

The regulatory role of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat leaves in field drought conditions

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Abstract The effects of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat (*Triticum aestivum* L.) leaves were investigated in field drought conditions. Silicon application improved the leaf relative water content and water potential under drought. The leaf net photosynthetic rate and stomatal conductance were significantly decreased between 7:30 and 17:30 under drought, whereas silicon application increased the leaf net photosynthetic rate between 7:30 and 15:30 with an exception at 9:30. Silicon application also increased the leaf stomatal conductance at 13:30 and 17:30 under drought. The leaf transpiration rate was decreased by drought but it was increased by silicon from 13:30 to 17:30. The intercellular CO₂ concentration was increased at 7:30 under drought, while it was decreased most of the time from midday to the afternoon. The leaf stomatal limitation was increased under drought from 11:30 to 17:30, whereas it was intermediate in silicon treated plants. The instantaneous water use efficiency was significantly increased by silicon application at 7:30 under drought. Silicon application slightly decreased the activity of ribulose-1, 5-bisphosphate carboxylase, but it increased the activity of phosphoenolpyruvate carboxylase and the concentration of

inorganic phosphorus under drought. These results suggest that silicon could improve the photosynthetic ability of wheat in field drought conditions, and both stomatal and non-stomatal factors were involved in the regulation. In the early morning (at 7:30), the non-stomatal factor was the main contributor; 9:30 was a turning point, after which the stomatal factor was the main contributor.

Keywords Drought · Photosynthetic carboxylation activity · Photosynthetic gas exchange · Silicon · Wheat (*Triticum aestivum* L.)

Introduction

The yield of wheat is affected by diversely adverse environment. Drought is one of the main causes of severe yield reductions. According to the prediction of current climate change models, the frequency and severity of drought will increase in several regions around the world (Shen et al. 2010). Increasing drought tolerance of wheat is a feasible way to overcome the drought problems.

Silicon is the second most abundant mineral element in the soil after oxygen and comprises 28% of the earth's crust (Epstein 1994). It is still hard to prove that silicon is an essential element for all higher plants, since it is ubiquitous. However, the beneficial effects of silicon have been observed in many plants, especially when they are subjected to environmental stresses (Liang et al. 2007).

There have been some research accumulation of silicon effects on drought tolerance of plants. Agarie et al. (1998) showed that silicon addition decreased the transpiration and membrane permeability of rice under water deficit induced by polyethylene glycerol. Hattori et al. (2001) found that silicon application increased the relative water

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content and dry mass of sorghum (*Sorghum bicolor* Moench), and their later studies suggested that the improvement of drought tolerance by silicon may be associated with enhancement of water uptake (Hattori et al. 2005, 2007). Silicon is also shown to be associated with osmotic adjustment (Crusciol et al. 2009). Our previous results suggested that the improvement of drought tolerance of plants by silicon was associated with the increase of antioxidant defense abilities (Gong et al. 2005; Pei et al. 2010). However, these studies were performed with potted seedlings or polyethylene glycol-stressed seedlings, in which the experimental duration was relatively short. However, little work has been done in the field. Under short-term water stress, the stress is usually abrupt and the plant responses are different from those in gradual water stress, such as field conditions (Zhu et al. 2005).

Photosynthesis is one of the first physiological processes affected by drought (Signarbieux and Feller 2011, and references therein). The positive effects of silicon on photosynthesis under drought have been observed in several plants. Hattori et al. (2005) reported that the photosynthetic rate and stomatal conductance of silicon-applied sorghum plants grown in pots were higher compared with those of plants without silicon application under drought. Lobato et al. (2009) reported the improvement of photosynthesis of pepper with applied silicon under water deficit and showed that the leaf relative water content, stomatal conductance, chlorophylls were maintained at higher levels compared with plants without applied silicon. Increased photosynthetic rate, transpiration, F_v/F_0 and F_v/F_m were also found in rice applied with silicon under drought (Chen et al. 2011). However, diurnal photosynthetic gas exchange as affected by silicon application in the field have not been investigated, nor were the silicon effects on the activities of photosynthetic carboxylases clear.

In this work, we report the regulative role of silicon on plant water relations, photosynthetic gas exchange and carboxylase activities of wheat in field drought conditions. The study could contribute to the understanding of silicon-induced improvement in plant drought tolerance in field conditions.

Materials and methods

Plant materials and experiment design

Plants of wheat (*Triticum aestivum* L. cv. Longchun 8139) were grown in the arid region (located in Lanzhou, Gansu Province, China). Before sowing, the field was irrigated with tap water and applied with farmhouse fertilizer. Before silicon application, the soil properties were as follows: pH 8.2, organic matter 2.9%, total nitrogen 0.1%,

available phosphorus 0.074 g kg^{-1} , available potassium 0.12 g kg^{-1} , slowly available potassium 0.93 g kg^{-1} , and available silicon 0.28 g kg^{-1} . Every treatment had three replications, each of which was $2.0 \times 1.5 \text{ m}$ in size. Drought treatment was applied by withholding irrigation and movable rainout shelters were used to shield the plants from rain after sowing, while the control plants were regularly well watered. The photosynthetic gas exchange of the recent fully expanded leaves was monitored 59 days after seedling emergence. For determination of carboxylase activities and inorganic phosphorus content, the leaves were collected 69 days after seedling emergence at 9 am and immediately frozen in liquid N_2 until analysis. The soil moisture content and plant water status were measured on both days.

Soil moisture content and leaf water status

The soil moisture content was measured gravimetrically based on soil samples after drying at $105\text{--}110^\circ\text{C}$. The relative water content (RWC) was determined using the following equation: $\text{RWC} (\%) = (\text{FW} - \text{DW})/(\text{SW} - \text{DW}) \times 100$, where FW is fresh weight, DW is dry weight, and SW is saturated weight in water. The dry matter of leaves was determined after drying for 72 h at 80°C . The leaf water potential was measured using the pressure chamber method as previously used (Gong et al. 2005).

Photosynthetic gas exchange

Leaf gas exchange parameters such as net CO_2 assimilation rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were assayed with a CI-301PS photosynthesis system (CID Inc., USA). Instantaneous water use efficiency (WUE) was calculated as the ratio of P_N to E . Stomatal limitation (L_s) was calculated according the following equation: $L_s = 1 - C_i/C_a$, where C_a is the ambient CO_2 concentration.

Activities of RUBPC and PEPC

Enzyme extraction for determination of ribulose-1, 5-bisphosphate carboxylase (RUBPC) and phosphoenolpyruvate carboxylase (PEPC) activities was carried out according to Wei et al. (2003).

The RUBPC activity was determined according to Wang et al. (2001) with slight modifications. The reaction mixture (1 mL) contained 50 mmol L^{-1} Tricine–NaOH (pH 7.9), 10 mmol L^{-1} KCl, 1 mmol L^{-1} EDTA, 2 mmol L^{-1} dithiothreitol, 0.2 mmol L^{-1} NADH, 5 mmol L^{-1} phosphocreatine, two units of creatine phosphokinase, four units of glyceraldehyde 3-P dehydrogenase and 3-P glycerate kinase, and enzyme extract. The mixture was incubated at

25°C for 5 min. Reactions were initiated by addition of 0.5 mmol L⁻¹ of RUBP.

The PEPC activity was determined according to Wei et al. (2003).

Protein content was measured according to Bradford's method (1976).

Inorganic phosphorus

Inorganic phosphorus in the leaves was extracted in 2% (w/v) trichloroacetic acid (g:mL = 1:10) with a mortar and pestle. The homogenates were centrifuged at 3,100g for 25 min. Inorganic phosphorus in the extract was determined following the method of Xu et al. (1994).

Statistical analysis

One-way ANOVA was used to analyze the data and comparisons were made at $P < 0.05$ using SPSS 15.0 for windows (SPSS, Chicago, IL, USA).

Results and discussion

The control field was maintained with soil moisture content of around 15% at both day 59 and day 69. At day 59, the soil moisture contents in the drought fields decreased to about 3, 7, 7.5 and 10% at depth of 10, 30, 60 and 100 cm, respectively. With drought progressing, the soil moisture contents at day 69 decreased further to about 2.5, 6.5, 7 and 9%, respectively. There was no obvious difference in the presence or absence of applied silicon under drought. The relative water content and water potential of wheat leaves were decreased under drought (Table 1). However, the silicon-applied plants could maintain better water status than those without added silicon under drought (Table 1). These indicate that silicon application improved the water

status of wheat in field drought conditions, which are consistent with the observations in pot and hydroponical conditions (Gong et al. 2005; Pei et al. 2010).

Silicon could improve plant photosynthesis under drought (Hattori et al. 2005). Sonobe et al. (2009) investigated the diurnal changes of gas exchange in sorghum grown hydroponically and found that silicon-applied plants had higher stomatal conductance (g_s), net CO₂ assimilation rate (P_N), and transpiration rate (E) than untreated plants under water stress. In this study, the photosynthetically active radiation and air temperature shared similar diurnal trends with maximum values at around 11:30 (Fig. 1A). The leaf P_N was decreased between 7:30 and 17:30 under drought, and silicon application alleviated the decrease between 7:30 and 15:30, except at 9:30 (Fig. 1B). By and large, the changes of g_s under drought showed similar trends with those of P_N (Fig. 1C). The leaf transpiration rate was decreased under drought, and it was increased by silicon application from 13:30 to 17:30 (Fig. 1D).

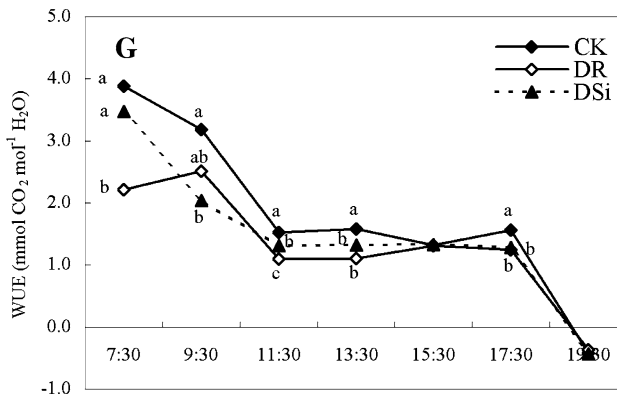
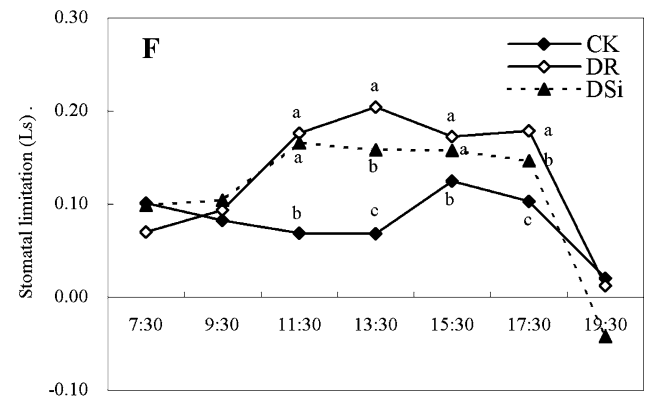
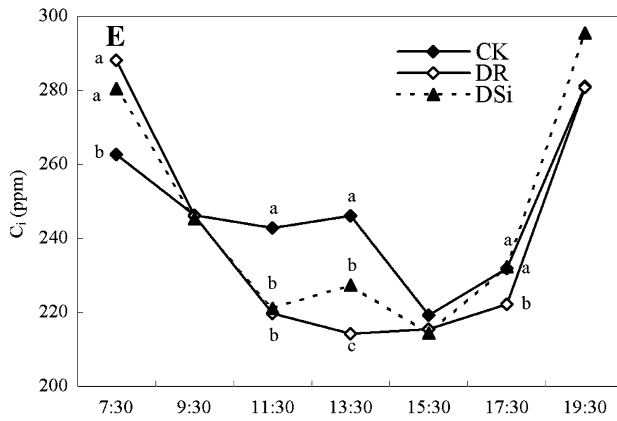
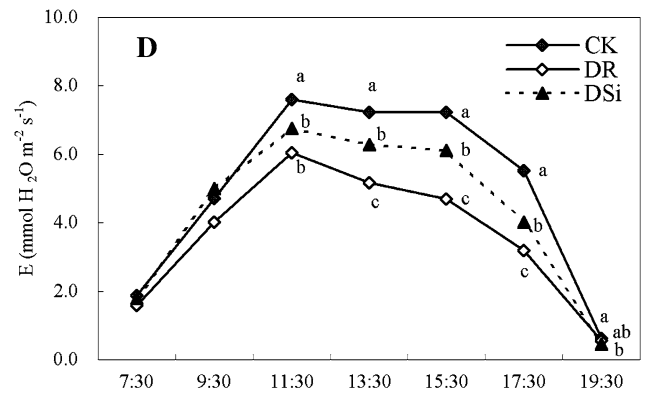
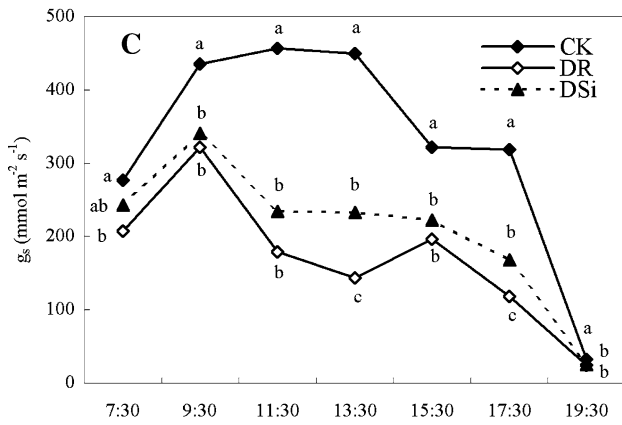
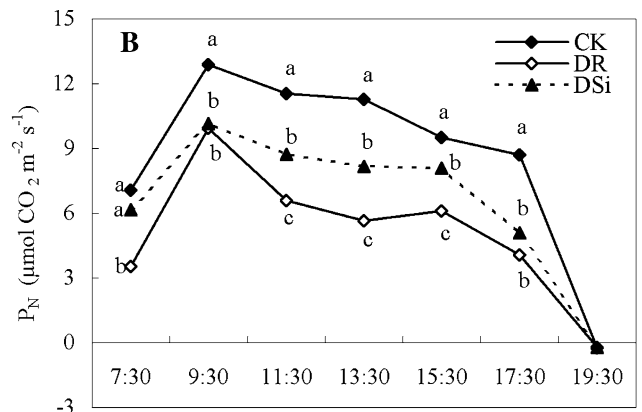
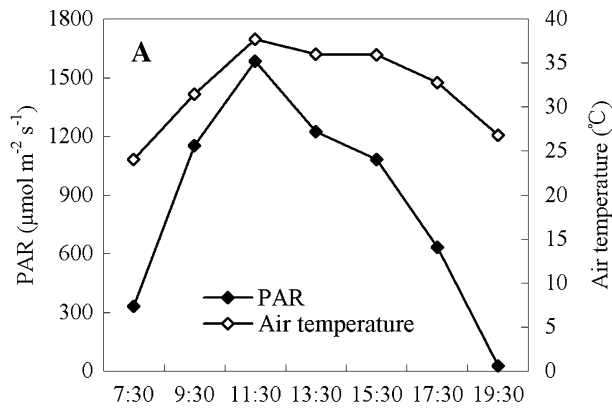
The beneficial effect of silicon on plant growth and water status has been linked to its effects on transpiration. It is suggested that silicon could decrease cuticular and stomatal transpiration (Yoshida 1965; Gao et al. 2006). Liang et al. (2008) proposed that accumulation of polar monosilicic acid and/or polymerized silicic acid in epidermal cell walls may form H bonds between H₂O and SiO₂·*n*H₂O, therefore making water molecules less easily to escape from leaf surface. However, in this study, both stomatal conductance and transpiration rate of wheat leaves were increased by silicon application under drought (Fig. 1C, D), which are in accordance with previous findings in sorghum (Hattori et al. 2005) and pepper (Lobato et al. 2009). Obviously, physical blockage cannot explain the silicon-mediated increase in transpiration and stomatal conductance in this study (Fig. 1C, D). In our previous pot experiment, we also found that silicon addition increased leaf transpiration and stomatal conductance of drought-

Table 1 Effect of silicon on the relative water content, water potential, carboxylation activities and inorganic phosphorus of wheat leaves under drought

	Day 59			Day 69		
	CK	DR	DSi	CK	DR	DSi
RWC (%)	94.3 ^a	89.6 ^c	93.0 ^b	94.3 ^a	83.7 ^c	86.1 ^b
WP (MPa)	-1.10 ^a	-1.95 ^c	-1.74 ^b	-1.35 ^a	-2.42 ^c	-2.16 ^b
RUBPC (nmol mg ⁻¹ protein min ⁻¹)	ND	ND	ND	138.5 ^a	139.9 ^a	128.6 ^b
PEPC (nmol mg ⁻¹ protein min ⁻¹)	ND	ND	ND	54.5 ^a	45.1 ^b	55.1 ^a
PEPC/RUBPC	ND	ND	ND	0.39 ^b	0.32 ^c	0.43 ^a
Inorganic phosphorus (μmol g ⁻¹ dw)	ND	ND	ND	42.7 ^a	34.5 ^b	43.7 ^a

CK control, DR drought, DSi drought plus silicon, RWC relative water content, WP water potential, RUBPC ribulose-1, 5-bisphosphate carboxylase, PEPC phosphoenolpyruvate carboxylase, ND not determined

Mean values followed by the same letters are not significantly different at $P = 0.05$. $n = 6$ for WP, 3 for the others



◀ **Fig. 1** Effect of silicon on the leaf net CO₂ assimilation rate (P_N), stomatal conductance (g_s), transpiration rate (E), intercellular CO₂ concentration (C_i), stomatal limitation (L_s) and water use efficiency (WUE) of wheat under drought. Water use efficiency (WUE) was calculated as the ratio of P_N/E . The gas exchange was assayed with a CI-301 PS photosynthesis system (CID Inc., USA). Stomatal limitation (L_s) was calculated according the following equation: $L_s = 1 - C_i/C_a$, where C_a is ambient CO₂ concentration. These measurements were made on the recent fully expanded leaves for each treatment ($n = 10$). *CK* control, *DR* drought, *DSi* drought plus silicon, *PAR* photosynthetically active radiation

stressed wheat (Gong et al. 2005). Hattori et al. (2007) found that silicon could facilitate water uptake and transportation under drought. Therefore, we speculate that the improvement of plant water status by silicon application in the present field drought conditions might be due to enhanced root water uptake, which needs further investigation.

In this study, compared with the control, the C_i of stressed plants was increased at 7:30, while it was decreased most of the time from midday to the afternoon. Silicon application increased the leaf C_i of stressed plants at 13:30 and 17:30 (Fig. 1E). There was no significant difference in the leaf stomatal limitation (L_s) between the control and stressed plants in the morning and evening (Fig. 1F). However, it markedly increased from 11:30 to 17:30 under drought. At 13:30 and 17:30, silicon-applied plants showed significantly lower L_s than untreated plants under drought.

The water use efficiency (WUE) for plants is very important, especially when they are subjected to drought. In this study, drought decreased the WUE, which was improved by silicon application in the morning (except at 9:30) (Fig. 1G). Increased WUE by silicon was also observed in wheat grown hydroponically under water stress (Ding et al. 2007). However, silicon did not affect the WUE in the afternoon, which might be due to the increase in transpiration (Fig. 1D).

The inhibition of plant photosynthesis by drought may be caused by stomatal and non-stomatal factors (Yu et al. 2009). From 11:30 to 17:30, the g_s and C_i were decreased under drought, while the L_s was significantly increased (Fig. 1C, E, F), indicating that the photosynthesis depression was mainly due to stomatal factors. However, before 9:30, although the g_s was decreased under drought, the C_i was even increased and the L_s value was not changed (Fig. 1C, E, F), suggesting the involvement of non-stomatal factors in photosynthetic inhibition. Therefore, both stomatal and non-stomatal factors were involved in silicon-induced improvement in photosynthesis in the present field drought conditions.

Photosynthetic carboxylation activity and inorganic phosphorus supply are two important non-stomatal factors for inhibition of photosynthesis. In this study, the leaf

RUBPC activity was not changed in the absence of added silicon under drought (Table 1). However, it was slightly decreased in silicon-applied plants. The PEPC activity was decreased by drought, while it was maintained at the control level in silicon-applied plants. As a result, the ratio of RUBPC/PEPC was decreased under drought, whereas it was increased in silicon-applied plants (Table 1). Adatia and Besford (1986) also reported that silicon addition could increase the chlorophyll content and RUBPC activity in cucumber grown hydroponically. In this work, the increases in PEPC activity and PEPC/RUBPC ratio suggest that the C₄ photosynthetic ability of wheat was obviously increased by silicon application under drought. We also speculate that the improved WUE by silicon at 7:30 and 11:30 in this study was mainly due to improved photosynthesis, which was associated with the increased C₄ carboxylase activity (Table 1).

Inorganic phosphorus is an important component and affects ATP synthesis in the chloroplast (dos Santos et al. 2006). In this study, silicon application increased the inorganic phosphorus concentration in the leaves under drought (Table 1). The increase in inorganic phosphorus concentration might facilitate ATP synthesis, which is needed in the cycle of CO₂ assimilation (Doubnerová and Ryšlavá 2011). dos Santos et al. (2006) also found that foliar spray of inorganic phosphorus up-regulated the recovery of photosynthesis after drought in common bean, although the effect was genotype dependent. How did silicon influence the concentration of inorganic phosphorus in the leaves? Sistani et al. (1997) found that application of rice hull ash, which contained about 8% silicon, increased the phosphorus uptake by 7.7% in the shoot. Therefore, we speculate that in this experiment, silicon application increased phosphorus uptake in the leaves. To our knowledge, up to date, little information is available about the effect of silicon on the photosynthetic carboxylation activity and inorganic phosphorus content in the leaves under drought. These results may help to understand the mechanism for the improvement of photosynthesis by added silicon under drought.

As a whole, the results obtained here suggest that silicon application could improve the water status of plants under drought and this effect could contribute to alleviation of photosynthetic damages. Both stomatal and non-stomatal factors contribute to the silicon-induced increase in net photosynthetic rate of wheat in field drought conditions: in the early morning (at 7:30), the non-stomatal factor is the main contributor; 9:30 is a turning point, after which the stomatal factor was the main contributor. The improvement in plant water status, and increases in PEPC activity and inorganic phosphorus concentration in the leaves are among the causes for improvement of photosynthesis by silicon under drought.

Author contributions statement Kunming Chen measured the water status of plants. Haijun Gong did other measurements, analyzed data, and wrote the paper.

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