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The Application of Melatonin and Organic Waste Derived from Vitamin C Industry Effectively Promotes Seed Germination and Seedling Growth of Cotton in Saline–Alkali Soil

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Abstract: Saline–alkali stress severely affects plant growth and productivity. Although melatonin can promote seed germination as a growth regulator, it cannot address the weak seedling growth caused by insufficient organic nutrients in saline–alkali soil. The RAE (residue after evaporation, an industrial waste from the industrial production of vitamin C) can enhance plant salt tolerance by stimulating vitamin C (ASA) synthesis and contains abundant small molecular organic acids. We hypothesized that the combined application of melatonin and RAE might synergistically enhance cotton germination and seedling growth. The cotton seeds used in this study were "Xin Lu Zhong No. 87"; a Petri dish simulation experiment and a pot experiment were conducted in 2023. Four treatments were set: control (CK), melatonin (MT), RAE (RAE), and the combined application of MT and RAE (MR). Compared to CK, MT significantly increased the germination rate of cotton seed (194.4%), while RAE significantly enhanced the underground biomass of cotton seedlings (40.3%) and ASA content (203.8%). Compared to MT and R, the combined application of melatonin and RAE significantly increased the ASA content (54.5%, 29.6%) in roots, superoxide dismutase (SOD) activity (220.3%, 89.6%) in roots, catalase (CAT) activity (15.8%, 97.5%) in leaves on the 15th day, soil cation exchange capacity (CEC) (57.2%, 9.7%), and total fresh weight (20.8%, 33.8%). Collectively, these findings indicate that the synergistic effect under the combined use of melatonin and RAE promotes cotton seed germination and seedling growth, offering a novel technical solution for salt–alkali soil cotton cultivation along with an innovative approach for the resource utilization of RAE.

Keywords: cotton seed germination; growth and development; melatonin; RAE; saline stress; soil environment improvement

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1. Introduction

Saline–alkali soil is a general term for various types of soils affected by salinization and alkalization, which is characterized by high concentrations of soluble salts, hindering the normal growth of plant and eventually causing agricultural economic losses and secondary disasters [1–3]. Statistics show that the global area of saline–alkali land exceeds nearly 1 billion hm^2, accounting for 24% of the total global arable land area [4]. China has approximately 3.6×10^7 hm^2 of saline–alkali land, accounting for 4.88% of the country's total available land base [5]. These saline–alkali lands have not yet been effectively utilized and represent a significant potential resource for agricultural development [5]. Unlocking the productive potential of saline–alkali land is not only a pivotal scientific concern at the forefront of international research but also a paramount issue in the pursuit of sustainable agricultural development [6]. Cotton is one of the most extensively cultivated cash crops globally; not only does it exhibit commendable tolerance to salt–alkali stress but it is also a pioneer crop for ameliorating and rehabilitating saline–alkali land [7]. However, the

growth and productivity of cotton are significantly hampered by the detrimental effects of salt–alkali stress, leading to a substantial decline in its survival rate and yield in saline–alkali land [8]. Therefore, addressing the imperative issue of enhancing cotton growth saline–alkali land remains a pressing concern for botanists to solve [9].

Cotton is a sugar-rich plant, and it exhibits enhanced tolerance to abiotic stresses compared to other major crops [10]. Despite being categorized as a crop with moderate salt tolerance, the cotton's growth, yield, and fiber quality are all adversely affected by salt stress [11]. Cotton exhibits higher susceptibility to salt stress during the stages of germination and seedling development compared to other growth stages [11]. To mitigate the impact of salt stress, cotton exhibits a delayed response at the germination and emergence stages [12]. The poor germination of cotton can result in diminished plant population, ultimately leading to a substantial decline in cotton yield [13]. Consequently, yield losses attributed to salt stress-induced damage can range between 50% and 90% during this critical period [7,13]. Therefore, enhancing the germination efficiency of cotton and ensuring its robust growth during the seedling stage constitute a fundamental prerequisite for achieving stable and high yields of cotton in saline–alkali environments.

In response to the constraints imposed by saline–alkali soil on cotton growth, current research has been primarily focused on employing plant growth regulators, such as melatonin (MT), gibberellin (GA), indole-3-acetic acid (IAA), salicylic acid (SA), and others, for seed treatment in order to alleviate seed dormancy and enhance germination rates [14–18]. Among them, MT, an indole heterocyclic compound, has garnered significant attention from researchers due to its remarkable efficacy in promoting cotton seed germination under salt stress [8,16,17,19,20]. However, existing studies have predominantly focused on the germination stage of seeds with inadequate consideration of the subsequent growth condition of seedlings. Salinity stress persistently occurs throughout the plant growth cycle, impacting normal plant development and metabolic processes through primary and secondary salinization effects [19]. Lei et al. proposed that successful germination encompasses not only the physical breakthrough of seeds through the seed coat but also encompasses the robust growth of seedlings [20]. Particularly during the seedling stage, cotton exhibits heightened sensitivity to saline–alkali soil stress, necessitating more than mere germination for ensuring optimal and healthy seedling development. Chen et al. found that the exclusive reliance on MT seed soaking treatment proves inadequate in mitigating the persistent salt stress encountered during the seedling growth stage in saline–alkali soil [17]. Furthermore, soil salt stress often coincides with issues of low fertility [21], significantly impeding plant growth and development. In our previous experiments, it was observed that while MT soaking facilitated the germination of cotton seeds, the growth of cotton seedlings, and their subterranean development, did not meet desired expectations. Therefore, we speculate that the employment of MT soaking as an independent method exhibits inherent limitations in effectively addressing the early growth challenges encountered by cotton seedlings, necessitating the further exploration of novel approaches to overcome these current obstacles.

Our previous research has demonstrated that the industrial waste from vitamin C production (RAE), which contains a lot of small molecular organic carbon, exhibits the potential to enhance crop growth in saline–alkali soil, particularly for perennial ryegrass and purslane [22–24]. This effect is accompanied by a significant increase in ascorbic acid (ASA) levels [25]. ASA is a predominant antioxidant in plant, exerting a crucial role in plant growth and development [26]. It is capable of eliminating free radicals through oxidation-reduction reactions, alleviating the detrimental effects caused by stress environments [27]. Numerous studies have emphasized the significance of ASA in enhancing the tolerance of plants to adverse conditions, and the accumulation of ASA can markedly enhance the plant's resistance to abiotic stress [28,29]. Moreover, the RAE significantly facilitated the growth of crop roots and enhanced the saline–alkali soil fertility [22–24,30], offering novel insights for cotton planting in saline–alkali soil.

In summary, melatonin can conspicuously facilitate seed germination under saline–alkali stress, whereas RAE can enhance the synthesis of plant vitamin C to promote crop growth in abiotic stress. Through promoting seed germination and plant growth under saline–alkali stress, the combined application of melatonin and RAE might not only foster the germination of cotton seeds but also stimulate the growth of seedlings under saline–alkali stress, establishing a solid foundation for high cotton yield. Therefore, the objective of this study was to investigate the effects of MT, RAE, and their combined application on cotton germination rate and seedling growth as well as to elucidate the underlying mechanisms. We hypothesized that the combined utilization of MT and RAE would enhance the seed germination rate and further promoting seedling growth, ultimately improving the germination rate and robust cotton seedling development. These research findings will provide novel theoretical foundations and practical guidance for cotton cultivation in saline–alkali soil.

2. Materials and Methods

2.1. Experimental Materials

The cotton seeds used in this experiment were "Xin Lu Zhong No. 87", which was provided by Kuqa Qiuci Seed Industry Co., Ltd., Kuqa, China. The experimental saline–alkali soil was collected from the cotton-growing region of Kuqa, China (40°41′ N, 84°25′ E). The main zonal soil in this area is salinized fluvo-aquic soils with blocky structure, and the average contents of sand, silt, and clay are 35.81%, 44.62%, and 19.57%, respectively. To ensure that the collected soil samples objectively reflect the actual soil conditions of the area with greater representativeness, twenty quadrats of 5 × 5 m were initially established. Subsequently, ten soil samples (each sample was about 5 kg) were also randomly collected at depths of 0–20 cm in each quadrat. The samples were evenly mixed, and the gravel and plant residues were removed. After natural air drying, the samples were screened through a 2 mm mesh for chemical properties determination and the pot trial. The cotton in this area was sown between 20 and 30 April and harvested between 20 and 30 September. The initial soil properties were as follows: soil pH was 8.20, soil salinity was 0.367%, soil organic matter content was 16.05 g/kg, total nitrogen content was 0.98 g/kg, available phosphorus content was 33.24 mg/kg, and available potassium content was 184 mg/kg. Melatonin was purchased from Shanghai Yuanye Co., Ltd., Shanghai, China, with an analytical purity of 99%. The RAE was provided by the Northeast Pharmaceutical Group Co., Ltd., Shenyang, China. The characteristics of RAE are listed in Table 1. The experiment was conducted in March 2023 in the constant temperature cultivation room (at a temperature of 25 ± 2 °C) of the Shenyang Institute of Applied Ecology, Chinese Academy of Sciences.

Table 1. The characteristics of RAE.

Indices	Value	Indices	Value
pH	0.29	AP ($mg \cdot L^{-1}$)	4.33
COD ($mg \cdot L^{-1}$)	1.18×10^6	AK ($mg \cdot L^{-1}$)	147.22
TOC ($g \cdot L^{-1}$)	177.52	2-KGA ($g \cdot L^{-1}$)	201.83
TN ($g \cdot L^{-1}$)	4.69	Oxalic acid ($g \cdot L^{-1}$)	26.52
TP ($mg \cdot L^{-1}$)	0.18	Formic acid ($g \cdot L^{-1}$)	3.41
TK ($mg \cdot L^{-1}$)	2.11	Valeric acid ($g \cdot L^{-1}$)	0.42
AN ($mg \cdot L^{-1}$)	113.82	Water content (%)	55.69

COD—chemical O_2 demand, TOC—total organic carbon, TN—total nitrogen, TP—total phosphorus, TK—total potassium, AN—available nitrogen, AP—available phosphorus, AK—available potassium, 2-KGA—2-keto-L-gulonic acid.

2.2. Experimental Treatments

2.2.1. Seed Disinfection and Soaking

Cotton seeds with the same morphological characteristics were selected and disinfected with 75% (v/v) alcohol for 15 min; then, they were rinsed thoroughly with distilled

water and air-dried at room temperature. The dried seeds were randomly divided into two groups: one group (approximately 500 seeds) was soaked in 500 mL of 20 μM melatonin solution, and the other group (approximately 500 seeds) was soaked in 500 mL of distilled water. Both groups were soaked for 24 h before being retrieved for subsequent use.

2.2.2. Experimental Design and Treatments

The experiments were divided into two parts: the Petri dish simulation experiment and the pot experiment.

For the Petri dish experiment, four treatment groups were designed: the control group (CK), which was soaked with distilled water and maintained with distilled water; the salt stress control group (CK-S), which was soaked with distilled water and moistened with 0.4% sodium chloride solution to simulate saline stress; the melatonin treatment group (MT), which was soaked with 20 μM melatonin solution and maintained with distilled water; and the melatonin plus stress treatment group (MT-S), which was soaked with 20 μM melatonin solution and moistened with 0.4% sodium chloride solution. Each treatment included five Petri dishes; each Petri dish contained 20 soaked seeds and was covered with two layers of filter paper with 10 mL of distilled water or 0.04% NaCl solution injected precisely using a syringe. During the cultivation process, water was supplemented daily by the weighing method. The greenhouse cultivation conditions were 25 ± 2 °C, light intensity of 1000–2000 μmol/m^2s, with a 16 h light and 8 h dark photoperiod, and the relative humidity maintained at 50 ± 10%. Each treatment was repeated five times.

For the pot experiment, four treatment groups were designed: the melatonin-only treated group (MT), the RAE-only treated group (R), the combined melatonin and RAE treated group (MR), and the control group (CK). Each group consisted of five pots with 10 seeds sown in each pot containing 1.5 kg of saline soil. The R and MR groups were watered with 200 mL of diluted RAE (diluted 200 times) on the fifth and tenth days after planting, while the CK and MT groups were watered with an equal amount of distilled water to maintain soil moisture. All pots were cultivated in a constant temperature chamber at 25 °C with light and other environmental conditions consistent with the Petri dish experiment. Each treatment was repeated five times.

2.3. Determination of Germination Rate, Seedling Establishment Rate, and Seed Vigor

The germination rate in the Petri dish simulation experiment was determined when the radicle broke through the seed coat, reaching half the length of the cotton seed [6].

Germination rate = the number of germinated seeds on the seventh day/total number of seeds applied.

Seedling establishment rate in the pot experiment was determined when the cotton seedling exceeded 3 cm above the soil surface.

Seedling establishment rate = the number of seedlings on the seventh day/total number of seeds sown.

Seed vigor was measured using the TTC method [31], which was represented by the rate of TTC reduction to TTF due to seed respiration over a unit of time.

2.4. Determination of Seedling Physiological Parameters and Soil Characteristics

Cotton tissues (leaves and roots) with the same parts, sampled and stored at −20 °C on days 5, 10 and 15, were used to determine the physical parameters (the activities of antioxidase, the contents of MDA and ASA; the contents of soluble sugar, soluble protein and proline). For biomass measurement, fresh samples were taken on the 15th day to determine the fresh weight. Subsequently, the fresh samples were placed in an oven at 105 °C for 2 h, and the dry weight was determined. The aboveground part of the cotton seedling was above the hypocotyl, and the underground part was below the hypocotyl. The plants cultivated in the pot on the 15th day were employed to determine the physical parameters using a photo-synthesizer (Licor6800, LI-COR, NE, USA). The fresh root samples taken on the 15th day were used to determine the root characteristics using a root scanner

(LC-4800, LEGENTSYS-SINTEK, CA). The fresh leaf samples taken on the 15th day were utilized to determine the content of photosynthetic pigments. Three replicates were set for each group.

2.4.1. Determination of Seedling Physiological Parameters

Cotton leaves from the intact plant in the pot on the 15th day were measured using the Licor6800, (LI-COR, NE, USA) photosynthesis meter to determine the net photosynthetic rate, the transpiration rate, the intercellular carbon dioxide concentration, the leaf total conductance to water vapor, the leaf total conductance to carbon dioxide, and the leaf area. The measurement was referred to Haghshenas et al. [32].

Cotton roots on the 15th day were washed and laid flat on the LC-4800 (LEGENTSYS-SINTEK, CA) for the precise scanning and quantification of the total root length, projected area, area, superficial area, volume, and average diameter.

Cotton seedling tissues (leaves and roots) that were 5, 10, and 15 days old were collected. The determination of photosynthetic pigments, soluble sugars, soluble proteins, proline, ASA, superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity, and malondialdehyde (MDA) followed the methods described by Chen et al. [33] and Gao et al. [22].

The detailed information of the experimental methods was described in the Supplementary Materials.

2.4.2. Analysis of Soil Characteristics

Soil characteristics were measured according to the method described by the published references [34–41]. Soil samples were collected on days 0, 8, 13 and 18 during the germination stage. The samples were dried in a ventilated place, screened and stored at −20 °C for testing.

Soil catalase was measured by the potassium permanganate titration method [34,36]. Sucrase was determined by 3,5-dinitrosalicylic acid (DNS) assay [35]. The content of 3-amino-5 nitrosalicylic acid was determined by ultraviolet spectrophotometer at 508 nm. The concentration of p-nitrophenol was determined by p-nitrophenyl phosphate (pNPP) assay with an ultraviolet spectrophotometer at 400–420 nm [37]. Protease was determined by the dansyl chloride method [34], and the NH_3-N content was determined by ultraviolet spectrophotometer at 500 nm. Urease was determined by the indigo carmine colorimetric method [39], and the NH_3-N concentration was determined by ultraviolet spectrophotometer at 578 nm.

Soil pH was measured using a pH meter; nitrate nitrogen content was measured by detecting the difference between 220 and 275 nm from UV/visible spectroscopic methods. The soil solution of ammonium nitrogen, available phosphorus and available potassium was extracted by a combined extraction and colorimetric method, and the absorbance was measured at 420 nm, 685 nm and 685 nm by adding the respective color developing agent. The content of soil organic matter was measured by the potassium dichromate oxidation method, and the absorbance was measured at 590 nm [40]. The soil cation exchange capacity (CEC) was measured by the the hexaamminecobalt trichloride method [41], and the absorbance was measured by ultraviolet spectrophotometer at 475 nm.

The detailed steps and conditions of the experimental methods are described in the Supplementary Materials.

2.5. Statistical Analysis

The data presented in the text represent the mean ± standard deviation of the biological replicates ($n \geq 3$). The variables were compared among the groups using a one-way analysis of variance (ANOVA) and Tukey's multiple comparisons. The correlations between the measured variables were assessed using Spearmen's method. All the data were statistically analyzed using GraphPad Prism software (v 9.5.1) and Origin 2021 software (v 9.8.0.200). The significance level was set at $p < 0.05$.

3. Results

3.1. Seed Germination and Seedling Establishment

As shown in Figure 1A, under non-saline stress, there was no significant difference in the average germination rate of cotton between the MT and CK groups ($p > 0.05$). However, under saline stress conditions, the average germination rate of cotton in the melatonin group (MT-S) was 75%, which was significantly higher than that in the control group (CK-S) (38%, $p < 0.05$). The results of the pot experiment (Figure 1B) indicated that the average seedling establishment rate of cotton in the melatonin-treated group (MT) was significantly higher than that of the non-melatonin-treated group (CK) (increased by 194%, $p < 0.05$). The result of seed vitality (Figure 1C) showed that on the last soaking day (0 d), the seed vitality in MT was 30.29% higher than that in CK. On the first day after sowing (1 d), the seed vitality of both groups dropped significantly, but the MT group's seed vitality remained significantly higher than that in CK ($p < 0.05$). From the second to the third day, the seed vitality in the two treatments gradually increased with the values in the MT group consistently and significantly higher than that in the CK group.

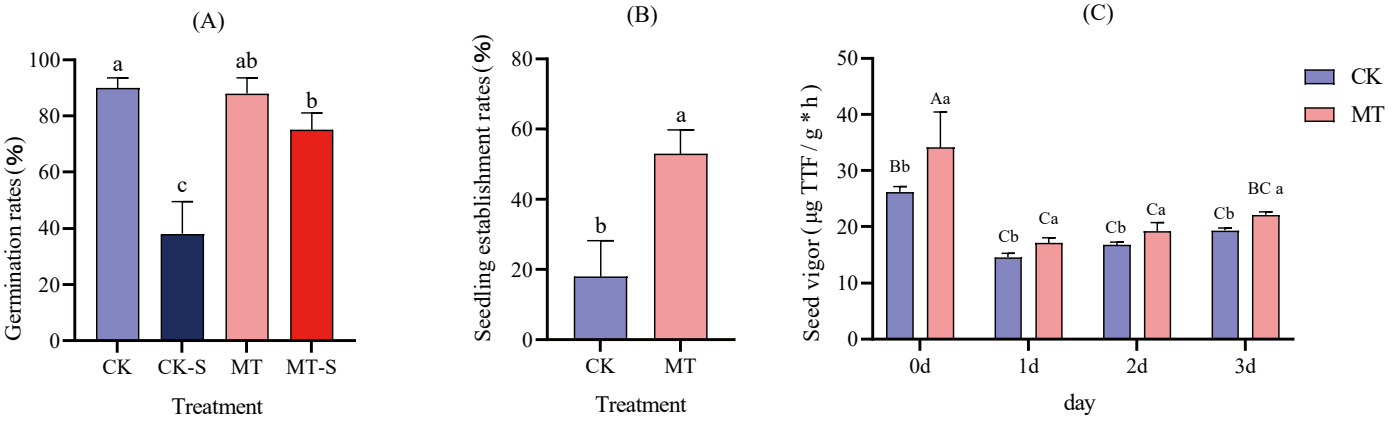

Figure 1. The germination rate, seedling establishment rate and seed vitality of cotton seed under different treatment. (**A**) In the simulation experiment, the germination rate of cotton seeds in different treatment. CK, the control group; CK-S, the control group in salt stress (0.4% NaCl, *w*/*v*); MT, the melatonin group; MT-S, the melatonin group in salt stress (0.4% NaCl, *w*/*v*). (**B**) In the pot experiment, seedling rate of cotton in different treatment. CK, the control group; MT, the melatonin group. (**C**) In the pot experiment, the seed vitality at the end of soaking (0 d) and after sowing for 3 days (1 d, 2 d, 3 d). CK, the control group; MT, the melatonin group. Different lowercase letters indicate statistically significant differences between different treatments at the same time, and uppercase letters represent the comparison between different times of the same treatment (n = 5, $p < 0.05$). Error bars represent the standard deviation.

3.2. Effects of Exogenous MT and RAE on the Antioxidase Activities, and Contents of MDA and ASA

As shown in (Figure 2A,C,E), during the cultivation period, the enzyme activities of POD and CAT in cotton seedling leaves in the melatonin groups (MT and MR) were significantly higher than that in CK and R groups, respectively. However, the enzyme activities of SOD in the melatonin groups (MT and MR) were significantly higher than that in CK and R groups, respectively, but this only occurred on the 10th day. On the 15th day, the enzyme activities of SOD, POD, and CAT in cotton seedling leaves in the R group were higher than those in the CK group but lower than those in the MT group, while the MR group exhibited the highest values among all groups. In the roots (Figure 2B,D,F), just like the leaves, the enzyme activities of POD and CAT in the melatonin groups (MT and MR) were significantly higher than those in the CK and R groups, respectively, during the cultivation period. On the 15th day (Figure 2B,D,F), the activities of SOD, POD, and CAT in leaves in the MT group were significantly higher than those in the CK group. However,

the activities of SOD and POD in the R group were significantly higher than those in the MT group; whereas the activities of SOD, POD, and CAT in the MR group were the highest values out of all the groups. Especially, the SOD activity in MR was significantly enhanced than that in R or MT on the 15th day.

The content of MDA in the cotton seedling leaves gradually decreased from the 5th day to the 15th day (Figure 2G), and there were no significant differences among the four treatments. On the 15th day, the MDA content in R and MR was significantly lower than that in CK, respectively, while MT was lower than CK (decreased by 52.7%, $p < 0.05$). In the roots (Figure 2H), the MDA content in the R and MR groups firstly decreased and then tended to be stable, and it was significantly lower than that in the MT and CK groups on the 15th day ($p < 0.05$).

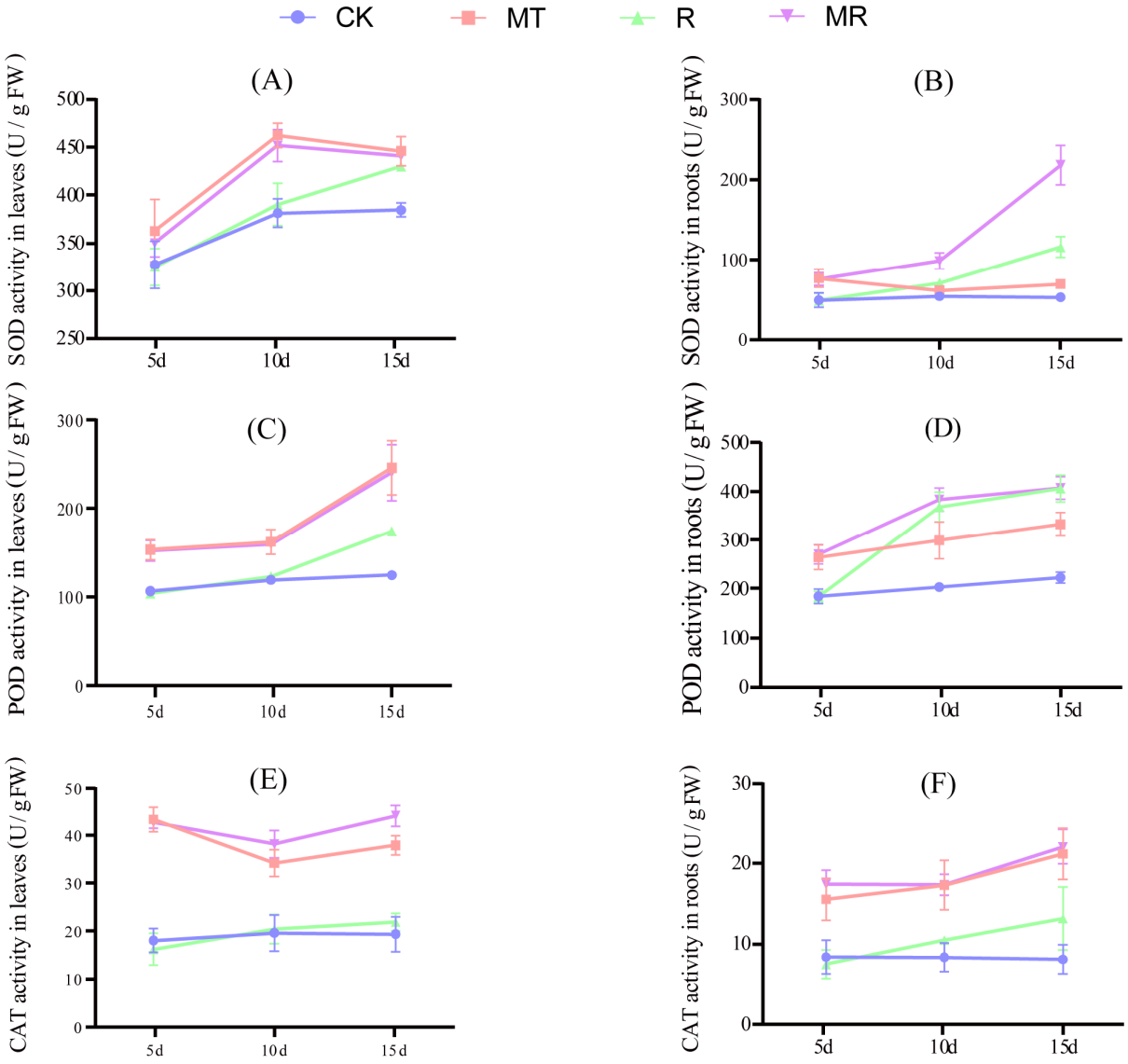

Figure 2. *Cont.*

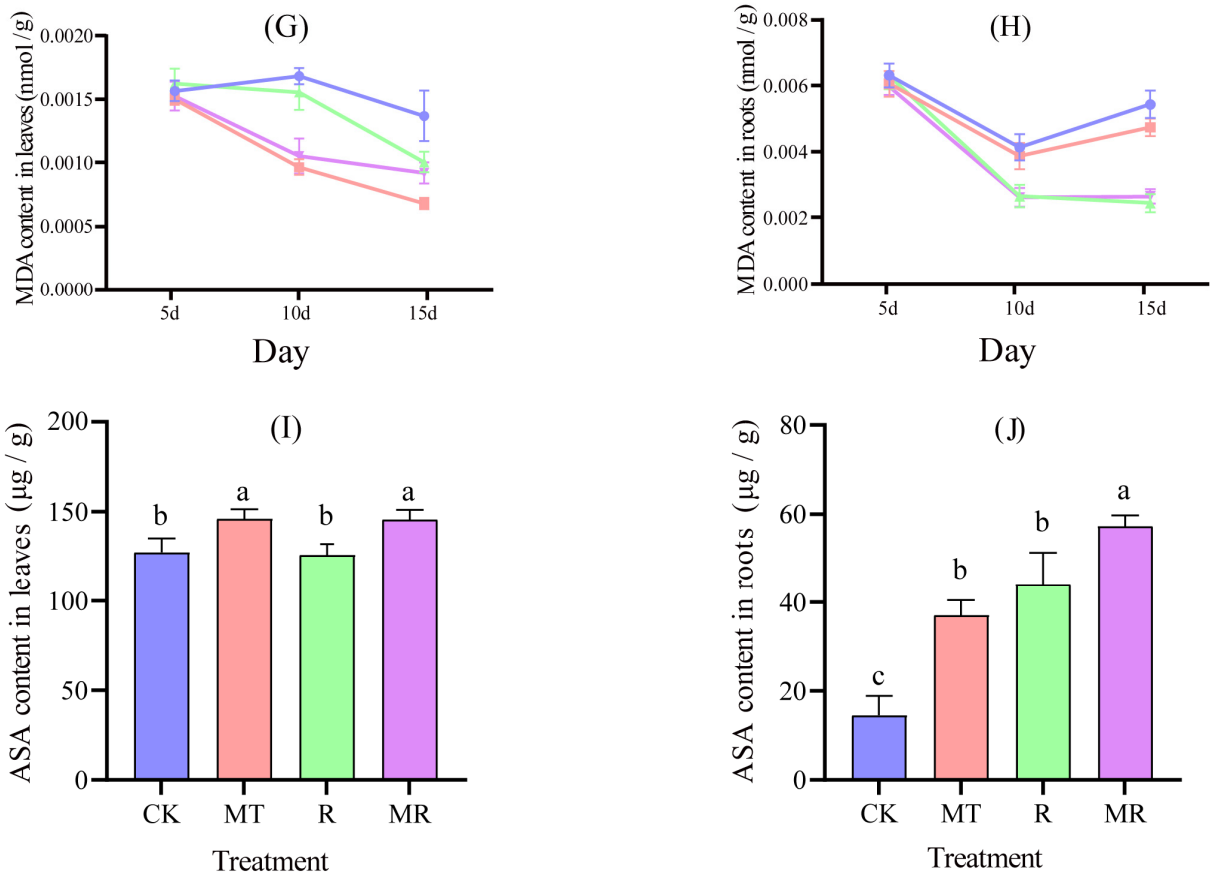

Figure 2. The levels of antioxidants and peroxide in the leaves (**A**,**C**,**E**,**G**,**I**) and roots (**B**,**D**,**F**,**H**,**J**) of cotton seedlings. SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; ASA, ascorbic acid; MDA, malondialdehyde. Different lowercase letters indicate statistically significant differences (n = 3, $p < 0.05$). Error bars represent the standard deviation.

In the cotton seedling leaves (Figure 2I), the ASA content in the MT and MR groups was significantly higher than that in the CK and R groups ($p < 0.05$); however, in the roots (Figure 2J), the MR group exhibited the highest ASA content (increased by 298.3% compared to CK, $p < 0.05$), while the ASA content in the MT and R groups was significantly higher than that in the CK group ($p < 0.05$), respectively.

3.3. Effects of Exogenous MT and RAE on the Contents of Soluble Sugar, Soluble Protein and Proline

As depicted in Figure 3A, the levels of soluble sugars in cotton leaves exhibited an initial decline followed by a subsequent increase in the CK, MT and MR groups, except for the R group, which displayed a consistent upward trend. On the 15th day, the soluble sugar content in three groups (MT, R and MR) was higher than that in CK, respectively, with the MR group showing the highest value (increased by150.7% compared to CK, $p < 0.05$); meanwhile, from the 5th to the 15th days, there was a gradual decrease in the soluble sugar content in the roots of all treatment groups (Figure 3D). On the 15th day, both the R and MR groups exhibited significantly higher levels of soluble sugar content compared to the MT and CK groups ($p < 0.05$).

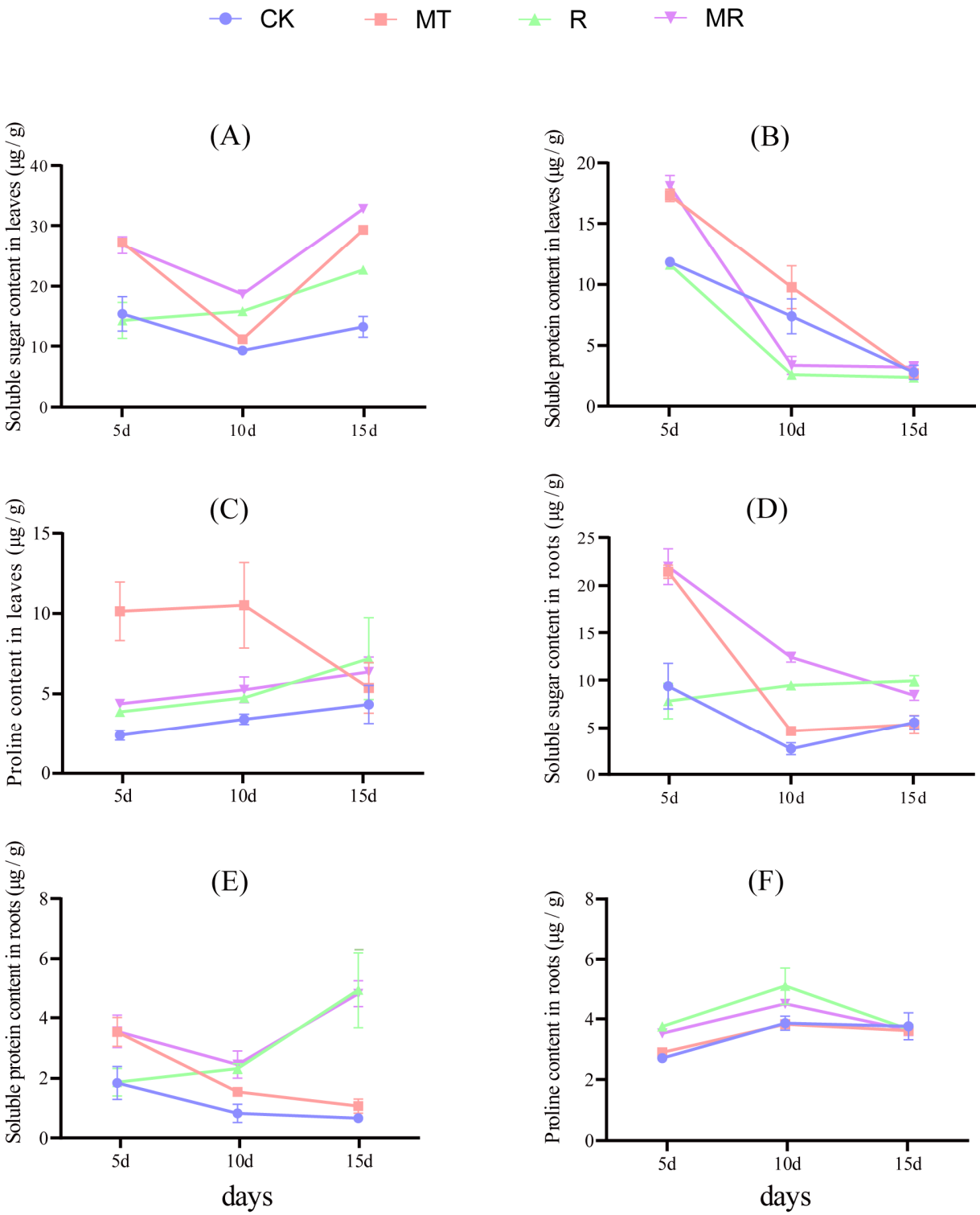

Figure 3. Content of osmotic regulators in different treatments: soluble sugar content in cotton seedling leaves (**A**) and roots (**D**); soluble protein content in cotton seedling leaves (**B**) and roots (**E**); proline content in cotton seedling leaves (**C**) and roots (**F**).

From the 5th to the 15th day, the soluble protein contents of the cotton seedling leaves in all treatment groups decreased (Figure 3B). On the 10th day, the soluble protein content in the MT group was significantly higher than that in the R and MR groups ($p < 0.05$). In the roots (Figure 3E), the soluble protein content in the MT and CK groups gradually decreased, while the R and MR groups fluctuated and then increased. On the 15th day, the

soluble protein content in the R and MR groups was significantly higher than that in the MT and CK groups ($p < 0.05$), respectively.

The proline content of the cotton seedling leaves in the MT group was significantly higher than that in the other groups on both the 5th and 10th day (Figure 3C), showing an increase of 215.5% on the 5th day and 154.8% on the 10th day compared to CK ($p < 0.05$). In terms of roots (Figure 3F), on the 10th day, both the MR and R groups exhibited significantly higher proline content compared to the MT and CK groups ($p < 0.05$).

3.4. Effects of Exogenous MT and RAE on the Root Characteristics

The results presented in Table 2 illustrated the root development in cotton seedlings on the 15th day for each treatment. Compared to the CK group, the MT group did not have a significant impact on the root growth ($p > 0.05$). However, both the MR and R groups significantly increased the total root length, the projected area, the surface area, and the volume of roots compared to the control group ($p < 0.05$), respectively. Additionally, except for the average diameter of the root, MR had a significantly more positive effect on all indicators of the root growth than MT and CK ($p < 0.05$) with each value being higher than that of R but without significant differences. There was no significant difference in the average diameter of the roots among the four groups ($p > 0.05$).

Table 2. The root characteristics of cotton seedlings on the 15th day.

Treatment	Total Root Length (cm)	Projected Area (cm^2)	Area (cm^2)	Superficial Area (cm^2)	Volume (cm^3)	Average Diameter (mm)
CK	68.65 ± 6.39 b	2.90 ± 0.24 b	2.77 ± 0.34 b	10.06 ± 1.94 b	0.17 ± 0.01 b	0.36 ± 0.05 a
MT	81.96 ± 3.21 b	3.05 ± 0.41 b	3.28 ± 0.20 b	9.95 ± 0.76 b	0.16 ± 0.01 b	0.37 ± 0.02 a
R	120.31 ± 3.45 a	4.22 ± 0.11 a	4.01 ± 0.07 a	13.27 ± 0.33 a	0.20 ± 0.01 b	0.36 ± 0.02 a
MR	119.61 ± 8.15 a	4.31 ± 0.32 a	4.10 ± 0.21 a	13.51 ± 0.64 a	0.29 ± 0.03 a	0.33 ± 0.03 a

Different lowercase letters indicate statistically significant differences (one-way analysis of variance (ANOVA) and Tukey's multiple comparisons, n = 3, $p < 0.05$).

3.5. Effects of Exogenous MT and RAE on the Content of Photosynthetic Pigments

As shown in Figure 4A,D, compared to CK, only the MR group exhibited a significant increase in the levels of chlorophyll A and carotenoids in cotton seedling leaves ($p < 0.05$), respectively. Among all four groups, MR displayed the highest content of chlorophyll A, chlorophyll B, total chlorophyll, and carotenoids. The MT group had a significantly decreased content of chlorophyll B compared to the CK group, but it had no significant effect on the levels of chlorophyll A, total chlorophyll, and carotenoids (Figure 4A–D).

It was observed that compared to CK (Table 3), the MT group had a significantly increased transpiration rate of the leaves and moderately enhanced net photosynthetic rate, intercellular CO_2 concentration, leaf area, total conductance to water vapor, and total conductance to CO_2 ($p < 0.05$), respectively. The R group had a significantly improved net photosynthetic rate and leaf chamber CO_2 concentration as well as moderately increased intercellular CO_2 concentration, intercellular CO_2 partial pressure, leaf area, stomatal conductance to water vapor, and boundary layer conductance to water vapor, but it had a notably decreased transpiration rate. Compared to the CK group, the MR group significantly enhanced the transpiration rate (49%, $p < 0.05$), net photosynthetic rate (47.1%, $p < 0.05$), leaf chamber CO_2 concentration (47%, $p < 0.05$), intercellular CO_2 concentration (80.3%, $p < 0.05$), intercellular CO2 partial pressure (33.3%, $p < 0.05$), leaf area (33.7%, $p < 0.05$), stomatal conductance to water vapor (42.9%, $p < 0.05$), boundary layer conductance to water vapor (50%, $p < 0.05$), total conductance to water vapor (34%, $p < 0.05$), and total conductance to CO_2 (39.6%, $p < 0.05$).

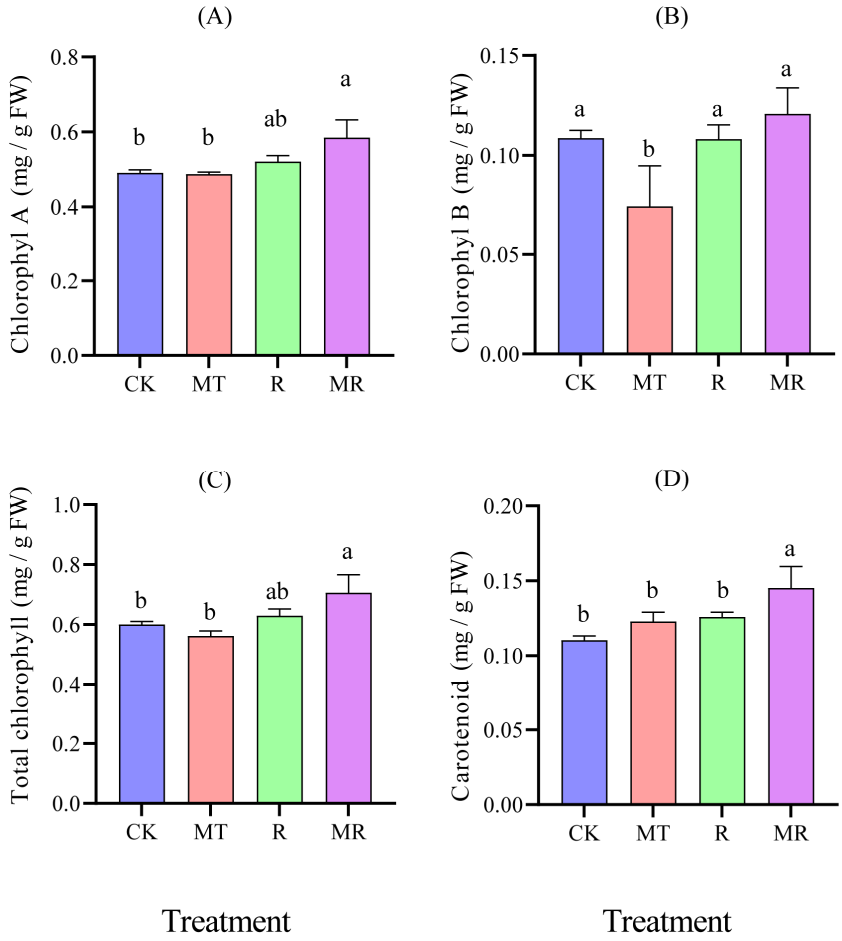

Figure 4. The photosynthetic pigment content in different treatment: (**A**) chlorophyll A; (**B**) chlorophyll B; (**C**) total chlorophyll; (**D**) carotenoid. Different lowercase letters indicate statistically significant differences (n = 3, $p < 0.05$).

Table 3. Photosynthetic and transpiration performance of leaves.

Treatment	Transpiration Rate (mol m^{-2} s^{-1})	Net Photosynthetic Rate (μmol m^{-2} s^{-1})	Intercellular CO_2 Concentration (μmol mol^{-1})	Leaves Area (cm^2)	Total Conductivity to Water Vapor (mol m^{-2} s^{-1})	Total Conductivity to CO_2 (mol m^{-2} s^{-1})
CK	9.23 ± 0.92 bc	134.71 ± 5.78 b	38.00 ± 5.16 b	7.48 ± 1.36 b	179.77 ± 25.36 b	29.04 ± 4.16 b
MT	19.82 ± 2.73 a	321.89 ± 73.80 ab	70.56 ± 14.51 a	10.94 ± 0.78 a	279.00 ± 57.40 a	41.70 ± 3.75 a
R	5.99 ± 3.19 c	439.58 ± 50.06 a	60.17 ± 4.43 ab	9.55 ± 0.96 ab	221.55 ± 9.37 ab	35.60 ± 1.67 ab
MR	13.75 ± 1.01 b	198.14 ± 55.54 b	68.53 ± 10.54 a	10.00 ± 0.48 a	241.02 ± 13.19 ab	40.54 ± 1.60 a

Different lowercase letters indicate statistically significant differences (one-way analysis of variance (ANOVA) and Tukey's multiple comparisons, n = 3, $p < 0.05$).

3.6. Effects of Exogenous MT and RAE on Biomass of Cotton Seedlings

In comparison CK, MT, R, and MR significantly enhanced both the aboveground and the total fresh weight of cotton seedlings (Figure 5A,C) ($p < 0.05$). Notably, the aboveground fresh weight and total fresh weight in the MR were markedly greater than those observed in MT and R ($p < 0.05$), respectively. Furthermore, relative to CK, MT, R, and MR significantly increased both the above-ground dry weight and total dry weight of cotton seedlings ($p < 0.05$) (Figure 5B,D,F); additionally, the total dry weight in MR was significantly higher than that recorded for all other treatments ($p < 0.05$), respectively.

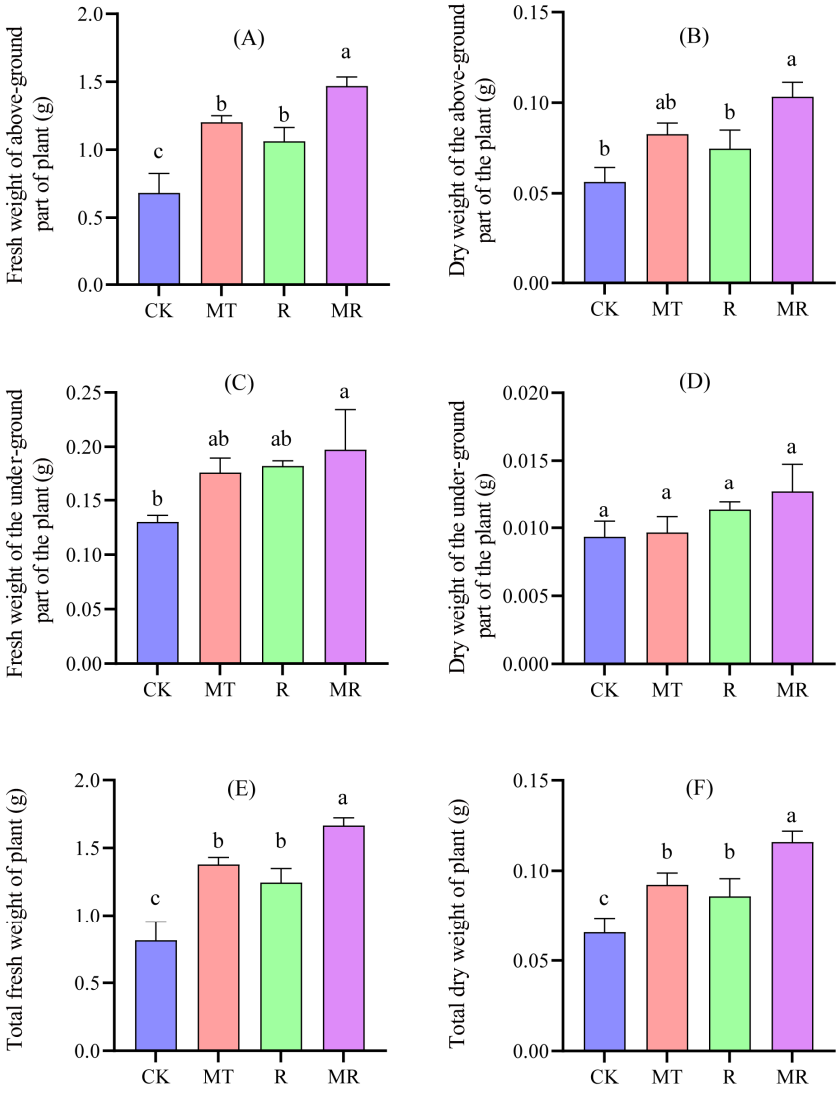

Figure 5. Biomass of cotton seedling on the 15th day in different treatments: (**A**) fresh weight of the aboveground parts; (**B**) dry weight of the aboveground parts; (**C**) fresh weight of the underground parts; (**D**) dry weight of the underground parts; (**E**) total fresh plant weight; (**F**) total dry weight of the plant. Different lowercase letters indicate statistically significant differences (n = 3, $p < 0.05$).

3.7. Effects of Exogenous MT and RAE on the Soil Nutrition

As depicted in Figure 6A, the ammonia nitrogen content in the soil of the R and MR groups on the 8th and 18th days exhibited a significant increase compared to that of the CK and MT groups, respectively. During the cultivation, the ammonia nitrogen content in the MT group showed no significant difference compared to that in the CK group (Figure 6A). MT did not exhibit a significant effect on nitrate nitrogen content, while R significantly reduced the soil nitrate nitrogen content compared with the CK group on the 8th and 18th days (Figure 6B). On the 0–18th days, the concentration of available phosphorus in the RAE groups (R and MR) sustained a comparative stability, whereas in the CK and MT groups, it underwent an initial increase followed by a decrement. Moreover, from the 13th day to the 18th day, the content of available phosphorus in the MT group was lower than that in the CK group (Figure 6C). Concomitant with the application of potassium chloride solution, there was a marked ascension in the available potassium content in the CK and MT groups over the course of the 0–13th days. Notably, the MT group experienced a subsequent significant decline on the 18th day. Conversely, the RAE group exhibited a consistent rise in available potassium from the 8th to the 18th day. A stable ascending trajectory was showed in MR from the 13th and the 18th days (Figure 6D). In comparison

with CK, both R and MR groups significantly increased the cation exchange capacity of soil on day 18 (Figure 6E). Compared with CK, the R group significantly reduced soil pH and reached the lowest value on day 8, and the three treatments (R, MT, MR) significantly reduced soil pH on day 13 and day 18 with the largest reduction in the R group (Figure 6G). There was no significant change in soil organic carbon content in all treatments (Figure 6F).

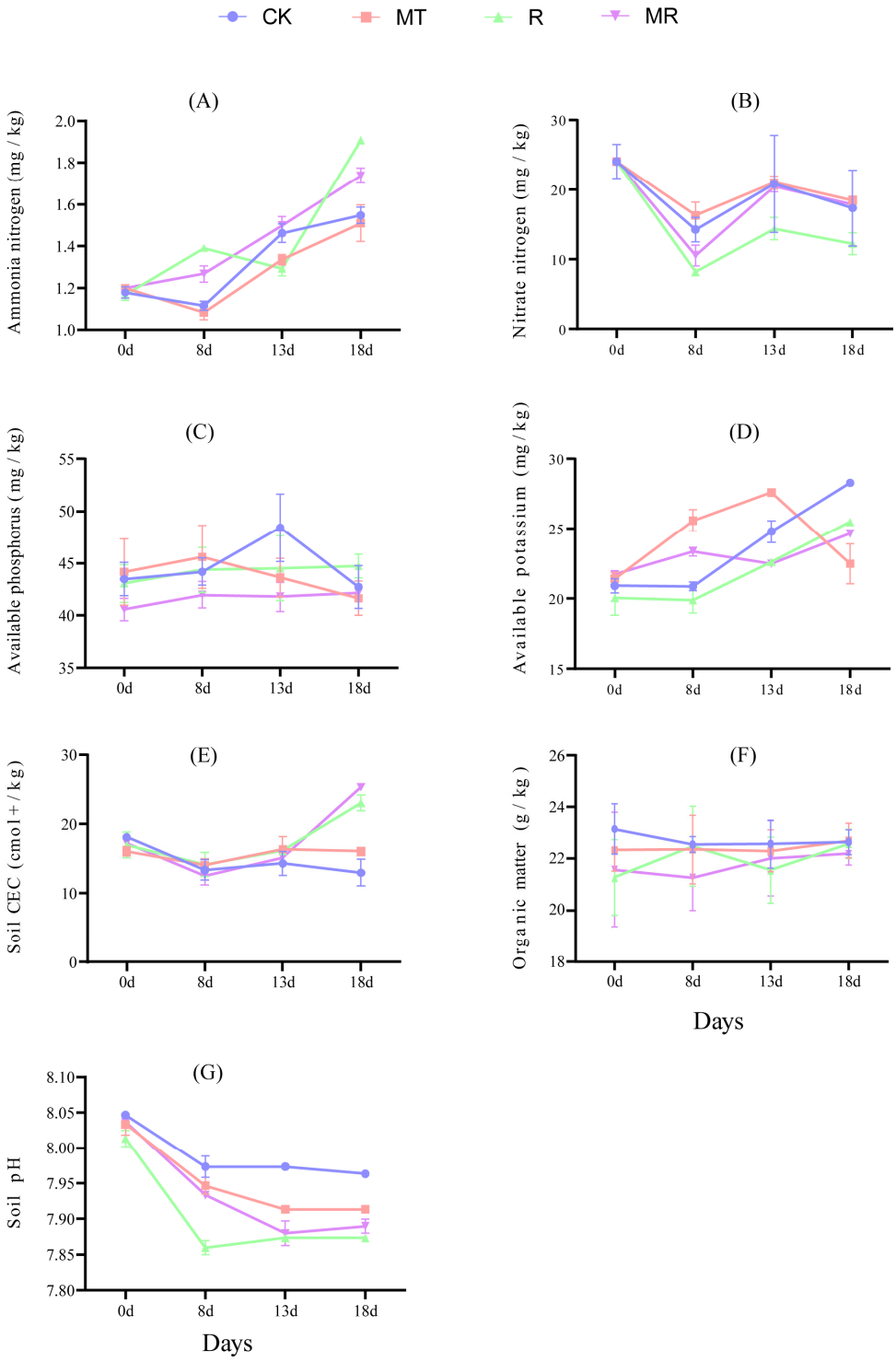

Figure 6. Effects of RAE and melatonin addition on fertility-related properties of bulk soil nutrition: (**A**) ammonia nitrogen; (**B**) nitrate nitrogen; (**C**) available phosphorus; (**D**) soil cation exchange capacity (CEC); (**E**) rapidly available potassium; (**F**) organic matter; (**G**) soil pH.

3.8. Effects of Exogenous MT and RAE to the Activity of Soil Enzyme

As shown in Figure 7, we measured the activities of protease, catalase, phosphomonoesterase, urease, and sucrase in the rhizosphere soil of cotton seedlings. In general, the activity of the five enzymes in each treatment showed a gradually increasing trend with the cultivation time. The protease enzyme activity in the MT group did not show a significant difference compared to the CK group. In contrast, the RAE groups (R and MR) exhibited a significant increase in protease enzyme activity on days 13 and 18 (Figure 7C). Similarly, there was no significant difference in catalase enzyme activity between the MT and the CK groups, while the RAE groups (R and MR) had significantly increased catalase enzyme activity on days 8 and 13 (Figure 7A). On day 13, the MR group showed significantly higher phosphomonase enzyme activity compared to the CK and MT groups (Figure 7D). Apart from a significant decrease in urease activity observed in the MT group on day 13, there were no significant differences in urease activities among the four treatments throughout the growth stage (Figure 7E). The sucrase enzyme activity in MR was notably lower than that in both CK and MT on day 13 and 18, whereas the sucrase activity reached its peak in the R group on day 18 (Figure 7B). From 13 to 18 days, the CK group's sucrase activity decreased, while the other groups continued to increase.

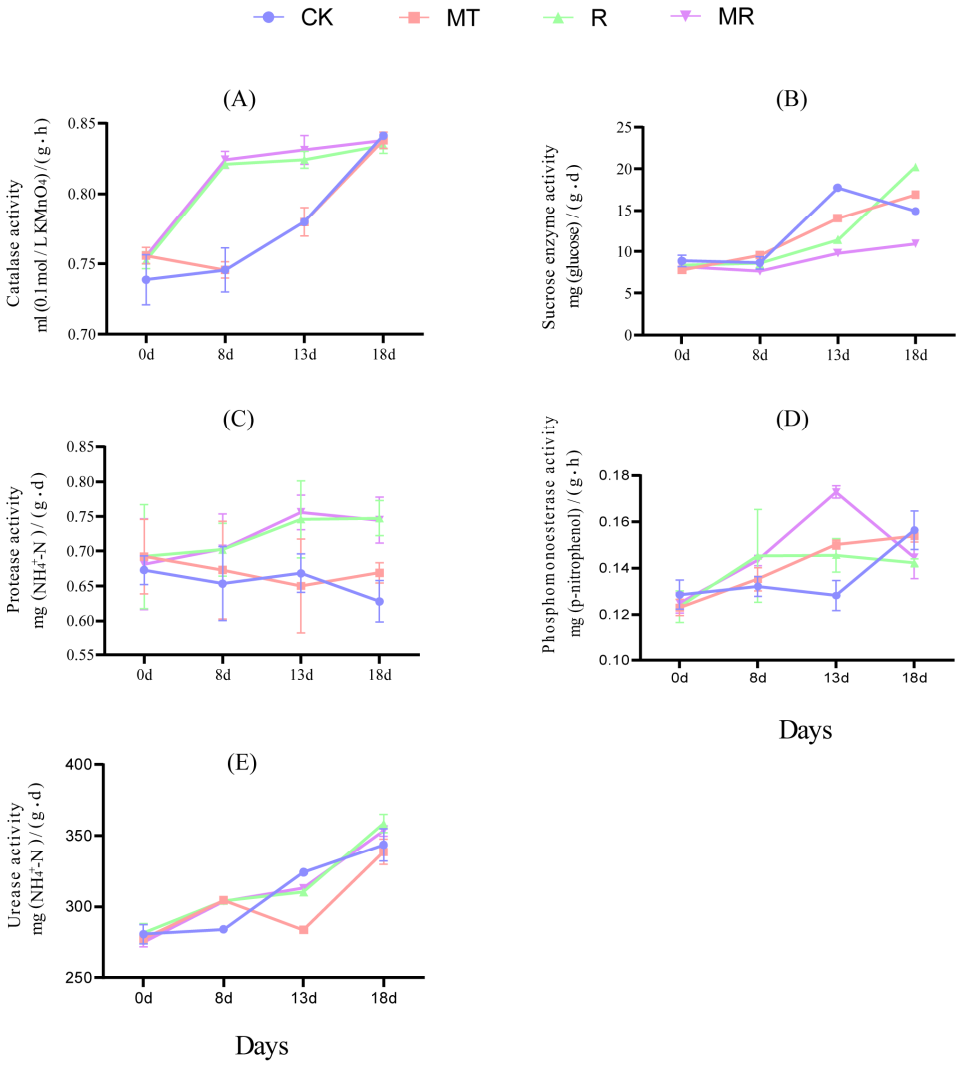

Figure 7. Soil enzymes activities during the cultivation of cotton seedlings in different treatments. (**A**) Protease, (**B**) catalase, (**C**) phosphomonoesterase, (**D**) urease (**E**) sucrase.

3.9. Correlation Analysis Between Physiological and Agronomic Indexes of Cotton Seedling and Physicochemical Properties of Rhizosphere Soil

In order to investigate the potential association between the measured variables under the influence of RAE and MT, Spearman's rank correlation coefficient was employed for the correlation analysis. As can be seen from Figure 8, the content of antioxidants, especially ASA, is positively correlated with the content of the main osmotic regulatory substances, the development of the primary nutritional absorption organs, the content of the main photosynthetic pigments, and the biomass of the cotton seedlings, and it is negatively correlated with the content of the membrane lipid peroxidation product MDA: ASA (leaves) and soluble sugar (leaves) ($r > 0.76$, $p < 0.05$); ASA (roots) and soluble protein (roots) ($r > 0.77$, $p < 0.05$); ASA (leaves) and leaves area ($r > 0.65$, $p < 0.05$); ASA (roots) and total root length ($r > 0.81$, $p < 0.05$); ASA (roots) and fresh weight (total) ($r > 0.90$, $p < 0.05$); MDA (leaves) and ASA (leaves) ($r < 0.50$, $p < 0.05$); and MDA (roots) and ASA (roots) ($r < -0.58$, $p < 0.05$).

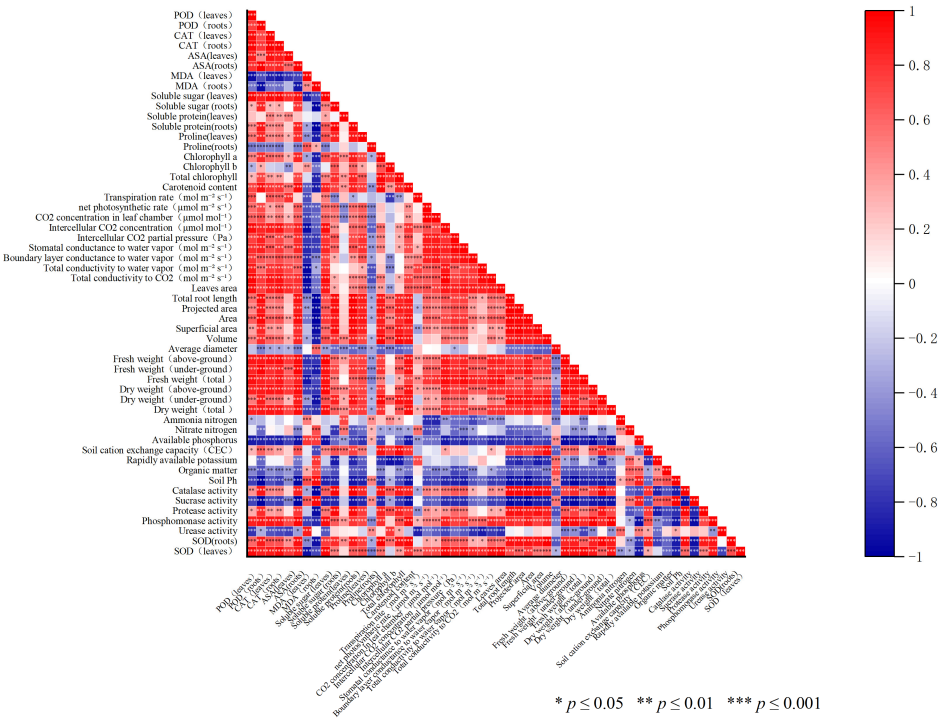

Figure 8. Correlation analysis between physiological and agronomic indexes of cotton seedling and physicochemical properties of rhizosphere soil. Spearman's correlation coefficient was used for the correlation analysis, $p < 0.05$.

The activity of the soil enzymes, especially catalase, protease, and phosphomonoesterase, is positively correlated with the antioxidant enzymes of the cotton seedlings, the photosynthetic pigments in the leaves, the development level of the cotton root system, and the biomass of the cotton seedlings, for catalase: CAT (roots) ($r > 0.90$, $p < 0.05$), total chlorophyll ($r > 0.72$, $p < 0.05$), total root length ($r > 0.59$, $p < 0.05$), total fresh weight ($r > 0.59$, $p < 0.05$); for protease: SOD (roots) ($r > 0.62$, $p < 0.05$), total chlorophyll ($r > 0.52$, $p < 0.05$), total root length ($r > 0.52$, $p < 0.05$), fresh weight (total) ($r > 0.42$, $p < 0.05$); for phosphomonoesterase: ASA (roots) ($r > 0.93$, $p < 0.05$), carotenoid content ($r > 0.80$, $p < 0.05$), total root length ($r > 0.64$, $p < 0.05$), fresh weight (total) ($r > 0.93$, $p < 0.05$).

4. Discussion

4.1. Effects of Melatonin Application on Seed Germination and Seedling Growth of Cotton

Our results showed that soaking cotton seeds with 20 μM melatonin could effectively improve the germination rate and seedling establishment rate of cotton seeds under salt

stress, but it could not reach the level without salt stress (Figure 1A). This finding is consistent with previous research results. Lei et al. pointed out that exogenous melatonin promoted the germination of cotton seeds under salt stress by regulating the internal hormone balance and the activity of antioxidant enzymes [20]. In addition, Chen et al. also conducted a similar experiment and found that under salt stress, melatonin could improve the germination of cotton seeds through the regulation of osmoregulatory substances and ion homeostasis, which further confirmed the potential role of melatonin in improving the seed germination rate [17]. Further, we measured seed vitality by the TTC method [31,42] and found that 20 μM melatonin treatment could significantly improve the germination potential and seed vigor, which was consistent with our results (Figure 1C). These studies together elucidated the mechanism of melatonin promoting seed germination, and they provide a scientific basis for us to deeply understand the role of melatonin in plant stress resistance. However, the effect of melatonin on the germination process of cotton seeds was not significant under the condition of no salt stress, which may indicate that the effect of melatonin on plants is regulated by abiotic stress conditions (drought, cold, heat, salinity, chemical pollutants, herbicides, UV irradiation, etc.) [43].

In this study, we observed that melatonin treatment had a significant impact on the development of cotton seedlings, which was reflected in the positive effect on the antioxidant system of cotton seedlings leaves, the regulation of photosynthesis and transpiration, the enhancement of nutrient absorption and the increase in aboveground biomass.

Firstly, we found that the application of melatonin significantly increased the antioxidant enzymes activities in cotton seedling leaves, which was consistent with the reported results [17]. Melatonin treatment increased the activity of antioxidant enzymes in leaves such as SOD, POD, and CAT (Figure 2A–F), and the content of non-enzymatic antioxidant ASA in the leaves and roots of cotton seedlings (Figure 2I,J), which can enhance the ability of cotton seedling leaves to remove reactive oxygen species (ROS) [21] and decrease the MDA content in cotton leaves (Figure 2G). SOD acts as the first step in the antioxidant reaction, converting superoxide anions into hydrogen peroxide and oxygen, while POD and CAT further break down hydrogen peroxide to produce water and oxygen. ASA acts as a non-enzymatic antioxidant that clears ROS directly and acts by regenerating other antioxidants such as carotenoids and vitamin E [44]. On the whole, melatonin can reduce the damage caused by oxidation under salt stress by increasing the antioxidant enzyme activity and ASA content of cotton seedlings, promoting the growth of cotton seedlings under salt stress.

Under salt stress, plants undergo "physiological drought" due to the reduction in turgor pressure of root tips and young leaf cells caused by salt stress, leading to stomatal closure and reduced transpiration [11]. This results in insufficient carbon accumulation in plants, ultimately inhibiting plant growth [45]. Our findings demonstrated that melatonin regulated the photosynthesis and transpiration of cotton seedlings: cotton seedlings treated with melatonin exhibited a higher intercellular carbon dioxide concentration (Table 2), which was potentially attributed to melatonin promoting stomatal opening and enhancing carbon dioxide entry, providing more substrates for photosynthesis [46]. Melatonin also enhanced photosynthetic pigment activity and photo enzyme activity while improving the efficiency of carbon dioxide fixation [47]. Additionally, melatonin enhanced the water vapor boundary layer conductance of cotton seedling leaves (Table 3), which may optimize stomatal regulation by balancing transpiration and water use efficiency while promoting photosynthesis. As a regulator of plant growth, melatonin modulates plant hormones such as gibberellins (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA) and their signaling pathways, and accelerated nutrient absorption and utilization have been shown to promote cell division and elongation [16]. This concept is further substantiated by the findings that melatonin enhances the uptake of soil ammonium nitrogen, phosphorus, and potassium by cotton seedlings (Figure 6A,C,D). The results of the correlation analysis (Figure 8) indicated a significant positive correlation between antioxidant content and the cotton seedling nutritional organs as well as biomass in cotton seedings. Consequently, we

speculated that melatonin may directly or indirectly modulate antioxidant levels, mitigating oxidative damage during leaf photosynthesis, transpiration, and other metabolic processes in cotton seedings, ultimately accelerating the utilization of soil nutrients and promoting seedling growth.

It is crucial to emphasize that in alignment with the findings of Chen et al., the application of melatonin is insufficient for enhancing plants' capacity to withstand continuous stress [17]. Furthermore, melatonin-induced alterations in nutrient uptake in plants have the potential to disrupt soil nutrient homeostasis, unbalancing the nutrients in the soil [12]. We propose that melatonin treatment should be integrated with additional resilience strategies to ensure more comprehensive protection and foster robust development of the cotton seedlings.

4.2. The Effects of RAE Application on Seed Germination and Seedling Growth of Cotton

Similar to the mechanisms of melatonin [48], RAE may effectively maintain the homeostasis of photosynthetic pigments under soil stress by enhancing antioxidant oxidase activity and increasing antioxidant content. The results in our study demonstrated that cotton seedlings treated with RAE exhibited significantly higher levels of SOD, POD enzyme activities, and ASA content in cotton seedling roots compared to that in CK. Building upon our research team's previous study [22], we have identified 2-keto-gulonic acid, which is one of the main substances in RAE, as a crucial precursor involved in ASA synthesis [22]. ASA serves not only as an important antioxidant but also as a key regulator of plant growth [22]. The substantial increase in ASA may be attributed to its involvement in leaf photoprotection mechanisms such as excess excitation energy dissipation through non-photochemical quenching (NPQ) processes [49].

The photosynthetic and transpiration processes of cotton seedlings were effectively regulated by RAE. The increase in net photosynthetic rate suggests that RAE may enhance leaf carbon fixation capacity, potentially through the activation of protease activity, accelerating the process of carbon dioxide fixation and conversion [50]. The decrease in the intercellular partial pressure of carbon dioxide may reflect the regulatory effect of RAE on stomatal aperture, facilitating improved photosynthetic efficiency and reduced water transpiration loss [51].

Under saline stress, the root system is the most vulnerable to be damaged compared to other plant parts, making it the most affected area for cotton seedlings [8]. The strength of the root system determines the plant's nutrient uptake capacity under stress, which ultimately reflects in underground and aboveground biomass [11]. The findings of this study demonstrate that the application of RAE significantly enhanced the root morphological development levels (e.g., total root length, root surface area, root volume) and underground biomass of cotton seedlings under salt stress (Table 2 and Figure 6C,D). According to the results of correlation analysis, this improvement may be attributed to a notable increase in the antioxidant ASA content of cotton seedling roots in RAE treatment (Figure 2J), while ASA serve as a regulatory substance for plant growth and signal transduction, influencing cell division and expansion regulation [52].

The RAE also plays a crucial role in enhancing the soil environment. The results in our study demonstrated that the application of RAE significantly enhanced the content of ammonium nitrogen and decreased the content of nitrate nitrogen in the tested soil, and it significantly decreased the pH value on the 8th to the18th day during the cultivation of cotton seedlings (Figure 6A,B,G). It may be associated with RAE's ability to regulate soil pH and promote soil microbial activity, finally accelerating the mineralization process of soil nutrients [22,23]. The results of the correlation analysis revealed a significant positive correlation between soil urease activity and ammonium nitrogen content (Figure 8), further substantiating the aforementioned hypothesis. Concurrently, RAE increased the soil cation exchange capacity (CEC), indicating that RAE enhanced the soil's ability to retain nutrient elements, which is crucial for plants in soil nutrients uptake and healthy growth [53]. The increase in soil CEC also enhanced the soil's adsorption capacity for sodium ions and

other metal ions in saline–alkali soils, reducing their detrimental effects on plants [54]. The correlation analysis results showed a significant positive correlation between soil CEC and the biomass of cotton seedlings (Figure 8), further confirming that the increased CEC may facilitate the cotton's growth. Furthermore, the enhanced catalase, protease, and phosphomonoesterase activities in RAE treatment are instrumental in enhancing the soil environment and stimulating the activation of soil nutrients [55–57], indicating their close association with cotton seedling growth and development, according to the positively correlated with cotton seedling biomass (Figure 8).

In summary, the RAE exerted promotive effects on cotton seedling growth and development by enhancing root growth, mitigating oxidative damage, and regulating photosynthesis efficiency. Furthermore, it also enhanced the soil microenvironment, soil fertility, and enzyme activity to provide an optimal soil environment for robust cotton seedling growth.

4.3. Synergistic Effects of Combined Application of Melatonin and RAE on Seed Germination and Seedling Growth of Cotton

The present study investigated the effects of the combined application of RAE and melatonin on cotton germination and seedling growth. Our results demonstrated that this combination not only lacked any antagonistic effect but also exhibited a positive "relay and complement" effect, suggesting a synergistic interaction between these compounds in plants.

The finds of this study indicate that the combined administration of melatonin and RAE significantly enhanced the SOD activities and ASA content in the roots of cotton seedlings, as well as CAT activities in their leaves, when compared to CK, R and MT, respectively. These results suggest a potential synergistic effect between these two compounds in bolstering the antioxidant defense system of cotton seedlings. This cooperative interaction is essential for equipping cotton plant roots to endure oxidative stress, protecting plant cells from damage caused by environmental stressors [58]. Under salt stress conditions, both plant and soil reactive oxygen species (ROS) levels are markedly elevated [59], leading to significantly greater stress than observed under non-saline conditions). Therefore, the combined application of melatonin and RAE may play a crucial role in enhancing the antioxidant capacity of cotton seedlings.

Furthermore, this combined treatment significantly increased the soluble sugar contents in the leaves and roots of cotton seedlings on the 10th day compared to the CK, R, and MT treatments, respectively. This potential enhancement is likely attributable to melatonin's modulatory effects on plant endogenous hormonal responses [48], coupled with the nutrient provision offered by RAE, which synergistically fortifies the plant's physiological mechanisms: maintaining cellular water balance and alleviating adverse effects caused by physiological drought induced by salt stress on cotton seedling growth.

We observed that the combined application resulted in a significant increase in the photosynthetic pigment (chlorophyl A, total chlorophyll and carotenoid) contents in cotton seedling leaves. This suggests that melatonin and RAE may synergistically promote photosynthetic pigment synthesis, leading to improved leaf photosynthetic efficiency (Figure 4). Additionally, this combined treatment positively influenced overall plant productivity [60], as evidenced by enhanced root development (Table 2) and increased biomass accumulation (Figure 5). The enhanced development of roots can improve the capacity for water and nutrient absorption, promoting robust plant growth [61]. Furthermore, nutrient uptake in the plant rhizosphere is primarily facilitated through two mechanisms: "interception" and "mass flow" [62,63]. The former depends on root development with interceptive capacity increasing as roots expand. The latter relies on crop transpiration to induce the movement of water and nutrients toward the root surface. Our findings indicated that the application of MT markedly elevated the transpiration rate in cotton seedlings, whereas RAE significantly bolstered the comprehensive root growth of these seedlings. Consequently, this substantially improved the root system's ability to absorb nutrients from the soil and ultimately resulted in enhanced biomass accumulation [64].

The plant rhizosphere, through the secretion of organic matter, works in conjunction with soil to resist ionic stress [65] through adsorption, chelation, and other mechanisms. As the first line of defense against ionic stress for crops [65], the adsorption capacity of rhizosphere soil for these ions is crucial, and this capacity is positively correlated with the soil's cation exchange capacity (CEC) value [63].

The organic matter secreted by plant roots, in conjunction with the soil, acts to mitigate ionic stress on plant growth through processes such as the adsorption and chelation of ions [65]. The adsorption capacity of the soil in the plant rhizosphere is positively correlated with its CEC value [53]. In this study, it was observed that the CEC in the MR treatment was significantly higher than that of other treatments on day 18 of cultivation (Figure 6E). Correlation analysis revealed a significant positive relationship between CEC and photosynthetic pigments, particularly chlorophyll A, underground dry weight, and root volume (Figure 8, $p < 0.05$). We propose a potential mechanism by which MR enhances CEC value: on one side, melatonin improved the photosynthetic pigment content (Figure 4), enhancing organic matter accumulation efficiency in plants and facilitating its transport to roots [66], promoting the release of organic matter from roots into soil including compounds like citric acid and oxalic acid, and consequently improving soil adsorption and the chelation of ions in the rhizosphere. On the other side, RAE contains abundant soluble low molecular weight organic acids (LMWOAs), such as 2-keto-L-gulonic acid and oxalic acid; these LMWOAs possess similar abilities to adsorb and chelate cations as those secreted by plant roots like citric acid or oxalic acid. Therefore, synergistic effects between melatonin and RAE ultimately lead to a significant increase in the soil CEC value.

In summary, the combined application of melatonin and RAE significantly augmented the SOD activity in roots and CAT activity in leaves of cotton seedlings compared to a single application. Moreover, it promoted root system development and enhanced the soil's capacity for nutrient transformation and retention, elevated photosynthetic pigment content in cotton seedling leaves, and consequently resulted in a substantial increase in biomass under salt–alkali environments. These findings suggest that the synergistic interactions between endogenous hormones and external additives can significantly enhance plant growth efficiency and productivity (Figure 9).

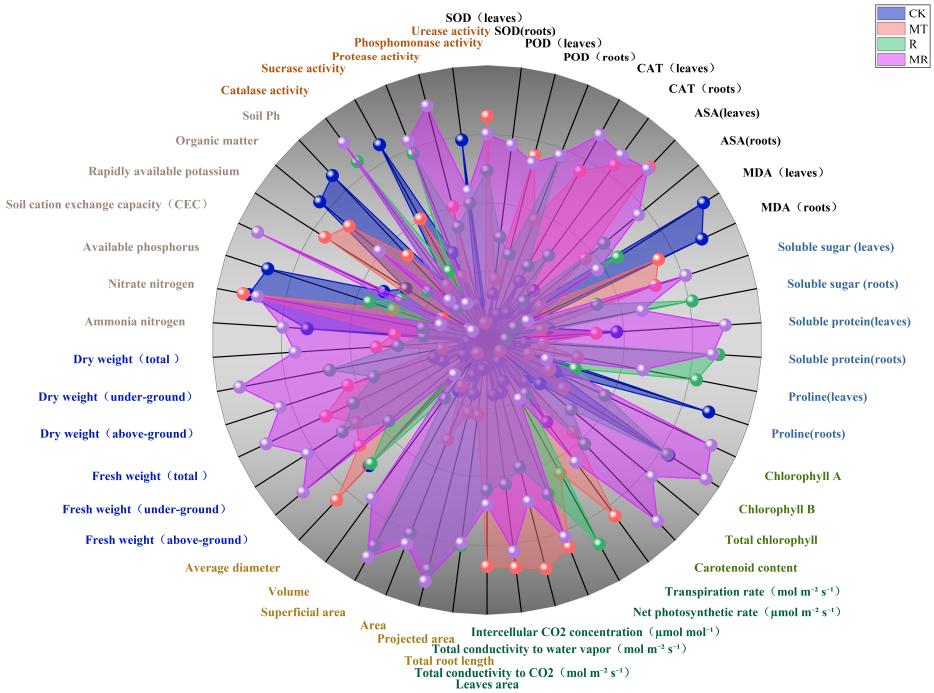

Figure 9. Physiological and agronomic indexes of cotton seedling and physicochemical properties of rhizosphere soil on 15th day.

5. Conclusions

In this study, we investigated the effects of melatonin and RAE application, both individually and in combination, on the seed germination and seedling growth of cotton in saline–alkali soil. Melatonin significantly enhanced the germination rate and seedling establishment under salt stress compared to the control group while also improving antioxidant capacity and photosynthetic efficiency in cotton seedlings. RAE improved root antioxidant capacity, promoted root growth, and effectively regulated nutrient levels in saline–alkali soil. The combined application of melatonin and RAE acted as a relay system for exogenous stimuli from seed germination to seedling growth by increasing the germination rate, enhancing the antioxidant capacity and osmotic regulation ability in cotton seedlings, promoting root and leaf development, significantly increasing biomass production, and improving the soil nutrients and environment, thus providing a solid foundation for the future growth and development of cotton plants. This study contributes to our understanding of cotton stress physiology while offering a novel theoretical basis along with an innovative approach for cultivating cotton in saline soils.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14122135/s1.

Author Contributions: X.Z., W.Y. and H.X. planned and designed the experiments; X.Z. and Y.W. performed the experiments; X.Z., H.S. and M.G. analyzed the data; X.Z. and W.Y. wrote the manuscript; W.Y. and H.X. eliminated grammatical errors. All authors have read and agreed to the published version of the manuscript.

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