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Effects of *Serendipita indica* on the Morphological and Physiological Characteristics of *Agrostis stolonifera* L. Under Drought Stress

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Abstract: This study investigates the effect of *Serendipita indica* inoculation on the growth, structural characteristics of leaf epidermis, photosynthetic parameters, and antioxidant and osmoregulation capacities of *Agrostis stolonifera* L. under different drought stresses (normal moisture management: at 70–75% of the field capacity, low drought: at 55–60% field capacity, moderate drought: at 40–45% of the field capacity, and severe drought: at 25–30% of the field capacity). The results showed that inoculation with *S. indica* significantly enhanced the growth potential of *A. stolonifera* compared to uninoculated controls, and then under drought stress conditions, inoculation with *S. indica* significantly alleviated the inhibition of the growth and development of *A. stolonifera*, especially under mild and moderate drought stresses. These improvements were evident in both aboveground and underground parts, leaf relative water content, total root length, and root surface area after 25 days of drought treatments. Inoculated plants also exhibited higher levels of photosynthetic pigments, net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) under drought conditions. Additionally, *S. indica* inoculation significantly increased the activities of catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), as well as the soluble sugar, soluble protein, and proline levels under drought-stressed and non-stressed conditions. In addition, the increases in the malondialdehyde (MDA) content and relative conductivity (RC) of leaves were significantly lower in the inoculated group compared to the control group. In conclusion, the symbiosis with *S. indica* promotes the growth of *A. stolonifera* under drought stress, likely by enhancing photosynthesis, osmoregulatory substances, and antioxidant enzyme activities.

Keywords: *Agrostis stolonifera* L.; *Serendipita indica*; drought stress; plant growth; physiological indices

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1. Introduction

Currently, drylands cover approximately 41% of the Earth's surface, with projections indicating a 23% increase by the end of the 21st century [1,2]. As global warming intensifies and environmental temperatures continue to rise, extreme droughts are expected to become more frequent, prolonged, and widespread. The increased evaporation, coupled with reduced surface runoff, have already had negative effects on grassland vegetation growth in the Yangtze River Basin [3], and the situation is even more critical in arid and semi-arid

regions where water is scarce [4]. Drought stress is now a major factor affecting plant growth; impairing stomatal movement, respiration, and photosynthesis; and ultimately disrupting physiological processes and metabolic functions [5,6]. As a result, drought has become one of the most significant environmental factors leading to plant death and grassland degradation. Consequently, finding effective strategies to mitigate the impact of drought on plant growth is a priority.

Serendipita indica, a plant endophytic fungus first isolated from shrub roots in 1998 by Verma et al. [7], shares similarities with arbuscular mycorrhizal fungi (AMFs) in its ability to colonize plant roots and enhance nutrient uptake from the soil [8]. Additionally, *S. indica* improves plant resistance to biotic and abiotic stresses [9–11]. Unlike AMFs, which have limitations in host plant selection and microbial preparation, *S. indica* has a broader applications range, being able to colonize Brassicaceae plants, such as *Brassica campestris* and *Chinese cabbage*, which AMFs cannot [12,13]. Moreover, *S. indica* can be cultured on artificial medium, facilitating its acquisition and study [14]. Previous studies have shown that inoculation with *S. indica* triggers a signaling cascade in plants, including the activation of oxidative signals, which promote plant growth and increase biomass and yield [15,16]. Moreover, *S. indica* significantly enhances plant resistance to stresses such as salinity, heavy metals, and drought [9,11,17]. Recent studies have shown that inoculating *Pinus taeda* roots with *S. indica* improves the biosynthesis of flavonoids and organic acids, thereby enhancing drought tolerance in seedlings [18]. Yu Wang et al. [19] also showed that *S. indica* mitigates oxidative damage in trifoliate orange caused by drought through the activation of its antioxidant defense systems. Similarly, it has been demonstrated that *S. indica* colonization improves the morphology, physiology, and metabolite levels of wheat, further promoting its growth, yield, and disease resistance [20]. In summary, *S. indica* holds significant potential for agricultural applications, particularly in improving nutrient uptake and reducing pesticide usage.

Agrostis stolonifera L., a cool-season turfgrass native to Eurasia, is one of the most important species globally for turf applications [21]. Known for its delicate texture, bright green color, cold hardiness, shade tolerance, and resistance to low pruning, *A. stolonifera* is a preferred choice for landscaping and golf greens [22,23]. However, its limited tolerance to heat and drought is a significant concern, particularly as drought stress becomes an increasing environmental challenge. Although previous studies have explored the physiological, molecular, transcriptomic, and proteomic responses of *A. stolonifera* to drought, research on mycorrhizal symbiosis remains limited [24–26]. Therefore, analyzing the different pathways of *A. stolonifera* that respond to drought threats and the molecules that play important roles in these pathways is crucial for current turfgrass breeding efforts. This study aims to investigate the symbiotic mechanisms between *A. stolonifera* and *S. indica*, with the goal of developing strategies to enhance drought resistance in *A. stolonifera*.

2. Materials and Methods

2.1. Preparation of S. indica

S. indica was provided by the Institute of Root Biology, Yangtze University and the subculture of fungi was carried out by the Germplasm Resources Evaluation and Innovation Center of *Phoebe* Nees and *Machilus* Nees, Yangtze University. To prepare the inoculum, 1 cm × 1 cm pieces of the fungal block were inoculated into liquid PDA (potato dextrose agar) medium and cultured in an incubator at 28 °C and 240 r/min for 7 days to obtain the fungal stock. Subsequently, the mycelium was then filtered using 20-mesh gauze and blended with distilled water to prepare a suspension at a concentration of 30 g/L.

2.2. Experimental Design

The experiment took place in mid-February 2024 in a glass greenhouse at the West Campus of Yangtzeu University in Jingzhou City, Hubei Province. A pot experiment was employed using full, uniformly sized seeds, which were purchased from Lvzhongcheng Seed Industry Co., Ltd., Nanjing, China, for sowing. Before sowing, the seeds were soaked in a 50% carbendazim solution (800 times dilution) for 15 min, rinsed 2–3 times with distilled water, dried, and set aside. Seeds were then sown in a sterilized substrate (*V* pastoral soil/*V* vermiculite/*V* perlite = 4:1:1), with 60 seeds sown per pot (upper caliber 11.5 cm, height 11 cm).

The experiment design included two inoculation treatments: inoculation with (+*Si*) and without (−*Si*) *S. indica*. Four moisture treatments were applied: normal moisture management (CK) at 70–75% of the field capacity, low drought (LD) at 55–60% of the field capacity, moderate drought (MD) at 40–45% of the field capacity, and severe drought (SD) at 25–30% of the field capacity. A completely randomized design was used, resulting in eight treatment combinations (CK − *Si*, LD − *Si*, MD − *Si*, SD − *Si*, CK + *Si*, LD + *Si*, MD + *Si*, and SD + *Si*), with five pots per treatment and three replicates, for a total of 15 pots per treatment.

2.3. Establishment of *S. indica*–*A. stolonifera* Symbiosis and the Drought Stress Treatment

S. indica inoculation started on the day of sowing. For the inoculated group (+*Si*), 50 mL of the mycorrhizal solution was added to each pot along the contact surface, while the non-inoculated group (−*Si*) received an equal volume of distilled water. Inoculations were repeated every 7 days. The colonization rate was determined after inoculation with three doses of *S. indica*, and after the fifth inoculation, the colonization rate was greater than 50% and then drought stress treatment was initiated.

The soil moisture content was determined using a soil moisture meter at the same time period each morning, with six randomized selected pots per treatment. Drought stress officially began when each of the four water treatments (CK, LD, MD, and SD) reached their target soil water content (recorded as 0 d). During the drought treatment, potted plants were watered manually every day according to weight loss using the pot weighing method. The stress period lasted for 25 days, and relevant morphological and physiological biochemical indices were determined.

2.4. Detection of *A. stolonifera* Colonization by *S. indica*

Root system colonization was examined using the Trypan blue staining method [27]. A total of 30 pots from the inoculated group were randomly selected, and the intact root system of three plants per pot were washed with distilled water. The 120 root segments were then stained with Trypan blue, decolorized, and prepared on glass slides. Colonization by *S. indica* was observed under a light microscope, and the colonization rate was determined. Colonization was recorded as successful when the number of colonized roots was equal to or greater than the number of roots expected to be colonized. The colonization rate (CR) was calculated using the following formula:

$$\text{CR}\ (\%) = (\text{NCR}/\text{TNOR}) \times 100\% \quad (1)$$

NCR is the number of root segments showing colonization, and TNOR is the total number of root segments observed.

2.5. Preparation of Freehand Sections for Observations of the Leaf Subepidermal Structure

The middle section of *A. stolonifera* leaves was randomly selected, rinsed with distilled water, and prepared using the freehand sectioning method described by Cheng [28]. The

sections were photographed under a light microscope, and the morphological characteristics of the lower epidermal cells and stomata in the middle leaf section were measured and recorded using ImageJ 1.53t image analysis software. The measured parameters included length, width, and density per unit area. The aspect ratio, density, and stomatal index were then calculated [29]. Each index was measured at least 50 times, and the experiment was repeated three times. And the stomatal index (I) was calculated using the formula:

$$\text{Stomatal index (I)} = S/(S + P) \times 100\% \quad (2)$$

The S is the number of stomatal apparatus per unit field of view and P is the number of epidermal cells per unit field of view.

2.6. Measurement of Physiological and Biochemical Indicators

Root systems were scanned using an EPSON scanner (v3.771), manufactured by Epson Co., Ltd., Tokyo, Japan, and root morphological parameters (total length, surface area, and mean diameter) were analyzed with WinRHIZO Pro 2007a. The fresh and dry weights of the aboveground parts for each treatment were measured using a precision balance.

The leaf relative water content (RWC) was determined using the drying method, The photosynthetic pigment content was measured using spectrophotometrically, superoxide dismutase (SOD) activity was determined by the nitrogen blue tetrazolium photo-oxidative reduction method, peroxidase (POD) activity was determined by the guaiacol method, the proline content was assessed using the ninhydrin method, the soluble sugar and protein contents were determined using anthrone colorimetry, the leaf relative conductivity was determined by the immersion method, and the malondialdehyde (MDA) content was determined using thiobarbituric acid colorimetric method. Then, the ascorbate peroxidase (APX) and soluble starch contents were assessed using Qiangsheng Wu's method [30]. Root vigor was determined using triphenyl tetrazolium chloride (TTC) staining [31], and catalase (CAT) activity was measured according to the method of Harper et al. [32]. A spectrophotometer was used to read the absorbance value of the sample, and then the corresponding content was calculated according to the formula in the method.

2.7. Data Analysis

The data were organized using Microsoft Excel 2010, and statistical analyses were performed using SPSS 26.0. Duncan's New Multiple Comparison Test was used to test the significance of differences between treatments. Graphs and images were processed using Origin 2022, Adobe Photoshop 2021, and ImageJ.

3. Results and Analysis

3.1. Colonization of S. indica

As shown in Figure 1A, the mycelium of *S. indica* exhibited a growth pattern that expanded concentrically from the center to the outer circle on solid PDA medium. Over time, the mycelium transitioned from white to light yellow and began producing a spore powder.

A microscopic examination of *A. stolonifera* roots was conducted after five consecutive inoculations with the *S. indica* solution. Thick-walled spores were observed in the roots colonized by *S. indica* (Figure 1B), while no such spores were found in uncolonized root systems (Figure 1C). Among the 90 randomly selected root segments from the inoculated group, colonization by *S. indica* was observed in 57 segments, resulting in a colonization rate of 63.3%. This successful colonization established the stage for subsequent drought stress treatments.

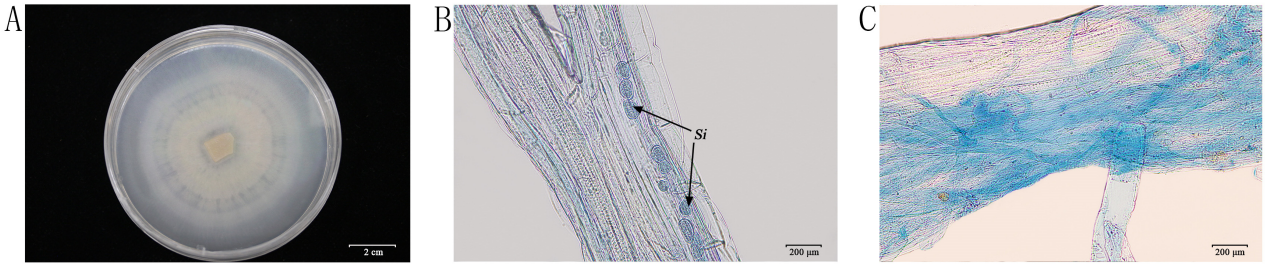

Figure 1. The colony morphology of *S. indica* and the microscopic examination of the root system. (**A**) The colony morphology of *S. indica*; (**B**) colonized roots; (**C**) uncolonized roots; *Si*: *Serendipita indica*.

3.2. Effects of Different Treatments on the Growth of *A. stolonifera*

Observations of *A. stolonifera* growth under various treatments revealed that as the drought intensity increased, plants exhibited wilting, tilting, yellowing, curling of leaves, and even drying and death (Figure 2A). Under normal water management conditions, the leaves of *S. indica*-inoculated plants were longer and greener. Low drought stress resulted in less severe wilting of inoculated plants, partially mitigating the negative effects of drought. However, under severe drought stress, the beneficial effects of *S. indica* were less pronounced, and plants continued to show signs of wilting and leaf dieback.

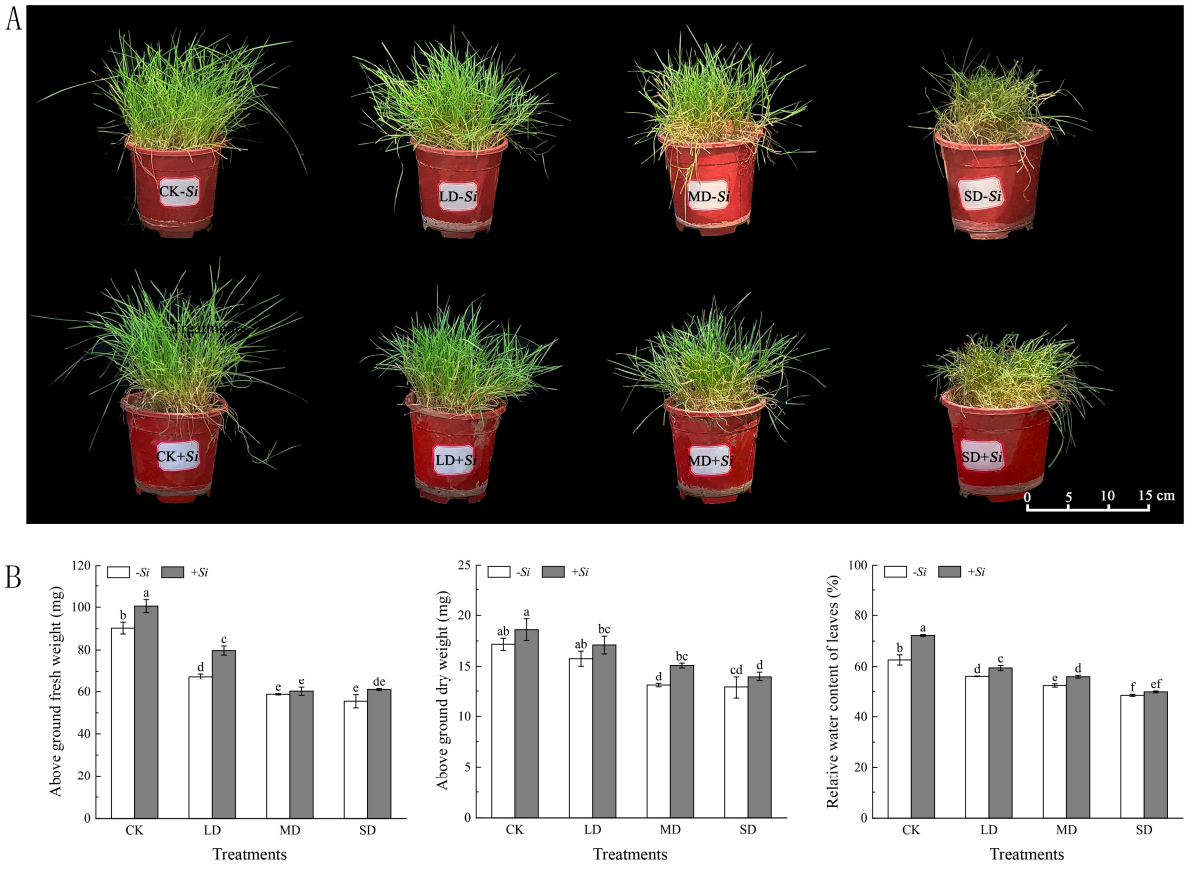

Figure 2. Effects of different treatments on the plant growth of *A. stolonifera*. (**A**) The phenotypic morphology of plants. (**B**) The above ground fresh weight, above ground dry weight, and relative water content of *A. stolonifera* under different treatments. The data are presented as the means ± SEs (n = 3); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought stress at 25–30% of the field capacity.

Under normal conditions, the fresh weight and leaf relative water content (RWC) of *A. stolonifera* inoculated with *S. indica* were significantly higher by 11.4% and 15.3%, respectively, compared to the non-inoculated group. Drought stress inhibited growth, with both fresh and dry weights, as well as the leaf RWC, decreasing as stress levels increased. However, *S. indica* inoculation mitigated the decline in the aboveground biomass under equivalent stress conditions. For example, in the LD + *Si* group, the fresh weight and leaf RWC increased by 18.3% and 5.8%, respectively, compared to the LD − *Si* group. Similar trends were observed under moderate drought stress, where the dry weight of the aboveground portion and leaf RWC increasing by 15.3% and 7.3%, respectively. Although the aboveground biomass in the SD + *Si* group was greater than that in the SD − *Si* group, the difference was not statistically significant.

3.3. Effects on the Root Development of A. stolonifera

Drought stress negatively impacted root development, with more severe stress leading to poorer root growth (Figure 3A). After the inoculation with *S. indica*, root development in *A. stolonifera* was significantly enhanced compared to the uninoculated treatment groups, with increases in both primary and fibrous root numbers. Under normal water management, inoculation with *S. indica* resulted in significant increases of 53.4%, 58.7%, and 25.8% in total root length, root surface area, and root vigor, respectively. In addition, under drought conditions, root indices generally decreased with increasing stress. However, the LD − *Si* group exhibited increases in total root length, root surface area, and mean root diameter compared to the CK − *Si* group, with the total root length increasing significantly by 5.9%. In the MD − *Si* group, the mean root diameter increased significantly by 48.2% compared to the LD − *Si* group. Inoculation with *S. indica* significantly improved root metrics under equivalent stress conditions. For instance, in the LD + *Si* group, the total root length, root surface area, and root vigor increased by 32.6%, 41.5%, and 7.0%, respectively. Similar improvements were observed under moderate drought stress, with increases of 30.2%, 31.4%, and 25.8%. However, the effects of *S. indica* were not significant under severe drought stress.

3.4. Effects of Different Treatments on the Morphology and Indices of the Lower Epidermis of A. stolonifera Leaves

As shown in Figure 4A, under drought stress, leaf epidermal cells gradually shriveled and lost water, the stomata were closed, and the density of lower epidermal cells and stomata per unit area increased, but the shrinkage of epidermal cells was slightly alleviated after inoculation with *S. indica*. The cellular and stomatal parameters of the lower epidermis of *A. stolonifera* leaves were determined, then the results are shown in Figure 4B. Under normal water management, inoculation with *S. indica* increased the cell width and stomatal length by 11.5% and 9.9%, respectively. Under drought stress, the cell length, width, and stomatal dimensions decreased with increasing stress levels, while the cell density, stomatal density, and stomatal index exhibited overall increases. Compared to the CK − *Si* group, the LD − *Si* group exhibited significant decreases in cell length and width of 15.4% and 7.7%, respectively, while cell and stomatal densities increased significantly by 35.9% and 36.4%. Similar trends were observed in the MD − *Si* and SD − *Si* groups.

Inoculation with *S. indica* effectively mitigated the decreases in cell and stomatal dimensions, alleviating some of the effects of cell water loss and crumpling under mild and moderate drought conditions. Specifically, the cell aspect ratio, stomatal length, and stomatal width in the MD + *Si* group increased significantly by 22.5%, 8.7%, and 17.3%, respectively, while the stomatal density and stomatal index decreased significantly by 19.5% and 16.0% compared to the MD − *Si* group. Some indices showed improvements but did not reach statistical significance under mild and moderate drought stress conditions.

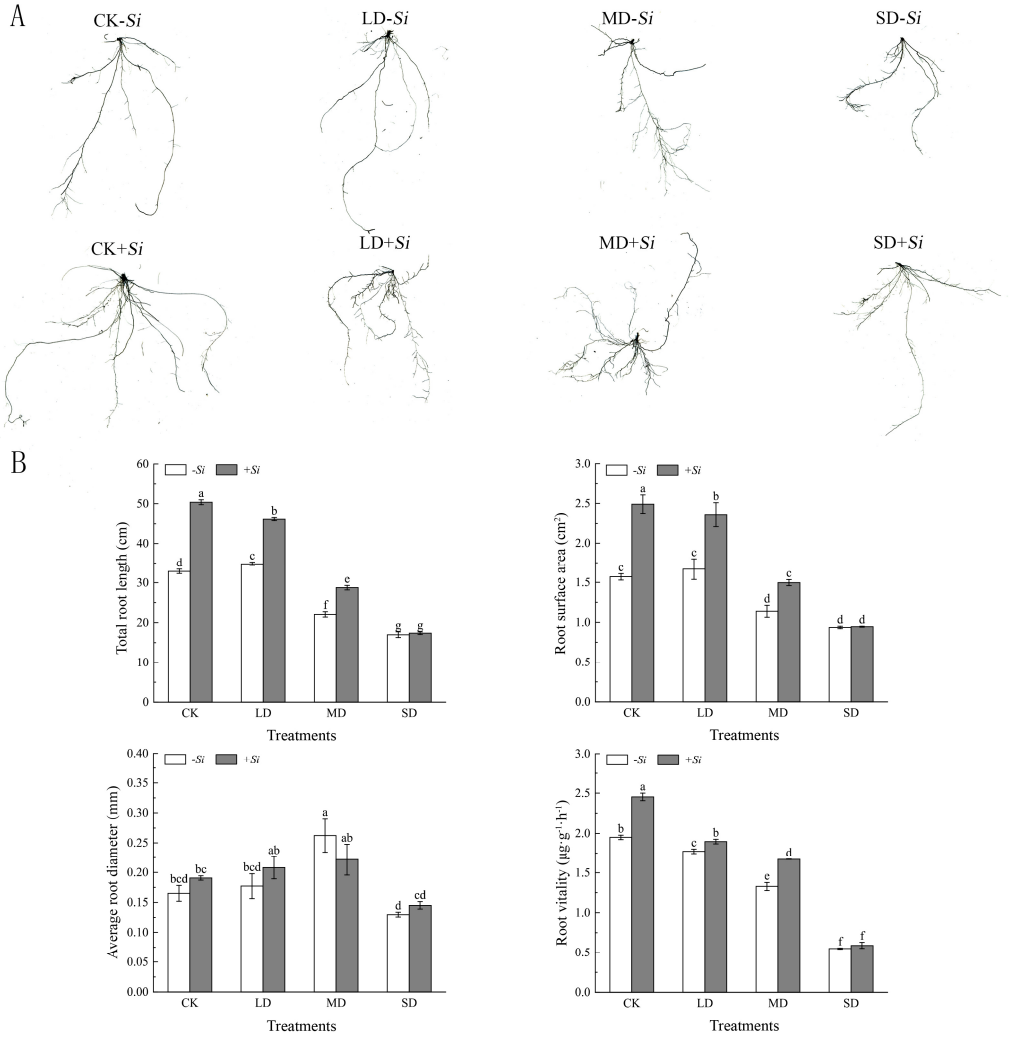

Figure 3. Effects of different treatments on the root morphology and development of *A. stolonifera*. (**A**) The root morphology of plants. (**B**) The total root length, root surface area, average root diameter, and root vitality of *A. stolonifera* under different treatments. The data are presented as the means ± SEs (n = 5); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought stress at 25–30% of the field capacity.

3.5. Effects of Different Treatments on Photosynthetic Indices of A. stolonifera

The photosynthetic parameters of *A. stolonifera* were determined. As shown in Figure 5, under normal conditions, inoculation with *S. indica* significantly increased the contents of photosynthetic pigment and various photosynthetic parameters. Specifically, Gs and Tr increased by 6.7% and 41.6%, respectively, compared with the CK − *Si* group. Under increasing drought stress, the chlorophyll a (Chl a) content initially increased but then declined, while the levels of chlorophyll b, carotenoids, and other photosynthetic parameters consistently decreased. Notably, the total chlorophyll (Tchl) content, carotenoid (Car) content, Pn, Gs, Ci (intercellular CO_2 concentration) and Tr were significantly reduced by 20.6%, 16.2%, 19.2%, 25.9%, 65.5%, and 63.4%, respectively, in the SD − *Si* group compared to the MD − *Si* group. In treatment groups inoculated with *S. indica*, the levels of photosynthetic pigments and parameters were consistently higher than in uninoculated treatment groups under the same stress conditions. Specifically, the Chl a content, Chl b content, Car content, Gs, Ci and Tr in the LD + *Si* group were significantly higher than those in the

LD − *Si* group, with increases of 6.9%, 16.4%, 8.0%, 4.0%, and 5.6%, respectively. Similar trends were observed under moderate and severe drought stress conditions.

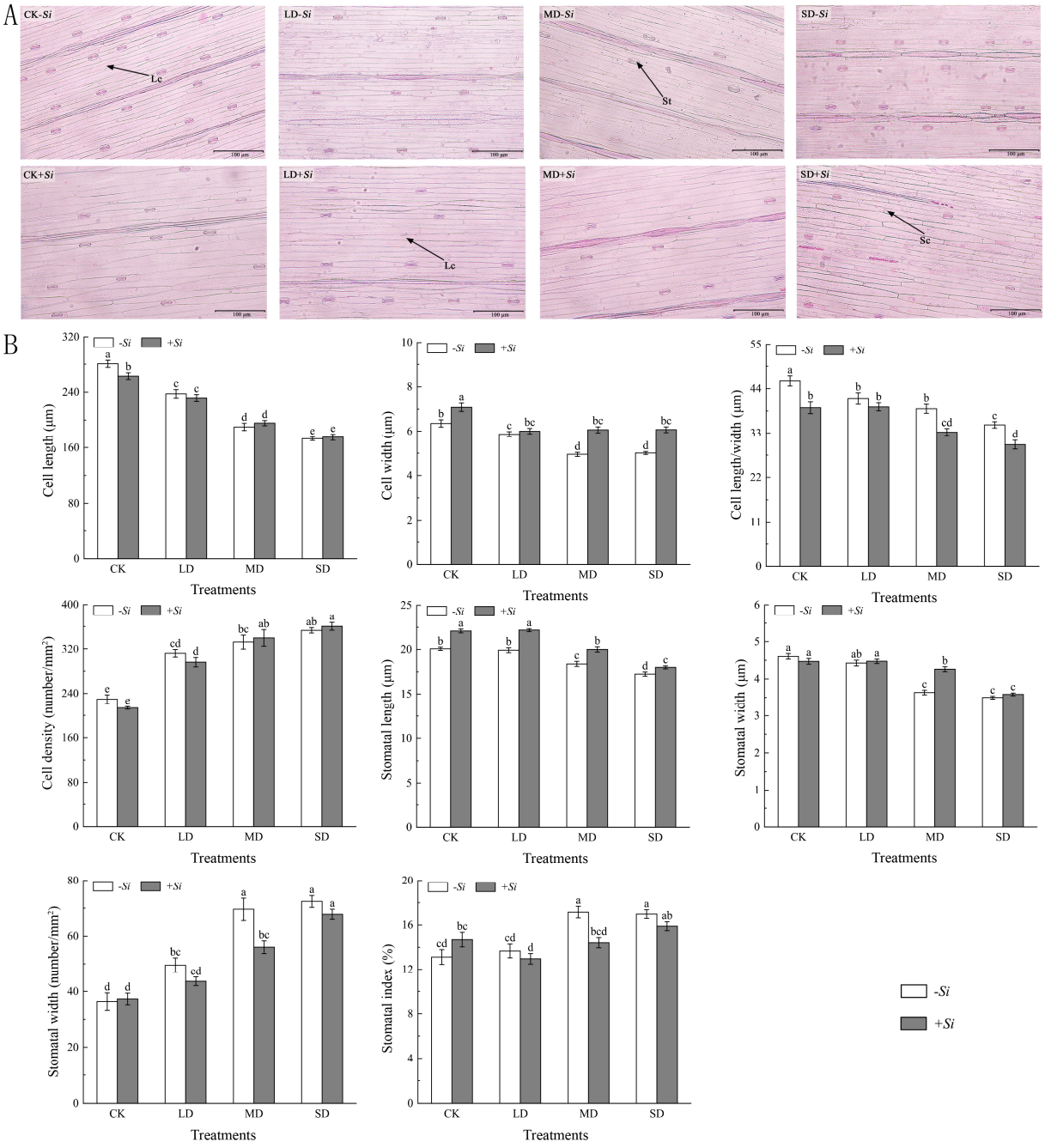

Figure 4. Effects of different treatments on lower epidermal characteristics of *A. stolonifera* leaves. (**A**) Lower epidermal structure of the leaves under a light microscope. (**B**) Parameters (length, width, and density) related to lower epidermal cells and stomata. The data are presented as the means ± SEs (n = 30); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: Lc—long cells, St—stomata, Sc—short cells, *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought stress at 25–30% of the field capacity.

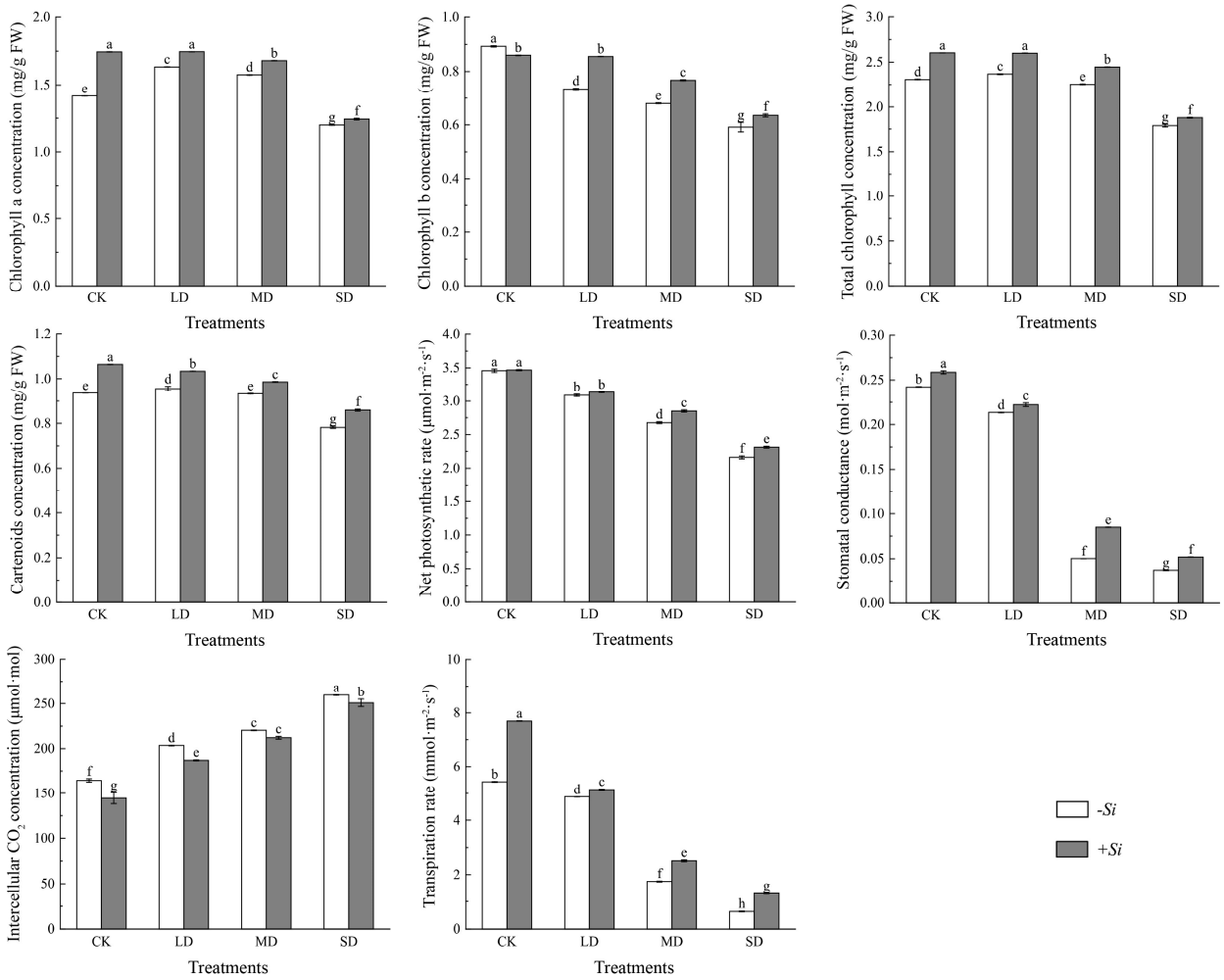

Figure 5. Effects of different treatments on photosynthetic pigment (Chl a, Chl b, Tchl, and Car) concentrations and gas exchange indices (Pn, Gs, Ci, and Tr) of *A. stolonifera*. The data are presented as the means ± SEs (n = 3); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought stress at 25–30% of the field capacity.

3.6. Effects of Different Treatments on Antioxidant Enzyme Activities in A. stolonifera

Drought stress induces plasma membrane peroxidation, leading to the excessive accumulation of reactive oxygen species (ROS) and a subsequent cellular osmotic imbalance. To mitigate oxidative damage, plants regulate the activity of their antioxidant enzymes to scavenge excess ROS [33]. As shown in Figure 6, under normal conditions, no significant differences in antioxidant enzyme activities were observed between the CK + *Si* and CK − *Si* groups. However, under drought stress, as the severity of the stress increased, CAT activity initially rose before declining, peaking under low drought stress. SOD activity decreased, with the LD − *Si* group showing a 45.9% reduction compared to the CK − *Si* group. Both CAT and APX activities increased, with larger increments observed under higher stress levels. Inoculation with *S. indica* significantly enhanced antioxidant enzyme activities. Specifically, CAT and APX activities were increased by 25.7% and 39.5%, respectively, in the LD + *Si* group compared to the LD − *Si* group, while POD activity increased by 20.3% in the SD + *Si* group compared to the SD − *Si* group.

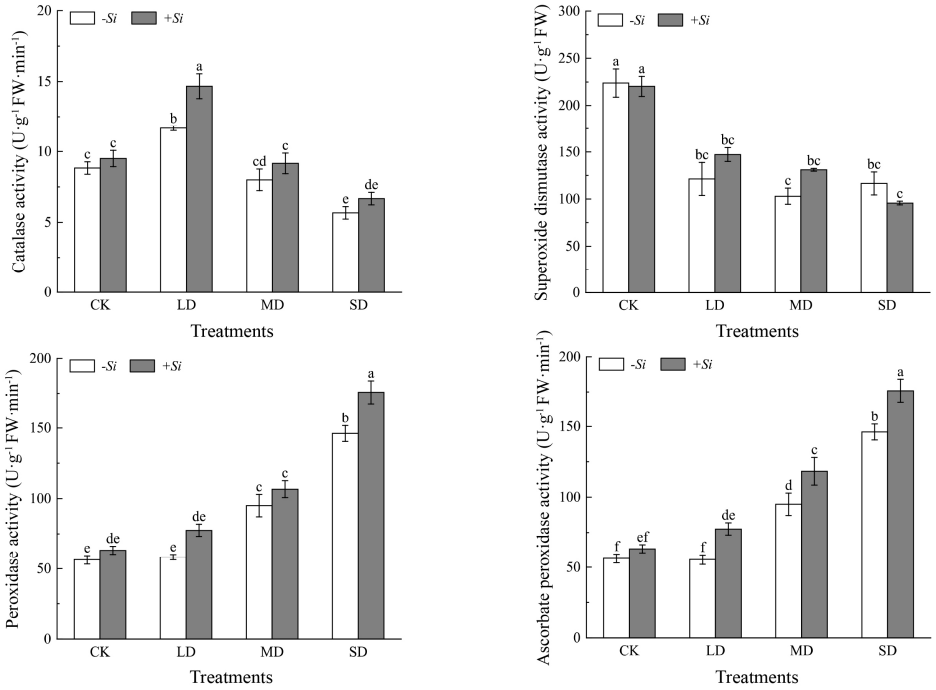

Figure 6. Effects of different treatments on antioxidant enzyme (CAT, SOD, POD, and APX) activities in *A. stolonifera*. The data are presented as the means ± SEs (n = 3); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought stress at 25–30% of the field capacity.

3.7. Effects of Different Treatments on Osmoregulatory Substances in A. stolonifera

Changes in osmoregulatory substances, such as soluble sugars and proline, help maintain osmotic balance under stress, thereby alleviating membrane damage [34]. As shown in Figure 7 under normal conditions, no significant differences were observed in the contents of soluble starch, soluble protein, and proline between the CK + *Si* and CK − *Si* groups. Under drought conditions, soluble protein content initially decreased but then increased, with a notable inflection point under mild stress. In addition, the soluble sugar, starch, and proline contents increased, with the MD − *Si* group showing significant increases of 72.3%, 24.5%, and 44.3%, respectively, compared to the LD − *Si* group. Inoculation with *S. indica* further enhanced the contents of soluble sugars, proteins, and proline while alleviating the increase in the soluble starch content. Specifically, the MD + *Si* group exhibited significant increases of 12.6% and 23.5% in the soluble sugar and protein contents, respectively, compared to the MD − *Si* group. Under severe drought stress, the SD + *Si* group showed significant increases of 9.7%, 11.3%, and 30.1% in the soluble sugar, protein, and proline contents, respectively, compared to the SD − *Si* group.

3.8. Effects of Different Treatments on the Malondialdehyde Content and Leaf Relative Conductivity in A. stolonifera

Drought stress destroys plant cells, leading to cytoplasmic leakage, which is indicated by an elevated MDA content and increased relative conductivity [35]. As shown in Figure 8, under normal conditions, the MDA content in the CK + *Si* group was significantly reduced by 13.4% compared to the CK − *Si* group. As the soil moisture content decreased, the MDA content and leaf relative conductivity increased, with the MD − *Si* group showing significant increases of 31.4% and 72.3%, respectively, compared to the LD − *Si* group. Moreover, the SD − *Si* group exhibited increases of 55.4% and 52.0%, respectively, compared

to the MD − *Si* group. Inoculation with *S. indica* significantly reduced the MDA content and relative conductivity under equivalent stress conditions. Specifically, the LD + *Si* group showed reductions of 24.5% and 9.8%, respectively, compared to the LD − *Si* group. Similar trends were observed under moderate and severe drought stresses.

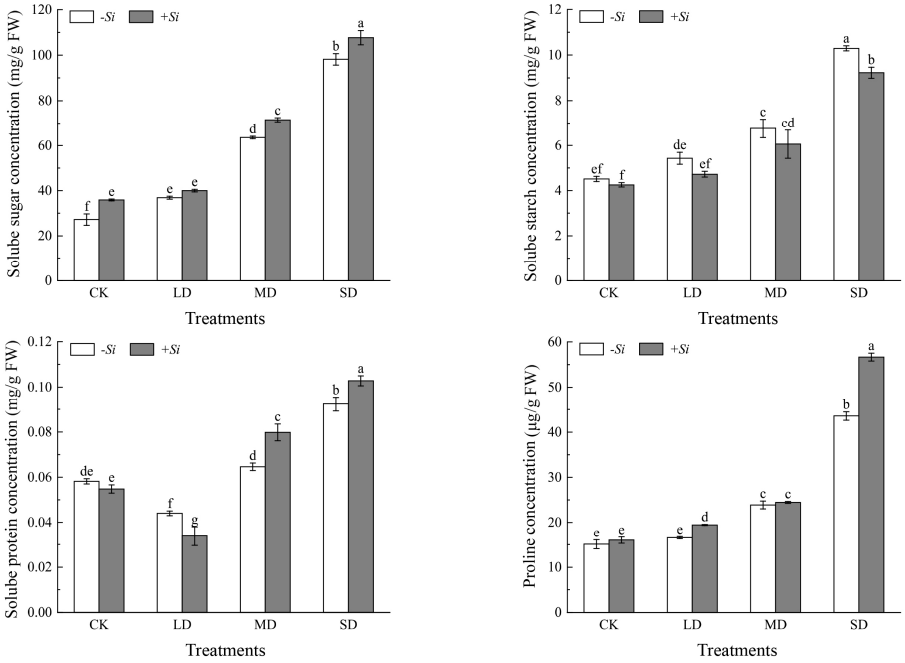

Figure 7. Effects of different treatments on osmotic regulatory substances (soluble sugar, soluble starch, soluble protein, and proline concentrations) in *A. stolonifera*. The data are presented as the means ± SEs (n = 3); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought at 25–30% of the field capacity.

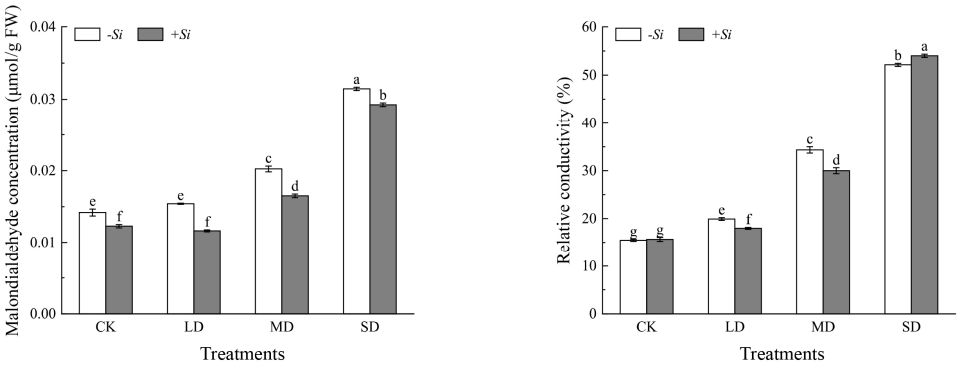

Figure 8. Effects of different treatments on the MDA contents and relative conductivity of *A. stolonifera*. The data are presented as the means ± SEs (n = 3); different letters above the bars indicate significant ($p < 0.05$) differences. Abbreviations: *Si*—*Serendipita indica*, CK—normal moisture management at 70–75% of the field capacity, LD—low drought stress at 55–60% of the field capacity, MD—moderate drought stress at 40–45% of the field capacity, SD—severe drought at 25–30% of the field capacity.

4. Discussion

Several root traits, such as root diameters, specific root length, and root length density, are associated with plant growth and productivity under drought stress [36]. Mild water scarcity prompts plant roots to elongate and increase their diameter and density to gain

more water from the soil; then, under severe drought stress for a long time, the plant root system will shrink, blacken, and gradually die [37]. So, the root system plays a critical in sensing drought signals, it is the first perceptual organ, and then it triggers morphological, physiological, and cellular responses in plants under drought stress [38]. *S. indica* forms a symbiotic relationship that improves root development and enhances nutrient absorption, leaf and shoot growth, and abiotic stress resistance [39]. Previous studies have shown that inoculation with *S. indica* increases the root length, lateral root numbers, and biomass, while also promoting aboveground growth parameters in *Brassica napus* L. [40], *Zea mays* L. [41], *Musa acuminata* [42], and *Chenopodium quinoa* [43]. In this study, as the soil moisture content decreased, *A. stolonifera* exhibited inhibited root growth, along with symptoms of wilting and yellowing. However, inoculated plants demonstrated better growth in plant height and biomass compared to uninoculated ones, indicating that *S. indica* had enhanced its drought tolerance. It is consistent with the results that inoculation of *S. indica* can alleviate the inhibition of aboveground and underground growth of *Citrullus lanatus* [44] and *Salvia officinalis* [45] seedlings under drought stress.

Leaves, as key organs for energy exchange, are particularly sensitive to environmental changes, and drought stress will cause plant leaf senescence and even death [46]. In response to a water deficit, plants close their stomata to reduce water consumption during transpiration, which leads to the shrinking of leaf cells and a reduction in intercellular gaps, causing the leaves to become thinner and wrinkled [47]. Drought conditions have been shown to reduce epidermal cell and stomatal dimensions while increasing cell and stomatal densities [48,49]. Then, under water stress, *S. indica* colonization can promote *Oryza sativa* L. stomata closure and increases the leaf surface temperature to increase water stress tolerance [50]. Research shows that an inoculation with *S. indica* will upregulate genes associated with chlorophyll synthesis, leading to increased levels of intermediates in leaves, which increase chlorophyll components, chlorophyll fluorescence parameters, and photosynthesis, improving plant growth under drought stress [51,52]. Our results showed that drought stress inhibited epidermal cell growth, resulting in higher cell and stomatal densities under stress. Inoculation with *S. indica* alleviated this damage, maintaining normal cell growth. Additionally, the contents of photosynthetic pigments decreased with increasing drought severity, particularly under severe stress, and inoculation with *S. indica* could only slow the downward trend to a certain extent, which aligns with the findings in *Dracocephalum moldavica* L. [53] and *Solanum melongena* L. [54]. Inoculation with *S. indica* also helps to maintain higher photosynthetic pigment levels, suggesting it has a protective effect on chloroplast damage and reduces chlorophyll degradation, consistent with the observations of Kaboosi et al. [55].

Changes in Pn, Gs, Ci and Tr indicated that decreased the photosynthetic capacity of *A. stolonifera* was primarily due to stomatal limitations [56]. Drought stress induced stomatal closure in *A. stolonifera*, limiting CO_2 uptake and reducing Pn, as similarly observed in *Zoysia* [57]. Moreover, our findings indicate that *S. indica* inoculation improves root access to soil moisture, providing more water for cell growth, reducing leaf crumpling, improving leaf hydration, and significantly alleviating declines in Cond, Pn and Tr, as reported in cotton studies by Saman et al. [58].

Reactive oxygen species (ROS) play dual roles in plant responses to abiotic stresses: they act an important signaling molecules but can also be toxic [47]. Excessive ROS accumulation during drought stress causes oxidative damage to cell membranes, with compounds like HO·, H_2O_2, and $O_2\cdot^-$ contributing to this damage [59]. Under mild stress, plants can mobilize their own antioxidant system to remove ROS, but when the stress is too severe, the system can become overwhelmed. Consequently, researchers have explored ways to enhance the oxidative defense system using exogenous substances. In this context,

some researchers found that *S. indica* can stimulate antioxidant enzymes and the expression of drought-related genes to help Chinese cabbage resist drought stress [60]. Then, *S. indica* inoculation was shown to increase the activity of antioxidant enzymes and the content of non-enzymatic antioxidants, thereby stimulating plant's antioxidant defense system and improving stress resistance [9,61]. Our study showed that SOD activity decreased with the soil moisture content, while POD and APX activities were also limited under severe stress. Inoculation with *S. indica* enhanced the activities of these antioxidant enzyme, effectively activating the antioxidant system in *A. stolonifera* and reducing membrane damage. MDA, a product of membrane peroxidation, and relative conductivity parameters increased with stress [62]. As we have found, *S. indica* inoculation significantly reduced MDA levels and conductivity under equivalent stress conditions. But this protective effect diminished as the stress severity increased, consistent with findings in studies of sorghum (*Sorghum hybrid sudangrass*) and Gerbera (*Gerbera jamesonii* L.) [63,64].

Osmoregulation plays a vital role in helping plants adapt to drought by maintaining the osmotic balance [34]. Our results showed increased levels of soluble sugars, starch, proteins, and proline in *A. stolonifera* under drought stress, with significant increases in the sugar and proline contents under severe stress. Inoculation with *S. indica* further enhanced the accumulation of these osmoregulatory substances, improving drought resistance, in line with Wu et al. [65].

5. Conclusions

Drought stress leads to cell crumpling, water loss, and cytosol leakage in *A. stolonifera*, disrupting the osmotic balance and exacerbating oxidative damage, which lead to reduced chlorophyll contents and a lower net photosynthetic rate. Inoculation with *S. indica* effectively mitigates these detrimental effects by enhancing antioxidant and osmoregulatory capacities, reducing cellular water loss, and proving more effective under low drought stress than under severe stress. After colonization, *S. indica* can promote the development of plant roots. On the one hand, it improved the ability of plant roots to obtain water from the soil; on the other hand, it could also promote the accumulation of osmotic pressure regulators, stimulate antioxidant enzyme activities, and then improve the stress resistance of plants.

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