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Effects of Exogenous Trehalose on Plant Growth, Physiological and Biochemical Responses in *Gardenia* Jasminoides Seedlings During Cold Stress

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Abstract: In order to explore the effect and mechanism of trehalose (Tre) on the growth and cold resistance of *gardenia jasminoides* Ellis under low-temperature stress, 15 mmol/L of Tre was used on *gardenia* seedlings under different degrees of low-temperature stress. The results show that −3 °C (low-temperature stress) significantly inhibited the growth of *gardenia*, while Tre at 15 mmol/L restored the plant height, number of leaves, total plant weight, and fresh weight of above ground and underground parts to 88.10%, 81.05%, 98%, 87.61% and 96.68% at 20 °C (normal temperature conditions). The total length of the root, number of lateral roots, total root surface area and root volume recovered to 88.48%, 74.08%, 104.03% and 83.77% under normal temperature at 20 °C. The chlorophyll content, chlorophyll fluorescence parameters and photosynthetic intensity parameters in the leaves of *gardenia jasminoides* were significantly decreased under low-temperature stress at −3 °C, while the contents of chlorophyll a, chlorophyll b and total chlorophyll were significantly increased under 15 mmol/L Tre treatment. Meanwhile, chlorophyll fluorescence parameters (φPSII, Fv′/Fm′ and qP increased by 18.60%, 73.17% and 81.82%, respectively) and photosynthetic intensity parameters (Pn, Gs, Ci and Tr increased by 33.33%, 70.86%, 14.83% and 116.50%, respectively) were also increased. At −3 °C, Tre treatment significantly increased the activities of SOD, POD and CAT in roots, and reduced the content of reactive oxygen species (H_2O_2 and superoxide anion). At the same time, the contents of root osmotic regulatory substances (proline and malondialdehyde) were decreased by 15.16% and 12.65%, respectively. At −3 °C, Tre significantly increased the root auxin content and significantly decreased the trans-zeanoside content, but had no significant effects on gibberellin and abscisic acid. Tre can also regulate the content of root respiratory metabolites under low-temperature stress, increase the malic acid content by 96.77% under −3 °C, and decrease the succinic acid content by 56.34%. In conclusion, Tre reduces the content of ROS and its damage by improving the antioxidant capacity in roots and enhances the osmoregulation and stability of cell membranes by reducing the content of osmoregulation substances, hormones and aerobic respiratory metabolites in the root, thus enhancing the cold resistance of *gardenia*.

Keywords: trehalose; low-temperature stress; *gardenia*; photosynthesis; hormone

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1. Introduction

Gardenia is suitable for growing in warm and humid environments, and is mainly distributed in tropical and subtropical regions. However, it is also found in temperate regions [1]. *Gardenia* is a type of four-season evergreen plant. The flowers have ornamental, medicinal, tea, dye extraction, oil extraction, spices and other uses. The fruits have gallbladder, liver protection, blood pressure reduction, swelling and sedation, antipyretic, anti-inflammatory and other functions [2].

As an abiotic stress, the low-temperatures often encountered in the process of crop growth and development cause physiological damage to crops, resulting in yield decline and economic losses. Short-term low-temperature stress slows the growth and development of crops and reduces the protoplasmic capacity. With the intensification of low-temperature stress, various tissues and organs in crops will be damaged, growth and metabolic activities will be inhibited, and cell death might even be caused [3]. The root system is an important organ in plants, as it absorbs water and nutrients [4,5]. After different degrees of low-temperature stress, the root system will show different changes. Under mild low-temperature stress, the root surface area and root volume increase, and the root tip cell structure remains intact. Under heavy and low-temperature conditions, root growth stops, the cell wall begins to disintegrate, and root tip cells become loose and cause death [6]. When plants are subjected to low-temperature stress, a large number of reactive oxygen species accumulate, destroying the dynamic balance between the production and removal of original free radicals in the cell [7]. Studies have confirmed that low-temperature stress significantly increases the production rate of reactive oxygen species in maize seedlings [8]. Superoxide dismutase (SOD), catalase (POD) and peroxidase (CAT) are a class of enzymatic systems that can effectively remove reactive oxygen species from plants [8]. Previous studies have shown that low-temperature stress significantly decreased the activities of three antioxidant enzymes (SOD, POD, CAT) in *Phalaenopsis* leaves, and that high-intensity low-temperature stress inhibited or destroyed the intracellular antioxidant enzyme system [9]. Under normal growth conditions, free radicals in rice undergo continuous generation and elimination, but under low-temperature conditions, the free radical generation rate in rice is far greater than the removal rate [10]. Osmotic adjustment is an important reflection of plants' adaptation to low temperatures. With a change in the external environment, the contents of the substances (proline, soluble protein and soluble sugar) in plants will change significantly, and their contents will be related to the tolerance of plants to low temperatures [11]. Research results have confirmed that the soluble protein content in plants is positively correlated with their ability to resist low temperatures [12]. As a protective substance in plant tissues, an increase in the soluble protein content can improve the water retention capacity of cells, reduce the freezing point of cells, and alleviate the damage caused by low-temperature stress to plants [12]. Proline can prevent water loss and protect membrane proteins, and its content changes greatly under the influence of temperature; it is of great significance for maintaining the integrity of cell membranes [13]. The soluble sugar content is the most sensitive index used to reflect plant metabolism under low-temperature stress [14].

Photosynthesis can promote the cold resistance of plants. The sugars (such as glucose) and amino acids produced by photosynthesis can directly increase the concentration of cell fluids, reduce the freezing point, and prevent the formation of intracellular ice crystals [15]. For example, cactus produces 'cactin' through photosynthesis, which can both resist drought and stabilize the cell membrane structure to resist low-temperature damage [16]. A high content of cis-unsaturated fatty acids can maintain the fluidity of the chloroplast membrane and ensure normal photosynthesis at low temperatures, thus enhancing cold tolerance [17]. Spraying potassium dihydrogen phosphate and other

fertilizers can improve the photosynthetic efficiency of leaves and increase the cell fluid concentration [18,19]. Exogenous substances supplement, such as the addition of Tre, can jointly improve the photosynthetic efficiency and cold resistance of plants, forming a virtuous cycle [20,21].

Tre can form a unique protective film on the cell surface under harsh environmental conditions such as high temperatures, low temperatures, high osmotic pressure and dry water loss, effectively protecting the structure of biomolecules from being destroyed, so as to maintain the life process and biological characteristics of living organisms [22]. Tre is a safe, stable and very reliable natural sugar that has a non-specific protective effect on biological macromolecules and organisms, especially under adverse conditions such as low temperatures, drought, salt damage and high heat, and has an efficient protective effect on a series of biological macromolecules that maintain normal plant life activities [23].

Exogenous Tre spray can protect plant proteins from damage under abiotic stress conditions such as low temperatures, drought and salt damage [24]. Studies have shown that exogenous Tre spray can reduce the malondialdehyde (MDA) content and relative permeability of the plasma membrane under salt stress, and increase SOD and POD activities [25]. Under high-temperature stress, exogenous Tre spray increased the ascorbic acid content, enhanced CAT and ascorbate peroxidase activities, and decreased the MDA and hydrogen peroxide content in wheat seedlings [26]. At the same time, the exogenous spray of Tre can improve POD and CAT activities and the ascorbic acid content of maize under drought stress, and alleviate oxidative damage under drought stress [27]. Under the condition of low-temperature treatment, exogenous Tre spray increased the relative water content, proline content and soluble sugar content of wheat, decreased the MDA content, and alleviated the damage caused by low-temperature stress [28]. Other studies have shown that after applying different concentrations of Tre, various indexes of muskmelon seedlings are improved and the root activity and soluble sugar content are increased, indicating that Tre has an alleviating effect on the physiological and biochemical characteristics of plants [29].

As a landscape plant, *gardenia* is suitable for growing in a warm and humid environment, but harsh climates such as a cold spring occur frequently, seriously affecting the growth of *gardenia*. We hypothesize that Tre can enhance the cold resistance of *gardenia* through various pathways such as photosynthesis, the antioxidant system, endogenous hormone metabolism and respiration. The main significance of this study is its provision of new technology and theoretical support for the cultivation of gardenia under low-temperature conditions.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment adopted a two-factor design: (1) Tre treatment (including 0 mmol/L Tre and 15 mmol/L Tre); (2) Low-temperature treatment (20 °C, 10 °C, 0 °C and −3 °C). The experiment consisted of 8 treatments with 5 replicates per treatment group (1 basin per replicate) and a total of 40 pots. In this experiment, *gardenia* seedlings (given by Jiangxi Academy of Forestry, Nanchang, China) were transplanted into plastic pots (with sterilized river sand) on 12 April 2023, and then the plastic pots were placed in an illumination incubator (18/6 h day/night) at four temperatures (20 °C, 10 °C, 0 °C and −3 °C). Hoaglang nutrient solution was irrigated from the day of planting, and the nutrient solution with a corresponding concentration of Tre was irrigated once every three days. Plants were harvested on 11 June 2023 after 2 months of Tre treatment at 4 temperatures and corresponding concentrations.

2.2. Plant Growth Index and Root System Configuration

Before harvest, the plant height was measured with a ruler (cm) and the number of fully unfolded leaves was measured by the counting method. After harvest, the total weight of the plants and the fresh weight of the above-ground parts and underground parts were measured by electronic balance. After the plants were harvested, root images were obtained using an Epson V700 color image scanner, and root configuration parameters (root length, number of lateral roots, total root surface area, root volume, etc.) were obtained using the root analyzer system (WinRHIZO, Regent Instruments Company, Québec City, QC, Canada).

2.3. Chlorophyll in Leaves, Chlorophyll Fluorescence Parameters and Photosynthetic Intensity Parameters

Before harvesting the plants, healthy and fully unfolded leaves were selected, and the content of chlorophyll a, chlorophyll b and total chlorophyll was measured after wiping the leaves with a clean wet cloth. The chlorophyll fluorescence parameters were determined by IMAGING-PAM (M-series modulated chlorophyll fluorescence meter, Heinz Walz GmbH, Nuremberg, Germany). Before harvesting, the *gardenia* treated with different treatments was completely unfolded and measured from 09:00 to 11:00. After dark adaptation treatment for 20 min, the blades were fixed on the loading platform. The actual photochemical efficiency (φPSII), maximum photochemical efficiency (Fv'/Fm'), non-photochemical quenching coefficient (NPQ) and photochemical quenching coefficient (qP) of the blades were measured to evaluate the light energy utilization efficiency. Before harvesting the plants, the photosynthetic parameters of the leaves were determined by a Li-6400 photosynthesator. Functional leaves at the 4th to 5th positions with a good physiological status were selected as the measurement objects, and parameters such as the transpiration rate (Tr), net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci) and stomatal conductance (Gs) were obtained.

2.4. Activities of Reactive Oxygen Species and Antioxidant Enzymes and Contents of Osmotic Substances in Roots

By using the hydroxylamine oxidation method, employing hydroxylamine (NH_2OH) and O2-specific reoxidation reaction, determining the absorbance at a 540 nm wavelength, and calculating the concentration of NO_2^- using the standard curve, the content of superoxide anion could be determined. Hydrogen peroxide (H_2O_2) is analyzed by titanium sulfate colorimetry. Titanium sulfate reacts with hydrogen peroxide to produce yellow precipitate. The precipitate can be used to detect the concentration of hydrogen peroxide by measuring the Optical Density (OD value) at 415 nm.

The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were determined by the nitrogen blue tetrazole photoreduction method, guaiacol color development method and ammonium molybdate color development method.

The proline (Pro) content was determined by sulfosalicylic acid extraction and the acid indanhydrin color development method. The product was obtained by this method and placed at a 520 nm wavelengths; then, the concentration of proline was converted by detecting the OD value of the wavelength. The soluble sugar was detected by the anthrone color development method. The product obtained by this method was placed at a 620 nm wavelength, and then the content of soluble sugar could be converted by detecting the OD value of the wavelength. The content of malondialdehyde was determined by the thiobarbituric acid (TBA) color development method. The concentration of malondialdehyde was obtained by determining the OD600, OD532 and OD450 of the reaction products on the spectrophotometer. The soluble protein content was determined by the Coomasil bright blue (G-250) staining method. Then, 20 μL of extract solution (enzyme solution) was added

to 80 μL, 0.05 mol·L^{-1} and pH 7.8 phosphate buffer. Then, 2.9 mL of Coomasil bright blue solution was added, and OD595 was measured after the reaction for 2 min. The soluble protein concentration was obtained by calculating the value.

2.5. Endogenous Hormones, Malic Acid and Succinic Acid in Roots

The endogenous hormones in roots were determined using various kits (Nanjing Jiancheng Bioengineering Research Institute Co., Ltd., Nanjing, China), such as the IAA ELISA kit, trans-zeaxin nucleoside (tZR) ELISA kit, abscisic acid (ABA) ELISA kit and gibberellin (GA3) ELISA kit. The above kits are based on the double antibody sandwich method, which detects the hormone content in plant samples. The malic acid and succinic acid contents were determined by high-performance liquid chromatography (HPLC): 1.00 g of *gardenia* root was accurately weighed and mixed with a blender, ground with 4 mL of extraction solution and centrifuged for 10 min at 10,000 r/min; the residue was added to 2 mL of extraction solution and then extracted, combined with the supernatant, dried in a water bath at 90 °C at a fixed volume of 10 mL, and then extracted with a disposable syringe after whirlpool mixing. It was filtered using a 0.45 μm filter membrane and analyzed using a machine.

2.6. Statistical Analysis

We performed a variance analysis (ANOVA, the GLM procedure) (SAS software 8.1v, SAS Institute, Gaston County, NC, USA) to statistically analyze the data. Microsoft Excel (Version 2013, Microsoft Institute, Redmond, WA, USA) was used for data processing and graphing, and Duncan's multirange experiment compared significant differences between treatments with $p < 0.05$.

3. Results

3.1. Effects of Trehalose on the Growth of Gardenia Under Low-Temperature Stress

The effect of Tre on the growth of *gardenia* under low-temperature stress is shown in Figure 1. The plant height, leaf number and fresh weight of *gardenia* were decreased by low-temperature stress. Compared with the 20 °C treatment, the 10 °C, 0 °C and −3 °C treatment significantly reduced the plant height by 9.12%, 13.78% and 20.54%, respectively (Table 1). Compared with 20 °C, the treatments at 10 °C and 0 °C had no significant effect on the number of leaves, total plant weight, and fresh weight of the above-ground and underground part, but treatment at −3 °C significantly reduced the number of leaves, total plant weight, and fresh weight of the above-ground and underground part by 43.81%, 24.48%, 22.39% and 23.46% (Table 1). In conclusion, low-temperature stress inhibited the growth of *gardenia*, especially at −3 °C.

Table 1. Effects of exogenous trehalose (Tre) on plant growth performance of *gardenia jasminoides* seedlings under different temperature conditions at 5 cm. Note: Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		Plant Height (cm)	Leaf Number (#)	Totol Weight (g)	Root Weight (g)	Shoot Weight (g)
0 mmol/L Tre	20 °C	29.16 ± 1.96 b	22.85 ± 1.12 b	12.01 ± 0.42 b	8.80 ± 0.58 b	4.22 ± 0.38 c
	10 °C	26.59 ± 1.89 c	22.83 ± 1.46 b	11.88 ± 0.88 b	7.83 ± 0.49 bc	4.24 ± 0.39 c
	0 °C	25.14 ± 2.34 c	23.85 ± 2.17 b	10.10 ± 0.79 c	6.84 ± 0.47 c	4.25 ± 0.21 c
	−3 °C	23.17 ± 1.78 d	12.84 ± 1.12 d	9.07 ± 0.63 d	6.83 ± 0.45 c	3.23 ± 0.22 d
15 mmol/L Tre	20 °C	32.04 ± 2.01 a	25.51 ± 1.54 a	15.68 ± 1.09 a	10.67 ± 1.01 a	6.02 ± 0.57 a
	10 °C	31.18 ± 2.36 a	23.59 ± 2.12 b	13.79 ± 1.28 ab	9.72 ± 0.58 ab	5.56 ± 0.48 ab
	0 °C	28.32 ± 1.75 b	22.54 ± 2.15 b	12.88 ± 1.07 b	8.75 ± 0.78 b	5.17 ± 0.39 b
	−3 °C	25.69 ± 2.17 c	18.52 ± 1.19 c	11.77 ± 1.06 b	7.71 ± 0.66 bc	4.08 ± 0.37 c

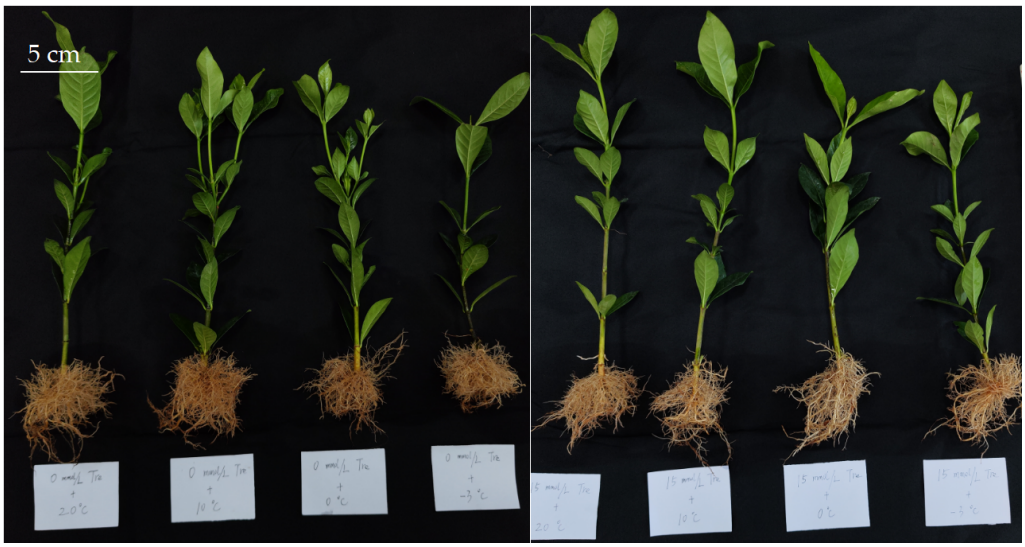


Figure 1. Whole plant morphology in *gardenia jasminoides* seedlings treated by exogenous trehalose (Tre) under different temperature conditions. Note: From left to right, these are 0 mmol/L Tre + 20 °C, 0 mmol/L Tre + 10 °C, 0 mmol/L Tre + 0 °C, 0 mmol/L Tre + −3 °C, 15 mmol/L Tre + 20 °C, 15 mmol/L Tre + 10 °C, 15 mmol/L Tre + 0 °C, 15 mmol/L Tre + −3 °C. Bar—5 cm.

It can also be seen from Table 1 that exogenous Tre significantly promoted the growth of *gardenia*. Compared with the 0 mmol/L Tre treatment, the 15 mmol/L Tre treatment significantly increased the plant height of *gardenia* at 20 °C, 10 °C, 0 °C and −3 °C by 9.88%, 17.26%, 12.65% and 10.88%, respectively (Table 1). Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment also increased the number of leaves, total plant weight, above-ground fresh weight and subsurface fresh weight of *gardenia* at different degrees, and the number of leaves increased by 11.64%, 3.33%, 5.49% and 44.24%, respectively. The total plant weight increased by 30.56%, 16.08%, 27.52% and 29.77%, the above-ground fresh weight increased by 21.25%, 24.14%, 27.92% and 12.88%, and the underground fresh weight increased by 42.65%, 31.13%, 21.65% and 26.32%, respectively (Table 1). It can be concluded that Tre can effectively promote the growth of *gardenia*.

What is noteworthy is that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment significantly alleviated the inhibitory effect of low-temperature stress on the growth of *gardenia*. Compared with the 20 °C treatment without Tre, the plant height, leaf number, total plant weight, above-ground and subsurface fresh weight of *gardenia* under low-temperature stress at −3 °C without Tre significantly decreased by 20.54%, 43.81%, 24.48%, 22.39% and 23.46%. However, the exogenous addition of 15 mmol/L Tre at −3 °C reduced the plant height, leaf number, total plant weight, and above-ground and subsurface fresh weight to 88.10%, 81.05%, 90.01%, 87.61% and 96.68% (Table 1). In conclusion, Tre can effectively alleviate the inhibitory effect of low-temperature stress on the growth of *gardenia*.

3.2. Influence of Trehalose on Root Configuration of Gardenia Under Low-Temperature Stress

The effects of Tre on the root configuration indexes of *gardenia* seedlings under low-temperature stress are shown in Figure 2. Low-temperature stress decreased the root length, lateral root number and root volume, but had no significant effect on the total root surface area. Compared with treatment at 20 °C, treatment at 10 °C, 0 °C and −3 °C reduced the total length of roots by 7.10%, 13.94% and 20.28%, respectively, and treatment at 10 °C, 0 °C and −3 °C reduced the number of lateral roots by 3.44%, 22.35% and 37.72%, respectively. Treatment at 10 °C, 0 °C and −3 °C reduced the root volume by 7.46%, 15.79% and 20.61%,

respectively (Table 2). It should be noted that compared with the 20 °C treatment, the root length, lateral root number and root volume were significantly reduced by 20.28%, 37.72% and 20.61% at −3 °C, but the total root surface area was not significantly affected (Table 2). In conclusion, low-temperature stress inhibited the root growth of *gardenia*, especially at −3 °C.

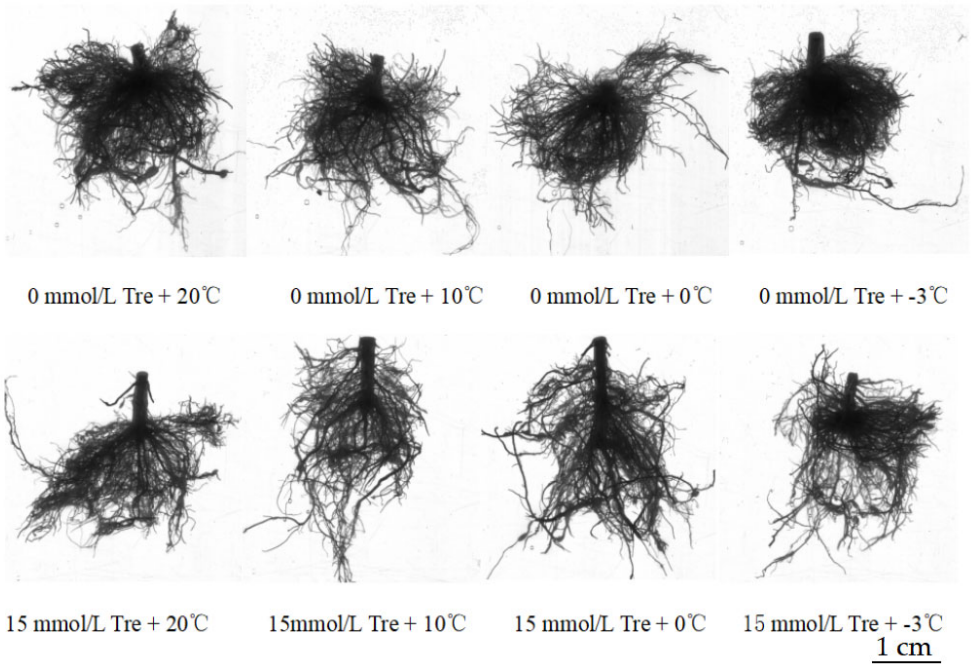


Figure 2. Root system architecture of *gardenia jasminoides* seedlings treated by exogenous trehalose (Tre) under different temperature conditions. Note: Bar—1 cm.

Table 2. Effects of exogenous trehalose (Tre) on root system architecture of *gardenia jasminoides* seedlings under different temperature conditions. Note: Data (means ± SD, n = 5) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		Total Root Length (cm/Plant)	Lateral Root Number (#)	Total Root Surface Area (cm^2/Plant)	Root Volume (cm^3/Plant)
0 mmol/L Tre	20 °C	142.73 ± 11.16 bc	58.75 ± 5.05 ab	10.41 ± 0.82 b	2.28 ± 0.12 a
	10 °C	132.59 ± 10.11 c	56.73 ± 5.36 b	10.51 ± 0.89 b	2.11 ± 0.20 ab
	0 °C	122.83 ± 10.14 c	45.62 ± 4.17 c	9.61 ± 0.63 b	1.92 ± 0.12 ab
	−3 °C	113.78 ± 10.12 d	36.59 ± 3.02 d	9.47 ± 0.83 b	1.81 ± 0.15 b
15 mmol/L Tre	20 °C	165.24 ± 14.05 a	60.68 ± 4.63 a	12.48 ± 1.09 a	2.26 ± 0.19 a
	10 °C	155.29 ± 12.06 ab	64.36 ± 5.84 a	13.08 ± 1.14 a	2.07 ± 0.18 ab
	0 °C	150.63 ± 13.09 b	63.37 ± 6.03 a	12.30 ± 1.17 a	2.05 ± 0.17 ab
	−3 °C	126.29 ± 10.07 c	43.52 ± 4.15 c	10.83 ± 1.02 b	1.91 ± 0.14 ab

Table 2 also shows that exogenous Tre significantly promoted the root development of *gardenia*. Compared with the 0 mmol/L Tre treatment, the 15 mmol/L Tre treatment significantly increased the total root length of *gardenia* at 20 °C, 10 °C, 0 °C and −3 °C by 15.77%, 17.12%, 22.63% and 10.99%, respectively (Table 2). Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment also increased the number of lateral roots, total root surface area and root volume to varying degrees, and the number of lateral roots increased by 3.29%, 13.45%, 38.91% and 18.94%, respectively. The total root surface area increased by 19.88%, 24.45%, 27.99% and 14.36%, and the root volume increased by 0.88%, 9.21%, 6.77% and 5.52%, respectively (Table 2).

What is noteworthy is that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment significantly alleviated the inhibitory effect of low-temperature stress on root growth and the development of *gardenia*. Compared with the treatment at 20 °C without

Tre, the total root length, lateral root number, total root surface area and root volume of *gardenia* under low-temperature stress at −3 °C without Tre significantly decreased by 20.28%, 37.72%, 9.03% and 20.61%. However, the total root length, lateral root number, total root surface area and root volume recovered to 88.48%, 74.08%, 104.03%, and 83.77% after the exogenous addition of 15 mmol/L Tre under −3 °C low=temperature stress (Table 2). In conclusion, Tre can effectively alleviate the inhibitory effect of low-temperature stress on the root growth of *gardenia*.

3.3. Effects of Trehalose on Chlorophyll Content of Gardenia Under Low-Temperature Stress

The effects of Tre on the chlorophyll content and chlorophyll fluorescence parameters of leaves of *gardenia* seedlings under low-temperature stress are shown in Table 3. The contents of chlorophyll a, chlorophyll b and total chlorophyll in *gardenia* were decreased by low-temperature stress. Compared with treatment at 20 °C, treatment at 10 °C, 0 °C and −3 °C reduced the chlorophyll a content by 3.81%, 35.24% and 49.52%, respectively, and 10 °C, 0 °C and −3 °C reduced the chlorophyll b content by 4.08%, 28.57% and 36.73%, respectively. Treatment at 10 °C, 0 °C and −3 °C reduced the total chlorophyll content by 1.32%, 32.45% and 43.71%, respectively (Table 3). It is interesting that compared with the 20 °C temperature treatment, the −3 °C treatment significantly reduced the contents of chlorophyll a, chlorophyll b and total chlorophyll by 49.52%, 36.73% and 43.71% (Table 3). In conclusion, low-temperature stress inhibited the synthesis of chlorophyll in leaves of *gardenia* to different degrees, especially at −3 °C.

Table 3. Effects of exogenous trehalose (Tre) on the chlorophyll content in the leaves of *gardenia jasminoides* seedlings under different temperature conditions. Note: Data (means ± SD, n = 5) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		Chlorophyl a (mg/g)	Chlorophyl b (mg/g)	Total Chlorophyll (mg/g)
0 mmol/L Tre	20 °C	1.05 ± 0.08 b	0.49 ± 0.04 ab	1.51 ± 0.12 b
	10 °C	1.01 ± 0.07 b	0.47 ± 0.04 ab	1.49 ± 0.11 b
	0 °C	0.68 ± 0.04 c	0.35 ± 0.03 c	1.02 ± 0.09 c
	−3 °C	0.53 ± 0.04 d	0.31 ± 0.02 c	0.85 ± 0.07 d
15 mmol/L Tre	20 °C	1.25 ± 0.11 a	0.55 ± 0.05 a	1.81 ± 0.12 a
	10 °C	1.12 ± 0.09 ab	0.50 ± 0.04 ab	1.61 ± 0.08 ab
	0 °C	1.04 ± 0.09 b	0.45 ± 0.04 b	1.50 ± 0.09 b
	−3 °C	1.01 ± 0.07 b	0.45 ± 0.03 b	1.46 ± 0.11 b

Table 3 showed that exogenous Tre significantly promoted the growth of *gardenia*. Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment significantly increased the chlorophyll a content of *gardenia* by 19.05%, 10.89%, 52.94% and 90.57%, respectively (Table 3). Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment also increased the chlorophyll b and total chlorophyll content to varying degrees, in which chlorophyll b increased by 12.24%, 6.38%, 28.57% and 45.16%, respectively. The total chlorophyll increased by 19.87%, 8.05%, 47.06% and 71.76%, respectively (Table 3). It can be concluded that Tre can effectively promote the synthesis of chlorophyll in *gardenia* leaves.

What is noteworthy is that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment significantly alleviated the effect of low-temperature stress on chlorophyll synthesis in *gardenia* leaves. Compared with the 20 °C treatment without Tre, low-temperature stress at −3 °C without Tre significantly reduced the chlorophyll a, chlorophyll b and total chlorophyll content of *gardenia* by 49.52%, 36.73% and 43.71%. However, the exogenous addition of 15 mmol/L Tre under −3 °C low-temperature stress caused the chlorophyll

a, chlorophyll b and total chlorophyll content to recover to 96.19%, 91.84% and 96.69% (Table 3). In conclusion, Tre can effectively alleviate the inhibition effect of low-temperature stress on chlorophyll synthesis in the leaves of *gardenia*.

3.4. Effects of Trehalose on Chlorophyll Fluorescence Parameters of Gardenia Under Low-Temperature Stress

The effects of Tre on the chlorophyll fluorescence parameters of *gardenia* under low-temperature stress are shown in Table 4. Low-temperature stress decreased the values of φPSII, Fv′/Fm′ and qP, but had no significant effect on NPQ. Compared with treatment at 20 °C, treatment at 10 °C, 0 °C and −3 °C can reduce φPSII by 3.23%, 25.81% and 30.65%, and Fv′/Fm′ by 6.17%, 28.39% and 49.38%, respectively. qP decreased by 6.45%, 58.06% and 64.52% at varying degrees (Table 4). Compared with the 20 °C treatment, although the 10 °C treatment had no significant effect on the φPSII, Fv′/Fm′ and qP, the −3 °C treatment significantly reduced the φPSII, Fv′/Fm′ and qP values (Table 4). It can be seen that the chlorophyll fluorescence parameters in leaves of *gardenia* were reduced to different degrees under low-temperature stress, especially under −3 °C low-temperature stress.

Table 4. Effects of exogenous trehalose (Tre) on the parameters of chlorophyll fluorescence in the leaves of *gardenia jasminoides* seedlings under different temperature conditions. Note: Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		φPSII	Fv′/Fm′	qP	NPQ
0 mmol/L Tre	20 °C	0.62 ± 0.06 a	0.81 ± 0.05 a	0.31 ± 0.02 a	0.81 ± 0.04 ab
	10 °C	0.60 ± 0.05 a	0.76 ± 0.06 ab	0.29 ± 0.02 a	0.82 ± 0.05 ab
	0 °C	0.46 ± 0.04 c	0.58 ± 0.04 c	0.13 ± 0.01 c	0.88 ± 0.05 a
	−3 °C	0.43 ± 0.03 c	0.41 ± 0.03 d	0.11 ± 0.01 c	0.89 ± 0.06 a
15 mmol/L Tre	20 °C	0.63 ± 0.04 a	0.82 ± 0.07 a	0.32 ± 0.02 a	0.79 ± 0.04 b
	10 °C	0.62 ± 0.04 a	0.78 ± 0.04 ab	0.30 ± 0.02 a	0.80 ± 0.06 b
	0 °C	0.59 ± 0.03 ab	0.72 ± 0.05 b	0.22 ± 0.02 b	0.82 ± 0.07 ab
	−3 °C	0.51 ± 0.04 b	0.70 ± 0.06 b	0.20 ± 0.01 b	0.84 ± 0.06 ab

Exogenous Tre improved the chlorophyll fluorescence parameters in leaves to varying degrees, as shown in Table 4. Compared with the 0 mmol/L Tre treatment, the φPSII values in the leaves of *gardenia* treated with 15 mmol/L Tre at 20 °C, 10 °C, 0 °C and −3 °C were increased by 1.61%, 3.33%, 28.26% and 18.60%, respectively (Table 4). Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the Fv′/Fm′ and qP values of the 15 mmol/L Tre treatment also increased by 1.23%, 2.63%, 24.14% and 70.73%, respectively. qP increased by 3.23%, 3.45%, 69.23% and 81.81%, respectively (Table 5). Compared with the 0 mmol/L Tre treatment, the 15 mmol/L Tre treatment at 20 °C, 10 °C, 0 °C and −3 °C reduced the NPQ value by 2.47%, 2.44%, 6.82% and 5.62%, respectively, but there were no significant differences (Table 4). It can be concluded that Tre can effectively improve chlorophyll fluorescence parameters in the leaves of *gardenia* and improve light use efficiency.

It is interesting that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment significantly alleviated the effect of low-temperature stress on chlorophyll fluorescence parameters. Compared with the 20 °C treatment without Tre, the values of φPSII, Fv′/Fm′ and qP were significantly reduced by 30.65%, 49.38% and 64.52% under low-temperature stress at −3 °C without Tre. However, the exogenous addition of 15 mmol/L Tre at −3 °C reduced the values of φPSII, Fv′/Fm′ and qP to 82.26%, 86.42% and 64.52% (Table 4). In conclusion, Tre can effectively alleviate the effect of low-temperature stress on chlorophyll fluorescence parameters in the leaves of *gardenia*, and enhance the light use efficiency.

Table 5. Effects of exogenous trehalose (Tre) on photosynthetic parameters in the leaves of *gardenia jasminoides* seedlings under different temperature conditions. Note: Data (means ± SD, n = 5) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		Pn (μmol/m^2.s)	Gs (μmol/m^2.s)	Ci (μmol/mol)	Tr (mmol/m^2.s)
0 mmol/L Tre	20 °C	8.11 ± 0.72 ab	2.81 ± 0.21 ab	250.03 ± 22.15 a	3.50 ± 0.29 a
	10 °C	7.84 ± 0.59 b	2.72 ± 0.13 b	245.08 ± 20.11 a	3.13 ± 0.29 ab
	0 °C	6.50 ± 0.34 c	1.82 ± 0.12 c	240.90 ± 18.19 a	2.24 ± 0.21 c
	−3 °C	5.19 ± 0.46 d	1.51 ± 0.11 d	209.07 ± 19.12 b	1.03 ± 0.10 d
15 mmol/L Tre	20 °C	8.91 ± 0.71 a	2.92 ± 0.27 a	255.68 ± 21.29 a	3.55 ± 0.21 a
	10 °C	8.02 ± 0.69 ab	2.85 ± 0.22 a	249.79 ± 15.18 a	3.22 ± 0.22 ab
	0 °C	7.32 ± 0.61 bc	2.69 ± 0.21 b	247.88 ± 16.37 a	2.85 ± 0.21 b
	−3 °C	6.92 ± 0.42 bc	2.58 ± 0.24 b	240.07 ± 14.22 a	2.23 ± 0.18 c

3.5. Effects of Trehalose on Photosynthesis of Gardenia Leaves Under Low-Temperature Stress

The effects of Tre on the photosynthetic intensity parameters of leaves of *gardenia* seedlings under low-temperature stress are shown in Table 5. Low-temperature stress decreased the Pn, Gs, Ci and Tr of *gardenia* at different degrees. Compared with treatment at 20 °C, treatment at 10 °C, 0 °C and −3 °C reduced the Pn by 3.33%, 19.85% and 36.00%, Gs by 3.20%, 35.23% and 46.26%, and Ci by 2.01%, 3.64% and 16.41%, respectively. Tr decreased by 10.57%, 36.01% and 70.57% at different degrees (Table 5). It is noteworthy that Pn, Gs, Ci and Tr at −3 °C showed the most serious decline, significantly decreasing by 36.00%, 46.26%, 16.41% and 70.57%, respectively, compared with the 20 °C treatment (Table 5). In conclusion, low-temperature stress inhibited the photosynthetic intensity and photosynthetic efficiency of *gardenia*, especially at −3 °C.

Table 5 demonstrates that exogenous Tre enhanced the photosynthetic intensity and photosynthetic efficiency of *gardenia* leaves at different degrees. Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment increased the leaf Pn by 9.86%, 2.3%, 12.62% and 33.33%. The leaf Gs of *gardenia* increased by 3.91%, 14.23%, 47.80% and 70.86%, the leaf Ci of *gardenia* increased by 2.26%, 1.92%, 2.90% and 14.83%, and the leaf Tr of *gardenia* increased by 1.43%, 2.88%, 27.23% and 116.50%, respectively (Table 5). In conclusion, Tre can effectively enhance the photosynthetic intensity, light and efficiency of *gardenia* leaves.

It is noticeable that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment significantly alleviated the inhibition of low-temperature stress on the photosynthetic intensity and photosynthetic efficiency of *gardenia* leaves. Compared with the 20 °C treatment without Tre, the Pn, Gs, Ci and Tr of *gardenia* leaves under low-temperature stress at −3 °C without Tre significantly decreased by 36.00%, 46.26%, 16.41% and 70.57%. However, the exogenous addition of 15 mmol/L Tre restored the Pn, Gs, Ci and Tr to 85.33%, 91.81%, 96.02% and 63.71% (Table 5). In conclusion, Tre can effectively alleviate the inhibition effect of low-temperature stress on the photosynthetic intensity and photosynthetic efficiency of *gardenia* leaves.

3.6. Effect of Trehalose on Active Oxygen Content of Gardenia Root Under Low-Temperature Stress

The effects of Tre on active oxygen species in the root system of *gardenia* after low-temperature stress are shown in Table 6. The contents of H_2O_2 and superoxide anion radical were increased by low-temperature stress. Compared with treatment at 20 °C, treatment at 10 °C, 0 °C and −3 °C increased the H_2O_2 content by 6.84%, 59.91% and 84.50%, respectively (Table 6). Compared with the treatment at 20 °C, although the treatment at 10 °C and 0 °C had no significant effect on the content of superoxide anion free radicals, the treatment at −3 °C significantly increased the content by 10.62% (Table 6). In conclusion,

low-temperature stress promoted ROS production in the roots of *gardenia*, especially at −3 °C, which resulted in the highest ROS content in the roots.

Table 6. Effects of exogenous trehalose (Tre) on the ROS contents in the roots of *gardenia* jasminoides seedlings under different temperature conditions. Note: Data (means ± SD, n = 5) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		H_2O_2 (μmol/g)	O_2^- (μmol/g)
0 mmol/L Tre	20 °C	104.13 ± 9.26 c	80.01 ± 2.15 b
	10 °C	111.25 ± 9.59 c	81.32 ± 0.16 b
	0 °C	166.51 ± 13.34 b	81.40 ± 0.17 b
	−3 °C	192.12 ± 14.98 a	88.51 ± 0.12 a
15 mmol/L Tre	20 °C	103.01 ± 10.01 c	76.52 ± 0.10 c
	10 °C	105.95 ± 9.36 c	80.53 ± 0.12 b
	0 °C	145.35 ± 13.79 b	80.52 ± 0.15 b
	−3 °C	152.76 ± 13.47 b	85.51 ± 0.14 ab

Table 6 indicates that exogenous Tre can regulate ROS metabolism and reduce ROS accumulation. Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment reduced the H_2O_2 content in the roots of *gardenia* by 1.08%, 4.76%, 12.71% and 20.49%, respectively. The treatment with 15 mmol/L Tre also reduced the content of superoxide anion radicals by 4.36%, 0.97%, 1.08% and 3.39%, respectively (Table 6). In conclusion, Tre can effectively regulate the metabolism of ROS and reduce the accumulation of ROS.

It is interesting that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment significantly alleviated the effect of low-temperature stress on the ROS content in the roots of *gardenia*. Compared with treatment at 20 °C with no Tre, the contents of H_2O_2 and superoxide anion radicals in the roots of *gardenia* under low-temperature stress at −3 °C with no Tre increased by 84.50% and 10.62%. However, the exogenous addition of 15 mmol/L Tre under −3 °C low-temperature stress only increased the content of H_2O_2 and superoxide anion radicals by 46.70% and 6.87%, respectively (Table 6). In conclusion, Tre can effectively regulate the effect of low-temperature stress on ROS metabolism in the roots of *gardenia*, reduce the accumulation of ROS, and protect the structure and function of cell membranes.

3.7. Effect of Trehalose on Antioxidant Oxidase Activity of Gardenia Root Under Low-Temperature Stress

The changes in the antioxidant enzyme activity in the roots are shown in Table 7. The activities of SOD, POD and CAT were increased by low-temperature stress. Compared with treatment at 20 °C, the 10 °C, 0 °C and −3 °C treatments increased the SOD activity by 2.01%, 23.92% and 42.04%, POD activity by 15.45%, 38.43% and 92.39%, and CAT activity by 2.07%, 8.29% and 9.65%, respectively (Table 7). Compared with the 20 °C treatment, although the −3 °C treatment had no significant effect on CAT activity, it significantly increased the SOD and POD activity by 42.04% and 92.39%, respectively (Table 7). In conclusion, low-temperature stress enhanced the activity of antioxidant enzymes in the roots of *gardenia*, especially at −3 °C.

Table 7 shows that exogenous Tre can enhance the activity of antioxidant enzymes in the root system of *gardenia*, which can promote the removal of free radicals and reactive oxygen species in the root system, thus protecting cells from oxidative damage. Compared with the 0 mmol/L Tre treatment, the 15 mmol/L Tre treatment significantly increased the SOD activity in the roots of *gardenia* at 20 °C, 10 °C, 0 °C and −3 °C by 10.36%, 10.15%, 42.23% and 12.75%, respectively (Table 7). Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment also enhanced the POD and

CAT activities to varying degrees, with POD activities increased by 9.22%, 7.99%, 28.98% and 32.64%, respectively. CAT activity was increased by 7.59%, 7.43%, 5.74% and 10.71%, respectively (Table 7). It can be concluded that Tre can effectively enhance the activity of antioxidant enzymes and effectively improve the ability of plants to remove reactive oxygen species and free radicals in vivo.

Table 7. Effects of exogenous trehalose (Tre) on the antioxidant enzyme activity in the roots of *gardenia jasminoides* seedlings under different temperature conditions. Note: Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		SOD (U/g)	POD (U/g)	CAT (U/g)
0 mmol/L Tre	20 °C	502.01 ± 40.12 e	13.01 ± 1.12 d	145.01 ± 11.12 b
	10 °C	512.08 ± 41.11 e	15.02 ± 1.32 cd	148.02 ± 12.42 b
	0 °C	622.08 ± 51.11 c	18.01 ± 1.71 c	157.03 ± 14.87 ab
	−3 °C	713.08 ± 57.23 b	25.03 ± 2.11 b	159.01 ± 12.62 ab
15 mmol/L Tre	20 °C	554.01 ± 40.32 d	14.21 ± 1.19 d	156.03 ± 13.18 ab
	10 °C	564.01 ± 48.12 d	16.22 ± 1.25 cd	159.02 ± 12.42 ab
	0 °C	714.01 ± 61.19 b	23.23 ± 1.14 b	166.05 ± 14.85 ab
	−3 °C	804.01 ± 70.92 a	33.20 ± 3.11 a	176.04 ± 13.11 a

It is noticeable that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment further enhanced the activities of antioxidant enzymes in the root system of *gardenia*, so as to promote the removal of free radicals and reactive oxygen species in the root system of *gardenia*. Compared with treatment at 20 °C with no Tre, the activities of SOD, POD and CAT in *gardenia* roots under −3 °C and no Tre increased by 42.04%, 92.39% and 9.65%. However, the activities of SOD, POD and CAT in *gardenia* roots increased by 60.16%, 155.19% and 21.39%, respectively, with the exogenous addition of 15 mmol/L Tre at a low-temperature stress of −3 °C (Table 7). In conclusion, Tre can effectively enhance the antioxidant enzyme activity in *gardenia* roots under low-temperature stress, maintain the REDOX balance in plant cells, protect the structure and function of cells, and effectively improve the defensive capacity of antioxidants.

3.8. Effects of Trehalose on the Content of Osmoregulatory Substances in Roots of Gardenia Under Low-Temperature Stress

The effects of Tre on the contents of osmoregulatory substances (Pro, MDA, soluble protein and soluble sugar) in the roots of *gardenia* seedlings under low-temperature stress are shown in Table 8. The contents of Pro, MDA, soluble protein and soluble sugar in the roots of *gardenia* were increased by low-temperature stress. Compared with treatment at 20 °C, treatment at 10 °C, 0 °C and −3 °C increased the Pro content by 0.71%, 14.80% and 25.65%, respectively, and the MDA content by 6.52%, 48.65% and 61.67%, but had no significant effect on soluble protein and soluble sugar (Table 8). In conclusion, low-temperature stress increased the content of osmoregulatory substances such as Pro and MDA in the roots of *gardenia*, especially at −3 °C.

Table 8 also shows that exogenous Tre can reduce the content of osmoregulatory substances in the roots of *gardenia*. Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment reduced the Pro content in the roots of *gardenia* by 11.58%, 8.07%, 8.89% and 15.16%, respectively. The MDA content in the roots decreased by 10.68%, 10.02%, 9.24% and 12.65%, respectively, while the soluble sugar content in the roots increased by 10.35%, 11.76%, 9.15% and 11.58%, respectively (Table 8). There was no significant change in the content of soluble protein in all treatments. In conclusion, Tre can effectively reduce the content of Pro and MDA in roots, but significantly

increase the content of soluble sugar. The content of soluble protein did not change regularly after the addition of Tre, indicating that it was not affected by Tre.

Table 8. Effects of exogenous trehalose (Tre) on the osmotic regulating substances in the roots of *gardenia jasminoides* seedlings under different temperature conditions. Note: Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		Pro (ug/g)	MDA (μmol/g)	Soluble Protein (mg/g)	Soluble Sugar (mg/L)
0 mmol/L Tre	20 °C	70.13 ± 6.26 bc	30.81 ± 2.15 c	12.01 ± 1.12 a	509.01 ± 40.12 b
	10 °C	70.65 ± 6.59 bc	32.82 ± 3.16 c	12.08 ± 1.11 a	512.08 ± 45.15 b
	0 °C	80.51 ± 7.34 ab	45.80 ± 3.17 ab	12.10 ± 1.19 a	522.10 ± 31.19 b
	−3 °C	88.12 ± 7.98 a	49.81 ± 4.12 a	12.27 ± 1.03 a	512.27 ± 41.03 b
15 mmol/L Tre	20 °C	62.01 ± 5.01 c	27.52 ± 2.19 c	11.68 ± 1.09 a	561.68 ± 53.09 a
	10 °C	64.95 ± 4.36 c	29.53 ± 2.16 c	12.29 ± 1.08 a	572.29 ± 54.08 a
	0 °C	73.35 ± 6.79 b	41.52 ± 4.11 b	11.88 ± 1.07 a	569.88 ± 41.07 a
	−3 °C	74.76 ± 5.47 b	43.51 ± 3.14 ab	11.57 ± 1.06 a	571.57 ± 43.06 a

It should be noted that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment can effectively alleviate the abnormal accumulation of osmoregulatory substances induced by low temperatures. Compared with the 20 °C treatment without Tre, the contents of Pro and MDA in *gardenia* were significantly increased by 25.65% and 61.67% under low-temperature stress at −3 °C without Tre. However, the exogenous addition of 15 mmol/L Tre under −3 °C low-temperature stress only increased the Pro and MDA contents by 6.60% and 41.22% (Table 8). In conclusion, Tre can effectively alleviate the abnormal accumulation of osmoregulatory substances induced by low temperatures, but extreme low temperature still have a significant effect on the osmoregulatory substances of *gardenia* root, and the protective strength of Tre is limited to a certain extent by the intensity of the low temperature.

3.9. Effect of Trehalose on Endogenous Hormone Content in Roots of Gardenia Under Low-Temperature Stress

The effect of Tre on the hormone concentration in the roots of *gardenia* is shown in Table 9. Low-temperature stress significantly decreased the contents of IAA in the root system and significantly increased the contents of tZR, GA3 and ABA in the root system. Compared with the 20 °C treatment, treatment at 10 °C, 0 °C and 3 °C significantly reduced the auxin content by 19.04%, 27.89% and 66.16%, respectively (Table 9). Compared with treatment at 20 °C, the 10 °C, 0 °C and −3 °C treatments significantly increased tZR by 17.97%, 37.77% and 37.89%, GA3 by 22.49%, 42.16% and 42.99%, and ABA by 19.15%, 31.94% and 37.01%, respectively (Table 9). In conclusion, low-temperature stress decreased the contents of IAA but increased the contents of tZR, GA3 and ABA in the roots of *gardenia*, especially at −3 °C, which severely inhibited the growth of plants.

Table 9 also shows the effects of exogenous Tre on the IAA, tZR, GA3 and ABA contents in the roots of *gardenia*. Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment significantly increased the auxin content of *gardenia* by 0.53%, 5.71%, 4.03% and 32.03%, respectively (Table 9). Compared with the 0 mmol/L Tre treatment, at 20 °C, 10 °C, 0 °C and −3 °C, the 15 mmol/L Tre treatment reduced the tZR and GA3 contents to varying degrees, and the tZR content decreased by 0.16%, 12.40%, 16.29% and 14.92%, respectively. The content of GA3 decreased by 32.96%, 40.70%, 38.97% and 7.76%, respectively (Table 9). However, the exogenous addition of 15 mmol/L Tre had no significant effect on the ABA content of *gardenia* root. In conclusion, Tre could increase the content of IAA but decrease the contents of tZR and GA3, and had no significant effect on the ABA content.

Table 9. Effects of exogenous trehalose (Tre) on the concentrations of root endogenous hormones in *gardenia* jasminoides seedlings under different temperature conditions. Note: Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		IAA (ng/g FW)	tZR (ng/g FW)	GA_3 (ng/g FW)	ABA (ng/g FW)
0 mmol/L Tre	20 °C	15.13 ± 1.26 a	500.81 ± 40.15 c	356.01 ± 30.12 c	156.80 ± 10.02 c
	10 °C	12.25 ± 1.19 b	590.82 ± 51.16 b	436.08 ± 40.11 b	186.83 ± 10.02 b
	0 °C	10.91 ± 1.04 c	689.96 ± 60.17 a	506.10 ± 40.19 a	206.88 ± 20.03 ab
	−3 °C	5.12 ± 0.48 e	690.55 ± 59.12 a	509.07 ± 20.13 a	214.83 ± 20.02 a
15 mmol/L Tre	20 °C	15.21 ± 1.01 a	500.01 ± 40.10 c	238.68 ± 20.09 d	156.97 ± 15.01 c
	10 °C	12.95 ± 1.06 b	517.53 ± 40.12 c	258.59 ± 22.08 d	187.02 ± 10.02 b
	0 °C	11.35 ± 1.09 bc	577.52 ± 50.12 b	308.88 ± 26.07 cd	207.11 ± 18.03 ab
	−3 °C	6.76 ± 0.47 d	587.51 ± 52.14 b	469.57 ± 31.06 ab	217.01 ± 19.01 a

What is noticeable is that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment alleviated the decreasing effect of low-temperature stress on the IAA content in the roots of *gardenia*, and weakened the increasing effect of low-temperature stress on the tZR and GA3 content. Compared with treatment at 20 °C without Tre, low-temperature stress at −3 °C without Tre reduced the IAA content by 66.16%, and increased the tZR and GA3 contents by 37.89% and 42.99%. However, the exogenous addition of 15 mmol/L Tre restored the IAA content to 44.68%. The tZR and GA3 contents only increased by 17.31% and 31.90% (Table 9). Similarly, the 15 mmol/L Tre treatment had no significant effect on the ABA content in the roots of *gardenia*. In conclusion, Tre can effectively alleviate the decreasing effect of low-temperature stress on IAA and weaken the increasing effect of low-temperature stress on the tZR and GA3 contents of *gardenia*.

3.10. Effect of Trehalose on Malic Acid and Succinic Acid Content of Gardenia Root Under Low-Temperature Stress

The effects of Tre on malic acid and succinic acid in the roots of *gardenia* under low-temperature stress are shown in Table 10. Low-temperature stress decreased the malic acid content and increased the succinic acid content in the roots of *gardenia* to different degrees. Compared with the treatment at 20 °C, the malic acid content at 10 °C, 0 °C and −3 °C was reduced by 9.21%, 43.42% and 59.21%, respectively (Table 9). Compared with the 20 °C treatment, the 10 °C, 0 °C and −3 °C treatments significantly increased the content of succinic acid by 12.26%, 52.83% and 61.32%, respectively (Table 10). In conclusion, low-temperature stress can reduce the malic acid content in the roots of *gardenia*, but significantly increase the succinic acid content, which affects the metabolism of carbohydrates in the plant and then affects the growth of *gardenia*.

Table 10. Effects of exogenous trehalose (Tre) on the contents of malic acid and succinic acid in the roots of *gardenia* jasminoides seedlings under different temperature conditions. Note: Data (means ± SD, $n = 5$) followed by different letters in the column indicate significant ($p < 0.05$) differences. The same applies below.

Treatments		Malic Acid (μmol/g FM)	Succinic Acid (μmol/g FM)
0 mmol/L Tre	20 °C	0.76 ± 0.06 a	1.06 ± 0.06 c
	10 °C	0.69 ± 0.05 ab	1.19 ± 0.11 b
	0 °C	0.43 ± 0.04 c	1.62 ± 0.12 a
	−3 °C	0.31 ± 0.02 d	1.71 ± 0.14 a
15 mmol/L Tre	20 °C	0.77 ± 0.06 a	1.05 ± 0.09 c
	10 °C	0.71 ± 0.06 a	1.16 ± 0.11 b
	0 °C	0.66 ± 0.05 ab	1.23 ± 0.12 b
	−3 °C	0.61 ± 0.04 b	1.31 ± 0.08 ab

Exogenous Tre promoted malic acid formation but inhibited succinic acid formation in the roots of *gardenia* (Table 10). Compared with the 0 mmol/L Tre treatment, the malic acid content in the roots of *gardenia* was increased by 1.32%, 2.90%, 53.49% and 96.77% under the 15 mmol/L Tre treatment at 20 °C, 10 °C, 0 °C and −3 °C, respectively (Table 10). Compared with the 0 mmol/L Tre treatment, the 15 mmol/L Tre treatment at 20 °C, 10 °C, 0 °C and −3 °C reduced the content of succinic acid by 0.94%, 2.52%, 24.07% and 23.40%, respectively. In conclusion, Tre can regulate the metabolism of endogenous acids such as malic acid and succinic acid, and affect the growth of *gardenia*.

It is interesting that under −3 °C low-temperature stress, the 15 mmol/L Tre treatment alleviated the effect of low-temperature stress on the reduction in malic acid content, but also weakened the effect of low-temperature stress on the increase in succinic acid content. Compared with treatment at 20 °C without Tre, the malic acid content significantly decreased by 59.21% and the succinic acid content significantly increased by 61.32% under low-temperature stress at −3 °C without Tre, while the malic acid content only decreased by 19.74% after the exogenous addition of 15 mmol/L Tre. Succinic acid increased by only 23.58% (Table 10). In conclusion, Tre can regulate the metabolism of endogenous acids such as malic acid and succinic acid in *gardenia*, and establish a dynamic balance between energy supply, osmotic protection and REDOX homeostasis, thereby alleviating the inhibitory effect of low-temperature stress on the growth of *gardenia*.

4. Discussion

The growth of *gardenia* is regulated not only by environmental conditions, but also by exogenous substances, which could help plants to resist the stress of adversity. Few studies have reported the effect of exogenous Tre on the growth of *gardenia* seedlings under low-temperature stress, but the regulation of other crops and adversity has been reported. Based on low-temperature stress experiments on *Catharanthus roseus*, which was used as the experimental material, Wei et al. [30] discovered that Tre effectively increased plant growth, improved the chlorophyll content and antioxidant enzyme activity of leaves, and improved their cold resistance. Aldesuquy et al. [31] found that the exogenous application of Tre appeared to mitigate the damaging effect of drought with different magnitudes by counteracting the negative effects of water stress on all growth criteria for wheat root and improving the turgidity of wheat leaf by decreasing the rate of transpiration, increasing the relative water content, decreasing the saturation water deficit, and increasing the water use efficiency for wheat economic yield. In this study, the exogenous application of Tre solution under low-temperature stress significantly improved the growth potential of *gardenia* seedlings, such as the plant height, leaf number, total plant weight, above-ground fresh weight, underground fresh weight, root total length, lateral root number, total root surface area and root volume, especially under −3 °C low-temperature stress. Exogenous Tre had the best effect on restoring the growth potential of *gardenia*. This is similar to the results of a study by Raza et al. [32] on exogenous Tre's effect on the cold tolerance of Rapeseed (*Brassica napus* L.) seedlings under low-temperature stress.

When plants encounter low-temperature stress, the chlorophyll will be destroyed, and the chlorophyll content will be forced to decrease, which will eventually weaken the photosynthetic capacity of plants and inhibit the carbon assimilation pathway, resulting in slow plant growth [33,34]. Tang et al. [35] proved that exogenous Tre (10 mmol·L^{-1}) could significantly increase the contents of chlorophyll a, chlorophyll b and total chlorophyll in the leaves of wheat seedlings under low-temperature stress. This is consistent with the results of this study: low-temperature stress inhibited the synthesis of chlorophyll in the leaves of *gardenia* to varying degrees, and exogenous Tre at 15 mmol/L could effectively alleviate the inhibition of low-temperature stress on chlorophyll synthesis in the

leaves of *gardenia* and moderately restore the contents of chlorophyll a, chlorophyll b and total chlorophyll.

When plant growth is subjected to abiotic stress, the inner membrane of chloroplasts is destroyed, thus affecting plant photosynthesis and growth and development [36]. The photosynthetic mechanism PSII on the chloroplast thylakoid membrane is particularly sensitive to environmental changes [36]. The chlorophyll fluorescence parameters φPSII, Fv′/Fm′, qP and NPQ can represent the initial photochemical capacity of PSII and are important indicators used to reflect the effects of environmental stress on photosynthesis [37]. In the photosynthetic apparatus PSII, Fv′/Fm′ is decreased when plants are subjected to photoinhibition, which indicates that photosystem II is destroyed [37]. Pilon-Smits [38] showed that Tre treatment significantly improved the Fv′/Fm′ value in leaves under abiotic stress and restored it to the control level, effectively alleviating the damage caused by abiotic stress to the PSII reaction center, indicating that Tre can alleviate or even restore the damage caused by abiotic stress to PSII. These results also showed that Tre increased the φPSII in leaves under abiotic stress, indicating that the actual photochemical utilization efficiency of leaves increased [38]. Tre also slows down the decrease in qP and ETR in leaves caused by abiotic stress, and decreases NPQ, indicating that Tre can alleviate problems such as the decrease in the photochemical efficiency and fluorescence yield in leaves caused by abiotic stress [38]. The above results were similar to the present study: the φPSII, Fv′/Fm′ and qP values of *gardenia* leaves decreased to varying degrees under low-temperature stress, and exogenous Tre treatment effectively restored these parameters to the control level, so as to improve the adverse effect of low-temperature stress on the PSII reaction center.

Photosynthesis is very sensitive to abiotic stresses, including strong light, water stress, high temperatures, salt damage, heavy metal toxicity, etc., that reduce the photosynthetic efficiency of plants and thus affect the normal growth of plants [39]. The photosynthetic intensity parameters can accurately reflect the photosynthetic intensity of plants, which mainly include the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci) and transpiration rate (Tr) [40]. Plants mainly rely on stomata to exchange external gases (CO_2 and H_2O_2), and CO_2 is the substrate of plant photosynthesis, so the level of Ci and Tr are affected by Gs, further affecting the strength of photosynthesis [40]. Tr represents the amount of water evaporated per unit leaf area of plant leaves within a certain period of time, and the transpiration pull generated by Tr can absorb and transport water, providing material sources for the plant photosynthesis process [40]. It has been reported that stress can affect the normal level of various photosynthetic intensity parameters [41]. With the aggravation of stress, the Pn, Tr and Gs of the leaves of *Leymus chinensis* showed a gradual decline, while Ci showed an upward trend [41]. In order to reduce the damage caused by stress to plant growth and development, researchers have added exogenous substances (such as Tre) to alleviate the adverse effects of stress on plant photosynthesis. Studies have shown that the exogenous addition of Tre can affect the parameters of photosynthetic intensity in wheat leaves under drought stress, and that the appropriate concentration of Tre can significantly increase the chlorophyll content and enhance the values of Pn, Tr and Gs in wheat leaves [42]. Razzaq et al. [43] showed that chromium (Cr) stress (100 uM) significantly reduced the Pn, Gs and Tr of *Zea mays* leaves, and that the increase in Cr concentration (500 uM) further exacerbated this adverse effect. However, exogenous Tre treatment can effectively reduce these adverse effects caused by Cr stress on *Zea mays* leaves, and the effect of 50 mM of Tre is better than that of 25 mM of Tre. These results are consistent with the results of this study: low-temperature stress inhibited the photosynthetic intensity parameters and photosynthetic efficiency of *gardenia*; in particular, a low-temperature stress of −3 °C significantly decreased Pn, Gs, Ci and Tr,

while 15 mmol/L Tre effectively mitigated the inhibition effects of low-temperature stress on the photosynthetic intensity and photosynthetic efficiency of *gardenia* leaves.

Plants will produce abundant ROS when they are subjected to environmental stress. This ROS could destroy macromolecular substances such as DNA, proteins and membrane structures in plant tissues [44–46]. In order to avoid the damage caused by excessive ROS accumulation to cells, plants relieve the damage via antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) [47–50]. Luo and Li [51] showed that heat stress can significantly increase the ROS content (hydrogen peroxide, superoxide anion radical, etc.) in wheat, while exogenous Tre can scavenge the ROS content (hydrogen peroxide, superoxide anion, etc.) by increasing the activities of antioxidant enzymes such as APX, SOD, and CAT, thus alleviating the damage caused by abiotic stress in wheat. Tre treatment significantly enhanced the activities of antioxidant-related enzymes such as APX, CAT, SOD, and GR, as well as the transcription levels of the *AsA-GSH* cycle-related gene, which led to a reduction in the ROS (such as hydrogen peroxide) content in peach during cold storage [52]. According to a study by Akram et al. [53] in radish (*Raphanus sativus* L.), spraying Tre (25 mM) can alleviate the damage caused by water stress to seedlings by enhancing the activities of SOD and POD. Zheng et al. [54] also confirmed that exogenous Tre (5.0 mM) could induce an increase in the antioxidant enzyme activity (such as SOD and POD) in tea plant under heat stress, indicating that Tre could further stimulate the enzymatic defense system of tea seedlings under heat stress, enhance the antioxidant capacity of plants, and alleviate the damage to cell membranes caused by high-temperature stress. These results are consistent with the results of this study: low-temperature stress promotes the production of excessive ROS in *gardenia*, and exogenous Tre can effectively enhance the activity of antioxidant enzymes in *gardenia* under low-temperature stress, improve its antioxidant defense ability to reduce the ROS content in vivo, maintain the reoxygen–reduction balance in cells, and protect the structure and function of cell membranes.

Plants under abiotic stress will produce a large number of osmoregulatory substances. Osmoregulatory substances can not only maintain cell turgor and prevent excessive water loss in the protoplasm, but also stabilize the organelle structure, regulate some physiological functions, and alleviate the damage caused to plants under stress. Proline (Pro), malondialdehyde (MDA), soluble protein and soluble sugar are osmoregulatory substances in plants. Hasanuzzaman et al. [55] confirmed that drought increased the Pro and MDA contents and altered the antioxidant and glyoxalase systems in three Brassica species (*B. napus*, *B. campestris* and *B. juncea*), while Tre reduced the MDA and Pro contents and activity of lipoxygenase enzymes (LOX), further enhancing their drought tolerance. This is consistent with the conclusions of this study: low-temperature stress increased the contents of osmoregulatory substances (Pro and MDA) in the roots of *gardenia*, and exogenous Tre effectively alleviated the abnormal accumulation of osmoregulatory substances induced by low temperatures, reduced the damage caused by membrane lipid peroxidation, and improved the cold resistance of *gardenia*. In addition, this study also found that soluble protein and soluble sugar were not affected by low-temperature stress and exogenous Tre. However, our results are not quite the same as Zheng et al. [54]: the contents of PRO and soluble sugar exhibited a significant increase, while the MDA content decreased following treatment with 5.0 mmol·L^{-1} Tre under 24 h high-temperature stress (38 °C/29 °C, 12 h/12 h). This may be due to the different responses of Tre to different plants under different abiotic stress conditions; the specific reasons for this need to be further explored.

Plant hormones play an important role in the plant stress response, and can cause adaptive regulatory responses in plants [56–58]. When plants encounter abiotic stress such as low temperatures, they can cope with environmental stress by regulating related

hormones in the plants [59,60]. Cui et al. [61] treated *Cabernet Sauvignon* seedlings with 15 mmol·L^{-1} Tre and determined and analyzed the contents of four endogenous hormones (tZR, GA3, IAA and ABA) under low-temperature stress. Compared with the control group, the contents of tZR, GA3 and ABA in the Tre treatment group at −3 °C were increased by 80.03%, 27.66% and 39.14%, respectively, while the contents of IAA were decreased to 0.94 ng·g^{-1} [61]. This is consistent with the findings of this study: low-temperature stress decreased the IAA content but increased the tZR, GA3 and ABA contents in the roots of *gardenia*; the 15 mmol/L Tre treatment alleviated the decreasing effect of low-temperature stress on the IAA content in the roots of *gardenia* and weakened the increasing effect of low-temperature stress on the tZR and GA3 contents, but had no significant effect on ABA. It can be concluded from the above that Tre treatment has a certain protective effect on the plant hormone synthesis system under low-temperature stress, and can restore the auxin content to a certain extent to restore plant growth.

The normal development of respiratory metabolism plays a vital role in the process of plant growth and development [62]. When plants face abiotic stress, the appropriate amount of intermediate metabolites is the basis for their adaptation to low temperatures [62]. As intermediate products of plant respiratory metabolism, succinic acid and malic acid are closely related to plant metabolism [63]. The succinic acid produced during the tricarboxylic acid cycle, under the action of SDH, produces fumaric acid, which is converted into malic acid by hydration [63]. A previous study showed that the concentration of root respiratory metabolites is significantly correlated with root activity in rhizosphere soil, and that malic acid and succinic acid as root respiratory metabolites can enhance root activity and promote plant growth [64]. However, Tre treatment in this study increased the content of malic acid in the roots of *gardenia* but decreased the content of succinic acid. This may be different from the response of different plants to Tre stimulation. *Gardenia* may respond to the stimulation of exogenous Tre in root respiration through malic acid. Therefore, the application of an appropriate concentration of Tre can promote the production of root respiratory metabolites, enhance root vitality, promote plant growth, and improve plant resistance to low temperatures.

5. Conclusions

Trehalose increased the content of chlorophyll, chlorophyll fluorescence parameters and the photosynthesis intensity under low-temperature stress. Trehalose can significantly improve the antioxidant enzyme activity in the roots of *gardenia* seedlings, enhance the antioxidant capacity of plants, reduce the content of reactive oxygen species to reduce the damage caused by reactive oxygen species, maintain the level of reactive oxygen metabolism, and enhance the cold resistance of *gardenia* seedlings. Trehalose also can effectively reduce the content of osmoregulatory substances in seedling roots, effectively enhance the osmoregulatory capacity of the roots of *gardenia* seedlings, reduce cell osmotic potential, maintain the cellular fluid concentration, prevent the osmotic damage caused to root cells by low-temperature stress, reduce the generation of harmful substances and oxidation products, and maintain cell membrane stability. Trehalose significantly promoted the biosynthesis of auxin and increased the content of respiratory metabolites (malic acid) in the roots of *gardenia* seedlings so as to increase their adaptability and resistance to low temperatures.

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