agriculture

MDPI

Article

Insights into Orris (*Iris pallida* Lam.) In Vivo Acclimatization and Response to Salt Stress via Exogenous Melatonin Application

Annalisa Meucci [1,*], Cristina Ghelardi [1], Rita Maggini [2], Fernando Malorgio [2], Beatrice Pezzarossa [3], Irene Rosellini [3] and Anna Mensuali [1]

[1] Institute of Crop Science, Sant'Anna School of Advanced Studies, Piazza Martiri della Libertà, 33, 56127 Pisa, Italy; cristina.ghelardi@santannapisa.it (C.G.); anna.mensuali@santannapisa.it (A.M.)
[2] Department of Agricultural, Food and Agro-Environmental Sciences, University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy; rita.maggini@unipi.it (R.M.); fernando.malorgio@unipi.it (F.M.)
[3] Institute of Research on Terrestrial Ecosystems (IRET), National Research Council (CNR), Via Moruzzi 1, 56124 Pisa, Italy; beatrice.pezzarossa@cnr.it (B.P.); irene.rosellini@cnr.it (I.R.)
* Correspondence: annalisa.meucci@santannapisa.it

Abstract: The loss of agricultural land is one of the main problems facing the global agricultural sector, and it is related to multiple phenomena; one of the main causes is soil salinization, induced both by natural processes and human activities. Among the strategies adopted to deal with soil salinization and its mitigation, the cultivation of species able to survive in saline soils seems to be an effective way of making salt-compromised lands usable. *Iris pallida* is a rustic plant and a species of high economic value that is mostly cultivated for perfume production. Consequently, the application of *I. pallida* to cover soils not suitable for crops traditionally cultivated for human and livestock nutrition could be considered; therefore, a preliminary test on the capacity of *I. pallida* to tolerate salinity during the acclimatization phase of micropropagated plants was conducted. Plantlets were treated with exogenous melatonin during the in vitro phase by adding it to the culture medium; therefore, during the acclimatization phase, crescent salt doses (150, 300, and 400 mM) were added to the soil every 14 days, administering melatonin to plants by a spray solution 24 h before each salt addition. At the end of the experiment, biometric measurements, chlorophylls, carotenoids, and macro-element contents were measured, and the relative water content (RWC) was determined in each salt addition. The results showed that orris plants can survive soil salt concentrations of up to 400 mM, and that the 50 µM melatonin spray treatment can protect orris rhizomes from salt side effects.

Keywords: in vitro; growth regulators; salinity; acclimatization; tolerance

Citation: Meucci, A.; Ghelardi, C.; Maggini, R.; Malorgio, F.; Pezzarossa, B.; Rosellini, I.; Mensuali, A. Insights into Orris (*Iris pallida* Lam.) In Vivo Acclimatization and Response to Salt Stress via Exogenous Melatonin Application. *Agriculture* **2024**, *14*, 2353. https://doi.org/10.3390/agriculture14122353

Academic Editor: Guisheng Zhou

Received: 2 December 2024
Revised: 17 December 2024
Accepted: 19 December 2024
Published: 21 December 2024

1. Introduction

Soil salinization is a huge issue affecting a wide range of environments around the world; it affects 833 million hectares globally, including both saline and sodic soils [1], and is getting worse over time, with a rate of 1–2 million hectares damaged every year [2]. Soil salinization can occur due to natural processes (primary salinization) that induce saltwater intrusion that is responsible for coastal forest loss, species invasions, and eutrophication phenomena [3], or it can be induced by human activities (secondary salinization), mainly with agricultural irrigations performed with saline waters. This type of irrigation, associated with poor drainage, inevitably leads to a lack of water availability in the soil and an imbalance between its biochemical and physiological characteristics, which heavily influences crop physiology and production [4,5]. The consequences related to these two types of salinization can be easily seen in European areas, where the lack of precipitation and irrigations made with low-quality waters caused the damage of hectares of soils [6]; today, 3.3% of global saline and sodic soils is represented by European land [1]. Over the

years, some strategies have been adopted to mitigate the salinization of soils traditionally cultivated with food crops [6], for example, using an excess of water during irrigation to drain the soil and wash away excess salt from the root zone [7], or by using drips and micro-sprinklers that, using low volumes of water, are capable of minimizing salt consequences on plant roots and to neutralize salt leaching towards the layers of soil below the root zone [8]. As salinization is spreading, causing soil degradation and consequently land abandoning, it appears necessary to reflect on alternative strategies to cover abandoned saline soils, trying to manage them with low inputs of water. Cultivating rustic species able to survive also on saline soils and that require low water inputs appears to be an effective way to protect land.

Among all the rustic species that represent the Mediterranean landscape, we find *Iris pallida*, belonging to the Iridaceae family, which for decades has been cultivated in the hilly and mountain areas of Italy and France and that has recently spread in Morocco and China as well [9,10]. This species is commonly known both for its landscape value and for its characteristic violet-like essence that is used largely in the perfumery sector and is extracted via the hydrodistillation of rhizomes previously broken and dried [11,12]. Orris essence is composed almost entirely of irones, responsible for its odorous characteristics, which are ketone compounds generated inside rhizomes due to oxidative phenomena, concerning iripallidal and iriflorental [13], that are activated in rhizomes stored for at least 2–3 years [13,14]; for this reason, the essence matures over a long time and its cost at point of sale is particularly high. The application of rustic plants tolerant to salinity with a high economic value was already considered to cover saline soils not suitable for crops traditionally cultivated for human and livestock nutrition [15–17]; even if *I. pallida* is traditionally considered a rustic plant, no information is available on its tolerance to salt stress, even if some studies conducted on other Iris species showed a reduction in biomass in *I. hexagona* plants subjected to salt [18] or the ability to maintain osmotic adjustments and biomass growth in *I. lactea* [19]. Moreover, Zhao et al. [20] demonstrated the salt tolerance of some cultivars of *I. germanica*.

Another solution to mitigate saline effects that has been widely investigated recently is the application of small compounds with biostimulating effects, such as proline, glycine betaine, glutathione, and melatonin [21]. Biostimulants play a crucial role in sustaining plant growth under stress conditions and can help eco-friendly crop production as substances of natural origin with subsequent low environmental impacts [22]. In particular, in this paper, we focused on the possible application of melatonin, being interested not only in its potential role in ameliorating the salinity tolerance of *Iris pallida*, but also on its effect as a growth regulator in the tissue culture micropropagation phase [23]. Melatonin (N-acetyl-5-methoxytryptamine), a derivative of tryptophan, is an indolic compound initially found in animals [24] and then in plants in 1995 [25]; it is biosynthesized inside plants chloroplasts and mitochondria [26] and is involved in important biological processes linked to growth [27], seed germination, photosynthetic activity, fruit maturation, water use efficiency, and ROS and RNS scavenging [28,29]. Moreover, it is involved in the regulation of carbohydrates, lipids, nitrogen, and sulphur compound pathways, and it works as a signalling molecule, enhancing antioxidant enzyme activity and inducing the mechanism of stress response in plants [30,31]. In this way, melatonin can contribute to mitigating the major abiotic stresses to which plants are subjected, such as drought stress [32–34], waterlogging stress [31,35], cold stress [36], heat stress [23,37], heavy metal toxicity [38], light stress [39,40], and salt stress. It has been reported that several crops, such as some cereals and horticultural species, show tolerance to salt stress thanks to endogenous melatonin [41–43]. Furthermore, treatments with exogenous melatonin showed to be efficient in mitigating salt stress, thanks to the accumulation of endogenous melatonin induced by exogenous melatonin [44–48]. The main positive effects of exogenous melatonin on salinity stress can be related to the improvement of antioxidant enzyme activity and antioxidant content [44,49], the strengthening of the root system and the reduction in Na^+ and Cl^- content in roots [45], the improvement of photosynthetic capacity [46,47], and ion

homeostasis [48]. The effects of exogenous melatonin were also tested in vitro, both on cell suspensions and plantlets [50]. On the basis of the aforementioned studies on melatonin application, we hypothesized a positive effect of this growth regulator on physiological and metabolic processes that influence in vitro plantlet acclimatization and subsequent plant growth in an unfavourable environment, such as saline soils. The aim of our research is therefore to test the capacity of *Iris pallida* to tolerate salinity during the acclimatization phase of micropropagated plants, helping plants with an exogenous application of melatonin, which has never been tested before on plants belonging to the Iris species. During the experiment, plant growth, leaf water content, and pigment modulation were monitored as markers of the physiological response to saline administration.

2. Materials and Methods

2.1. In Vitro Melatonin Treatment

The plantlets used for the experiment were obtained by somatic embryogenesis performed on flower buds belonging to *I. pallida* plants grown in the experimental fields of the Department of Agricultural, Food and Agro-Environmental Sciences of the University of Pisa (43.7121439043662, 10.413284264300806), following the method of Meucci et al. [51]. In vitro regenerated plantlets were multiplied by cultivation in Magenta® containers (Merck Life Science S.r.l., Milan, Italy), with 4 plantlets per container, with Murashige and Skoog macro and micro salts [52] supplemented with Gamborg B5 vitamins [53], 500 mg L^{-1} of 2-(N-morpholino) ethane sulfonic acid (MES), 300 mg L^{-1} of reduced glutathione (GSH), 3.0 g L^{-1} of Gelrite® (Merck Life Science S.r.l., Milan, Italy) (basal medium), and 30 g L^{-1} of sucrose; the pH was adjusted to 5.9 with 1N potassium hydroxide (KOH). The control medium was therefore complemented with 0.1 mg L^{-1} of 6-benzyladenine (BA), and 0.1 mg L^{-1} of α-naphthaleneacetic acid (NAA), while the media of the treated plantlets were supplemented with two different doses of melatonin (5 and 50 µM) without other growth regulators, chosen according to the literature [54]. Melatonin was added to culture media after their autoclavation (120 °C for 20 min). At the beginning of the experiment, all plantlets were deprived of roots and old leaves and transferred to melatonin medium and control medium for three weeks. The plantlets were then removed from the media, the weighted and number of roots and new leaves produced, the length of the roots and the height of the leaves were measured. Thereafter, the plantlets were transferred to new melatonin medium and control medium and subjected to a pre-acclimatization phase consisting of using ventilated caps for three weeks.

Subsequently, the plantlets underwent the transition from in vitro to in vivo conditions; they were transplanted into plastic vessels with perlite and placed in a climatic chamber with sodium lamps (400 W), at 22 °C and a 16 h photoperiod (400 µmol $m^{-2}s^{-1}$); they were then put in a plastic bag and administered with a sprayed nutrient solution containing Murashige and Skoog macro and micro salts [52] and Gamborg B5 [53] vitamins, and gradually became accustomed to the environmental conditions of the growth chamber by shedding the plastic film every day for an increasing number of hours until it was completely removed. After one month, the plants were transplanted into a mixture of peat and perlite (1:1 ratio) with the following characteristics: E.C. 20.8 mS cm^{-1}, Ca^{2+} 4.9 g kg^{-1}, Mg^{2+} 1.04 g kg^{-1}, K^{+} 4.5 g kg^{-1}, and Na^{+} 1.7 g kg^{-1}. They were also watered with tap water (E.C. 1024 µS cm^{-1}) every five days inside the growth chamber.

2.2. In Vivo Melatonin and Salt Treatment

In this second step of the experiment, the plants were subjected to increasing doses of salt (150, 300 and 400 mM) while the control plants were administered only with distilled water; each salt treatment lasted 14 days and the plants were sprayed with a melatonin solution 24 h before each salt addition. Plants subjected to 5 µM of melatonin during the in vitro cultivation were sprayed with a solution containing 50 µM melatonin, and those subjected to 50 µM in vitro were sprayed with 100 µM of melatonin. The melatonin dose was chosen within the range of doses used in other works to face salinity stress [42,43]. In the meantime,

the control plantlets were sprayed with distilled water, separating them at each sprayed treatment to avoid contamination. At the end of the experiment, analyses were conducted to assess the levels of macro-elements, sodium content, and electrical conductivity (E.C.) of the substrates used, providing insights into their salinization condition.

2.3. Growth and Biochemical Determinations

At the end of the experiment, biometric measurements including the quantification of root and leaf numbers and lengths, as well as the determination of roots, rhizomes, and leaf dry weights, were performed. Additionally, analyses were conducted to measure chlorophylls, carotenoids, macro-elements content, and relative water content (RWC). RWC was also monitored at the end of each salt treatment and was conducted on leaf discs with a 10 mm diameter, collected using a cork borer and weighted to determine their fresh weight (FW); then, discs were soaked in Petri® dishes with 2 mL of distilled water for 24 h to obtain their turgid weight (TW). Therefore, the discs were put in the oven at 50 °C to determine their dry weight (DW). The following formula was used to calculate the RWC:

$$\mathrm{RWC} = (\mathrm{FW} - \mathrm{DW}) / (\mathrm{TW} - \mathrm{DW})$$

Chlorophyll and carotenoid content was performed starting from 20 mg of leaf tissue for each replicate (6 replicates for each treatment), which were extracted and placed in 1.5 mL of methanol (100% (*v/v*)) for 24 h at 4 °C in darkness. Absorbance was read at λ = 665.2 nm and λ = 652.4 nm for chlorophylls a and b, respectively, and at λ = 470 nm for carotenoids. Pigment levels were calculated using Lichtenthaler's formula [55] and expressed as tissue fresh weight.

Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} content was investigated on leaves, rhizomes, and roots via microwave digestion. The tissues were oven dried at 50 °C for 10 days, finely minced, weighted, and pre-digested overnight in closed vessels with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The samples in vessels were then treated with two cycles of microwave digestion (Milestone Ethos 900, Bergamo, Italy) at 250 Watt for 10 min, followed by 5 min of ventilation. Then, the samples were filtered using a Whatman® paper filter and diluted with milli-Q water. Ca^{2+}, Mg^{2+}, and K^{+} content was finally analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES 5900, Agilent, Santa Clara, CA, USA) with the following wavelengths: 766.790 nm (K^{+}), 315.887 nm (Ca^{2+}), and 279.079 nm (Mg^{2+}), while Na^{+} determination was performed by fast-sequential atomic absorption spectrometer (AA240 FS, Agilent Technologies, Santa Clara, CA, USA), reading samples at λ = 589 nm.

2.4. Statistical Analysis

Student's t-test or analysis of variance (ANOVA) was performed at a $p \leq 0.05$ significance level. The mean values were separated using the Tukey post-test ($p < 0.05$). Statistical analysis was carried out with GraphPad Prism (version 10.00 for Windows, GraphPad Software, La Jolla, San Diego, CA, USA).

3. Results

The application of melatonin as a growth regulator during the final phase of in vitro culture multiplication of *I. pallida* did not compromise the ultimate survival of plantlets during acclimatization when used at a low dosage (100% survival). However, at higher concentrations, it slightly reduced survival rates (62.5%), albeit without yielding any significant differences compared to the control medium containing IBA and BA (70% survival). The in vitro treatment improved the quality of root apparatus, increasing root length (Figure 1); this trend was already evident at the end of the multiplication phase (T0) and remained so during the in vitro pre-acclimatization phase (T0-T1) and the first phase of acclimatization in perlite (T1-T2). At the end of the acclimatization phase (TF), no differences can be appreciated in plants treated with melatonin in comparison with control plants grown in a culture medium with NAA and BA (Ctr medium).

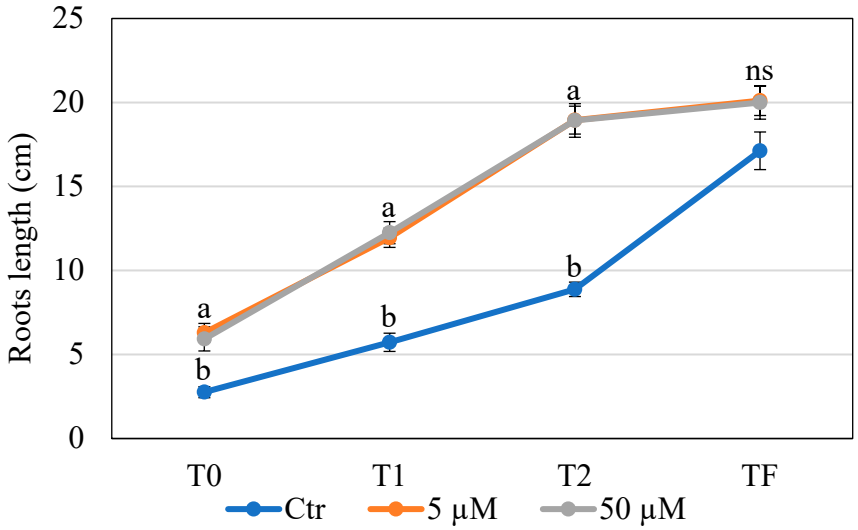

Figure 1. Root development (cm) monitored during the different steps of the treatment: plants transferring to Magenta® vessels with ventilated caps (T0), transferring to perlite (T1), to peat and perlite (T2), and their length at the end of the acclimatization phase (TF). Data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$) and ns indicates no significance among the treatments.

Melatonin also slightly influenced leaf length at the end of plant cultivation on perlite (T2), which resulted higher than control plantlets (Figure 2). After the salt treatment of acclimatized plants a significant reduction in n° of roots was observed in all treatments containing salt (Figure 3). Conversely, a marginal effect of 50 µM melatonin was observed in the number of leaves of salt-treated plants, although this effect was not statistically significant.

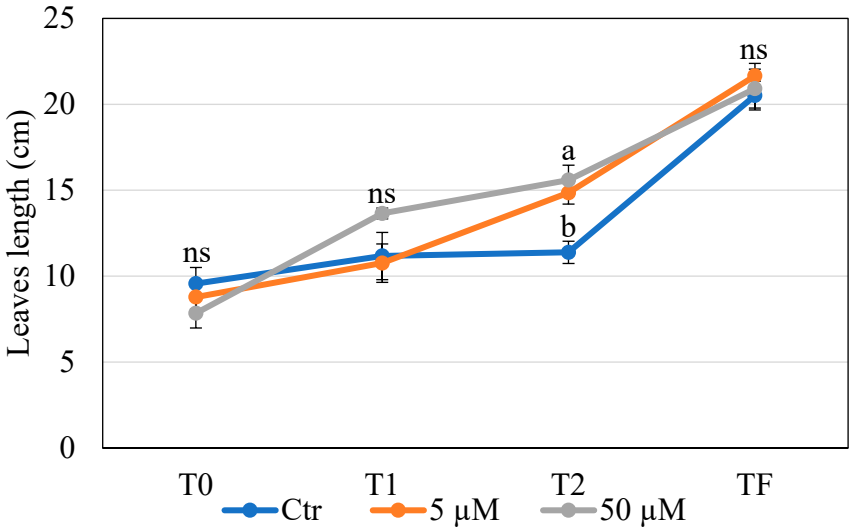

Figure 2. Leaf development (cm) monitored during the different steps of the treatment: plants transferred to containers with ventilated caps (T0), transferred to perlite (T1), and transferred to peat and perlite (T2), and their length at the end of the acclimatization phase (TF). The data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$) and ns indicates no significance among the treatments.

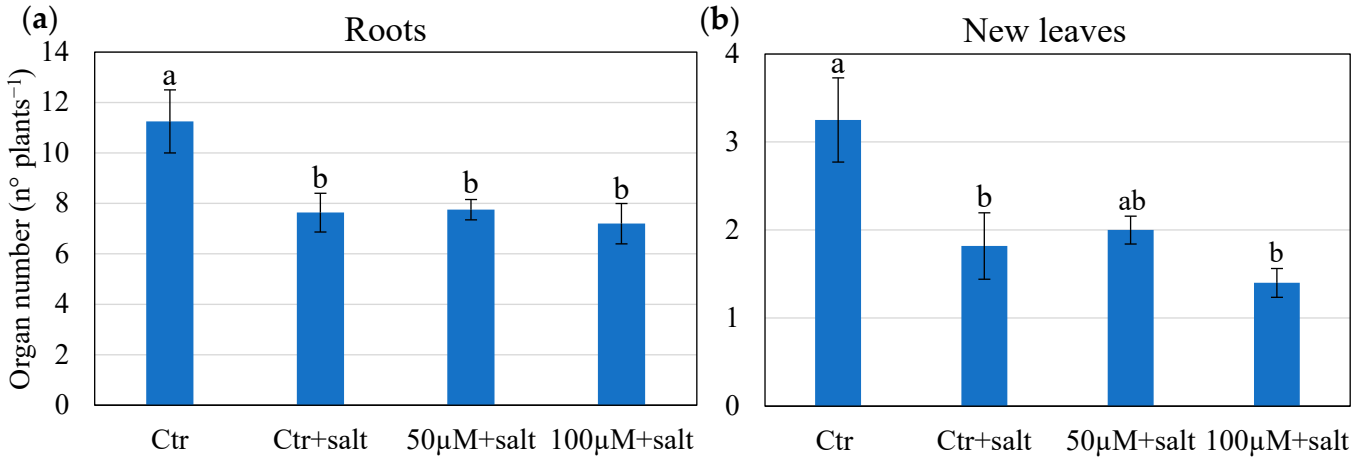

Figure 3. Number of roots (**a**) and new leaves (**b**) of *I. pallida* plants treated with 400 mM of salt (Ctr + salt) and with both melatonin and salt and (50–100 µM + salt) compared to control plants (Ctr). Data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), and the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$).

The increasing doses of salt treatment notably impacted rhizome and leaf dry weight. Additionally, a modest mitigating effect of melatonin on salt stress was observed in the rhizome dry weights of plants treated with both melatonin doses (Figure 4).

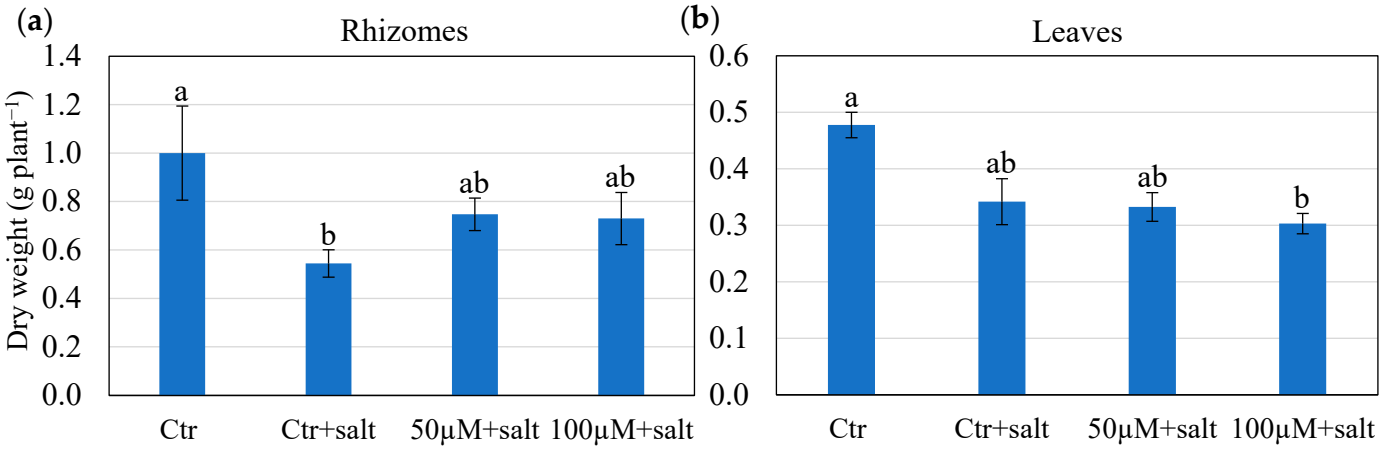

Figure 4. Dry weight (g $plant_{dw}$ $^{-1}$) of rhizomes (**a**) and leaves (**b**) of *I. pallida* plants treated with 400 mM of salt (Ctr + salt) and with both melatonin and salt (50–100 µM + salt) compared to control plants (Ctr). The data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), and the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$).

Concerning the relative water content (RWC), at the end of the experiment, plants treated with up to 400 mM showed a RWC reduction; in this case, melatonin sprayed on plants seemed to significantly help them maintain their RWC at the same level as that of control plants (Figure 5).

The findings (Figure 6) suggest that salt treatment increases the total chlorophyll and carotenoid contents in leaves. However, when plants are sprayed with 50 µM of melatonin, they exhibit the same pigment content as control plants, indicating that melatonin at this concentration does not significantly affect pigment levels. On the other hand, when plants are treated with 100 µM melatonin, there is a reduction in chlorophyll and carotenoid levels in the leaf tissues of the treated plants. This suggests that higher concentrations of melatonin might have an inhibitory effect on pigment production or retention in plants under salt stress (Figure 6).

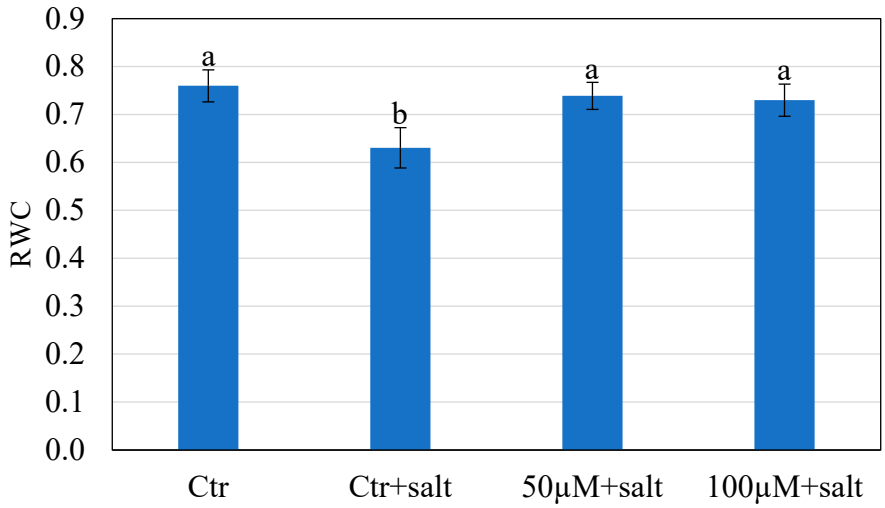

Figure 5. Relative water content (RWC) of leaves of *I. pallida* plants treated with 400 mM of salt (Ctr + salt) and with both melatonin and salt (50–100 µM + salt) compared to control plants (Ctr). The data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), and the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$).

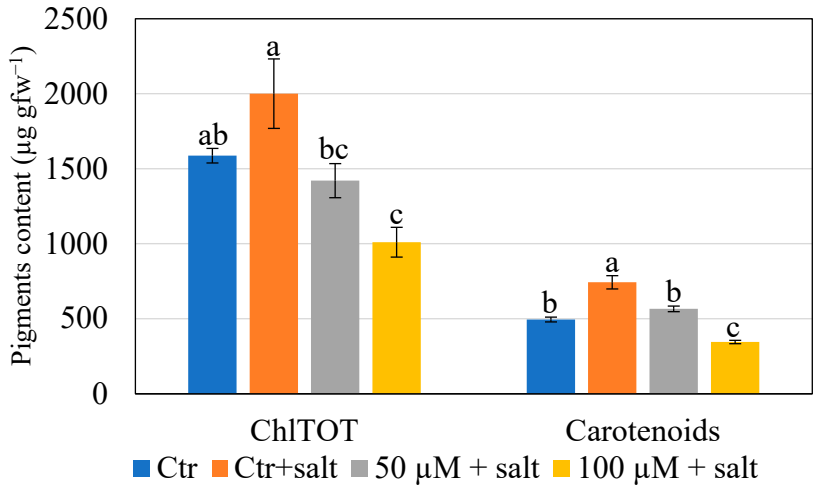

Figure 6. Total chlorophyll (ChlTOT) and carotenoid content (µg $g_{fw}{}^{-1}$) in leaves of *I. pallida* plants treated with 400 mM of salt (Ctr + salt) and with both melatonin and salt (50–100 µM + salt) compared to control plants (Ctr). The data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), and the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$).

The E.C. values increased significantly in the salt-treated substrates contained in pots used for *I. pallida* cultivation if compared to control ones, with measurements of 20 mS cm^{-1} for the control and 120 mS cm^{-1} for the salt-treated substrates. Additionally, the sodium content also increased in the salt-treated substrates, although specific data are not provided in this excerpt. Figure 7 likely presents data on the mineral content of the roots, leaves, and rhizomes of the plants in response to substrate modifications. These data could provide insights into how the salt treatments influenced the uptake and distribution of minerals within different parts of the plants. Salt treatment and melatonin did not affect K^+ content in roots, rhizomes, or leaves; Ca^{2+} and Mg^{2+} content did not change in roots and rhizomes, but decreased in the leaves of plants treated with salt. The presence of melatonin, particularly with 100 µM via spray administration, seemed to mitigate Na^+ accumulation, which occurred in plants treated with NaCl, both in rhizomes and leaves. (Figure 7).

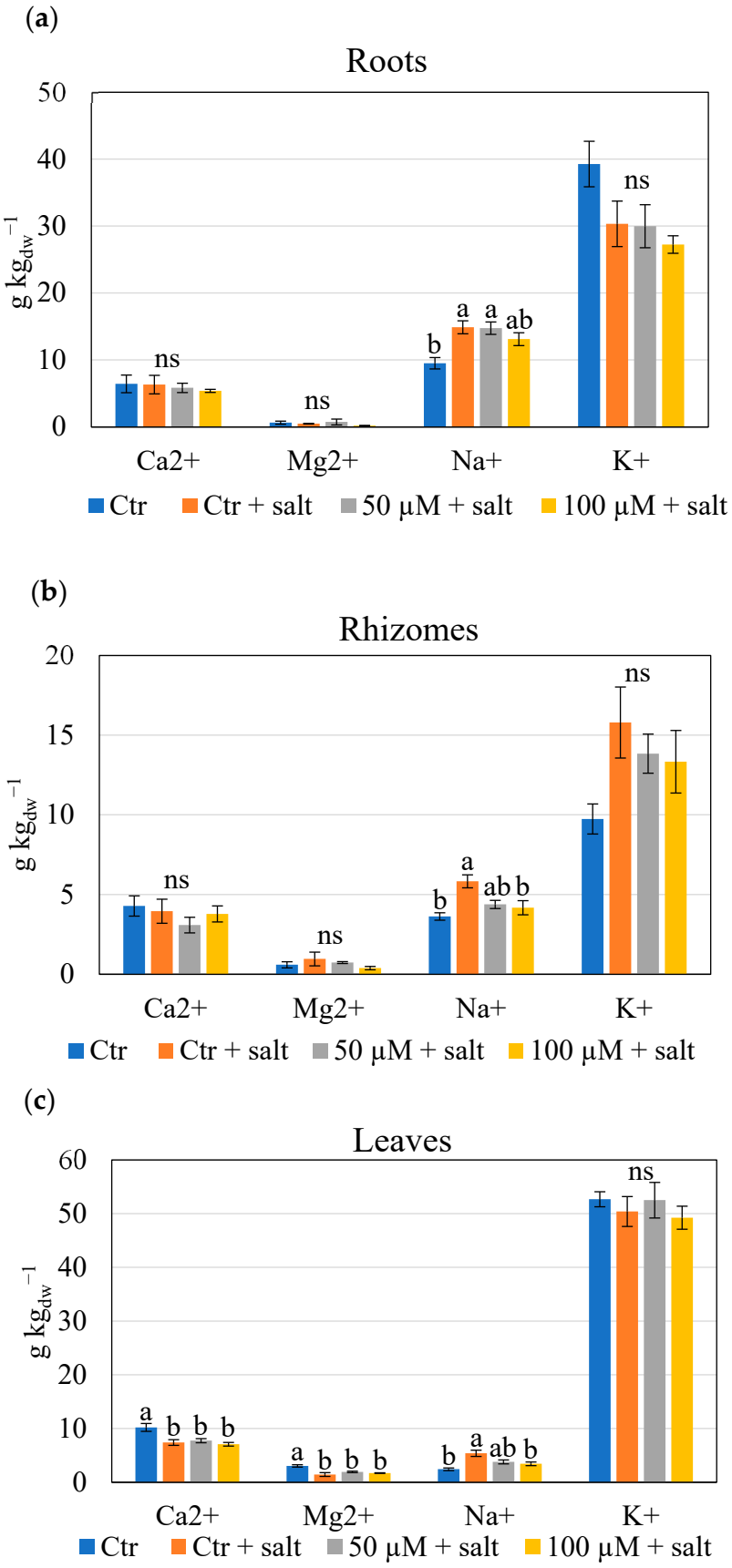

Figure 7. Macro-elements content (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} g kg_{dw}^{-1}) in roots (**a**), rhizomes (**b**), and leaves (**c**) of *I. pallida* plants treated only with 400 mM of salt and with both melatonin and salt

(50–100 µM + salt) compared to control. The data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$) and ns indicates no significance among the treatments.

Sodium accumulation resulted in changes in the potassium-to-sodium ratio, both in the roots and leaves of plants treated only with salt and sprayed with melatonin, as reported in Table 1. This suggests that the salt treatment, also in combination with melatonin application, influenced the balance between potassium and sodium in these plant parts. However, no significant differences were observed in the rhizome, indicating that the effects were more pronounced in the roots and leaves. This alteration in the potassium-to-sodium ratio could have implications for various physiological processes within the plant, such as osmotic regulation, ion balance, and stress responses.

Table 1. Potassium sodium ratio (K^+/Na^+) of roots, rhizomes, and leaves of *I. pallida* plantlets. The data, reported as mean values ± S.E., were subjected to analysis of variance (ANOVA), the different letters indicate significant differences among means (Tukey post-test, $p \leq 0.05$) and ns indicates no significance among the treatments.

K^+/Na^+	Roots	Rhizomes	Leaves
Ctr	4.13 ± 0.7 a	2.74 ± 0.37 ns	21.74 ± 1.74 a
Ctr + salt	2.03 ± 0.34 b	2.76 ± 0.46 ns	9.37 ± 0.61 b
50 µM + salt	2.03 ± 0.16 b	3.19 ± 0.35 ns	13.89 ± 1.36 b
100 µM + salt	2.13 ± 0.26 b	3.15 ± 0.14 ns	14.31 ± 1.3 b

4. Discussion

In the last decade, melatonin was employed in different culture systems in relation to its role as a plant growth regulator. In the literature, many examples can be found on the positive action of melatonin on cells and callus cultures [54]. Its role on the production of secondary metabolites in vitro [56] and on the root organogenesis of in vitro cultured plantlets [57] has already been widely elucidated. Using melatonin as a component of culture media for micropropagation is less studied in comparison to its other roles and applications in plant physiology and growth regulation. However, there is some research interest in exploring its potential in this context, as demonstrated in species like *Malus prunifolia*, which may benefit from improved root development during micropropagation [58]. During the acclimatization phase, plants undergo a transition from controlled laboratory conditions to the external environment, which often involves exposure to various stress factors. This phase is critical for plants to adapt and establish themselves successfully in their new surroundings [59]. The potential role of melatonin in aiding plants during the acclimatization phase, especially concerning its involvement in the response to abiotic stresses, remains unexplored. Melatonin's role in regulating various physiological processes in response to stressors like drought [34] or waterlogging stress [35] suggest that it could indeed have implications for plant acclimatization. Our results indicate that melatonin supplementation in culture media led to an overall improvement in the root apparatus of plants at the end of the in vitro culture period, particularly by increasing root length. This suggests that melatonin may be more effective than other hormones like 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA), which are commonly used in culture media for propagating *I. pallida* [51]. The positive effect of melatonin on root elongation is maintained also during the following steps of the acclimatization, both in plants grown on media with 5 and 50 µM. In *Malus prunifolia*, 1.29 µM melatonin added to a culture medium, where shoots remained for two days before being transferred to a medium without growth regulators, significantly promoted the elongation of roots; this effect was verified to be related to an increase in indole-3-acetic acid (IAA) levels in the tissues [58].

Orris plants are not classified as halophytes, generally defined as plants able to grow in presence of at least 200–500 mM of salt solution; one example of a halophyte is *Salicornia*

europaea [60], considered one of the most tolerant species as it reaches a salt tolerance limit of 1000 mM of NaCl. The resistance of the Iridaceae family to salinity stress was already investigated [61,62]; specifically, Zhao et al. [20] reported severe foliar damage to *I. germanica*, *I. ensataI,* and *I. pseudacorus* plants subjected to salt doses higher than 150 mM; on the contrary, plants belonging to *I. lactea* did not show damage even if subjected to 175 mM of salt. On the basis of this previous evidence, we decided to set the lower salt concentration at 150 mM, increasing salt concentration up to the doses considered the thresholds for tolerant (200 mM) and halophyte (400 mM) plants. In our study, no visual damage was detected on plants subjected up to 400 mM of salt, significantly above the threshold of 200 mM indicated by Flowers et al. [63] for the definition of tolerant plants, proving that *I. pallida* species has a higher salinity tolerance if compared to the other ornamental Iris species cited above that showed behaviour analogous to that of typical halophytes [64]. However, an evident reduction in rhizome growth was observed in plants treated with salt if compared to control plants, as observed in *Curcuma longa* rhizomes [65]; moreover, no significant differences were detected on the growth of the aerial part of the plant, despite a reduction in the relative water content (RWC) of leaves, that is one of the most efficient markers of plants water balance, usually impaired by salinity stress.

Also, no damage was observed in chlorophyll and carotenoid content, which resulted in higher levels if compared to control plant levels. This behaviour was also observed by Wang and Nil [66], who have reported that chlorophyll content increases under conditions of salinity in *Amaranthus* sp., while a chlorophyll and carotenoid decrease was usually detected in most of the plants treated with NaCl [67,68]. The high carotenoid content in leaves treated with salt may act as a protective mechanism against oxidative damage caused by salt, helping also to maintain chlorophyll synthesis [16]. Melatonin treatment performed on plants under salinity stress allowed for the maintenance of pigment concentration at the same level as that of the control, confirming its role in protecting plant photosynthesis apparatus [46]. However, we could also observe a melatonin dose effect; in fact, at high doses of administration (100 μM), it decreases pigment concentration, as observed in other studies [69]. However, we cannot suggest a toxicity effect because in other species the same dosage (100 μM) significantly improved photosynthetic pigment concentration under salt stress [43].

The acclimatized *I. pallida* plants treated with increasing concentrations of salt exhibited significant accumulation of Na^+ ions in all the analyzed organs, as observed in other Iris species [20], while K^+ values showed no changes in content; the ability to maintain a constant K^+ content or even increase it in the presence of salinity stress is a marker of salt tolerance [70]. This evidence suggests that iris plantlets can survive not because they are able to restrict Na^+ uptake but, rather, because of their ability to withstand Na^+ in their tissues [71]. Na^+ and K^+ homeostasis is critical for salt tolerance, and salt sensitivity was associated with the inability to maintain an optimal cellular K^+/Na^+ homeostasis. Maintaining the proper cytosolic K^+/Na^+ ratio is difficult because it is kept under control by K^+-release channels such as KORs and by Na^+ uptake [72]. In *Iris pallida*, high salt concentration reduced the K^+ and Na^+ ratio in roots and leaves, but not in the rhizome, demonstrating different mechanisms contributing to salt tolerance in the two organs [20]. When plants are sprayed with melatonin, mostly at 100 μM of administration, sodium accumulation is maintained at the control level, even if is not sufficient at maintaining the K^+/Na^+ ratios of both roots and leaves. Further investigations are needed to deepen our knowledge on the ion transport cellular mechanisms responsible for *Iris pallida* resistance to salinity stress.

It is reported in the literature that the effects of salt on plants treated with melatonin are the following: the enhancement of photosynthetic efficiency, the modification of ion fluxes, the reduction in ROS production, the improvement of antioxidant activity, and the accumulation of compatible solutes [28]. On the basis of our results, the main mechanisms with which melatonin copes with salt stress in *Iris pallida* seem to be related to a reduction in Na^+ content in all the organs that avoids unbalancing ion levels [73] and the supply

of chlorophylls and carotenoids, which is kept stable also in the presence of salt, due to melatonin's capacity to maintain normal chloroplast structures, inhibiting chlorophyll degradation [74].

5. Conclusions

In summary, melatonin presents itself as a promising addition to media used in in vitro production, aiding in the preparation of plantlets for the acclimatization phase by enhancing root development. Additionally, its application via spraying has shown potential in helping plants mitigate the detrimental effects of salt stress by reducing sodium uptake and maintaining leaf hydration levels, but also enhancing plant resilience and productivity. Melatonin could be a feasible and cost-effective choice for its large scale application if compared to other biostimulants currently used (glutathione, proline, glycine, betaine) thanks to the low dosage required (50 μM), the simplicity of the administration method, and its non-toxicity to humans and the environment. Further studies are necessary to better elucidate the optimal dose for its application in fields and to monitor if its efficacy varies with different soil types or climatic conditions.

This study sheds a light on the impact of salinity on *Iris pallida*, showing its ability to cope with saline concentrations up to 400 mM and suggesting the possibility of cultivating this species on saline soils, or of using saline irrigation water in field conditions.

However, the translation of these results into real-world agricultural practises requires further investigation. Long-term field trials are desirable for determining the practical feasibility of melatonin application, its cost–benefit ratio, and its overall impact on crop yield and soil health. Such studies could provide concrete recommendations for sustainable saline soil management, particularly in areas affected by soil degradation, salinity, or erosion.

By bridging the gap between experimental findings and practical implementation, this research lays the foundation for innovative agricultural practises that can revitalize abandoned or marginal lands introducing rustic species such as *I.pallida* while contributing to more resilient and sustainable crop production systems.

Author Contributions: Conceptualization and methodology, A.M. (Anna Mensuali) and A.M. (Annalisa Meucci); formal analysis and investigation, A.M. (Annalisa Meucci), I.R., R.M. and C.G.; data curation, A.M. (Annalisa Meucci); writing—review and editing, A.M. (Anna Mensuali), A.M. (Annalisa Meucci), B.P. and F.M.; supervision, A.M. (Anna Mensuali); funding acquisition, A.M. (Anna Mensuali). All authors have read and agreed to the published version of the manuscript.

Funding: This study was carried out within the Agritech National Research Center and received funding from European Union Next-GenerationEU (PNRR) AGRITECH, PNRR M4C2, id CN00000022 (CUP J53C22001610007). This manuscript reflects only the authors' views and opinions, and neither the European Union nor the European Commission can be considered responsible for them.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: Our thanks go to Giorgiana Chietera and the LMR-International Flavors and Fragrances Industry (LMR-IFF), based in Grasse (France), for providing part of the plant material.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO. The World Map of Salt Affected Soil [WWW Document]. FOOD Agric. Organ UNITED NATIONS. 2021. Available online: https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/global-map-of-salt-affected-soils/en/ (accessed on 18 December 2024).
2. Hopmans, J.W.; Qureshi, A.S.; Kisekka, I.; Munns, R.; Grattan, S.R.; Rengasamy, P.; Ben-Gal, A.; Assouline, S.; Javaux, M.; Minhas, P.S. Critical knowledge gaps and research priorities in global soil salinity. *Adv. Agron.* **2021**, *169*, 191.
3. Tully, K.L.; Weissman, D.; Wyner, W.J.; Miller, J.; Jordan, T. Soils in transition: Saltwater intrusion alters soil chemistry in agricultural fields. *Biogeochemistry* **2019**, *142*, 339–356. [CrossRef]

4. Smith, P.; Cotrufo, M.F.; Rumpel, C.; Paustian, K.; Kuikman, P.J.; Elliott, J.A.; McDowell, R.; Griffiths, R.I.; Asakawa, S.; Bustamante, M.; et al. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. *Soil* **2015**, *1*, 665–685. [CrossRef]
5. Decock, C.; Lee, J.; Necpalova, M.; Pereira, E.I.P.; Tendall, D.M.; Six, J. Mitigating N_2O emissions from soil: From patching leaks to transformative action. *Soil* **2015**, *1*, 687–694. [CrossRef]
6. Panagea, I.S.; Daliakopoulos, I.N.; Tsanis, I.K.; Schwilch, G. The application of three promising technologies for soil salinity amelioration in Timpaki (Crete): A participatory approach. *Solid Earth* **2016**, *7*, 177–190. [CrossRef]
7. Andreu, L.; Moreno, F.; Jarvis, N.J.; Vachaud, G. Application of the model MACRO to water movement and salt leaching in drained and irrigated marsh soils, Marismas, Spain. *Agric. Water Manag.* **1994**, *25*, 71–88. [CrossRef]
8. Taylor, R.; Zilberman, D. Diffusion of drip irrigation: The case of California. *Appl. Econ. Perspect. Policy* **2017**, *39*, 16–40. [CrossRef]
9. Ieri, F.; Vignolini, P.; Urciuoli, S.; Pinelli, P.; Romani, A. The Cultivation of *Iris pallida* as an Opportunity for the Enhancement of Tuscan Agro-Biodiversity and a Resource for the Local Economy. In *Innovation, Quality and Sustainability for a Resilient Circular Economy, AISME, Circular Economy and Sustainability*; Lagioia, G., Paiano, A., Amicarelli, V., Gallucci, T., Ingrao, C., Eds.; Springer: Berlin/Heidelberg, Germany, 2022.
10. Belletti, G.; Fani, E.; Marescotti, A.; Scaramuzzi, S. The role of traditional products in the valorisation of marginal rural areas: The case of *Iris pallida*. *Span. J. Rural. Dev.* **2013**, *4*, 11–22. [CrossRef]
11. Başer, K.H.; Demirci, B.; Orhan, I.E.; Kartal, M.; Sekeroglu, N.; Sener, B. Composition of volatiles from three Iris species of Turkey. *J. Essent. Oil Res.* **2011**, *23*, 66–71. [CrossRef]
12. Roger, B.; Jeannot, V.; Fernandez, X.; Cerantola, S.; Chahboun, J. Characterisation and quantification of flavonoids in *Iris germanica* L. and *Iris pallida* Lam. resinoids from Morocco. *Phytochem. Anal.* **2012**, *23*, 450–455. [CrossRef] [PubMed]
13. Krick, W.; Marner, F.J.; Jaenicke, L. Isolation and structure determination of the precursors of α and β-irones and homologous compounds from *Iris pallida*. *Z. Naturforschung* **1983**, *38*, 179–184. [CrossRef]
14. Bicchi, C.; Rubiolo, P. Analysis of constituents of Iris rhizomes. Part I: High performance liquid chromatographic-particle beammass spectral analysis of iridals from *Iris pallida* rhizomes. *Phytochem. Anal.* **1993**, *4*, 171–177.
15. Long, X.H.; Chi, J.H.; Liu, L.; Li, Q.; Liu, Z.P. Effect of seawater stress on physiological and biochemical responses of five Jerusalem artichoke ecotypes. *Pedosphere* **2009**, *19*, 208–216. [CrossRef]
16. Li, Z.; Li, G.; Qin, P. The prediction of ecological potential for developing salt-tolerant oil plants on coastal saline land in Sheyang Saltern, China. *Ecol. Eng.* **2010**, *36*, 27–35. [CrossRef]
17. Wu, X.H.; Zhang, H.S.; Li, G.; Liu, X.C.; Qin, P. Ameliorative effect of castor bean (*Ricinus communis* L.) planting on physico-chemical and biological properties of seashore saline soil. *Ecol. Eng.* **2012**, *38*, 97–100. [CrossRef]
18. Van Zandt, P.A.; Tobler, M.A.; Mouton, E.; Hasenstein, K.H.; Mopper, S. Positive and negative consequences of salinity stress for the growth and reproduction of the clonal plant, *Iris hexagona*. *J. Ecol.* **2003**, *91*, 837–846. [CrossRef]
19. Bai, W.B.; Li, P.F.; Li, B.G.; Fujiyama, H.; Fan, F.C. Some physiological responses of Chinese iris to salt stress. *Pedosphere* **2008**, *18*, 454–463. [CrossRef]
20. Zhao, Z.; Li, T.; Cheng, Y.; Wang, F.; Zhao, X. Morphological and metabolic responses of four Iris germanica cultivars under salinity stress. *Sci. Hortic.* **2021**, *281*, 109960. [CrossRef]
21. Khalid, F.; Rasheed, Y.; Asif, K.; Ashraf, H.; Maqsood, M.F.; Shahbaz, M.; Zulfiqar, U.; Sardar, R.; Haider, F.U. Plant Biostimulants: Mechanisms and Applications for Enhancing Plant Resilience to Abiotic Stresses. *J. Soil Sci. Plant Nutr.* **2024**. [CrossRef]
22. Nawaz, K.; Chaudhary, R.; Sarwar, A.; Ahmad, B.; Gul, A.; Hano, C.; Abbasi, B.H.; Anjum, S. Melatonin as master regulator in plant growth, development and stress alleviator for sustainable agricultural production: Current status and future Perspectives. *Sustainability* **2020**, *13*, 294. [CrossRef]
23. Iqbal, N.; Fatma, M.; Gautam, H.; Umar, S.; Sofo, A.; D'ippolito, I.; Khan, N.A. The crosstalk of melatonin and hydrogen sulfide determines photosynthetic performance by regulation of carbohydrate metabolism in wheat under heat stress. *Plants* **2021**, *10*, 1778. [CrossRef]
24. Lerner, A.B.; Case, J.D.; Takahashi, Y.; Lee, T.H.; Mori, W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Am. Chem. Soc.* **1958**, *80*, 2587. [CrossRef]
25. Dubbels, R.; Reiter, R.J.; Klenke, E.; Goebel, A.; Schnakenberg, E.; Ehlers, C.; Schiwara, H.W.; Schloot, W. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J. Pineal Res.* **1995**, *18*, 28–31. [CrossRef] [PubMed]
26. Tan, D.X.; Reiter, R.J. An evolutionary view of melatonin synthesis and metabolism related to its biological functions in plants. *J. Exp. Bot.* **2020**, *71*, 4677–4689. [CrossRef] [PubMed]
27. Ribeiro, J.E.D.S.; Silva, A.G.D.; Coêlho, E.D.S.; Oliveira, P.H.D.A.; Silva, E.F.D.; Oliveira, A.K.D.; Santos, G.L.D.; Lima, J.V.L.; Silva, T.I.D.; Silveira, L.M.D. Melatonin mitigates salt stress effects on the growth and production aspects of radish. *Rev. Bras. Eng. Agrícola Ambient.* **2024**, *28*, e279006. [CrossRef]
28. Hassan, M.U.; Mahmood, A.; Awan, M.I.; Maqbool, R.; Aamer, M.; Alhaithloul, H.A.S.; Huang, G.; Skalicky, M.; Brestic, M.; Pandey, S.; et al. Melatonin-Induced Protection Against Plant Abiotic Stress: Mechanisms and Prospects. *Front. Plant Sci.* **2022**, *13*, 902694. [CrossRef] [PubMed]
29. Pardo-Hernández, M.; López-Delacalle, M.; Rivero, R.M. ROS and NO regulation by melatonin under abiotic stress in plants. *Antioxidants* **2020**, *9*, 1078. [CrossRef]

30. Arnao, M.B.; Hernández-Ruiz, J. Melatonin as a plant biostimulant in crops and during post-harvest: A new approach is needed. *J. Sci. Food Agric.* **2021**, *101*, 5297–5304. [CrossRef] [PubMed]
31. Zhang, Q.; Liu, X.; Zhang, Z.; Liu, N.; Li, D.; Hu, L. Melatonin improved waterlogging tolerance in alfalfa (*Medicago sativa*) by reprogramming polyamine and ethylene metabolism. *Front. Plant Sci.* **2019**, *10*, 44. [CrossRef]
32. Shi, H.; Jiang, C.; Ye, T.; Tan, D.-X.; Reiter, R.J.; Zhang, H.; Liu, R.; Chan, Z. Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L). Pers.] by exogenous melatonin. *J. Exp. Bot.* **2015**, *66*, 681–694. [CrossRef] [PubMed]
33. Moustafa-Farag, M.; Mahmoud, A.; Arnao, M.B.; Sheteiwy, M.S.; Dafea, M.; Soltan, M.; Elkelish, A.; Hasanuzzaman, M.; Ai, S. Melatonin-I nduced water stress tolerance in plants: Recent Advances. *Antioxidants* **2020**, *9*, 809. [CrossRef] [PubMed]
34. Hosseini, M.S.; Samsampour, D.; Zahedi, S.M.; Zamanian, K.; Rahman, M.M.; Mostofa, M.G.; Tran, L.S.P. Melatonin alleviates drought impact on growth and essential oil yield of lemon verbena by enhancing antioxidant responses, mineral balance, and abscisic acid content. *Physiol. Plant.* **2021**, *172*, 1363–1375. [CrossRef]
35. Gu, X.; Xue, L.; Lu, L.; Xiao, J.; Song, G.; Xie, M.; Zhang, H. Melatonin enhances the waterlogging tolerance of *Prunus persica* by modulating antioxidant metabolism and anaerobic respiration. *J. Plant Growth Regul.* **2020**, *40*, 2178–2190. [CrossRef]
36. Wang, D.; Chen, Q.; Chen, W.; Guo, Q.; Xia, Y.; Wu, D.; Jing, D.; Liang, G. Melatonin treatment maintains quality and delays lignification in loquat fruit during cold storage. *Sci. Hortic.* **2021**, *284*, 110126. [CrossRef]
37. Wang, X.; Zhang, H.; Xie, Q.; Liu, Y.; Lv, H.; Bai, R.; Ma, R.; Li, X.; Zhang, X.; Guo, Y.-D.; et al. SlSNAT interacts with HSP40, a molecular chaperone, to regulate melatonin biosynthesis and promote thermotolerance in tomato. *Plant Cell Physiol.* **2020**, *61*, 909–921. [CrossRef] [PubMed]
38. Jahan, M.S.; Guo, S.; Baloch, A.R.; Sun, J.; Shu, S.; Wang, Y.; Ahammed, G.J.; Kabir, K.; Roy, R. Melatonin alleviates nickel phytotoxicity by improving photosynthesis, secondary metabolism and oxidative stress tolerance in tomato seedlings. *Ecotoxicol. Environ. Saf.* **2020**, *197*, 110593. [CrossRef]
39. Wei, W.Z.; Li Chao, L.C.; Gao TengTeng, G.T.; Zhang ZhiJun, Z.Z.; Liang BoWen, L.B.; Lv ZuoSen, L.Z.; Ma FengWang, M.F. Melatonin increases the performance of Malus hupehensis after UV-B exposure. *Plant Physiol. Biochem.* **2019**, *139*, 630–641. [CrossRef]
40. Yang, S.J.; Huang, B.; Zhao, Y.Q.; Hu, D.; Chen, T.; Ding, C.B.; Chen, Y.E.; Yuan, S.; Yuan, M. Melatonin enhanced the tolerance of Arabidopsis thaliana to high light through improving anti-oxidative system and photosynthesis. *Front. Plant Sci.* **2021**, *12*, 752584. [CrossRef] [PubMed]
41. Liang, C.; Zheng, G.; Li, W.; Wang, Y.; Hu, B.; Wang, H.; Qian, Y.; Zhu, X.; Tian, D.; Chu, C. Melatonin delays leaf senescence and enhances salt stress tolerance in rice. *J. Pineal Res.* **2015**, *59*, 91–101. [CrossRef]
42. Zhou, X.; Zhao, H.; Cao, K.; Hu, L.; Du, T.; Baluška, F.; Zou, Z. Beneficial roles of melatonin on redox regulation of photosynthetic electron transport and synthesis of D1 protein in tomato seedlings under salt stress. *Front. Plant Sci.* **2016**, *7*, 1823. [CrossRef]
43. Chen, Z.; Gu, Q.; Yu, X.; Huang, L.; Xu, S.; Wang, R.; Shen, W. Hydrogen peroxide acts downstream of melatonin to induce lateral root formation. *Ann. Bot.* **2018**, *121*, 1127–1136. [CrossRef] [PubMed]
44. Yan, F.; Wei, H.; Ding, Y.; Li, W.; Liu, Z.; Chen, L.; Tang, S.; Ding, C.; Jiang, Y.; Li, G. Melatonin regulates antioxidant strategy in response to continuous salt stress in rice seedlings. *Plant Physiol. Biochem.* **2021**, *165*, 239–250. [CrossRef]
45. Li, X.; Yu, B.; Cui, Y.; Yin, Y. Melatonin application confers enhanced salt tolerance by regulating Na^+ and Cl^- accumulation in rice. *Plant Growth Regul.* **2017**, *83*, 441–454. [CrossRef]
46. Shen, J.; Chen, D.; Zhang, X.; Song, L.; Dong, J.; Xu, Q.; Hu, M.; Cheng, Y.; Shen, F.; Wang, W. Mitigation of salt stress response in upland cotton (*Gossypium hirsutum*) by exogenous melatonin. *J. Plant Res.* **2021**, *134*, 857–871. [CrossRef]
47. Yang, H.; Dai, L.; Wei, Y.; Deng, Z.; Li, D. Melatonin enhances salt stress tolerance in rubber tree (*Hevea brasiliensis*) seedlings. *Ind. Crops Prod.* **2019**, *145*, 111990. [CrossRef]
48. Li, C.; Wang, P.; Wei, Z.; Liang, D.; Liu, C.; Yin, L.; Jia, D.; Fu, M.; Ma, F. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *J. Pineal Res.* **2012**, *53*, 298–306. [CrossRef] [PubMed]
49. Khan, T.A.; Saleem, M.; Fariduddin, Q. Recent advances and mechanistic insights on melatonin-mediated salt stress signaling in plants. *Plant Physiol. Biochem.* **2022**, *188*, 97–107. [CrossRef] [PubMed]
50. Iqbal, R.; Khan, T. Application of exogenous melatonin in vitro and in planta: A review of its effects and mechanisms of action. *Biotechnol. Lett.* **2022**, *44*, 933–950. [CrossRef] [PubMed]
51. Meucci, A.; Ghelardi, C.; Chietera, G.; Mensuali, A. Synthetic Seed Production and Slow Growth Storage of In Vitro Cultured Plants of *Iris pallida* Lam. *Horticulturae* **2024**, *10*, 272. [CrossRef]
52. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* **1962**, *15*, 473. [CrossRef]
53. Gamborg, O.L.; Miller, R.; Ojima, K. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* **1968**, *50*, 151–158. [CrossRef] [PubMed]
54. Ullah, M.A.; Tungmunnithum, D.; Garros, L.; Drouet, S.; Hano, C.; Abbasi, B.H. Effect of ultraviolet-C radiation and melatonin stress on biosynthesis of antioxidant and antidiabetic metabolites produced in in vitro callus cultures of *Lepidium sativum* L. *Int. J. Mol. Sci.* **2019**, *20*, 1787. [CrossRef] [PubMed]
55. Lichtenthaler, H.K. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. In *Methods Enzymol*; Academic Press: Cambridge, MA, USA, 1987; Volume 148, pp. 350–382.

56. Khan, T.; Ullah, M.A.; Garros, L.; Hano, C.; Abbasi, B.H. Synergistic effects of melatonin and distinct spectral lights for enhanced production of anti-cancerous compounds in callus cultures of *Fagonia indica*. *J. Photochem. Photobiol. B Biol.* **2019**, *190*, 163–171. [CrossRef]
57. Murch, S.J.; Campbell, S.S.; Saxena, P.K. 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.). *Vitr. Cell Dev. Biol.-Plant* **2021**, *37*, 786–793. [CrossRef]
58. Mao, J.; Niu, C.; Li, K.; Chen, S.; Tahir, M.M.; Han, M.; Zhang, D. Melatonin promotes adventitious root formation in apple by promoting the function of MdWOX11. *BMC Plant Biol.* **2020**, *20*, 536. [CrossRef] [PubMed]
59. Kozai, T. Acclimatization of Micropropagated Plants. In *High-Tech and Micropropagation I Biotechnology in Agriculture and Forestry 17*; Bajaj, Y.P.S., Ed.; Springer: Berlin/Heidelberg, Germany, 1991.
60. Lv, S.; Jiang, P.; Chen, X.; Fan, P.; Wang, X.; Li, Y. Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. *Plant Physiol. Biochem.* **2012**, *51*, 47–52. [CrossRef] [PubMed]
61. Li, L.M.; Wang, R.G. Effects of NaCl stress on physiological and biochemical characteristics of *Belamcanda chinensis* and zirs germanic two ground cover plants. *Chin Agric Sci Bull* **2012**, *28*, 181–184.
62. Zhang, Y. Study on Tolerant Ability of Four Species of Iris l. Under NaCl Stress. Master's Thesis, Nanjing Forestry University, Nanjing, China, 2008.
63. Flowers, T.J.; Munns, R.; Colmer, T.D. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot.* **2015**, *115*, 419–431. [CrossRef]
64. Mann, A.; Lata, C.; Kumar, N.; Kumar, A.; Kumar, A.; Sheoran, P. Halophytes as new model plant species for salt tolerance strategies. *Front. Plant Sci.* **2023**, *14*, 1137211. [CrossRef] [PubMed]
65. Bonacina, C.; da Cruz, R.M.S.; Nascimento, A.B.; Barbosa, L.N.; Gonçalves, J.E.; Gazim, Z.C.; Magalhães, H.M.; de Souza, S.G.H. Salinity modulates growth, oxidative metabolism, and essential oil profile in *Curcuma longa* L.(Zingiberaceae) rhizomes. *South Afr. J. Bot.* **2022**, *146*, 1–11. [CrossRef]
66. Wang, Y.; Nii, N. Changes in chlorophyll, ribulose bisphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. *J. Hortic. Sci. Biotechnol.* **2000**, *75*, 623–627. [CrossRef]
67. Parida, A.K.; Das, A.B. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.* **2005**, *60*, 324–349. [CrossRef] [PubMed]
68. Trivellini, A.; Lucchesini, M.; Ferrante, A.; Carmassi, G.; Scatena, G.; Vernieri, P.; Mensuali-Sodi, A. Survive or die? A molecular insight into salt-dependant signaling network. *Environ. Exp. Bot.* **2016**, *132*, 140–153. [CrossRef]
69. Lin, S.; Song, X.F.; Mao, H.T.; Li, S.Q.; Gan, J.Y.; Yuan, M.; Zhang, Z.W.; Yuan, S.; Zhang, H.Y.; Su, Y.Q.; et al. Exogenous melatonin improved photosynthetic efficiency of photosystem II by reversible phosphorylation of thylakoid proteins in wheat under osmotic stress. *Front. Plant Sci.* **2022**, *13*, 966181. [CrossRef]
70. Tester, M.; Davenport, R. Na^+ tolerance and Na^+ transport in higher plants. *Ann. Bot.* **2003**, *91*, 503–527. [CrossRef] [PubMed]
71. Bernstein, L. Osmotic adjustment of plants to saline media. *II. Dyn. Phase. Am. J. Bot.* **1963**, *50*, 360–370. [CrossRef]
72. Shabala, S.; Pottosin, I. Regulation of potassium transport in plants under hostile conditions: Implications for abiotic and biotic stress tolerance. *Physiol. Plant.* **2014**, *151*, 257–279. [CrossRef] [PubMed]
73. Jiang, C.; Cui, Q.; Feng, K.; Xu, D.; Li, C.; Zheng, Q. Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta Physiol. Plant.* **2016**, *38*, 82. [CrossRef]
74. Liu, C.; Mao, B.; Yuan, D.; Chu, C.; Duan, M. Salt tolerance in rice: Physiological responses and molecular mechanisms. *Crop J.* **2022**, *10*, 13–25. [CrossRef]