in mitigating the reduction of chlorophyll content and photosynthetic rate in cucumbers under salt stress (Wang et al., 2016). It helps stabilize electron transfer processes within the PSII system, including the donor, acceptor, and reaction center. By doing so, melatonin effectively reduces the detrimental impact of salt stress on the photosynthetic machinery (Jahan et al., 2021).

In cotton, melatonin treatment enhanced seed germination by influencing the expression of phyto-melatonin signaling genes, thereby affecting metabolic processes and reducing stress in seedlings under saline conditions (Chen et al., 2021). Furthermore, phyto-melatonin has been found to decrease abscisic acid (ABA) levels while increasing gibberellic acid (GA) concentrations during the initial stages of germination compared to NaCl treatment (N. Zhang et al., 2017). While in cucumber seedlings under saline conditions, exogenous MT has been shown to significantly increase endogenous levels of ABA and GA, thereby enhancing their resistance to salinity (H.J.Zhang et al., 2014).

2.3.2.1. Impact of melatonin on growth and antioxidant responses in horticultural plants under saline condition:

Numerous investigations have confirmed that melatonin effectively alleviates salt stress in various horticultural crops (N. Zhang et al., 2017). Supplementation with melatonin has been shown to enhance growth traits and decrease ROS, MDA, and electrolyte leakage (EL) by upregulating both enzymatic and non-enzymatic antioxidant activities (Zhan et al., 2019). Hu et al. (2021) reported significant reductions in MDA and ROS levels, alongside increases in antioxidant activities, melatonin, proline, and pigment contents under saline conditions. Under similar conditions, melatonin treatment reduced H2O2 and O2 levels while improving ROS scavenging in both cucumber (Zhang et al., 2017) and tomato plants (Yin et al., 2019). Melatonin treatment notably improved the growth of tomato seedlings under NaCl toxicity, resulting in reduced chlorophyll degradation, lower ROS levels, and decreased glycolate oxidase activity, while simultaneously increasing glycine betaine, proline content, and antioxidant enzyme activity (Siddiqui et al., 2022) as well as, enhanced gas exchange parameters, relative water content (RWC), and overall growth characteristics (Ali et al., 2021). The application of melatonin has been shown to boost the yield and quality of strawberry fruit in saline environments (Zahedi et al., 2020).

2.3.2.2. Melatonin biosynthesis and gene regulation in salt tolerance

Phyto-melatonin-related genes, such as COMT which has efective role in phyto-melatonin biosynthesis, are involved in alleviating the negative effects of salt stress and promoting crop growth and yield (Li et al., 2017). Liu et al., (2019) discovered that salinity stress elevated the transcript levels of SlCOMT1 in tomato leaves, which inversely correlated with melatonin production. The upregulation of SlCOMT1 aided in maintaining Na^+/K^+ balance and minimizing ion damage, resulting in increased antioxidant enzyme levels (CAT, SOD, and POD) and higher concentrations of AsA and GSH. Furthermore, the overexpression of SlCOMT1 was linked to improved regulation of mineral nutrient fluxes (Sun et al., 2020). In citrus, the combined application of ascorbic acid (AsA) and melatonin influenced the expression of CaMIPS, CaSLAH1, and CaMYB73, triggering glucose metabolism, ion homeostasis, and enhanced salt adaptation (Kostopoulou et al., 2015).

Effective regulation of Na^+, Cl^-, and K^+ absorption and transport is essential for minimizing salt damage (X. Li et al., 2017). Melatonin increased NO and ATP levels, as well as H^+ pump activity, contributing to the maintenance of Na^+/K^+ balance and reduction of hydrogen peroxide content in rice roots under salt stress (Yan et al., 2020; Yan et al., 2021). Several studies have indicated that melatonin regulates stress tolerance in plants through interactions with other hormones. Combined transcriptomic and metabolic analyses have revealed that melatonin activates the AP2/EREBP-HB-WRKY transcriptional cascade and plant hormone signaling pathways (e.g., auxin and ABA), as well as metabolic networks including linoleic acid metabolism and amino acid metabolism, which are crucial for melatonin-mediated salt tolerance (Xie et al., 2021). Previous research has demonstrated that melatonin promotes ethylene biosynthesis, with genes such as ACS1 and MYB108A being significantly induced during grapevine ripening under salt stress (Xu et al., 2017).

Thus, a comprehensive understanding of melatonin's multifaceted roles in plant drought and salt defense necessitates an exploration of the regulatory mechanisms underlying these physiological processes.

2.4. *Ranunculus asiaticus* L., Fam. *Ranunculacea*

Ranunculus asiaticus L. Interest in ornamental geophytes has increased significantly over time, largely because of their substantial influence across various market sectors, including cut flowers, potted plants, propagation materials, and landscaping. While ornamental geophytes have experienced periods of popularity followed by downturns, the so-called "minor geophytes" continue to play a crucial role in the domestic flower industry. Notably, *Ranunculus* has emerged as a key player among these plants.

The *Ranunculaceae* family is relatively extensive, comprising 59 genera and approximately 2,525 species (Stevens and Davis, 2001). A majority of these plants are perennial, predominantly herbaceous geophytes (Tamura, 1995b). Many genera within this family feature large, vibrant flowers that are often cultivated for ornamental purposes. Additionally, some species possess medicinal properties and are utilized in traditional medicine (Tamura, 1993). The genus *Ranunculus*, comprising 600 species, is the most extensive within the *Ranunculaceae* family (Tamura, 1995a). It originates from the Eastern Mediterranean and was brought to Western Europe in the 16th century. In the 18th century, these plants became popular as garden specimens in Europe, leading to significant breeding efforts in England during the 19th century, which produced over 500 cultivars.

Ranunculus can be found across all continents, although it is primarily located in non-tropical areas (Tamura, 1995b). *Ranunculus* has established itself as a key contender in the global flower industry, especially in areas such as Liguria, Italy, where it stands as the leading cut flower (Beruto et al.,2019). Among the various species, *R. asiaticus* stands out as the most widely cultivated for ornamental purposes (Meynet, 1993). This species is particularly significant in Mediterranean countries, where it serves as a key cut flower, blooming from February to April (Beruto and Debergh, 2004s; Meynet, 1993). *R. asiaticus* has been a subject of research since the early symposia in the 1970s. This species showcases a diverse palette of flower colors, including white, yellow, orange, red, pink, fuchsia, and green, often featuring attractive bicolor combinations. Its aesthetic appeal is complemented by a favorable postharvest performance, although variations exist among different cultivars (Scariot et al., 2009). Additionally, the economic significance of cut flowers from the *Ranunculaceae* family is notable, as evidenced by Dutch auction statistics reporting 67 million stems of *R. asiaticus* sold in 2009, with increasing interest in its potential as a potted plant. Despite its prominence, achieving consistent production quality and standardization remains a challenge. Ongoing research and innovation are essential to address these issues and sustain its competitive market presence.

R. asiaticus is grown across various Mediterranean nations, including Italy, France, and the Netherlands, as well as in Israel, South Africa, California, Japan, and to a lesser extent in

Ecuador, Ethiopia, Kenya, Tunisia, and Turkey. In 2014, Flora Holland in the Netherlands reported that *Ranunculus* represented 0.4% of the overall turnover in cut flowers and foliage. Additionally, the Hyères market in France noted that approximately 1 million stems of *Ranunculus* were sold in 2015. Italy leads global production with 132 million stems harvested from around 300-350 hectares (International Statistics Flowers and Plants (ISPF), 2014). *Ranunculus* exemplifies an ornamental geophyte that effectively endures summer droughts and high temperatures, aligning well with production needs.

2.4.1. Propagation

In a cool and moist summer climate, seeds can be planted in May for blooms by early November (Meynet, 1993). However, in Mediterranean climates, this requires artificially cooling the soil to 18 °C. Consequently, in hot and dry summers, sowing is typically delayed until July or August, with early autumn being preferable. This timing allows the embryos to naturally break dormancy and mature. About two months post-sowing, the plants start to grow vigorously, leading to flowering in the spring. In horticultural practices involving seed propagation, it is essential to allow for the formation of tuberous roots after one growing season as seedlings. While it is possible to propagate by dividing these tuberous roots, the annual multiplication rate tends to be low, and there is a significant risk of disease transmission (Meynet, 1993; Beruto and Debergh, 2004). Tissue culture has emerged as an effective method for rapidly propagating selected healthy genotypes. This approach led to the creation of the "Ranunculus clone," which demonstrates better agronomic performance compared to conventional seedling production. A structured production chain has been developed to maintain consistently high-quality standards, addressing factors such as plant health, pest management, and cultivation practices (Beruto et al.,2019). However, similar to other methods, the tuberous roots obtained at the end of the growing cycle *of ex vitro* plantlets serve as the cultivation units provided to growers for winter production (Beruto and Debergh, 2004).

At the conclusion of the first growing season, tuberous roots are collected, dried, and sorted for commercial sale. In horticultural practices, these roots are classified into small (2-3 cm) and large (5-7 cm) categories, which correspond to different flowering times: the larger roots typically bloom from mid-October, while the smaller ones flower in January or February. It is recommended to hydrate the tuberous roots prior to planting, and various methods have been documented in the literature to ensure optimal growth (Ohkawa, 1986; Dhooghe et al., 2012; Cerveny et al., 2012). During growth, the plants require low temperatures, with an optimal night/day temperature range of 5–10°C at night and 12–25°C during the day, with an ideal daytime temperature of 16°C. Additionally, they have a medium to high light intensity requirement for optimal development. *R. asiaticus* is classified as a quantitative long-day (LD) plant in terms of its photoperiodic flowering requirements (Horovitz, 1985). The process of flowering is regulated by both the thermal history of tuberous roots and the photoperiod during plant growth. Specifically, cold treatments (vernalization) of tuberous roots promote early sprouting, leaf rosette formation, and flowering (Beruto et al., 2009). As a result, using vernalized propagation material is a common practice in cut-flower production for precise scheduling, similar to other geophytic flowering plants.

2.4.2. Storage and handling of tuberous roots

As mentioned earlier, *Ranunculus* is grown in Mediterranean regions using tuberous roots harvested at the end of the initial growth cycle of seedlings or *ex vitro* plantlets. To promote more consistent plant growth, these tuberous roots are typically hydrated before planting. Beruto et al., (2019) noted that cold treatment is crucial for breaking the summer dormancy of *Ranunculus*, even in natural conditions. Furthermore, the ability of tuberous roots to sprout can be influenced by their storage environment; those stored at a low temperature (2°C) in either open or modified atmospheres (2% O_2 and 4% CO_2) retained their sprouting potential for up to a year, although variations were observed depending on the genotype (Beruto et al., 2019).

In Mediterranean climates, the cultivation cycle for *Ranunculus* begins in August with the planting of tuberous roots and concludes around April to May. This species is classified as a long-day plant and can thrive in various environments, including greenhouses and open fields, making it relatively hardy. It has been observed to respond well to fertigation (Hassan et al., 1985). However, the quality of irrigation water can impact the viability of tuberous roots. Hassan et al. (1985) found that Ranunculus is particularly sensitive to salinity. Their research demonstrated that salinity levels (EC 4-6 dS m^{-1}) combined with alkaline pH led to reduced plant growth and lower flower yields. Additionally, they suggested that salinity might affect the sprouting of tuberous roots by inducing a greater dormancy, potentially linked to higher concentrations of ABA in the root tissues.

This study will thoroughly examine the impact of drought and salt stress on the growth of *R. asiaticus*. It will also explore how the application of exogenous melatonin (MT) can alleviate the detrimental effects of these abiotic stresses and promote overall plant growth.

3. MATERIAL AND METHODS

3.1. Materials and conditions for planting

The pot experiment was conducted in the research greenhouse of the Floriculture and Dendrology Department at the Hungarian University of Agriculture and Life Sciences (MATE) in Budapest, Hungary. Planting commenced on October 1, 2021, with harvesting taking place 150 days later. The greenhouse maintained an average temperature range of 20-15 °C during the day and night, along with a relative humidity of 60%. Healthy seedlings of *Ranunculus asiaticus*, aged 30 days and exhibiting four to five fully developed true leaves measuring 5-6 cm, were transplanted into plastic pots measuring 9x9x10 cm.

3.1.1. Drought stress experiments

Each pot contained one plant, contributing to a total of 160 plants allocated across two treatment groups: well-watered and drought-stressed conditions. Each group was further divided into four subgroups corresponding to varying concentrations of melatonin (20 plants per subgroup). The growing medium utilized was a consistent mixture of Klassmann TS3 Baltic peat, which included urea (46.5% N), calcium superphosphate (15.5% P_2O_5), potassium sulfate (48.0% K_2O), augmented with 2 g/L Osmocote Exact, a potassium-dominant controlled-release fertilizer with a duration of 3-4 months, and 1 g/L Futor (soluble calcium carbonate).

3.1.1.1. Experimental design and irrigation treatments

Following transplantation, standard irrigation practices were employed until the plants reached the eighth true leaf stage, utilizing 100% of the field capacity. After establishing the transplants for 30 days, irrigation treatments commenced. The experimental design employed a completely randomized design (RCD) featuring two independent variables: irrigation and foliar application of melatonin. The first variable involved two irrigation levels: well-irrigated plants maintained soil moisture content between 50 and 120 millibar, while drought-stressed plants had moisture content ranging from 180 to 200 millibar. These were combined with four levels of melatonin application (refer to Table 1). Daily tensiometer readings (Blumat Digital PRO Plus, Blumat GmbH & Co. KG, Telfs, Austria) were recorded to monitor changes in soil moisture and water capacity at a depth of 5cm.

3.1.2. Salinity stress experiment

In this trial, one plant was placed in each pot, totaling 135 plants. The experimental design followed a randomized complete block design (RCBD) with a split-plot arrangement. Main plots consisted of three salinity levels:

1. Control group receiving typical watering at 80% field capacity without melatonin or salt treatment.
2. Salinity treatment at an electrical conductivity (EC) of 4.5 dS/m.
3. Salinity treatment at an EC of 5.5 dS/m.

Sub-plots included various levels of melatonin treatments associated with the salinity levels at ECs of 4.5 and 5.5 dS/m (15 plants per subgroup). The growing medium for each pot was a uniform blend of Klassmann TS3 Baltic peat (detailed constituents are listed in Table S1), supplemented with 3 kg/m³ Osmocote Exact Potassium Dominant (Scotts, NSW, Australia) and 1 kg/m³ soluble carbonate.

3.1.2.1. Salinity treatment preparation

Before initiating salinity treatments, the seedlings of *Ranunculus* were cultivated in separate pots for four weeks. The preparation of the salt solution involved dissolving sodium chloride (NaCl) and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in a molar ratio of 2:1 to achieve desired electrical conductivity values of 4.5 and 5.5 dS/m (Rauter et al., 2021; see Table 2). The electrical conductivity of the prepared solution was measured using a handheld EC meter (Milwaukee EC 60 Inc., Szeged, Hungary).

3.2. MT application

The selection of melatonin doses was informed by studies conducted by Y.P.Zhang et al. (2017) and Bidabadi et al. (2020). Melatonin solutions were procured from Thermo Fisher (Geel, Belgium) and prepared in accordance with the methodology outlined by H.Li et al. (2017) This involved dissolving the melatonin in ethanol and subsequently diluting it with Milli-Q water at a ratio of 1:10,000 (ethanol/water v/v), followed by application using a manual pump. After 30 days of cultivation under salt and drought treatments, various concentrations of exogenous melatonin (0, 50, 100, and 200 μM) were applied via foliar spraying at four intervals: 45, 60, 75, and 90 days post-planting of *R. asiaticus*. A handgun sprayer was used to ensure that the leaves were thoroughly wetted until dripping occurred. For the control treatment in both salinity and drought stress conditions, tap water was utilized for regular irrigation.

Table 1 Design experiment of drought stress**.**

Factor 1: watering conditions	Group 1: Well-watered (50–120 mbar)				Group 2: Drought-stressed (180–200 mbar)			
Factor 2: Melatonin Concentration (µM)	0	50	100	200	0	50	100	200

Table 2 Design experiment of salinity stress.

Factor :1 (Salinity conditions)	Group 1: (Control)	Group 2: Moderate Salinity ($4.5\ dS.m^{-1}$)				Group 3: High Salinity (5.5 dS.m−1)			
Factor:2 Melatonin Concentration (µM)	0	0	50	100	200	0	50	100	200

3.3. Morphological characteristics

Following the fourth foliar treatment, plant samples were collected after two weeks to assess various growth parameters. The height of each plant (in cm) was measured from the surface of the growing medium to the apex of the shoot using a meter rod. The total number of leaves per plant was counted manually. Fresh and dry weights of vegetative parts (shoots and leaves) were recorded in grams, with five representative plants selected from each treatment group for this measurement. Leaf area (in cm^2) was determined using a leaf area meter (Area Meter 350, ADC Bioscientific Ltd., UK). Additionally, the number of days until flower bud appearance was noted from the time of planting.

3.4. Physiological and biochemical characteristics

To evaluate chlorophyll content, carotenoid levels, electrolyte leakage, relative water content, proline concentration, and antioxidant activity (specifically POD), tissue samples were collected from the five uppermost fully expanded leaves of at least five independent plants at the conclusion of the experiment. These samples were immediately cooled in liquid nitrogen at -80 °C and stored until analysis. Photosynthetic pigments were assessed using fresh leaf samples (0.1 g) from each treatment group. The leaves were homogenized in 80% acetone for extraction. The resulting homogenates were centrifuged at 14,000 g for 5 minutes, and 1.5 mL of the supernatant was used for pigment analysis. A spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, USA) measured light absorption at specific wavelengths: 644 nm and 663 nm for total chlorophyll, and 480 nm for carotenoids, following the methods established by (Arnon, 1949).

The total chlorophyll and carotenoid contents were calculated using the following formulas:

$$\text{Total chlorophyll mg g}^{-1}\text{ FW} = [20.2(A_{644}) + 8.02\,(A_{663})] * V/W$$

$$\text{Carotenoid mg g}^{-1}\text{ FW} = 5.01 * A_{480} * V/W$$

3.5. Relative water content (RWC) assessment

The relative water content (RWC) in leaves was determined following the methodology outlined by Turk and Erdal (2015). RWC was calculated using the formula:

$$\text{RWC} = \left(\frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}}\right) \times 100$$

This calculation involved five replicates. A portion of the leaves was submerged in distilled water overnight to obtain their turgid weight (TW). Subsequently, the leaf samples were dried in an oven at 75 °C to determine their dry weight (DW). The fresh weight (FW) of the fifth leaf was recorded at the time of sampling.

3.6. Electrolyte leakage (EL) evaluation

Electrolyte leakage (EL) was measured using the method described by Turk and Erdal (2015). Leaf discs with a diameter of 0.5 cm were placed in tubes containing 40 mL of distilled deionized water at 10 °C for 24 hours. After this incubation period, the initial electrical conductivity (EC1) of the solution was measured using a conductivity meter (Cole-Parmer Instrument Co., Chicago). To extract total electrolytes from the samples, they were autoclaved for 20 minutes at 121 °C. After allowing the samples to rest at 21 °C overnight, the final electrical conductivity (EC2) of the dead tissues was determined. The percentage of electrolyte leakage was calculated using the formula (Reddy et al., 2004): $\text{EL}(\%) = \left(\frac{\text{EC1}}{\text{EC2}}\right) \times 100$

3.7. Peroxidase activity determination

POD activity was assessed by preparing an enzyme extract from leaf samples. One gram of leaf tissue was homogenized with 2 mL of phosphate buffer (pH 7.0) in a pre-cooled mortar. The homogenate was then centrifuged at 12,000 × g for 20 minutes at 4 °C, and the supernatant was used for measuring POD activity. The procedure followed that described by He et al. (2014).The reaction mixture consisted of 2.9 mL of 50 mM phosphate buffer (pH 5.5), 1 mL of

0.6 M hydrogen peroxide, 1 mL of 50 mM guaiacol, and 0.1 mL of the enzyme extract. This mixture was incubated at 37 °C for 15 minutes and subsequently stopped by adding 2 mL of 20% (v/v) trichloroacetic acid. The change in absorbance due to guaiacol oxidation was measured at a wavelength of 470 nm. The activity of POD was calculated using the following equation: POD activity $(\mu g - 1FW) = \frac{\Delta A470 \times Vt}{W \times Vs \times 0.01 \times t}$

Where:

- $\Delta A470$ = Change in absorbance
- Vt = Total volume of the reaction mixture
- W = Fresh weight of the sample
- Vs = Volume of crude enzyme extract
- t = Reaction time (in minutes)

3.8. Proline content determination

The proline content (PC) in fresh leaf samples was measured following the method established by (Ábrahám et al., 2010). Initially, 0.1 g of fresh leaf tissue was ground and homogenized in 10 mL of 3% aqueous sulfosalicylic acid. The resulting mixture was then centrifuged at 4 °C for 10 minutes at 14,000 rpm to obtain a clear supernatant.

Next, 100 µL of the supernatant was transferred into test tubes containing 200 µL of acidic ninhydrin solution and 200 µL of glacial acetic acid. The tubes were placed in a water bath set to 90 °C for one hour to facilitate the reaction. After this incubation period, the reaction was halted by transferring the tubes to an ice bath.

Following this step, 4 mL of toluene was added to the reaction mixture, and the contents were vortexed for 20 seconds to ensure thorough mixing. The mixture was allowed to separate into toluene and aqueous phases for at least 20 minutes in the dark at room temperature. The toluene phase, which contained the colored complex, was carefully collected into clean test tubes.

The absorbance of the colored solutions was measured at 520 nm using a Genesys 10S UV-VIS Spectrophotometer (Waltham, MA, USA). The concentration of proline was calculated on a fresh weight basis by referencing a standard curve created from known concentrations of proline.

3.9. Drought and salt tolerance index (DTI and STI)

The Drought Tolerance Index (DTI) and Salt Tolerance Index (STI) were calculated as percentages for each of the traits analyzed, following the methodology described by (Sbei et al., 2014), with slight modifications in the notation used.

3.9.1. Drought tolerance index (DTI)

The DTI was determined using the formula: DTI= $\left(\frac{T\ drought}{Tcont}\right) \times 100$

In this equation, T_{drought} represents the average value of the characteristic under water deficit stress, while T_{cont} denotes the average value of the characteristic under well-watered conditions (as referenced by Rafi et al. (2019).

3.9.2. salt tolerance index (STI)

Similarly, the STI was calculated using the formula: STI= $\left(\frac{TSalt}{Tcont}\right) \times 100$

Here, T_{salt} indicates the average value of the characteristic under saline stress conditions induced by electrical conductivity (EC) of 5.5 dS/m, while T_{cont} represents the average value of the characteristic under unstressed (control) conditions.

3.10. NaCl content determination

The sodium chloride (NaCl) content in leaf samples was assessed using Mohr's titration method, as described by (Korkmaz, 2017) and is expressed as grams per 100 g dry weight (g 100 g^{-1} DW). A standardized silver nitrate solution (0.1 M) was employed for the titration process. Prior to titration, the pH of the testing solution was adjusted to near neutrality by adding sodium bicarbonate powder. During the titration with silver nitrate, potassium chromate (5%, 1 mL) served as the indicator. The endpoint of the titration was identified by the formation of a red brick precipitate, indicating the presence of silver chromate. Double-distilled water was used as a blank control in the procedure. The NaCl content in the leaf sample was calculated based on the amount of chloride ions that were titrated, allowing for an accurate determination of salt concentration within the samples.

3.11. Statistical analysis

The current study employed a completely randomized design for the experimental trials. The analysis was conducted using Two-Way MANOVA, followed by UNIANOVA with Bonferroni's correction applied to all dependent variables. The factors included two levels:

1. Treatments:

- Drought experiment: Irrigation and Dry conditions
- Salinity experiment: Control (no salinity and no melatonin treatment) and Salinity (EC 4.5 and EC 5.5)

2. Melatonin concentrations: Levels of 0, 50, 100, and 200 µM.

For the Drought Tolerance Index (DTI) and Salt Tolerance Index (STI) characteristics, a One-Way MANOVA was utilized.

3.11.1. Assumptions for treatments

The normality of residuals for most dependent variables was confirmed using the Kolmogorov-Smirnov test ($p > 0.05$) in both experiments. Exceptions included the number of leaves, height DTI, number of leaves DTI, and EL DTI, which were accepted based on Skewness and Kurtosis values, where the absolute values were less than 1 (West et al., 1995). However, for the variable carotenoids, normality was not met; thus, a logarithmic transformation of the data was applied in the drought experiment.

The homogeneity of variances was assessed using Levene's F test, which indicated that most dependent variables satisfied this assumption ($p > 0.05$) across both experiments. Nonetheless, violations were noted for plant height, leaf area, dry weight (DW), RWC DTI, and carotenoids DTI in the drought experiment ($p < 0.05$). Despite these violations, they were deemed acceptable based on the variance ratio test ($F = \text{Max variance} / \text{Min variance}$), where the ratio of maximum sample size to minimum sample size was less than 1.5 and the ratio of maximum variance to minimum variance was less than 6 (Brown and Forsythe, 1974).

3.11.2. Post Hoc testing

For comparisons between factor levels, Tukey’s post hoc test was employed (Garson, 2012; and Tabachnick and Fidell, 2013). Additionally, Dunnett's test was utilized to compare control treatments with the two irrigation groups in the drought experiment and the two salinity groups in the salinity experiment. Pairwise within-subject effects were analyzed using Bonferroni's method. All statistical analyses were performed using IBM SPSS Statistics version 27 (Armonk, 2020).

4. RESULTS

MANOVA Results:

The results of the drought and salinity trials, based on both one-way and two-way MANOVA analyses, revealed a highly significant multivariate main effect of the examined factors, as indicated by Wilk's lambda values of less than 0.001. In the two-way MANOVA, the interaction effect between the factor levels was also significant (Wilk's lambda < 0.001) (Olson, 1976).

In the drought experiment, we conducted pairwise comparisons of different irrigation levels within each hormone concentration level, followed by an assessment of hormone concentration effects across various irrigation conditions. Similarly, in the salinity experiment, comparisons were performed between the two salinity levels and the control to evaluate the impact of melatonin (MT) concentrations. Additionally, we examined the effects of different MT concentrations under the applied salinity conditions.

Following these comparisons for both stress trials, a univariate ANOVA was conducted on various variables, applying Bonferroni's correction to identify significant differences in all individual variables with a significance level of $p > 0.05$ (Barbara and Linda, 2013).

Post-Hoc Testing

Post-hoc tests were performed for drought and salinity experiments on all significant variables influenced by the melatonin concentrations, as well as by irrigation or salinity levels. Different letters were assigned to indicate distinct groups based on the Tukey/Games-Howell test ($p < 0.05$). Lowercase letters were used to denote comparisons of melatonin concentrations under fixed treatment conditions, whereas uppercase letters represented comparisons of irrigation levels in the drought experiment and salinity levels in the salinity experiment under fixed melatonin concentration levels.

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

4.1.1. Vegetative parameters

Vegetative parameters, such as plant height, leaf area, and biomass, are crucial indicators of plant growth and development. These parameters reflect the overall health and vigor of plants under various environmental conditions, including abiotic stresses like drought and salinity. Changes in vegetative traits can provide insights into how plants adapt to stress, often through physiological mechanisms like enhanced water retention or nutrient uptake. Monitoring these parameters helps assess the effectiveness of treatments, such as exogenous melatonin application, in improving stress tolerance and promoting plant growth.

4.1.1.1. The Influence of melatonin on morphological traits in response to drought stress.

The results of this study indicated that drought stress significantly reduced various morphological parameters. However, the application of melatonin (MT) treatment had a notable positive impact on plant growth and development. As a result of the interaction between different concentrations of MT and irrigation levels, these effects were significant. Under well-irrigated conditions, foliar spraying with MT at concentrations of 0, 50, 100, and 200 µM significantly enhanced fresh and dry vegetative weights, shoot length, leaf number, and leaf area compared to the control group with no treatment (W0MT).

In untreated and drought-stressed plants (D0MT), the morphological parameters were adversely affected: shoot length decreased to 13.97 cm, leaf number dropped to 4.60, leaf area was reduced to 18.15 cm², and both fresh weight (FW) and dry weight (DW) of shoots decreased to 11.92 g and 1.47 g, respectively, when compared to well-watered plants (W0MT).

In contrast, plants that received foliar spraying with MT (specifically at the 50 µM concentration) exhibited slight improvements in all measured vegetative traits compared to untreated drought-stressed plants (D0MT). More pronounced enhancements were observed with the higher concentrations of MT (100 µM and 200 µM), which resulted in significant increases in plant development metrics: shoot length increased to 16.78 cm and 17.09 cm, respectively; leaf number rose to 5.73 and 5.93; leaf area expanded to 28.78 cm² and 29.56 cm²; fresh weight increased to 15.92 g and 17.01 g; and dry weight improved to 1.85 g and 2.01 g, respectively (see; Figure 5; Table 3). These beneficial effects were evident in a concentration-dependent manner.

Figure 4 Morphological changes in *R. asiaticus* plants under well-irrigated and drought stress conditions after exogenous MT treatment.

Table 3 Effects of exogenous melatonin application on the vegetative growth traits of *R. asiaticus* plants with/without drought stress

Treatments		Shoot Length (cm)	No. of Leaves	Area/Leaf (cm^2)	Fresh Shoot Weight (g)	Dry Shoot Weight (g)
Effect of MT (μM) under well-irrigated conditions	0	18.87 ± 0.18 bA	6.67 ± 0.49 bA	47.97 ± 0.05 dA	18.19 ± 0.39 cA	3.12 ± 0.19 bA
	50	19.07 ± 0.27 bA	7.33 ± 0.49 aA	54.49 ± 0.11 cA	18.15 ± 0.15 cA	3.12 ± 0.07 bA
	100	21.49 ± 0.35 aA	7.67 ± 0.49 aA	55.31 ± 0.08 bA	18.82 ± 0.26 bA	3.17 ± 0.14 bA
	200	21.55 ± 0.34 aA	7.73 ± 0.46 aA	55.60 ± 0.18 aA	21.73 ± 0.27 aA	3.12 ± 0.19 aA
Effect of MT (μM) under drought conditions	0	13.97 ± 0.47 cB	4.60 ± 0.51 cB	18.15 ± 0.20 dB	11.92 ± 0.17 dB	1.47 ± 0.06 dB
	50	15.75 ± 0.37 bB	5.20 ± 0.56 bB	21.61 ± 0.16 cB	13.99 ± 0.09 cB	1.63 ± 0.09 cB
	100	16.78 ± 0.30 aB	5.73 ± 0.70 abB	28.78 ± 0.22 bB	15.92 ± 0.13 bB	1.85 ± 0.06 bB
	200	17.09 ± 0.39 aB	5.93 ± 0.46 aB	29.56 ± 0.17 aB	17.01 ± 0.10 aB	2.01 ± 0.12 aB

MT (μM) (exogenous melatonin) at different concentrations: 0, 50, 100, and 200 μM. Well-irrigated: plants under well irrigation conditions; Drought: plants under drought conditions. The values represent the mean ± standard deviation of at least 10 replicates. Lowercase letters: comparison of MT concentrations under fixed irrigation treatments; uppercase letters: comparison of the irrigation treatment groups under fixed MT concentration levels (Tukey/Games–Howell test, $p < 0.05$)

4.1.1.2. Influence of melatonin on plant morphology under salinity stress

The application of salinity stress at both levels significantly negatively affected the growth parameters of buttercup plants, as illustrated in Figure 5 and Table 4. When comparing the control group (non-stressed plants) to salt-stressed plants at EC 4.5 (0 MT) and EC 5.5 (0 MT), there were notable reductions in various growth metrics: shoot length decreased by 31.78% and 35.64%, the number of leaves by 32.23% and 34.03%, total leaf area by 58.34% and 60.98%, shoot fresh weight (FW) by 29.32% and 36.61%, and shoot dry weight (DW) by 42.15% and 46.08%, respectively. As a result of the interaction between different concentrations of MT and salinity levels, these effects were significant.

In contrast, foliar applications of melatonin at concentrations of 50, 100, and 200 µM significantly improved plant development and enhanced all vegetative parameters under salinity stress. Notably, plants treated with 200 µM MT demonstrated the most favorable outcomes across both salinity levels, showing substantial increases in shoot length (23.37% and 30.04%), leaf numbers (28.32% and 21.14%), total leaf area (58.01% and 58.79%), shoot FW (29.92% and 42.33%), and shoot DW (45.20% and 41.82%) when compared to the stressed plants at EC 4.5 MT0 and EC 5.5 MT0, respectively, relative to the non-MT treated plants (Table 4).

Table 4 Effects of exogenous melatonin application on growth parameters of *Ranunculus asiaticus* under salinity stress.

Treatments	**Shoot length (cm)**	**No of leaves**	**Leaf area (cm^2)**	**Fresh shoot Weight (g)**	**Dry shoot Weight (g)**
Control (non-stressed)	18.88±0.2aA	6.67±0.15aA	47.96±0.2aA	18.11±0.2aA	3.06±0.02aA
S1					
MT (0)	12.88±0.12eB	4.52±0.12eB	19.98±0.2eB	12.80±0.1eB	1.77±0.02eB
MT (50)	14.29±0.22dB	5.07±0.11dB	22.47±0.2dB	13.90±0.1dB	2.07±0.01dB
MT (100)	15.62±0.02cB	5.40±0.18cB	27.99±0.1cB	15.72±0.2cB	2.25±0.01cB
MT (200)	15.89±1.3bB	5.80±0.02bB	31.57±0.2bB	16.63±0.2bB	2.57±0.02bB
S2					
MT (0)	12.15±0.2eC	4.40±0.02eC	18.71±0.2eC	11.48±0.2eC	1.65±0.02eC
MT (50)	14.20±0.1dC	4.87±0.01dC	20.60±0.3dC	13.64±0.3dC	1.80±0.01dC
MT (100)	15.55±0.2cC	5.27±0.01cC	26.25±0.12cC	14.67±0.1cC	2.24±0.02cC
MT (200)	15.80±0.1bC	5.33±0.11bC	29.71±0.4bC	16.34±0.2bC	2.34±0.02bC

Control (no stress – no melatonin), **S1**: plants under salt stress EC 4.5 ds.m^{-1} were sprayed with MT (0 µM, 50 µM, 100 µM, and 200 µM), **S2**: plants under salt stress EC 5.5 ds.m^{-1} were sprayed with MT (0 µM, 50 µM, 100 µM and 200 µM).; different letters are for significantly different groups (Tukey/Dunnett $p<0.05$). The lowercase letters are for significant differences amongst melatonin concentrations under fixed salinity treatments, while the uppercase letters for significant differences the salinity treatments with the control group under fixed melatonin concentration levels ($n \geq 10$).

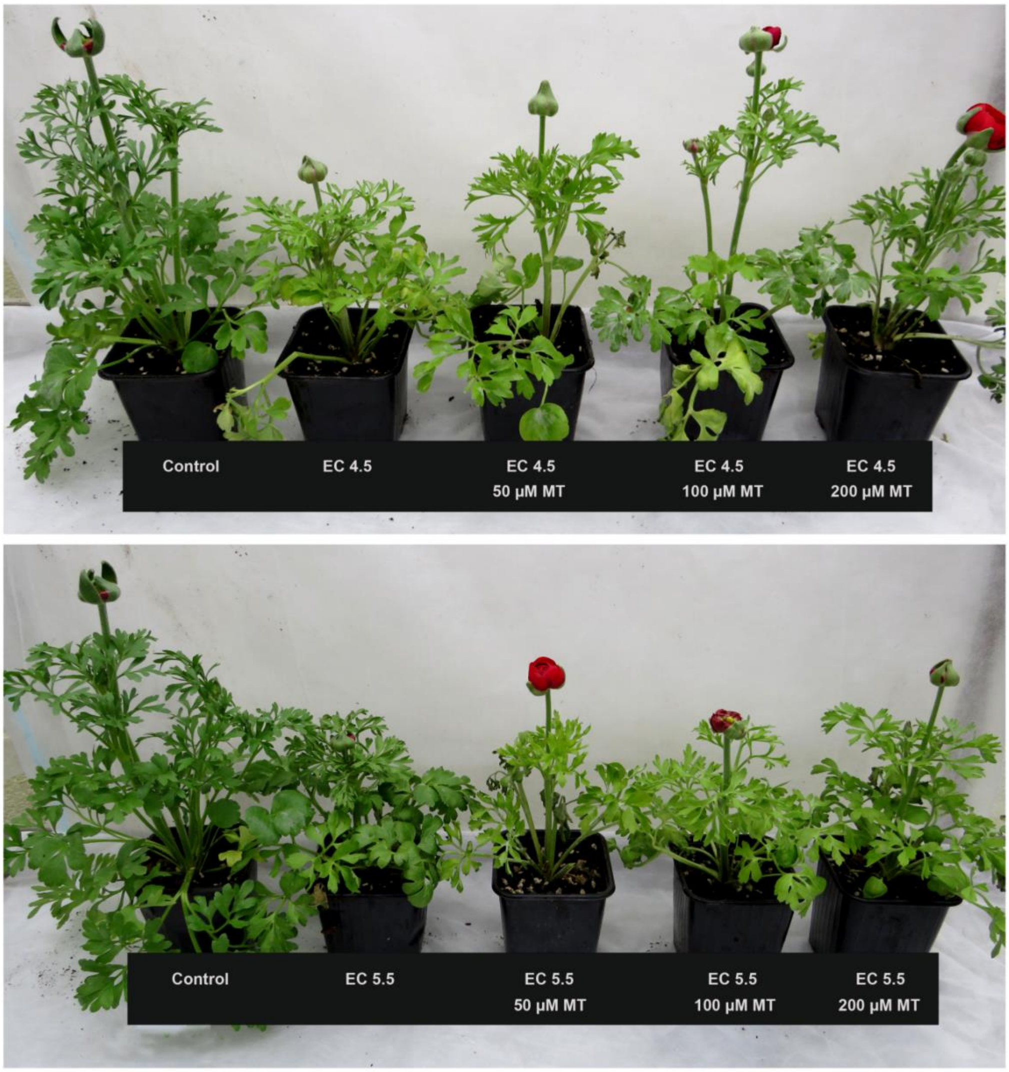


Figure 5 Morphological changes in *R. asiaticus* plants treated with varying concentrations of exogenous melatonin under salinity stress

4.1.2. Emergence of flower buds

4.1.2.1. Effect of exogenous melatonin on flower bud emergence and morphological responses under drought stress.

Exogenous melatonin (MT) was applied at various concentrations: M0, M50, M100, and M200, corresponding to 0, 50, 100, and 200 µM, respectively. The term "well-irrigated" refers to plants maintained under optimal irrigation conditions, while "drought" denotes plants subjected to water deficit conditions. The interaction between different concentrations of MT and irrigation levels resulted in significant effects, with the findings suggesting that drought stress accelerated the timing of flower bud emergence. Specifically, the control plants (D0MT) exhibited flower bud emergence 29 days earlier than their well-watered counterparts. In comparison, the D50 MT, D100 MT, and D200 MT-treated plants showed flower bud emergence 23, 19, and 21 days earlier, respectively, relative to adequately watered plants with or without melatonin treatment (see Figure 6).

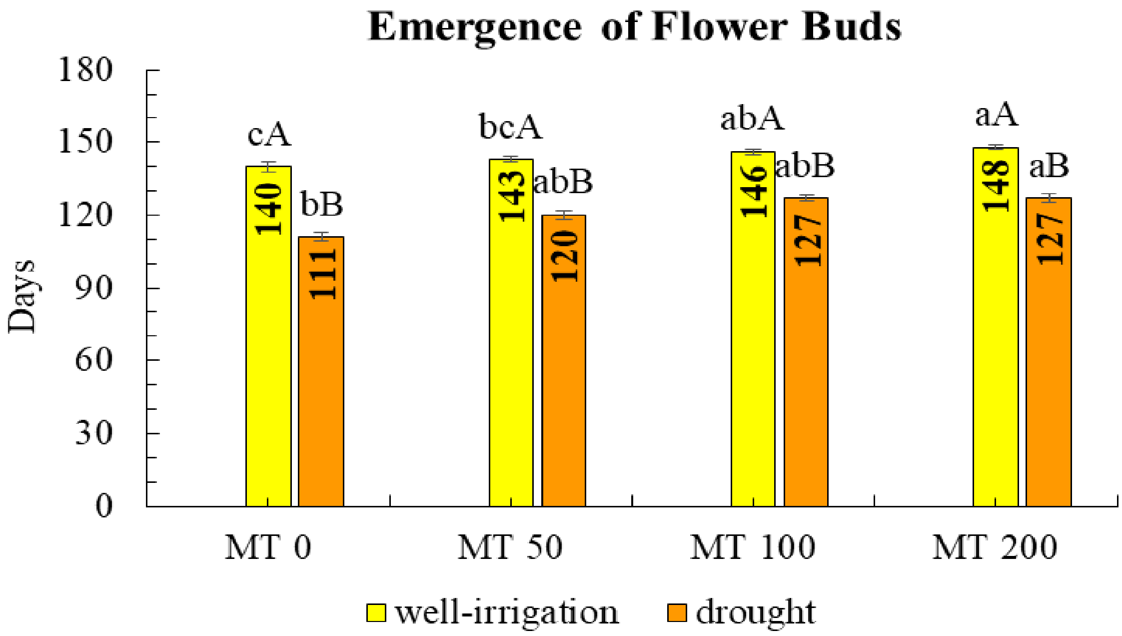


Figure 6 Effect of exogenous melatonin on the emergence of flower buds in *R. asiaticus* plants cultivated under well-irrigated and drought stress conditions. MT0, well-watered and drought-stressed plants without MT treatment; MT50, MT100, and MT200, treatment with MT at different concentrations (50, 100, and 200 µM, respectively) under well-irrigated and drought circumstances. Lowercase letters: comparison of MT concentrations under fixed irrigation treatments; uppercase letters: comparison of the irrigation treatment groups under fixed MT concentration levels (Tukey/Games–Howell test, $p < 0.05$). The values represent the mean ± standard deviation of at least 10 replicates.

4.1.2.2. Alterations in flower bud emergence under salinity stress and melatonin treatment.

The results revealed that salinity stress (S1 and S2) significantly accelerated the emergence of flower buds (Figure 7). In plants exposed to salt stress with irrigation at electrical conductivities of 4.5 and 5.5 dS m^{-1} without melatonin treatment, flowering occurred 29 and 36 days earlier than in the control group, respectively. As a result of the interaction between different concentrations of MT and salinity stress levels, these effects were found to be significant, with the application of melatonin effectively delayed this premature flowering.

In the S1 treatment, the timing of flowering was postponed by 22, 24, and 15 days with the application of 50, 100, and 200 µM MT, respectively, compared to control plants. Similarly, in the S2 treatment, melatonin application at concentrations of 50, 100, and 200 µM resulted in delays of 17, 8, and 10 days in flowering, respectively, when compared to non-stressed control plants.

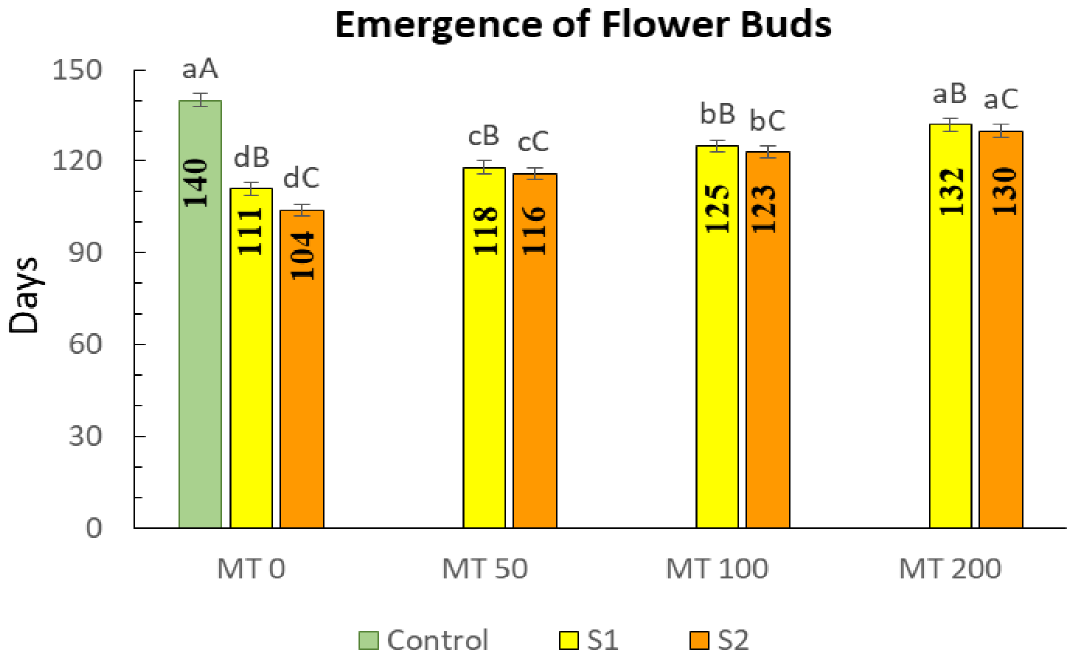


Figure 7 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on flower bud Emergence timing in Ranunculus asiaticus under saline conditions. Control: no-stress and no melatonin, S1: plants under salt stress EC 4.5 ds.m-1, S2: plants under salt stress EC 5.5 ds.m-1 different letters are for significantly different groups (Tukey/Dunnett $p<0.05$). The lowercase letters are for significant differences amongst melatonin concentrations under fixed salinity treatments, while the uppercase letters for significant differences the salinity treatments with the control group under fixed melatonin concentration levels ($n \geq 5$).

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

4.2.1. Photosynthetic pigments.

Photosynthetic pigments, such as chlorophylls and carotenoids, are essential for capturing light energy during photosynthesis.The balance and concentration of these pigments can be influenced by environmental stressors, such as drought and salinity, affecting overall plant photosynthetic efficiency

4.2.1.1.Alterations in photosynthetic pigments under drought stress and melatonin treatment.

The impact of foliar applications of melatonin (MT) at varying concentrations (0, 50, 100, and 200 µM) on the photosynthetic pigments in *R. asiaticus* plants subjected to drought stress (with soil moisture levels between 180–200 mbar) is summarized in Table 5 and illustrated in Figure 8 (A and B). The results indicate a significant reduction in the leaf pigment components, specifically total chlorophyll (Chl) and carotenoids (Car), when compared to well-irrigated plants that were neither stressed nor treated with MT. In non-MT-treated plants experiencing drought stress, the decrease in chlorophyll and carotenoid content was measured at 68.29% and 51.72%, respectively, relative to non-stressed plants without MT treatment. The interaction between different concentrations of MT and drought stress levels significantly influenced the results. Specifically, foliar application of MT, particularly at the 200 µM concentration, notably enhanced chlorophyll and carotenoid levels under drought conditions, with increases of 75% and 50%, respectively, compared to non-MT-treated drought-stressed plants (D0MT).

Table 5 Effect of exogenous melatonin on the total chlorophyll and carotenoid content of *R. asiaticus* under drought stress conditions.

Treatments		**Chlorophyll content (mg g^{-1} FW)**	**Carotenoide content (mg g^{-1} FW)**
Effect of MT (µM) under well-irrigated conditions	0	1.64 ± 0.02 cA	0.29 ± 0.01 bA
	50	1.66 ± 0.04 cA	0.30 ± 0.01 bA
	100	1.92 ± 0.05 bA	0.31 ± 0.03 bA
	200	2.37 ± 0.01 aA	0.52 ± 0.01 aA
Effect of MT (µM) under drought conditions	0	0.52 ± 0.02 cB	0.14 ± 0.00 dB
	50	0.56 ± 0.02 cB	0.16 ± 0.00 cB
	100	0.66 ± 0.04 bB	0.18 ± 0.01 bB
	200	0.91 ± 0.01 aB	0.21 ± 0.01 aB

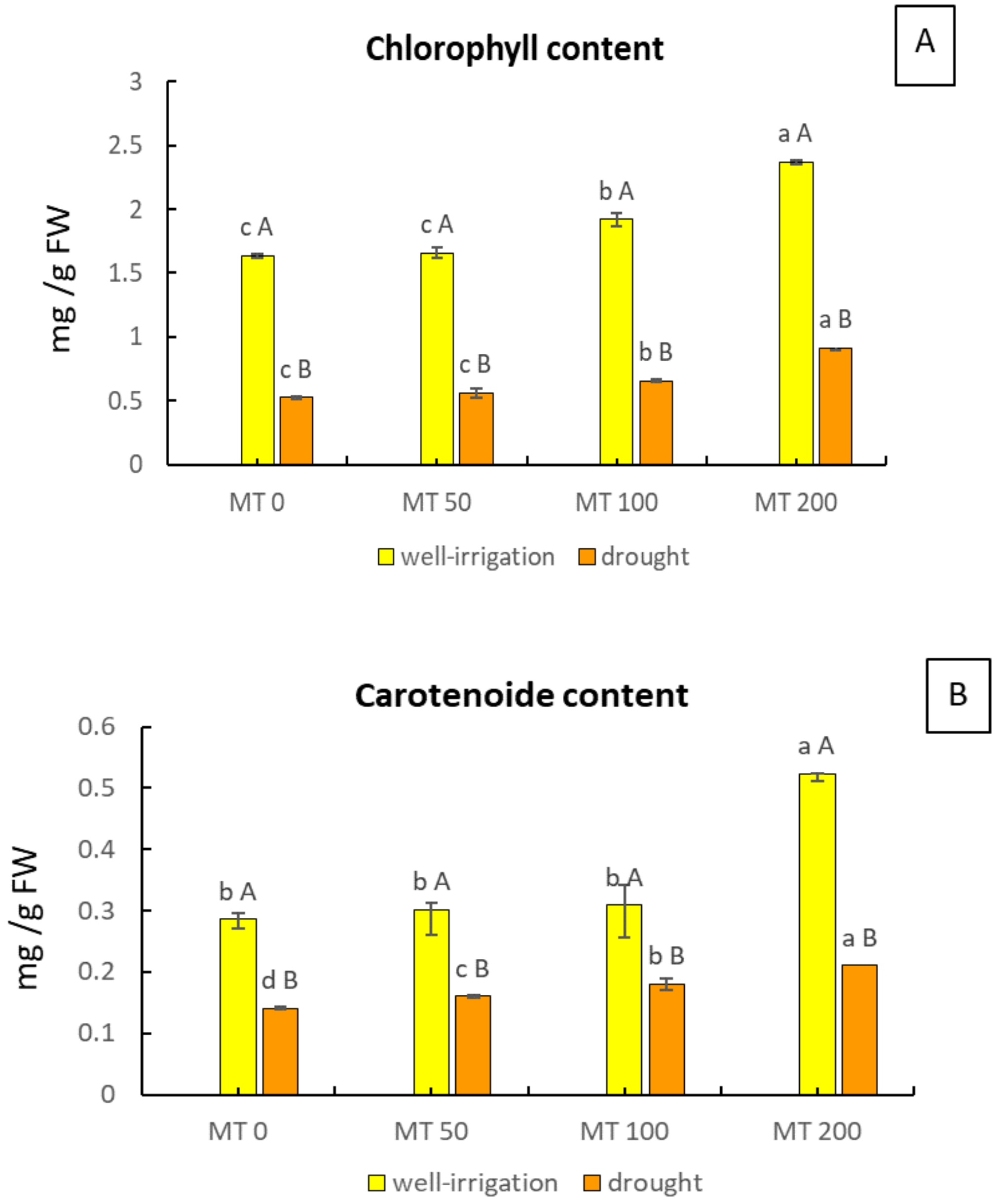


Figure 8 Effect of exogenous melatonin on the total chlorophyll (mg/g FW) (**A**) and carotenoid (mg/g FW) (**B**) content of *R. asiaticus* under drought stress conditions. MT0, well-watered and drought-stressed plants without MT treatment; MT50, MT100, and MT200, treatment with MT at different concentrations (50, 100, and 200 µM, respectively) under well-irrigated and drought conditions. Lowercase letters: comparison of MT concentrations under fixed irrigation treatments; uppercase letters: comparison of the irrigation treatment groups under fixed MT concentration levels (Tukey/Games-Howell test, $p < 0.05$). The values represent the mean ± standard deviation of at least five replicates.

4.2.1.2. Alterations in photosynthetic pigments in leaf tissues under salinity stress and melatonin application.

Saline conditions had a detrimental impact on the total chlorophyll (Chl.) and carotenoid (Car.) content in buttercup plants, with the most pronounced effects observed at the higher NaCl concentration of EC 5.5 (Table 6; Figure 9 A and B). The Chl. and Car. content in non-MT-treated plants under both salinity levels (S10MT and S20MT) showed reductions of 45.12% and 55.49% for chlorophyll, and 17.24% and 44.82% for carotenoids, respectively.

In contrast, the application of exogenous melatonin (MT) at various concentrations (50, 100, and 150 µM) significantly enhanced the levels of photosynthetic pigments (total Chl. and Car.) in *R. asiaticus* plants compared to stressed plants without MT treatment under both salinity levels. Notably, foliar application of 200 µM MT at both S1 and S2 levels resulted in substantial increases in Chl. and Car., with enhancements of 68.89% and 83.56%, respectively, compared to salinity-stressed plants without MT application (S10MT and S20MT). This provides evidence of a significant interaction between the phytohormone MT and stress levels, suggesting that melatonin plays a crucial role in modulating plant responses under saline conditions. These findings suggest that 200 µM MT effectively mitigates the influx of harmful ions into plant cells, thereby protecting cellular integrity.

Table 6 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on chlorophyll and carotenoid content in *Ranunculus asiaticus* under saline conditions.

Treatments	**Chlorophyll content (mg g^{-1} FW)**	**Carotenoide content (mg g^{-1} FW)**
Control (non-stressed)	1.64 ±0.015aA	0.29±0.003aA
S1		
MT (0)	0.90±0.010dB	0.24±0.002cB
MT (50)	1.23±0.015cB	0.24±0.002cB
MT (100)	1.24±0.010cB	0.24±0.002cB
MT (200)	1.52±0.006bB	0.25±0.004bB
S2		
MT (0)	0.73±0.021eC	0.16±0.005dC
MT (50)	0.85±0.030dC	0.18±0.003cC
MT (100)	0.95±0.031cC	0.19±0.001cC
MT (200)	1.34±0.031bC	0.21±0.001bC

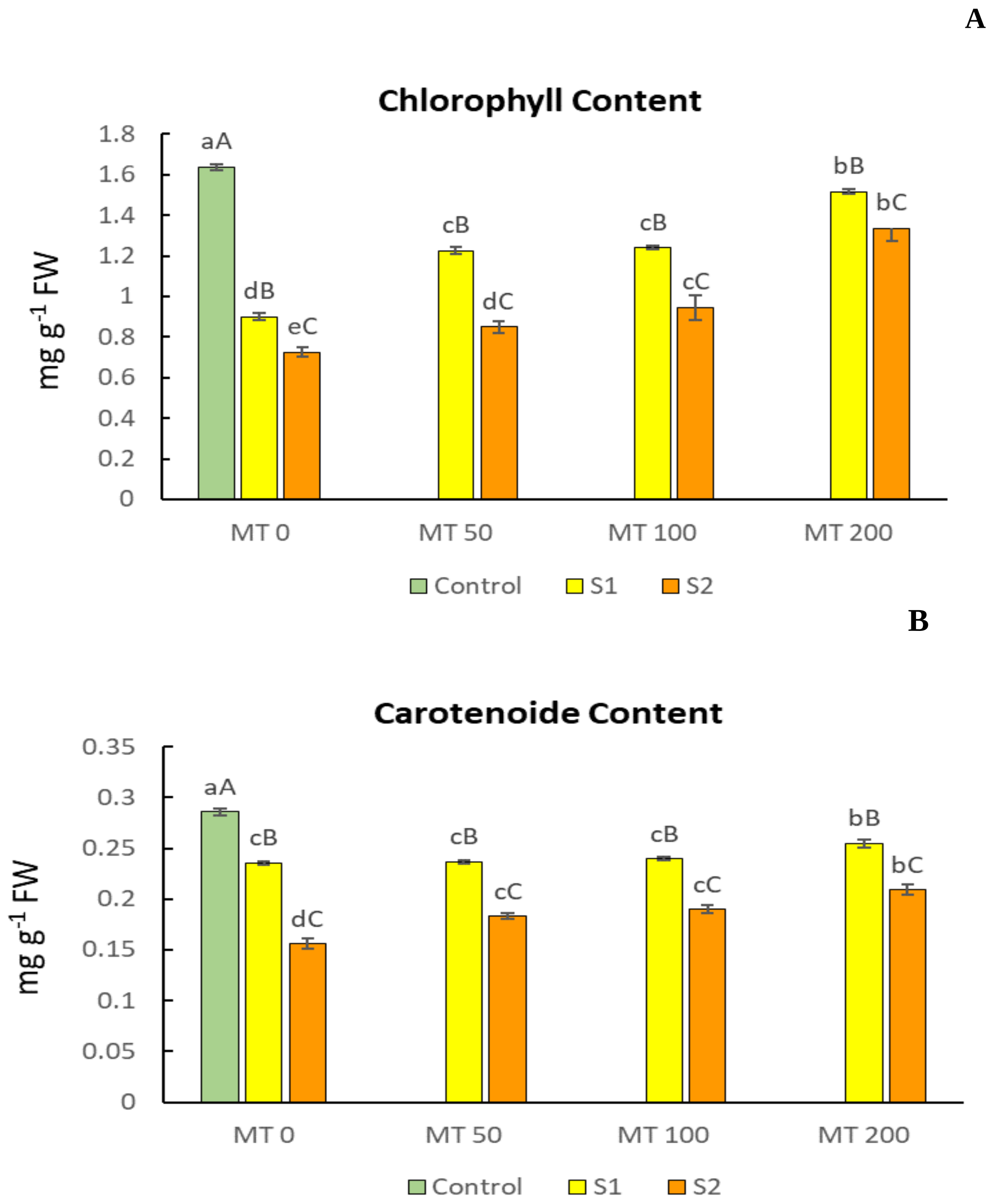


Figure 9 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on chlorophyll content (A), and carotenoid Content (B) in *Ranunculus asiaticus* under saline conditions. Control: no-stress and no melatonin, S1: plants under salt stress EC 4.5 ds.m-1, S2: plants under salt stress EC 5.5 ds.m-1 different letters are for significantly different groups (Tukey/Dunnett $p<0.05$). The lowercase letters are for significant differences amongst melatonin concentrations under fixed salinity treatments, while the uppercase letters for significant differences the salinity treatments with the control group under fixed melatonin concentration levels ($n \geq 5$).

4.2.2. Relative water content and proline accumulation in leaves.

The accumulation of osmolytes in plants serves as an effective defense mechanism against osmotic stress induced by drought (Sahin et al., 2018). Consequently, evaluating the levels of specific accumulated solutes can provide insight into a plant's water stress status. The relative water content (RWC) of plant tissues is widely regarded as a reliable indicator of water status and survival capacity under stressful conditions.

4.2.2.1. Exogenous melatonin modulates relative water content (RWC) and proline accumulation under drought stress.

In this study, a significant reduction in RWC was observed under drought stress, but this decline was alleviated by melatonin (MT) treatment. According to the significant interaction between exogenous MT treatment and drought treatments, we obtained the following results. Untreated and drought-stressed plants (D0MT) exhibited a notable decrease in RWC, with a reduction of 7.1% compared to well-watered untreated plants (W0MT). In contrast, MT-treated plants demonstrated significantly improved RWC under stress, with the most substantial enhancement occurring at 200 µM MT, which resulted in a 6.4% increase in RWC compared to untreated drought-stressed plants (D0MT) (see Table 7 and Figure 10A).

Proline functions as a compatible solute that helps maintain membrane integrity, enhances cytoplasmic osmotic pressure, and protects cells during dehydration. The current investigation revealed that proline levels in *R. asiaticus* plants experiencing drought stress were higher than those in well-irrigated plants (Table 7 and Figure 10B). Overall, proline levels were influenced by both drought stress and MT application. Under drought conditions, proline content in plants treated with MT at 50 µM increased slightly by 17.8% compared to stressed plants without MT treatment (D0MT). Furthermore, plants treated with MT at concentrations of 100 µM and 200 µM exhibited significant increases in proline content of 28.6% and 32.1%, respectively, relative to untreated drought-stressed plants (D0MT). Additionally, applying MT at concentrations of 0, 50, 100, and 200 µM under non-stressed conditions resulted in a gradual increase in proline content in well-irrigated plants (W0MT), although these levels were lower than those observed in drought-stressed plants.

These findings suggest that proline plays a crucial role in the leaves of *R. asiaticus* plants by employing various mechanisms to mitigate the adverse effects of drought. Therefore, the application of exogenous MT may help restore these changes and enhance protective responses.

Table 7 Impact of exogenous melatonin on relative water content (%) and proline accumulation in *Ranunculus asiaticus* under drought stress conditions**.**

Treatments		**Relative water content %**	**Proline content (µmol g^{-1}/FW)**
Effect of MT (µM) under well-irrigated conditions	0	83.96 ± 0.27 dA	0.27 ± 0.0001 dB
	50	84.48 ± 0.36 cA	0.39 ± 0.0002 cB
	100	84.56 ± 0.26 bA	0.40 ± 0.0004 bB
	200	86 ± 0.16 aA	0.41 ± 0.0003 aB
Effect of MT (µM) under drought conditions	0	78 ± 0.16 dB	0.56 ± 0.0002 dA
	50	80.54 ± 0.95 cB	0.66 ± 0.0002 cA
	100	81.98 ± 0.19 bB	0.72 ± 0.0002 bA
	200	82.96 ± 0.15 aB	0.74 ± 0.0003 aA

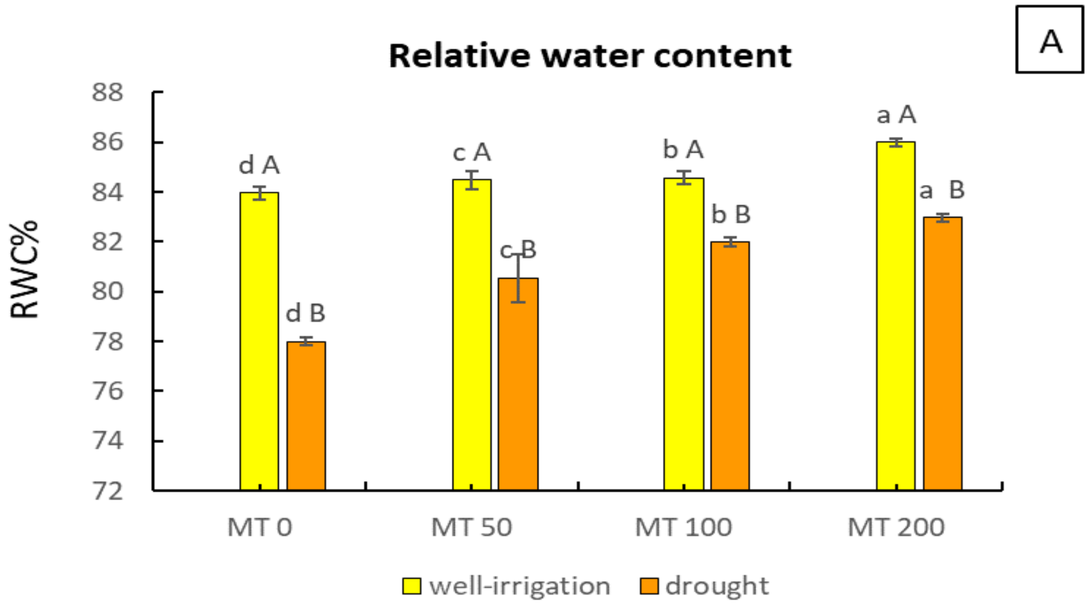


Figure 10. A. Impact of exogenous melatonin on the relative water content (%) of *R. asiaticus* under drought stress conditions.

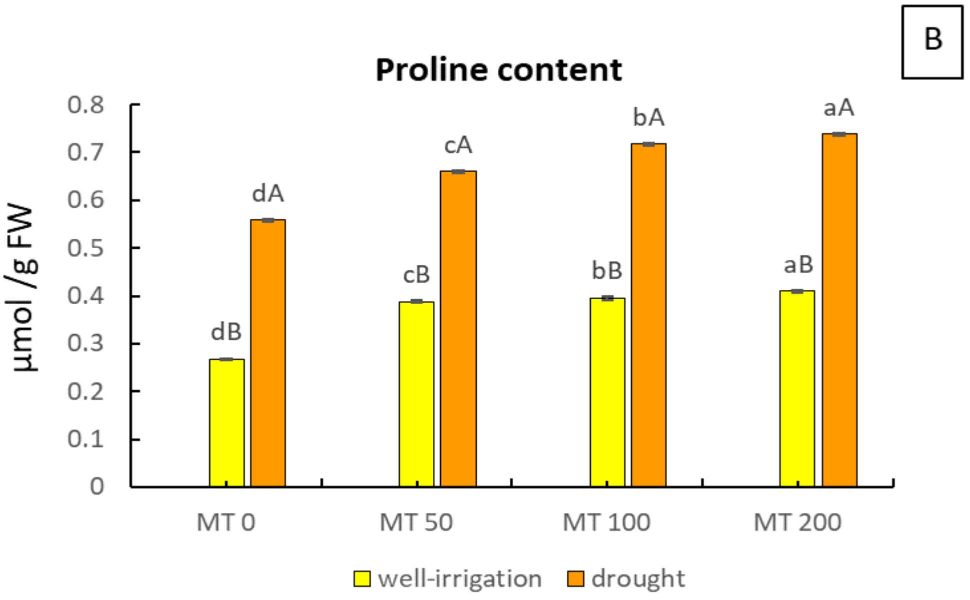


Figure 10 B. Impact of exogenous melatonin on proline accumulation (µmol /g FW) of *R. asiaticus* under drought stress conditions. MT0, well-watered and drought-stressed plants without MT treatment; MT50, MT100, and MT200, treatment with different MT concentrations (50, 100, and 200 µM, respectively) under non-stressful and stressful conditions. The values represent the mean ± standard deviation of at least five replicates. Lowercase letters: comparison of MT concentrations under fixed irrigation treatments; uppercase letters: comparison of the irrigation treatment groups under fixed MT concentration levels (Tukey/Games–Howell test, $p < 0.05$).

4.2.2.2. Alterations in relative water Content (RWC) and proline accumlation in leaves of *R. asiaticus* under salinity stress.

Our data indicated that saline conditions adversely affected the RWC of leaves, particularly at EC 5.5 dS m^{-1}, which exhibited the most pronounced negative impact (Table 8, Figure 11A). Stressed plants (S10MT and S20MT) showed significant decreases in RWC of 21.1% and 28.3%, respectively, compared to non-stressed control plants. Conversely, foliar melatonin treatment resulted in a slight improvement in RWC under saline conditions. Increases in RWC were observed with the application of 100 µM and 200 µM melatonin compared to non-MT-treated stressed plants; however, these values remained lower than those of control plants (Table 8, Figure 11B). This highlights the significant interaction between exogenous MT application and salt stress, leading to the following results. These results suggest that foliar application of melatonin has beneficial effects on plant biomass and development under salt stress, with the 200 µM concentration providing better protection for the plants compared to the lower concentrations of 100 µM and 50 µM.

Furthermore, our results indicated that saline conditions significantly elevated proline content in leaves, with the highest levels observed in the S2 treatment (EC 5.5). Stressed plants (S10MT and S20MT) demonstrated an approximate 50% increase in proline content relative to control plants. Additionally, foliar application of melatonin at concentrations of 50 µM, 100 µM, and 200 µM resulted in a gradual increase in proline levels under saline conditions compared to non-MT-treated stressed plants (Table 8; Figure 11B).

Table 8 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on relative water content (RWC) and proline level under salt stress.

Treatments	**Relative water content (%)**	**Proline content (µmol g^{-1}/FW)**
Control (non-stressed)	83.65±0.27aA	0.27±0.001eC
S1		
MT (0)	66.00±0.15dB	0.54±0.004dB
MT (50)	70.00±0.19cB	0.57±0.004cB
MT (100)	74.33±0.16bB	0.59±0.000bB
MT (200)	75.13±0.22bB	0.61±0.002aB
S2		
MT (0)	60.00±0.16dC	0.55±0.003dA
MT (50)	67.00±0.18cC	0.60±0.002cA
MT (100)	73.00±0.20bC	0.63±0.003bA
MT (200)	75.13±0.23bC	0.64±0.003aA

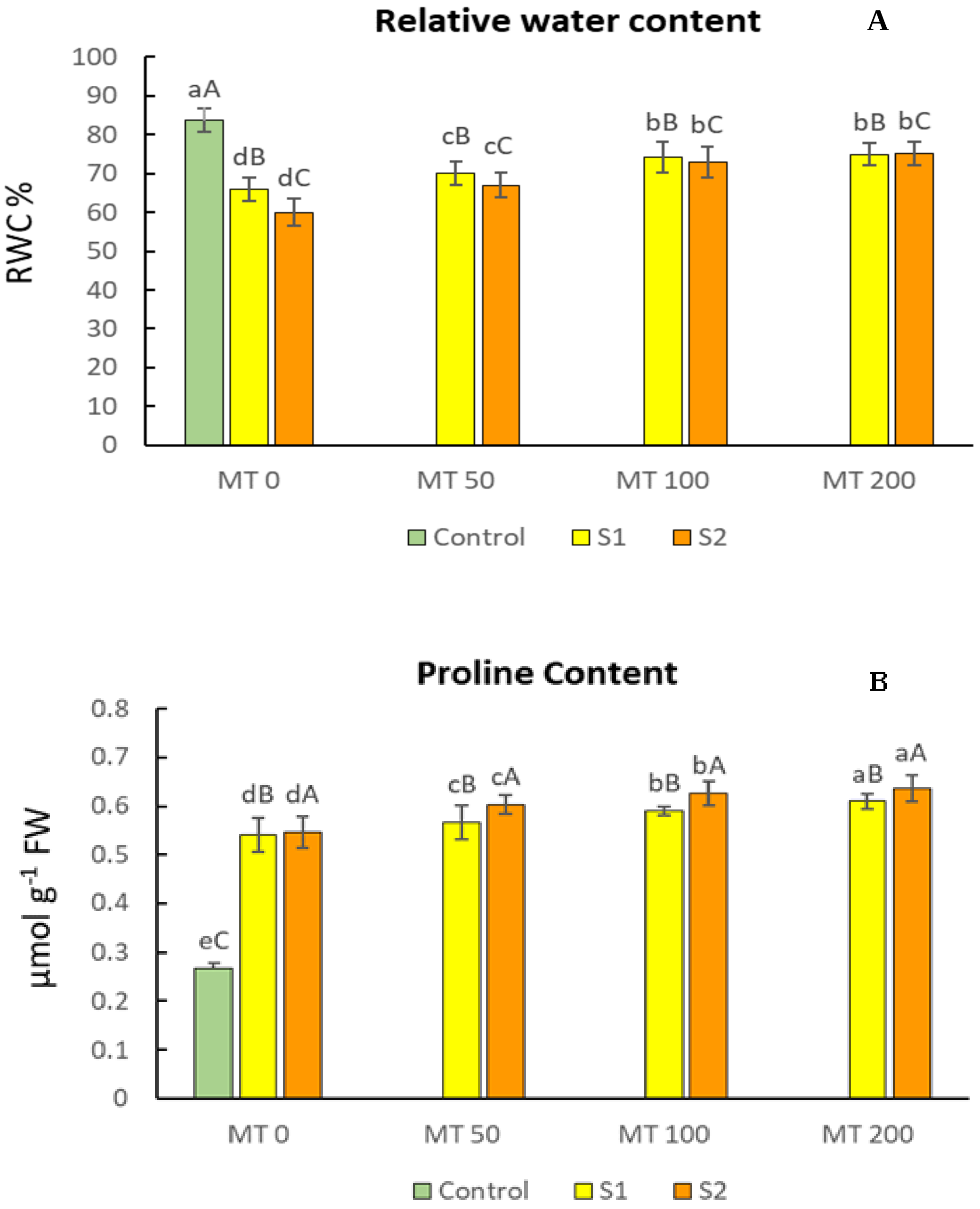


Figure 11 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on relative water content (RWC) (A) and proline accumlation (B),Control: no-stress and no melatonin, S1: plants under salt stress EC 4.5 ds.m-1, S2: plants under salt stress EC 5.5 ds.m-1 different letters are for significantly different groups (Tukey/Dunnett $p<0.05$). The lowercase letters are for significant differences amongst melatonin concentrations under fixed salinity treatments, while the uppercase letters for significant differences the salinity treatments with the control group under fixed melatonin concentration levels ($n \geq 5$).

4.2.3. Alterations in sodium ion levels in leaves under salinity stress.

Our findings demonstrated that *R. asiaticus* exhibits sensitivity to salinity, as evidenced by an increase in sodium ion (Na^+) concentration. The Na^+ levels in dry leaves rose by 280% and 311% at electrical conductivities of 4.5 (S1) and 5.5 (S2) dS m^{-1}, respectively, when compared to control plants (Figure 12). Notably, the interaction between exogenous melatonin (MT) application and salinity stress played a crucial role in mitigating these adverse effects. The application of exogenous melatonin (MT) at varying concentrations (50, 100, and 150 µM) significantly reduced Na^+ content in the leaf samples of *R. asiaticus* plants subjected to both salinity levels (Table 9, Figure 12). Specifically, foliar application of 200 µM MT at both S1 and S2 levels resulted in a substantial reduction of Na^+ content by 50.4% and 20.5%, respectively, compared to salinity-stressed plants without MT treatment (S10MT and S20MT).

Table 9 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on Sodium Ion (Na+) levels in *R. asiaticus* leaves.

Treatments	**NaCl content (g 100g^{-1} DW)**
Control (non-stressed)	0.65 ±0.21dC
S1	
MT (0)	2.47±0.06aB
MT (50)	2.21±0.09bB
MT (100)	2.14±0.06bB
MT (200)	1.23±0.06cB
S2	
MT (0)	2.67±0.06aA
MT (50)	2.60±0.18bA
MT (100)	2.46±0.14bcA
MT (200)	2.13±0.10cA

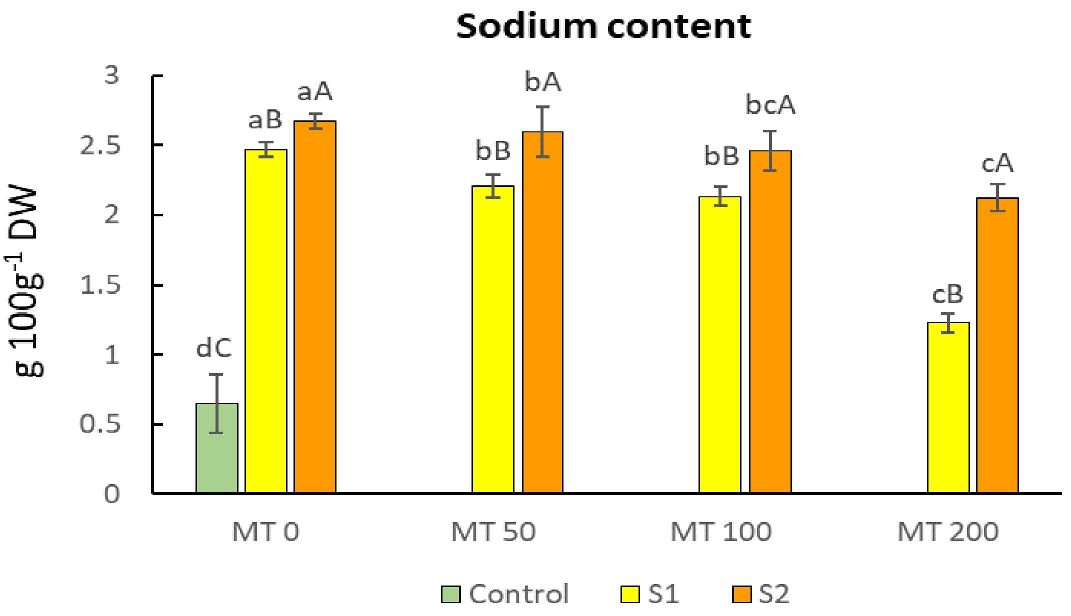


Figure 12 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on sodium ion (Na+) levels**,** Control: no-stress and no melatonin, S1: plants under salt stress EC 4.5 ds.m^{-1}, S2: plants under salt stress EC 5.5 ds.m^{-1} different letters are for significantly different groups (Tukey/Dunnett $p<0.05$). The lowercase letters are for significant differences amongst melatonin concentrations under fixed salinity treatments, while the uppercase letters for significant differences the salinity treatments with the control group under fixed melatonin concentration levels ($n \geq 5$).

4.2.4. Oxidative stress and membrane stability of *R. asiaticus* under stressful conditions.

Drought and salinity stress leads to an increase in ROS, which, when present in excess, can compromise cell membrane permeability. Electrolyte leakage (EL) serves as a measure of this oxidative damage (H.J. Zhang et al., 2014).

4.2.4.1.Melatonin modulates peroxidase enzyme activity (POD) and regulates electrolyte leakage (El) under drought stress.

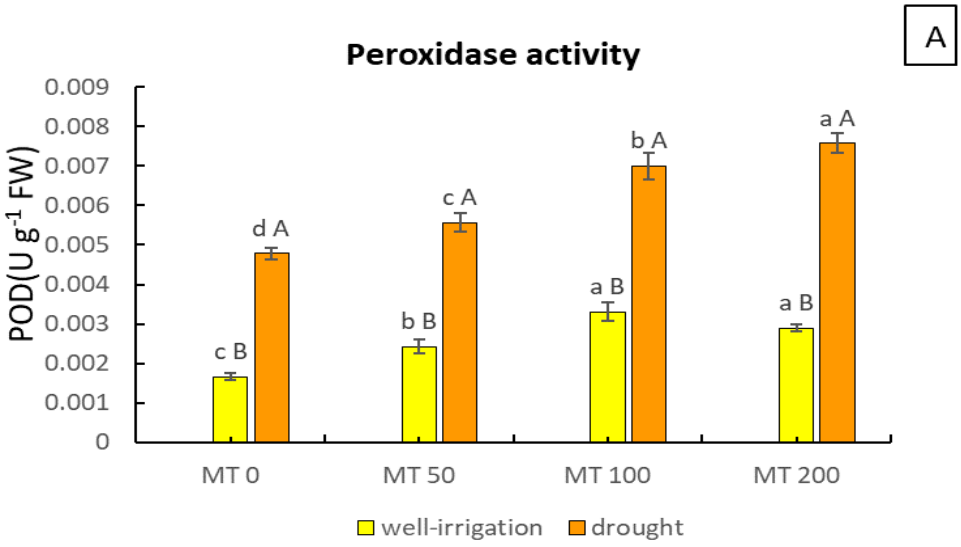
In our experiments, we examined the correlation between EL and POD activity to assess the role of POD in maintaining membrane integrity (Table 7 and Figure 8A and B). Melatonin (MT) acts as an activator of antioxidant enzymes, providing protection to plants against oxidative stress (Na. Zhang et al., 2015). This study investigated the impact of drought stress on POD activity in *R. asiaticus* plants, both with and without MT treatment (Table 10 and Figure 13A).

The results indicated that the absence of MT significantly reduced POD activity in plants under both well-irrigated and drought conditions compared to those treated with MT. Additionally, POD activity was found to be lower in well-watered plants than in drought-stressed plants, exhibiting a concentration-dependent response. Specifically, the POD activity in untreated/stressed plants (D0MT) increased by 64.6% compared to untreated/well-irrigated plants (W0MT). In non-stressed conditions, MT treatment at concentrations of 50 µM and 100 µM resulted in gradual increases in antioxidant enzyme activity (41.2% and 94.1%, respectively), while a slight decrease was observed at 200 µM MT (70.6%), which still exceeded the levels seen in untreated plants (W0MT).

In contrast, following drought exposure, MT-treated plants at concentrations of 50 µM, 100 µM, and 200 µM demonstrated progressive increases in POD activity of 16.6%, 45.8%, and 58.3%, respectively, compared to untreated plants (D0MT). The effects of water deficit and exogenous MT on membrane integrity and EL are depicted in Figure 5B. Under stressful conditions, EL was significantly elevated. However, when compared to non-MT-stressed plants, those treated with MT at concentrations of 50, 100, and 200 µM exhibited substantial reductions in EL of 5.7%, 7.7%, and 14.3%, respectively. Aligned with the highly significant interaction between drought stress and MT treatment, these findings suggest that foliar application of MT effectively mitigates ROS accumulation, with the most pronounced effect observed at 200 µM.

Table 10 Effect of exogenous melatonin on peroxidase activity and electrolyte leakage % in *R. asiaticus* plants subjected to drought stress conditions.

Treatments		**EL%**	**POD (U g^{-1} FW)**
Effect of MT (µM) under well-irrigated conditions	0	29.09 ± 0.126 aB	0.0017± 0.0001 c B
	50	25.66 ± 0.016 bB	0.0024± 0.0002 b B
	100	23.40 ± 0.016 cB	0.0033± 0.0002 a B
	200	23.05 ± 0.063 dB	0.0029± 0.0001 a B
Effect of MT (µM) under drought conditions	0	35.05 ± 0.061 aA	0.0048± 0.0002 d A
	50	33.05 ± 0.028 bA	0.0056± 0.0002 c A
	100	32.35 ± 0.008 cA	0.0070± 0.0003 b A
	200	30.02 ± 0.024 dA	0.0076± 0.0003 a A



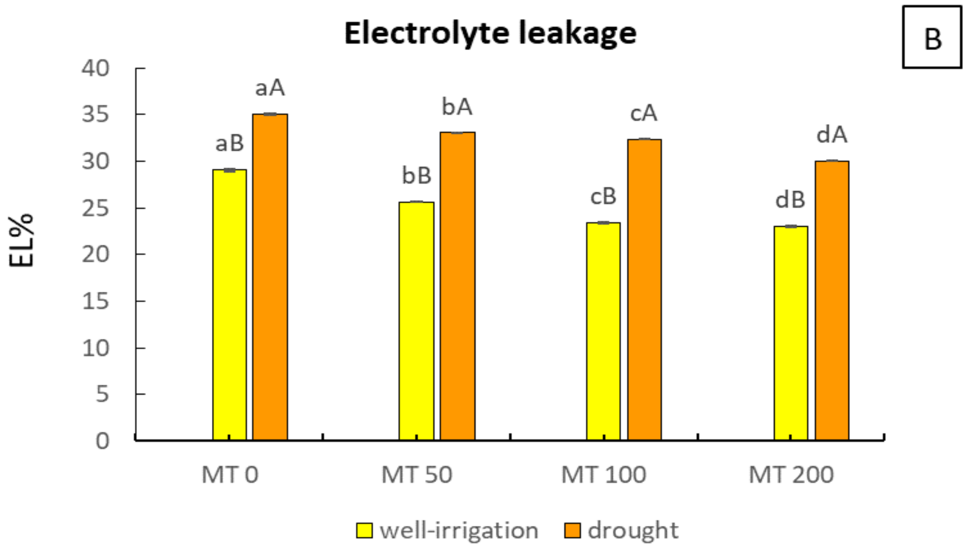


Figure 13 Impact of exogenous melatonin on peroxidase activity [POD (U g−1 FW)] (**A**) and electrolyte leakage [El (%)] (**B**) in *R. asiaticus* plants subjected to drought stress conditions. MT0, well-watered and drought-stressed plants without MT treatment; MT50, MT100, and MT200, treatment with different MT concentrations (50, 100, and 200 µM, respectively) under non-stressful and stressful conditions. The values represent the mean ± standard deviation of at least five replicates. Lowercase letters: comparison of MT concentrations under fixed irrigation treatments; uppercase letters: comparison of the irrigation treatment groups under fixed MT concentration levels (Tukey/Games–Howell test, $p < 0.05$).

4.2.4.2. Alterations in peroxidase enzyme activity (POD) and electrolyte leakage (EL) under salinity stress.

A notable increase ($p < 0.05$) in POD was observed in buttercup plants exposed to both salinity stress conditions (Table 11; Figure 14 A). Specifically, POD levels rose by 61.9% and 82.2% when the plants were irrigated with saline water at electrical conductivities of 4.5 and 5.5 dS m^{-1}, respectively, compared to non-stressed control plants.

Under both S1 and S2 salinity conditions, all melatonin treatments (50 µM, 100 µM, and 200 µM) significantly increased ($p < 0.05$) POD activity, with the highest levels recorded following the application of 200 µM MT. In particular, a 48.8% increase in antioxidant enzyme activity was observed in plants under S1 conditions, while a 46.7% increase was noted under S2 conditions with the same MT treatment, relative to non-melatonin-treated stressed plants at both salinity levels. These results indicate that NaCl stress enhances POD activity to a certain extent, and that the foliar application of melatonin further amplifies this activity in a linear concentration-dependent manner, thereby contributing to cellular protection against the accumulation of ROS.

Additionally, the effects of salinity stress and exogenous melatonin application on membrane integrity and electrolyte leakage (EL) are depicted in Figure 14 B. Saline conditions induced by NaCl concentrations of 4.5 and 5.5 dS m^{-1} led to significant increases in electrolyte leakage, with values rising by 64.01% and 67.74%, respectively, compared to control plants. Conversely, in stressed plants treated with foliar applications of melatonin at concentrations of 50 µM, 100 µM, and 200 µM, there was a notable and concentration-dependent reduction in electrolyte leakage. Specifically, the application of 200 µM MT resulted in the most substantial decreases in EL, with reductions of 28.93% and 20.57% under the S1 and S2 salinity levels, respectively, when compared to salt-stressed plants without MT treatment (Table 11; Figure 14B). The significant interaction between salinity stress and MT application was evident in both POD activity and EL, further supporting the protective role of melatonin in mitigating oxidative damage and maintaining membrane stability under saline conditions.

Table 11 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on peroxidase enzyme activity and electrolyte leakage in *Ranunculus asiaticus* under saline conditions.

Treatments	**POD (U g^{-1} FW)**	**El %**
Control (non-stressed)	0.0008±0.0002aC	29.08 ±0.029aA
S1		
MT (0)	0.0021±0.0001eB	80.74±0.020bB
MT (50)	0.0026±0.0002dB	76.04±0.010cB
MT (100)	0.0033±0.0001cB	71.11±0.015dB
MT (200)	0.0041±0.0002bB	57.38±0.010eB
S2		
MT (0)	0.0024±0.0002dA	90.07±0.020bC
MT (50)	0.0027±0.0002dA	82.24±0.002cC
MT (100)	0.0038±0.0001cA	75.31±0.002dC
MT (200)	0.0045±0.0001bA	71.54±0.020eC

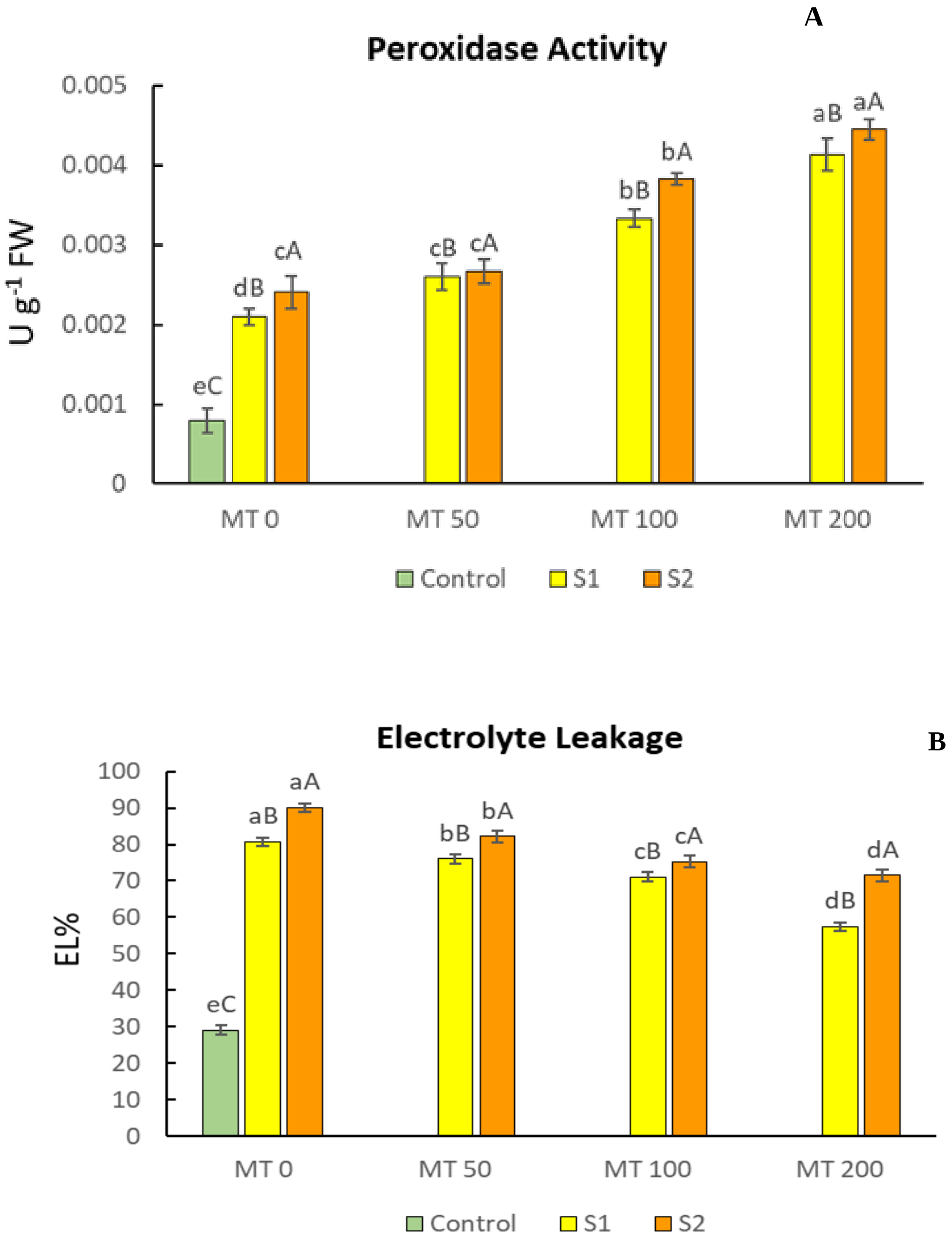


Figure 14 Effects of exogenous melatonin application (0, 50, 100, and 200 µM) on peroxidase enzyme activity (**A**) and electrolyte leakage levels (**B**) in *Ranunculus asiaticus* under saline conditions. Control: no-stress and no melatonin, S1: plants under salt stress EC 4.5 ds.m-1, S2: plants under salt stress EC 5.5 ds.m-1 different letters are for significantly different groups (Tukey/Dunnett $p<0.05$). The lowercase letters are for significant differences amongst melatonin concentrations under fixed salinity treatments, while the uppercase letters for significant differences the salinity treatments with the control group under fixed melatonin concentration levels ($n \geq 5$).

4.2.5. Stress response index

4.2.5.1. Drought tolerance index (DTI).

Table 12 presents the drought tolerance index (DTI) of the studied plants, expressed as a percentage of all measured variables in both non-MT-treated plants grown under well-irrigated and drought stress conditions. The DTI% indicates that POD activity was the most responsive trait to drought stress, with a DTI value of 282.35%, surpassing the DTI% of other traits. Proline content followed as the second most sensitive trait, recording a DTI of 207.40%, while electrolyte leakage (El) had a DTI of 120.49%.

Furthermore, other traits exhibited DTI values below 100% but above 50%. Notably, carotenoids, shoot dry weight, leaf area, and total chlorophyll (Chl) demonstrated the least sensitivity to drought conditions, with DTI values of 49.12%, 47.20%, 37.84%, and 32.00%, respectively. Consequently, total chlorophyll content and leaf area emerged as the most sensitive traits to drought stress, whether or not MT was applied, as both were reduced by more than 60%.

Table 12 Drought tolerance index (DTI%) of all investigated *R. asiaticus* characteristics**.**

Traits	**DTI (%)**
Shoot length	74.02 ± 2.83 ef
Leaf number	69.37 ± 9.35 ef
Leaf area	37.84 ± 0.39 gh
Shoot fresh weight	65.53 ± 1.73 f
Shoot dry weight	47.20 ± 3.48 g
Time of flower bud emergence	79.31 ± 2.30 ed
Total chlorophyll content	32.00 ± 1.02 h
Carotenoid content	49.12 ± 0.58 g
Relative water content (RWC)	92.90 ± 0.45 d
Proline content	207.40 ± 0.30 b
Peroxidase activity (POD)	282.35 ± 10.53 a
Electrolyte leakage (El)	120.49 ± 0.72 c

The values represent the mean ± standard deviation of at least five replicates. Different letters indicate significantly different groups (post hoc Tukey’s test, $p < 0.05$). Lowercase letters: comparison of the tolerance index of all estimated traits under drought stress without MT application.

4.2.5.2.Salt tolerance index (STI)

Table 13 presents the salt tolerance index (STI) as a percentage of all assessed variables comparing non-melatonin-treated plants grown under unstressed conditions to those subjected to S2 salinity levels (EC 5.5 dS m^{-1}). The STI% analysis revealed that leaf Na^+ content was the most sensitive trait to saline conditions, with a value of 437.9%, surpassing the STI% of other variables. This was followed by POD activity and electrolyte leakage (EL), which recorded values of 321.3% and 309.9%, respectively. Proline content ranked third in terms of salt response, achieving an STI% of 202.5%. Additionally, the remaining traits exhibited STI% values below 100% but above 50%, with the exception of total chlorophyll, which demonstrated the lowest responsiveness to salinity, registering an STI% of only 44.4%.

Table 13 Salt tolerance index (STI) % of *R. asiaticus* traits**.**

Traits	**STI %**
Shoot length	64.4±0.2 d
Number of leaves	65.9±0.2 d
Total leaf area	39.0±0.03 d
Shoot fresh weight (FW)	63.4±0.0 d
Shoot dry weight (DW)	53.9±0.3 d
Emergence of Flower buds	74.3±2.5 d
Total Ch. content	44.4±1.2 d
Total Car. content	54.5±2.1 d
Relative water content (RWC)	71.7±0.3 d
Proline content	202.5±2.1 c
Leaves Na^+ content	383.1±23.7 a
Peroxidase activity (POD)	309.9±0.3 bc
Electrolyte leakage (El)	321.3±28.5 b

The values represent the mean of at least five replicates for comparison of tolerance index of all estimated traits under salt stress induced by EC 5.5 ds.m^{-1} without melatonin application. Lowercase letters: comparison of the tolerance index of all estimated traits under salt stress without MT application.

5. DISCUSSIONS

Water scarcity and salinity are among the most significant environmental stressors affecting plant growth and productivity, particularly in arid and semi-arid regions. Both stressors induce a range of physiological, anatomical, and morphological alterations, negatively impacting plant development (M. Liu et al., 2015).

In this study, drought stress markedly reduced all vegetative growth parameters in *R. asiaticus*, with increasing stress severity exacerbating these reductions (Table 3 and figure 4). The observed stunted growth under drought conditions is a morphological adaptation that plants employ to minimize water loss by reducing the area available for transpiration. Previous studies (Elewa et al., 2017; Sadiq et al., 2018; Dawood et al., 2019; Sadak et al., 2020) have shown that drought stress adversely affects desirable traits in crops such as mung bean, flax, sunflower, and *Moringa oleifera*, while also diminishing their water retention capabilities. The authors attributed these changes to drought-induced diseases and the formation of ROS (Dawood and Sadak, 2014; Sadak et al., 2020). The reductions in plant height observed following drought exposure can be explained by decreases in cell elongation, turgor pressure, and overall cell growth (Banon et al., 2006).Water deficit reduces the water content in plant shoots, leading to osmotic stress that inhibits cell development and division, ultimately resulting in stunted overall plant growth (Bakry et al., 2012; Alam et al., 2014). Additionally, a reduction in leaf area under stress conditions diminishes water loss and carbon assimilation, degrades photosynthetic pigments, and adversely affects photosynthesis, thereby negatively impacting plant growth (Naeem et al., 2018).

Moreover, salinity stress negatively impacts plant performance due to osmotic, ionic, and nutritional disorders, which are reflected in the morphological characteristics of affected plants. Ranunculus, particularly as a cut flower species, is known to be sensitive to salinity stress (Valdez-Aguilar et al., 2009 ; Rauter et al., 2021). In this study, exposing *R.asiaticus* to salinity stress, particularly at higher electrical conductivities (EC 4.5 and 5.5), significantly inhibited plant growth, with the highest EC level proving most detrimental (Table 4 Figures 5). These reductions align with previous findings on *Ranunculus*, where increasing EC levels from 2 to 3 dS•m^{-1} impaired shoot development, dry weight accumulation, leaf count, and flowering stem diameter (Valdez-Aguilar et al., 2009). Moreover, it has been documented that leaf-related parameters are significantly affected by salinity. The findings of this study underscore the sensitivity of ranunculus to salinity stress, highlighting the importance of managing salinity levels in cultivation practices for optimal growth and development (Poorter et al., 2010).

Melatonin (MT), a well-documented plant growth regulator, has been widely recognized for its dual role in promoting plant development and enhancing stress tolerance (Imran et al., 2021). Research has consistently demonstrated MT's effectiveness in enhancing plant resilience and supporting growth under challenging environmental conditions (Szafrańska et al., 2016; Han et al., 2017; Kamiab, 2020; Sadak et al., 2020; Imran et al., 2021). Melatonin (MT) plays a crucial role in regulating the biochemical and physiological functions of plants (Wei et al., 2015; El-Awadi et al., 2017; Li et al., 2018; Sadak et al., 2020; Ahmad et al., 2021a), operating effectively even in trace amounts (N. Zhang et al., 2015). As an indoleamine, MT shares a metabolic precursor with indole-3-acetic acid (IAA), which may explain its role in promoting cell elongation, division, and overall vegetative growth (Hernández-Ruiz et al., 2004 and Szafrańska et al., 2016).

In this study on drought stress, MT treatment progressively enhanced vegetative growth traits under non-stress conditions as the concentration of MT increased. Furthermore, the negative effects of drought stress on *R. asiaticus* traits were alleviated, leading to improved growth performance (as illustrated in Table 3 and Figure 4). Specifically, plants treated with 100 µM and 200 µM MT exhibited significant enhancements in shoot length and leaf number compared to untreated drought-stressed plants. Additionally, the highest fresh and dry weights were recorded in plants treated with 200 µM MT, surpassing those treated with 100 µM and 50 µM under drought stress (Table 3, Figures 4). These findings support the enhancing role of melatonin in improving morpho-physiological traits under drought conditions, aligning with the findings of Imran et al. (2021); Sadak and Ramadan (2021); Altaf et al. (2022), who reported that exogenous melatonin (MT) enhanced the growth of soybean, lupin, and tomato plants under drought conditions. Similarly, under salinity stress, our findings align with numerous studies that confirm MT's effectiveness as a dose-dependent enhancer of vegetative traits, as evidenced by our evaluation of *Ranunculus* plants under saline conditions, where a foliar spray of 200 µM MT proved to be most effective in mitigating stress impacts across various traits. This concentration also mirrors recent findings on cotton seedlings, emphasizing the importance of applying exogenous melatonin at species-specific optimal levels (Bajwa et al., 2014). Furthermore, these results suggest that elevated levels of MT may enable plants to withstand prolonged periods of stress, thereby significantly boosting their yield potential (Wei et al., 2015).

The protective role of MT is likely linked to its regulatory function in stress response pathways. Studies indicate that MT is recognized for its role as an activator of antioxidant enzymes, providing protection against oxidative stress (Na. Zhang et al., 2015). Furthermore, Han et al. (2017) demonstrated that the exogenous application of MT in soybean plants led to increased accumulation of ABA, enhancing their resistance to drought stress. This indicates a potential interaction between MT and other plant hormones. Additionally, exogenous MT has been shown to upregulate the expression of defense-related components; for instance, a transcriptome analysis in *Arabidopsis* revealed that auxin-responsive genes were both upregulated and downregulated in response to MT treatment (Weeda et al., 2014; Li et al., 2018).

In our study, drought and salt-stressed plants exhibited earlier flower bud emergence compared to those receiving adequate irrigation (Figure 6 and 7). This early development of flower buds appears to be a strategic response to stressful conditions, allowing plants to adapt their growth patterns accordingly (Sharp et al., 2009). Such adaptations may enable plants to ensure the continuation of their species even in challenging environments (Wada and Takeno, 2010). Previous research has also indicated that shoots under drought stress tend to develop flower buds earlier and on shorter stems than those subjected to less stressful conditions (Chimonidou-Pavlidou, 2001).

Melatonin (MT) has been identified as a key modulator of flowering responses under abiotic stress conditions. While drought and salinity stress may trigger early or delayed flowering as survival mechanisms, MT application can regulate these responses by influencing key genetic pathways involved in flowering (Zhang et al., 2019; Ruiz et al., 2020). In *Arabidopsis*, exogenous MT application has been shown to delay flowering by activating *Flowering Locus C* (FLC), which suppresses the transcription of *Flowering Locus T* (FT), a key promoter of flowering (Shi et al., 2016; Lozano-Juste and León, 2011). This mechanism suggests that MT plays a crucial role in maintaining optimal flowering time, potentially counteracting the premature flowering observed under drought stress while mitigating the negative effects of salinity-induced delays.

Photosynthetic pigments, including chlorophyll and carotenoids, are essential for vital physiological functions such as light absorption, energy transmission, and overall photosynthetic efficiency (Arnao and Hernández-Ruiz, 2009). However, abiotic stresses such as drought and salinity significantly impact pigment biosynthesis and stability, leading to reduced photosynthetic capacity and impaired plant growth.

Drought stress has been shown to significantly ($p < 0.05$) reduce chlorophyll and carotenoid content in *R. asiaticus* (Table 5, Figure 8A and B). This decline is primarily attributed to dehydration-induced damage to mesophyll cells and the inhibition of enzymes responsible for glucose metabolism, ultimately leading to impaired pigment biosynthesis and degradation (Din et al., 2011; Pandey et al., 2012). Carotenoids, known for their photo-protective and antioxidant properties, play a critical role in stabilizing chlorophyll and mitigating oxidative damage under stress conditions (Szafrańska et al., 2016; Emiliani et al., 2018).

Similarly, under salinity stress, total chlorophyll and carotenoid levels decreased, particularly under the S2 condition (Table 6 and Figure 9A and B). The reduction in pigments is largely associated with excessive Na+ accumulation, which disrupts pigment synthesis and accelerates chlorophyll degradation, leading to compromised photosynthetic efficiency (Muller et al., 2014).

Despite the adverse effects of drought and salinity stress on pigment levels, foliar melatonin application significantly enhanced chlorophyll and carotenoid content under both normal and stress conditions. The present findings demonstrate that *R. asiaticus* plants subjected to drought and salinity stress exhibited higher pigment retention and photosynthetic efficiency when treated with melatonin compared to untreated plants, suggesting that MT pretreatment supports chlorophyll biosynthesis while minimizing degradation (Liang et al., 2019). Specifically, 200 µM MT was the most effective concentration in preventing pigment degradation and maintaining carotenoid levels under drought conditions. Similarly, under salinity stress, exogenous melatonin significantly boosted chlorophyll and carotenoid levels in *Ranunculus* plants, with the highest pigment accumulation observed under the S1 (4.5 EC) condition. This aligns with previous findings showing that melatonin application enables salt-stressed plants to sustain chlorophyll levels comparable to those in unstressed conditions, as observed in various species, including melon (*Cucumis melo* L.), pistachio, maize seedlings, and spearmint plants (Castañares and Bouzo, 2019; Kamiab, 2020; Ahmad et al., 2021b; Gohari et al., 2023).

This protective effect of MT has been widely reported in previous research, including in rice (*Oryza sativa*) and *Moringa oleifera*, where MT application under abiotic stress conditions enhanced chlorophyll stability and photosynthetic performance (Han et al., 2017; Sadak et al., 2020). The mechanism underlying this effect is attributed to melatonin's strong antioxidant capacity, which reduces reactive oxygen species (ROS) accumulation and modulates the expression of chlorophyll-degrading genes such as *chlorophyllase*, *pheophorbide oxygenase*, and *red chlorophyll catabolite reductase* (Ruiz et al., 2020). Additionally, MT has been found to upregulate carotenoid biosynthesis while mitigating photooxidative damage, thereby enhancing stress resilience in plants exposed to drought and salinity (Szafrańska et al., 2016; Liang et al., 2019).

Relative water content (RWC) is widely recognized as a rapid and reliable indicator of stress levels in various plant species, closely associated with physiological changes at both the leaf and whole-plant levels (Wang et al., 2019; Oraee and Tehranifar, 2020). Maintaining high RWC is a critical strategy in breeding programs aimed at enhancing stress tolerance, as it plays a vital role in sustaining plant metabolism (Soltys-Kalina et al., 2016). A decline in RWC typically indicates a loss of turgor pressure and reduced water availability for cellular growth. The extent of RWC reduction in stressed plants largely depends on their ability to absorb water and regulate stomatal function to minimize water loss (Keyvan, 2010).

In this study, RWC significantly decreased in *R. asiaticus* plants subjected to drought stress compared to well-irrigated controls, confirming that drought-exposed plants experienced substantial water deficit (Table 7, Figure 10 A). Similarly, under salinity conditions (Table 8, Figure 11 A) RWC was also reduced due to toxic ion accumulation, particularly Na^+ and Cl^-, which induced leaf shrinkage and stomatal closure, ultimately decreasing internal CO_2 partial pressure and exacerbating dehydration, reduced cell growth, and a cascade of metabolic changes resembling those caused by water deficit (Liu et al., 2015; Liang et al., 2015). Such effects have been reported in various salt-sensitive species, including melon (*Cucumis melo* L.) and pepper cultivars (Castañares and Bouzo, 2019; Hand et al., 2017).

Despite these adverse effects, foliar application of melatonin significantly improved RWC in *R. asiaticus* plants under both drought and salinity stress. This enhancement is likely due to MT's role in stabilizing cell membranes and improving water absorption efficiency (Castañares and Bouzo, 2019; Hu et al., 2022). Previous studies have demonstrated that melatonin can strengthen the plant cuticle, thereby reducing transpirational water loss and contributing to turgor maintenance (Liu et al., 2015). In drought-stressed plants, MT-treated individuals exhibited higher RWC levels, suggesting that melatonin aids in drought tolerance by enhancing water retention and mitigating dehydration. Similarly, under salinity conditions, increasing MT concentrations progressively improved RWC, with the highest MT dose yielding the most pronounced effect across both salinity levels, S1 (4.5 EC) and S2 (5.5 EC). These findings align with previous research on borage (*Borago officinalis*), which also demonstrated improved water balance following MT treatment (Farouk and Al-Huqail, 2022).

Proline accumulation is a crucial adaptive response that enhances plant resilience under adverse environmental conditions, including both drought and salinity stress. As a multifunctional osmolyte, proline plays a significant role in stabilizing cellular structures, scavenging reactive oxygen species (ROS), maintaining redox homeostasis, and preserving membrane integrity (Seki et al., 2007; Ferchichi et al., 2018). In this study, the accumulation of proline was observed under both stress conditions, reinforcing its importance as a universal stress tolerance mechanism.

In the drought experiment, the negative correlation between plant water status and proline accumulation in untreated plants suggested a compensatory role of proline in osmotic adjustment. However, the foliar application of melatonin (MT) significantly enhanced both proline content and relative water content (RWC), aligning with previous findings (P. Zhang et al., 2021). The highest proline levels were recorded in drought-stressed plants, with a gradual and substantial increase following exogenous MT treatment, particularly at 200 µM, which proved to be the most effective concentration (Table 7 and Figure 10 B). This aligns with previous studies that highlight melatonin's dual role as both an antioxidant and a regulator of osmotic balance by potentially inhibiting proline degradation (Sarropoulou et al., 2012) and maintaining cellular hydration under drought conditions (Wang et al., 2009; Jiang et al., 2021).

Similarly, under salinity stress, proline functioned as an osmoprotectant, contributing to membrane stabilization, protein protection, and redox regulation (Zhu, 2001; Chegah et al., 2013; Sheikhalipour et al., 2022). The observed increase in proline content under saline conditions further supports its role in enhancing osmotic adjustment and stress tolerance. As noted in the drought stress discussion, melatonin-induced proline accumulation was likely mediated by the upregulation of key genes such as BADH and P5CS, which are involved in proline biosynthesis (Castañares and Bouzo, 2019; P. Zhang et al., 2021). Additionally, melatonin has been reported to stimulate root growth and enhance aquaporin activity, thereby improving water uptake and distribution, a critical factor for sustaining hydration under salinity stress (Qiao et al., 2020). Consistent with our findings (Table 8 and Figure 11B), melatonin-treated plants exhibited significantly higher proline levels under both salinity conditions compared to untreated plants. These results align with those of Sheikhalipour et al. (2022), who observed that melatonin application led to increased proline accumulation and improved RWC in salt-stressed plants. Taken together, these findings highlight the pivotal role of proline in conferring stress tolerance in *R. asiaticus* under both drought and salinity stress. The interaction between melatonin and stress-induced proline accumulation suggests a conserved mechanism through which melatonin enhances plant resilience.

Under salinity conditions, excessive levels of cytoplasmic NaCl disrupt ion balance, inhibiting plant growth and development (Yuan et al., 2016). To enhance salt tolerance effectively, plants must be capable of absorbing and compartmentalizing ion. They achieve this by redistributing cytoplasmic salt ions into vacuoles or storing them in various tissues to manage salinity stress (Zhu, 2003 ; Yuan et al., 2015). As a result, a higher accumulation of Na^+ is typically observed in the leaves. In our experiment, the Na^+ content in the leaves significantly increased under both saline conditions of EC 4.5 and 5.5 $dS \cdot m^{-1}$ (Table 10 and Figure 11A). Similar findings were reported in cotton leaves (Jiang et al., 2021) and in pepper cultivars 'Granada' and 'Nobili' (Hand et al., 2017). However, exogenous melatonin (MT) treatment enabled *Ranunculus* plants to maintain lower Na^+ levels in their leaves compared to non-MT-treated stressed plants. Notably, a greater reduction in Na^+ content was observed under S1 conditions as MT levels increased. These results are consistent with the studies by Castañares and Bouzo (2019) and L.Wei et al., (2022) which demonstrated that MT treatment mitigated ion toxicity in melons and rice, respectively, when subjected to salt stress. This study further demonstrated that a concentration of 200 µM melatonin supports ion stability when plants are subjected to saline conditions. Melatonin's influence under salt stress involves up-regulating key transporter genes, NHX1 and AKT1 (Li et al., 2012), essential for maintaining ionic balance.

Melatonin (MT) plays a critical role in enhancing plant stress tolerance by boosting antioxidant defense mechanisms that mitigate oxidative damage caused by reactive oxygen species (ROS) (Na. Zhang et al., 2015). ROS homeostasis is essential for maintaining cellular integrity, as excessive accumulation leads to oxidative stress and irreversible cellular damage (Liang et al., 2020 ; Wei et al., 2022). The antioxidant defense system in plants comprises various antioxidant compounds and enzymes, including peroxidase (POD), which scavenges ROS by converting hydrogen peroxide (H_2O_2) into water after superoxide dismutase (SOD) catalyzes the conversion of $O_2^{\bullet -}$ to O_2 and H_2O_2 (Hu et al., 2016).

In this study, we evaluated POD activity under both drought and salinity stress conditions to determine the extent to which melatonin mitigates oxidative stress. Under drought conditions, a significant increase in POD activity was observed in MT-treated plants, with the highest levels recorded at 200 µM MT (Table 10, Figure 13A). This suggests that melatonin effectively enhances the plant's enzymatic antioxidant defense, aligning with previous findings in *Brassica napus* (Li et al., 2018), pansy (Oraee and Tehranifar, 2020), and tomato (Altaf et al., 2022), where MT application improved drought tolerance by reducing oxidative damage and maintaining membrane stability. Additionally, MT has been reported to translocate from treated tissues to untreated areas via vascular bundles, thereby extending its protective effects throughout the plant (Na. Zhang et al., 2015). Moreover, MT enhances stress resilience by promoting the transcription of antioxidant genes, reducing ROS accumulation, and increasing antioxidant enzyme activity (Shi, 2015a; Varghese et al., 2019).

Similarly, under salinity stress, ROS accumulation serves as a critical marker of oxidative damage induced by salt stress (Ahanger et al., 2020). As observed in the drought experiment, plants depend on their antioxidant defense systems to maintain ROS homeostasis, thereby preventing cellular deterioration (Liang et al., 2020; L. Wei et al., 2022). Our findings indicate that MT significantly reduced ROS levels under salinity stress by upregulating POD activity, with the highest enzyme activity recorded under the most severe salinity conditions S2 (EC 5.5) following 200 µM MT application (Table 11, Figure 14 A). These results corroborate the findings of H.J. Zhang (2014) and Shi et al., (2015), who demonstrated that MT enhances salt tolerance by upregulating antioxidant enzyme genes and stabilizing macromolecules, leading to increased antioxidant enzyme activity in high-salinity environments. Similar protective effects of MT have been reported in rice (Y. Wei et al., 2022), naked oat (Gao et al., 2019), and rapeseed (Zeng et al., 2018).

Membrane stability, commonly evaluated through electrolyte leakage (EL), is a key indicator of cellular damage caused by abiotic stress (Saneoka et al., 2004). Both drought and salinity stress negatively affect membrane integrity, leading to increased ion leakage and loss of cellular homeostasis. Drought stress, in particular, results in irreversible potassium (K^+) loss, impairing the plant's ability to maintain osmotic balance (Parkash and Singh, 2020). Similarly, salt-induced damage primarily disrupts the plasma membrane, further reducing K^+ retention capacity and compromising cell function (Ashraf and Ali, 2008). Given the critical role of membrane stability in stress tolerance, EL measurements serve as a reliable parameter for assessing plant resilience under adverse environmental conditions.

Under drought conditions, our findings demonstrated that melatonin (MT) treatment significantly reduced EL, thereby providing protective effects against membrane damage (Table 10, Figure 13B). This aligns with studies indicating that MT regulates the antioxidant enzyme system, enhancing enzyme activity either directly or indirectly to mitigate oxidative stress (Rodriguez et al., 2004). Notably, soybean plants subjected to water deficits exhibited reduced oxidative damage in leaves when treated with exogenous MT, as evidenced by increased peroxidase

(POD) activity and decreased EL levels compared to untreated drought-stressed plants (Imran et al., 2021). Among the tested concentrations, 200 µM MT proved to be the most effective in reducing EL and preserving membrane stability (Table 10, Figure 13A).

Likewise, salt stress significantly increased EL, with the highest leakage observed at EC 5.5 dS m^{-1} (Table 11, Figure 14B). However, melatonin application mitigated this effect, reinforcing its role in protecting membrane integrity. Similar findings have been reported in sugar beets and tomatoes, where MT treatment effectively reduced EL and minimized oxidative damage under salinity stress (T. Khan et al., 2022; Zhang et al., 2021). One potential mechanism underlying MT's protective function is its interaction with lipid bilayers. Positioned on the hydrophilic side of the membrane, MT can diffuse through lipid membranes and cytoplasm, thereby stabilizing membrane structures (Saneoka et al., 2004; Catala, 2007; Huang et al., 2019). The organization of MT within the bilayer appears to be concentration-dependent: at lower doses, MT aligns parallel to lipid tails, while at higher doses, it integrates more deeply into the membrane, reinforcing its structural stability (Huang et al., 2019). This suggests that MT plays a pivotal role in safeguarding cell membranes by counteracting oxidative stress and maintaining ionic equilibrium under drought and salt conditions.

Assessing plant stress responses through tolerance indices provides a valuable approach for identifying key physiological and biochemical traits associated with drought and salinity resilience. In this study, Drought Tolerance Index (DTI%) and Salinity Tolerance Index (STI%) were evaluated to classify traits based on their relative sensitivity or adaptability under stress conditions.

Under drought stress, traits were categorized into three distinct groups based on DTI% values (Table 12). The first category includes variables with a DTI% greater than 100%, such as peroxidase (POD), proline, and electrolyte leakage (EL). The second category encompasses variables with DTI% values between 50% and 100%, including the timing of flower bud emergence, shoot fresh weight, number of leaves, and shoot length, listed in descending order. The final category consists of variables with DTI% values below 50%, which includes carotenoid content, shoot dry weight, leaf area, and chlorophyll levels. Given the elevated DTI% values for POD activity, leaf proline content, and EL, these traits could serve as effective markers for assessing drought stress responses in *R. asiaticus* plants. Previous studies have explored the use of DTI as an indicator of drought response in various species such as *Malva sylvestris*, *Althea rosea*, *Callistephus chinensis*, *Rudbeckia hirta* (Rafi et al., 2019), and *Helianthus annuus* (Ahmad et al., 2009), where stress was evaluated based on the stress index of multiple traits like plant height, dry matter accumulation, root length, and relative root volume and density.

Similarly, under severe salinity stress at EC 5.5 (S2 treatment), traits were classified into three STI% categories (Table 13). The first category includes variables with STI% values above 400%, as seen with Na content. The second group comprises traits with STI% over 200%, including POD activity, EL, and proline. The final category consists of traits with STI% below 100%, including flower bud emergence time, RWC, leaf count, shoot length, fresh and dry shoot weight, carotenoids, total chlorophyll, and leaf area, listed in descending order. The high STI% values for Na content, followed by POD activity, EL, and proline, suggest that these traits may serve as reliable indicators of *Ranunculus* response to salt stress. Some studies have debated using STI% as a marker for stress response and instead focused on whole-plant biomass, as in chickpea (Mann et al., 2019) and asian barley (Sbei et al., 2014). However, in this study, both DTI% and STI% were calculated across a broad range of *Ranunculus* traits, following a similar approach to Roshdy et al. (2021) in strawberry. To our knowledge, this is the first study to comprehensively assess these indices in *R. asiaticus*, offering valuable insights for future research in plant physiology and breeding strategies.

6. CONCLUSION AND RECOMMENDATIONS

6.1. 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.

6.2. 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.

7. NEW SCIENTIFIC RESULTS

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.

8. THESIS SUMMERY

The current research focuses on the role of melatonin in enhancing drought and salinity tolerance in *Ranunculus asiaticus* by investigating its effects on physiological and biochemical parameters under abiotic stress conditions.

The thesis seeks to:

1. Assess the effects of drought and salinity stress on *R. asiaticus*.
2. Evaluate melatonin's efficacy in improving the plant's tolerance under stress by testing various concentrations (0, 50, 100, 200 µM).
3. Investigate melatonin’s impact on growth, antioxidant defense, and other physiological responses under stress conditions.

Experiments were conducted in controlled greenhouse conditions to simulate drought and salinity stresses. Melatonin was applied at four concentrations (0, 50, 100, and 200 µM) in a randomized design. Key measurements included plant growth metrics, chlorophyll content, electrolyte leakage, and oxidative stress markers to quantify stress alleviation effects.

- The drought stress experiment:

1. Morphological Traits: Drought significantly reduced shoot length, leaf number, leaf area, and plant weights. Melatonin treatment, especially at higher concentrations (100 µM and 200 µM), enhanced these traits under both well-watered and drought conditions.

2. Flower Bud Emergence: Drought stressed plants flowered earlier than well-watered ones. MT treatment delayed this early emergence, with higher concentrations showing the most improvement in aligning bud emergence to well-watered timing.

3. Photosynthetic Pigments: Drought lowered chlorophyll and carotenoid levels. However, melatonin, particularly at 200 µM, preserved pigment content under stress, nearly matching well-watered plant levels.

4. Relative Water Content (RWC) and Proline Accumulation: Melatonin effectively increased RWC and proline, crucial for drought tolerance, with the highest MT concentrations yielding the greatest protective effects.

5. Antioxidant Activity: Melatonin boosted peroxidase activity and reduced electrolyte leakage under drought, helping maintain membrane integrity. Higher MT concentrations led to stronger antioxidant responses.

6. Drought Tolerance Index (DTI): Antioxidant activity and proline levels were most responsive to drought, suggesting these as critical indicators of MT's protective effects.

Overall, melatonin at higher concentrations (100 and 200 µM) significantly mitigated the impacts of drought, highlighting its potential as a biostimulant for enhancing drought tolerance in *R. asiaticus*.

- The salinity stress experiment:

The study found that salinity stress significantly decreased growth metrics, chlorophyll content, and water retention in *R. asiaticus* while increasing sodium levels, electrolyte leakage, and peroxidase activity. Key findings include:

1. Plant Morphology: Salinity stress notably reduced shoot length, leaf area, and biomass. However, melatonin (MT) applications, particularly at 200 µM, significantly improved these parameters under both levels of salinity stress.

2. Flowering Time: Salinity accelerated flower bud emergence, but melatonin delayed this early flowering, with the 100 µM MT concentration showing the most consistent postponement.

3. Sodium and Water Retention: Salinity increased sodium ions in leaves and reduced relative water content (RWC). Melatonin application, especially at 200 µM, reduced sodium buildup and improved RWC.

4. Photosynthetic Pigments: Chlorophyll and carotenoid levels dropped under salinity, but melatonin at 200 µM increased these pigments, countering salinity's adverse effects.

5. Membrane Stability and Antioxidant Response: Salinity raised El and POD activity, indicating oxidative stress. Melatonin reduced leakage and enhanced POD activity, improving cell integrity and stress tolerance.

6. Salt tolerance index (STI): Sodium content, POD activity, and electrolyte leakage were highly responsive to salinity, highlighting these as critical indicators for evaluating salt stress tolerance.

These results show that melatonin, especially at higher concentrations, effectively mitigates salinity stress, enhancing growth, water retention, and antioxidant defense in *R. asiaticus*.

The findings of this study underscore the pivotal role of melatonin in enhancing *Ranunculus asiaticus*' resilience to drought and salinity stresses. The results clearly demonstrate that exogenous melatonin, particularly at concentrations of 100 µM and 200 µM, significantly mitigates the adverse effects of abiotic stresses by preserving plant morphology, maintaining photosynthetic pigment levels, and enhancing antioxidant defenses. Notably, melatonin improved relative water content and reduced oxidative stress markers, such as El and POD activity, thereby maintaining cellular integrity. The delay in premature flowering under stress conditions further highlights melatonin’s potential to regulate reproductive responses in stressed environments. These findings collectively position melatonin as a promising biostimulant for optimizing horticultural practices under challenging environmental conditions, offering a sustainable solution to the growing impacts of climate-induced stress factors on crop production. Future studies could further elucidate the molecular mechanisms driving these beneficial effects, paving the way for broader applications of melatonin in stress management across diverse plant species.

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10. 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 . https://doi.org/10.1007/978-1-60761-702-0_20.

Abdelrahman, M., Jogaiah, S., Burritt, D. J., and Tran, L. P. (2018): Legume genetic resources and transcriptome dynamics under abiotic stress conditions. Plant Cell Environ. 41, 1972–1983. doi: 10.1111/pce.13123.

Ahammed, G.J. and Li, X. (2022) : Melatonin-induced detoxification of organic pollutants and alleviation of phytotoxicity in selected horticultural crops, Horticulturae, 8(12), p. 1142.

Ahanger, M., Aziz, U., Alsahli, A., Alyemeni, M., and Ahmad, P. (2020). Influence of Exogenous Salicylic Acid and Nitric Oxide on Growth, Photosynthesis, and Ascorbate-Glutathione Cycle in Salt Stressed Vigna angularis. Biomolecules, 10. https://doi.org/10.3390/biom10010042.

Ahmad, I. G., Zhou, G., Song, X., Hussein Ibrahim, M. E., Ibrahim Salih, E. G., Hussain, S., and Younas, M. U. (2022): Pivotal role of phytohormones and their responsive genes in plant growth and their signaling and transduction pathway under salt stress in cotton. International Journal of Molecular Sciences, 23(13), p. 7339. https://doi.org/10.3390/ijms23137339.

Ahmad, I., Zhu, G., Zhou, G., Liu, J., Younas, M. U., and Zhu, Y (2023) : Melatonin Role in Plant Growth and Physiology under Abiotic Stress. International Journal of Molecular Sciences, 24(10), p. 8759. https://doi.org/10.3390/ijms24108759.

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. https://doi.org/10.1016/j.sajb.2023.10.045.

Ahmad, R., Manzoor, M., Muhammad, H., Altaf, M., and Shakoor, A., (2023): Exogenous Melatonin Spray Enhances Salinity Tolerance in Zizyphus Germplasm: A Brief Theory. *Life*, 13. https://doi.org/10.3390/life13020493.

Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M., and Waraich, E. A.. (2009): Sunflower (*Helianthus annuus L.*) response to drought stress at germination and seedling growth stages.Pak.

J. Bot, 41(2), pp. 647–654.

Ahmad, S., Muhammad, I., Wang, G. Y., Zeeshan, M., Yang, L., Ali, I., and Zhou, X. B. (2021a): Ameliorative effect of melatonin improves drought tolerance by regulating growth, photosynthetic traits and leaf ultrastructure of maize seedlings. BMC Plant Biology, 21(1), p. 368.https://doi.org/10.1186/s12870-021-03160-w.

Ahmad, S., Cui, W., Kamran, M., Ahmad, I., Meng, X., Wu, X., Su,W., Javed,T.,El Serehy, H., Jia, Z., and Han, Q. (2021b): Exogenous application of melatonin induces tolerance to salt stress by improving the photosynthetic efficiency and antioxidant defense system of maize seedling. Journal of Plant Growth Regulation, 40(3), pp. 1270–1283. https://doi.org/10.1007/s00344-020-10187-0.

Ahmad, S. , Wang, F., Ahmad, A., Ercisli, S., Chen, J., Guan, Y., Zhao , K.,Zho, Y., Lan ,S., Liu,Z. and Peng, D. (2023): Suggesting a prospect for melatonin-mediated orchid flowering under extreme conditions. Scientia Horticulturae, 321, p. 112340. https://doi.org/10.1016/j.scienta.2023.112340.

Ahmed, J. ,Wang, H., Song, J., Nuerawuti, M., Zhang, X., Yang, W., Ma, L. and Li, XX. (2021): The tolerance of an exhtensive collection of garlic (*Allium sativum* l.) germplasms to salt stress – a sustainable solution to salt stress. Applied Ecology and Environmental Research, 19(3), pp. 2281–2303. https://doi.org/10.15666/aeer/1903_22812303.

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.

Ali, M., Kamran, M., Abbasi, G., Saleem, M., Ahmad, S., Parveen, A., Malik, Z., Afzal, S., Ahmar, S., Dawar, K., Ali, S., Alamri, S., Siddiqui, M., Akbar, R., & Fahad, S.,. (2021): Melatonin-Induced Salinity Tolerance by Ameliorating Osmotic and Oxidative Stress in the Seedlings of Two Tomato (*Solanum lycopersicum* L.) Cultivars. Journal of Plant Growth Regulation, 40(5), pp. 2236–2248. https://doi.org/10.1007/s00344-020-10273-3.

Altaf, M.A., Shahid, R., Ren, M.X., Naz, S., Altaf, M.M., Khan, L.U., Tiwari, R.K., Lal, M.K., Shahid, M.A., Kumar, R., Nawaz, M.A., Jahan, M.S., Jan, B.L. and Ahmad, P. (2022): Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. Antioxidants, 11(2), p. 309. https://doi.org/10.3390/antiox11020309.

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.

Antoniou, C., Chatzimichail, G., Xenofontos, R., Pavlou, J. J., Panagiotou, E., Christou, A., and Fotopoulos, V. (2017): Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. Journal of Pineal Research, 62(4). https://doi.org/10.1111/jpi.12401.

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.

Armonk, N.Y. (2020): IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0.

Arnao, M.B. (2014)a: Phytomelatonin: Discovery, Content, and Role in Plants. Advances in Botany, 2014, pp. 1–11. https://doi.org/10.1155/2014/815769.

Arnao, M.B., Cano, A. and Hernández-Ruiz, J. (2022): Phytomelatonin: an unexpected molecule with amazing performances in plants. Journal of Experimental Botany. Edited by D. Ort, 73(17), pp. 5779–5800. Available at: https://doi.org/10.1093/jxb/erac009.

Arnao, M.B. and Hernández-Ruiz, J. (2009): Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. Journal of Pineal Research, 46(1), pp. 58–63.https://doi.org/10.1111/j.1600-079X.2008.00625.x.

Arnao, M.B. and Hernández-Ruiz, J. (2014)b:Melatonin: plant growth regulator and/or biostimulator during stress. Trends in Plant Science, 19(12), pp. 789–797. https://doi.org/10.1016/j.tplants.2014.07.006.

Arnao, M.B. and Hernández-Ruiz, J. (2015): Functions of melatonin in plants: a review. Journal of Pineal Research, 59(2), pp. 133–150. https://doi.org/10.1111/jpi.12253.

Arnao, M.B. and Hernández-Ruiz, J. (2018) : Melatonin and its relationship to plant hormones. Annals of Botany, 121(2), pp. 195–207. https://doi.org/10.1093/aob/mcx114.

Arnao, M.B. and Hernández-Ruiz, J. (2019): Melatonin: A New Plant Hormone and/or a Plant Master Regulator. Trends in Plant Science, 24(1), pp. 38–48.

https://doi.org/10.1016/j.tplants.2018.10.010.

Arnao, M.B. and Hernández-Ruiz, J. (2020): Melatonin in flowering, fruit set and fruit ripening. Plant Reprod. **33**: 77-87.

Arnao, M.B. and Hernández-Ruiz, J. (2007): Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. Journal of Pineal Research, 42(2), pp. 147–152. https://doi.org/10.1111/j.1600-079X.2006.00396.x.

Arnon, D.1. 1949: Copper enzymes inisolated chaloroplasts. Poly-phenoloxidase in *Beta vulgaris*. Plant Physiology. 24:1-15.

Ashraf, M. and Ali, Q. (2008) : Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). Environmental and Experimental Botany, 63, pp. 266–273.

Ayyaz, A., Shahzadi, A. K., Fatima, S., Yasin, G., Zafar, Z. U., Athar, H. R., and Farooq, M. A. (2022): Uncovering the role of melatonin in plant stress tolerance. Theoretical and Experimental Plant Physiology, 34(3), pp. 335–346. https://doi.org/10.1007/s40626-022-00255-z.

Back, K., Tan, D. and Reiter, R.J. (2016):Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts, Journal of Pineal Research, 61(4), pp. 426–437. https://doi.org/10.1111/jpi.12364.

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.

Bakry, B.A., El-Hariri, DM, Sadak, M.S. and El-Basiouny, H.M.S. (2012): Drought stress mitigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed sandy soil, J Appl Sci Res, 8(7), pp. 3503–3514.

Banon, S., Ochoa, J., Franco, J. A., Alarcón, J. J., and Sánchez-Blanco, M. J. (2006): Hardening of oleander seedlings by deficit irrigation and low air humidity. Environ Exp Bot., 56(1), pp. 36–43.

Bano, A., and Fatima, M. (2009): Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. Biology and Fertility of Soils, *45*, 405–413.

Barbara, G.T. and Linda S.F. (2013): Using multivariate statistics. , 7th Edition. NY, NY : Pearson.ISBN-13: 9780135350904 ISBN-10: 0134790545.

Bartwal, A., Mall, R., Lohani, P., Guru, S. K., and Arora, S. (2013): Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. journal of Plant Growth Regulation, 32(1), pp. 216–232. https://doi.org/10.1007/s00344-012-9272-x.

Bera, K., Dutta, P. and Sadhukhan, S. (2022): Plant responses under abiotic stress and mitigation options towards agricultural sustainability. in Plant stress*:* challenges and management in the new decade. Springer, pp. 3–28.

Beruto, M. and Debergh, P. (2004): Micropropagation of *Ranunculus asiaticus*: a review and perspectives. Plant Cell, Tissue and Organ Culture, 77(3), pp. 221–230. https://doi.org/10.1023/B:TICU.0000018416.38569.7b.

Beruto, M., Fibiani, M., Rinino, S., Lo Scalzo, R., and Curir, P. (2009): Plant development of *Ranunculus asiaticus* L. tuberous roots is affected by different temperature and oxygen conditions during storage period. Isr. J. Plant Sci. 57, 377–388. doi: 10.1560/IJPS.57.4.377

Beruto, M., Martini, P. and Viglione, S. (2019): *Ranunculus asiaticus*: From research to production. Acta Horticulturae, 1237(Im), pp. 117–128. https://doi.org/10.17660/ActaHortic.2019.1237.16.

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. https://doi.org/10.1016/J.JAFREARSCI.2021.104134.

Bhardwaj,S., Sharma, D., Singh, S., Ramamurthy, P. C., Verma, T., Pujari, M., ... and Prasad, R. (2023): Physiological and molecular insights into the role of silicon in improving plant performance under abiotic stresses. Plant and Soil, 486(1–2), pp. 25–43. https://doi.org/10.1007/s11104-022-05395-4.

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.

Blask, D.E. (2004) :Melatonin uptake and growth prevention in rat hepatoma 7288CTC in response to dietary melatonin: melatonin receptor-mediated inhibition of tumor linoleic acid metabolism to the growth signaling molecule 13-hydroxyoctadecadienoic acid and the potential rol, Carcinogenesis, 25(6), pp. 951–960. https://doi.org/10.1093/carcin/bgh090.

Brown, M.B. and Forsythe, A.B. (1974): Robust tests for the equality of variances. Journal of the American statistical association, 69(346), pp. 364–367.

Byeon, Y. and Back, K. (2014) :An increase in melatonin in transgenic rice causes pleiotropic phenotypes, including enhanced seedling growth, delayed flowering, and low grain yield. Journal of Pineal Research, 56(4), pp. 408–414. https://doi.org/10.1111/jpi.12129.

Cao, S., Shao, J., Shi, L., Xu, L., Shen, Z., Chen, W., and Yang, Z (2018): Melatonin increases chilling tolerance in postharvest peach fruit by alleviating oxidative damage. Scientific Reports, 8(1), p. 806. https://doi.org/10.1038/s41598-018-19363-5.

Cao, Y., Du, P., Ji, J., He, X., Zhang, J., Shang, Y., and Liang, B. (2022):Ionomic combined with transcriptomic and metabolomic analyses to explore the mechanism underlying the effect of melatonin in relieving nutrient stress in apple, International Journal of Molecular Sciences, 23(17), p. 9855. https://doi.org/10.3390/ijms23179855.

Carter, C.T. and Grieve, C.M. (2008) :Mineral nutrition, growth, and germination of *Antirrhinum majus* l. (snapdragon) when produced under increasingly saline conditions, HortScience, 43(3), pp. 710–718. https://doi.org/10.21273/HORTSCI.43.3.710.

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.

Castañares, L.J. and Bouzo, A.C. (2019) : Effect of Exogenous Melatonin on Seed Germination and Seedling Growth in Melon (*Cucumis melo* L.) Under Salt Stress, Horticultural Plant Journal, 5(2), pp. 79–87. https://doi.org/10.1016/j.hpj.2019.01.002.

Catala, A. (2007): The Ability of Melatonin to Counteract Lipid Peroxidation in Biological Membranes. *Current Molecular Medicine*, 7(7), pp. 638–649. https://doi.org/10.2174/156652407782564444.

Cerveny, C.B., Miller, W.B., Björkman, T., and Mattson, N.S. (2012): Soaking Temperature of Dried Tuberous Roots Influences Hydration Kinetics and Growth of *Ranunculus asiaticus* (L.)', HortScience, 47(2), pp. 212–216. https://doi.org/10.21273/HORTSCI.47.2.212.

Chegah, S., Chehrazi, M. and Albaji, M. (2013): Effects of drought stress on growth and development frankinia plant (*Frankinia leavis*).', *Bulgar. J. Agric. Sci.*, 19, pp. 659–665.

Chen, G. Huo, Y., Tan, D. X., Liang, Z., Zhang, W., and Zhang, Y. (2003) :Melatonin in Chinese medicinal herbs, *Life Sciences*, 73(1), pp. 19–26. https://doi.org/10.1016/S0024-3205(03)00252-2.

Chen, K., Hu, Q., Ma, X., Zhang, X., Qian, R., and Zheng, J. (2024): The effect of exogenous melatonin on waterlogging stress in Clematis. Frontiers in Plant Science, 15, p. 15:1385165. https://doi.org/10.3389/fpls.2024.1385165.

Chen, L. Lu, B., Liu, L., Duan, W., Jiang, D., Li, J., Zhang, K., Sun, H., Zhang, Y., Li, C. and Bai, Z. (2021): Melatonin promotes seed germination under salt stress by regulating ABA and GA3 in cotton (*Gossypium hirsutum* L.). Plant Physiology and Biochemistry, 162, pp. 506–516. https://doi.org/10.1016/j.plaphy.2021.03.029.

Chen, Q., Qi, W. B., Reiter, R. J., Wei, W., and Wang, B. M (2009): Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea.* Journal of Plant Physiology, 166(3), pp. 324–328. https://doi.org/10.1016/j.jplph.2008.06.002.

Chen, Q. and Arnao, M.B. (2022):Phytomelatonin: an emerging new hormone in plants, Journal of Experimental Botany. Oxford University Press UK, pp. 5773–5778.

Chen, Z. , Gu, Q., Yu, X., Huang, L., Xu, S., Wang, R. and Shen, W. (2018): Hydrogen peroxide acts downstream of melatonin to induce lateral root formation. Annals of Botany, 121(6), pp. 1127–1136. https://doi.org/10.1093/aob/mcx207.

Cherono, S., Ntini, C., Wassie, M., Mollah, M.D., Belal, M.A., Ogutu, C., and Han, Y. (2021): Exogenous application of melatonin improves drought tolerance in coffee by regulating photosynthetic efficiency and oxidative damage. Journal of the American Society for Horticultural Science, 146(1), pp. 24–32. https://doi.org/10.21273/JASHS04964-20.

Chimonidou-Pavlidou, D. (2001): Effect of irrigation and shading at the stage of flower bud appearance. Acta Horticulturae, (547), pp. 245–251. https://doi.org/10.17660/ActaHortic.2001.547.29.

Dawood, M. G., El-Awadi, M. E. S., Sadak, M. S., and El-Lethy, S. R. (2019): Research article comparison between the physiological role of carrot root extract and β-carotene in inducing *Helianthus annuus* L. drought tolerance. *Asian J Biol Sci* , *12* , 231-241

Dawood, M.G. and Sadak, M.S. (2014): Physiological role of glycinebetaine in alleviating the deleterious effects of drought stress on canola plants (*Brassica napus* L.)’, *Middle East J. Agric. Res*, 3(4), pp. 943–954.

Debnath, B., Islam, W., Li, M., Sun, Y., Lu, X., Mitra, S., Hussain, M., Liu, S., and Qiu, D. (2019): Melatonin mediates enhancement of stress tolerance in plants. International Journal of Molecular Sciences, 20(5), p. 1040. https://doi.org/10.3390/ijms20051040.

Denancé, N., Sánchez-Vallet, A., Goffner, D., and Molina, A. (2013): Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Frontiers in Plant Science, 4. https://doi.org/10.3389/fpls.2013.00155.

Dhooghe, E., Grunewald, W., Reheul, D., Goetghebeur, P.,and Van Labeke, M. C. (2012) : Floral characteristics and gametophyte development of *Anemone coronaria* L. and *Ranunculus asiaticus* L. (Ranunculaceae). Scientia Horticulturae, 138, pp. 73–80. https://doi.org/10.1016/j.scienta.2011.10.004.

Din, J., Khan, S. U., Ali, I., and Gurmani, A. R. . (2011): Physiological and agronomic response of canola varieties to drought stress. J. An Plant Sci., 21, pp. 78–82.

Ding, F., Wang, G., Wang, M., and Zhang, S. (2018): Exogenous melatonin improves tolerance to water deficit by promoting cuticle formation in tomato plants. Molecules, 23(7), p. 1605. https://doi.org/10.3390/molecules23071605.

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.

Dubbels, R. Reiter, R. J., Klenke, E., Goebel, A., Schnakenberg, E., Ehlers, C.and Schloot, W. (1995): Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry, Journal of Pineal Research, 18(1), pp. 28–31. https://doi.org/10.1111/j.1600-079X.1995.tb00136.x.

El-Awadi, M. , Sadak, M., Dawood, M. G., Khater, M. A., and Elashtokhy, M. M. (2017): Amelioration the adverse effects of salinity stress by using γ- radiation in *Faba bean* plants. Bull NRC, 41(1), pp. 293–310.

Elewa, T.A., Sadak, M.S. and Saad, A.M. (2017): Proline treatment improves physiological responses in quinoa plants under drought stress. Bioscience Research, 14(1), pp. 21–33.

Emiliani, J., D'Andrea, L., Lorena Falcone Ferreyra, M., Maulión, E., Rodriguez, E., Rodriguez-Concepción, M., and Casati, P (2018): A role for β,β-xanthophylls in Arabidopsis UV-B photoprotection. Journal of Experimental Botany, 69(20), pp. 4921–4933. https://doi.org/10.1093/jxb/ery242.

FAO (2016) FAO *soils portal*. https://doi.org/http://www.fao.org/soils-portal/soilmanagement/management-of-some-problem-soils/salt-affected-soils/moreinformation- on-salt-affected-soils/en/a.

FAO and ITPS (2015): Status of the World's Soil Resources—Main Report, in *FAO* (Rome: FAO and ITPS). eds. L. Montanarella, M. Badraoui, V. Chude, I. D. S. B. Costa, T. Mamo and M. Yemefack, et al.

Farooq, M. , Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. M. ((2009): Plant Drought Stress: Effects, Mechanisms and Management. in Sustainable Agriculture. Dordrecht: Springer Netherlands, pp. 153–188. https://doi.org/10.1007/978-90-481-2666-8_12.

Fazal, H., Abbasi, B.H., Ahmad, N., and Ali, M. (2018): Exogenous melatonin trigger biomass accumulation and production of stress enzymes during callogenesis in medicinally important *Prunella vulgaris* L. (Selfheal). Physiology and Molecular Biology of Plants, 24(6), pp. 1307–1315. https://doi.org/10.1007/s12298-018-0567-7.

Feng, X. , Wang, M., Zhao, Y., Han, P.,and Dai, Y. (2014): Melatonin from different fruit sources, functional roles, and analytical methods. Trends in Food Science & Technology, 37(1), pp. 21–31. https://doi.org/10.1016/j.tifs.2014.02.001.

Ferchichi, S. Hessini, K., Dell'Aversana, E., D'Amelia, L., Woodrow, P., Ciarmiello, L.F.,and Carillo, P. (2018): *Hordeum vulgare* and *Hordeum maritimum* respond to extended salinity stress displaying different temporal accumulation pattern of metabolites. Funct Plant Biol., 45(11), pp. 1096–109. https://doi.org/https:// doi. org/ 10. 1071/ FP180 46.

Gao, W., Feng, Z., Bai, Q., He, J.,and Wang, Y. (2019): Melatonin-mediated regulation of growth and antioxidant capacity in salt-tolerant naked oat under salt stress. Int. J. Mol. Sci., p. 20:1176.

Garson, G.D. (2012): Testing statistical assumptions. in Asheboro, NC: Statistical Associates Publishing.

Giridhar, A.R.P. and Ravishankar, G.A. (2009): Indoleamines and calcium channels influence morphogenesis in in vitro cultures of *Mimosa pudica* L.Plant Signaling & Behavior, 4(12), pp. 1136–1141. https://doi.org/10.4161/psb.4.12.10101.

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.

Han, Q., Huang, B., Ding, C., Zhang, Z., Chen, Y., Hu, C., Zhou, L., Huang, Y., Liao, J., Yuan, S., and Yuan, M., 2017. Effects of melatonin on anti-oxidative systems and photosystem ii in cold-stressed rice seedlings. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.00785.

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. https://doi.org/10.15835/NBHA45210928.

Hassan, H.A. , Agina, E. A., Koriesh, E. M., and Mohamed, S. M (1985): Physiological studies on *Anemone coronaria* L. and *Ranunculus asiaticus* L. 2. Effect of gibberellic acid', Annals of Agricultural Science, Moshtohor, 22(2), pp. 583-595.

Hassan, M.U. , Mahmood, A., Awan, M., Maqbool, R., Aamer, M., Alhaithloul, H., Huang, G., Skalický, M., Brestič, M., Pandey, S., Sabagh, E., and Qari, S.(2022): Melatonin-Induced Protection Against Plant Abiotic Stress: Mechanisms and Prospects', *Frontiers in Plant Science,* 13, p. 902694. https://doi.org/10.3389/fpls.2022.902694.

Hattori, A. Migitaka, H., Iigo, M., Itoh, M., Yamamoto, K., Ohtani-Kaneko, R. and Reiter, R. J. (1995) : Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochemistry and molecular biology international, 35(3), pp. 627–34. http://www.ncbi.nlm.nih.gov/pubmed/7773197.

He, Y., Tang, R.H., Hao, Y., Stevens, R.D., Cook, C.W., Ahn, S.M., Jing, L., Yang, Z., Chen, L., Guo, F. (2004): Nitric oxide represses the *Arabidopsis* floral transition. Science.305: 1968-

1971.

He, J. Ren, Y., Chen, X., and Chen, H. (2014): Protective roles of nitric oxide on seed germination and seedling growth of rice (*Oryza sativa* L.) under cadmium stress. Ecotoxicology and Environmental Safety, 108, pp. 114–119. https://doi.org/10.1016/j.ecoenv.2014.05.021.

Hernandez-Ruiz, J., Cano, A. and Arnao, M.B. (2004): Melatonin: a growth-stimulating compound present in lupin tissues. Planta, 220(1), pp. 140–144. https://doi.org/10.1007/s00425-004-1317-3.

Hernández-Ruiz, J., Cano, A. and Arnao, M.B. (2005): Melatonin acts as a growth-stimulating compound in some monocot species. Journal of Pineal Research, 39(2), pp. 137–142. https://doi.org/10.1111/j.1600-079X.2005.00226.x.

Horovitz, A. (1985): "Ranunculus," in *Handbook of Flowering*, Vol. 4, ed. A. Halevy (Boca Raton FL: CRC Press), 155–161.

Hu, E. , Liu, M., Zhou, R., Jiang, F., Sun, M., Wen, J., Zhu, Z., and Wu, Z. (2021): Relationship between melatonin and abscisic acid in response to salt stress of tomato. Scientia Horticulturae, 285, p. 110176. https://doi.org/10.1016/j.scienta.2021.110176.

Hu, W., Tie, W., Ou, W., Yan, Y., Kong, H., Zuo, J., Ding, X., Ding, Z., Liu, Y., Wu, C., Guo, Y., Shi, H., Li, K., and Guo, A., (2018): Crosstalk between calcium and melatonin affects postharvest physiological deterioration and quality loss in cassava. Postharvest Biology and Technology. https://doi.org/10.1016/J.POSTHARVBIO.2018.02.007.

Hu, W., Zhang, J., Wu, Z., Loka, D. A., Zhao, W., Chen, B., Wang, Y., Meng, Y., Zhou, Z. and Gao, L. (2022): Effects of single and combined exogenous application of abscisic acid and melatonin on cotton carbohydrate metabolism and yield under drought stress. Industrial Crops and Products, 176, p. 114302. https://doi.org/10.1016/j.indcrop.2021.114302.

Hu, Z., Fan, J., Xie, Y., Aombo, E., Liu, A., Gitau, M. M., Khaldun, A.B.M., Chen, L. and Fu, J. (2016): Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin. Plant Physiology and Biochemistry, 100, pp. 94–104. https://doi.org/10.1016/j.plaphy.2016.01.008.

Huang, B., Chen, Y., Zhao, Y., Ding, C., Liao, J., Hu, C., Zhou, L., Zhang, Z., Yuan, S., and Yuan, M., 2019. Exogenous melatonin alleviates oxidative damages and protects photosystem ii in maize seedlings under drought stress. Frontiers in Plant Science, 10.

https://doi.org/10.3389/fpls.2019.00677.

Hussain, Q., Asim, M., Zhang, R., Khan, R., Farooq, S., and Wu, J., (2021): Transcription factors interact with aba through gene expression and signaling pathways to mitigate drought and salinity stress. Biomolecules, 11(8), p. 1159. https://doi.org/10.3390/biom11081159.

Ibrahim, M.F.M., Elbar, O., Farag, R., Hikal, M., Elkelish, A., El-Yazied, A., Alkahtani, J., and El-Gawad, H. (2020): Melatonin counteracts drought induced oxidative damage and stimulates growth, productivity and fruit quality properties of tomato plants. Plants, 9(10), p. 1276. Available at: https://doi.org/10.3390/plants9101276.

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.

Jackson, W.T. (1967) : Control of events of cell cycle in endosperm cells by isopropyl N-phenyl carbamate and melatonin. Am J Bot., p. 54: 633. https://doi.org/10.1016/j.hpj.2023.03.011.

Jafari, M. and Shahsavar, A. (2021) 'The Effect of Foliar Application of Melatonin on Changes in Secondary Metabolite Contents in Two Citrus Species Under Drought Stress Conditions', *Frontiers in Plant Science*, 12. Available at: https://doi.org/10.3389/fpls.2021.692735.

Jahan, M., Guo, S., Sun, J., Shu, S., Wang, Y., El-Yazied, A., Alabdallah, N., Hikal, M., Mohamed, M., Ibrahim, M., and Hasan, M. (2021): Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress.Plant physiology and biochemistry *:* PPB, 167, 309-320 . https://doi.org/10.1016/j.plaphy.2021.08.002.

Jan, S. , Singh, B., Bhardwaj, R., Singh, R., Mansoor, S., and Ahmed, P. (2023): Recent advances on the pragmatic roles of phytomelatonin and its exogenous application for abiotic stress management in plants. Journal of Plant Growth Regulation, 42(8), pp. 4885–4900. https://doi.org/10.1007/s00344-022-10766-3.

Ji, R., Min, J., Wang, Y., Kronzucker, H. J., and Shi, W.. (2022) : The role of plant growth regulators in modulating root architecture and tolerance to high-nitrate stress in tomato. Frontiers in Plant Science, 13, p. 864285. https://doi.org/10.3389/fpls.2022.864285.

Jia, C. et al. (2020) : Application of Melatonin-Enhanced Tolerance to High-Temperature Stress in Cherry Radish (*Raphanus sativus* L. var. radculus pers). Journal of Plant Growth Regulation.

39(2), pp. 631–640. https://doi.org/10.1007/s00344-019-10006-1.

Jiang, D. Lu, B., Liu, L., Duan, W., Meng, Y., Li, J., Zhang, K., Sun, H., Zhang, Y., Dong, H., Bai, Z., and Li, C. (2021): Exogenous melatonin improves the salt tolerance of cotton by removing active oxygen and protecting photosynthetic organs. BMC Plant Biology, 21(1), p. 331. https://doi.org/10.1186/s12870-021-03082-7.

Kamiab, F. (2020): Exogenous melatonin mitigates the salinity damages and improves the growth of pistachio under salinity stress. Journal of Plant Nutrition, 43(10), pp. 1468–1484. https://doi.org/10.1080/01904167.2020.1730898.

Kanwar, M.K., Yu, J. and Zhou, J. (2018): Phytomelatonin: Recent advances and future prospects, Journal of Pineal Research, 65(4). https://doi.org/10.1111/jpi.12526.

Karaca, P. and Cekic, F.Ö. (2019) ‘Exogenous melatonin-stimulated defense responses in tomato plants treated with polyethylene glycol’, *International Journal of Vegetable Science*, 25(6), pp. 601–609. Available at: https://doi.org/10.1080/19315260.2019.1575317.

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.

Khan, S., Sehar, Z., Fatma, M., Mir, I., Iqbal, N., Tarighat, M., Abdi, G., and Khan, N. (2022): Involvement of ethylene in melatonin-modified photosynthetic-N use efficiency and antioxidant activity to improve photosynthesis of salt grown wheat. Physiologia Plantarum, 174(6). https://doi.org/10.1111/ppl.13832.

Khan, T.A., Saleem, M. and Fariduddin, Q. (2022):Melatonin Influences Stomatal Behavior, Root Morphology, Cell Viability, Photosynthetic Responses, Fruit Yield, and Fruit Quality of Tomato Plants Exposed to Salt Stress’, *Journal of Plant Growth Regulation* [Preprint]. Available at: https://doi.org/10.1007/s00344-022-10713-2.

Khosravi, S., Haghighi, M. and Mottaghipisheh, J. (2023) ‘Effects of melatonin foliar application on hot pepper growth and stress tolerance’, *Plant Stress*, 9, p. 100192. Available at: https://doi.org/10.1016/j.stress.2023.100192.

Kolář, J., Machackova, I., Illnerova, H., Prinsen, E., van Dongen, W. and Van Onckelen, H. (1995): Melatonin in higher plant determined by radioimmunoassay and liquid chromatography-mass spectrometry. Biological Rhythm Research. 26, 406–409.

Kolář, J., Johnson, C. H., and Macháčková, I. (2003): Exogenously applied melatonin (N-acetyl-5-methoxytryptamine) affects flowering of the short-day plant Chenopodium rubrum. Physiologia Plantarum, 118(4), 605-612.

Kołodziejczyk, I., Dzitko, K., Szewczyk, R., and Posmyk, M. M. (2016): Exogenous melatonin expediently modifies proteome of maize (*Zea mays* L.) embryo during seed germination.Acta Physiologiae Plantarum, 38(6), p. 146. https://doi.org/10.1007/s11738-016-2166-y.

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.

Kostopoulou, Z., Therios, I., Rouumeliotis, E., Kanellis, A. K.,and Molassiotis, A. (2015): Melatonin combined with ascorbic acid provides salt adaptation in *Citrus aurantium* L. seedlings. Plant Physiology and Biochemistry, 86, pp. 155–165. https://doi.org/10.1016/j.plaphy.2014.11.021.

Kumar, G., Saad, K. R., Arya, M., Puthusseri, B., Mahadevappa, P., Shetty, N. P., and Giridhar, P. (2021): The Synergistic role of serotonin and melatonin during temperature stress in promoting cell division, ethylene and isoflavones biosynthesis in Glycine max. Current Plant Biology, 26, p. 100206. https://doi.org/10.1016/j.cpb.2021.100206.

Langaroudi, I.K., Piri, S., Chaeikar, S. S., and Salehi, B. (2023): Evaluating drought stress tolerance in different *Camellia sinensis* L. cultivars and effect of melatonin on strengthening antioxidant system. Scientia Horticulturae, 307, p. 111517. https://doi.org/10.1016/j.scienta.2022.111517.

Lee, H.Y. and Back, K. (2017): Melatonin is required for $H_2O_2^-$and NO^- mediated defense signaling through MAPKKK 3 and OXI 1 in *Arabidopsis thaliana*. Journal of Pineal Research, 62(2). https://doi.org/10.1111/jpi.12379.

Lee, H.Y. and Back, K. (2020): The phytomelatonin receptor (PMRT1) Arabidopsis Cand2 is not a bona fide G protein–coupled melatonin receptor. Melatonin Research, 3(2), pp. 177–186. Available at: https://doi.org/10.32794/mr11250055.

Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., and Mori, W. (1958):Isolation of melatonin, the pineal gland factor that lightens melanocyteS1. Journal of the american chemical society, 80(10), pp. 2587–2587. https://doi.org/10.1021/ja01543a060.

Li, C. , Wang Ping, W. P., Wei ZhiWei, W. Z., Liang Dong, L. D., Liu ChangHai, L. C., Yin LiHua, Y. L.and Ma FengWang, M. F. (2012):The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*., J. Pineal.Res., 53, pp. 298–306.

Li, H., Chang, J., Chen, H., Wang, Z., Gu, X., Wei, C., Zhang, Y., Ma, J., Yang, J., and Zhang, X. (2017): Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. Frontiers in Plant Science, 8(March), pp. 1–9. https://doi.org/10.3389/fpls.2017.00295.

Li, H., Guo, Y., Lan, Z., Zhang, Z., Ahammed, G. J., Chang, J., and Zhang, X. (2021): Melatonin antagonizes ABA action to promote seed germination by regulating Ca^{2+} efflux and H2O2 accumulation. Plant Science, 303, p. 110761. https://doi.org/10.1016/j.plantsci.2020.110761.

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.

Li, J., Liu, J., Zhu, T., Zhao, C., Li, L., and Chen, M. (2019): The Role of Melatonin in Salt Stress Responses, International Journal of Molecular Sciences, 20(7), p. 1735. https://doi.org/10.3390/ijms20071735.

Li, R. Yang, R., Zheng, W., Wu, L., Zhang, C., and Zhang, H. (2022): Melatonin promotes sgt1-involved signals to ameliorate drought stress adaption in rice. International Journal of Molecular Sciences, 23(2), p. 599. https://doi.org/10.3390/ijms23020599.

Li, X., Wei, J. P., Scott, E. R., Liu, J. W., Guo, S., Li, Y.,and Han, W. Y. (2015): Exogenous melatonin alleviates cold stress by promoting antioxidant defense and redox homeostasis in *Camellia sinensis* L. Molecules, 23(1), p. 165. https://doi.org/10.3390/molecules23010165.

Li, X., Tan, D. X., Jiang, D.,and Liu, F. (2016): Melatonin enhances cold tolerance in drought-primed wild-type and abscisic acid-deficient mutant barley, Journal of Pineal Research, 61(3), pp. 328–339. https://doi.org/10.1111/jpi.12350.

Li, X. Yu, B., Cui, Y., and Yin, Y. (2017): Melatonin application confers enhanced salt tolerance by regulating Na^+ and Cl^- accumulation in rice. Plant Growth Regulation, 83(3), pp. 441–454. https://doi.org/10.1007/s10725-017-0310-3.

Li, X., Ahammed, G. J., Zhang, X. N., Zhang, L., Yan, P., Zhang, L. P., and Han, W. Y. (2021): Melatonin-mediated regulation of anthocyanin biosynthesis and antioxidant defense confer tolerance to arsenic stress in *Camellia sinensis* L, Journal of Hazardous Materials, 403, p. 123922.https://doi.org/10.1016/j.jhazmat.2020.123922.

Liang, C. , Zheng, G., Li, W., Wang, Y., Hu, B., Wang, H., and Chu, C. (2015): Melatonin delays leaf senescence and enhances salt stress tolerance in rice. Journal of Pineal Research, 59(1), pp. 91–101. https://doi.org/10.1111/jpi.12243.

Liang, C., Li, A., Yu, H., Li, W., Liang, C., Guo, S., and Chu, C. (2017): Melatonin regulates root architecture by modulating auxin response in rice. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.00134.

Liang, D., Gao, F., Ni, Z., Lin, L., Deng, Q., Tang, Y.,and Xia, H. (2018) : Melatonin improves heat tolerance in kiwifruit seedlings through promoting antioxidant enzymatic activity and glutathione s-transferase transcription. Molecules, 23(3), p. 584.https://doi.org/10.3390/molecules23030584.

Liang, D., Ni, Z., Xia, H., Xie, Y., Lv, X., Wang, J., and Luo, X (2019): Exogenous melatonin promotes biomass accumulation and photosynthesis of kiwifruit seedlings under drought stress.Scientia Horticulturae, 246(March 2018), pp. 34–43.https://doi.org/10.1016/j.scienta.2018.10.058.

Liang, L., Li, D., Chen, Y., Cheng, J., Zhao, G., Fahima, T.,and Yan, J. (2020) : Selenium mitigates salt-induced oxidative stress in durum wheat (*Triticum durum* Desf.) seedlings by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. 3 Biotech, 10, p. (8):368.

Liu, D. D., Sun, X. S., Liu, L., Shi, H. D., Chen, S. Y., and Zhao, D. K. (2019): Overexpression of the melatonin synthesis-related gene slcomt1 improves the resistance of tomato to salt stress.Molecules, 24(8), p. 1514.https://doi.org/10.3390/molecules24081514.

Liu, J. , Wang, W., Wang, L.,and Sun, Y. (2015) : Exogenous melatonin improves seedling health index and drought tolerance in tomato. Plant Growth Regulation, 77(3), pp. 317–326. https://doi.org/10.1007/s10725-015-0066-6.

Liu, K. Jing, T., Wang, Y., Ai, X., and Bi, H.(2022) :Melatonin delays leaf senescence and improves cucumber yield by modulating chlorophyll degradation and photoinhibition of PSII and PSI, Environmental and Experimental Botany, 200, p.104915.https://doi.org/10.1016/j.envexpbot.2022.104915.

Liu, M., Li, M., Liu, K., and Sui, N. (2015): Effects of drought stress on seed germination and seedling growth of different maize varieties. Journal of Agricultural Science, 7(5), p. 231.

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. https://doi.org/10.1016/j.scienta.2014.10.049.

Liu, Y. Wang, X., Lv, H., Cao, M., Li, Y., Yuan, X., and Zhang, N. (2022): Anabolism and signaling pathways of phytomelatonin, Journal of Experimental Botany, 73(17), pp. 5801–5817.

Lozano-Juste, J., León, J. (2011) :Nitric oxide regulates DELLA content and PIF expression to promote photomorphogenesis in Arabidopsis. Plant Physiol. 156: 1410-1423.

Luboshitzky, R., Yanai, D., Shen-Orr, Z., Israeli, E., Herer, P.,and Lavie, P. (1998): Daily and seasonal variations in the concentration of melatonin in the human pineal gland. Brain Research Bulletin, 47(3), pp. 271–276. https://doi.org/10.1016/S0361-9230(98)00105-1.

Ma, W., Xu, L., Gao, S., Lyu, X., Cao, X., and Yao, Y. et al. (2021):Melatonin alters the secondary metabolite profile of grape berry skin by promoting VvMYB14-mediated ethylene biosynthesis', Horticulture Research, 8(1), p. 43. https://doi.org/10.1038/s41438-021-00478-2.

Ma, X., Zhang, J., Burgess, P., Rossi, S.,and Huang, B. (2018): Interactive effects of melatonin and cytokinin on alleviating drought-induced leaf senescence in creeping bentgrass (*Agrostis stolonifera*). Environmental and Experimental Botany, 145, pp. 1–11. https://doi.org/10.1016/j.envexpbot.2017.10.010.

Machado, R. M. A., and Serralheiro, R. P. (2017):Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae 3:30. doi: 10.3390/horticulturae3020030.

Mann, A., Kaur, G., Kumar, A., Sanwal, S. K., Singh, J., and Sharma, P. C. (2019): Physiological response of chickpea (*Cicer arietinum L.*) at early seedling stage under salt stress conditions. Legume Research - an International Journal, 42(of), pp. 625–632. https://doi.org/10.18805/LR-4059.

Margherita, B., Giampiero, C. and Pierre, D. (1996) : *Field performance of tissue-cultured plants* of *Ranunculus asiaticus* L., Scientia Horticulturae, 66 (3–4), pp. 229–239.

https://doi.org/10.1016/S0304-4238(96)00916-8.

Matthews, J.S.A., Vialet-Chabrand, S. and Lawson, T. (2018) :Acclimation to Fluctuating Light Impacts the Rapidity of Response and Diurnal Rhythm of Stomatal Conductance, Plant Physiology, 176(3), pp. 1939–1951. https://doi.org/10.1104/pp.17.01809.

Meng, J.F. , Shi, T. C., Song, S., Zhang, Z. W., and Fang, Y. L (2017) : Melatonin in grapes and grape-related foodstuffs: A review. Food Chemistry, 231, pp. 185–191. https://doi.org/10.1016/j.foodchem.2017.03.137.

Meng, J. , Xu, T.F., Wang, Z.Z., Fang, YL, Xi, ZM, and Zhang, Z.W. (2014) :The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. Journal of Pineal Research, 57(2), pp. 200–212. https://doi.org/10.1111/jpi.12159.

Meynet, J. (1993) :Ranunculus', in M. (Eds. . In: De Hertogh, A., Le Nard (ed.) The Physiology of Flower Bulbs. The Netherlands: Elsevier Science Publishers, pp. 603–610.

Mittler, R. (2002): Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science, 7(9), pp. 405–410. https://doi.org/10.1016/S1360-1385(02)02312-9.

Moustafa-Farag, M., Elkelish, A., Dafea, M., Khan, M., Arnao, M. B., Abdelhamid, M. T., and Ai, S. (2020): Role of melatonin in plant tolerance to soil stressors: salinity, ph and heavy metals. Molecules, 25(22), p. 5359. https://doi.org/10.3390/molecules25225359.

Muhammad, I. Yang, L., Ahmad, S., Mosaad, I. S., Al-Ghamdi, A. A., Abbasi, A. M., and Zhou, X. B. (2022) : Melatonin application alleviates stress-induced photosynthetic inhibition and oxidative damage by regulating antioxidant defense system of maize: A Meta-Analysis', Antioxidants, 11(3), p. 512. https://doi.org/10.3390/antiox11030512.

Muller, O., Cohu, C. M., Stewart, J. J., Protheroe, J. A., Demmig-Adams, B.,and Adams III, W. W.(2014) : Association between photosynthesis and contrasting features of minor veins in leaves of summer annuals loading phloem via symplastic versus apoplastic routes. Physiol Plant, 152, pp. 174–183.

Munné-Bosch, S., Mueller, M., Schwarz, K., and Alegre, L. (2001) : Diterpenes and antioxidative protection in drought-stressed Salvia officinalis plants. Journal of Plant Physiology, 158(11), pp. 1431–1437. https://doi.org/10.1078/0176-1617-00578.

Munns, R. (2002): Comparative physiology of salt and water stress. Plant, Cell & Environment, *25*, 239–250.

Munns, R. and Tester, M. (2008): Mechanisms of Salinity Tolerance. Annual Review of Plant Biology, 59(1), pp. 651–681. Available at: https://doi.org/10.1146/annurev.arplant.59.032607.092911.

Murch, S.J., Campbell, S.S.B. and Saxena, P.K. (2001):The role of serotonin and melatonin in plant morphogenesis: Regulation of auxin-induced root organogenesis in in vitro-cultured explants of st. John's Wort (*Hypericum perforatum* L.*). In Vitro Cellular & Developmental Biology - Plant,* 37(6), pp. 786–793. https://doi.org/10.1007/s11627-001-0130-y.

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.

NaveenKumar, S.K. Hemshekhar, M., Jagadish, S., Manikanta, K., Vishalakshi, G.J., Kemparaju, K.,and Girish, K.S. (2020): Melatonin restores neutrophil functions and prevents apoptosis amid dysfunctional glutathione redox system. Journal of Pineal Research, 69(3). https://doi.org/10.1111/jpi.12676.

Netondo, G. W., Onyango, J. C., and Beck, E. (2004): Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Science, *44*, 806–811.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y. I., Neukermans, J., Marquez-Garcia, B., Queval, G. and Foyer, C. H. (2012): Glutathione in plants: an integrated overview. Plant, Cell & Environment, 35(2), pp. 454–484. https://doi.org/10.1111/j.1365-3040.2011.02400.x.

Ohkawa, K. (1986): Growth and flowering of *Ranunculus asiaticus*. Acta horticulturae, (177), pp. 165–172. https://doi.org/10.17660/ActaHortic.1986.177.22.

Okazaki, M. and Ezura, H. (2009): Profiling of melatonin in the model tomato (*Solanum lycopersicum* L.) cultivar Micro-Tom. Journal of Pineal Research, 46(3), pp. 338–343. https://doi.org/10.1111/j.1600-079X.2009.00668.x.

Olson, C.L. (1976): On choosing a test statistic in multivariate analysis of variance. Psychological bulletin, 83(4), p. 579.

Oraee, A. and Tehranifar, A. (2020): Evaluating the potential drought tolerance of pansy through its physiological and biochemical responses to drought and recovery periods. Scientia

Horticulturae, 265(August 2019), p. 109225. https://doi.org/10.1016/j.scienta.2020.109225.

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.

Pandolfi, C., Mancuso, S. and Shabala, S. (2012): Physiology of acclimation to salinity stress in pea (*Pisum sativum*). Environmental and Experimental Botany, 84, pp. 44–51. https://doi.org/10.1016/j.envexpbot.2012.04.015.

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. https://doi.org/10.3390/antiox10050775.

Park, S. Lee, K., Kim, Y. S., and Back, K. (2012): Tryptamine 5-hydroxylase-deficient Sekiguchi rice induces synthesis of 5-hydroxytryptophan and N -acetyltryptamine but decreases melatonin biosynthesis during senescence process of detached leaves, Journal of Pineal Research, 52(2), pp. 211–216. https://doi.org/10.1111/j.1600-079X.2011.00930.x.

Park, S. Le, T. N. N., Byeon, Y., Kim, Y. S., and Back, K. (2013) : Transient induction of melatonin biosynthesis in rice (*Oryza sativa* L.) during the reproductive stage. Journal of Pineal Research, 55(1), pp. 40–45.https://doi.org/10.1111/jpi.12021.

Park, S. and Back, K. (2012): Melatonin promotes seminal root elongation and root growth in transgenic rice after germination. Journal of Pineal Research, 53(4), pp. 385–389. https://doi.org/10.1111/j.1600-079X.2012.01008.x.

Parkash, V. and Singh, S. (2020):A review on potential plant-basedwater stress indicators for vegetable crops, *Sustainability (Switzerland)*, 12(10). Available at: https://doi.org/10.3390/SU12103945.

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.

Porcel, R., Aroca, R. and Ruiz-Lozano, J.M. (2012): Salinity stress alleviation using arbuscular mycorrhizal fungi. A review: Agronomy for Sustainable Development, 32(1), pp. 181–200. https://doi.org/10.1007/s13593-011-0029-x.

Posmyk, M.M. , Kuran, H., Marciniak, K.,and Janas, K. M. (2008): Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. Journal of Pineal Research, 45(1), pp. 24–31. https://doi.org/10.1111/j.1600-079X.2007.00552.x.

Posmyk, M.M. , Bałabusta, M., Wieczorek, M., Sliwinska, E., and Janas, K. M. (2009): Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. Journal of Pineal Research, 46(2), pp. 214–223. https://doi.org/10.1111/j.1600-079X.2008.00652.x.

Qi, Z.Y. , Wang, K. X., Yan, M. Y., Kanwar, M. K., Li, D. Y., Wijaya, L., and Zhou, J. (2018): Melatonin alleviates high temperature-induced pollen abortion in solanum lycopersicum. Molecules, 23(2), p. 386. https://doi.org/10.3390/molecules23020386.

Qiao, Y., Ren, J., Yin, L., Liu, Y., Deng, X., Liu, P., and Wang, S. (2020): Exogenous melatonin alleviates PEG-induced short-term water deficiency in maize by increasing hydraulic conductance. BMC Plant Biol., 20, p. 204:218.

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.

Rauter, S., Sun, Y. and Stock, M. (2021): Visual quality, gas exchange, and yield of anemone and ranunculus irrigated with saline water. HortTechnology. (Boca Rato. Edited by ed. A. Halevy, 31(6), pp. 763–770. https://doi.org/10.21273/HORTTECH04930-21.

Reddy, A.R., Chaitanya, K.V. and Vivekanandan, M. (2004): Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of plant physiology, 161(11), pp. 1189–1202.

Roy, S. J., Negrão, S., and Tester, M. (2014): Salt resistant crop plants. Curr. Opin. Biotechnol. 26, 115–124. doi: 10.1016/j.copbio.2013.12.004.

Rajendran, K., Tester, M., and Roy, S. J. (2009): Quantifying the three main components of salinity tolerance in cereals. Plant Cell Environ. 32, 237–249. doi: 10.1111/j.1365-3040.2008.01916.x

Ren, S., Jiang, G.-L. and Rutto, L. (2019): Melatonin priming enhances symbiotic nitrogen fixation in soybean, *Glycine max* L.J. Biotech Res., 10, pp. 136–144.

Rodriguez, C., Mayo, J.C., Sainz, R.M., Antolín, I., Herrera, F., Martín, V.,and Reiter, R.J. (2004): Regulation of antioxidant enzymes: a significant role for melatonin. Journal of Pineal

Research, 36(1), pp. 1–9. https://doi.org/10.1046/j.1600-079X.2003.00092.x.

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.

Ruiz, M.B.A.J.H., Arnao, M.B. and Hernández-Ruiz, J. (2020) :Melatonin in flowering, fruit set and fruit ripening. Plant Reproduction, 33(2), pp. 77–87. https://doi.org/10.1007/s00497-020-00388-8.

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.

Sadak, M.S. and Ramadan, A.A.E.-M. (2021):Impact of melatonin and tryptophan on water stress tolerance in white lupine (*Lupinus termis* L.). Physiology and Molecular Biology of Plants, 27(3), pp. 469–481. https://doi.org/10.1007/s12298-021-00958-8.

Sadiq, M., Akram, N.A. and Ashraf, M. (2018):Impact of exogenously applied tocopherol on some key physio-biochemical and yield attributes in mungbean [*Vigna radiata* (L.) Wilczek] under limited irrigation regimes. Acta Physiologiae Plantarum, 40(7), pp. 1–14.

Sahin, U., Ekinci, M., Ors, S., Turan, M., Yildiz, S.,and Yildirim, E. (2018): Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea var. capitata*)’, *Scientia Horticulturae*, 240, pp. 196–204. https://doi.org/10.1016/j.scienta.2018.06.016.

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.

Sarropoulou, V.N., Therios, I.N. and Dimassi-Theriou, K.N. (2012) :Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* × *P. canescens*), and MxM 60 (*P. avium* × *P. mahaleb*).Journal of Pineal Research, 52(1), pp. 38–46. https://doi.org/10.1111/j.1600-079X.2011.00914.x.

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.

Scariot, V. Larcher, F., Caser, M., Costa, E., Beruto, M., and Devecchi, M. (2009): Flower longevity in ten cultivars of cut *Ranunculus asiaticus* L. as affected by ethylene and ethylene inhibitors. European Journal of Horticultural Science, 74(3), p. 137.

Seki, M., Umezawa, T., Urano, K., and Shinozaki, K. (2007): Regulatory metabolic networks in drought stress responses. Curr. Opin. Plant Biolol. 10, pp. 296–302.

Shabala, S. and Cuin, T.A. (2008): Potassium transport and plant salt tolerance. Physiologia Plantarum, 133(4), pp. 651–669. https://doi.org/10.1111/j.1399-3054.2007.01008.x.

Sharif, R., Xie, C., Zhang, H., Arnao, M. B., Ali, M., Ali, Q., Muhammad, I., Shalmani, A., Nawaz, M. A., Chen, P., and Li, Y. (2018): Melatonin and its effects on plant systems. Molecules, 23(9), 2352. https://doi.org/10.3390/molecules23092352

Sharma, A., Wang, J., Xu, D., Tao, S., Chong, S., Yan, D., Li, Z., Yuan, H. Zheng, B. (2020): Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted *Carya cathayensis* plants. Science of The Total Environment, 713, p. 136675.https://doi.org/10.1016/j.scitotenv.2020.136675.

Sharma, P. Thakur, N., Mann, N. A.,and Umar, A.(2024) :Melatonin as plant growth regulator in sustainable agriculture, Scientia Horticulturae, 323(September 2023), p. 112421. https://doi.org/10.1016/j.scienta.2023.112421.

Sharp, R.G., Else, M. A., Cameron, R. W., and Davies, W. J. (2009): Water deficits promote flowering in *Rhododendron* via regulation of pre and post initiation development', *Scientia Horticulturae*, 120(4), pp. 511–517. https://doi.org/10.1016/j.scienta.2008.12.008.

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.

Shi, H., Jiang, C., Ye, T., Tan, D. X., Reiter, R. J., Zhang, H., Liu, R. and Chan, Z. (2015)a:

Comparative physiological, metabolomic, and transcriptomic analyse sreveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). Pers.] by exogenous melatonin. J. Exp. Bot., 66, pp. 681–694.

Shi, H., Reiter, R. J., Tan, D. X., and Chan, Z. (2015)b: INDOLE -3- ACETIC ACID INDUCIBLE 17 positively modulates natural leaf senescence through melatonin-mediated pathway in Arabidopsis. Journal of Pineal Research, 58(1), pp. 26–33. https://doi.org/10.1111/jpi.12188.

Shi, H., Chen, Y., Tan, D. X., Reiter, R. J., Chan, Z., and He, C. (2015)c: Melatonin induces nitric oxide and the potential mechanisms relate to innate immunity against bacterial pathogen infection in Arabidopsis. Journal of Pineal Research, 59(1), pp. 102–108.

Shi, H., Wei, Y., Wang, Q., Reiter, R. J., and He, C. (2016) : Melatonin mediates the stabilization of DELLA proteins to repress the floral transition in Arabidopsis. J. Pineal Res., 60, pp. 373–379.

Siddiqui, M.H., Mukherjee, S.,Kumar, R., Alansi, S., Shah,A.A., Kalaji , H.M., Javed , T., Raza, A. (2022): Potassium and melatonin-mediated regulation of fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7- bisphosphatase (SBPase) activity improve photosynthetic efficiency, carbon assimilation and modulate glyoxalase system accompanying tolerance to cad’, *Plant Physiology and Biochemistry*, 171, pp. 49–65. https://doi.org/10.1016/j.plaphy.2021.12.018.

Smith, P., Calvin, K., Nkem, J., Campbell, D., Cherubini, F., Grassi, G., Korotkov, V., Hoang, A., Lwasa, S., McElwee, P., Nkonya, E., Saigusa, N., Soussana, J., Taboada, M., Manning, F., Nampanzira, D., Arias-Navarro, C., Vizzarri, M., House, J., Roe, S., Cowie, A., Rounsevell, M., and Arneth, A.(2020): Which practices co-deliver food security, climate change mitigation and adaptation, and combat land degradation and desertification. Global Change Biology, 26(3),1532–1575.p. https://doi.org/10.1111/gcb.14878.

Socaciu, A.I. Ionuţ, R., Socaciu, M. A., Ungur, A. P., Bârsan, M., Chiorean, A., and Râjnoveanu, A. G. (2020) : Melatonin, an ubiquitous metabolic regulator: functions, mechanisms and effects on circadian disruption and degenerative diseases, Reviews in Endocrine and Metabolic Disorders, 21(4), pp. 465–478. https://doi.org/10.1007/s11154-020-09570-9.

SoltysKalina, D. Plich, J., Strzelczyk-Żyta, D., Śliwka, J., and Marczewski, W. (2016) :Effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of

‘Katahdin’-derived potato cultivars. Breed. Sci., 66(2), pp. 328–331.

Song, L. Tan, Z., Zhang, W., Li, Q., Jiang, Z., Shen, S.,and Chen, X. (2023) : Exogenous melatonin improves the chilling tolerance and preharvest fruit shelf life in eggplant by affecting ROS- and senescence-related processes, Horticultural Plant Journal, 9(3), pp. 523–540. https://doi.org/10.1016/j.hpj.2022.08.002.

Su, J. Yang, X., Shao, Y., Chen, Z.,and Shen, W. (2021) :Molecular hydrogen–induced salinity tolerance requires melatonin signalling in *Arabidopsis thaliana* , Plant, Cell & Environment, 44(2), pp. 476–490.https://doi.org/10.1111/pce.13926.

Sun, Q. Zhang, N., Wang, J., Zhang, H., Li, D., Shi, J., and Guo, Y. D. (2015) :Melatonin promotes ripening and improves quality of tomato fruit during postharvest life, Journal of Experimental Botany, 66(3), pp. 657–668. https://doi.org/10.1093/jxb/eru332.

Sun, Q. Zhang, N., Wang, J., Cao, Y., Li, X., Zhang, H., and Guo, Y. D. (2016) :A label-free differential proteomics analysis reveals the effect of melatonin on promoting fruit ripening and anthocyanin accumulation upon postharvest in tomato, Journal of Pineal Research, 61(2), pp. 138–153. https://doi.org/10.1111/jpi.12315.

Sun, S.,Wen, D., Yang, W., Meng, Q., Shi, Q., and Gong, B.(2020) : Overexpression of caffeic acid o-methyltransferase 1 (comt1) increases melatonin level and salt stress tolerance in tomato plant’, Journal of Plant Growth Regulation, 39(3), pp. 1221–1235.https://doi.org/10.1007/s00344-019-10058-3.

Sun, C., Liu, L., Wang, L., Li, B., Jin, C., and Lin, X. (2021). Melatonin: A master regulator of plant development and stress responses. Journal of Integrative Plant Biology, 63(1), 126-145.

Stevens, P. F.,and Davis, H. (2001): Angiosperm phylogeny website (Vol. 9). St. Louis: Missouri Botanical Garden.

Szafrańska, K., Reiter, R.J. and Posmyk, M.M. (2016): Melatonin Application to *Pisum sativum L.* Seeds Positively Influences the Function of the Photosynthetic Apparatus in Growing Seedlings during Paraquat-Induced Oxidative Stress. Frontiers in Plant Science, 7, p. 1663. https://doi.org/10.3389/fpls.2016.01663.

Tabachnick, B. and Fidell, L. (2013): IBM SPSS statistics for windows. Using Multivariate Statistics. Pearson, Boston. IBM Corp. Released 2020. IBM SPSS Statistics for Windows,Version 27.0. Armonk, NY: IBM Corp.

Tabassum, S. , Ossola, A., Marchin, R., Ellsworth, D., and Leishman, M., (2021): Assessing the relationship between trait-based and horticultural classifications of plant responses to drought. Urban Forestry & Urban Greening, 61, p. 127109. https://doi.org/10.1016/j.ufug.2021.127109.

Tamura, M. (1993): Ranunculaceae. in Flowering Plants · Dicotyledons. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 563–583. https://doi.org/10.1007/978-3-662-02899-5_67.

Tamura, M. (1995a): Die Natür lichen Pflanzenfamilien. Bd. 17 a IV Angiospermae. in K. (Eds. . Systematic part. In: Engler, A., Prantl (ed.) Ordnung Ranunculales. Fam. Ranunculaceae. Berlin: Duncker & Humblot, pp. 220–519.

Tamura, M. (1995b): Geographical distribution. in In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamilien. Bd. 17a IVAngiospermae.Ordnung Ranunculales. Fam. Ranunculaceae. Berlin: Duncker & Humblot, pp. 89–96.

Tan, D.X. Manchester, L. C., Liu, X., Rosales-Corral, S. A., Acuna-Castroviejo, D., and Reiter, R. J. (2013): Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin’s primary function and evolution in eukaryotes, Journal of Pineal Research, 54(2), pp. 127–138. https://doi.org/10.1111/jpi.12026.

Tan, D.X. Hardeland, R., Back, K., Manchester, L. C., Alatorre-Jimenez, M. A., and Reiter, R. J.(2016): On the significance of an alternate pathway of melatonin synthesis via 5-methoxytryptamine: comparisons across species, Journal of pineal research, 61(1), pp. 27–40.

Tiwari, R.K., Lal, M. K., Naga, K. C., Kumar, R., Chourasia, K. N., Subhash, S., and Sharma, S. (2020) : Emerging roles of melatonin in mitigating abiotic and biotic stresses of horticultural crops, Scientia Horticulturae, 272, p. 109592. https://doi.org/10.1016/j.scienta.2020.109592.

Tiwari, R.K., Lal, M. K., Kumar, R., Chourasia, K. N., Naga, K. C., Kumar, D.,and Zinta, G. (2021): Mechanistic insights on melatonin-mediated drought stress mitigation in plants, Physiologia Plantarum, 172(2), pp. 1212–1226. https://doi.org/10.1111/ppl.13307.

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.

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.

Underwood, H. (1989): The pineal and melatonin: Regulators of circadian function in lower vertebrates. Experientia, 45(10), pp. 914–922. https://doi.org/10.1007/BF01953048.

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.

Vantassel, D.L., Li, J.A. and Oneill, S.D. (1993): Melatonin-identification of a potential dark signal in plants. in Plant Physiology, p. 117.

Vantassel, D.L., Roberts, N.J. and Oenill, S.D. (1995): Melatonin from higher-plants-isolation and identification of N-acetyl 5-methoxytryptamine. in Plant physiology108 (2). , p. 101. 15501 MONONA DRIVE, ROCKVILLE, MD 20855: AMER SOC PLANT PHYSIOLOGISTS

Varghese, N., Alyammahi, O., Nasreddine, S., Alhassani, A., and Gururani, M. A. (2019): Melatonin positively influences the photosynthetic machinery and antioxidant system of avena sativa during salinity stress. Plants, 8(12), p. 610. https://doi.org/10.3390/plants8120610.

Wada, K.C. and Takeno, K. (2010): Stress-induced flowering. Plant Signaling & Behavior, 5(8), pp. 944–947. https://doi.org/10.4161/psb.5.8.11826.

Wang, F. Zeng, B., Sun, Z.,and Zhu, C. (2009): Relationship between proline and Hg^{2+}-induced oxidative stress in a tolerant rice mutant. Archives of Environmental Contamination and Toxicology, 56(4), pp. 723–731. https://doi.org/10.1007/s00244-008-9226-2.

Wang, J., Sun, H., Sheng, J., Jin, S., Zhou, F., Hu, Z., and Diao, Y. (2019): Transcriptome, physiological and biochemical analysis of *Triarrhena sacchariflora* in response to flooding stress. BMC Genetics, 20(1), p. 88. https://doi.org/10.1186/s12863-019-0790-4.

Wang, K. Cai, S., Xing, Q., Qi, Z., Fotopoulos, V., Yu, J., and Zhou, J. (2022): Melatonin delays dark-induced leaf senescence by inducing miR171b expression in tomato.Journal of Pineal Research, 72(3). https://doi.org/10.1111/jpi.12792.

Wang, L.-F., Lu, K. K., Li, T. T., Zhang, Y., Guo, J. X., Song, R. F., and Liu, W. C.(2022) :Maize phytomelatonin receptor1 functions in plant tolerance to osmotic and drought stress, Journal of Experimental Botany. Edited by M. Jones, 73(17), pp. 5961–

5973.https://doi.org/10.1093/jxb/erab553.

Wang, L., Feng, C., Zheng, X., Guo, Y., Zhou, F., Shan, D., Liu, X. and Kong, J. (2017): Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. Journal of Pineal Research, 63(3). https://doi.org/10.1111/jpi.12429.

Wang, L.Y., Liu, J.L., Wang, W.X., and Sun, Y. (2016): Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. Photosynthetica, 54(1), pp. 19–27. https://doi.org/10.1007/s11099-015-0140-3.

Wang, M., Zhang, T. and Ding, F. (2019): Exogenous melatonin delays methyl jasmonate-triggered senescence in tomato leaves. Agronomy, 9(12), p. 795. https://doi.org/10.3390/agronomy9120795.

Wang, P., Yin, L., Liang, D., Li, C., , F., and Yue, Z., (2012): Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate–glutathione cycle. Journal of Pineal Research, 53. https://doi.org/10.1111/j.1600-079X.2011.00966.x.

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. https://doi.org/10.1016/j.hpj.2023.03.011.

Wang, Y. , Zhang, X., Chen, J., Chen, A., Wang, L., Guo, X., Niu , Y., Liu , S., Mi, G., Gao, Q. (2019): Reducing basal nitrogen rate to improve maize seedling growth, water and nitrogen use efficiencies under drought stress by optimizing root morphology and distribution. Agric. Water Manag., 212, pp. 328–337.

Weeda, S., Zhang, N., Zhao, X., Ndip, G., Guo, Y., Buck, G. A., Fu, C. and Ren, S. (2014): Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. PLoS One, p. 9:e93462.

Wei, J., Li, D. X., Zhang, J. R., Shan, C., Rengel, Z., Song, Z. B., and Chen, Q. (2018): Phytomelatonin receptor PMTR 1-mediated signaling regulates stomatal closure *in Arabidopsis thaliana*. Journal of pineal research, 65(2), e12500.https://doi.org/10.1111/jpi.12500.

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.

Wei, L. Zhao, H., Wang, B., Wu, X., Lan, R., Huang, X., and Zheng, Q.(2022): Exogenous melatonin improves the growth of rice seedlings by regulating redox balance and ion homeostasis under salt stress, Journal of Plant Growth Regulation, 41(6), pp. 2108–2121. https://doi.org/10.1007/s00344-021-10417-z.

Wei, W., Li, Q. T., Chu, Y. N., Reiter, R. J., Yu, X. M., Zhu, D. H., and Chen, S. Y. (2015): Melatonin enhances plant growth and abiotic stress tolerance in soybean plants, Journal of Experimental Botany, 66(3), pp. 695–707. https://doi.org/10.1093/jxb/eru392.

Wei, Y., Liu, G., Chang, Y., Lin, D., Reiter, R. J., He, C.,and Shi, H. (2018): Melatonin biosynthesis enzymes recruit WRKY transcription factors to regulate melatonin accumulation and transcriptional activity on W-box in cassava. Journal of pineal research, 65(1), e12487.: https://doi.org/10.1111/jpi.12487.

West, S.G., Finch, J.F. and Curran, P.J. (1995) :Structural equation models with nonnormal variables: Problems and remedies. in R.H. Hoyle (ed.) Structural equation modeling: Concepts, issues, and applications. Sage Publications, Inc, pp. 56–75.

Wolf, K., Kolář, J., Witters, E., van Dongen, W., van Onckelen, H., Macháčková, I. (2001) : Daily profile of melatonin levels in *Chenopodium rubrum* L. depends on photoperiod. J. Plant Physiol. 158: 1491-1493.

Wu, P., Y., Ahammed, G., Hao, B., Chen, J., Wan, W., Zhao, Y., Cui, H., Xu, W., Cui, J., and Liu, H. (2022): Insights into melatonin-induced photosynthetic electron transport under low-temperature stress in cucumber. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.1029854.

Xia, H., Ni, Z., Hu, R., Lin, L., Deng, H., Wang, J., Tang, Y., Sun, G., Wang, X., Li, H., Liao, M., Lv, X., and Liang, D. (2020): Melatonin alleviates drought stress by a non-enzymatic and enzymatic antioxidative system in kiwifruit seedlings. International Journal of Molecular Sciences, 21(3), p. 852. https://doi.org/10.3390/ijms21030852.

Xia, X.-J. , Zhou, Y. H., Shi, K., Zhou, J., Foyer, C. H., and Yu, J. Q. (2015): Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. Journal of Experimental Botany, 66(10), pp. 2839–2856. https://doi.org/10.1093/jxb/erv089.

Xiao, S, Liu, L., Wang, H., Li, D., Bai, Z., Zhang, Y., Sun, H., Zhang, K. and Li, C. (2019): Exogenous melatonin accelerates seed germination in cotton (*Gossypium hirsutum* L.). PLOS ONE. Edited by T. Roach, 14(6), p. e0216575. Available at: https://doi.org/10.1371/journal.pone.0216575.

Xie, Q. Zhang, Y., Cheng, Y., Tian, Y., Luo, J., Hu, Z. and Chen, G. (2022) :The role of melatonin in tomato stress response, growth and development, Plant Cell Reports, 41(8), pp. 1631–1650. https://doi.org/10.1007/s00299-022-02876-9.

Xie, Z. , Wang, J., Wang, W., Wang, Y., Xu, J., Li, Z., Zhao, X. and Fu, B. (2021) :Integrated analysis of the transcriptome and metabolome revealed the molecular mechanisms underlying the enhanced salt tolerance of rice due to the application of exogenous melatonin. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.618680.

Xu, L. , Yue, Q., Bian, F. E., Sun, H., Zhai, H., and Yao, Y. (2017): Melatonin enhances phenolics accumulation partially via ethylene signaling and resulted in high antioxidant capacity in grape berries. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.01426.

Yan, F. , Wei, H., Li, W., Liu, Z., Tang, S., Chen, L., Ding, C., Jiang, Y., Ding, Y. and Li, G. (2020): Melatonin improves K^+ and Na^+ homeostasis in rice under salt stress by mediated nitric oxide. Ecotoxicology and Environmental Safety, 206, p. 111358. https://doi.org/10.1016/j.ecoenv.2020.111358.

Yan, F., Wei, H., Ding, Y., Li, W., Chen, L., Ding, C., Jiang, Y., Ding, Y. and Li, G. (2021): Melatonin enhances Na^+/K^+ homeostasis in rice seedlings under salt stress through increasing the root H^{+-}pump activity and Na^+/K^+ transporters sensitivity to ROS/RNS. Environmental and Experimental Botany, 182, p. 104328. https://doi.org/10.1016/j.envexpbot.2020.104328.

Yang, X. Chen, J., Ma, Y., Huang, M., Qiu, T., Bian, H., and Wang, J. (2022) : Function, Mechanism, and Application of Plant Melatonin: An Update with a Focus on the Cereal Crop, Barley (*Hordeum vulgare* L.), Antioxidants, 11(4), p. 634.https://doi.org/10.3390/antiox11040634.

Yang, X.L., Xu, H., Li, D., Gao, X., Li, T. L., and Wang, R. (2018): Effect of melatonin priming on photosynthetic capacity of tomato leaves under low-temperature stress. Photosynthetica, 56(3), pp. 884–892. https://doi.org/10.1007/s11099-017-0748-6.

Ye, J., Yang, W., Li, Y., Wang, S., Yin, L., and Deng, X. (2020): Seed pre-soaking with melatonin improves wheat yield by delaying leaf senescence and promoting root development. Agronomy, 10(1), p. 84. https://doi.org/10.3390/agronomy10010084.

Yin, Z., Lu, J., Meng, S., Liu, Y., Mostafa, I., Qi, M., and Li, T. (2019): Exogenous melatonin improves salt tolerance in tomato by regulating photosynthetic electron flux and the ascorbate–glutathione cycle. Journal of Plant Interactions, 14(1), pp. 453–463.

https://doi.org/10.1080/17429145.2019.1645895.

Yuan, F., Lyu, M. J. A., Leng, B. Y., Zheng, G. Y., Feng, Z. T., Li, P. H.,and Wang, B. S. (2015): Comparative transcriptome analysis of developmental stages of the *Limonium bicolor* leaf generates insights into salt gland differentiation.', *Plant. Cell Environ.*, 38, pp. 1637–1657. https://doi.org/10.1111/pce.12514

Yuan, F. Lyu, M. J. A., Leng, B. Y., Zhu, X. G., and Wang, B. S. (2016) : The transcriptome of NaCl-treated Limonium bicolor, leaves reveals the genes controlling salt secretion of salt gland. Plant. Mol. Biol., 91, pp. 241–256. https://doi.org/10.1007/s11103-016-0460-0

Zahedi, S.M., Hosseini, M. S., Abadía, J.,and Marjani, M. (2020): Melatonin foliar sprays elicit salinity stress tolerance and enhance fruit yield and quality in strawberry (*Fragaria* × *ananassa Duch.*). Plant Physiology and Biochemistry, 149, pp. 313–323.https://doi.org/10.1016/j.plaphy.2020.02.021.

Zamani, Z., Amiri, H. and Ismaili, A. (2020):Improving drought stress tolerance in fenugreek (*Trigonella foenum-graecum*) by exogenous melatonin', *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, 154(5), pp. 643–655. https://doi.org/10.1080/11263504.2019.1674398.

Van Zelm, E., Zhang, Y. and Testerink, C. (2020): Salt Tolerance Mechanisms of Plants. Annual Review of Plant Biology, 71(1), pp. 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005.

Zeng, L., Cai, J. S., Li, J. J., Lu, G. Y., Li, C. S., Fu, G. P., and Cheng, Y. (2018): Exogenous application of a low concentration of melatonin enhances salt tolerance in rapeseed (*Brassica napus* L.) seedlings. Journal of Integrative Agriculture, 17, pp. 328–335. https://doi.org/10.1016/S2095-3119(17)61757-X.

Zhan, H., Nie, X., Zhang, T., Li, S., Wang, X., Du, X., Tong, W., and Song, W. (2019): Melatonin: A small molecule but important for salt stress tolerance in plants. International Journal of Molecular Sciences, 20(3), 709. https://doi.org/10.3390/ijms20030709

Zhang, H. J. , Zhang, N. A., Yang, R. C., Wang, L., Sun, Q. Q., Li, D. B., and Guo, Y. D. (2014): Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA 4 interaction in cucumber (*Cucumis sativus* L.). Journal of Pineal Research, 57(3), pp. 269–279. https://doi.org/10.1111/jpi.12167.

Zhang, J., Shi, Y., Zhang, X., Du, H., Xu, B., & Huang, B. (2017): Melatonin suppression of heat-induced leaf senescence involves changes in abscisic acid and cytokinin biosynthesis and signaling pathways in perennial ryegrass (*Lolium perenne* L.). Environmental and Experimental Botany, 138, pp. 36–45. https://doi.org/10.1016/j.envexpbot.2017.02.012.

Zhang, L., Zhu, M., Ren, L., Li, A., Chen, G., and Hu, Z. (2018):The SlFSR gene controls fruit shelf-life in tomato. Journal of Experimental Botany, 69(12), pp. 2897–2909. https://doi.org/10.1093/jxb/ery116.

Zhang, N. , Zhao, B., Zhang, H. J., Weeda, S., Yang, C., Yang, Z. C.,and Guo, Y. D. (2013): Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). Journal of Pineal Research, 54(1), pp. 15–23.https://doi.org/10.1111/j.1600-079X.2012.01015.x.

Zhang, N. , Zhang, H.J., Zhao, B., Sun, Q.Q., Cao, Y.Y., Li, R., and Guo, Y.D. (2014)a : The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. Journal of Pineal Research, 56(1), pp. 39–50. https://doi.org/10.1111/jpi.12095.

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.

Zhang, N., Zhang, H.J., Sun, Q.Q., Cao, Y.Y., Li, X., Zhao, B.,and Guo, Y.D.. (2017): Proteomic analysis reveals a role of melatonin in promoting cucumber seed germination under high salinity by regulating energy production. Scientific Reports, 7(1), p. 503.https://doi.org/10.1038/s41598-017-00566-1.

Zhang, P. , Liu, L., Wang, X., Wang, Z., Zhang, H., Chen, J.,and Li, C (2021): Beneficial effects of exogenous melatonin on overcoming salt stress in sugar beets (*Beta vulgaris* L.). Plants, 10(5), p. 886. https://doi.org/10.3390/plants10050886.

Zhang, R., Sun, Y., Liu, Z., Jin, W., and Sun, Y.(2017) : Effects of melatonin on seedling growth, mineral nutrition, and nitrogen metabolism in cucumber under nitrate stress. Journal of Pineal Research, 62(4). https://doi.org/10.1111/jpi.12403.

Zhang, R., Yue, Z., Chen, X., Wang, Y., Zhou, Y., Xu, W., and Huang, R. (2021): Foliar applications of urea and melatonin to alleviate waterlogging stress on photosynthesis and antioxidant metabolism in sorghum seedlings. Plant Growth Regulation, pp. 1-10..

https://doi.org/10.1007/s10725–021-00705–9.

Zhang, Y. , Fan, Y., Rui, C., Zhang, H., Xu, N., Dai, M., and Ye, W. (2021): Melatonin improves cotton salt tolerance by regulating ros scavenging system and Ca^{2+} signal transduction. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.693690.

Zhang, Y.P., Yang, S.J. and Chen, Y.Y. (2017): Effects of melatonin on photosynthetic performance and antioxidants in melon during cold and recovery. Biologia Plantarum, 61(3), pp. 571–578.

Zhang, Z. , Hu, Q., Liu, Y., Cheng, P., Cheng, H., Liu, W., and Chen, F. (2019): Strigolactone represses the synthesis of melatonin, thereby inducing floral transition in Arabidopsis thaliana in an FLC-dependent manner., J. Pineal Res., p. 67(2):e12582. https://doi.org/10.1111/jpi.12582.

Zhao, H. , Zhang, K., Zhou, X., Xi, L., Wang, Y., Xu, H.,and Zou, Z. (2017) :Melatonin alleviates chilling stress in cucumber seedlings by up-regulation of CsZat12 and modulation of polyamine and abscisic acid metabolism. Scientific Reports, 7(1), p. 4998. https://doi.org/10.1038/s41598-017-05267-3.

Zhu, J.K. (2001): Plant salt tolerance. Trends in Plant Science, 6(2), pp. 66–71. https://doi.org/doi: 10.1016/s1360-1385(00)01838-0.

Zhu, J.K. (2003): Regulation of ion homeostasis under salt stress. Curr. Opin. Plant. Boil., 6, pp. 441–445. https://doi.org/10.1016/S1369-5266(03)00085-2

Zhu, Z. and Lee, B. (2015) : Friends or Foes: New insights in jasmonate and ethylene co-actions. Plant and Cell Physiology, 56(3), pp. 414–420. https://doi.org/10.1093/pcp/pcu171.

Zinta, G., Khan, A., AbdElgawad, H., Verma, V., and Srivastava, A. K. (2016): Unveiling the redox control of plant reproductive development during abiotic stress. Frontiers in Plant Science, 7. https://doi.org/10.3389/fpls.2016.00700.

Zuo, Z., Sun, L., Wang, T., Miao, P., Zhu, X., Liu, S., Song, F., Mao, H.,and Li, X.. (2017): Melatonin improves the photosynthetic carbon assimilation and antioxidant capacity in wheat exposed to nano-ZnO stress. Molecules, 22(10), p. 1727. https://doi.org/10.3390/molecules22101727.