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Research article

Silicon-mediated changes in polyamine and 1-aminocyclopropane-1-carboxylic acid are involved in silicon-induced drought resistance in *Sorghum bicolor* L

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ABSTRACT

The fact that silicon application alleviates drought stress has been widely reported, but the mechanism it underlying remains unclear. Here, morphologic and physiological changes were investigated in sorghum (*Sorghum bicolor* L.) seedlings treated with silicon and exposed to PEG-simulated drought stress for seven days. Drought stress dramatically decreased growth parameters (biomass, root/shoot ratio, leaf area, chlorophyll concentration and photosynthetic rate), while silicon application reduced the drought-induced decreases in those parameters. Leaf relative water content and transpiration rate were maintained at high levels compared to those in seedlings without silicon. The soluble sugar contents were increased, but the proline contents and the osmotic potential were decreased, showing that osmotic adjustment did not contribute to the silicon induced-drought resistance. Furthermore, levels of both free and conjugated polyamines (PAs) levels, including putrescine, spermidine and spermine, were all found to be increased by silicon under drought stress both in leaf and root. Meanwhile, 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene, was markedly decreased by supplemental silicon. Several key PA synthesis genes were upregulated by silicon under drought stress. These results suggest that silicon improves sorghum drought resistance by mediating the balance of PAs and ethylene levels. In leaf, the increased PAs and decreased ACC help to retard leaf senescence. In root, the balance between PAs and ACC participates in the modulation of root plasticity, increases the root/shoot ratio, and contributes to an increase in water uptake. These results suggest that silicon increases drought resistance through regulating several important physiological processes in plants.

1. Introduction

Silicon is the second most abundant element in soil after oxygen, comprising 31% of the earth's crust (Epstein, 1999). To date, silicon has not been considered an essential element for higher plants, through the uptake of silicon has been widely found to be beneficial in increasing plant resistance to stresses of various kinds (Ma, 2004; Liang et al., 2007; Gonzalo et al., 2013; Gottardi et al., 2012; Liu et al., 2013; Mateos-Naranjo et al., 2013; Yin et al., 2013). Numerous studies have shown that silicon is effective in improving plant drought resistance in various species, including wheat, sorghum, maize, soybean, and rice (Gong et al., 2005; Hattori et al., 2005; Gao et al., 2006; Shen et al., 2010; Nolla et al., 2012).

Silicon's effect in enhancing drought resistance has been shown to reduce evaporation or control stomata conductance when taken up and deposited in leaf cuticle, thereby reducing transpiration (Gao et al., 2006; Matoh et al., 1991); to decrease osmotic potential through influencing the accumulation of proline, inorganic ions and other osmotic solutes (Ming et al., 2012); to minimize drought-induced oxidative damage through enhancing enzymatic and non-enzymatic antioxidant capacities (Gong et al., 2005; Shen et al.,

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2010); and to enhance the activities of photosynthetic enzymes such as ribulose bisphosphate carboxylase and $NADP^+$-dependent glyceroldehyde-3-phosphate dehydrogenase (Gong et al., 2005). However, the mechanisms underlying the effects of silicon are still unclear, especially with regard to the physiological processes of the plant.

Sorghum (*Sorghum bicolor* L.) is an important crop that can take up and accumulate abundant silicon (2–3% DW) in its tissues. It is considered a median silicon accumulator (Hattori et al., 2005). Previous researches has shown that silicon administration can improve drought resistance in sorghum (Hattori et al., 2005; Sonobe et al., 2009), perhaps through accumulation in leaves, where, in certain other plants such as rice, maize and cucumber, it decreases the transpiration rate (Gao et al., 2006; Matoh et al., 1991; Hattori et al., 2008). In general, controlling transpiration through regulating the stomata is an important way to improve plant drought resistance (Bartels and SunKar, 2005). In sorghum, the decrease in transpiration rate associated with drought stress is smaller in silicon treated-plants than in untreated plants; meanwhile, the leaf water content was maintained at a higher level under drought stress in silicon-treated plants than in untreated plants10]. These indicates that silicon improves sorghum drought resistance through improving the plant's water uptake. The fact that sorghum responds to silicon treatment differently from rice, maize and cucumber implies that silicon's effect on drought resistance occurs not only through its effects on transpiration, but also through its role in mediating water uptake.

In our previous research, we found that silicon mediated levels of polyamines (PAs) and 1-aminocyclopropane-1-carboxylic acid (ACC, the precursor of ethylene) levels under salt stress (Yin et al., 2011). It is known that salt and drought stress have some stress factors in common, and also that similar changes in PA and ethylene accumulation occur when plants are confronted with salt or drought stress. First, both drought stress and salt stress result in osmotic stress, which limits the water availability, which in turn affects water status (Munns and Tester, 2008). Second, both drought stress and salt stress commonly cause changes in PA and ethylene accumulation in a wide variety of plants, and a tight relationship between levels of PAs and ethylene resistance to drought and salt stress resistance has been established (Groppa and Benavides, 2008; Alcázar et al., 2010a; Cvikrova et al., 2013). Considering the important role of PAs and ethylene in mediating drought resistance, the question of whether PAs and ethylene are involved in silicon-induced drought resistance needs to be clarified. Therefore, the influence of silicon on PA and ethylene levels, and their relations with silicon-mediated changes in morphogenesis and physiology were investigated in sorghum.

2. Results

2.1. Plant growth, photosynthesis parameters, chlorophyll concentration and relative water content (RWC)

Plant growth parameters were investigated under drought and in the presence of silicon treatment. Under the non-stress condition, silicon application had no effect on dry weight, ratio of root/shoot and leaf area. Drought stress significantly decreased those parameters after seven days of treatment. However, the inhibiting effects of drought stress on sorghum growth were substantially alleviated by the application of silicon. The total dry weight under drought stress was increased by 43% in the silicon-treated plants compared with untreated plants. It is worth noting that the ratio of root/shoot under drought stress conditions was likewise was increased by silicon. Similarly, the drought-induced decreases in photosynthetic rate, transpiration rate and stomatal conductance were significantly mitigated by silicon after one, three and seven days of treatment (Figs. 1 and 2). Moreover, drought stress decreased the chlorophyll concentration after three and seven day's treatment, but silicon application kept the chlorophyll concentration higher than is was in untreated plants. Likewise, the leaf RWC of untreated plants without silicon was 92, 86, and 82% after one, three, and seven days, respectively, of drought stress; in silicon-treated plants, in contrast, it was 96, 91, and 88% (Fig. 3). These results show that the application of silicon improves sorghum's drought resistance, and that silicon-treated plants maintain a higher water status than that untreated plants under drought stress.

2.2. Osmotic potential and osmotic solute contents

The osmotic potential of leaf and root were decreased by drought stress both in silicon-treated plants and in untreated plants. In silicon-treated plants, however, they were maintained at

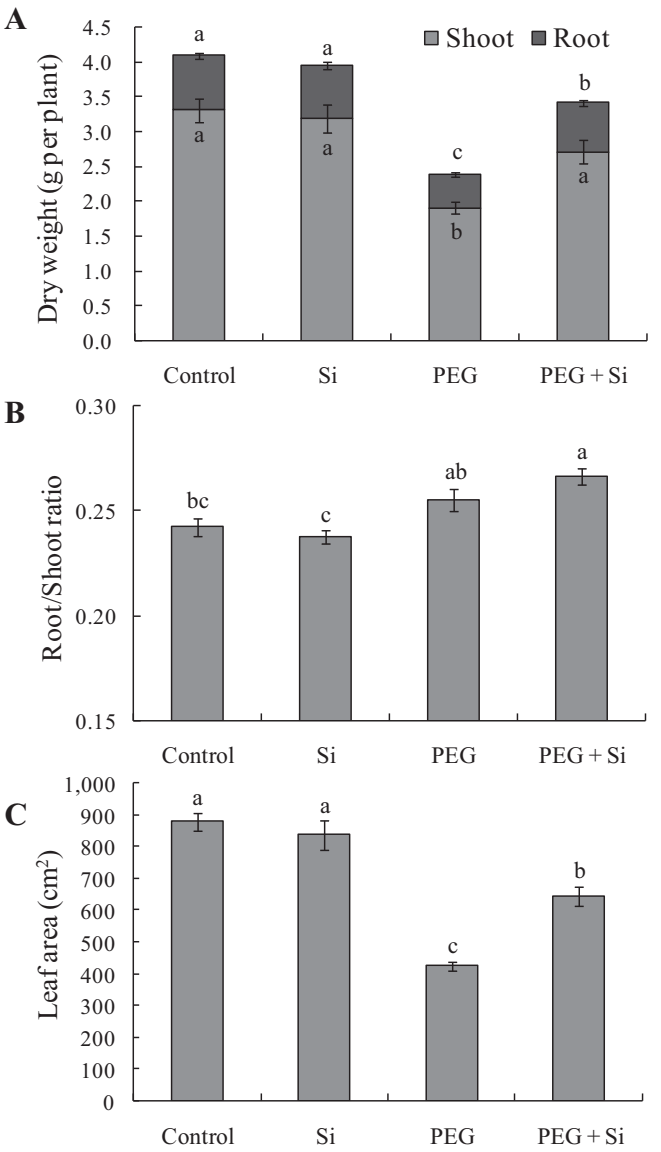

Fig. 1. Effects of silicon (Si, 0.83 mM) on dry weight (A), root/shoot ratio (B) and leaf area (C) of sorghum plants grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after seven days of treatment. Values are the means of six replicates ±SE. Different letters in one measure indicate statistically significant differences at $P < 0.05$.

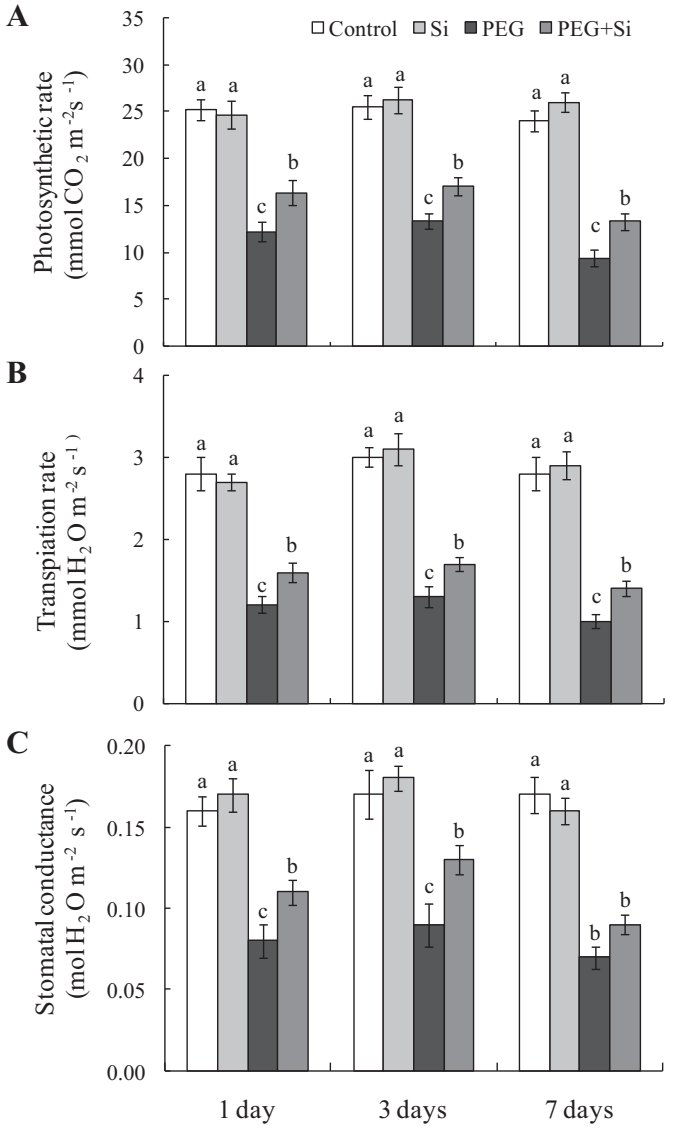

Fig. 2. Effects of silicon (Si, 0.83 mM) on photosynthetic rate (A), transpiration rate (B) and stomatal conductance (C) of sorghum plants grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after one, three and seven days of treatment. Values are the means of six replicates ±SE. Different letters in one measure indicate statistically significant differences at $P < 0.05$.

higher levels under drought stress than in untreated plants (Fig. 4). These results showed that the decrease in osmotic potential did not contribute to silicon-induced drought resistance. In order to further investigate the effect of silicon on osmotic adjustment, the main osmotic solutes, soluble sugar and proline, were measured (Table 1). Under normal conditions, silicon had no effects on the total sugar contents; under drought stress, however, it increased the total sugar contents in both leaf and root. The increase in sugar concentration in leaf under drought stress caused by silicon application was not significant; in root, however, sugar concentration was increased two to three times after three and seven days treatment. The sucrose, fructose and glucose levels were all increased by silicon, but sucrose and fructose increased to a greater degree than glucose. Proline, the other main osmotic adjustment solute, was increased by drought stress and decreased by silicon (Table 1). These results show that silicon promotes the soluble sugar accumulation in root, but not the proline accumulation under drought stress.

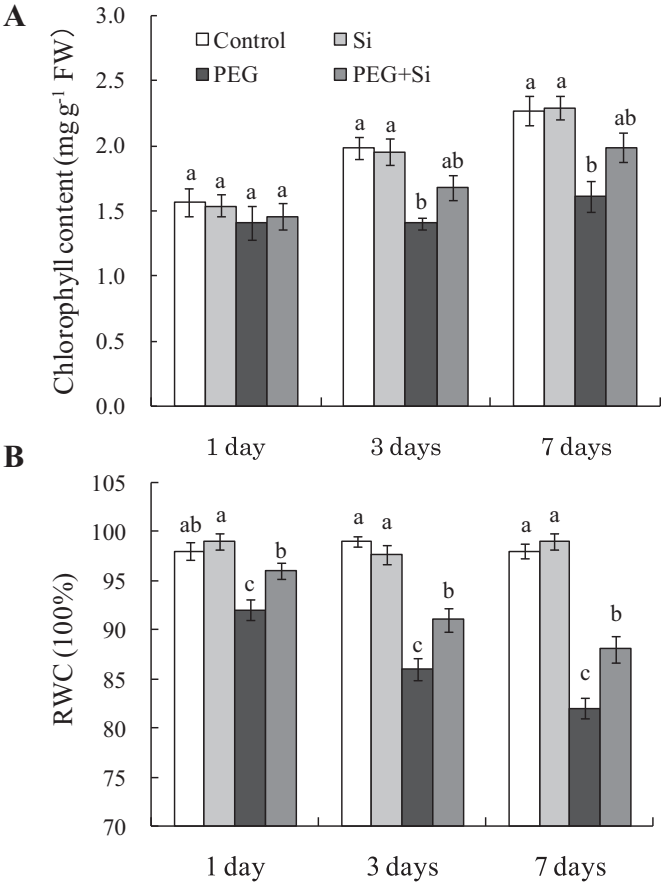

Fig. 3. Effects of silicon (Si, 0.83 mM) on chlorophyll content (A) and leaf relative water content (RWC) (B) of sorghum plants grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after one, three and seven days of treatment. Values are the means of six replicates ±SE. Different letters in one measure indicate statistically significant differences at $P < 0.05$.

2.3. Polyamine contents

Both free and conjugated types of PAs were investigated in leaf and root after treatment for one, three and seven days. In sorghum leaf, spermine (Spm) is the major free PA, accounting for 50–70% of total free PAs; in root, however, spermidine (Spd) is the major free PA, accounting for 50–65% of total free PAs. As for conjugated PAs, on the one hand, Spd is the major one in both leaf and root, accounting for 50–70% of total conjugated PAs (Fig. 5, Supplementary Table 1). Drought stress markedly decreased the total free and conjugated PAs in leaf. In root, in contrast, the total free PAs increased while the total conjugated PAs did not substantially change. Silicon application significantly increased the levels of free putrescine (Put), Spd and Spm both in leaf and root under drought treatment. Compared with levels of free PAs, levels of conjugated PAs were less strongly affected by silicon under drought stress in both leaf and root. Nevertheless, the total PAs (both free and conjugated type together) under drought stress were increased markedly by silicon application both in leaf and root after one, three and seven days treatment.

2.4. 1-aminocyclopropane-1-carboxylic acid (ACC) contents

In sorghum, malony-ACC (MACC, conjugated ACC) concentrations were much higher than free ACC concentrations (about 10- to

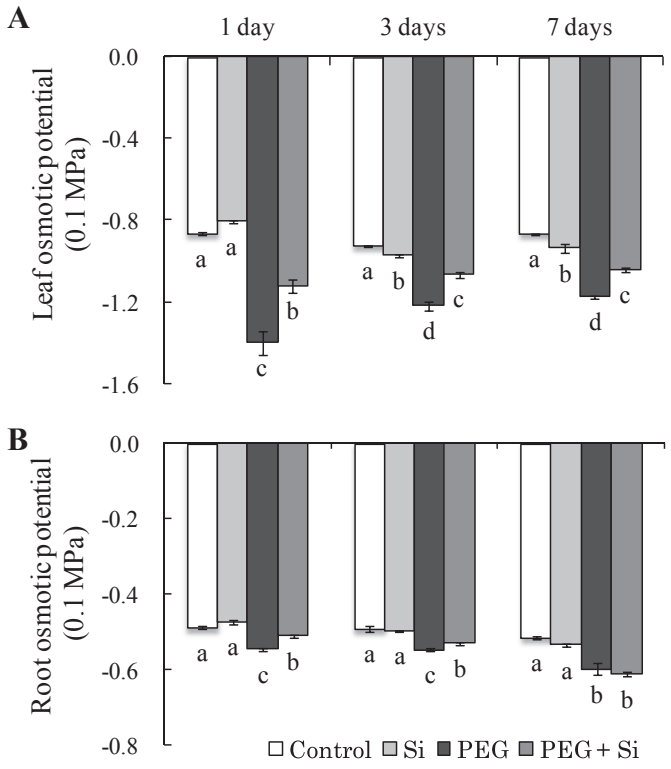

Fig. 4. Effects of silicon (Si, 0.83 mM) on leaf (A) and root (B) osmotic potential in sorghum plants grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after one, three and seven days of treatment. Values are the means of three replicates ±SE. Different letters in one measure indicate statistically significant differences at $P < 0.05$.

15-fold). Under non-stress conditions, silicon application decreased the ACC concentrations both in leaf and root, but the total ACC was not changed. Drought stress dramatically increased ACC and MACC production in both leaf and root, with final concentration two to five times those seen in control conditions. Silicon application significantly decreased this drought-induced accumulation of ACC and MACC. In leaf, silicon application decreased the ACC concentration by 40, 18 and 74%, and the MACC concentration by 3, 27 and 40% after one, three and seven days, respectively. Meanwhile, silicon application decreased the total ACC concentration under drought stress by 24 and 42% after three and seven days treatment, respectively. In root, the ACC concentration was not affected by silicon application, but the levels of MACC and total ACC were substantially decreased after treatment for one, three and seven days (Fig. 6).

2.5. Transcriptional profiling of genes involved in PA and ACC synthesis

We examined the expression levels of 11 genes involved in PA and ACC synthesis in sorghum by RT-qPCR (Fig. 7). Drought stress decreased the expression levels of the ornithine decarboxylase (*ODC1, ODC2 and ODC3*) and significantly enhanced the expression levels of S-adenosyl-L-methionine decarboxylase (*SAMDC04*) and 1-aminocyclopropane-1-1-carboxylic acid synthase (*ACS1 and ACS2*). However, the expression levels of almost all tested PA synthesis-related genes (including *ADC, CAP, ODC1, ODC2, ODC3, SAMDC04, SAMDC06 and SPDS*) were up-regulated by silicon application under drought stress, with the exception of *SAMDC02*. In contrast, the expression levels of the ACC synthesis-related genes, *ACS1* and *ACS2*, were decreased markedly by silicon application.

Table 1
Effect of silicon (Si, 0.83 mM) on concentrations of sucrose, fructose, glucose and proline in leaf and root of sorghum grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after one, three and seven days of treatment.

		(μmol g^{-1} FW)	Control	Si	PEG	PEG + Si
1 day	Leaf	Sucrose	2.78 ± 0.06^{b}	3.27 ± 0.02^{b}	11.64 ± 0.36^{a}	11.78 ± 1.25^{a}
		Fructose	0.66 ± 0.02^{c}	1.00 ± 0.04^{c}	4.16 ± 0.08^{b}	5.88 ± 0.66^{a}
		Glucose	19.15 ± 0.38^{b}	20.59 ± 0.53^{b}	34.21 ± 2.17^{a}	32.22 ± 0.97^{a}
		Total sugar	22.59 ± 0.41^{b}	24.86 ± 0.59^{b}	50.01 ± 2.44^{a}	49.88 ± 2.81^{a}
		Proline	0.14 ± 0.02^{c}	0.18 ± 0.01^{c}	13.58 ± 0.32^{a}	9.06 ± 0.83^{b}
	Root	Sucrose	1.42 ± 0.24^{ab}	1.06 ± 0.05^{b}	0.59 ± 0.02^{c}	1.58 ± 0.11^{a}
		Fructose	0.48 ± 0.08^{ab}	0.36 ± 0.01^{b}	0.19 ± 0.01^{c}	0.57 ± 0.02^{a}
		Glucose	3.19 ± 0.26^{a}	3.04 ± 0.15^{a}	3.27 ± 0.09^{a}	2.91 ± 0.13^{a}
		Total sugar	5.08 ± 0.47^{a}	4.46 ± 0.21^{ab}	4.05 ± 0.10^{b}	5.06 ± 0.21^{a}
		Proline	0.07 ± 0.01^{c}	0.06 ± 0.00^{c}	3.52 ± 0.20^{a}	2.12 ± 0.13^{b}
3 days	Leaf	Sucrose	3.36 ± 0.05^{b}	2.90 ± 0.09^{b}	11.42 ± 0.30^{a}	11.12 ± 0.51^{a}
		Fructose	0.71 ± 0.04^{c}	0.68 ± 0.03^{c}	3.08 ± 0.19^{b}	5.58 ± 0.36^{a}
		Glucose	18.21 ± 0.19^{c}	15.11 ± 0.35^{d}	25.81 ± 0.96^{a}	21.67 ± 0.10^{b}
		Total sugar	22.28 ± 0.24^{c}	18.69 ± 0.45^{d}	40.31 ± 0.36^{a}	38.37 ± 0.28^{b}
		Proline	0.24 ± 0.01^{c}	0.31 ± 0.02^{c}	4.91 ± 0.39^{a}	3.13 ± 0.34^{b}
	Root	Sucrose	1.67 ± 0.03^{c}	1.36 ± 0.04^{d}	1.83 ± 0.05^{b}	5.31 ± 0.03^{a}
		Fructose	0.49 ± 0.04^{b}	0.60 ± 0.01^{b}	0.56 ± 0.04^{b}	2.39 ± 0.14^{a}
		Glucose	2.87 ± 0.11^{b}	2.85 ± 0.11^{b}	2.69 ± 0.02^{b}	3.92 ± 0.03^{a}
		Total sugar	5.03 ± 0.15^{b}	4.81 ± 0.15^{b}	5.07 ± 0.11^{b}	11.62 ± 0.08^{a}
		Proline	0.05 ± 0.01^{c}	0.05 ± 0.00^{c}	2.46 ± 0.03^{a}	1.41 ± 0.08^{b}
7 days	Leaf	Sucrose	4.24 ± 0.06^{c}	3.64 ± 0.05^{c}	7.31 ± 0.59^{b}	9.96 ± 0.53^{a}
		Fructose	0.79 ± 0.02^{c}	0.96 ± 0.05^{c}	1.82 ± 0.12^{b}	2.54 ± 0.17^{a}
		Glucose	14.07 ± 0.05^{d}	15.12 ± 0.17^{c}	27.97 ± 0.36^{b}	29.60 ± 0.37^{a}
		Total sugar	19.10 ± 0.09^{c}	19.72 ± 0.17^{c}	37.10 ± 0.78^{b}	42.10 ± 0.35^{a}
		Proline	0.24 ± 0.01^{c}	0.28 ± 0.01^{c}	3.55 ± 0.22^{a}	1.82 ± 0.11^{b}
	Root	Sucrose	0.90 ± 0.01^{c}	1.77 ± 0.08^{b}	2.23 ± 0.04^{b}	11.50 ± 0.50^{a}
		Fructose	0.42 ± 0.01^{b}	0.41 ± 0.03^{b}	0.76 ± 0.10^{b}	4.43 ± 0.24^{a}
		Glucose	2.06 ± 0.02^{b}	2.40 ± 0.10^{b}	2.48 ± 0.11^{b}	5.05 ± 0.21^{a}
		Total sugar	3.38 ± 0.02^{c}	4.57 ± 0.19^{bc}	5.47 ± 0.22^{b}	20.98 ± 0.94^{a}
		Proline	0.07 ± 0.01^{c}	0.07 ± 0.00^{c}	2.08 ± 0.10^{a}	1.37 ± 0.07^{b}

Values are the means of three replicates ±SE. Means within the same line are comparable, and values marked by different letters are significantly different ($P < 0.05$).

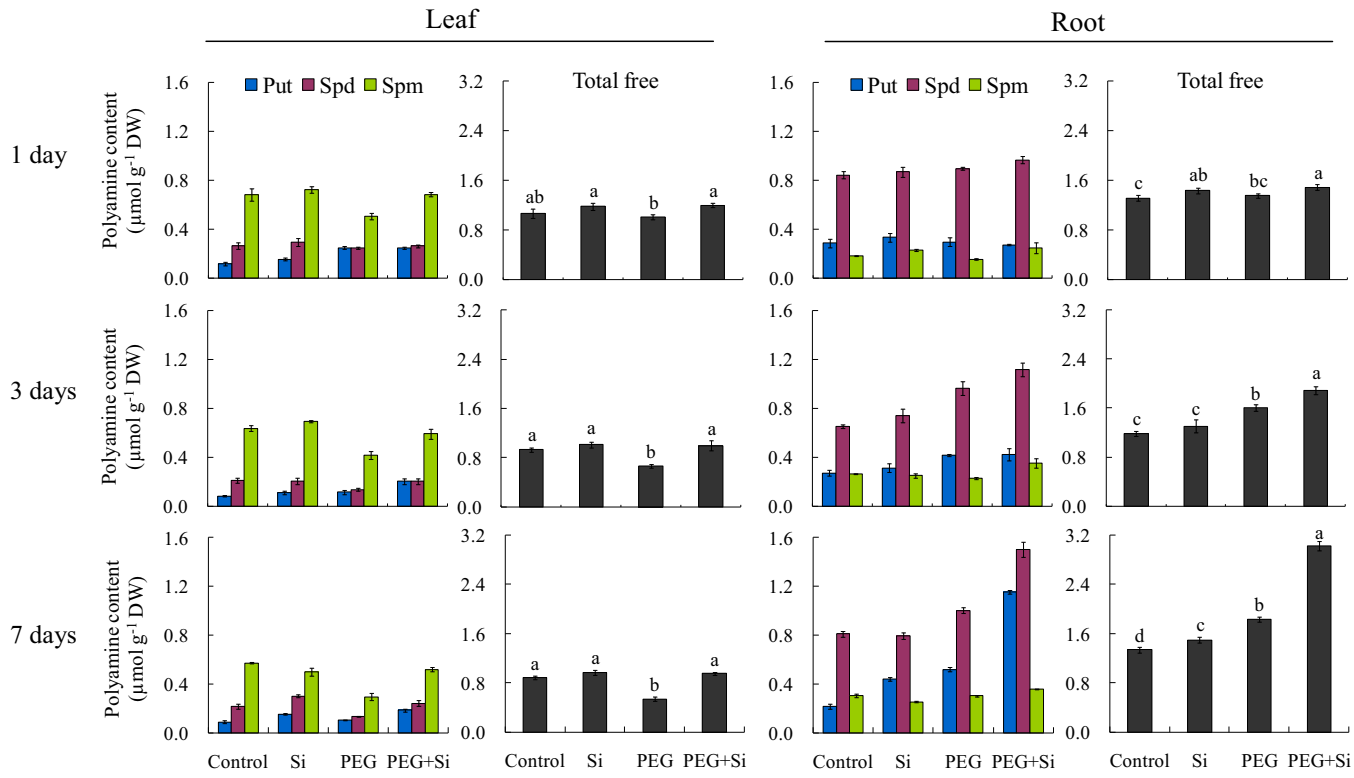

Fig. 5. Effects of silicon (Si, 0.83 mM) on polyamines (Put, Spd and Spm) contents in leaf and root of sorghum plants grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after one, three and seven days of treatment. Values are the means of three replicates ±SE. Different letters indicate statistically significant differences at $P < 0.05$.

3. Discussion

3.1. Silicon retarded leaf senescence and enhanced root water uptake under drought stress

To minimize injury due to water scarcity, plants have developed multiple strategies by which they resist or avoid drought stress. These mechanisms include adjusting the stomatal closures to reduce transpiration; reducing leaf and stem growth rates; maintaining or increasing root and shoot hydraulic conductance; synthesizing osmotic solutes that are involved in the maintenance of cell turgor; and synthesizing antioxidant proteins to prevent chlorophyll disaggregation (Wilkinson and Davies, 2010). In this study, plant dry weights, chlorophyll levels and photosynthetic rates stayed higher in silicon-treated plants than in untreated plants, indicating that silicon application shows the processes of leaf retardance and growth inhibition that are otherwise induced by drought stress. In general, reducing water loss from the leaves and/or increasing water uptake from the roots are essential goals of most plants' drought resistance strategies. In the current study, the transpiration rates and leaf areas of plants treated with silicon were higher than those of untreated plants, though plants treated with silicon also maintained a higher RWC than untreated plants did. Thus, the results indicated that silicon moderates plant water status through improving root water uptake under drought stress.

3.2. Promotion of PAs and inhibition of ACC synthesis are involved in silicon-induced retardance of leaf senescence

Changes in PAs and ethylene accumulation are a common response of plants to lack of water and are considered to be correlated with plant drought resistance. The role of PAs in improving drought resistance has been examined from several perspectives, focusing on such as PA functions as improving antioxidant ability (Shi et al., 2010); working as stress-signaling regulators (Kasukabe et al., 2004); inducing stomatal closure (Liu et al., 2000); and improving leaf water status (Alcázar et al., 2010a). In the present study, Si application resulted in the upregulation of PA synthesis-related genes, meanwhile, PA levels in plants tissues were significantly under drought stress, including both free and conjugated PAs (Figs. 5 and 7, Supplementary Table 1). It has been established that a decline in PAs leads to a decrease in chlorophyll concentration and increased rated of leaf senescence, while an increase in PAs prevents chlorophyll loss and thus delays leaf senescence under stress condition (Shi et al., 2010; Capell et al., 2004; Alcázar et al., 2010b). In this study, the chlorophyll concentrations were found to be significantly increased by silicon under drought stress (Fig. 3). PAs levels, meanwhile, were also increased, indicating that PAs may play an important role in the maintenance of chlorophyll concentration and the retardance of leaf senescence, and thus than PSs may participate in silicon-induced improvement of drought resistance. Moreover, it is worth mentioning here that, in contrast to previous finding in maize (Yin et al., 2013), although the RWC was high in silicon-treated plants, the stomatal conductance was also high in those plants, showing that the alleviation water stress was not due to the regulation of stomata closure by increased PAs in this study.

Ethylene is usually considered a senescing hormone in plants, and inhibition of ethylene generation retards leaf senescence (John et al., 1995). In classical terrestrial plants, ethylene and PA pathways are considered to be in competition with each other (Pandey et al., 2000). Therefore, the balance between PAs and ethylene will influence leaf senescence. ACC, the precursor of ethylene, was investigated instead of ethylene in this study. Silicon application

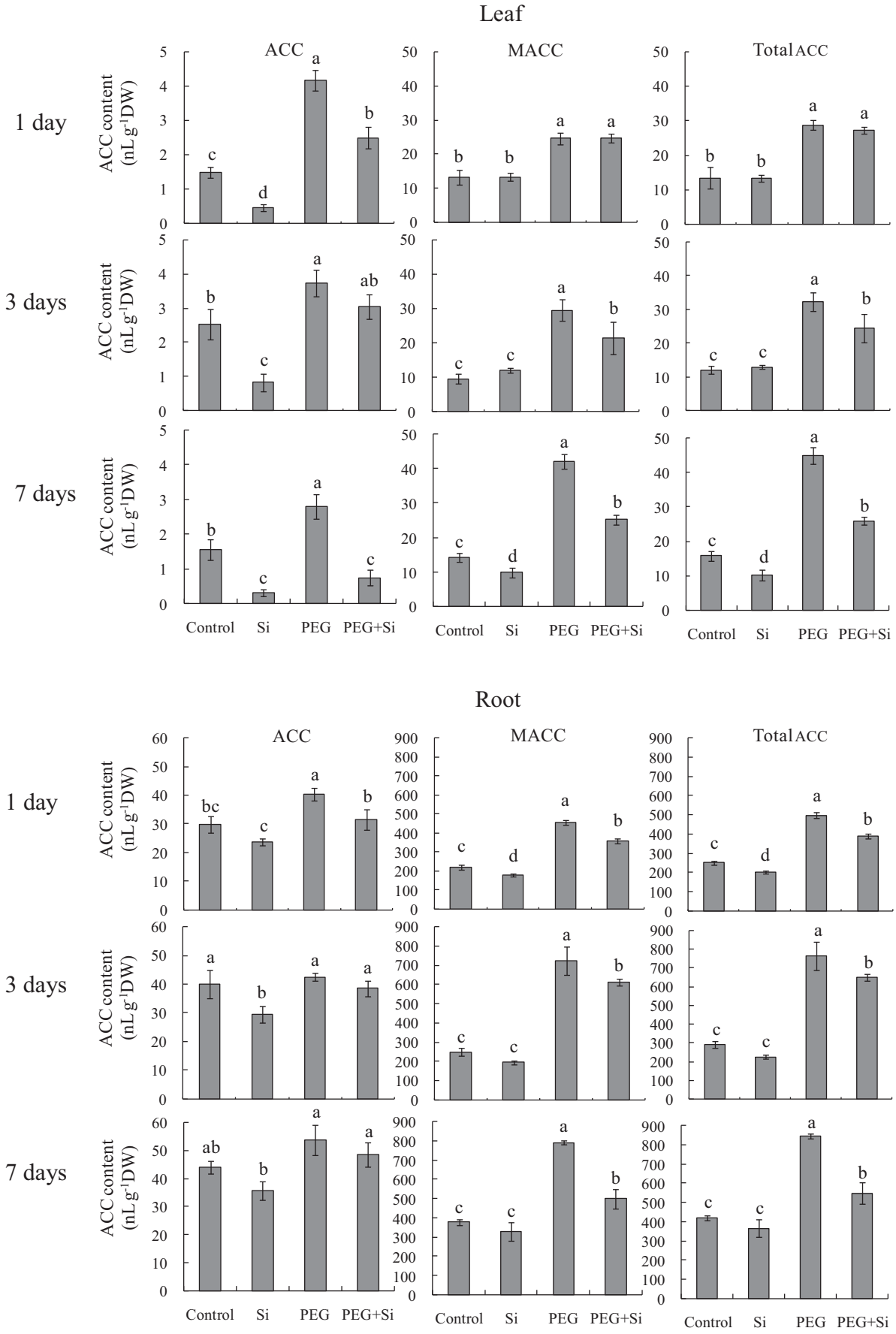

Fig. 6. Effects of silicon (Si, 0.83 mM) on ACC and MACC contents in leaf and root of sorghum plants grown under control and PEG-simulated drought stress (10% PEG). All parameters were measured after one, three and seven days of treatment. Values are the means of three replicates ±SE. Different letters in one measure indicate statistically significant differences at $P < 0.05$.

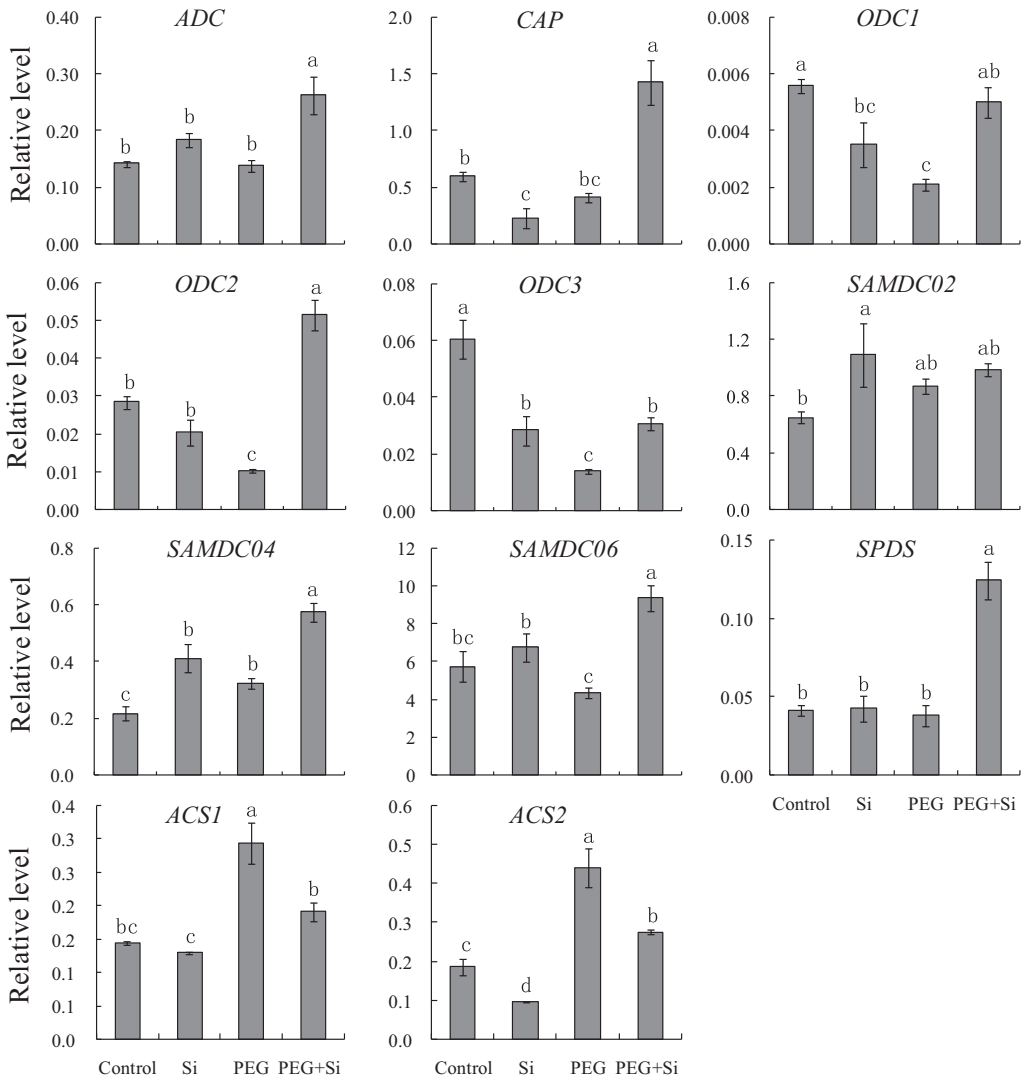

Fig. 7. Effects of silicon (Si, 0.83 mM) on relative expression level of polyamine and ethylene biosynthesis-related genes encoding arginine decarboxylase (*ADC*), N-carbamoylputrescine amidohydrolase (*CAP*), ornithine decarboxylase (*ODC1, ODC2, ODC3*), S-adenosyl-L-methionine-decarboxylase (*SAMDC02, SAMDC04, SAMDC06*), spermidine synthase (*SPDS*) and 1-aminocyclopropane-1-carboxylic acid synthase (*ACS1, ACS2*) in root of sorghum grown under control and PEG-simulated drought stress (10% PEG) for 24 h. Relative expressions were analyzed by real-time qPCR and are normalized to the host *Actin1* gene. Values are the means of three replicates ±SE. Different letters indicate statistically significant differences at $P < 0.05$.

enhanced the Spd and Spm levels, and decreased the ACC levels under drought stress, indicating that silicon changed the balance between PAs and ethylene to favor PA synthesis. Furthermore, the transcript levels of the S-adenosyl-L-methionine decarboxylase (*SAMDC*) gene, which encodes a key enzyme in PAs and ACC biosynthesis (Alcázar et al., 2010b), were markedly increased by silicon under drought stress, indicating that silicon shifted the utilization of S-adenosyl-L-methionine (SAM, the same precursor of both ACC and Spd) in favor of PA biosynthesis. Meanwhile, the chlorophyll concentration and photosynthetic rate were kept at higher levels in silicon-treated plants than in untreated plants, suggesting that silicon retarded leaf senescence by affecting the balance between PAs and ACC synthesis.

3.3. Changes in the PA-ethylene balance are involved in the silicon-induced increase in root water uptake

In roots, water uptake capacity mainly depends on the driving force, root surface, root anatomy and root hydraulic conductivity (Steudle, 2000; Vandeleur et al., 2009; Sutka et al., 2011). Under normal conditions, the driving force originates from the hydrostatic pressure gradient and the osmotic gradient; under drought stress, on the other hand, the dominating driving force for water uptake is hydrostatic pressure, and the osmotic gradient may be also conducive to water uptake (Steudle, 2000; Javot and Maurel, 2002). In the present study, the concentrations of some osmotic solutes, such as soluble sugar and polyamines, were increased by silicon under drought stress, while those of others, such as proline, were significantly decreased. As a result, the osmotic potential was not decreased, indicating that the osmotic gradient does not contribute to the silicon-mediated enhancement of root water uptake (Fig. 4, Table 1).

Upon long-term (>three days) exposure to drought stress, roots can respond with changes in root surface and anatomical structures, which in turn cause profound changes in their water uptake (Javot and Maurel, 2002). In this study, root weight and the ratio of root/shoot were increased by silicon after seven days treatment (Fig 1), suggesting that the silicon-mediated

modification of root morphology may account for the increased water uptake ability. It is well known that both PAs and ethylene are involved in regulating the phenotypic plasticity of root architecture. PAs positively regulate primary and lateral root, whereas ethylene inhibits lateral root elongation and promotes root hair development (Tang and Newton, 2005; Lewis et al., 2011; Saini et al., 2013). In this study, we did not distinguish and quantify the primary, lateral and root hair, but the root weight and the ratio of root/shoot was higher in silicon-treated plants than in untreated plants under drought stress. A positive correlation was found between root weight and PA levels, and a negative correlation was found between root weight and ACC levels, suggesting that silicon may regulate root morphogenesis through mediating the balance of PA and ethylene accumulation under stress conditions.

3.4. A simple model describing how PAs and ethylene are involved in silicon-induced drought resistance

This study showed that silicon could mediate PA and ACC synthesis and could thereby enhance drought tolerance. Combining the present finding with those of previous research, we developed a model describing one way in which PAs and ethylene could be involved in silicon-mediated drought resistance. (1) Under drought stress, silicon upregulates the transcription levels of PA synthesis genes, especially *SAMDC*, leading to an increase in the synthesis and accumulation of PAs and an inhibition of ACC production; (2) in leaf, high levels of PAs and low levels of ethylene could promote the maintenance of chlorophyll contents, leading to the retardance of leaf senescence and the maintenance of a high photosynthetic rate; (3) in root, high PA levels, as well as low ethylene levels may modulate root architecture, promoting root development and enhancing the ratio of root/shoot; the higher root/shoot ratio could increase the plant's water uptake capacity, permitting it to maintain its water status; The overall effect of silicon in this model is that the resulting high levels of PAs and low levels of ethylene retarded leaf senesces and promote root water uptake, resulting in the improvement of drought resistance. The results of this study suggest that silicon could increase drought resistance through regulating the important metabolic processes in plants.

4. Materials and methods

4.1. Plant material and growth conditions

Seeds of sorghum (*Sorghum bicolor* L. Moench cv. Gadambalia) were germinated in a Petri dish with a wet filter paper for three days at 28 °C in an incubator under dark conditions. After three days' germination, seedlings were transplanted into plastic containers with 20-L of a Hoagland solution and then placed in an environmentally controlled growth chamber. Hoagland solution was changed every three days. The growth chamber was set to a 14/10 h day/night cycle at a day/night temperature of 28/23 °C with 40% relative humidity. The amount of photosynthetically active radiation (PAR) of upper plant was about 800 μmol m^{-2} s^{-1}.

4.2. Drought and silicon treatment

After 17days of growth, uniform plants were selected for drought and silicon treatment. The silicon solution was prepared according to the method of Sonobe et al. (Sonobe et al., 2009). 10% PEG-6000 in Hoagland solution was used to simulate medial drought stress. Sorghum seedlings were exposed to 0 mM (control) or 10% PEG and combined with 0 mM or 0.83 mM H_2SiO_3. Each treatment was continued for seven days. The culture solution was renewed every three days, and the pH was adjusted every day to 6.0 with 0.1 M HCl or KOH.

4.3. Dry weight and leaf area

After seven days of treatment, plants were harvested and separated into leaf, shoot and root. The leaf area was measured with a leaf area meter (AAL-410, Hayashi Denko Inc., Tokyo, Japan). Each sample was dried and the dry weight was measured. Each treatment was administered to six 6 replicates.

4.4. Photosynthetic rate, transpiration rate and stomatal conductance

Before drought treatment, the upper expanded leaf was marked. Net photosynthetic rate, transpiration rate, and stomatal conductance of the marked leaf were measured between 9:00 and 11:00 after treated for one, three and seven days using a portable photosynthesis system (LI-6400, LI-COR., Lincoln, NE, USA). The photo flux density was set at 1000 μmol m^{-2} s^{-1} PAR, and the flow rate through the chamber was 500 mL s^{-1}. Six plants were measured in each treatment. Meanwhile, part plants were washed, sampled (the upper three fully expanded leaves and 3 cm root tips) and frozen into liquid nitrogen for measurement of osmotic potential, PA and ACC concentrations, and gene transcript levels.

4.5. Leaf relative water content (RWC) and leaf chlorophyll concentration

After one, three and seven days of treatment, ten leaf discs (each 9 mm in diameter) from fully expanded leaves were weighed immediately for fresh weight (FW). The discs were floated in distilled water for 6 h, then dried with filter paper and weighed for total weight (TW). Dry weight (DW) was measured after drying the discs at 70 °C for 24 h. The relative water content was calculated as: $RWC = (FW - DW)/(TW - DW) \times 100\%$. The chlorophyll was extracted and analyzed according to Knudson et al. (Knudson et al., 1997).

4.6. Measurement of leaf and root osmotic potential

The upper three fully expanded leaves and 3 cm root tips were collected and frozen into liquid nitrogen for measurement of osmotic potential. The osmotic potential was measured according to the method of Yin et al. (Yin et al., 2013) using a dew point microvolt meter (Model 5600, Wescor, Logan, UT, USA).

4.7. Measurement of osmotic solute contents determination

The proline content was determined according to the method of Bates et al. (Bates et al., 1973). A plant sample weighting 0.5 g was extracted with 5 mL sulfosalicylic acid (3%). The 1 mL extraction volume was mixed with 1 mL of a mixture of glacial acetic acid and 6 M orthophosphoric acid (3:2, v/v) and 50 mg ninhydrin. After incubation for 1 h at 100 °C, the tube was cooled down to room temperature and 5 mL toluene was added, and the absorbance of the upper phase was spectrophotometrically determined at 520 nm. The proline concentration was determined using a standard curve. The soluble sugar was measured according to the method of Yin et al. (Yin et al., 2013) using the Shimadzu sugar analysis system (HPLC, Shimadzu, Kyoto, Japan).

Table 2
Genes and oligonucleotides used in the real-time quantitative PCR experiment.

Genes	Loci	Encoded proteins	Primers (5′–3′)
Actin1	Sb01g010030	Actin	TGTTCCCTGGGATTGCTG
			GCCGGACTCATCGTACTCA
SAMDC02	Sb02g025110	*S*-adenosyl-Met	TGTGGGTACTCGATGAATG
		decarboxylase	ACGGCAACTGAGAACTCC
SAMDC04	Sb04g025720	*S*-adenosyl-Met	CTGTTCATGGCCCTGCTT
		decarboxylase	CCCGCTCACCGAAGATAG
SAMDC06	Sb06g021540	*S*-adenosyl-Met	TTTTGACTTTGAACCCTGTG
		decarboxylase	AGCCCATAACCTCATAACT
ODC1	Sb02g01570	Ornithine	GGCCTCTACGGCTCGCTCAA
		decarboxylase	CCAGTCGCCCACGCTCATCT
ODC2	Sb02g031560	Ornithine	TCTACGGCTCGCTGAACTGC
		decarboxylase	GGTAATCGGTCACCATCCTGTC
ODC3	Sb05g021830	Ornithine	CTACGGCTCGCTCAACAACG
		decarboxylase	CGGTCACCACCTGGTCCTG
ADC	Sb10g002070	Arginine	CGGGGAGAAGGGCAAGT
		decarboxylase	CTGGGAGCCAATGTGGAAGT
CPA	Sb04g021790	N-carbamoylputrescine	CTTGGTCCCTCTAGTTGC
		amidohydrolase	TTCCTCGTCTTTGTCGTT
ACS1	Sb06g026160	1-aminocyclopropane-	GCCAACTTCCAGGACTACCACG
		1-carboxylic acid	GAGGCAGAAGGCCAGAGTGT
		synthase	
ACS2	Sb01g009450	1-aminocyclopropane-	GAGCTTCGGCCTGGTGTCGT
		1-carboxylic acid	TGTTGACCCAGCAGAAGAGCC
		synthase	
SPDS	Sb10g020570	Spermidine synthase	ATCGCCTTACCAAGAAAT
			CACCTCCAACAACCAAAA

4.8. PAs quantification

The PA contents were analyzed by means of high performance liquid chromatography (HPLC: LC-10A, Shimadzu, Kyoto, Japan) according to the method (Flores and Galston, 1982). Plant samples each weighting 1 g were ground and homogenized in 5 mL of 5% $HClO_4$ and extracted on a shaker at room temperature overnight. After centrifuging, the extracted supernatant was used for measurement of the free-type PAs. To measure the combined-type PAs (PA conjugates with macromolecules), the residues of plant extracts were washed with 5% $HClO_4$, then subjected to hydrolysis in 6 M HCl at 110 °C for 15 h. The filtrated hydrolyzates were allowed to evaporate to dryness, and the residues were dissolved in 5% $HClO_4$ for the measurement of combined-type of PAs.

4.9. ACC measurements

The ACC extraction and chemical conversion to ethylene were performed according to the method of Concepcion et al. (1979). Briefly, 0.5 g (fresh weight) plant samples were extracted with 2 mL of 85% ethanol. After the extracted supernatant was dried, 1 mL of chloroform and 1 mL of distilled water were added separately. Then the aqueous phase was collected and $HgCl_2$ and NaOCl/saturated NaOH (2:1) were added. After incubation and intermittent agitation, 2 mL of airspace was extracted with a gas syringe and injected into a gas chromatograph (G-3000; Hitachi, Tokyo, Japan). For malony-ACC (MACC, conjugated ACC) measurement, the aqueous phase was subjected to hydrolysis in 6 M HCl at 100 °C for 3 h. The amount of MACC was calculated by subtracting the amount of ACC from that of total ACC.

4.10. Real-time qPCR assay

Total RNA was extracted using an RNeasy® Plant Mini Kit (Qiagen), and treated with Recombinant DNase I (RNase-free; Takara). Reverse transcription was performed with an iScript™ cDNA Synthesis Kit (Bio-Rad). The iQ™ SYBR® Green Supermix (Bio-Rad) was used for the RT-qPCR on a LightCycler 480 II (Roche). The specific primers used are shown in Table 2. To further confirm that the single peaks in the melting analysis corresponded to a unique amplification product of the correct size, the PCR reactions were run on 1% agarose gel, and the single peak for each pair of primers was confirmed. The expression levels of target genes were normalized to that of the internal control gene *Actin1* using the $2^{\Delta Ct}$ methods. Each treatment includes three replications and each replication includes two technical replications.

4.11. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS version 8.0) software. Differences between the means were compared by the Tukey–Kramer test ($P < 0.05$). Each experiment was repeated at least twice.

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Author contribution

Lina Yin conducted the experiment, collected and analyzed the data, and prepared the draft. Shiwen Wang planned the experiment and revised the manuscript. Peng Liu, Wenhua Wang and Dan Cao helped measurements of polyamine contents and osmotic potential. Xiping Deng and Suiqi Zhang helped in drafting the manuscript and interpretation of the results.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2014.04.014.

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