

Silicate application increases the photosynthesis and its associated metabolic activities in Kentucky bluegrass under drought stress and post-drought recovery

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Abstract Drought stress is the most pervasive threat to plant growth, which disrupts the photosynthesis and its associated metabolic activities, while silicate (Si) application may have the potential to alleviate the damaging effects of drought on plant growth. In present study, the role of Si in regulating the photosynthesis and its associated metabolic events in Kentucky bluegrass (cv. Arcadia) were investigated under drought stress. Drought stress and four levels (0, 200, 400, 800 mg L⁻¹) of Si (Na₂SiO₃·9H₂O) were imposed on 1-year-old plants removed from field and cultured under glasshouse conditions. After 20 days of drought stress, the plants were re-watered to reach soil field capacity for the examination of recovery on the second and the seventh day. The experiment was arranged in completely randomized design replicated four times. Drought stress severely decreased the photosynthesis, water use efficiency, stomatal conductance, chlorophyll contents, Rubisco activity, and Rubisco activation state in Kentucky bluegrass. Nevertheless, application of Si had a positive influence on all these attributes, particularly under stress conditions. As compared to control, Si application at 400 mg L⁻¹ recorded 78, 64, and 48 % increase in photosynthesis, Rubisco initial activity, and Rubisco total activity, respectively, at 20 days of drought. Higher

photosynthesis and higher Rubisco activity in Si-applied treatments suggest that Si may have possible (direct or indirect) role in maintenance of more active Rubisco enzyme and Rubisco activase and more stable proteins for carbon assimilation under stress conditions, which needs to be elucidated in further studies.

Keywords Drought stress · Leaf soluble protein · Photosynthesis · Rubisco activity · Silicate · Turfgrass

Introduction

Drought stress is considered one of the main causes for limiting plant growth among major abiotic factors in many areas of the world, which is progressively increasing due to global warming (Yang et al. 2010). Drought stress persuades several physiological, biochemical, and molecular processes in plants, which may facilitate their adaptations to limiting environmental conditions (Fahad et al. 2014a; Saud et al. 2014). The photosynthetic inhibition in plants upon exposure to drought stress predominantly occurred due to low CO₂ availability in response to stomatal closure (stomatal limitation) or/and the modifications of carbon assimilation (metabolic limitation) metabolism (Flexas et al. 2004). Under drought stress, stomatal closure is one of the most quick responses of plants to lessen the amount of water reduction in water-limiting environments; nevertheless, it also restricts the diffusion of CO₂ into the leaves leading to decreased net photosynthetic rate (Comic, 2000). Furthermore, along with stomatal closure, mesophyll conductance also performs a vital responsibility in limiting photosynthesis under drought stress (Fry and Huang 2004, Fahad et al. 2016), and diffusional limitations of a leaf are attributed to both of these factors.

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Drought stress severely limits photosynthetic activity is known to be associated with low carboxylation efficiency, perturbed regeneration of ribulose-1,5-bisphosphate (RuBP), and alterations in capacity of ribulose-1,5-bisphosphate carboxylase:oxygenase (Rubisco) (Parry et al. 2002, Pena-Rojas et al. 2004). Rubisco is a chloroplast-specific photosynthetic protein, accounting 40–80 % of all soluble proteins in plant leaves. Activity of Rubisco is highly mediated by several metabolites, Rubisco activase enzyme, and phosphate ions (Servaites and Geiger, 1995). Drought stress and plant species influence the Rubisco activity ranging from low to severe reduction (Parry et al. 2002) to modest or no enzyme inhibition (Pelloux et al. 2001). Some early researches have reported that the impairment of Rubisco did not inhibit photosynthesis until severe or long-term drought stress occurred (Medrano et al., 1997; Flexas et al., 2006b). In previous decades, a number of studies have been carried out mainly in annual crops to differentiate the role of stomatal and non-stomatal limitations in drought-induced inhibition of photosynthesis (Lawlor and Cornic 2002). Flexas et al. (2004) suggested that determination of maximal CO₂ assimilation rate may let the assessment of non-stomatal constraints and, hence, the degrees of drought resistance of the photosynthetic machinery.

Silicon (Si) is one of the most important and second plentiful elements on earth crust and is known to have beneficial influences on plant performance (Gong et al. 2005). It acts as a physical/mechanical barrier in plants and keenly takes part in many physiological or/and metabolic attributes. Under both biotic and abiotic stresses when plants grown in Si-deficient environment demonstrate the sign of poor growth and also influence their tolerance (Datnoff et al. 2001). However, under Si condition, plants showed high tolerance to drought condition by retaining leaf water potential, stomatal conductance, photosynthetic activity, and structure of xylem vessels under high transpiration rates (Hattori et al. 2005). All these traits are known to be used as physiological indicators for the selection of drought-tolerant plant materials (Liu et al. 2008). Si application can enhance the photosynthetic activity in plants grown under water deficit conditions by reducing the electrolyte leakage from rice leaves (Agarie et al. 1998). Furthermore, Si strongly manipulates the stomata movement and, therefore, affects transpiration rate through stomata (Gao et al. 2006). Matoh et al. (1991) stated that application of Si resulted in formation of a silica-cuticle double layer on leaf epidermal tissue, which is responsible for higher leaf water potential and induced stress tolerance under drought. It is reported that some drought-tolerant cultivars mainly from the family Gramineae can accumulated silicon in endodermal tissues (Lux et al. 2003). Hattori et al. (2005) observed that higher root growth and water transport in Si-applied sorghum were responsible for higher water flux to lowered hydraulic resistance under drought stress. However, information is still limited to clarify the mechanism of improving metabolism in

plants by adding silicon, especially for the role of silicon in rubisco activity under drought condition.

The observed climatic changes have been typically summarized by escalating atmospheric and mounting temperatures and fluctuating precipitation models likely guiding to more frequent and relentless drought occurrences (Gornall et al. 2010). About one third of the area globally is facing the drought dilemmas, and in China, this ratio is more than 47 % (Wei et al. 1997). The comprehension of how Rubisco and its related attributes respond to Si application under drought stress and whether induced alterations in Rubisco activity influence the net photosynthesis in plants is of fundamental significance to envisage possible outcomes of future climate on agriculture and natural ecosystems.

Kentucky bluegrass, a member of Gramineae family, possesses a relatively excellent adaptability to various environments, is used often to cover barren soil and with an increasing interest in the quality of life and green environment, there has been an expansion of its utility and areas of use. Though numerous researchers have investigated several features of photosynthetic limitations under drought stress and analyzed the changes in stomatal and non-stomatal factors (Hu et al. 2010); nevertheless, little is known about photosynthetic responses in Kentucky bluegrass to different levels of Si under drought stress particularly in relation to metabolic limitations. Therefore, present study was carried out in North China to ascertain the influence of Si on photosynthesis and its associated Rubisco activity and its related components in Kentucky bluegrass under drought stress. Such information will be of worth for further understanding adaptation and survival mechanisms and developing drought-resistant grass germplasm for environments with limited water resources.

Materials and methods

Plant Material and growth conditions

Sods (10-cm diameter) of Kentucky bluegrass cv. “Arcadia” were collected from turf field with a metal hollow drill on the sixth of June, 2013. Grasses were grown in polyvinyl chloride (PVC) pots (11-cm diameter and 40-cm height) filled with 3.6-kg mixture of vermiculite and loam soil (1:4 v/v; with 625 mg kg⁻¹ of Si) under glasshouse conditions. “Arcadia” is a well known, lower water and nitrogen input cultivar and can adapt to the climatic and environment in the North China. In this study, the seeds of “Arcadia” were supported by USDA (USA) in 2012. Grasses were maintained for 2 months for establishing the canopy and roots. An average daily day and night temperature was 25 ± 2 and 15 ± 2 °C, respectively. Relative humidity of 75 ± 5 %, and natural sunlight was maintained during the study. Plants were cut to 10 cm every week, watered everyday, and were fertilized at fortnightly interval

(26 N-10P-15 K, Jinzhengda Fertilizer Co. Shangdong, China) during the experimental period.

Treatments

Silicate applying treatments were imposed to the plants during 2 months cultured under glasshouse conditions before the start of drought stress. Four concentration levels of silicate ($\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$) at 0 mg L^{-1} (Si-0), 200 mg L^{-1} (Si-200), 400 mg L^{-1} (Si-400), and 800 mg L^{-1} (Si-800) were sprayed to the leaf surface on every 3 days intervals with 5 times. Treatment without silicate application and drought stress was maintained as control for comparison. Three days after of the last time silicate supply, drought stress was start given by completely withholding irrigation for 20 days. Soil water contents were monitored and measured at day 0, 10, 20, 22, and 27 by TDR200 (Spectrum, USA) combined with weighting pots. After 20 days of drought stress, the plants were re-watered to reach soil field capacity for the examination of recovery on the second and the seventh day.

Data recorded

The plants were at the four-leaf stage at the beginning of the experiment, and all leaves collected for measurement were located as the second leaf from the top of the plant. The leaves being the youngest were fully expanded. Net photosynthesis (A) and stomatal conductance (g_s) were determined using Li-6400 (Li-6400, LICOR, Inc., Lincoln, NB, USA) following the method described in Hu et al. (2010). A fully expanded leaf was taken separate from six plants in each pot at 0, 10, 20, 22, and 27 days. The leaves were placed in a leaf chamber with a built-in red and blue light source of the Li-6400, and all measurements were taken on at the level of $800 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetic photon flux density, which was the light saturation point for Kentucky bluegrass leaves. Photosynthetic pigments (chlorophyll contents) were extracted by soaking leaves (0.1 g) in dimethyl sulfoxide solution for 48 h. Absorbance of extracts was read out at 663 and 645 nm wave length in a UV-spectrophotometer (UV-T6, Shanghai, China). These are expressed as mg g^{-1} dry leaf weight (Fu and Huang, 2001). Green color index (GCI) was measured by a turf color meter (TCM500, NDVI, USA). Soil water contents (SWC) were measured using time domain reflectometry (TDR200, Soil Moisture Equipment, Spectrum, USA) by inserting the 15-cm-long wave guide probe to monitor the soil water deficit in the top 15-cm soil profile. Leaf water potential (LWP) of fully expanded leaves was determined with a WP4 Dewpoint PotentiaMeter (Decagon Devices, Inc. Pullman, Wa, USA).

Rubisco extraction and activity assays were measured following the method of Hu et al. (2010) with slight modifications. The collected leaves (0.2 g) were immediately ground in liquid nitrogen with 3-mL extraction buffer containing

50 mM Hepes-KOH (pH=7.5 at 25 °C), 10 mM MgCl_2 , 2 mM ethylene diamine tetraacetic acid, 10 mM dithiothreitol, 10 % glycerol (v/v), 1 % bovine serum albumin (w/v), and 1 % Triton X-100 (v/v), (Sigma, St. Louis, MO, USA). The supernatant was isolated by centrifugation at 14,000g for 10 min at 4°C and used immediately for Rubisco activity assays. Rubisco activity was measured by adding RuBP to the assay solution [100 mM Bicine (pH=8.0), 25 mM KHCO_3 , 20 mM MgCl_2 , 3.5 mM ATP, 5 mM phosphocreatine, 5 units glyceraldehyde-3-phosphate dehydrogenase, 5 units 3-phosphoglyceric phosphokinase, 17.5 units creatine phosphokinase and 0.25 mM NADH], and absorbance was determined at 340 nm with a spectrophotometer (Helios Alpha, Thermospectronic, Rochester, NY). Initial activity was measured after adding RuBP for 40 s, and total activity was determined after incubating samples at 25 °C for 5 min. Rubisco activation state was assessed by the ratio of initial to total activity. A 50 μL of leaf extract was used to determine leaf soluble protein concentration (Bradford, 1976). Total leaf soluble protein was estimated from standard curves prepared with bovine serum albumin and used to calculate Rubisco activity expressed as micromoles CO_2 per second per gram fresh weight. Rubisco activation state was assessed by the ratio of initial to total activity (Hu et al., 2010). Data from control group without silicate and drought stress as expression basis of the percentage of soluble protein and Rubisco activity (Flexas et al., 2006b).

The amount of Rubisco protein was determined based on the method of Servaites et al. (1995) with minor modification. Coomassie brilliant blue R stained Rubisco protein and quantification following by SDS-PAGE and spectrophotometric measurement of the eluted dye. 0.1 g frozen fresh leaves cut into 2 mm length were homogenized in a buffer contains of 50 mM Tris-HCl (pH 8.0), 5 mM DTT, and 5 mM MgCl_2 . Further assay procedures were performed according to Campbell et al. (1988)). Purified Rubisco from Kentucky bluegrass was used as the standard.

Experimental design and statistical analyses

The experiment was arranged in a completely randomized design with four replicates. All the studies were repeated 3 times during 2013 and 2014. Since the results of all temporal repeated were similar; therefore, the data of three individual repeats were averaged. The collected data were statistically analyzed by means of variance analysis using the computer software SPSS (version 12, SPSS, Chicago, IL, USA). The mean values were compared with the least significance difference test at 0.05 probability level. The relationships between different attributes were evaluated by using quadratic regression analysis.

Results

Drought stress severely hampered the net photosynthesis and transpiration rate in Kentucky bluegrass (Fig. 1). When plants were subjected to drought, net photosynthesis and transpiration rate was progressively reduced with the passage of time until recovery stage. Across different Si application treatments, 83 and 53 % reductions in net photosynthesis and transpiration rate, respectively, were recorded after 20 days of drought stress. At this phase of drought stress, the application of Si at 400 and 800 mg L⁻¹ results in 44 and 39 % higher photosynthesis in comparison to control. Transpiration rate was unaffected by Si application under drought conditions (Fig. 1). Upon re-watering, both these attributes were

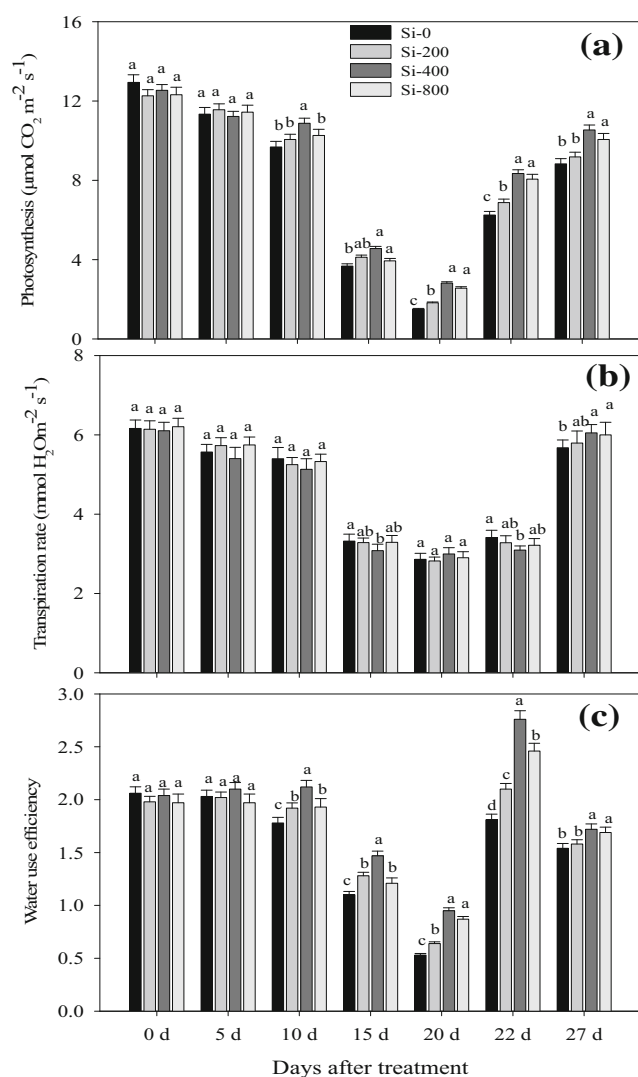


Fig. 1 Influence of various Si application treatments on **a** net photosynthesis and **b** transpiration rate, and **c** water use efficiency of Kentucky bluegrass under drought stress conditions. Error bars denote \pm S.E. The different small letters above means indicate significant differences ($P < 0.05$) between treatments at the same harvest time as determined by least significant difference (LSD) test

increased particularly in Si applied treatments. Water use efficiency was almost similar for all treatments at initial stages of drought. Nonetheless, it was severely reduced after 10 days of drought stress. In response to Si application, water use efficiency of Kentucky bluegrass was higher throughout the experiment. When plants were re-watered, sudden increase in water use efficiency was observed in all treatments.

Drought stress progressively decreased the soil water content (SWC) and leaf water potential (LWP), and such a decrease was more pronounced in control (Si-0) treatment (Table 1a, b). At the initiation of drought stress treatment, SWC in all the treatments was at the field capacity level (about 27 %). SWC declined in the range of 50–59 and 80–85 % at 10 and 20 days of drought, respectively. Upon re-watering,

Table 1 Influence of various Si application treatments on (a) soil water contents (SWC) and (b) leaf water potential (LWP), (c) green color index (GCI), (d) chlorophyll contents and (e) stomatal conductance of Kentucky bluegrass under drought stress conditions

Treatments	Days after treatment				
	0	10	20	22	27
(a) Soil water contents (%)					
Si-0	27.32 [†]	11.19b	4.18b	27.38a	26.67a
Si-200	27.16a	12.72a	4.35b	26.29a	27.49a
Si-400	26.56a	13.32a	5.33a	27.93a	27.03a
Si-800	27.60a	11.84b	4.93a	26.74a	26.45a
(b) Leaf water potential (–MPa)					
Si-0	1.26a	1.49d	2.38c	1.53b	1.27b
Si-200	1.32a	1.65c	2.47bc	1.57b	1.29b
Si-400	1.33a	1.90a	2.98a	1.63a	1.36a
Si-800	1.29a	1.79b	2.57b	1.48c	1.32ab
(c) Green color index					
Si-0	9.57a	6.53d	3.38c	4.52c	7.37c
Si-200	9.38a	7.43c	4.41b	5.43b	8.04b
Si-400	9.55a	8.35a	5.03a	5.83a	8.92a
Si-800	9.48a	7.75b	4.53b	5.74a	8.33b
(d) Chlorophyll contents (mg g ⁻¹ DW)					
Si-0	10.35a	8.16d	6.13d	7.54c	7.93d
Si-200	10.33a	8.71c	6.55c	8.10b	8.48c
Si-400	10.34a	9.84a	7.63a	8.45a	9.02a
Si-800	10.33a	9.19b	7.16b	8.14b	8.73b
(e) Stomatal Conductance (mol H ₂ O m ⁻² s ⁻¹)					
Si-0	0.38a	0.22a	0.12c	0.18b	0.26a
Si-200	0.37a	0.20b	0.13b	0.21a	0.24b
Si-400	0.37a	0.18c	0.14a	0.21a	0.23c
Si-800	0.38a	0.19bc	0.14a	0.20a	0.24b

[†] The different small letters within a column and at the same harvest time indicate significant differences ($P < 0.05$) between treatments as determined by least significant difference (LSD) test. All data were collected at 0, 10, 20, 22, and 27 days. At 20 days, all the treatments were re-watered for recovery

SWC returned to the field capacity level. Likewise, LWC was declined by 24 and 50 % (avg. across Si application treatments) at 10 and 20 days of drought, respectively, and after recovery stage, LWP becomes normal as before drought stress. Maximum decrease in SWC and LWP was observed in Si-0, while Si-400 was the most effective treatment for maintaining these attributes (Table 1a, b).

Drought stress led to a substantial reduction in green color index and chlorophyll contents of Kentucky bluegrass (Table 1c, d). After 20 days of drought stress, green color index and chlorophyll contents of Kentucky bluegrass were decreased by 47–65 and 26–40 %, respectively. Application of Si remained effective in alleviating the adverse effects of drought on all of these traits. As compared to control (Si-0), Si-400 recorded 49 and 25 % higher green color index and chlorophyll contents, respectively at 20 days of drought. In control treatment plants, both of these attributes were also significantly lower than in Si applied treatments even after re-watering (Table 1).

The stomatal conductance of Kentucky bluegrass at initial stages (0 days after drought) was almost similar for all treatments. Nonetheless, when Kentucky bluegrass plants were subjected to drought, stomatal conductance was considerably decreased, until plants were re-watered (Table 1e). Therefore, >40 and >60 % reductions in stomatal conductance were recorded at 10 and 20 days of drought stress, respectively. Applying Si to plants after 10 days of drought resulted in decreased stomatal conductance values across all Si-

concentrations when compared to control plants. The intermediate concentration of 400 mgL⁻¹ decreased stomatal conductance the most, while 200 and 800 mgL⁻¹ resulted in a smaller decrease in stomatal conductance. Adding Si in concentrations of 400 and 800 mgL⁻¹ to plants after 20 days of drought, resulted in significantly higher stomatal conductance values than that of 200 mgL⁻¹ of Si and the control plants. After re-watering, stomatal conductance in plants treated with all concentrations of Si recovered faster than the control plants. This indicates that applying Si to plants can delay stomatal occlusion, thus alleviating plant damage caused by drought stress.

Data regarding leaf soluble protein and Rubisco protein (Table 2) revealed that there were no significant differences among Si application treatments at the initiation stage of drought stress. Nevertheless, pronounced variations were observed for these two attributes at 10 and 20 days of drought under the influence of Si application treatments (Table 2). With the passage of time, drought stress increased the leaf soluble proteins; however, no considerable variations were observed for Rubisco protein (%) due to drought stress. At 10 and 20 days of drought stress, Si application exhibited 6.6–12.8 and 2.6–9.4 % decline in leaf soluble protein as compared to the control without Si treatment, respectively. Furthermore, application of Si increased the Rubisco protein (%) in Kentucky bluegrass under drought conditions (Table 2).

Rubisco activity (initial and total) and activation state were considerably decreased in Kentucky bluegrass upon exposure

Table 2 Influence of various Si application treatments on leaf soluble protein content, Rubisco protein content, Rubisco initial activity, Rubisco total activity, and Rubisco activation state of Kentucky bluegrass under drought stress conditions

Treatments	Harvest time (day)	Leaf soluble protein (% control)	Rubisco protein (%)	Rubisco Initial activity (% control)	Rubisco total activity	Rubisco activation state (%)
Si-0	0	100.0 [†]	51.4a	100.0b	100.0c	60.3c
Si-200	0	97.6a	50.2a	117.2a	114.6a	61.4bc
Si-400	0	100.7a	52.6a	113.8a	108.3b	63.7a
Si-800	0	98.4a	52.3a	113.8a	110.4b	62.2ab
Si-0	10	185.7a	50.7b	75.9c	89.6c	51.5d
Si-200	10	173.0b	52.4a	89.7b	97.9b	54.8c
Si-400	10	161.9b	52.9a	100.0a	104.2a	57.7a
Si-800	10	168.3b	50.4b	93.1b	102.1a	55.6bc
Si-0	20	234.9a	51.3b	37.9c	56.3c	40.2d
Si-200	20	223.8b	52.3a	51.7b	72.9b	43.4bc
Si-400	20	212.7c	52.7a	62.1a	83.3a	45.3a
Si-800	20	228.6b	52.6a	55.2b	77.1b	42.8c
Si-0	27	147.6a	51.4b	89.7d	100.0d	53.8b
Si-200	27	144.4a	51.8b	100.0c	110.4c	54.6ab
Si-400	27	137.3a	53.8a	117.2a	127.1a	55.8a
Si-800	27	141.3a	52.8ab	106.9b	116.7b	55.3a

[†] The different small letters within a column and at the same harvest time indicate significant differences ($P < 0.05$) between treatments as determined by least significant difference (LSD) test. All data were collected from leaves harvested at 0-, 10-, 20-, and 27-day treatments. At 20 days, all the treatments were re-watered for recovery

to drought stress (Table 2). Nonetheless, application of Si was effective in improving Rubisco activity as well as activation state during the entire experimental period. At 20 days of drought, Rubisco initial activity and Rubisco total activity were decreased by 37.9–48.3 and 16.7–27.1 %, respectively. Moreover, activation state was declined by 24.9–28.9 % at 20 days of drought stress with respect to control group. Application of Si-400 outperformed than rest of Si application treatments, as it recorded maximum increase in Rubisco activity and activation state during the entire course of study (Table 2).

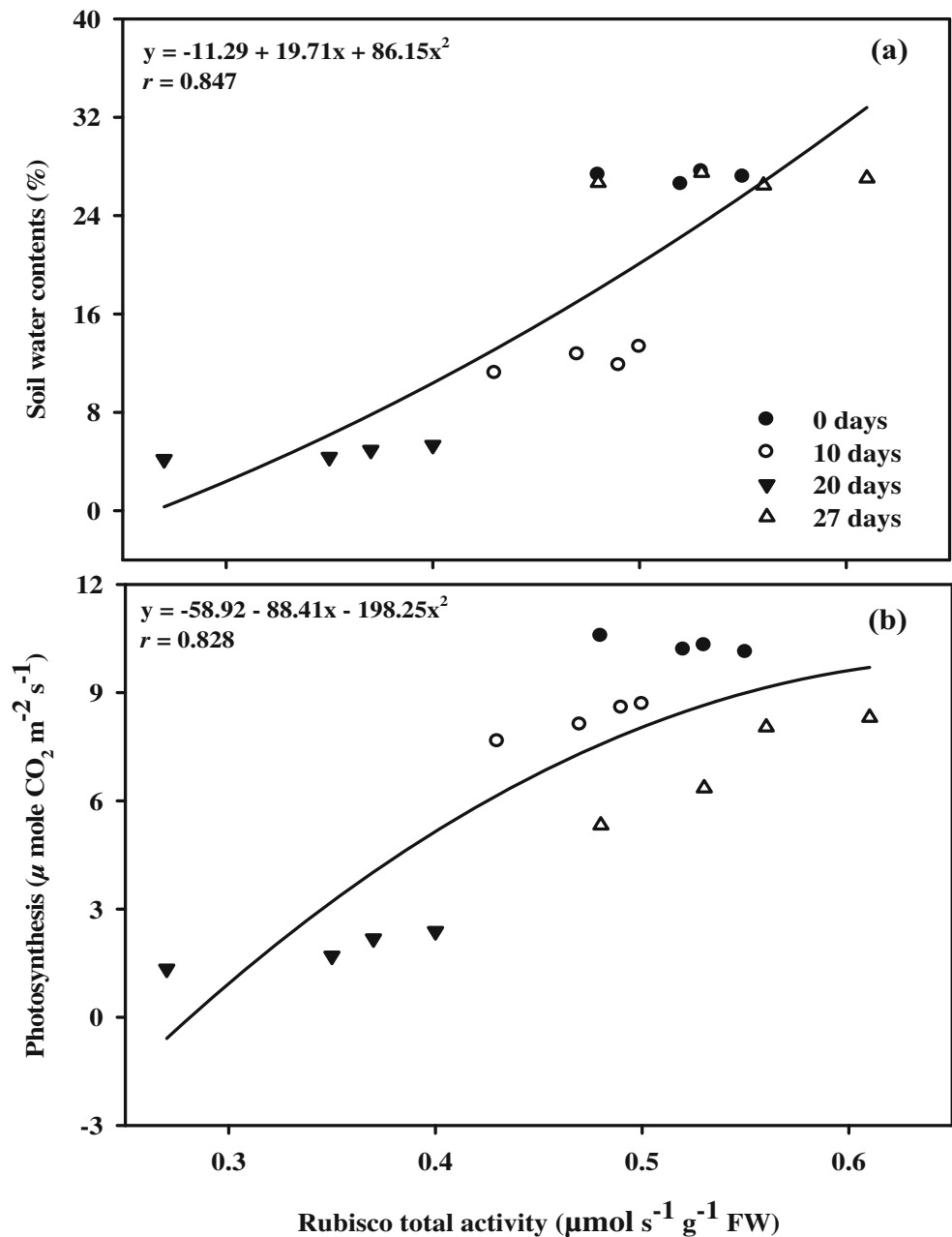
Quadratic regression analysis (Polynomial) of Rubisco total activity with SWC revealed strong positive relationship

showing 85 % association (Fig. 2a). Likewise, the relationship of Rubisco total activity with net photosynthesis was also positive and quadratic equation depicted 83 % variations between these two attributes (Fig. 2b). The relationships of Rubisco total activity with SWC as well as net photosynthesis were stronger under drought conditions (10 and 20 days of drought stress).

Discussion

Present study depicted that drought stress severely hampered the net photosynthesis and its associated metabolic events in

Fig. 2 Relationships of Rubisco total activity with **a** soil water content and **b** photosynthesis in Kentucky bluegrass under drought stress using quadratic regression analysis



Kentucky bluegrass. Decline in net photosynthesis was mainly associated with decrease in Rubisco activity and Rubisco activation state under drought stress (Table 2, Fig. 1). Previously, Flexas et al. (2004) stated that drought stress limits photosynthesis predominantly through increases in the leaf resistances to CO₂ transport, while at severe stress metabolic impairment also occurs. Nevertheless, variability in Rubisco related parameters as observed in present study indicates some controversy, which might be attributed to differences in the stress intensity and to species specific responses (Flexas et al. 2006a; Galmés et al. 2011).

At 10 days of drought stress, decline in photosynthesis rate was more than that of Rubisco activity, which suggests that the earlier inhibition of photosynthesis was presumably due to rapid stomatal closure. At early stage of drought initiation (10 days), the stress may be moderate; therefore, stomatal limitations were more dominant than metabolic impairment. Because at 10 days of drought stress, decrease in stomatal conductance was much higher than that of Rubisco activity. More pronounced reductions in Rubisco activity at severe stress (20 days of drought stress) support the findings of Flexas et al. (2004) and (Flexas et al., 2006b).

Metabolic impairment particularly decreases in the activity of Rubisco in response to drought stress, has previously reported in many plant species (Lawlor and Cornic, 2002; Bota et al., 2004). Initial and total Rubisco activities were decreased by about 48.3 and 27.1 % at 20 days of drought in control treatment, whereas, Rubisco protein (%) was not significantly modified. The decrease in Rubisco activity might be attributed to de-carbamylation and/or binding of inhibitory sugar phosphates, because Rubisco activation state, the indicator of these both, was severely hampered by drought stress (Table 2). Previously, Eichelmann et al. (2009) argued that along with carbamylation, presence of various unknown inhibitors regulate the Rubisco activity in plants. Under severe drought stress, decrease in the initial activity of Rubisco was more than that in total activity (Table 2). Galme's et al. (2011) pointed out that inactivation of Rubisco decreases its initial activity due to de-carbamylation of catalytic sites, possibly intervened by the lesser CO₂ availability under drought stress. Under severe drought, inhibitor tightly binds the catalytic sites of Rubisco which limit the concentration of sites catalytically accessible for carboxylation (Galmés et al. 2011). Moreover, an enhancement in the concentration of tight-binding inhibitors has been reported in various varieties under moderate-to-severe drought conditions (Parry et al. 2002). Tezara et al. (1999) stated that reduced level of ATP influences the production of RuBP under drought stress, which results in inhibitors binding to Rubisco sites. Parry et al. (2008) hypothesized that tight-packed inhibitors interaction with Rubisco stop the degradation by proteases of the Rubisco especially under stress condition.

In the present study, accumulation of leaf soluble protein in Kentucky bluegrass was progressively increased with drought

stress, but decreased after re-watering (Table 2). This might be due to de novo synthesis of stress proteins provoked by drought stress. Soluble proteins are non-toxic in nature which protect and maintain the integrity of biological membranes from oxidative nature of drought (Ashraf and Foolad 2007; Fahad et al. 2014a; Fahad et al. 2014b;). Increased concentration of osmolytes like proteins enhances the plant tolerance to drought condition, and not only assists in sustaining the tissue but also take part in osmoregulation (Farooq et al. 2009; Fahad and Bano 2012; Fahad et al. 2015a, b, c, d). We found that the accumulation of proteins increased with the increase in duration and severity of drought conditions, which enabled the plant to withstand better under drought associated with maintenance of relatively higher water contents. Our results are supported by the findings of Queiroz et al. (2011) who reported that increased protein accumulation with prolongation of drought conditions, helped the plants to maintain tissue turgidity in sugarcane. Previously, Vendruscolo et al. (2007) found positive correlations of osmolyte accumulation with drought duration and drought tolerance.

Application of Si remained effective in alleviating the negative effects of drought stress on photosynthesis and its associated metabolic events. Rubisco activity and Rubisco activation state were significantly improved by Si application under drought stress. This indicated that proper silicate application could enhance the enzyme activities including Rubisco more efficient at fixing CO₂ in initial process of photosynthesis in Kentucky bluegrass, accelerate CO₂ binding with ribulose biphosphate (RuBP). Furthermore, increase in Rubisco protein suggests that Si application not only maintains more active Rubisco enzyme and Rubisco activase but was also able to maintain more stable Rubisco proteins for carbon assimilation during severe drought compared with control, which may contribute to producing much more photosynthesis products during Calvin Cycle which are used to make sucrose and starch for plants growth and better drought tolerance of plants (Fry and Huang, 2004).

Although silicate is not traditionally considered as an essential element in plants; nevertheless, several studies have documented the positive role of Si on plant growth under drought conditions in many crop species (Gong et al. 2005; Lux et al. 2003, Hattori et al. 2005; Shen et al. 2010) and grasses (Eneji et al., 2008). Increased photosynthetic rate of Kentucky bluegrass under drought stress by Si application predominately occurred due to improved water uptake, root growth, and leaf erectness by application of Si (Hattori et al. 2005). Gong et al. (2005) found that application of 2.11 mmol Na₂SiO₃ increased net assimilation rate by ~37 mmol C m⁻² s⁻¹ under water-deficit conditions in wheat. Accumulation of silicate in the leaves increases leaf blade erectness, which in turns facilitates light penetration, reduces transpirational losses, and promotes photosynthesis. Chen et al. (2010) found that applying 1.5 mM silicate to drought stressed rice plants increased leaf surface area and

photosynthetic rate on a per-leaf basis. Hattori et al. (2005) observed that growth rate of Si-applied (1.66 mM K_2SiO_3) sorghum was higher under drought conditions as compared to control. While studying on soybean, Shen et al. (2010) also concluded that application of Si was effective in improving the photosynthesis and antioxidant activities under drought conditions.

Results revealed that at 7 days of re-watering, SWC and Rubisco related parameters were recovered to the pre-stress stage, demonstrating that plants were entirely rehydrated upon re-watering. Improvement in net photosynthetic rate upon re-watering reflected that a lesser amount of damage in photosynthesis systems during drought stress was occurred particularly in Si applied treatments. Thimmanaik et al. (2002) observed that just 2 days after re-watering, numerous enzymes were approximately entirely recover in the Calvin cycle. In the present study, even though all the metabolic events were fully recovered, but incomplete recovery was observed in photosynthesis, which emerged to be largely associated with lingering diffusion limitations (stomatal and mesophyll) after re-watering. In the present study, stomatal conductance could not fully recover even after re-watering (Table 1). Previously, Gallé and Feller (2007) also pointed out that drought induced stomatal occlusion of waxes may contribute to the remaining stomatal limitation after re-watering by blocking stomatal aperture.

Conclusively, our results indicated that drought stress severely diminished the photosynthesis and its associated metabolic activities in Kentucky bluegrass. Si application particularly in concentration of 400 mgL^{-1} counteracted the damaging effects of drought on these attributes suggesting possible (direct or indirect) role in maintenance of more active Rubisco enzyme and Rubisco activase and stable proteins for carbon assimilation under stress conditions; which needs to be elucidated in further studies. From this investigation, we may suggest that manipulation of endogenous Si through genetic or biotechnological means may result in improved photosynthesis and better drought tolerance in Kentucky bluegrass.

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