agronomy

MDPI

Article

Contribution of Putrescine and Glutamic Acid on γ-Aminobutyric Acid Accumulation of *Malus baccata* Borkh. Roots under Suboptimal Low Root-Zone Temperature

Xiaochen Lu [1], Meiqi Zhao [1], Enda Zhou [1], Huaiyu Ma [1,2,*] and Deguo Lyu [1,2,*]

[1] College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China; luxiaochen9726@163.com (X.L.); zhaomeiqi0909@163.com (M.Z.); zhouenda163@163.com (E.Z.)
[2] Key Lab of Fruit Quality Development and Regulation of Liaoning Province, Shenyang 110866, China
* Correspondence: 2006500038@syau.edu.cn (H.M.); lvdeguo@syau.edu.cn (D.L.)

Abstract: GABA (γ-aminobutyric acid) is found in plants and accumulates rapidly under stresses. However, the contributions of glutamic acid and a (Glu)-derived pathway and polyamines (PAs) catabolism pathway on GABA accumulation and the regulatory effects of exogenous putrescine (Put) on a GABA shunt under suboptimal low root-zone temperatures remain unknown. Our results showed that suboptimal low root-zone temperatures (treatment L) significantly increased GABA contents and GABA transaminase (GABA-T) activities. The contribution rate of the PAs catabolism pathway increased from 20.60% to 43.31%. Treatment L induced oxidative stress in *Malus baccata* Borkh. roots. Exogenous Put increased the contents of endogenous Put, spermine (Spm), and spermidine (Spd), promoted the transformation of PAs, increased the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), and decreased the contents of hydrogen peroxide (H_2O_2), superoxide anion ($O_2 \cdot^-$), and malondialdehyde (MDA). Meanwhile, contrasting results were observed after aminoguanidine (AG, an inhibitor of diamine oxidase) application. These findings revealed that the Glu-derived pathway is the main route of GABA synthesis. The contribution rate of the Pas catabolism pathway increased gradually with the extension of treatment time, and the treatment of exogenous Put significantly improved the tolerance of *Malus baccata* Borkh. Roots to suboptimal low temperature by regulating the transformation of Pas, GABA shunt, and the antioxidant system.

Keywords: γ-aminobutyric acid; polyamine; putrescine; glutamic acid; *Malus baccata* Borkh.; suboptimal low root-zone temperature

Citation: Lu, X.; Zhao, M.; Zhou, E.; Ma, H.; Lyu, D. Contribution of Putrescine and Glutamic Acid on γ-Aminobutyric Acid Accumulation of *Malus baccata* Borkh. Roots under Suboptimal Low Root-Zone Temperature. *Agronomy* **2023**, *13*, 1989. https://doi.org/10.3390/agronomy13081989

Academic Editor: Alfonso Albacete

Received: 28 April 2023
Revised: 19 July 2023
Accepted: 26 July 2023
Published: 27 July 2023

1. Introduction

GABA (γ-aminobutyric acid), first discovered in potato tubers in 1947, is a four-carbon non-protein amino acid and is ubiquitous in organisms [1,2]. GABA is also a small-molecule nitrogen-containing compound with a high level of physiological activity and plays an important role in plant growth and development [3]. The content of GABA in various tissues and organs of plants is at a low level under normal circumstances but accumulates rapidly and massively when exposed to stresses, even more than some protein amino acids [4–7]. Thus, GABA is considered to be a regulatory substance to resist stresses.

The GABA pathway in plants is shown in Figure 1, based on Shelp et al. and Wuddineh et al. [8,9]. GABA accumulation in plant cells is considered to be the main pathway in which Glu is converted to GABA by glutamate decarboxylase (GAD); however, the polyamine catabolism pathway also contributes. Arginine is converted to putrescine via alternative multi-step routes: arginine decarboxylase (ADC); arginase and ornithine decarboxylase (ODC). The specific process of the polyamine catabolism pathway starts with putrescine (Put), which is the main substrate for GABA. Put is not only converted to Δ1-pyrroline by diamine oxidase (DAO) but also to spermidine (Spd) and spermine (Spm) via spermidine

synthase and spermine synthase, respectively. Polyamine oxidases (POD) are responsible for catalyzing Spd to Δ1-pyrroline and Spm to 1,3-Diaminopropane, respectively. Then, 1,3-Diaminopropane is converted to Δ1-pyrroline. Finally, Δ1-pyrroline is further converted to GABA by the influence of aldehyde dehydrogenase. GABA derived from both Put and Glu is converted to succinate acid (Suc), which is a natural intermediate of the tricarboxylic acid cycle, via GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH). Consequently, GABA is closely related to a variety of physiological pathways in plants and participates in the metabolic regulation process to improve plant resistance under stress. In addition, it is worth mentioning that aminoguanidine (AG) is often used in experiments as a specific inhibitor of DAO to evaluate the effect of PAs on GABA shunts [10,11].

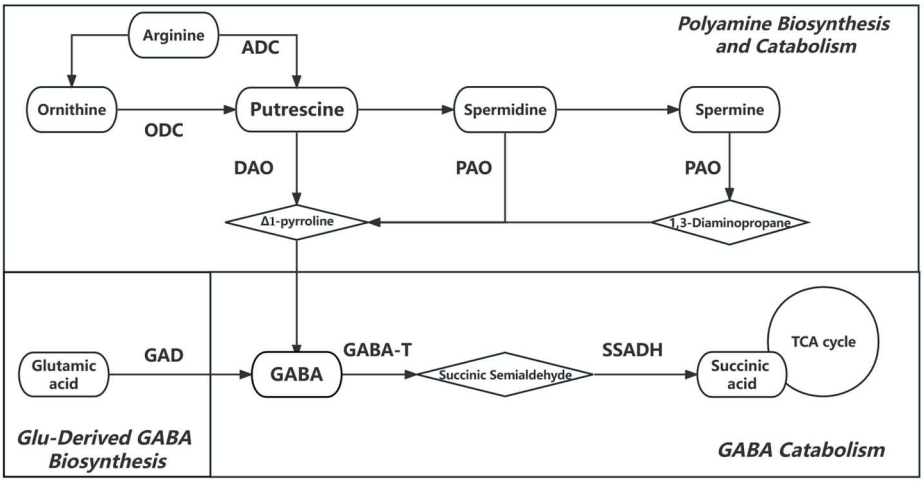

Figure 1. The γ-aminobutyric acid (GABA) pathway in plants is derived from Shelp et al. and Wuddineh et al. [8,9]. The primary metabolites under consideration here are indicated in the oval frames. Abbreviations: ADC, arginine decarboxylase; ODC, ornithine decarboxylase; DAO, diamine oxidase; PAO, polyamine oxidase; GAD, glutamate decarboxylase; GABA-T, GABA transaminase; SSADH, succinic semialdehyde dehydrogenase.

Currently, Glu-derived GABA biosynthesis receives more attention under abiotic stresses. However, the contribution of the polyamine catabolism pathway cannot be ignored. Polyamines (PAs), which have potent biological activities usually including Put, Spm, and Spd in plants, are ubiquitous in all cells and play an important role in plant growth and development [12,13]. Hu et al. found that exogenous GABA played an important role in alleviating $Ca(NO_3)_2$-induced injury to muskmelon seedlings by the physiological regulation of PAs [14]. However, the physiological relationship between abiotic stress and PAs in plants was met with debate [15,16]. On the one hand, PAs can enhance antioxidant enzyme activities and deal with oxidative radicals to protect plants; at the same time, the metabolism of PAs is closely connected to many other metabolic pathways to improve drought tolerance [17], salt [18,19], low temperature [20], and other stress [21]. On the other hand, PAs might be a potential cell-damaging factor due to hydrogen peroxide (H_2O_2) produced by their catabolism [22,23]. Many researchers have attempted to investigate the response of PAs metabolism to abiotic stress by exogenously adding PAs and their synthesis inhibitors. Yang et al. found that exogenous Spd effectively responded to high temperature by inhibiting stomatal opening and density, thus improving the net photosynthetic rate and biomass in lettuce [24]. Many studies also indicated that the function of PAs under stress can differ among plants and even parts of the same plant [25,26].

Suboptimal root-zone temperature occurs in early spring, which is the period when fruit trees sprout in the northeast of China caused by the inconsistencies between soil temperature and air temperature. *Malus baccata* Borkh., as one of the apple rootstocks, is widely used in the apple-producing areas of northeastern China. It has been found that

a suboptimal low root-zone temperature disturbs the growth and function of the roots of *Malus baccata* Borkh.; subsequently, this adversely affects the growth and development of the leaves [27,28]. We also found that suboptimally low root-zone temperatures can cause endogenous GABA accumulation in *M. baccata* Borkh. seedlings, and exogenous GABA had the effect of enhancing the antioxidant capacity of the roots [29]. However, the contribution and relationship between the Glu-derived pathway and the PAs catabolism pathway on GABA accumulation under low-temperature stress, respectively, are still unclear. Exogenous Put and AG were applied in this experiment to clarify the contribution of the PAs catabolism pathway to GABA accumulation under suboptimal root-zone temperature and to explore the effect of PAs metabolic pathway on the seedlings. Results from this study will provide some theoretical basis for the physiological metabolism of apple rootstocks in the early growing season to improve the tolerance of the fruit species in northern China.

2. Materials and Methods

2.1. Plant Materials, Culture Conditions and Treatments

Malus baccata Borkh. was used in this experiment. The seedlings with 4~5 leaves were cultivated in a 13 × 12 cm plastic pot filled with a soil mix containing 50% (V/V) garden soil, 25% (V/V) river sand, and 25% (V/V) substrate. When the seedlings had 15 leaves, the ones with consistent growth and no disease and insect pests were selected for experimental treatment. The whole experiment required an artificial climate chamber and low thermostatic-temperature baths. For the former, the photoperiod was set for 14 h/10 h, and the day and night temperatures were 18 °C/8 °C. Meanwhile, the latter was used to control root zone temperature. The low thermostatic temperature baths were equipped with a heat insulation board outside and a water circulation system inside, so that the root zone temperature could be kept at about 5 ± 0.5 °C. The upper part was still in the above artificial climate-chamber culture.

To adapt to the cultural conditions before the experimental treatment began, the selected-*M. baccata* Borkh. seedlings were transferred to an artificial climate chamber. Precultivation was performed for 2 days. Then the seedlings were divided into four groups, and the four treatments were defined as follows in Table 1.

Table 1. Conditions required for each treatment.

Treatment	Irrigation (100 mL/pot)	Upper Part	Potted Part
Control	dH_2O	18 °C (14 h)/8 °C (10 h)	18 °C (14 h)/8 °C (10 h)
L	dH_2O	18 °C (14 h)/8 °C (10 h)	5 ± 0.5 °C (24 h)
L + Put	0.1 mmol·L^{-1} Put	18 °C (14 h)/8 °C (10 h)	5 ± 0.5 °C (24 h)
L + AG	10 mmol·L^{-1} AG	18 °C (14 h)/8 °C (10 h)	5 ± 0.5 °C (24 h)

Control, not subjected to any treatments; L, suboptimal root-zone temperature; L + Put, suboptimal root-zone temperature + 0.1 mmol·L^{-1} putrescine (Solarbio, Beijing, China) solution; L + AG, suboptimal root-zone temperature + 10 mmol·L^{-1} aminoguanidine (Solarbio) solution. Irrigation, each seedling was watered with 100 mL liquid before treating; upper part and potted part, the temperature during treating.

The root samples were collected at days 0, 1, 4, and 7 of treatment. First, they were rinsed with dH_2O, then frozen in liquid nitrogen. The frozen sample was ground in a ball mill (MM400; Retsch, GmbH, Haan, Germany) into powder using liquid nitrogen, and stored at −80 °C for physiological and metabolic analysis.

2.2. Determination of γ-Aminobutyric Acid and Glutamic Acid Content

The γ-aminobutyric acid (GABA) and glutamic acid (Glu) were extracted according to the procedure of Baum et al. with some modifications [30]. We added 1 mL of extracting solution, containing 25% chloroform (V/V), 60% methanol (V/V), and 15% dH_2O (V/V), to the frozen root powder s (~300 mg), and mixed it at 4 °C for 1 h, then centrifuged it at 12,000× *g* for 5 min at 4 °C. After the supernatant was transferred, we added 375 µL of chloroform and 625 µL of dH_2O step by step, and vortexed for 20 s. The mixtures were

centrifuged in the same conditions as above. The organic phase containing GABA and Glu was dried using the vacuum concentrator (Eppendorf GmbH, Hamburg, Germany), redissolved in 1 mL of HPLC-grade acetonitrile, and filtered through a 0.22 μm membrane for further analysis.

The GABA and Glu were treated using an ACQUITY UPLC BEH Amide column (1.7 μm, 100 mm × 2.1 mm) at 35 °C. The mobile phase consisting of solvent A (0.5% formic acid in acetonitrile) and solvent B (0.5% formic acid in 20 mM ammonium acetate solution) was delivered at the flow rate of 0.6 mL·min^{-1}. The gradient elution was as follows: A% = 100, 100, 96, 80, 62, 55, 100, and 100 (0, 0.5, 2.5, 3.5, 4, 6, 6.5, and 8 min). The compound was detected by a Xevo TQ-D triple quadrupole mass spectrometer (Waters, Milford, MA, USA). The detection conditions refer to Helmond et al., with modification as follows: the positive-ion mode; source temperature, 150 °C; capillary voltage, 3.00 kV; cone voltage, 20 V; desolvation, 800 L·h^{-1}; desolvation temperature, 400 °C [31]. MassLynx version 4.1 analytical software was used for system control and data processing.

2.3. Determination of Polyamines Contents

Free polyamines (PAs) were extracted and derived as described in the methods of Gong and Liu, with slight modifications [32]. The frozen root samples (~500 mg) were homogenized in an extraction solution containing 5% perchloric acid. After being shaken in a thermomixer at 4 °C for 1 h at 1500 rpm, the mixture was centrifuged at 12,000× *g* for 30 min at 4 °C. The supernatant was transferred to another 10 mL tube for derivation. We added 2 mL of 2M NaOH and 15 μL of benzoyl chloride into the supernatant step by step, and vortexed for 20 s to mix them. After they were placed in a water bath at 37 °C for 20 min, 2 mL of saturated NaCl and 2 mL of ethyl ether were added to the tube. Subsequently, the mixture was vortexed slightly for several seconds and centrifuged at 8000× *g* for 5 min. The ethyl ether phase containing benzoyl-polyamines was transferred, vacuum-dried in a concentrator, then redissolved with 200 μL of HPLC-grade methanol. The resulting solution was filtered through a 0.22 μm membrane and collected into a 2 mL Waters screw top vial (Waters, Milford, MA, USA) for subsequent analysis.

Ultrahigh-performance liquid chromatography analysis of the free PAs was performed on an ACQUITY UPLC H-Class Waters instrument equipped with a Xevo TQ-D mass spectrometer (Waters); the specific instrument setting conditions refer to Tsutsui et al. [33] and Takayama et al. [34]. Chromatographic separation was performed on a Waters ACQUITY UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 mm), with the mobile phase composed of double distilled H_2O (ddH_2O) containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). Next, 2 μL of each resulting solution was loaded into the system. The flow rate of 0.30 mL·min^{-1} was applied, with a gradient elution mode (B% = 50, 50, 75, 100, 100, 50, and 50; at 0, 2, 10, 13, 16, 16.1, and 19 min). An MS system fitted with an electrospray ionization (ESI) source worked in a positive ion mode and scan mode for multiple-reaction monitoring (MRM). ESI ionization conditions were as follows: source temperature, 150 °C; capillary voltage, 3.00 kV; desolvation temperature, 500 °C; as curtain and auxiliary gas, high-purity nitrogen (>99.999%) was used to a flow of 1000 L·h^{-1}. The identity of PAs compounds was confirmed by accurate mass measurements and authentic standards using Masslynx version 4.1 software (Waters).

2.4. Determination of Enzyme Activities

Enzymes, the key enzymes in the GABA pathway in plants i.e., glutamate decarboxylase (GAD), γ-aminobutyric acid transaminase (GABA-T), arginine decarboxylase (ADC), ornithine decarboxylase (ODC), diamine oxidase (DAO), and polyamine oxidase (PAO), were extracted from the root samples in a phosphate buffer (pH 7.2–7.4). After being centrifuged at 5000× *g* for 20 min at 4 °C, the supernatant was collected for further analysis. The enzyme activities were measured using enzyme-linked immunosorbent assay (ELISA) detection kits (Mlbio, Enzyme-linked Biotechnology Co., Shanghai, China) on an ELISA instrument at 450 nm according to the instructions of the kits.

2.5. Determination of Hydroperoxide Content, Superoxide Anion Content and Lipid Peroxidation (Malondialdehyde Content)

The hydroperoxide (H_2O_2) content was determined by measuring the absorbance of the titanium-peroxide complex at 415 nm as described by Patterson et al. [35]. According to Verma and Mishra [36], the superoxide anion ($O_2 \cdot^-$) content was measured by monitoring the absorbance of hydroxylamine oxidation at 530 nm. The degree of lipid peroxidation in roots was assessed by the malondialdehyde (MDA) contents. The MDA content was determined spectrophotometrically at 450, 532, and 600 nm according to the methods of Liu et al. [37].

2.6. Analysis of Antioxidative Enzyme Activities

Enzymes were extracted from the root samples (~200 mg) in 4 mL of 100 mM potassium phosphate buffer containing 200 mg polyvinylpolypyrrolidone and 0.5% (*v*/*v*) Triton X-100 as described by He et al. [38]. After being centrifuged at 12,000× *g* for 20 min at 4 °C, the supernatant was used for the following enzyme assays. The activities of SOD (EC 1.15.1.1), POD (EC 1.11.1.7), and CAT (EC 1.11.1.6) were measured using the previously published method by Khan et al. [39].

2.7. Statistical Analysis

Data represent means ± standard error (SE) of three biological replicates. Data were processed in IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA). All data were subjected to a two-way analysis of variance (ANOVA) with Time and Treatment as factors for significant changes ($p < 0.05$, *; $p < 0.01$, **; ns, not significant). A posteriori means comparisons were performed by the Tukey-HSD method. Significance analysis was performed among the four treatments or time points, and significant differences were annotated with lowercase letters and capital letters, respectively. Differences were considered significant at $p < 0.05$.

3. Results

3.1. γ-Aminobutyric Acid (GABA) Content and GABA Transaminase (GABA-T) Activity

Compared with the control, γ-aminobutyric acid (GABA) content under suboptimal low temperature root-zone (treatment L) was significantly increased by 57.97% on day 1, and then decreased significantly with the extension of treating time. The changing trends of exogenous Put application and exogenous AG application (treatment L + Put, treatment L + AG) were similar to that of treatment L, both of which had the maximum value on day 1. GABA content in treatment L + Put was higher than that of treatment L. However, GABA content in treatment L + AG was between the treatments L and L + Put (Figure 2a).

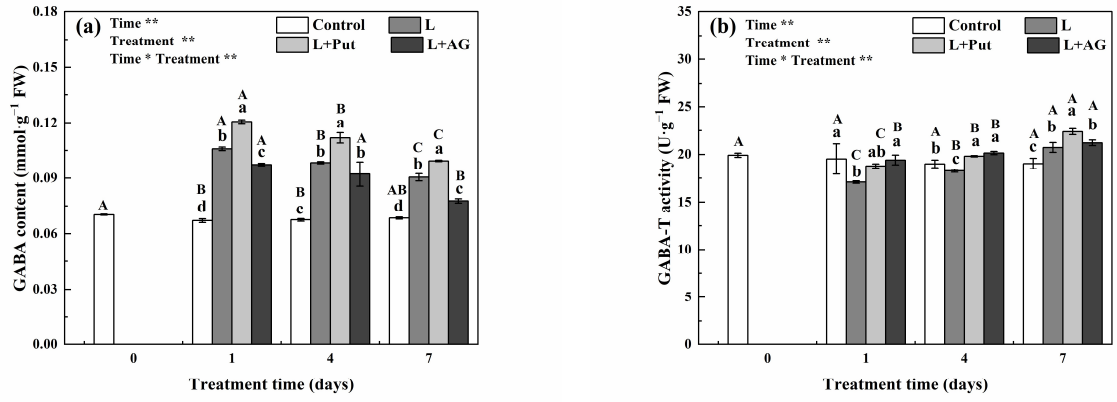

Figure 2. Effects of exogenous Put or AG application on (**a**) γ-aminobutyric acid (GABA) content and (**b**) GABA transaminase (GABA-T) activity in *Malus baccata* Borkh. roots exposed to suboptimal low

root-zone temperature from days 0 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment. Two-way ANOVA with Time and Treatment as factors for significant changes are indicated. $p < 0.01$, **. Lowercase letters above the columns indicate significant differences among treatments at the $p < 0.05$. Capital letters above the columns indicate significant differences among time points at the $p < 0.05$. Control, not subjected by any treatments; L, suboptimal root-zone temperature; L + Put, suboptimal root-zone temperature + 0.1 mmol·L^{-1} Put solution; L + AG, suboptimal root-zone temperature + 10 mmol·L^{-1} AG solution.

Treatment L significantly inhibited GABA transaminase (GABA-T) activity during days 1 to 4, and GABA-T activity in treatment L was significantly increased on day 7 compared to the control. GABA-T activity in treatments L + Put and L + AG had a similar trend to that of treatment L, that is, GABA-T activity was increased with treatment time. Moreover, exogenous Put (days 4 to 7) or AG (days 1 to 4) application significantly enhanced GABA-T activity compared with the treatment L (Figure 2b).

3.2. Glutamic Acid (Glu) Content and Glutamate Decarboxylase (GAD) Activity

Glutamic acid (Glu) content in the control remained stable and showed an increasing trend in the other three treatments. Treatment L significantly increased Glu content on day 7, increasing by 64.51% compared to the control. Compared to treatment L, Glu content in treatment L + Put was significantly increased during days 1 to 4 and was slightly decreased on day 7; however, Glu content in treatment L + AG was significantly increased in the whole treatment time (Figure 3a).

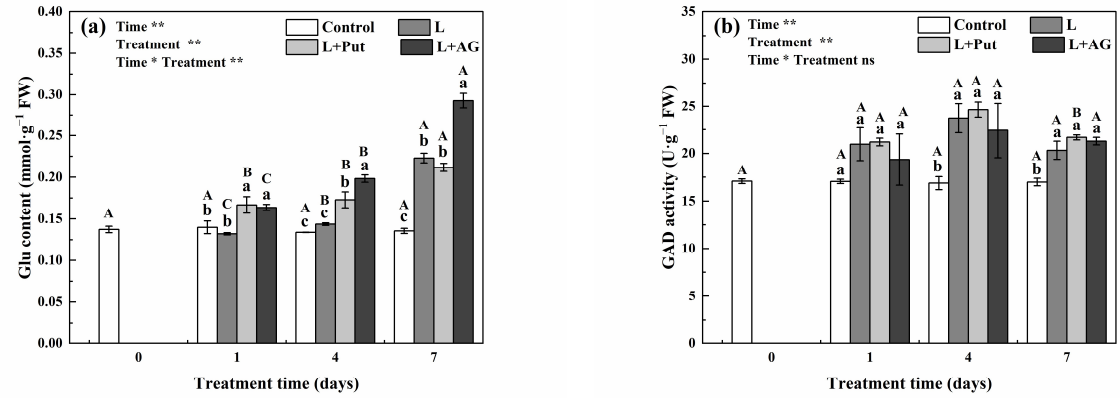

Figure 3. Effects of exogenous Put or AG application on (**a**) glutamic acid (Glu) content and (**b**) glutamate decarboxylase (GAD) activity in Malus baccata Borkh. roots exposed to suboptimal low root-zone temperature from days 0 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment. Two-way ANOVA with Time and Treatment as factors for significant changes are indicated. $p < 0.01$, **; ns, not significant. Lowercase letters above the columns indicate significant differences among treatments at the $p < 0.05$. Capital letters above the columns indicate significant differences among time points at the $p < 0.05$. Treatments legend as in Figure 2.

Compared to the control, glutamate decarboxylase (GAD) activity in treatment L was significantly enhanced during days 1 to 7, reaching the maximum value on day 4 and increasing by 40.62%. Similar trends existed in treatments L + Put and L + AG, that is, the maximum value appeared on day 4 and then decreased slightly. Compared to treatment L, GAD activity in treatment L + Put was induced from days 1 to 7; GAD activity in treatment L + AG was inhibited from days 1 to 4 and was higher than that of treatment L (Figure 3b).

3.3. Polyamines (PAs) Contents

As shown in Figure 4, the contents of (Figure 4a) putrescine (Put), (Figure 4b) spermidine (Spd), and (Figure 4c) spermine (Spm) in the control remained at a stable level from days 0 to 7. Treatment L significantly induced the accumulation of Put, Spd and Spm

contents compared to the control; Put and Spd contents reached the maximum on day 1, increasing by 82.91% and 75.39%, respectively; Spm content kept increasing from day 1 to day 7. Compared to treatment L, treatment L + Put further increased the Put and Spd contents with similar change trends. However, Spm content in treatment L + Put showed a trend of first increasing and then decreasing, reaching the maximum value on day 4, which increased by 43.56% compared to treatment L, and was significantly lower than that of treatment L on day 7. Put and Spd contents showed similar trends in treatment L + AG, reaching the highest level on day 4 compared with treatment L. Spm content in treatment L + AG had the maximum value on day 1, increasing by 37.42% and then gradually decreasing by 18.96% on day 7 compared with treatment L (Figure 4a–c).

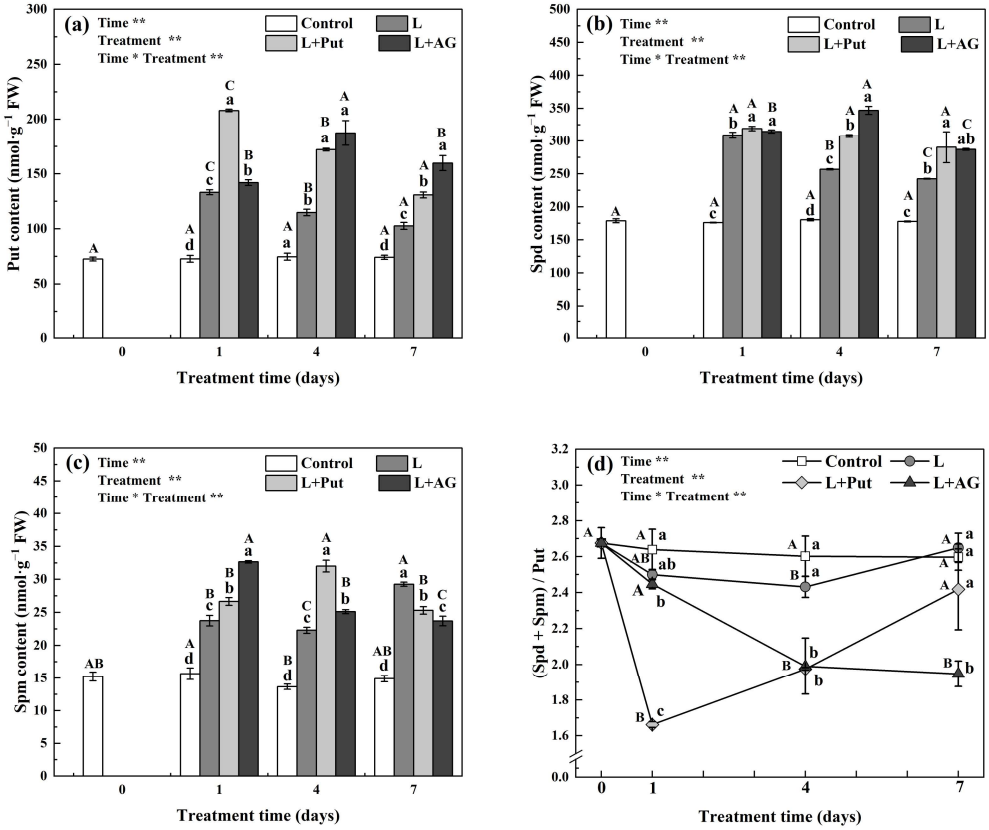

Figure 4. Effects of exogenous Put or AG application on the contents of (**a**) putrescine (Put), (**b**) spermidine (Spd) and (**c**) Spermine (Spm) in *Malus baccata* Borkh. roots exposed to suboptimal low root-zone temperature from days 0 to 7. (**d**) Effects of exogenous Put or AG application on the ratio of Spd and Spm with respect to Put [(Spd + Spm)/Put ratio] in *Malus baccata* Borkh. roots exposed to suboptimal low root-zone temperature from days 0 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment. Two-way ANOVA with Time and Treatment as factors for significant changes are indicated. $p < 0.01$, **. Lowercase letters above the columns indicate significant differences among treatments at the $p < 0.05$. Capital letters above the columns indicate significant differences among time points at the $p < 0.05$. Treatments legend as in Figure 2.

The ratio of Spd and Spm with respect to Put [(Spd + Spm)/Put ratio] in treatment L was lower than that of the control from days 0 to 4, and then gradually increased, higher than that of the control on day 7. Compared to treatment L, exogenous Put and AG application decreased the (Spd + Spm)/Put ratio. (Spd + Spm)/Put ratio in treatment L + Put had a minimum value on day 1, and then gradually increased after day 1. However, (Spd + Spm)/Put ratio in treatment L + AG decreased during days 0 to 7 (Figure 4d).

3.4. The Enzyme Activities of Putrescine Biosynthetic and Catabolic Pathway

Compared with the control, treatment L significantly increased arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) activities with similar trends from days 0 to 7. The activities of ADC and ODC in treatment L were the maximum value on day 4, 2.37-fold and 3.06-fold that of the control, respectively. Exogenous Put application further significantly enhanced the ADC activity. However, ODC activity in treatment L + Put was significantly higher than that of treatment L on day 1 and then decreased slightly, which was lower than that in treatment L from days 4 to 7. Compared to treatment L, ADC activity in treatment L + AG was decreased on day 1 and increased on days 4 and 7; ODC activity in treatment L + AG was the opposite, increasing on day 1 and decreasing on days 4 and 7 (Figure 5a,b).

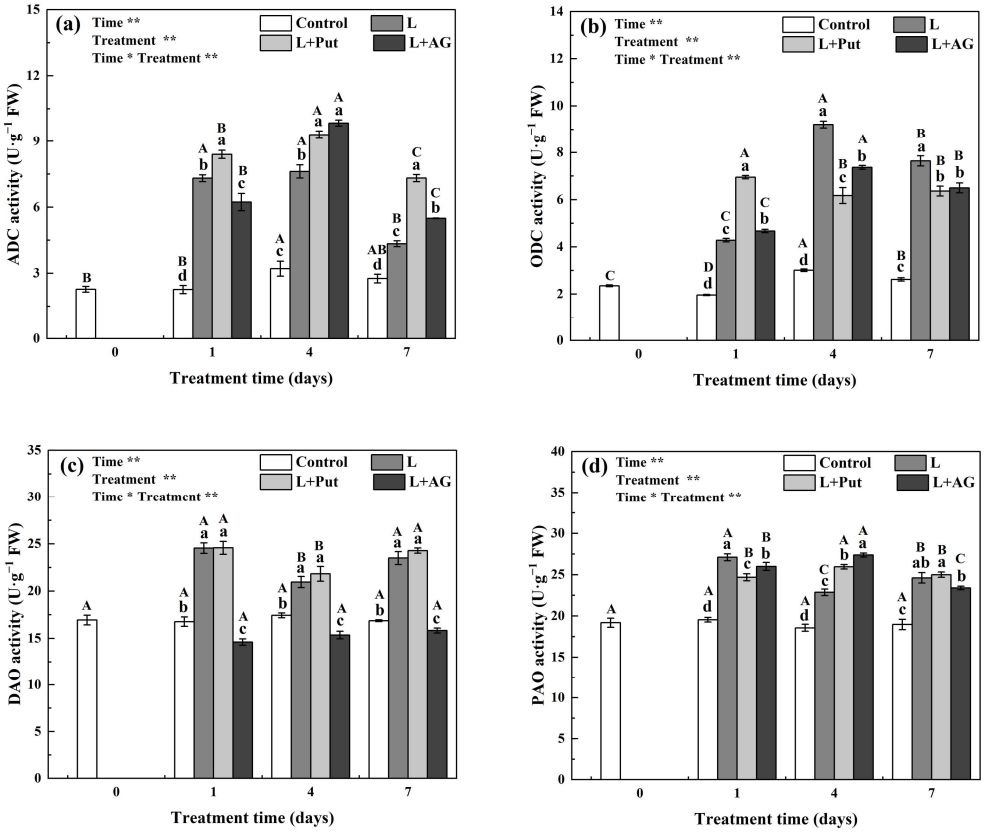

Figure 5. Effects of exogenous Put or AG application on the activities of (**a**) arginine decarboxylase (ADC), (**b**) ornithine decarboxylase (ODC), (**c**) diamine oxidase (DAO) and (**d**) polyamine oxidase (PAO) in *Malus baccata* Borkh. roots exposed to suboptimal low root-zone temperature from days 0 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment. Two-way ANOVA with Time and Treatment as factors for significant changes are indicated. $p < 0.01$, **. Lowercase letters above the columns indicate significant differences among treatments at the $p < 0.05$. Capital letters above the columns indicate significant differences among time points at the $p < 0.05$. Treatments legend as in Figure 2.

The activities of diamine oxidase (DAO) and polyamine oxidase (PAO) were significantly induced by treatment L relative to the control. Compared to treatment L, treatment L + Put further increased DAO and PAO activities from days 4 to 7. AG, as the DAO inhibitor, significantly inhibited DAO activity throughout the experimental treatment. However, PAO activity after AG application decreased on days 1 and 7 and increased on day 4 by 19.71% compared with treatment L (Figure 5c,d).

3.5. Contributions of Glu-Derived Pathway and PAs Catabolism Pathway to GABA Accumulation

In order to explore the effects of the Glu-derived pathway and the PAs catabolism pathway on GABA accumulation under suboptimal low root-zone temperature, we calculated the contribution rate by referring to the method of Yang et al. [40]. AG was the specific inhibitor of DAO. As Figure 5c shows, treatment L + AG significantly reduced DAO activities from day 1 to day 7 compared to treatment L. Meanwhile, GABA contents in treatment L + AG decreased compared to treatment L as in Figure 2a. Therefore, it could be inferred that the contributing rates of the Glu-derived pathway and PAs catabolism pathway had an effect on GABA accumulation. As shown in Figure 6, the Glu-derived pathway was the main pathway for GABA accumulation compared to the PAs catabolism pathway under suboptimal low root-zone temperature. However, the contributing rates of PAs catabolism pathway increased from 20.60% to 43.31% with the extension of treatment time.

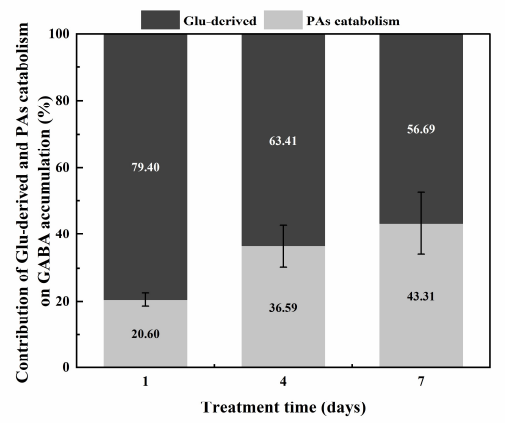

Figure 6. Contributions of Glu-derived pathway and PAs catabolism pathway on GABA accumulation in *Malus baccata* Borkh. roots exposed to suboptimal low root-zone temperature from days 1 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment.

3.6. The Contents of Hydroperoxide (H_2O_2), Superoxide Anion ($O_2\cdot^-$), and Malondialdehyde (MDA)

In the control, the contents of hydroperoxide (H_2O_2), superoxide anion ($O_2\cdot^-$), and malondialdehyde (MDA) remained at a relatively stable level from days 0 to 7; however, the response to each treatment was different. Compared to the control, treatment L significantly increased H_2O_2 from days 1 to 4, $O_2\cdot^-$ and MDA content from days 1 to 7, which increased by 29.02%, and 36.25% for H_2O_2, $O_2\cdot^-$ at day 1, respectively, and then decreased slightly, while MDA content continued to increase after day 1. After treatment L + Put, H_2O_2 content was lower on day 1 and higher on days 4 and 7 than that of treatment L; $O_2\cdot^-$ and MDA content were reduced throughout the experimental period compared to treatment L. Treatment L + AG further induced H_2O_2 and MDA accumulation compared to treatment L, and a similar changing trend occurred in treatment L, respectively. $O_2\cdot^-$ content in treatment L + AG significantly decreased on day 1 compared to treatment L and significantly increased on day 7 relative to treatment L + Put (Figure 7).

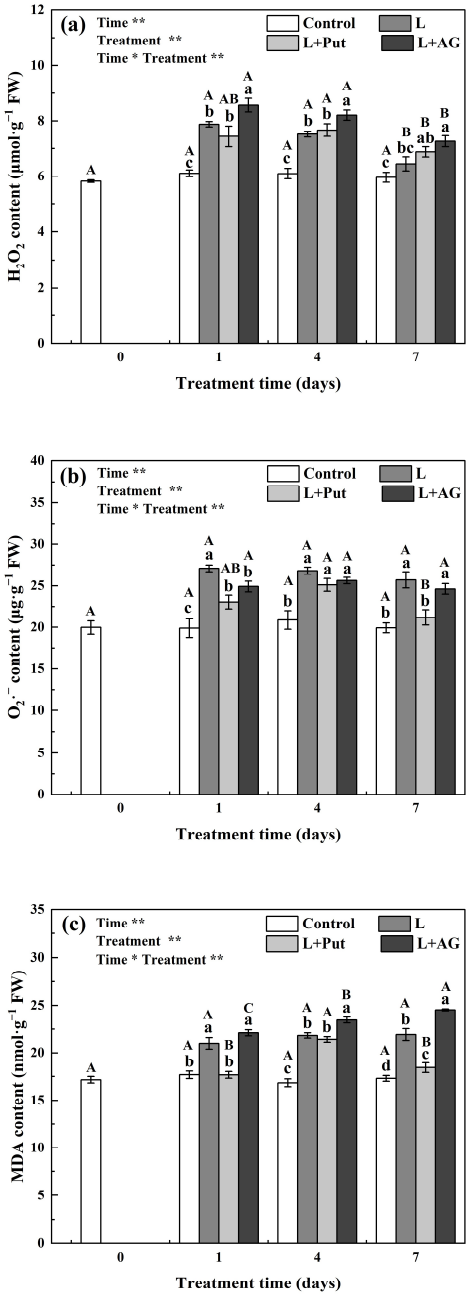

Figure 7. Effects of exogenous Put or AG application on the contents of (**a**) hydroperoxide (H_2O_2), (**b**) superoxide anion ($O_2\cdot^-$), and (**c**) malondialdehyde (MDA) in *Malus baccata* Borkh. roots exposed to suboptimal low root-zone temperature from days 0 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment. Two-way ANOVA with Time and Treatment as factors for significant changes are indicated. $p < 0.01$, **. Lowercase letters above the columns indicate significant differences among treatments at the $p < 0.05$. Capital letters above the columns indicate significant differences among time points at the $p < 0.05$. Treatments legend as in Figure 2.

3.7. Antioxidant Enzyme Activity

Compared to the control, superoxide dismutase (SOD) activity under treatment L was slightly increased on day 1 by 3.09% and significantly decreased after day 1; the activities of peroxidase (POD) and catalase (CAT) under treatment L were reduced on day 1, and then gradually enhanced from days 4 to 7, increased by 6.93% and 20.57% on day 7, respectively. Compared to treatment L, SOD and CAT activities in treatment L + Put

were significantly increased from days 1 to 7, and POD activity only on day 4. After AG application, the changing trend of SOD activity was similar to that in treatment L, but significantly enhanced from day 4 compared to treatment L; POD activity was lower than that of treatment L throughout the whole process; the changing trend of CAT activity was contrary to that of treatment L, significantly higher than that of treatment L on day 1 and then gradually decreased (Figure 8).

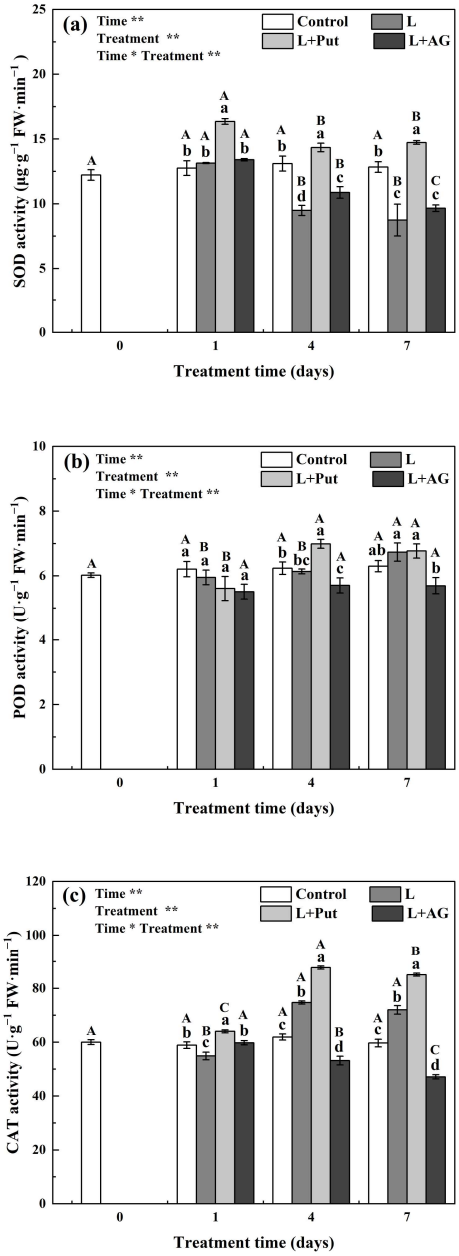

Figure 8. Effects of exogenous Put or AG application on the activities of (**a**) superoxide dismutase (SOD), (**b**) peroxidase (POD), and (**c**) catalase (CAT) in *Malus baccata* Borkh. roots exposed to suboptimal low root-zone temperature from days 0 to 7. Each value is the mean ± SE of three replicates for independent experiments under each treatment. Two-way ANOVA with Time and Treatment as factors for significant changes are indicated. $p < 0.01$, **. Lowercase letters above the columns indicate significant differences among treatments at the $p < 0.05$. Capital letters above the columns indicate significant differences among time points at the $p < 0.05$. Treatments legend as in Figure 2.

4. Discussion

The GABA shunt is an important pathway for plants to rapidly respond to stresses, which could be activated in a short period [41]. In this study, suboptimal low root-zone temperature (treatment L) significantly increased endogenous GABA contents, and GABA-T enzyme activities significantly increased with the extension of treatment time. This was consistent with the results of the anthurium cut flowers responding to the low postharvest temperature. [42]. Exogenous Put application (treatment L + Put) further increased endogenous GABA contents and GABA-T activities compared to treatment L. These indicated that treatments L and L + Put significantly enhanced GABA accumulations and GABA shunt activities. Compared to treatment L, endogenous GABA was significantly reduced after adding AG, which is the diamine oxidase inhibitor. However, GABA-T activities still increased. This may be the reason that endogenous GABA comes from two pathways. As we all know, the Glu-derived pathway and PAs catabolism pathway could cause GABA accumulation under stresses [43]. Therefore, we further investigated the contributions of two pathways to GABA accumulation under suboptimal low root-zone temperature. Compared to the control, treatment L significantly enhanced the activities of GAD and DAO and increased the contents of Glu (day 7), Put, Spd, and Spm, indicating that both pathways were activated by suboptimal low root-zone temperature. This provided further evidence for the source of endogenous GABA accumulation. Yang et al. found that AG, which is the specific inhibitor of DAO, could slightly decrease GAD activity, consistent with our study [44]. AG application (treatment L + AG) significantly decreased DAO activities from days 1 to 7, resulting in decrease of the GABA contents compared to treatment L. The contribution rate of the PAs catabolism pathway could be calculated using these data [40]. We found that the PAs catabolism pathway provided about 20~40% of GABA formation under treatment L. Hence, it could be inferred that the Glu-derived pathway was the main route of GABA accumulation, and the contribution rate of the PAs catabolism pathway continued to increase as processing time was prolonged under suboptimal low root-zone temperature.

Reactive oxygen species (ROS) could be produced in various subcellular compartments under low temperature stress and are precisely controlled by enzymatic and non-enzymatic antioxidant defense systems [45–47]. Excessive accumulation of ROS will cause membrane lipid peroxidation, which was evaluated by the MDA content in roots [48,49]. Obviously, the suboptimal low root-zone temperature caused oxidative stress to the roots in this study, which agreed with our previous research [27]. SOD, as the first defense in the antioxidant system of plants, can convert excessive $O_2 \cdot^-$ into H_2O_2; and then H_2O_2 was reduced to H_2O and O_2 by POD and CAT in the cytoplasm [50–53]. The antioxidant enzymes could regulate and cooperate with each other in the process of ROS clearance [54]. Zhang et al. found that SOD and CAT played a greater role in decomposing ROS than POD [55], and similar results were found in this study, especially that CAT activities were significantly enhanced from days 4 to 7. However, this did not reverse the trend towards oxidative stress in roots. Many previous studies have shown that PAs are related to plant stress resistance. PAs are essential biomolecules involved in the regulation of many developmental and growth processes as well as their response to different environmental stimuli. While PAs can act as osmoregulatory substances to maintain cell osmotic balance and against ROS, their catabolism is known to generate ROS. Therefore, maintaining the cellular pools of PAs concentration and interconversion between different PAs is critical to accomplish their normal functions [13,22,56,57]. Kielkowska et al. found that exogenously applied PAs maintained the viability of *B. oleracea* L. var. capitata protoplasts by alleviating oxidative stress and stimulating mitotic activity, which further affected the plant regeneration process [58]. Pretreatment with putrescine induces the unique expression of various general stress-related genes [59]. In this study, exogenous Put application significantly decreased the contents of $O_2 \cdot^-$ and MDA, indicating that it could relieve oxidative stress due to suboptimal low root-zone temperature. However, H_2O_2 contents were increased from days 4 to 7, which may be the result of Put catabolism. Our results showed that (Spd + Spm)/Put

ratio continued to increase from days 1 to 7 after Put application, indicating that exogenous Put promoted the transformation of Put to Spd and Spm. Many studies have reported that the (Spd + Spm)/Put ratio was positively correlated with the activity of antioxidant enzymes, and exogenous Put could reduce ROS by enhancing the activities of SOD, POD, and CAT, consistent with the results of this study [58,60–62]. DAO is the key enzyme of the synthesis of GABA by the PAs catabolism pathway. After AG application, the H_2O_2 contents significantly increased and aggravated membrane peroxidation, indicating that inhibiting the flow of PAs to GABA leads to increased damage of the suboptimal low root-zone temperature.

5. Conclusions

Suboptimal low root-zone temperature activates the GABA shunt and causes GABA accumulation. In this process, the Glu-derived pathway dominates, but the contribution rate of the PAs catabolism pathway increases gradually with the extension of treatment time. Suboptimal low root-zone temperature causes oxidative stress, and exogenous Put could reduce the damage by increasing endogenous PAs contents, GABA shunt activities, and antioxidant enzyme activities.

Author Contributions: Conceptualization, H.M. and D.L.; methodology, X.L., M.Z. and E.Z.; software, X.L. and E.Z.; validation, X.L. and M.Z.; formal analysis, X.L.; investigation, X.L. and M.Z.; resources, H.M. and D.L.; data curation, X.L. and M.Z.; writing—original draft preparation, X.L.; writing—review and editing, H.M.; visualization, X.L. and M.Z.; supervision, H.M. and D.L.; project administration, H.M.; funding acquisition, H.M. and D.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Basic Scientific Research Foundation of Liaoning Province Education Department-General Project [Grant number LJKMZ20221022], and the China Agriculture Research System of MOF and MARA [Grant No. CARS-27].

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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

References

1. Dent, C.E.; Stepka, W.; Steward, F.C. Detection of the free amino-acids of plant cells by partition chromatography. *Nature* **1947**, *160*, 682. [CrossRef] [PubMed]
2. Fait, A.; Fromm, H.; Walter, D.; Galili, G.; Fernie, A.R. Highway or byway: The metabolic role of the GABA shunt in plants. *Trends Plant Sci.* **2008**, *13*, 14–19. [CrossRef] [PubMed]
3. Shelp, B.J.; Bozzo, G.G.; Trobacher, C.P.; Chiu, G.; Bajwa, V.S. Strategies and tools for studying the metabolism and function of γ-aminobutyrate in plants. I. Pathway structure. *Botany* **2012**, *90*, 651–668. [CrossRef]
4. Antonio, C.; Papke, C.; Rocha, M.; Diab, H.; Limami, A.M.; Obata, T.; Fernie, A.R.; van Dongen, J.T. Regulation of primary metabolism in response to low oxygen availability as revealed by carbon and nitrogen isotope redistribution. *Plant Physiol.* **2016**, *170*, 43–56. [CrossRef]
5. Nonaka, S.; Arai, C.; Takayama, M.; Matsukura, C.; Ezura, H. Efficient increase of gamma-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Sci. Rep.* **2017**, *7*, 7057. [CrossRef]
6. Scholz, S.S.; Malabarba, J.; Reichelt, M.; Heyer, M.; Ludewig, F.; Mithofer, A. Evidence for GABA-induced systemic GABA accumulation in *Arabidopsis* upon wounding. *Front. Plant Sci.* **2017**, *8*, 388. [CrossRef]
7. Zarei, A.; Chiu, G.Z.; Yu, G.H.; Trobacher, C.P.; Shelp, B.J. Salinity-regulated expression of genes involved in GABA metabolism and signaling. *Botany* **2017**, *95*, 621–627. [CrossRef]
8. Shelp, B.J.; Bozzo, G.G.; Trobacher, C.P.; Zarei, A.; Deyman, K.L.; Brikis, C.J. Hypothesis/review: Contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. *Plant Sci.* **2012**, *193–194*, 130–135. [CrossRef]
9. Wuddineh, W.; Minocha, R.; Minocha, S.C. Polyamines in the context of metabolic networks. In *Methods Molecular Biology*; Springer: Clifton, NJ, USA, 2018; Volume 1694, pp. 1–23.
10. Xing, S.G.; Jun, Y.B.; Hau, Z.W.; Liang, L.Y. Higher accumulation of gamma-aminobutyric acid induced by salt stress through stimulating the activity of diamine oxidases in *Glycine max* (L.) Merr. roots. *Plant Physiol. Biochem.* **2007**, *45*, 560–566. [CrossRef]
11. Liao, J.R.; Wu, X.Y.; Xing, Z.Q.; Li, Q.H.; Duan, Y.; Fang, W.P.; Zhu, X.J. Gamma-aminobutyric acid (GABA) accumulation in tea (*Camellia sinensis* L.) through the GABA shunt and polyamine degradation pathways under anoxia. *J. Agric. Food Chem.* **2017**, *65*, 3013–3018. [CrossRef]

12. Vera-Sirera, F.; Minguet, E.G.; Singh, S.K.; Ljung, K.; Tuominen, H.; Blazquez, M.A.; Carbonell, J. Role of polyamines in plant vascular development. *Plant Physiol. Biochem.* **2010**, *48*, 534–539. [CrossRef]
13. Romero, F.M.; Maiale, S.J.; Rossi, F.R.; Marina, M.; Ruiz, O.A.; Garriz, A. Polyamine metabolism responses to biotic and abiotic Stress. In *Methods in Molecular Biology*; Springer: Clifton, NJ, USA, 2018; Volume 1694, pp. 37–49.
14. Hu, X.; Xu, Z.; Xu, W.; Li, J.; Zhao, N.; Zhou, Y. Application of gamma-aminobutyric acid demonstrates a protective role of polyamine and GABA metabolism in muskmelon seedlings under $Ca(NO_3)_2$ stress. *Plant Physiol. Biochem.* **2015**, *92*, 1–10. [CrossRef]
15. Minocha, R.; Majumdar, R.; Minocha, S.C. Polyamines and abiotic stress in plants: A complex relationship. *Front. Plant Sci.* **2014**, *5*, 175. [CrossRef]
16. Chen, D.; Shao, Q.; Yin, L.; Younis, A.; Zheng, B. Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Front. Plant Sci.* **2019**, *9*, 1945. [CrossRef]
17. Yong, B.; Xie, H.; Li, Z.; Li, Y.P.; Zhang, Y.; Nie, G.; Zhang, X.Q.; Ma, X.; Huang, L.K.; Yan, Y.H.; et al. Exogenous application of GABA improves PEG-induced drought tolerance positively associated with GABA-shunt, polyamines, and proline metabolism in white clover. *Front. Physiol.* **2017**, *8*, 1107. [CrossRef]
18. Xu, J.J.; Liu, T.; Yang, S.C.; Jin, X.Q.; Qu, F.; Huang, N.; Hu, X.H. Polyamines are involved in GABA-regulated salinity-alkalinity stress tolerance in muskmelon. *Environ. Exp. Bot.* **2019**, *164*, 181–189. [CrossRef]
19. Liu, J.; Yang, R.; Jian, N.; Wei, L.; Ye, L.; Wang, R.; Gao, H.; Zheng, Q. Putrescine metabolism modulates the biphasic effects of brassinosteroids on canola and Arabidopsis salt tolerance. *Plant Cell Environ.* **2020**, *43*, 1348–1359. [CrossRef]
20. Wang, D.; Li, L.; Xu, Y.; Limwachiranon, J.; Li, D.; Ban, Z.; Luo, Z. Effect of exogenous nitro oxide on chilling tolerance, polyamine, proline, and gamma-aminobutyric acid in bamboo shoots (*Phyllostachys praecox* f. prevernalis). *J. Agric. Food Chem.* **2017**, *65*, 5607–5613. [CrossRef]
21. Pal, M.; Csavas, G.; Szalai, G.; Olah, T.; Khalil, R.; Yordanova, R.; Gell, G.; Birinyi, Z.; Nemeth, E.; Janda, T. Polyamines may influence phytochelatin synthesis during Cd stress in rice. *J. Hazard. Mater.* **2017**, *340*, 272–280. [CrossRef]
22. Mohapatra, S.; Minocha, R.; Long, S.; Minocha, S.C. Putrescine overproduction negatively impacts the oxidative state of poplar cells in culture. *Plant Physiol. Biochem.* **2009**, *47*, 262–271. [CrossRef]
23. Gerlin, L.; Baroukh, C.; Genin, S. Polyamines: Double agents in disease and plant immunity. *Trends Plant Sci.* **2021**, *26*, 1061–1071. [CrossRef] [PubMed]
24. Yang, X.; Han, Y.; Hao, J.; Qin, X.; Liu, C.; Fan, S. Exogenous spermidine enhances the photosynthesis and ultrastructure of lettuce seedlings under high-temperature stress. *Sci. Hortic.* **2022**, *291*, 110570. [CrossRef]
25. Sen, S.; Ghosh, D.; Mohapatra, S. Modulation of polyamine biosynthesis in *Arabidopsis thaliana* by a drought mitigating pseudomonas putida strain. *Plant Physiol. Biochem.* **2018**, *129*, 180–188. [CrossRef] [PubMed]
26. Islam, M.A.; Pang, J.H.; Meng, F.W.; Li, Y.W.; Xu, N.; Yang, C.; Liu, J. Putrescine, spermidine, and spermine play distinct roles in rice salt tolerance. *J. Integr. Agr.* **2020**, *19*, 643–655. [CrossRef]
27. Li, L.J.; Lu, X.C.; Ma, H.Y.; Lyu, D.G. Jasmonic acid regulates the ascorbate-glutathione cycle in *Malus baccata* Borkh. roots under low root-zone temperature. *Acta Physiol. Plant.* **2017**, *39*, 174. [CrossRef]
28. Li, L.J.; Ma, H.Y.; Lu, X.C.; Lyu, D.G. TMT-Based quantitative proteomic revealed metabolic changes of Jasmonic Acid in regulating the response of *Malus baccata* (L.) Borkh. roots to low root-zone temperature. *J. Plant Growth Regul.* **2022**, *41*, 596–611. [CrossRef]
29. Lu, X.C.; Li, L.J.; Hu, X.L.; Ma, H.Y.; Lyu, D.G. Effects of exogenous GABA on antioxidant function of *Malus Baccata* Borkh. roots under suboptimal root-zone temperature. *Acta Bot. Boreali-Occident. Sin.* **2019**, *39*, 0285–0293.
30. Baum, G.; Lev-Yadun, S.; Fridmann, Y.; Arazi, T.; Katsnelson, H.; Zik, M.; Fromm, H. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. *EMBO J.* **1996**, *15*, 2988–2996. [CrossRef]
31. Helmond, W.; Puit, M. Amino acid profiling from fingerprints using amide stationary-phase UPLC-MS. In *Amino Acid Analysis: Methods and Protocols*; Springer: Berlin/Heidelberg, Germany, 2019; Volume 2030, pp. 439–450.
32. Gong, X.Q.; Liu, J.H. Detection of free polyamines in plants subjected to abiotic stresses by High-Performance Liquid Chromatography (HPLC). In *Plant Stress Tolerance: Methods and Protocols*; Springer: Berlin/Heidelberg, Germany, 2017; Volume 1631, pp. 305–311.
33. Tsutsui, H.; Mochizuki, T.; Inoue, K.; Toyama, T.; Yoshimoto, N.; Endo, Y.; Todoroki, K.; Min, J.Z.; Toyo'oka, T. High-throughput LC-MS/MS based simultaneous determination of polyamines including N-acetylated forms in human saliva and the diagnostic approach to breast cancer patients. *Anal. Chem.* **2013**, *85*, 11835–11842. [CrossRef]
34. Takayama, T.; Tsutsui, H.; Shimizu, I.; Toyama, T.; Yoshimoto, N.; Endo, Y.; Inoue, K.; Todoroki, K.; Min, J.Z.; Mizuno, H.; et al. Diagnostic approach to breast cancer patients based on target metabolomics in saliva by liquid chromatography with tandem mass spectrometry. *Clin. Chim. Acta* **2016**, *452*, 18–26. [CrossRef]
35. Patterson, B.D.; MacRae, E.A.; Ferguson, I.B. Estimation of hydrogen peroxide in plant extracts using titanium(IV). *Anal. Biochem.* **1984**, *139*, 487–492. [CrossRef]
36. Verma, S.; Mishra, S.N. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. *J. Plant Physiol.* **2005**, *162*, 669–677. [CrossRef]

37. Liu, Y.J.; Zhao, Z.G.; Si, J.; Di, C.X.; Han, J.; An, L.Z. Brassinosteroids alleviate chilling-induced oxidative damage by enhancing antioxidant defense system in suspension cultured cells of *Chorispora bungeana*. *Plant Growth Regul.* **2009**, *59*, 207–214. [CrossRef]
38. He, J.L.; Qin, J.J.; Long, L.Y.; Ma, Y.L.; Li, H.; Li, K.; Jiang, X.N.; Liu, T.X.; Polle, A.; Liang, Z.S.; et al. Net cadmium flux and accumulation reveal tissue-specific oxidative stress and detoxification in *Populus* × *canescens*. *Physiol. Plantarum* **2011**, *143*, 50–63. [CrossRef]
39. Khan, J.; Malangisha, G.K.; Ali, A.; Mahmoud, A.; Yang, J.; Zhang, M.; Hu, Z. Nitric oxide alleviates lead toxicity by inhibiting lead translocation and regulating root growth in watermelon seedlings. *Hortic. Environ. Biote.* **2021**, *62*, 701–714. [CrossRef]
40. Yang, R.; Chen, H.; Gu, Z. Factors influencing diamine oxidase activity and gamma-aminobutyric acid content of fava bean (*Vicia faba* L.) during germination. *J. Agric. Food Chem.* **2011**, *59*, 11616–11620. [CrossRef]
41. Shelp, B.J.; Aghdam, M.S.; Flaherty, E.J. γ-Aminobutyrate (GABA) regulated plant defense: Mechanisms and opportunities. *Plants* **2021**, *10*, 1939. [CrossRef]
42. Aghdam, M.S.; Naderi, R.; Jannatizadeh, A.; Babalar, M.; Sarcheshmeh, M.A.; Faradonbe, M.Z. Impact of exogenous GABA treatments on endogenous GABA metabolism in anthurium cut flowers in response to postharvest chilling temperature. *Plant Physiol. Biochem.* **2016**, *106*, 11–15. [CrossRef]
43. Shelp, B.J.; Zarei, A. Subcellular compartmentation of 4-aminobutyrate (GABA) metabolism in Arabidopsis: An update. *Plant Signal. Behav.* **2017**, *12*, e1322244. [CrossRef]
44. Yang, R.Q.; Guo, Q.H.; Gu, Z.X. GABA shunt and polyamine degradation pathway on gamma-aminobutyric acid accumulation in germinating fava bean (*Vicia faba* L.) under hypoxia. *Food Chem.* **2013**, *136*, 152–159. [CrossRef]
45. You, J.; Chan, Z. ROS regulation during abiotic stress responses in crop plants. *Front. Plant Sci.* **2015**, *6*, 1092. [CrossRef]
46. Puglia, G.D.; Balestrasse, K.; Bustos, J.S.; Huarte, H.R. New insights into the role of alternating temperatures and cyanide in the ROS-mediated cardoon seed dormancy termination. *Horticulturae* **2022**, *8*, 960. [CrossRef]
47. Pospisil, P. Production of reactive oxygen species by photosystem II as a response to light and temperature Stress. *Front Plant Sci.* **2016**, *7*, 1950. [CrossRef] [PubMed]
48. Sanchez, B.; Rasmussen, A.; Porter, J.R. Temperatures and the growth and development of maize and rice: A review. *Glob. Change Biol.* **2014**, *20*, 408–417. [CrossRef]
49. Erdal, S.; Genisel, M.; Turk, H.; Dumlupinar, R.; Demir, Y. Modulation of alternative oxidase to enhance tolerance against cold stress of chickpea by chemical treatments. *J. Plant Physiol.* **2015**, *175*, 95–101. [CrossRef] [PubMed]
50. Bhandari, K.; Sharma, K.D.; Hanumantha Rao, B.; Siddique, K.H.M.; Gaur, P.; Agrawal, S.K.; Nair, R.M.; Nayyar, H. Temperature sensitivity of food legumes: A physiological insight. *Acta Physiol. Plant.* **2017**, *39*, 68. [CrossRef]
51. Akbulut, M.; Cakir, S. The effects of Se phytotoxicity on the antioxidant systems of leaf tissues in barley (*Hordeum vulgare* L.) seedlings. *Plant Physiol. Biochem.* **2010**, *48*, 160–166. [CrossRef]
52. Sekmen, A.H.; Turkan, I.; Tanyolac, Z.O.; Ozfidan, C.; Dinc, A. Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. *Environ. Exp. Bot.* **2012**, *77*, 63–76. [CrossRef]
53. Nouairi, I.; Ben Ammar, W.; Ben Youssef, N.; Ben Miled, D.D.; Ghorbal, M.H.; Zarrouk, M. Antioxidant defense system in leaves of Indian mustard (*Brassica juncea*) and rape (*Brassica napus*) under cadmium stress. *Acta Physiol. Plant.* **2008**, *31*, 237–247. [CrossRef]
54. Yan, Q.Y.; Duan, Z.Q.; Mao, J.D.; Li, X.; Dong, F. Low root zone temperature limits nutrient effects on cucumber seedling Growth and induces adversity physiological response. *J. Integr. Agric.* **2013**, *12*, 1450–1460. [CrossRef]
55. Zhang, Y.P.; Jia, F.F.; Zhang, X.M.; Qiao, Y.X.; Shi, K.; Zhou, Y.H.; Yu, J.Q. Temperature effects on the reactive oxygen species formation and antioxidant defence in roots of two cucurbit species with contrasting root zone temperature optima. *Acta Physiol. Plant.* **2011**, *34*, 713–720. [CrossRef]
56. Tiburcio, A.F.; Alcazar, R. Potential applications of polyamines in agriculture and plant biotechnology. In *Methods in Mmolecular Biology*; Springer: Clifton, NJ, USA, 2018; Volume 1694, pp. 489–508.
57. Bueno, M.; Cordovilla, M.P. Polyamines in Halophytes. *Front. Plant Sci.* **2019**, *10*, 439. [CrossRef]
58. Kielkowska, A.; Adamus, A. Exogenously applied polyamines reduce reactive oxygen species, enhancing cell division and the shoot regeneration from *Brassica oleracea* L. var. capitata protoplasts. *Agronomy* **2021**, *11*, 735. [CrossRef]
59. Pal, M.; Majlath, I.; Nemeth, E.; Hamow, K.A.; Szalai, G.; Rudnoy, S.; Balassa, G.; Janda, T. The effects of putrescine are partly overlapping with osmotic stress processes in wheat. *Plant Sci.* **2018**, *268*, 67–76. [CrossRef]
60. Sardar, R.; Ahmed, S.; Yasin, N.A. Role of exogenously applied putrescine in amelioration of cadmium stress in *Coriandrum sativum* by modulating antioxidant system. *Int. J. Phytoremediat.* **2022**, *24*, 955–962. [CrossRef]
61. Collado-Gonzalez, J.; Pinero, M.C.; Otalora, G.; Lopez-Marin, J.; Amor, F.M.D. The Effect of foliar putrescine application, ammonium exposure, and heat stress on antioxidant compounds in cauliflower waste. *Antioxidants* **2021**, *10*, 707. [CrossRef]
62. Izadi, Z.; Nejad, A.R.; Abadia, J. Foliar applications of thidiazuron and putrescine increase leaf iron and chlorophyll concentrations in iron-deficient pot marigold (*Calendula officinalis* L.). *Acta Physiol. Plant.* **2021**, *43*, 122. [CrossRef]