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Exogenous Si Alleviation of Autotoxicity in Cucumber (*Cucumis sativus* L.) Seed Germination is Correlated with Changes in Carbohydrate Metabolism

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Abstract
Benzoic and cinnamic acid derivatives such as 3-phenylpropionic acid (PA) are the main autotoxins identified from cucumber root exudates. In this study, we investigated the effects of silicon (Si) supplemented on cucumber seed germination under PA-induced stress. Cucumber bud seedlings were harvested at 0, 4, 8, 12, 24, 36, and 48 h after treatment and assessed for plant growth, amylase activity and gene expression, and starch metabolism. Results revealed that PA significantly reduced the seed germination rate, seed vigor, radicle length, number of lateral roots, fresh weight, and soluble sugar content of cucumber bud seedlings. However, PA increased the starch content. The supplement of silicon promoted the growth of PA-treated plants and significantly increased the germination rate, seed vigor, and soluble sugar content of cucumber bud seedlings. Compared with those of the control plants, the levels and activities of α- (*AMY*) and β-amylase (*BMY*) during germination significantly decreased after PA treatment. The supplement of silicon significantly improved amylase activity, and total amylase and β-amylase activities reached maximum values at 36 h. PA significantly down-regulated the relative expression levels of *AMY* and *BMY*, whereas exogenous silicon significantly increased their transcript levels. These results suggest that supplement of Si alleviated cucumber autotoxicity caused by PA during seed germination at both the physiological and molecular levels.

Keywords Silicon · Autotoxicity, (PA) · Starch · Soluble sugar · α, β-Amylase · *AMY* and *BMY* gene expression

Introduction

Autotoxicity is an intraspecific allelopathy, which occurs when one crop species releases chemical substances that restrain the seed germination and growth of a congener crop species (Singh and others 1999; Yu and others 2000). The secondary metabolites of allelopathic plants have been identified as allelochemicals, which are released into the air and the soil, particularly the rhizosphere (Singh and others 1999). Among these allelochemicals, benzoic and cinnamic acid derivatives have been consistently determined from root exudates or rhizosphere soil (Yu and Matsui 1994; Blum 1996; Inderjit and Duke 2003). Phenylcarboxylic acids, such as benzoic and cinnamic acids, restrain ion uptake and increase the occurrence of *Fusarium* wilt via oxidative stress in *Cucumis sativus* (Yu and Matsui 1994, 1997; Ye and others 2004, 2006; Ding and others 2007). Moreover, root growth subjected to toxicity by benzoic and cinnamic acids has been widely detected in alfalfa, barnyardgrass (Chon and others 2002), *Ophiopogon japonicus* Ker-Gawler (Iqbal and others 2004), lettuce, pigweed, red clover, timothy-grass, bok choy (Hiradate and others 2005), *Phragmites australis* (Rudrappa and others 2007), mung bean (Batish and others 2008), and cucumber (Zhang and others 2009). Furthermore, physiological and biochemical reactions of plants, including photosynthesis, respiration, water and nutrient uptake, and oxidative stress may be affected by allelochemicals (Hejl and others 1993; Hejl and Koster 2004; Gonzalez and others 1997; Yu and Matsui 1997; Ding and others 2007). Allelochemicals also altered the gene expression (Golisz and others 2008; Zhang and others 2009). Phenylcarboxylic acid derivatives increased the levels of mitosis and endoreduplication, and they down-regulated the cell-cycle-related genes in cucumber roots (Zhang and others 2009). Autotoxicity

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is a major problem associated with continuous cropping, particularly in protected vegetable cultivation. Therefore, strategies for alleviating autotoxicity in protected vegetable cultivation must be developed.

Although silicon (Si) is the second most abundant element both in the Earth's crust and in the soil (Gottardi and others 2012), and it has not yet been considered as an essential element for higher plants (Epstein 1999). However, numerous studies have demonstrated that exogenous Si improved the ability of plants to tolerate environmental stresses through increased photosynthetic activity (Feng and others 2010), reduced heavy metal toxicity (Ma and others 2015; Tripathi and others 2012; Song and others 2009), improved nutrient balance (Epstein 1999), enhanced drought tolerance (Chen and others 2011; Gong and others 2005), improved salt tolerance (Yin and others 2016; Liu and others 2015), enhanced frost tolerance (Liang and others 2008), and increased insect and disease resistance (Van Bockhaven and others 2013; Ma 2004). These advantages of Si have been primarily associated with its easy accumulation in plant tissues and its strengthening of cell rigidity (Ma and Yamaji 2006; Jones and Handreck 1967; Jarvis 1987; Epstein 1994). The beneficial effects of Si demonstrated two main trends: (1) the effects varied with the plant species and were more obvious in plants that accumulated high Si levels in their shoots, such as rice; and (2) the effects were more apparent when the plants were exposed to various environmental stresses (Ma and others 2006; Epstein 2009).

Plants are subjected to various abiotic and biotic stresses. One such abiotic stress is autotoxicity, which is the main challenge in the continuous cropping of vegetable crops, particularly cucurbit crops. Therefore, the mechanisms of autotoxicity must be investigated to establish effective control measures. Cucumber is a major culinary vegetable used worldwide, and it actively absorbs Si (Liang and others 2005a). Previous reports indicated that exogenous Si increased the tolerance of cucumber to various abiotic and biotic stresses (Maksimović and others 2012; Chérif and others 1994; Kauss and others 2003; Zhu and others 2004; Shi and others 2005). In addition, Si increased antioxidant enzyme activity and promoted seedling photosynthesis in the continuous cropping of cucumber (Zhang and others 2014). Nonetheless, the mechanisms underlying the effects of Si on autotoxicity remain unclear. Seed germination is the most crucial phase of plant life. In this process, the embryo acquires vital nutrients produced by the degradation of carbohydrates stored in the endosperm. Amylases are hydrolytic enzymes involved in the decomposition of α-1,4-linked sugar polymers, such as starch and glycogen, into oligosaccharides. Autotoxicity is one of the most serious challenges in seed germination. To date, few studies have demonstrated that exogenous Si improved the tolerance of cucumber against autotoxic stress during seed germination. To clarify the underlying mechanisms, we analyzed the effects of Si on artificially induced autotoxicity in cucumber seedlings by determining the amylase activity and the starch metabolism in Si-treated and control plants. This study aimed to determine the mechanisms by which exogenous Si alleviated autotoxic stress during seed germination in cucumber.

Materials and Methods

Plant Materials, Growth Conditions, and Treatments

Cucumber (*Cucumis sativus* L. cv. "Xinchun No. 4") seeds were obtained from Gansu Academy of Agricultural Sciences. The seeds were germinated at 25 °C in darkness and placed in 9 cm Petri dishes ($n = 20$ per treatment group) on two layers of filter paper saturated with 10 mL distilled water or treatment solutions for 72 h. The solutions were renewed and the numbers of germinated seeds were counted every day to calculate the germination rates.

Autotoxicity was initiated by adding 2 mM 3-phenylpropionic acid (PA; Sigma, St Louis, MO, USA). The PA concentration was determined by preliminary experiments (data not shown). Si treatments (0–32 mM) were performed by adding sodium silicate ($Na_2SiO_3 \cdot 9H_2O$; AR). The experiments were conducted in a manual climatic box in the Vegetable Cultivation and Physiology Lab of Gansu Agricultural University in Lanzhou, Gansu (northwest China).

Experiment A: Preliminary Experiments

Experiment A involved three independent replications of simultaneous treatment with PA and Si at various concentrations: (1) control plants (distilled water with neither PA nor Si added); (2) 2 mM PA + 0 mM Si; (3) 2 mM PA + 1 mM Si; (4) 2 mM PA + 2 mM Si; (5) 2 mM PA + 4 mM Si; (6) 2 mM PA + 8 mM Si; (7) 2 mM PA + 16 mM Si; and (8) 2 mM PA + 32 mM Si. The cucumber bud seedlings were excised at 72 h after treatment and assayed for plant growth. The PA concentration was selected based on the results of preliminary experiments (data not shown).

Experiment B

Experiment B consisted of three independent replications of the following PA and Si treatments: (1) control plants (distilled water with neither PA nor Si added); (2) 2 mM PA; (3) 2 mM PA + 2 mM Si; and (4) 2 mM PA + NaOH (the NaOH concentration was adjusted so that the pH was maintained equal to that of the 2 mM PA + 2 mM Si treatment). The cucumber bud seedlings were excised at 0, 4, 8, 12, 24, 36, and 48 h after treatment. The seedlings were assessed for starch metabolism at 36 h after treatment, for α-amylase

(*AMY*) and β-amylase (*BMY*) relative gene expression and seed vigor at 48 h after treatment, and for plant growth at 72 h after treatment.

Determination of Radicle Length, Number of Lateral Roots, and Fresh Weight

During seed germination, the standard plantlet length was considered to be half of the full length of the cucumber seed. The fresh weights of 20 bud seedlings from each treatment were measured and used to calculate the average fresh weights for the control and treated groups. Three replicates were performed for each treatment. The radicle length was measured from the root tip to the radicle and hypocotyl junction (with embossment) of each seedling by using a ruler. The lateral roots were those attached to the taproot and visible to the naked eye. Twenty seedlings were evaluated for each treatment, and the average radicle length and number of lateral roots were determined.

Determination of Seed Vigor

The cucumber bud seedlings were cut into two parts along the central line of the embryo at 48 h after exposure to the following treatments: (1) control (distilled water); (2) 2 mM PA; (3) 2 mM PA + 2 mM Si; and (4) 2 mM PA + NaOH. The cut seedlings were immersed in a Petri dish filled with 0.5% (w/v) triphenyl tetrazolium chloride (TTC) solution and incubated for 1 h at 30 °C for preservation (Kittock and Law 1968). Then, the TTC solution was removed, and the seedlings were rinsed with water. The colors of the embryos were observed and photographed (Casio Exilim-Z750). Forty half-seedlings were analyzed for each treatment with three replicates.

Determination of Soluble Sugar and Starch Contents

The soluble sugar and starch contents were determined by the anthrone colorimetric method, as previously described by Vasquez-Tello and others (1990). The soluble sugar content was obtained by the anthrone–sulfuric acid method. A 0.5 g fresh weight of the cucumber bud seedlings was homogenized with deionized water. The extract was filtered and treated with 2% anthrone-ethyl acetate and 98% sulfuric acid. The mixture was boiled in a water bath for 1 min and cooled to room temperature. Then, the absorbance was measured at 630 nm by using a spectrophotometer (Beijing Purkinje General Instrument TU-1900). The residue of the extract filtered for sugar content was dried, weighted, and boiled with deionized water for 15 min. The supernatant was used to measure the starch content. The soluble sugar and starch contents were expressed as μg g^{-1} FW.

Determination of Amylase Enzyme Activity

Amylase enzyme activities were assayed by adding 0.5 mL of enzyme in buffer solution to 0.5 mL of 1.0 wt% of soluble starch in water (15 min; 100 °C; continuous mixing). The used buffers were 0.1 M phosphate buffer at pH 6.0 for *AMY*, 0.1 M acetate buffer at pH 4.8 for *BMY*, and 0.1 M acetate buffer at pH 4.5 for glucoamylase. After 15 min of incubation at 37 °C (*AMY* and *BMY*) or 55 °C (glucoamylase), the reaction was terminated by adding 2 mL of DNS (3,5-dinitrosalicylic acid) reagent, and the reducing sugar concentration was determined by the DNS method (Miller 1959). Enzyme activity was expressed as a unit organization liberating 1 mg of maltose per minute (μg g^{-1} FW min^{-1}) from starch at 37 °C. Three replicates were performed for each treatment.

RNA of AMY (α-amylase) and BMY (β-amylase) Extraction and Transcript-Level Estimation with qRT-PCR

The total RNA was extracted from cucumber bud seedlings from different treatment groups by using TRIzol® reagent (TaKaRa, China) in accordance with the manufacturer's instructions at 36 h after treatment. After extraction, the total RNA was dissolved in diethyl-pyrocarbonate-treated water.

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to determine the relative transcript level of each cDNA. Gene-specific primers for qRT-PCR were designed based on the expressed sequence tag sequences for *AMY* and *BMY*. Table 1 lists the specific sets of primers used for amplifying each cDNA. The first-strand cDNA used as a template for qRT-PCR was synthesized from 2 μg of the total RNA by using an oligo$(dT)_{18}$ 10 mM first-strand cDNA synthesis kit (Fermentas). Total RNA was purified by using an RNeasy Mini Kit (Qiagen). qRT-PCR was performed with a real-time PCR detection

Table 1 Primers used for relative quantitative real-time PCR assays

Gene	Encoding protein	Accession No.	Primer pair (5′–3′)
AMY	α-Type starch hydrolysis	XM_004151148	F:CACGGTTATTACACCCAGGACT
			R:TAAATCATCTTCGTTGCCCAT
BMY	β-Type starch hydrolysis	XM_004138543	F:GGTGTCAAGTGGTAGCAACAATAAC
			R:TGTCCTCTCTTTCTCTTCTAATGGTCT

system (ABI StepOne™ Plus, CA, USA). Each reaction (20 µL total volume) consisted of 10 µL of 2 × qPCR mix, 1 µL of diluted cDNA, and 10 µM of forward and reverse primers. The PCR cycling conditions were as follows: 95 °C for 5 min, 40 cycles of 95 °C for 10 s, and 60 °C for 30 s. The fluorescence data were collected during the 60 °C step. The cucumber actin gene was used as an internal control gene. The relative gene expression was computed according to a previously described method by Livak and Schmittgen (2001).

Statistical Analysis

The seeds were randomly arranged in a manual climatic box with three replicates per treatment. The data were presented as the mean ± SE of three replicates for each group. Statistical differences between treatments were analyzed by Duncan's multiple range tests. Differences were considered statistically significant at a probability level of $P < 0.05$. All statistical analyses were performed on the Statistical Package for Social Sciences (SPSS) software for Windows (version 17.0; SPSS, Inc., Chicago, IL, USA).

Results

Changes in Plant Growth and Seed Vigor

Si was applied at seven concentrations (0, 1, 2, 4, 8, 16, and 32 mM) in three replicates. As shown in Fig. 1, exogenous Si significantly promoted radicle elongation and increased the number of lateral roots. Compared with the control (distilled water) plants, the 2 mM PA treatment significantly inhibited radicle elongation (67.06% lower than that in the control plants) and the number of lateral roots (42.56% lower than that in the control plants) after 72 h of treatment (Fig. 1a, b). The inhibitory effect of PA was diminished by the addition of exogenous Si. Moreover, as the Si concentration was increased, its effect was intensified. Si at 2 mM exerted the best alleviating effect on the 2 mM PA-induced stress and was thus considered the optimal Si concentration (Fig. 1a, b), after which the effect declined. Therefore, we used 2 mM Si concentration for the subsequent experiments.

As shown in Table 2, compared with the control, the 2 mM PA treatment significantly reduced the germination rate, the radicle elongation, the number of lateral roots, and the fresh weight, which were 17.24, 81.39, 42.41, and 33.33%, respectively, lower than that in the control after

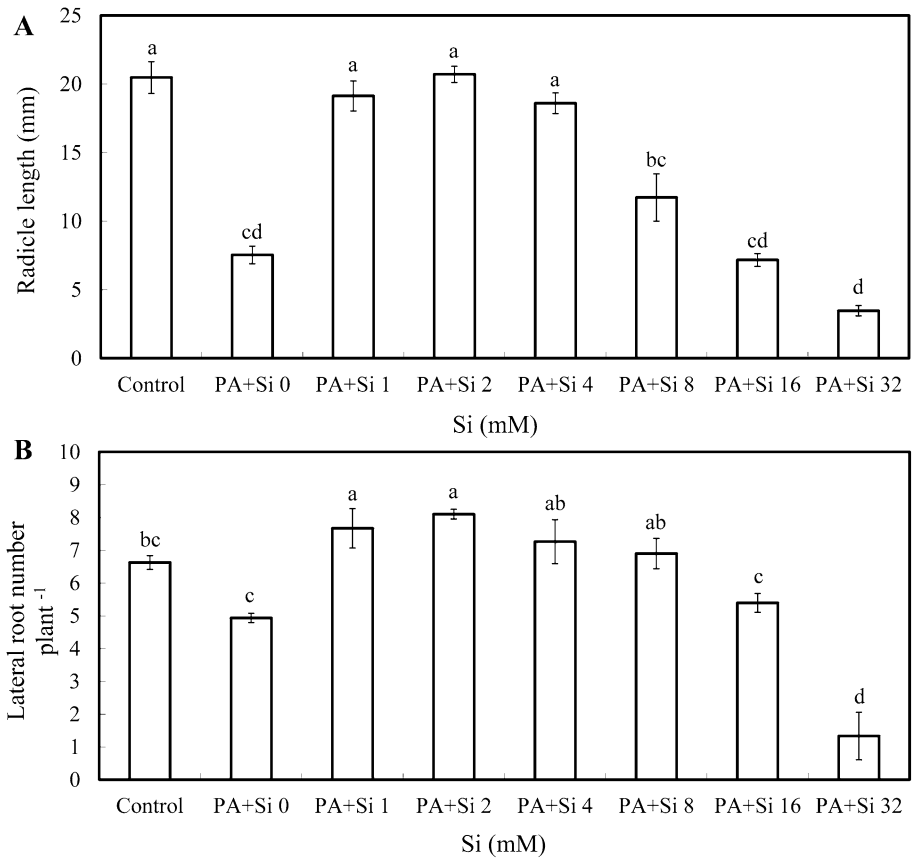

Fig. 1 Effects of different concentrations of Si supplement on radicle length (**a**) and lateral root number (**b**) of cucumber bud seedlings under PA-stress. The seeds were placed randomly in 2 mM PA with different Si concentrations for 72 h. Eight treatments: (1) control (distilled water); (2) 2 mM PA; (3) 2 mM PA + 1 mM Si; (4) 2 mM PA + 2 mM Si; (5) 2 mM PA + 4 mM Si; (6) 2 mM PA + 8 mM Si; (7) 2 mM PA + 16 mM Si; and (8) 2 mM PA + 32 mM Si. The values (mean ± SE) represent the average of three replicates ($n = 3$, each consisting of 20 cucumber bud seedlings). Different lowercase letters indicate significant differences by Duncan's multiple range tests ($P \leq 0.05$)

Table 2 Effect of PA and Si on germination rate, seed vigor, radicle long, lateral root number, and fresh weight of cucumber bud seedlings

Treatments	Germination rate (%)	Seed vigor (%)	Radicle long (mm)	Lateral root number ($plant^{-1}$)	Fresh weight (g)
Control	96.67 ± 1.67 a	68.33 ± 0.83 a	32.46 ± 1.48 a	6.72 ± 0.24 a	0.09 ± 0.0014 a
PA	80.00 ± 2.89 b	40.83 ± 1.67 d	6.09 ± 0.46 d	3.87 ± 0.13 c	0.06 ± 0.0003 c
PA + Si	93.33 ± 1.67 a	56.67 ± 0.83 b	15.31 ± 0.51 b	5.72 ± 0.09 b	0.08 ± 0.0019 b
PA + NaOH	85.00 ± 2.89 b	50.83 ± 0.83 c	9.12 ± 0.53 c	4.18 ± 0.07 c	0.06 ± 0.0006 c

Four treatments: (1) control (distilled water), (2) PA 2 mM, (3) PA 2 mM + Si 2 mM, (4) PA 2 mM + NaOH (the pH was adjusted to be the same as the 2 mM PA + 2 mM Si treatment). The values (mean ± SE) are the average of three replicates ($n = 3$, each consisting of 20 cucumber bud seedlings). Numbers with different letters in the same column refer to significant differences between the treatments ($P \leq 0.05$)

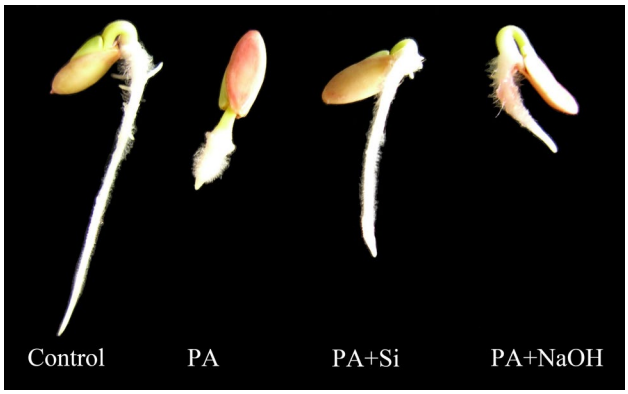

Fig. 2 Effect of PA (2 mM) and Si (2 mM) on the phenotype of cucumber bud seedlings. Four treatments: (1) control (distilled water); (2) 2 mM PA; (3) 2 mM PA + 2 mM Si; and (4) 2 mM PA + NaOH (the pH was adjusted to be the same as that of the 2 mM PA + 2 mM Si treatment). Photographs were captured at 72 h after treatment

72 h. By contrast, exogenous Si significantly increased the germination rate, the radicle elongation, the number of lateral roots, and the fresh weight, which were 16.66, 151.40, 47.80, and 33.33%, respectively, higher than that in PA treatment alone despite the presence of PA (Table 2; Fig. 2). Compared with the PA + NaOH treatment, the PA + Si treatment significantly promoted cucumber seed germination. As shown in Table 2, compared with the control, the 2 mM PA treatment for 48 h significantly reduced the number of red-stained cucumber seeds after being treated with TTC solution (40.25% lower than that in the control). By contrast, the PA + Si treatment increased the number of red-stained cucumber seeds by 38.80% from that in the PA treatment alone, suggesting that Si improved seed vigor (Table 2).

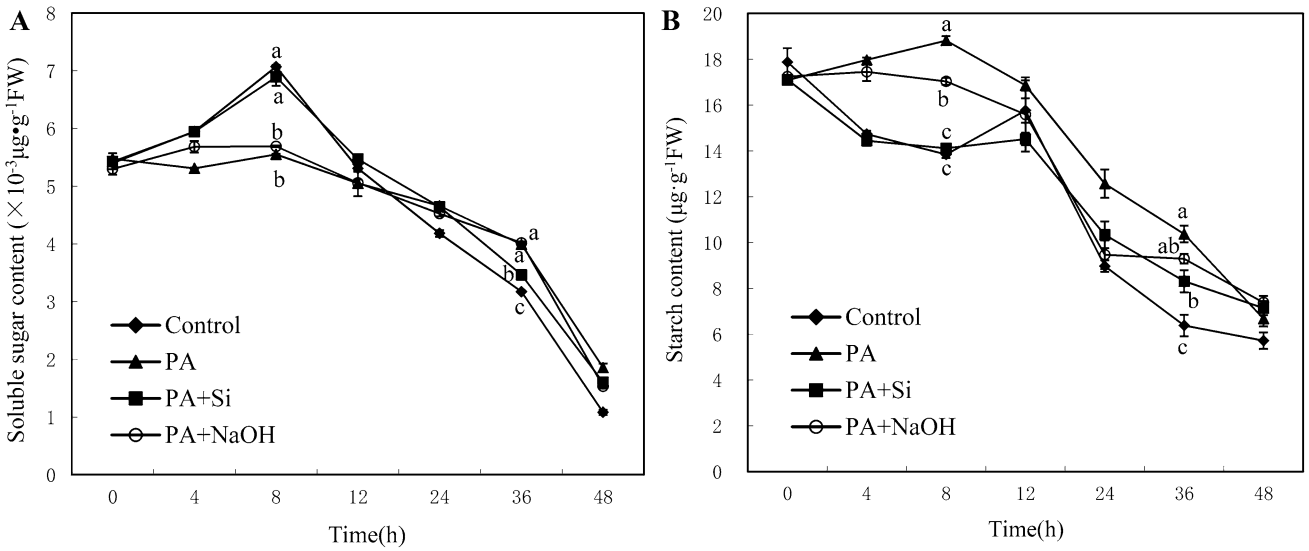

Fig. 3 The contents of soluble sugar (**a**) and starch (**b**) in cucumber bud seedlings at 0, 4, 8, 12, 24, 36, and 48 h after exposure to different treatments. Treatments consisted of (1) control (distilled water with neither PA nor Si added); (2) 2 mM PA; (3) 2 mM PA + 2 mM Si; and (4) 2 mM PA + NaOH (the pH was adjusted to be the same as that of the 2 mM PA + 2 mM Si treatment). Vertical bars represent the mean ± SE value from three independent experiments. Different lowercase letters above the vertical bars at 8 and 36 h after treatment indicate significant differences by Duncan's multiple range tests ($P \leq 0.05$)

Changes in Starch and Soluble Sugar Contents

More starch and less soluble sugar accumulated in the PA-treated cucumber seedlings than in the PA + Si-treated seedlings (Fig. 3a, b). The starch content remained at a steady level in all treatment groups from 0 to 12 h; after which, it declined. By contrast, PA treatment produced a starch content that was significantly higher than that in the control throughout the test period. Therefore, Si significantly reduced the starch content under PA-induced stress (Fig. 3b).

More soluble sugar accumulated in the PA + Si-treated seedlings than in the PA-treated seedlings from 4 to 8 h (Fig. 3a). The soluble sugar contents in the control and PA + Si-treated seedlings initially increased and then decreased, attaining the maximum at 8 h. The soluble sugar contents of all treatment groups declined after 12 h, and no significant difference was observed between the PA and PA + Si treatments from 12 to 48 h.

Changes in Total Amylase, and α- and β-Amylase Activities

Figure 4 shows the changes in amylolytic enzyme activity. These changes were determined by assaying the quantities of maltose produced via hydrolysis with starch as the substrate. The activities of total amylase and *BMY* in all

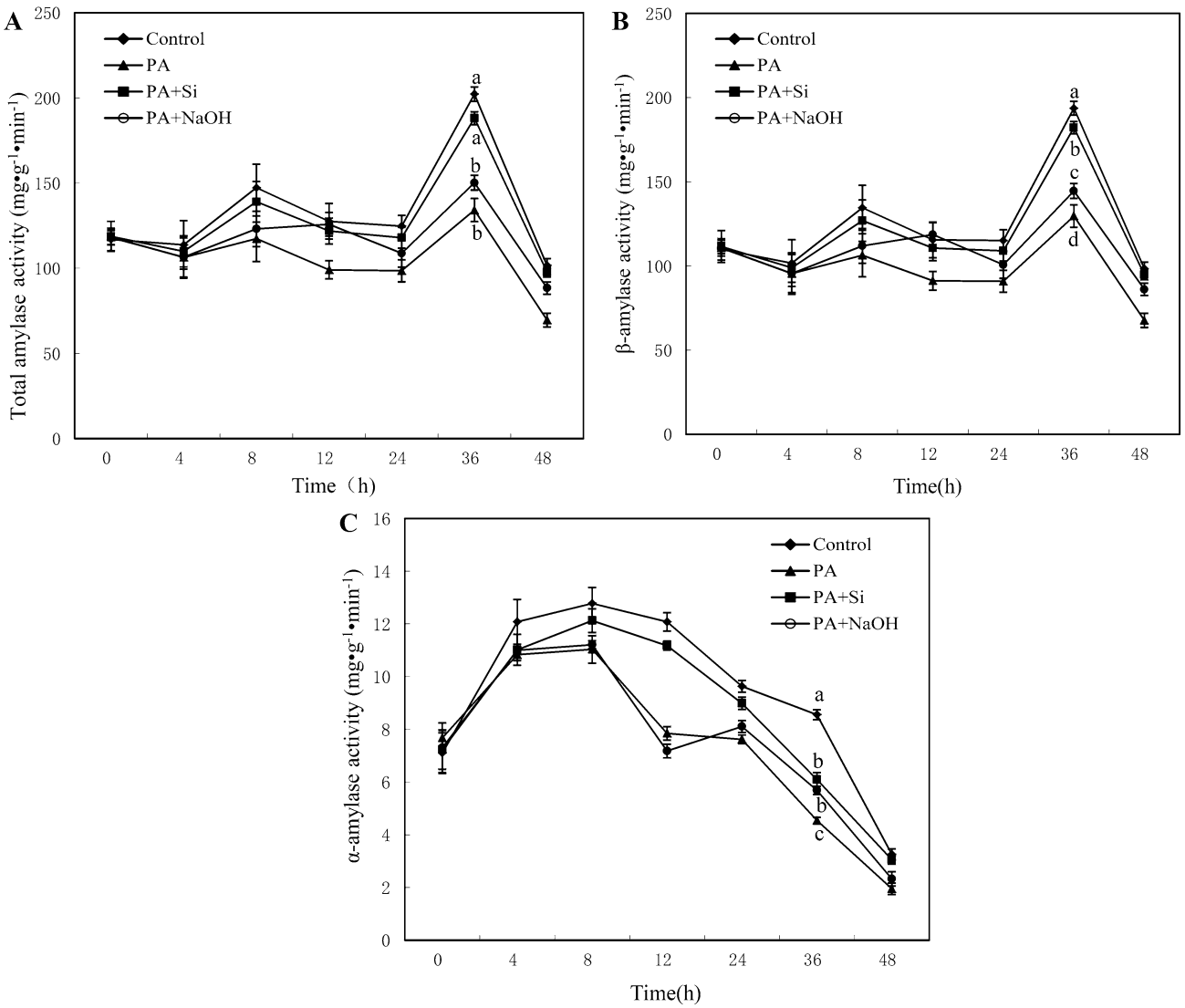

Fig. 4 Effects of the activity of total amylase (**a**), α-amylase (**b**), and β-amylase (**c**) in cucumber bud seedlings at 0, 4, 8, 12, 24, 36, and 48 h after exposure to different treatments. Treatments consisted of (1) control (distilled water with neither PA nor Si added); (2) 2 mM PA; (3) 2 mM PA + 2 mM Si; and (4) 2 mM PA + NaOH (the pH was adjusted to be the same as that of the 2 mM PA + 2 mM Si treatment). Vertical bars represent mean ± SE value from three independent experiments. Different lowercase letters above the vertical bars at 36 h after treatment indicate significant differences by Duncan's multiple range tests ($P \leq 0.05$)

treatment groups initially increased and then decreased, attaining their maximum at 36 h. The activities of total amylase and *BMY* in the PA-treated seedlings were significantly reduced compared with that in the control (Fig. 4a, b). However, the addition of exogenous Si significantly increased the activities of total amylase and *BMY* under PA-induced stress (Fig. 4a, b).

Similarly, the *AMY* activity in all treatment groups initially increased and then decreased, attaining the maximum at 8 h. PA-induced stress significantly decreased the *AMY* activity in the seedlings, particularly after 12 h (Fig. 4c). By contrast, exogenous Si significantly increased the *AMY* activity in cucumber bud seedlings under PA-induced stress (Fig. 4c).

Changes in AMY and BMY Gene Expression

To gain insight into the mechanism by which Si regulates amylase activity under PA-induced stress, gene expression of *AMY and BMY* in the cucumber bud seedlings was examined after 36 h of treatment. The transcript levels of *AMY and BMY* were significantly reduced after exposure to 2 mM PA. However, exogenous Si significantly increased the expression levels, particularly that of the *BMY* gene after 36 h of treatment, even in the presence of PA when compared with that in the control. No significant difference was observed between the PA and PA + NaOH treatments (Fig. 5).

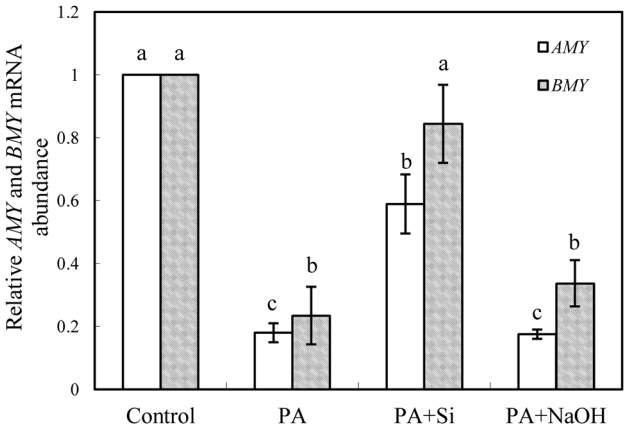

Fig. 5 Expression of *AMY* and *BMY* genes in cucumber bud seedlings at 36 h after exposure to different treatments. Treatments consisted of (1) control (distilled water with neither PA nor Si added); (2) 2 mM PA; (3) 2 mM PA + 2 mM Si; and (4) 2 mM PA + NaOH (the pH was adjusted to be the same as that of the 2 mM PA + 2 mM Si treatment). Expression levels by qRT-PCR are expressed as a ratio of the control, which is set as 1. Vertical bars represent the means ± SE from three independent experiments. Different lowercase letters above the vertical bars indicate significant differences by Duncan's multiple range tests ($P \leq 0.05$)

Discussion

Seed germination is the first step of plant growth and development, making it a vital stage in the plant lifecycle. The success of seed germination influences plant survival, the plant growth period in agricultural ecosystems, and yield (Weitbrecht and others 2011). Plants at the seed germination stage are fragile and susceptible to mechanical damage, disease, and environmental stresses (Rajjou and others 2012). Autotoxicity induced by benzoic and cinnamic acid derivatives, such as PA, is considered an abiotic stress (Yu and Matsui 1994). These autotoxins adversely affect seed germination, radicle elongation, ion uptake, membrane permeability, active oxygen metabolism, photosynthesis, and transpiration (Yu and Matsui 1994, 1997; Yu and others 2000; Ye and others 2006). Asaduzzaman and Asao (2012) reported that allelochemicals identified from root exudates demonstrated an excellent inhibiting effect on the root and shoot length of *P. vulgaris* and *V. faba* even at low concentrations. Our results confirmed that cucumber seed germination and radicle elongation were inhibited by autotoxin PA treatment (Table 2; Fig. 1). The application of exogenous substances is a feasible strategy for mitigating the inhibition of seed germination caused by autotoxicity.

Silicon (Si) alleviates abiotic stresses, such as salinity, drought, high temperature, freezing, and heavy metal toxicity (Zhu and Gong 2014; Van Bockhaven and others 2013; Romero-Aranda and others 2006; Liang and others 2007, 2005b; Kim and others 2011). Shi and others (2014) found that Si alleviated the reduction of the tomato germination index under water deficit stress. Exogenous Si also improved the seed germination of *Momordica charantia* under salt stress (Wang and others 2010). In the present study, an appropriate concentration of exogenous Si exerted a significantly positive effect on radicle elongation and the number of lateral roots of cucumber seedlings under PA-induced stress (Table 2). Moreover, Si enhanced the seed vigor of cucumber under autotoxic stress, implying the potential of using Si fertilizer to overcome the obstacles encountered in the continuous cropping of cucumber under protected cultivation.

Seed germination is a hyperaction phase that requires tremendous energy. Starch, which is one of the main stored substances in the cucumber seed, provides the needed energy and material for seed germination and seedling growth. Starch metabolism is regulated by a series of enzyme activations and gene expression. The key enzymes in the starch hydrolytic process are amylases, including *AMY* and *BMY* (Akazawa and Hara-Nishimura 1985). Researchers have reached two opposing views as regards the changes in amylase activity under stress conditions.

Jacobsen and others (1986) maintained that water-induced stress enhanced the activity and expression of *AMY* in barley leaves. Todaka and others (2000) also believed that *BMY* activity was enhanced, thereby causing sucrose accumulation in cucumber cotyledons under PEG-induced stress. Other studies disagreed with this viewpoint and presented different results. The activities of *AMY* in *Cicer arietinum* cotyledons (Kaur and others 1998, 2000) and of *AMY* and *BMY* in *Medicago sativa* germinated seeds (Zeid and Shedeed 2006) were reduced by PEG treatment. Our results indicated that *AMY* and *BMY* activities and gene expression were decreased under PA-induced stress over 0–48 h, and a significant difference was observed only at 36 h (Figs. 4, 5). Many studies have explored the physiological mechanisms of Si-mediated alleviation of abiotic stress, such as decreasing lipid peroxidation (Moussa 2006; Soylemezoglu and others 2009), improving photosynthesis (Yoshida 1965), regulating plant hormone levels (Kim and others 2013), and balancing mineral uptake (Sonobe and others 2011). However, limited works have been devoted to the effect of exogenous Si on starch metabolism at the germinated stage under abiotic stress. Our results revealed general trends of increased *AMY* and *BMY* activities of cucumber caused by Si application under PA-induced stress over 0–48 (Fig. 4b, c), suggesting that exogenous Si relieved autotoxic stress by regulating starch metabolism. We also found that Si application increased the down-regulated *AMY* and *BMY* relative gene expression in cucumber bud seedlings at 36 h after treatment under PA-induced stress (Fig. 5), indicating that Si regulated the physiological and metabolic processes by altering the expression of key genes.

Soluble sugar is a plant nutrient and osmotic regulator. Many studies have shown that soluble sugar content accumulated in many plants grown under abiotic stress (Dai and others 2012; Sakamoto and Murata 2002). In the present study, soluble sugar content was decreased under PA-induced stress for 0–12 h and increased after 12 h when compared with the control. Exogenous Si always mediated the effect of autotoxic stress (Fig. 3a). The probable reason is that soluble sugar served as an energy source at the early stage and as an osmotic substance at the latter period of seed germination. Further studies are needed to confirm this hypothesis.

We investigated the roles of Si in increasing the tolerance to autotoxic stress during cucumber seed germination and obtained reliable results. Exogenous supplement of NaOH was designed to eliminate the effect of neutralization, because sodium silicate ($Na_2SiO_3 \cdot 9H_2O$) was alkaline, whereas PA was acidic. The results demonstrated that exogenous Si under PA-induced stress alleviated the adverse effects. In future works, we will investigate whether Si mediated the autotoxic stress at the cucumber seedling stage and verify the physiological and molecular mechanisms.

Conclusions

Supplemented Si significantly alleviated the PA-induced phytotoxicity in cucumber bud seedlings. Supplement of Si to PA increased the germination rate, the radicle elongation, the number of lateral roots, the fresh weight, and the seed vigor. Moreover, the activities of total amylase, α- and β-amylase were significantly improved by Si application, which concurrently increased the *AMY* and *BMY* gene expression. Si addition significantly decreased the starch content of PA-stressed bud seedlings. These results suggested that the Si alleviation of PA autotoxic stress in germinating cucumber seedlings was correlated with the changes in carbohydrate metabolism. Overall, the results were consistent with the findings of previous studies that Si protected plant tissues, in this case, the radical growth of cucumber seedlings, from PA-induced stress.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest in this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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