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Poly-Glutamic Acid Promotes the Growth and the Accumulation of Main Medicinal Components in *Salvia miltiorrhiza*

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Abstract: *Salvia miltiorrhiza* Bunge is a traditional medicinal plant in China and poly-glutamic acid (PGA) is a valuable biopolymer. However, it is unclear whether PGA promotes growth and the accumulation of main medicinal components in *S. miltiorrhiza*. To elucidate this scientific question, the influences of PGA on the growth, physiological characteristics, and accumulation of main medicinal components in *S. miltiorrhiza* were explored through a pot experiment. The results revealed that PGA significantly promoted basal diameter, plant height, shoot and root biomass, as well as root volume, compared with control. PGA also increased SPAD value, net photosynthetic rate, actual and maximum photochemical efficiency of photosynthetic system II, photochemical quenching, and electronic transfer rate. Meanwhile, PGA increased transpiration rate, stomatal conductance, water use efficiency, leaf relative water content, and the contents of soluble protein, soluble sugar, and proline. Furthermore, PGA increased the activities of antioxidant enzymes and the contents of antioxidants. The above findings imply that PGA facilitated *S. miltiorrhiza* growth by enhancing photosynthetic performance, water metabolism, and antioxidant capacity. Additionally, PGA significantly improved the yield of rosmarinic acid, salvianolic acid B, dihydrotanshinone, cryptotanshinone, tanshinone I, and tanshinone IIA in roots by up-regulating the transcript levels of genes responsible for their biosynthesis. Our findings indicated that PGA promoted *S. miltiorrhiza* growth and the accumulation of main medicinal components in roots.

Keywords: growth; antioxidant capacity; photosynthetic performance; transcript level; medicinal component

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1. Introduction

Salvia is a genus of plants in the Labiatae family. This genus comprises several medicinal plants, such as *Salvia abrotanoides* (Karl.), *S. yangii*, and *S. miltiorrhiza* [1,2]. The roots of these *Salvia* species include water-soluble medicinal ingredients and fat-soluble medicinal ingredients. The main water-soluble medicinal ingredients include rosmarinic acid (RosA) and salvianolic acid B (SalB) [2]. Fat-soluble medicinal ingredients mainly include dihydrotanshinone (DHT), tanshinone I (Tan I), cryptotanshinone (CTS), and tanshinone IIA (Tan IIA) [3–5]. The above medicinal components can dissipate blood stasis by activating blood circulation, prevent atherosclerosis, lower blood pressure and fat, and have anti-inflammation and antitumor effects [6,7]. In China, *S. miltiorrhiza* is one of the major medicinal materials commonly used in clinical practice, with a long history of more than 2000 years. Its dry roots also contained above water-soluble and fat-soluble main medicinal ingredients, including RosA, SalB, DHT, Tan I, CTS and Tan IIA [8]. In *S. miltiorrhiza*, the water-soluble ingredients have anticoagulation, cytoprotection, antiviral, antioxidation and other pharmacological effects [9]. The fat-soluble ingredients have antibacterial, anti-inflammatory, antitumor and other pharmacological effects [10,11]. Thus,

the roots have been extensively used to treat inflammation, cardiovascular diseases, chronic renal failure, angina pectoris and dysmenorrhea in many regions in the world, mainly including Asia, Europe and the United States [2]. Due to the above reasons, *S. miltiorrhiza* is a very useful medicinal plant not only in China but all over the world. Therefore, *S. miltiorrhiza* has become a kind of bulk medicinal material. Recently, the demand for *S. miltiorrhiza* has increased significantly in the world. Thus, it is necessary to improve the growth and the accumulation of the main medicinal components in *S. miltiorrhiza* roots to meet the market demand. More and more research has demonstrated that exogenous substances improved the growth and the yield of main medicinal components in medical plants. For *S. miltiorrhiza*, Zhang et al. [12] found that nano-selenium improved the growth and the contents of tanshinones (Tans) and salvianolic acids (SAs). Han et al. [13] displayed that cerous nitrate promoted the growth and Tans production. Sun et al. [14,15] found that karrikins improved SalB content. Shi et al. [16] reported that abscisic acid (ABA) increased the contents of phenolic acids and Tans. For other medical plants, Islam et al. [17] showed that putrescine enhanced ginsenosides content in the sprouts of *Panax ginseng*. Chang et al. [18] reported that melatonin enhanced secondary metabolite contents and the yield of *Prunella vulgaris*. The above previous studies indicate that we could regulate the growth and the yield of medicinal components in medical plants through the application of exogenous substances. Thus, it is worth exploring the influences of exogenous substances on the growth and the accumulation of main medicinal components in *S. miltiorrhiza* roots.

It has been documented that the application of biopolymers in plant or plant organ cultures could improve plant growth and the accumulation of medicinal components [19–29]. Chang et al. [24] showed that biopolymer polylactic acid (PLA) stimulated the growth of soybeans. Patil et al. [27] reported that different biopolymers, agar, cellulose, alginate, psyllium gaur gum, and bacterial exopolysaccharide (EPS), all improved the seedling growth of *Gossypium herbaceum* L. Zagzog et al. [29] also demonstrated that chitosan improved the vegetative growth of mangos. Wierzchowski et al. [23] found that PLA could promote biomass proliferation of *Rindera graeca* hairy root. Additionally, previous studies have also proved that biopolymers could also improve the accumulation of medicinal components in the culture of plant hairy root [19–23,25,26]. Kawka et al. [21] showed that polyurethane foam (PUF) enhanced rinderol production in *R. graeca* hairy root. Wierzchowski et al. [23] found that poly(glycerol sebacate) (PGS) could cause deoxyshikonin production in *R. graeca* hairy root. Jose et al. [20] showed that polypropylene (PP) caused the production of plumbagin in *Plumbago rosea* L. hairy root. The above studies clearly indicate that biopolymers play vital roles in promoting plant growth and the accumulation of medicinal components in medicinal plants, which shows the wide application of biopolymers in plant science.

Poly-glutamic acid (PGA) is a valuable biopolymer. Being non-toxic, edible, and biodegradable, PGA is widely applied in food, biomedicine, and agriculture [30]. In agriculture, PGA has been extensively used to act as slow-release fertilizer, increase the relative abundance of plant-growth-promoting bacteria in soil, decrease excessive chemical fertilizer usage, enhance crop stress tolerance, and promote plant growth, etc. [31]. It has been reported that PGA improved the growth of *Brassica rapa* L. [32]. The application of PGA could also strengthen the antioxidant capacity of *B. napus* L. by reinforcing the activities of superoxide dismutase (SOD) and catalase (CAT) [33]. Meanwhile, the previous reports demonstrated that PGA reinforced the photosynthesis of *B. rapa* L. and maize (*Zea mays* L.) [34,35]. It has been displayed that PGA could promote maize growth by improving the photosynthesis, water metabolism, and the antioxidant capacity [36]. The above previous reports indicated that PGA promoted crops' growth by regulating the photosynthesis, water metabolism, and the antioxidant capacity, whereas it is still unclear whether, and how, PGA affects the photosynthetic performance, water metabolism, the antioxidant capacity, and the growth of *S. miltiorrhiza*. In addition, the influence of PGA on the yield of main medicinal components in *S. miltiorrhiza* roots is also unclear. Hence, it is worth investigating the role of PGA in regulating the photosynthetic performance,

water metabolism, the antioxidant capacity, the growth, and the yield of main medicinal components in *S. miltiorrhiza* roots, which is the main novelty of this study.

In this research, we supposed that PGA improved the growth and the accumulation of main medicinal components of *S. miltiorrhiza*. To prove the correctness of this hypothesis, we explored the influences of PGA on growth parameters such as basal diameter, plant height, shoot and root biomass, root volume, SPAD value, net photosynthetic rate (Pn), chlorophyll fluorescence parameters (actual photochemical efficiency of photosynthetical system II (Y(II)), maximum photochemical efficiency of photosynthetical system II in dark (Fv/Fm) and in light (Fv′/Fm′), photochemical quenching (qP), non-photochemical quenching (NPQ), electronic transfer rate (ETR)), transpiration rate (Tr), water use efficiency (WUE), stomatal conductance (Gs), leaf relative water content (LRWC), the activities of antioxidant enzymes (SOD, peroxidase (POD), CAT, ascorbate peroxidase (APX) and glutathione reductase (GR)), the contents of main osmotic adjustment substances (soluble protein (SP), soluble sugar (SS), proline (Pro)), malondialdehyde (MDA), reduced ascorbate (AsA), and reduced glutathione (GSH), as well as the yield of main medicinal components and the expression of gene coding enzymes in charge of the above medicinal components' biosynthesis at the transcript level. The purpose of our research was to elucidate whether and how PGA affected the growth and the accumulation of main medicinal components of *S. miltiorrhiza*, which can provide more knowledge for PGA applications in facilitating the yield of main medicinal components.

2. Material and Methods

2.1. Plant Material and Treatment

Five-month-old seedlings of purple *S. miltiorrhiza* were supplied by the planting base in Danyang city, Jiangsu Province, China. Seedlings with similar height and growth status were planted in gallon pots (30.0 cm height, 26.5 cm upper caliber and 25.5 cm lower caliber) with 2 seedlings per pot. Each pot contained 14.0 kg of garden soil and peat (6:1, *w*/*w*). Seedlings were cultivated in the open air. All the pots were placed under the rain shelter in rainy weather. Field water capacity (FC) of culture soil was 28% (*w*/*w*). During the whole experiment, the water content of the culture soil was controlled to 60% FC by weighing method from March to November 2022. In order to provide enough mineral nutrition, we added 4.44 g compound fertilizer (Stanley Fertilizer Co., Ltd., Linyi, China) to the culture soil per pot. The ratio of N:P:K in the compound fertilizer is 15:15:15.

To investigate the effects of PGA (Nanjing Shineking Biotech Co., Ltd., Nanjing, China), the seedlings were, respectively, treated by 0.022, 0.044, and 0.088 g PGA per pot through root irrigation method and expressed as T1, T2, and T3. Before treatment, different dosages of PGA per pot were prepared by dissolving PGA in 300 mL distilled water, respectively. Meanwhile, the control seedlings were irrigated by 300 mL distilled water alone. Each treatment was repeated nine times. We treated the seedlings on 15 April and 15 May 2022, respectively. Among nine replicates, the first three replicates were applied to investigate the effects of PGA on corresponding parameters in June in Table 1. The second three replicates were applied to investigate the effects of PGA on corresponding parameters in August in Table 1. The third three replicates were used to investigate the influences of PGA on final shoot and root dry weight, as well as the final yield of medicinal components in root shown in Table 1.

Table 1. The indicators measured in June, August, and November.

Time	June	August	November
Growth parameters	Basal diameter, plant height, shoot dry weight, root dry weight, root volume, root/shoot ratio	Basal diameter, plant height, shoot dry weight, root dry weight, root volume, root/shoot ratio	Shoot dry weight, root dry weight
Photosynthetic performance	SPAD, Pn, Y(II), Fv/Fm, Fv′/Fm′, qP, NPQ, ETR	SPAD, Pn, Y(II), Fv/Fm, Fv′/Fm′, qP, NPQ, ETR	—

Table 1. *Cont.*

Time	June	August	November
Water metabolism	Tr, WUE, Gs, LRWC, SP, SS, Pro	Tr, WUE, Gs, LRWC, SP, SS, Pro	—
Antioxidant capacity	SOD, POD, CAT, APX, GR, MDA, AsA, GSH	SOD, POD, CAT, APX, GR, MDA, AsA, GSH	—
Yield of medicinal components	—	—	RosA, SalB, Tan I, Tan IIA, DHT, CTS
Expression of genes	*HMGR*, *DXS2*, *FPPS*, *GGPPS*, *CPS*, *RAS10*, *CYP98A75*, *CYP98A14*	*HMGR*, *DXS2*, *FPPS*, *GGPPS*, *CPS*, *RAS10*, *CYP98A75*, *CYP98A14*	—

2.2. Assays of Growth Parameters

Basal diameter and plant height were measured by using the digital vernier scale (Shanghai Constant Measuring Tools Co., Ltd., Shanghai, China) and meter ruler (Hangzhou Weiwei Trading Co., Ltd., Hangzhou, China), respectively. Whole plants were taken out by the broken barrel method and each plant was then divided into root and shoot. Then, root volume was determined through drainage method using 500 mL volumetric cylinder (Shanghai Leigu Instrument Co., Ltd., Shanghai, China). Then, the root and shoot were dried for 72 h at 80 °C. Finally, the dry weight was calculated using the electronic balance (Shanghai Yajin Electronic Technology Co., Ltd., Shanghai, China). The accuracy of the electronic balance was 0.01 g. Root/shoot ratio was expressed as the quotient of root dry weight and shoot dry weight. In November, whole plants were also taken out by the broken barrel method and each plant was also divided into root and shoot. Then, their dry weight was measured as above. Above indicators of each treatment were all measured three replicates.

2.3. Assays of SPAD, Pn, and Chlorophyll Fluorescence Parameters

The SPAD value of the top fully expanded leaf was measured by using SPAD-502 Plus Chlorophyll Meter (Konica Minolta, Tokyo, Japan). The Pn value was measured by using Licor-6400 photosynthetic instrument (Lincoln, NE, USA). The conditions in leaf chamber were set as light intensity of 1000 μmol(photon) m^{-2} s^{-1}, CO_2 concentration of 400 ppm, and leaf temperature of 25.0 °C. The top fully expanded leaves were first equilibrated and then a steady-state value of Pn was recorded. The leaves used to measure Pn value were also used to measure chlorophyll fluorescence parameters Fv/Fm, Fv′/Fm′, NPQ, qP, Y (II), and ETR by a PAM-2500 portable modulated chlorophyll fluorometer (Walz, Effeltrich, Germany). Minimum fluorescence (F0) was measured under a weak modulating radiation (0.5 μmol(photon) m^{-2} s^{-1}), and maximum fluorescence (Fm) was induced by a saturating pulse of radiation (2400 μmol(photon) m^{-2} s^{-1}). For dark adaptation, the leaves were covered for 30 min. Then, the above chlorophyll fluorescence parameters were measured by the fluorometer. All above indicators were measured from 9:30 to 11:00. Above indicators of each treatment were all measured three replicates.

2.4. Assays of SP, SS, and Pro Contents in Leaves

SS content was measured according to Wei [37] by using the anthrone-sulfuric acid colorimetry method. SP content was measured according to Bradford [38] by using the Coomassie brilliant blue G-250 method. Pro content was analyzed by using the acid-ninhydrin chromogenic agent method described by Bates et al. [39]. Above indicators of each treatment were all measured three replicates.

2.5. Assays of Tr, Gs, WUE, and LRWC

The leaves used to measure Pn value were also used to measure Tr, Gs, and WUE, along with Pn, by using Licor-6400 photosynthetic instrument (Lincoln, NE, USA) from 9:30 to 11:00. The conditions in leaf chamber were set as light intensity of 1000 μmol(photon) m^{-2} s^{-1}, CO_2

concentration of 400 ppm, and leaf temperature of 25.0 °C. The top fully expanded leaves were first equilibrated and then steady-state values of Tr, Gs, and WUE were recorded. The value of LRWC was calculated using below equation: LRWC = [(FW − DW)/(SW − DW)] × 100%. In this equation, FW, DW, and SW stand for fresh weight, dry weight, and saturated weight of leaves. Briefly, fresh leaves were weighed and recorded as FW. Fresh leaves were immersed in water for 12 h and recorded as SW. Then, the leaves were dried in an oven (Shanghai Yilin Scientific Instrument Co., Ltd., Shanghai, China) at 105 °C for 5 min followed by 65 °C until a constant weight, which was recorded as DW. Above indicators of each treatment were all measured three replicates.

2.6. Assays of Antioxidant Enzymes and MDA Content in Leaves

The activities of SOD (EC 1.15.1.1), POD (EC 1.11.1.7), and CAT (EC 1.11.1.6) were, respectively, measured according to Giannopolitis and Ries [40], Scebba et al. [41], and Kato and Shimizu [42]. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition in the nitro blue tetrazolium chloride monohydrate reduction. One unit of POD activity was defined as an increase in absorbance of 0.001 per min at 470 nm. One unit of CAT activity was defined as a decrease in absorbance of 0.001 per min at 240 nm. The activities of APX (EC 1.11.1.11) and GR (EC 1.6.4.2) were measured according to Shan and Liang [38]. One unit of APX and GR activities was defined as a decrease in absorbance of 0.01 per min at 290 nm and 340 nm, respectively. The activities of the above enzymes were all expressed as U g^{-1} FW. MDA content was also measured according to Shan and Liang [43] by using the thiobarbituric acid method. Above indicators of each treatment were all measured three replicates.

2.7. Determination of AsA and GSH Contents in Leaves

Briefly, each leaf sample (0.5 g) was homogenized with 5 mL of 5% (m/v) ice-cold meta-phosphoric acid (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China). The homogenates were then centrifuged at 12,000× *g* for 20 min at 4 °C. Then, the supernatants were immediately used to measure the contents of AsA and GSH. The contents of AsA and GSH were, respectively, measured by using the method described by Hodges et al. [44] and Griffith [45]. Above indicators of each treatment were all measured three replicates.

2.8. Real-Time Quantitative PCR (qRT-PCR) of Key Genes Coding Enzymes Responsible for the Biosynthesis of Main Medicinal Components in Root

Total RNA was extracted from frozen root samples by using RNA extraction kit (Bioteke Corporation Co., Ltd., Wuxi, China). The quality of RNA extraction was assessed via BioDrop (Shanghai Representative Office of Biochrom Ltd., Shanghai, China) and agarose gel electrophoresis. According to the protocol of a cDNA synthesis kit (TransGen Biotech Co., Ltd., Beijing, China), cDNA was synthesized and then used as the template of qRT-PCR. Specific primers for key gene coding enzymes responsible for the biosynthesis of main medicinal components and the internal reference gene *Actin* were designed as in Table 2. The above primers were all synthesized by Sangon Biotech. PCR reactions (10 μL) included 0.5 μL cDNA, 0.2 μL each of upstream and downstream primers, 5 μL 2×PerfectStart Green qPCR SuperMix (TransGen Biotech Co., Ltd., Beijing, China), and 4.1 μL H_2O. Reactions were performed by using an ABI Quant Studio 6 Flex real-time PCR system (EPBAIS Applied Biosystems Trading (Shanghai) Co., Ltd., Shanghai, China) under below conditions: 95 °C for 30 s, 1 cycle; 95 °C for 5 s and 58 °C for 30 s, 40 cycles. The expression of each gene relative to average Ct value of the internal reference gene was determined and analyzed by using ABI 6 Flex System Sequence Detection Software version 1.3. The relative change in gene transcription level was quantified according to $2^{-\Delta\Delta Ct}$ method. For control samples, the mean relative expression level of the assayed gene was assigned a value of 1.0, and the relative expression level of all samples calculated relative to it. Each treatment was performed three replicates.

Table 2. Primers for real-time quantitative PCR.

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Genbank ID
Actin	CTACGAGCTTCCCGATGGAC	AGCCACCACTGAGGACAATG	DQ243702
HGMR	AGATCTATGGATATCCGCCGGAGGC	GGTGACCTCAGGAGCCAATCTTCGTG	EU680958
DXS2	AGATCTATGGCGTCGTCTTGTGGAGTTAT	GGTCACCTTACAAGTTGTTGATGAGATGAA	FJ643618
FPPS	ATGGCGAATCTGAACGGAGA	TTATTTCTGCCTCTTGTATA	DQ991431
GGPPS	AAGGATCCATGAGATCTATGAATCTGGT	CCGAGCTCTTAGTTCTGCCT ATGTGCAA	FJ643617
CPS	ATGGCCTCCTTATCCTCTAC	TCACGCGACTGGCTCGAAAAG	EU003997
RAS10	ATGAAGATCGATATCACAGACTCG	TCAAATATCATAAAACAACTTC	KM575933
CYP98A75	ATGGCGCCTCTCGCTCTCCTCCT	GTCGACGGCGACACGCTCGTAC	KP337735
CYP98A14	ATGGCAGCTCTCCTCCTCGCCCCCGTC	GGTGTCGACAGCAACGCGCTTGTA	HQ316179

2.9. Assays of the Yield of Main Medicinal Components per Plant in Root

The dried roots were pulverized and 50 mg of sample powder was dissolved in 5 mL 70% methanol (Thermo Fisher Scientific (China) Inc., Shanghai, China) and sonicated for 45 min. Then, the solution was centrifuged at 12,000× *g* for 10 min. The solution was filtered through a 0.22 mm microporous membrane (Zhejiang Tailin Biotechnology Co., Ltd., Hangzhou, China) and then used to measure the contents of DHT, CTS, Tan I, Tan IIA, RosA, and SalB in roots by using Waters e2695 binary high-performance liquid chromatography (Waters Technology (Shanghai) Co., Ltd., Shanghai, China) with Waters 2998 ultraviolet detector (Waters Technology (Shanghai) Co., Ltd., Shanghai, China) and Waters Sunfire C18 chromatographic column (Waters Technology (Shanghai) Co., Ltd., Shanghai, China) by comparison with authentic standards based on retention times and peak areas. The standards of DHT, CTS, Tan I, Tan IIA, RosA, and SalB were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The column temperature was 30 °C and sample volume was 20 µL. The volume flow rate was 1 mL/min, and the sample ran for 96 min. The chromatographic detection wavelength for Tans (DHT, CTS, Tan I, and Tan IIA) was 270 nm. The chromatographic detection wavelength for phenolic acids (RosA and SalB) was 288 nm. The mobile phase is chromatography-grade acetonitrile (Thermo Fisher Scientific (China) Inc., Shanghai, China) and 0.02% phosphoric acid (Thermo Fisher Scientific (China) Inc., Shanghai, China) aqueous solution. The elution gradient program was set according to Wang et al. [46]. The contents of main medicinal components were calculated by using the standard curves. The final yield of main medicinal components per plant was expressed as the product of the contents of main medicinal components and the dry weight of root per plant. Above indicators of each treatment were all measured three replicates.

2.10. Statistical Analysis

Data in figures and Tables were the mean of three replicates and expressed as mean ± standard deviation. Figures were drawn by using Excel software 2021. Statistical analyses were performed by one-way analysis of variance by using SPSS software 25.0. Significant difference was compared by Duncan's multiple range test at 5% level. Correlation analysis and regression analysis were also performed by using SPSS software 25.0.

3. Results

3.1. Influences of PGA on Growth Parameters

In comparation with control, different dosages of PGA all increased the growth parameters of basal diameter, plant height, shoot and root biomass, and root volume in June and August (Figure 1). PGA increased the root/shoot ratio in June, but reduced root/shoot ratio in August. Among different dosages of PGA, T2 treatment significantly increased the above growth parameters except the root/shoot ratio, especially in August. In August, T2 treatment increased the basal diameter, plant height, shoot and root biomass, and root volume by 36.8%, 39.9%, 68.3%, 46.4%, and 41.7%, in comparation with the control group. The above findings suggest that PGA application could facilitate the growth of *S. miltiorrhiza*.

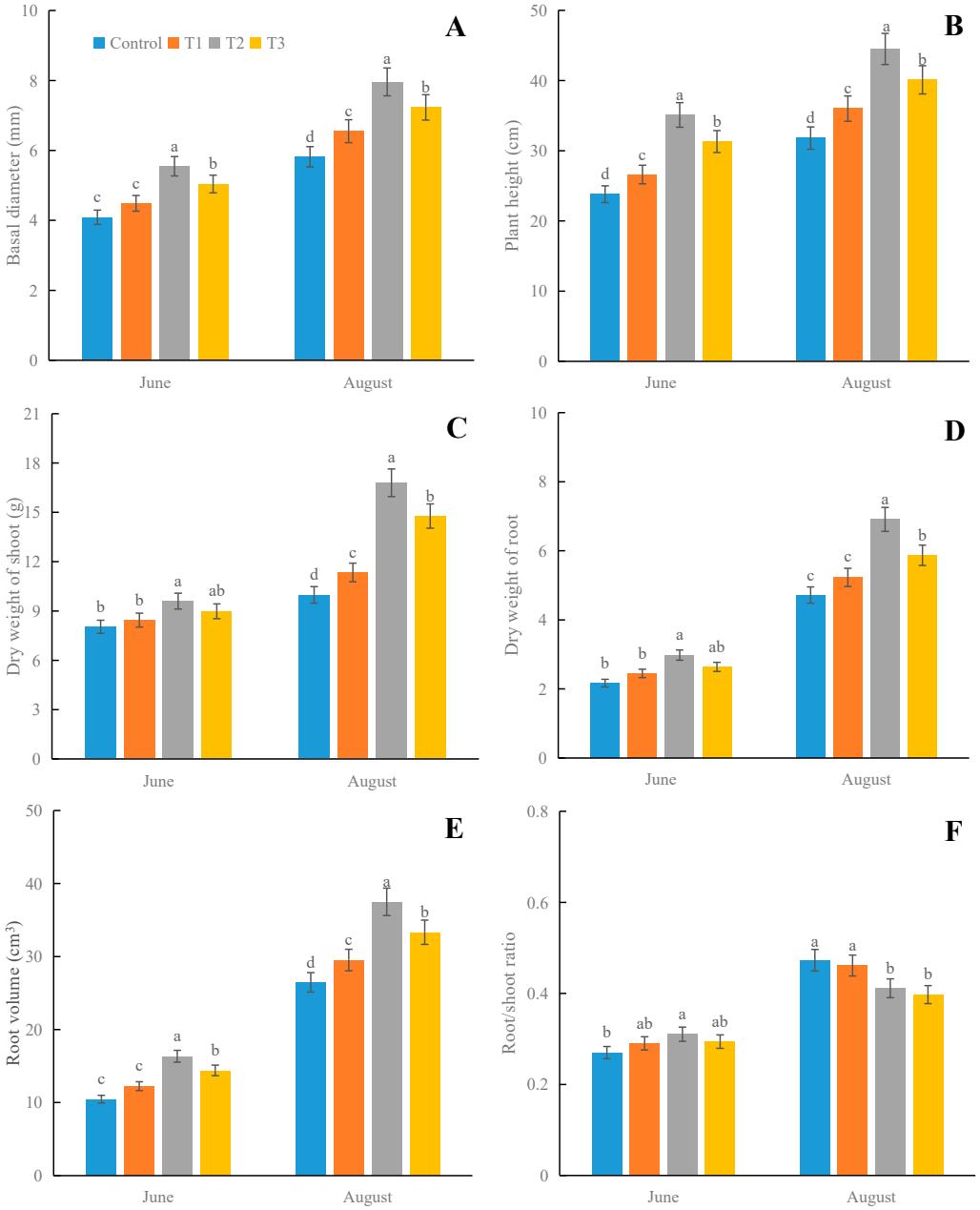

Figure 1. Influences of PGA on the growth parameters of basal diameter (**A**), plant height (**B**), biomass of shoot (**C**) and root (**D**), root volume (**E**), and root/shoot ratio (**F**) of *S. miltiorrhiza*. Different letters stand for significant difference at $p < 0.05$.

3.2. Effects of PGA on Photosynthetic Performance

Compared with the control, different dosages of PGA all increased the SPAD value, Pn, Y(II), Fv/Fm, Fv′/Fm′, qP, and ETR of *S. miltiorrhiza* in June and August (Table 3 and Figure 2). Meanwhile, different dosages of PGA decreased NPQ. Among different dosages of PGA, T2 treatment significantly affected the above parameters. In August, T2 treatment decreased NPQ by 26.9% and increased the SPAD value, Pn, Y(II), Fv/Fm, Fv′/Fm′, qP, and ETR by 30.5%, 51.1%, 27.5%, 27.4%, 34.9%, 24.6%, and 34.4%, in comparation with the control group. The above findings suggest that PGA application could improve the photosynthetic performance, which further facilitates the growth of *S. miltiorrhiza*.

Table 3. Influences of PGA on SPAD value and net photosynthetic rate of *S. miltiorrhiza*. Different letters stand for significant difference at $p < 0.05$.

Treatments	SPAD Value		Net Photosynthetic Rate (μmol m^{-2} s^{-1})	
	June	August	June	August
Control	38.0 ± 1.90 c	34.7 ± 1.39 d	16.4 ± 0.66 d	13.9 ± 0.76 d
T1	43.6 ± 1.91 b	37.9 ± 1.59 c	19.0 ± 0.95 c	15.7 ± 0.72 c
T2	51.6 ± 2.32 a	45.3 ± 2.04 a	24.4 ± 1.10 a	21.0 ± 1.11 a
T3	46.5 ± 2.32 b	41.2 ± 1.90 b	20.6 ± 0.91 b	18.8 ± 0.83 b

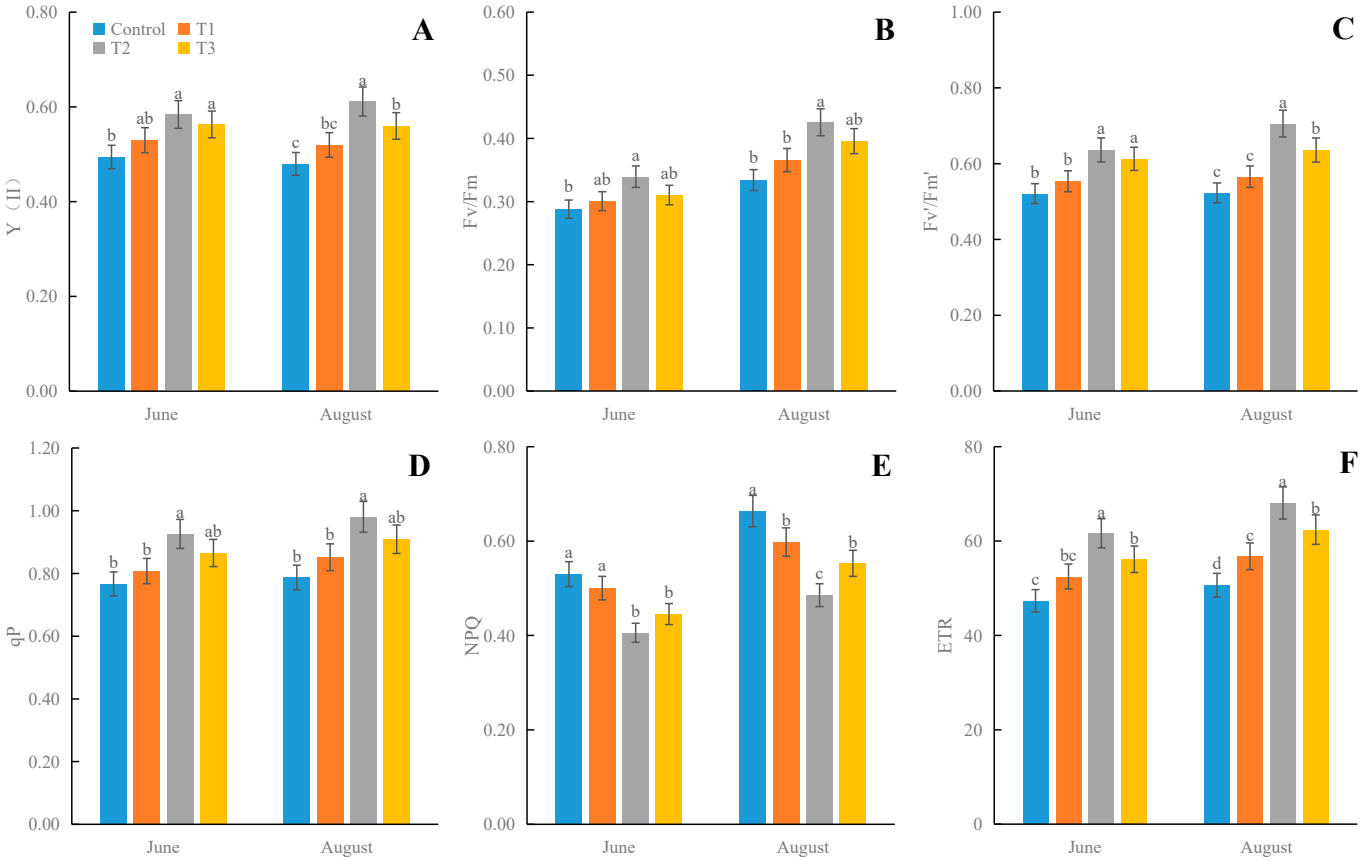

Figure 2. Influences of PGA on Y (II) (**A**), Fv/Fm (**B**), Fv′/Fm′ (**C**), qP (**D**), NPQ (**E**), and ETR (**F**) of *S. miltiorrhiza*. Different letters stand for significant difference at $p < 0.05$.

3.3. Effects of PGA on Water Physiological Characteristics

Compared with the control, different dosages of PGA increased Tr, WUE, Gs, and LRWC in June and August (Figure 3). Meanwhile, different dosages of PGA also increased the contents of osmolytes SP, SS, and Pro (Table 4). Among different levels of PGA, T2 treatment significantly increased the above parameters. In August, T2 treatment increased Tr, WUE, Gs, LRWC, SP, SS, and Pro by 34.9%, 12.3%, 40.2%, 11.6%, 29.5%, 37.8%, and 32.0%, in comparation with the control group. The above findings suggest that PGA application could promote the accumulation of osmolytes, which further improves water retention capacity and maintains the water balance of *S. miltiorrhiza*. In this way, PGA improves the water metabolism and water use efficiency, which further facilitates the growth of *S. miltiorrhiza*.

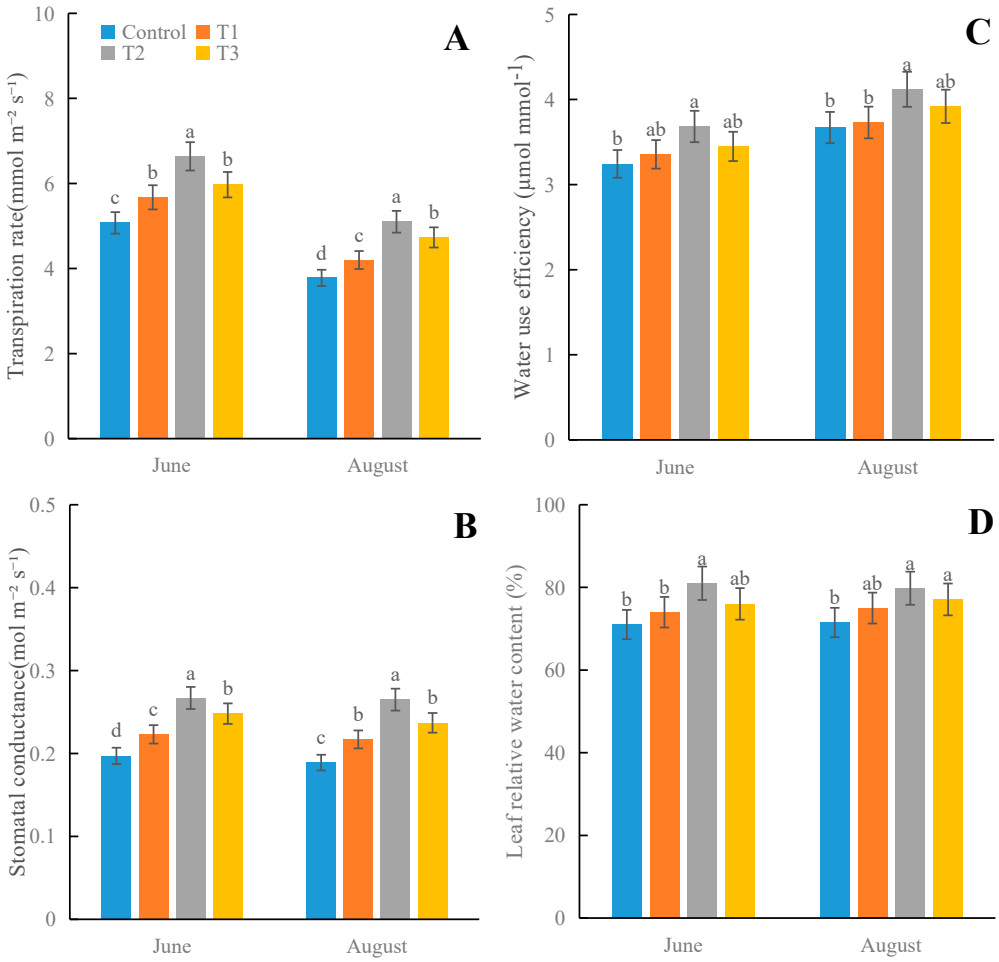

Figure 3. Influences of PGA on water physiological parameters of Tr (**A**), Gs (**B**), WUE (**C**), and LRWC (**D**) of *S. miltiorrhiza*. Different letters stand for significant difference at $p < 0.05$.

Table 4. Influences of PGA on the contents of osmolytes SP, SS, and Pro in *S. miltiorrhiza* leaves. Different letters stand for significant difference at $p < 0.05$.

Treatments	Soluble Protein Content (mg g^{-1} FW)		Soluble Sugar Content (mg g^{-1} FW)		Proline Content (μg g^{-1} FW)	
	June	August	June	August	June	August
Control	94.5 ± 4.35 c	57.7 ± 2.42 c	15.8 ± 0.74 c	17.2 ± 0.85 c	45.0 ± 1.80 c	50.3 ± 2.31 c
T1	102.5 ± 5.13 bc	62.9 ± 3.21 bc	17.8 ± 0.89 b	19.0 ± 1.01 c	54.5 ± 2.51 b	56.0 ± 2.52 b
T2	119.9 ± 4.80 a	74.7 ± 2.99 a	20.9 ± 0.84 a	23.7 ± 1.07 a	65.2 ± 2.80 a	66.8 ± 3.20 a
T3	107.3 ± 5.58 b	65.9 ± 2.96 b	19.0 ± 0.99 ab	21.3 ± 0.94 b	58.7 ± 2.76 b	58.9 ± 2.89 b

3.4. Influences of PGA on the Antioxidant Capacity

In comparation with the control group, different dosages of PGA enhanced the antioxidant capacity by promoting the activities of antioxidant enzymes and the contents of antioxidants, which further lowered the MDA content in June and August (Tables 5 and 6, Figure 4). Among different dosages of PGA, T2 treatment significantly strengthened the antioxidant capacity. In August, T2 treatment increased the activities of SOD, POD, CAT, APX, and GR by 33.3%, 29.9%, 40.1%, 36.6%, and 42.0%, respectively, in comparation with the control group. In addition, T2 treatment increased the contents of AsA and GSH by 39.9% and 30.1%, respectively. Meanwhile, T2 treatment decreased the MDA content by 41.1%. The above findings indicate that PGA application enhances the antioxidant capacity,

which further maintains the redox homeostasis of *S. miltiorrhiza.* In this way, PGA facilitates the growth of *S. miltiorrhiza*.

Table 5. Influences of PGA on the contents of AsA and GSH in *S. miltiorrhiza* leaves. Different letters stand for significant difference at $p < 0.05$.

Treatments	AsA Content (μg g^{-1} FW)		GSH Content (μg g^{-1} FW)	
	June	August	June	August
Control	160.0 ± 7.04 d	188.4 ± 9.42 d	412.4 ± 17.73 c	454.9 ± 18.19 d
T1	179.5 ± 7.18 c	208.4 ± 10.21 c	455.9 ± 23.10 b	505.5 ± 19.20 c
T2	230.0 ± 11.50 a	263.6 ± 10.80 a	515.7 ± 25.78 a	591.7 ± 22.85 a
T3	198.9 ± 8.35 b	231.5 ± 11.57 b	484.3 ± 21.30 ab	547.1 ± 21.61 b

Table 6. Influences of PGA on the activities of APX and GR in *S. miltiorrhiza* leaves. Different letters stand for significant difference at $p < 0.05$.

Treatments	APX Activity (U g^{-1} FW)		GR Activity (U g^{-1} FW)	
	June	August	June	August
Control	1.80 ± 0.11 d	2.24 ± 0.10 d	1.35 ± 0.09 d	1.57 ± 0.08 d
T1	1.91 ± 0.10 cd	2.46 ± 0.10 c	1.55 ± 0.10 c	1.76 ± 0.10 c
T2	2.64 ± 0.12 a	3.06 ± 0.15 a	2.01 ± 0.10 a	2.23 ± 0.13 a
T3	2.32 ± 0.11 b	2.76 ± 0.14 b	1.78 ± 0.12 b	1.98 ± 0.11 b

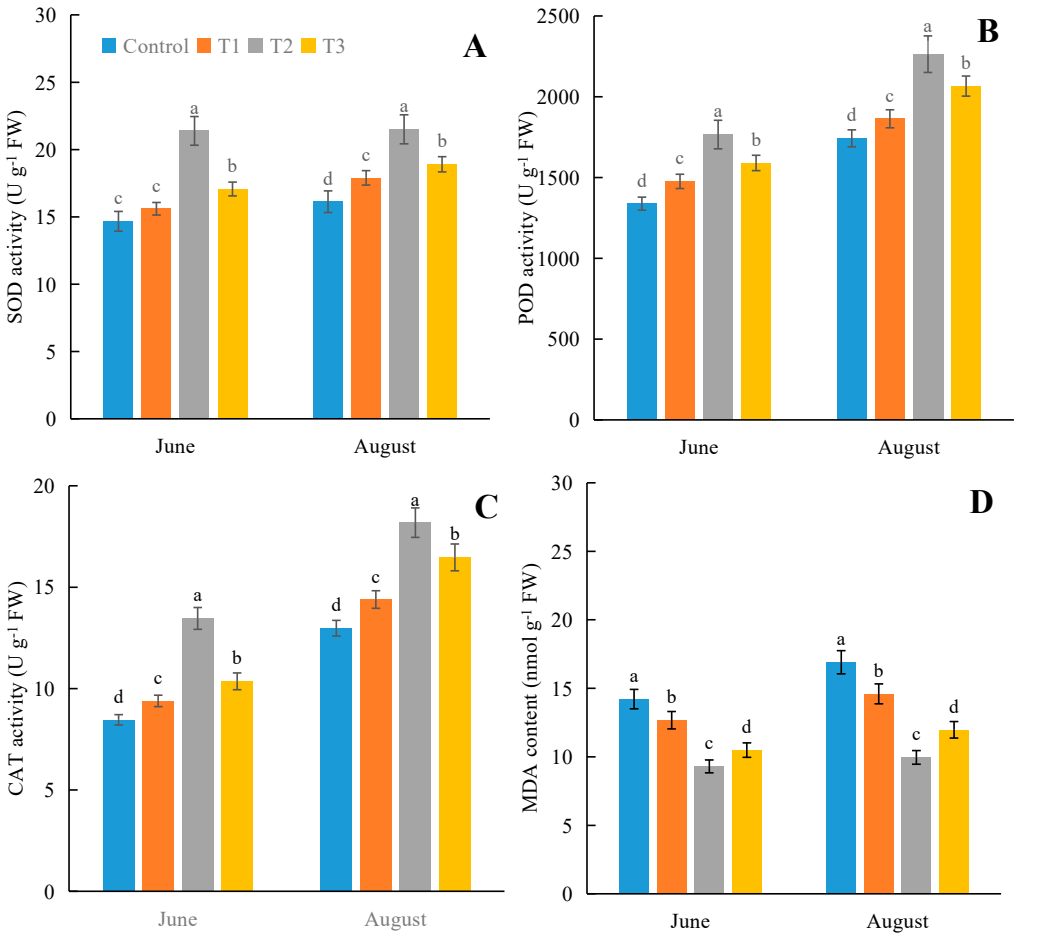

Figure 4. Influences of PGA on the activities of SOD (**A**), POD (**B**) and CAT (**C**), and MDA content (**D**) in *S. miltiorrhiza* leaves. Different letters stand for significant difference at $p < 0.05$.

3.5. Effects of PGA on the Yield of RosA and SalB per Plant and the Expression of Genes Coding Enzymes Responsible for Their Biosynthesis in S. miltiorrhiza Root

In comparation with control, different dosages of PGA had better influences on the yield of RosA and SalB per plant and the expression of gene coding enzymes responsible for their biosynthesis in roots in June and August (Figures 5 and 6). Among different dosages of PGA, T2 treatment significantly enhanced the expression of gene coding enzymes responsible for RosA and SalB biosynthesis, including *RAS10*, *CYD98A75*, and *CYD98A14*, which further increased the yield of RosA and SalB in roots. In August, T2 treatment up-regulated the expression of *RAS10*, *CYD98A75*, and *CYD98A14* by 111.0%, 200.0%, and 163.6%, in comparation with control. At the same time, T2 treatment facilitated the yield of RosA and SalB per plant by 74.8% and 130.4%. These results suggest that PGA application promotes RosA and SalB biosynthesis in roots by enhancing the expression of gene coding enzymes in charge of their biosynthesis at the transcript level. In this way, PGA facilitates the accumulation of water-soluble medicinal ingredients in roots.

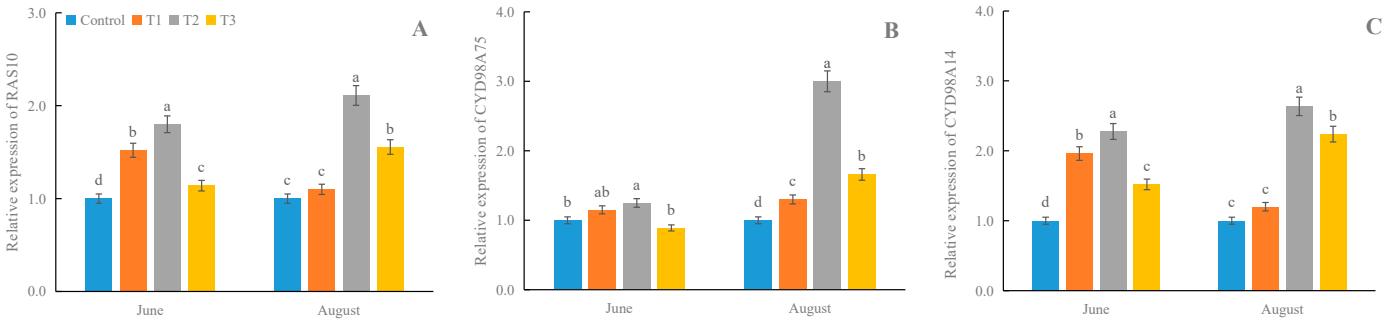

Figure 5. Influence of PGA on the expression of *RAS10* (**A**), *CYD98A75* (**B**), and *CYD98A14* (**C**) in root. Different letters stand for significant difference at $p < 0.05$.

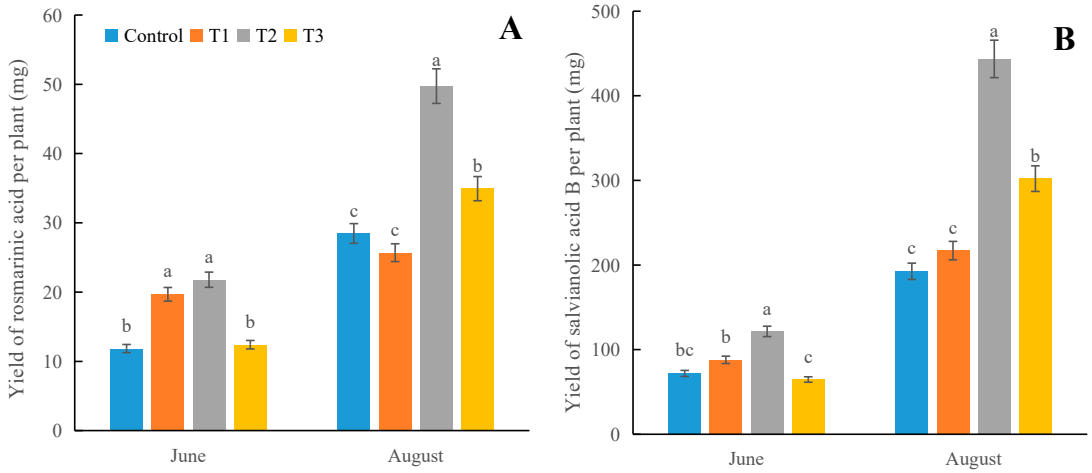

Figure 6. Influence of PGA on the yield of rosmarinic acid (**A**) and salvianolic acid B (**B**) per plant in *S. miltiorrhiza* root. Different letters stand for significant difference at $p < 0.05$.

3.6. Effects of PGA on the Yield of Main Tanshinones per Plant and the Expression of Genes Coding Enzymes Responsible for Their Biosynthesis in S. miltiorrhiza Root

In comparation with control, different dosages of PGA also had better influences on the yield of main tanshinones per plant and the expression of gene coding enzymes responsible for their biosynthesis in roots in June and August (Figures 7 and 8), especially in August. Among different dosages of PGA, T1 significantly enhanced the expression of *FPPS*, *HGMR*, and *GGPPS*. T2 significantly enhanced the expression of *DXS2*, *CPS*, *HGMR*, and *GGPPS*, which further increased the yield of main tanshinones in *S. miltiorrhiza* roots, including

DHT, CTS, Tan I, and Tan IIA. In August, T1 treatment up-regulated the expression of *HGMR*, *DXS2*, *FPPS*, *GGPPS*, and *CPS* by 125.0%, 150.0%, 233.0%, 399.0%, and 266.0%, T2 treatment up-regulated the expression of *HGMR*, *DXS2*, *FPPS*, *GGPPS*, and *CPS* by 136.0%, 222.0%, 157.0%, 411.0%, and 310.0%, T3 treatment up-regulated the expression of *HGMR*, *DXS2*, *FPPS*, *GGPPS*, and *CPS* by 80.0%, 191.0%, 160.0%, 271.0%, and 211.0%, compared with the control. Meanwhile, T1 treatment increased the yield of DHT, CTS, Tan I, and Tan IIA by 74.0%, 113.2%, 86.4%, and 46.6%, T2 treatment increased the yield of DHT, CTS, Tan I, and Tan IIA by 99.7%, 44.8%, 90.5%, and 27.1%. T3 treatment increased the yield of DHT, CTS, Tan I, and Tan IIA by 85.2%, 49.4%, 71.3%, and 13.3%, compared with the control. The above findings suggest that PGA application could facilitate the biosynthesis of DHT, CTS, Tan I, and Tan IIA in roots by enhancing the expression of gene coding enzymes in charge of their biosynthesis at the transcript level. In this way, PGA facilitates the accumulation of fat-soluble medicinal ingredients in roots.

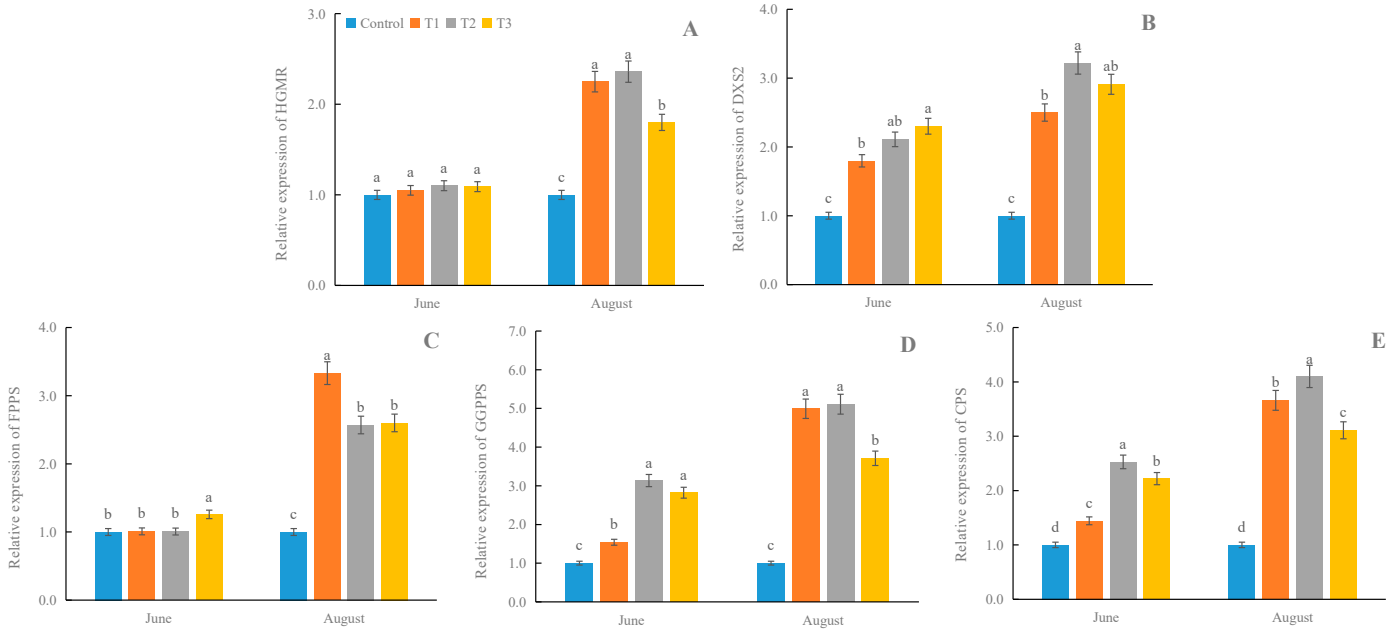

Figure 7. Influence of PGA on the expression of *HGMR* (**A**), *DXS2* (**B**), *FPPS* (**C**), *GGPPS* (**D**), and *CPS* (**E**) in *S. miltiorrhiza* root. Different letters stand for significant difference at $p < 0.05$.

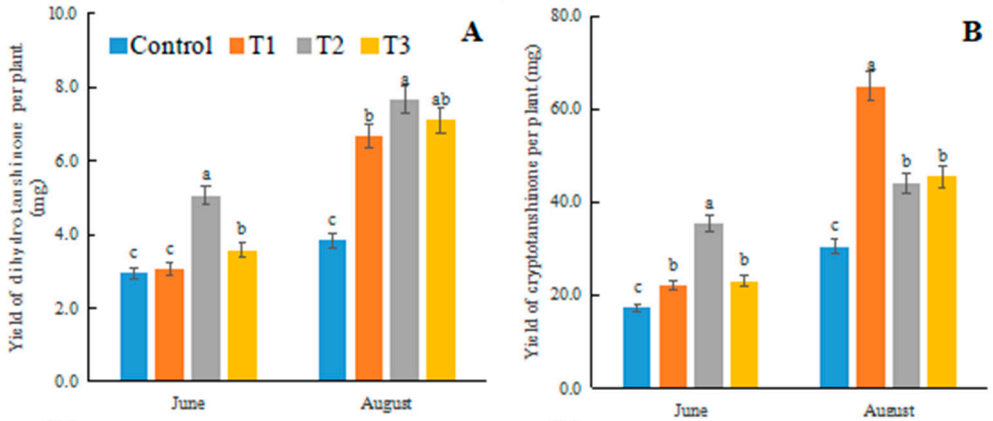

Figure 8. *Cont.*

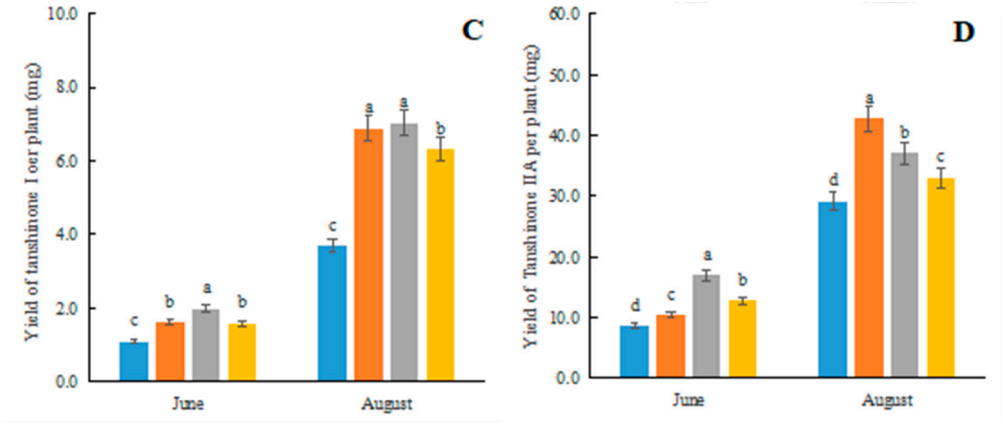

Figure 8. Influence of PGA on the yield of dihydrotanshinone (**A**), cryptotanshinone (**B**), tanshinone I (**C**), and tanshinone IIA (**D**) per plant in *S. miltiorrhiza* root. Different letters stand for significant difference at $p < 0.05$.

3.7. Effects of PGA on Final Biomass and the Final Yield of Main Medicinal Components per Plant

In comparation with the control group, different dosages of PGA had significant effects on the final biomass of the shoots and roots and the yield of RosA, SalB, DHT, CTS, Tan I, and Tan IIA per plant (Table 7 and Figure 9), especially with T2 and T3 treatment. Compared with control, T2 treatment improved the final biomass of the shoots and roots by 65.4% and 63.0%, T3 treatment improved the final biomass of the shoots and roots by 48.2% and 35.9%. Meanwhile, T2 treatment apparently improved the yield of RosA, SalB, DHT, CTS, Tan I, and Tan IIA per plant by 123.0%, 208.8%, 65.5%, 14.7%, 42.5%, and 22.2%, compared with the control. T3 treatment apparently improved the yield of RosA, SalB, DHT, CTS, Tan I, and Tan IIA per plant by 49.1%, 100.7%, 68.9%, 43.3%, 109.9%, and 86.5%. These results once again suggest that the application of PGA could improve *S. miltiorrhiza* growth and enhance the accumulation of main medicinal components in root.

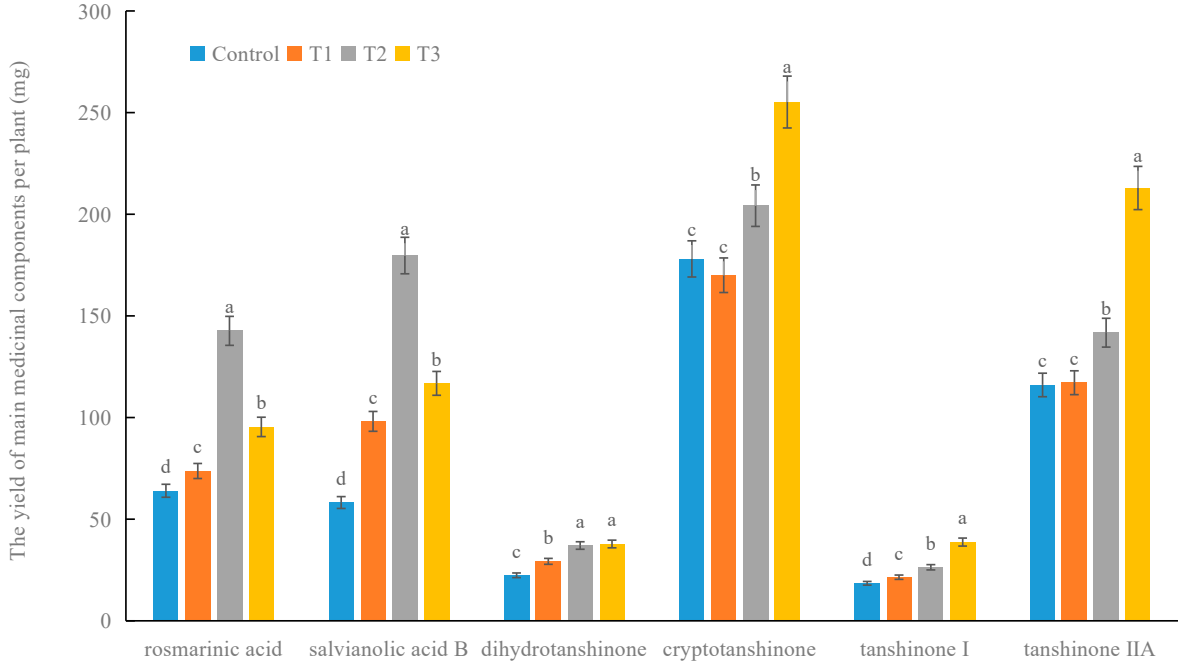

Figure 9. Influence of PGA on final yield of main medicinal components per plant in *S. miltiorrhiza* root. Different letters stand for significant difference at $p < 0.05$.

Table 7. Influences of PGA on final dry weight of shoot and root of *S. miltiorrhiza*. Different letters stand for significant difference at $p < 0.05$.

Treatments	Shoot Dry Weight per Plant (g)	Root Dry Weight per Plant (g)
Control	12.10 ± 0.70 d	18.70 ± 1.12 d
T1	14.75 ± 0.89 c	21.01 ± 1.18 c
T2	20.01 ± 1.10 a	30.49 ± 1.52 a
T3	17.93 ± 1.04 b	25.41 ± 1.21 b

Through Pearson correlation analysis (Table 8), we found that shoot and root dry weight and the yield of RosA, SalB, DHT, CTS, Tan I, and Tan IIA were all positively correlated with PGA dosage, which indicated that PGA had positive effects on plant growth and the yield of above main medicinal components in roots. Through regression analysis (Table 9), we found that shoot and root dry weight and the yield of RosA, SalB, and DHT increased when PGA dosage was during the range from 0 to 0.044 g per pot, but tended to be decreased under higher PGA dosage. While the yield of CTS, Tan I, and Tan IIA increased when PGA dosage was during the range from 0 to 0.088 g per pot. Therefore, our above results clearly suggested that there was a suitable dosage of PGA in promoting the growth and the yield of main medicinal components in roots.

Table 8. Correlation analysis between PGA dosage and final dry weight of shoot and root, and final yield of main medicinal components of *S. miltiorrhiza*. One asterisk (*) stands for the significant correlation at the level of 0.05 (double-tailed), two asterisk (**) stands for the significant correlation at the level of 0.01 (double-tailed).

	PGA Dosage	Shoot Dry Weight	Root Dry Weight	RosA	SalB	DHT	CTS	Tan I	Tan II A
PGA dosage	1.000	0.734	0.612	0.463	0.510	0.892	0.945	0.990 **	0.958 *
shoot dry weight	0.734	1.000	0.979 *	0.929	0.958 *	0.956 *	0.610	0.650	0.555
root dry weight	0.612	0.979 *	1.000	0.984 *	0.979 *	0.882	0.520	0.528	0.439
RosA	0.463	0.929	0.984 *	1.000	0.977	0.783	0.384	0.374	0.287
SalB	0.510	0.958 *	0.979 *	0.977 *	1.000	0.839	0.366	0.405	0.295
DHT	0.892	0.956 *	0.882	0.783	0.839	1.000	0.762	0.826	0.742
CTS	0.945	0.610	0.520	0.384	0.366	0.762	1.000	0.975 *	0.989 *
Tan I	0.990 *	0.650	0.528	0.374	0.405	0.826	0.975 *	1.000	0.989 *
Tan II A	0.958 *	0.555	0.439	0.287	0.295	0.742	0.989 *	0.989 *	1.000

Table 9. Regression analysis between PGA dosage and final dry weight of shoot and root, and final yield of main medicinal components of *S. miltiorrhiza*.

Variable Y	Variable X	Regression Equation	Degree of Fit
shoot dry weight	PGA dosage	$Y = -2100.4X^2 + 259.53X + 11.543$	$R^2 = 0.8958$
root dry weight		$Y = -3287.5X^2 + 384.37X + 17.458$	$R^2 = 0.7674$
RosA		$Y = -24011X^2 + 2620.1X + 54.057$	$R^2 = 0.6748$
SalB		$Y = -39378X^2 + 4276.3X + 48.622$	$R^2 = 0.8542$
DHT		$Y = -3171X^2 + 461.81X + 21.89$	$R^2 = 0.9816$
CTS		$Y = 967.04X + 164.63$	$R^2 = 0.8934$
Tan I		$Y = 235.43X + 17.166$	$R^2 = 0.9806$
Tan IIA		$Y = 1161.9X + 102.22$	$R^2 = 0.9181$

4. Discussion

In recent years, researchers have shown that PGA improves the growth of Chinese cabbage [32], maize [20], rape [47], spinach (*Spinacia oleracea* L.) [48], cotton (*Gossypium hirsutum* L.) [49], lettuce (*Lactuca sativa* var. ramosa Hort.) [50], wheat (*Triticum aestivum* L.) [51], cucumber (*Cucumis sativus* L.) [52], and *Solanum nigrum* [53]. In our study, the findings demonstrated that PGA significantly improved *S. miltiorrhiza* growth, as indicated by basal diameter, plant height, shoot and root biomass, and root volume, which agreed

with previous results in other plants [36,50]. Previous research has also documented that plant growth had close relationships with photosynthesis, water metabolism, and the antioxidant capacity [34,36]. Therefore, it will be interesting to explore the influences of PGA on the photosynthesis, water metabolism, and the antioxidant capacity of *S. miltiorrhiza*, which can elucidate the physiological mechanism of PGA in promoting the growth of *S. miltiorrhiza*. For photosynthesis, previous studies have shown that PGA improved Pn and the contents of photosynthetic pigments in maize, rape and Chinese cabbage [33–35]. It has been reported that PGA could decrease the chlorophyll fluorescence parameter NPQ value and increase the values of Fv/Fm, ETR, qP, and Y (II) [34]. In *S. miltiorrhiza*, we found that PGA decreased NPQ and improved the Pn, SPAD value, Fv/Fm, ETR, qP, and Y (II), which was consistent with previous studies in other plants [34,35]. For water metabolism, Cao et al. [36] reported that PGA could promote stomatal opening, WUE, and the content of osmotic regulator proline in maize. Quan et al. [34] reported that PGA increased Tr, Gs, and proline content in cabbage. Xu et al. [33] found that PGA improved RWC and proline content in rape. Liang et al. [49] showed that PGA enhanced WUE of cotton crop. Lei et al. [54] demonstrated that PGA could increase proline content in rape. In *S. miltiorrhiza*, we found that PGA also promoted Tr, Gs, WUE, and proline content, which agreed with previous results in above other plants [34,36]. Additionally, we also found that PGA could increase LRWC and the contents of osmotic regulators SP and SS, which indicated that PGA could enhance water metabolism by maintaining water balance through osmoregulation in *S. miltiorrhiza*. For the antioxidant capacity, Xu et al. [33] showed that PGA enhanced the activities of SOD and CAT in rape. Quan et al. [34] found that PGA enhanced the activities of SOD, POD, CAT, and APX in Chinese cabbage. Cao et al. [36] demonstrated that PGA could improve the activities of SOD, POD, and APX in maize. Guo et al. [52] showed that PGA enhanced the activities of SOD, POD, and CAT in wheat. For *S. miltiorrhiza*, our study also displayed that PGA strengthened the antioxidant capacity by enhancing the activities of SOD, POD, CAT, and APX, which agreed with previous results in above other plants [34,36]. In addition, our findings demonstrated that PGA increased GR activity and the contents of antioxidants AsA and GSH in leaves, which further enhanced the antioxidant capacity of *S. miltiorrhiza*, which is one novelty for this study. As APX and GR are in charge of the operation of the ascorbate–glutathione (AsA-GSH) cycle, our current findings indicated that PGA could enhance the antioxidant capacity by improving the AsA-GSH cycle in *S. miltiorrhiza*. Shan and Liang [38] have displayed that the contents of AsA and GSH could be regulated through the enzymes responsible for their regeneration and biosynthesis. In the AsA-GSH cycle, APX, GR, DHAR, and MDHAR are in charge of AsA and GSH regeneration [55]. L-galactono-1,4-lactone dehydrogenase (GalLDH) and gamma-glutamylcysteine synthetase (γ-ECS) are in charge of AsA and GSH biosynthesis, respectively [43]. In the study, the findings showed that PGA increased the contents of AsA and GSH in *S. miltiorrhiza*, whereas our study only explored the influences of PGA on the activities of APX and GR. We did not explore the influences of PGA on the activities of DHAR, MDHAR, GalLDH, and γ-ECS. Therefore, it is worth exploring the influences of PGA on the activities of DHAR, MDHAR, GalLDH, and γ-ECS, which can provide more information on the roles of PGA in enhancing the antioxidant capacity of *S. miltiorrhiza*. Furthermore, Xu et al. [47] displayed that brassinolide (BR), jasmonic acid (JA), Ca^{2+}, and H_2O_2 were involved in PGA regulating the antioxidant capacity of rape through cross-talk. However, it is still unclear whether BR, JA, Ca^{2+}, and H_2O_2 were involved in PGA regulating the antioxidant capacity of *S. miltiorrhiza* alone or through cross-talk. Therefore, it is worth investigating the roles of BR, JA, Ca^{2+}, and H_2O_2 in PGA regulating the antioxidant capacity of *S. miltiorrhiza*, which can clarify more mechanisms of the roles of PGA in enhancing the antioxidant capacity of *S. miltiorrhiza*. The above results of the current study are the first to report the function of PGA in facilitating *S. miltiorrhiza* growth by enhancing photosynthesis, water metabolism, and antioxidant capacity (Figure 10), which provides more theoretical basis for PGA applications in promoting the growth of *S. miltiorrhiza*.

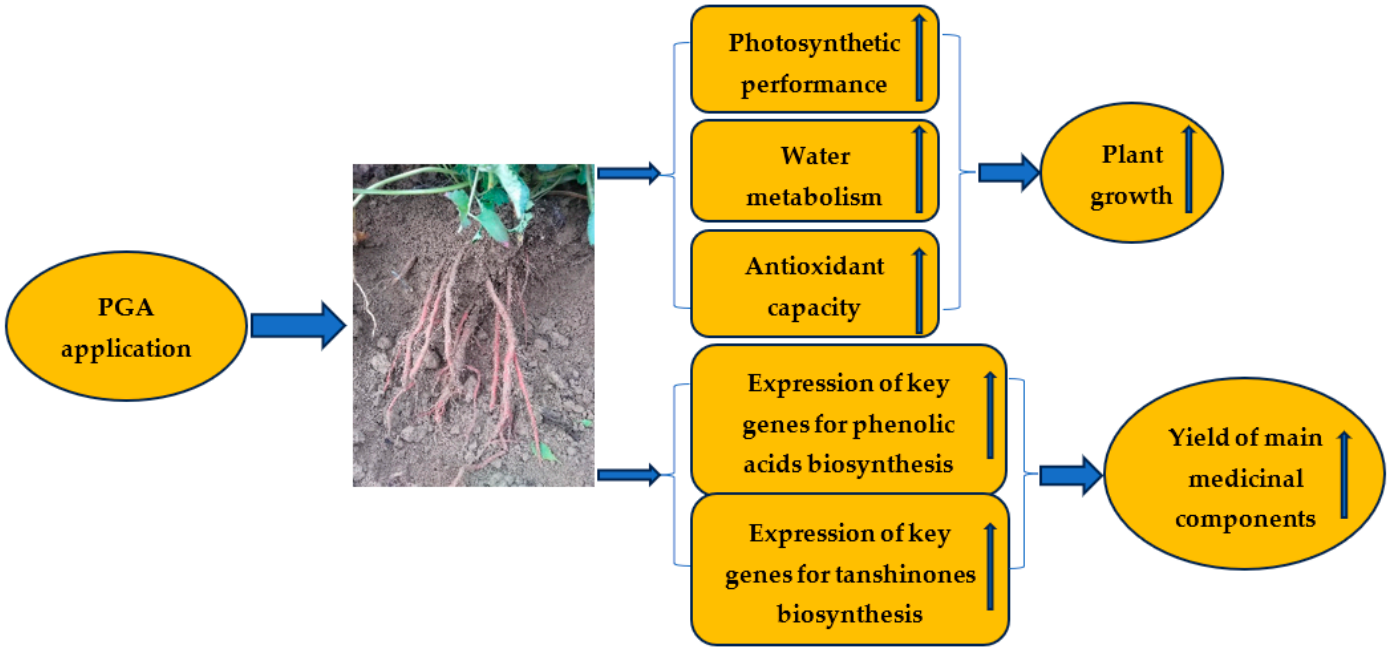

Figure 10. Synthetic summary of the influences of PGA on the growth, physiological characteristics, and accumulation of main medicinal components in *S. miltiorrhiza*.

Tans and SAs are the main medicinal components in *Salvia* species, including *S. miltiorrhiza*. In the root of *S. miltiorrhiza*, Tans mainly include DHT, CTS, Tan I, and Tan IIA, and SAs mainly include RosA and SalB [8]. For the current research, our findings demonstrate that different dosages of PGA had significant influences on the final yield of DHT, CTS, Tan I and Tan IIA, RosA, and SalB in *S. miltiorrhiza* roots, especially for the treatments with 0.044 and 0.088 g PGA per pot. The biosynthetic pathways of phenolic acids include the phenylpropanoid pathway and tyrosine-derived pathway [2,3]. The key enzymes in the phenylpropanoid pathway mainly include phenylalanine ammonia lyase (PAL) and 4-coumarate:CoA ligase (4CL). In the tyrosine-derived pathway, the key enzymes mainly include tyrosine aminotransferase (TAT) and 4-hydroxyphenylpyruvate reductase (HPPR). Rosmarinic acid synthase (RAS) can couple products from the above two pathways to synthesize 4-coumaroyl-3′,4′-dihydroxyphenyllactic acid, which is then hydroxylated by CYP98A to produce RosA. SalB is then derived from RosA [2,3]. Thus, the contents of the phenolic acids RosA and SalB have close relationships with the expression of *PAL*, *4CL*, *TAT*, *RAS*, and *CYP98A* [2,3]. In this study, we found that PGA could up-regulate the expression of *RAS10*, *CYD98A75*, and *CYD98A14*, which further increased the accumulation of the water-soluble ingredients RosA and SalB in roots. These results clearly indicated that PGA could improve the production of RosA and SalB by enhancing their anabolism through the up-regulation of *RAS* and *CYD98A* at the transcript level. The biosynthetic pathways of Tans include the mevalonate (MVA) pathway in cytoplasm and the methylerythritol phosphate (MEP) pathway in plastids [56]. In the MVA pathway, 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) is a key upstream enzyme. In the MEP pathway, 1-deoxy-D-xylulose-5-phosphate synthase (DXS) is a key upstream enzyme [56,57]. The products of the above two pathways are then catalyzed by the key midstream and downstream enzymes. The key midstream enzymes include geranylgeranyl diphosphate synthase (GGPPS), farnesyl pyrophosphate synthase (FPPS), and copalyl diphosphate synthase (CPS). The key downstream enzymes include kaurene synthase-like (KSL) and a cytochrome P450 enzyme (CYP76AH1) [58]. Thus the contents of Tans have also close relationships with the expression of their biosynthetic genes, including *DXS*, *HMGR*, *FPPS*, *GGPPS*, *CPS*, and *KSL*. The current findings also demonstrated that PGA could up-regulate the expression of *HGMR*, *DXS2*, *FPPS*, *GGPPS*, and *CPS*, which

further increases the accumulation of the fat-soluble ingredients DHT, CTS, Tan I, and Tan IIA in roots. The present study clearly indicates that PGA could increase the yield of the main medicinal components in roots by enhancing the expression of key upstream and midstream gene coding enzymes in charge of their biosynthesis at the transcript level (Figure 10), which provides knowledge for the roles of PGA in regulating the accumulation of medicinal components and PGA applications in *S. miltiorrhiza* production. This is the other novelty of this study. In the current study, we also found that the treatment with 0.044 g PGA per pot had better effects on the yield of the water-soluble medicinal ingredients RosA and SalB in *S. miltiorrhiza* roots, while the treatment with 0.088 g PGA per pot had better effects on the yield of the fat-soluble medicinal ingredients CTS, Tan I, and Tan IIA in roots. Thus, the above results suggest that we should select the suitable dosage of PGA according to market demand for medicinal ingredients in *S. miltiorrhiza* production. It has been documented that methyl jasmonate (MJ) promotes Tans biosynthesis in *S. miltiorrhiza* [59]. Increasing research has also shown that JA could be involved in smoke-isolated butenolide-regulated Tans biosynthesis in *S. miltiorrhiza* [60]. Additionally, MJ and JA could regulate phenolic acid accumulation in *S. miltiorrhiza* [3,14]. The previous research above indicated that jasmonates (JAs) could regulate Tans and phenolic acids biosynthesis. Meanwhile, previous studies showed that JA could regulate SalB content in *S. miltiorrhiza* through cross-talk with brassinolide, Ca^{2+}, and H_2O_2 [10,11]. A previous study displayed that JA participated in PGA regulating the antioxidant capacity of *S. miltiorrhiza* [47]. However, it is still unclear whether JA participated in PGA-regulated Tans and phenolic acid biosynthesis in *S. miltiorrhiza* alone or through cross-talk with other signal molecules. In addition, ABA has been proven to be a vital signal molecule in regulating the accumulation of Tans and phenolic acids in *S. miltiorrhiza* [61,62]. While it is still unknown whether ABA participated in PGA-regulated Tans and phenolic acid biosynthesis in *S. miltiorrhiz*. If so, it will also be worth investigating the cross-talk between ABA and the other signal molecules above in PGA-regulated Tans and phenolic acid biosynthesis, which will provide more knowledge on the mechanisms of PGA in improving the accumulation of main medicinal components in *S. miltiorrhiza*.

5. Conclusions

To conclude, our findings indicated that PGA significantly promoted the growth and the accumulation of main medicinal components in *S. miltiorrhiza*. The application of PGA improved the growth of *S. miltiorrhiza* by promoting photosynthesis, water metabolism, and the antioxidant capacity. Meanwhile, PGA also improved the accumulation of main medicinal components by enhancing the expression of gene coding enzymes in charge of Tans and phenolic acid biosynthesis in *S. miltiorrhiza* at the transcript level. The above results suggest that PGA could be used to improve the biomass and the yield of main medicinal components in the production of *S. miltiorrhiza*.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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

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