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REGULAR ARTICLE

Low Si combined with drought causes reduced transpiration in sorghum *Lsi1* mutant

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Abstract

Background and aims High and stable plant productivity is a major aim in agricultural research. Silicon fertilization improves yields of various crops under stress. Nonetheless, broad application of silicon is inhibited by the lack of a mechanism explaining this effect.

Experimental System To study the role of silicon in soil-grown plants under drought, we utilized a sorghum (*Sorghum bicolor*) mutant plant lacking the key silicon root channel – Low silicon 1 (SbLsi1). The *sblsi1* mutant plants absorb 1/15 of the silicon absorbed by wild type plants, making them a suitable tool to examine silicon physiology in soil and under field conditions.

Results In mutant plants grown in pots, significant reductions in momentary and accumulated whole plant transpiration, photosynthesis rate, and stomatal conductance were found only under water stress. Root structure, root hydraulic conductance, and stomatal density were similar between wild type and *sblsi1* plants. Similar leaf water contents between the genotypes suggested that the water uptake was balanced with transpiration.

Conclusions The similarity between the genotypes under benign conditions are in accordance with minor to no effects of silicon fertilization in non-stressed plants, and support the minor pleiotropic effects of the mutation. Early stomatal closure in the mutant plants under drought stress caused the reduced transpiration. Thise early response suggests that silicon may delay the onset of drought physiology by either reduced stress signaling or reaction.

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Introduction

Agricultural research focuses on increasing yields of crops under the changing climate and the unstable and unpredictable weather conditions. Water deficiency is a major abiotic stress that damages crops worldwide, causing significant yield loss. One of the ways to increase plant tolerance to drought is by fertilization with silicon as soluble monosilicic acid [$Si(OH)_4$] or solid amorphous hydrated silica ($SiO_2{\cdot}nH_2O$). Since alumino-silicate minerals dominate the soil solid phase, Si treatments are suggested as sustainable ways to enhance plant tolerance to drought without contaminating the environment with foreign materials. Natural silicic acid concentration in soil solutions normally varies between 0.1 to 0.6 mM, depending on its dynamic equilibrium with the soil particles (Epstein 1994; Savant et al. 1999). Some plant species absorb silicic acid actively, through the combined activity of a specialized aquaporin channel which belongs to the nodulin 26-like intrinsic proteins (NIPs) (Deshmukh and Bélanger 2016), termed Low silicon 1 (Lsi1), and a Si transporter driven by proton gradient, termed Low silicon 2 (Lsi2) (Ma and Yamaji 2015). Silicic acid is also absorbed through the apoplastic pathway even in plants that do not express Lsi1 and Lsi2. Consequently, all plants contain some silicon in their tissues (Hodson et al. 2005).

Si has a positive general effect on plants only under stress (Liang et al. 2007; Van Bockhaven et al. 2015; Vivancos et al. 2015; Coskun et al. 2019), and therefore it is considered as a beneficial "quasi-essential" element (Epstein 1999). Under drought stress, the reduction in growth and yields of plants with access to Si are smaller in comparison to plants deprived of Si (Gong et al. 2003; Hattori et al. 2005; Gao et al. 2006; Shi et al. 2016). However, the physiological effects of Si are varied and many times opposite, depending on plant species and varieties, growing conditions, and method of Si supplementation (Zhu and Gong 2014; Cooke and Leishman 2016; Verma et al. 2021).

A specific example is transpiration under drought. In rice and maize, Si-fed plants exhibit reduced transpiration, primarily by reduction of stomatal conductance without much change in root conductivity (Agarie et al. 1999; Gao et al. 2006). The explanation of Si drought amelioration was related to a reduction in stomatal opening, possibly by incorporation of silica in the stomata cell walls. However, in sorghum and wheat, leaf transpiration and stomatal conductance are increased under drought in Si treated plants, allowing for increased production under the stress (Hattori et al. 2007; Gong and Chen 2012). The contradicting evidence may indicate a change in water availability under Si treatments. Indeed, biogenic amorphous silica increases the soil water holding capacity, irrespective of plant water status nor Si uptake. However, fertilization with sodium silicate reduces water available for soil-planted plants (Schaller et al. 2020). The intricate response to silicon fertilization, combining physiological and environmental influences, calls for the development of an experimental system that separates soil and plant effects.

In this work, we avoided the influence of soil silica fertilization, by studying a sorghum mutant defective in Lsi1 (Markovich et al. 2019). The *sblsi1* knockout mutant contains about 1:40 silica content that of wt plants, making it an ideal model to focus on the influence of Si on plant physiological processes. We thus studied water uptake and transpiration in well-watered and water stressed sorghum plants, comparing the wild type (wt) to the *sblsi1* knockout mutant.

Materials and methods

Growth conditions

Net house experiment *Sorghum bicolor* BTx623 wt and *sblsi1* mutants (Markovich et al. 2019) were sowed in a plot was 500 m^2 of sandy soil at the experimental farm of The Hebrew University of Jerusalem (Rehovot, Israel) inside an insect-proof net-house protected by a polyethylene top. A randomized block design with eight replicates was employed. Each block consisted of twenty plants planted 10 cm apart in a single row. The plots were well watered via a drip irrigation system. At flowering, randomly selected inflorescences were covered with paper bags, and were later used to assess seed yield. Sorghum panicles were harvested and oven-dried at 35 °C for 48 h.

The panicles were then manually threshed and seeds were collected and weighed.

Open field experiment *Sorghum bicolor* BTx623 wt and *sblsi1* mutants were grown in an experimental design identical to the net house experiment but without any cover, mimicking commercial field conditions. Drought was induced to one-month-old plants at the vegetative growth stage, by discontinuing irrigation for three weeks. Biomass and grain yields were measured in mature plants.

Greenhouse experiments *Sorghum bicolor* BTx623 wt and *sblsi1* mutants were seeded in 4 L pots with commercial soil (Shacham g-a, Israel), in a greenhouse (The Hebrew University, Faculty of Agriculture, Rehovot, Israel), and grown for three months under natural light and temperatures. The plants were irrigated twice a day by water supplemented with fertilizer (N.P.K 5.3.8). Water stress was applied to one-month-old plants by arrest of irrigation for 13 days, after which irrigation was resumed.

Hydroponics experiments *Sorghum bicolor* BTx623 wt and *sblsi1* mutant seeds were rinsed with sodium hypochlorite, and germinated on wet filter paper. The young seedlings were transplanted into a hydroponic solution containing macronutrients (0.5 mM KNO_3, 0.15 mM $Ca(NO_3)_2$, 0.1 mM $MgSO_4$, and 0.1 mM $NH_4H_2PO_4$), and micronutrients (2 μM Ethylenediaminetetraacetic acid iron(III) sodium (EDFS), 4.6 μM H_3BO_3, 0.06 μM Na_2MoO_4, 0.9 μM $MnCl_2$, 0.16 μM $ZnSO_4$, and 0.03 μM $CuSO_4$), with the pH adjusted to 5.8. Plants were grown in a growth room at 25 °C, and 16/8 h light/dark cycle. The nutrient solution was renewed every 4 days.

Lysimeter drought experiments Lysimeter experiments were conducted in two different systems and locations. The first system was located at The Gilat Research Center in the northwestern Negev, Israel. Three week old sorghum seedlings of wt and *sblsi1* mutant were transplanted to 24 barrel-shaped weighing-drainage lysimeters each with a 0.2 m radius and 0.6 m depth placed on an automated rotating system (Lazarovitch et al. 2006). Each pot contained four plants that were measured together. The lysimeters incorporated a 70 cm drainage extension of highly conductive media (rockwool) to ensure negative soil water potential at the soil lower boundary without influencing water flow through the system (Ben-Gal and Shani 2002). The lysimeters were filled to a depth of 0.55 m with sandy soil (91% sand, 1% silt, 8% clay). Every day, the average evapotranspiration (ET) (the sum of soil evaporation and plant transpiration) was calculated according to the formula: $ET = I - D - \Delta S$, where I (kg) is irrigation, D (kg) is drainage, and ΔS (kg) is the change in water stored in the soil, measured by the difference in the lysimeter weight between the beginning and end of the period over which the water balance was determined.

The second system was located in a greenhouse at The Hebrew University, Faculty of Agriculture, Rehovot, Israel. Wt and *sblsi1* mutant plants were planted in 4 L pots and grown under controlled conditions (30/18 °C day/night under natural day length and light). Each pot was placed on a temperature-compensated load cell with digital output (Vishay Tedea-Huntleigh, Netanya, Israel) and was sealed from the growth medium to prevent evaporation from growth medium surface. Whole-plant transpiration rates and stomata conductance were determined using a lysimeters-array (Plantarray gravimetric prototype system, Plant-DiTech Ltd. Rehovot, Israel), as described in detail in Halperin et al. (2017). Daily transpiration (weight loss between predawn and 18:00 h) was normalized to plant biomass and leaf area. We report the average value for a given genotype and treatment over all plants.

Mineral content of flowering shoots

A batch of sample (about 300 mg) was digested in 5 ml HNO 3.65% and 1 mL HCl 30%. Digestion was carried out in quartz vessels with Teflon liners using a "Discover" sample digestion system at high temperature and pressure (CEM, USA). Vessels were cooled down and the volume was made up to 25 ml with deionized water. Element concentration was measured in the clear solutions using an End-On-Plasma ICP-AES model 'ARCOS' from Spectro GMBH, Germany. Measurements were calibrated with standards for ICP from Merck. The continuing calibration verification standard was measured to check the instrument stability.

Leaf silicon content

Dry ashing of the samples followed Marcovich et al. 2019 (Markovich et al. 2019). Leaves from mature well-watered plants that grew in the net house were collected and dried in an oven at 60ºC for 48 h. The plant material was shredded and weighed in a crucible. Samples were heated to 580ºC overnight, let to cool, washed thrice with HCl 1 M, thrice with double distilled water, and let dry in an oven at 60ºC for 48 h. Sample weight was recorded and the percent residual weight per initial dry weight was calculated. We assumed that the acid insoluble fraction contained only silica.

Leaf relative water content

Leaf fresh weight (FW) was measured immediately after detachment. Leaves were soaked for 8 h in double distilled water at room temperature in the dark, and their turgid weight (TW) was recorded. Leaf dry weight (DW) was recorded after fully drying the leaves at 70 °C. Leaf relative water content (RWC) was calculated as (FW—DW)/(TW—DW) × 100.

Gas-exchange measurements

Gas-exchange measurements were conducted with LI-6400 portable photosynthesis and fluorescence measurement system (LI-6400–40 leaf-chamber fluorometer; LICOR Inc., Nebraska, USA). The measuring chamber enclosed a circular 2 cm^2 leaf area and evaluated gas fluxes on both sides of the leaf. Light intensity was monitored prior to each measurement and kept constant at 1200 µmol photons m^{-2} s^{-1} (10% blue light) (LICOR 6400–40 LCF). Air flow-rate was kept constant at 500 µmol s^{-1}, and the reference CO_2 concentration was 400 ppm.

Leaf stomatal density

Transparent nail polish was applied to the abaxial leaf epidermis of mature wt and mutant leaves of one-month-old plants grown in the greenhouse, and let dry for about 30 min. Transparent sticky tape was applied onto the leaf and pulled gently, removing the dry nail polish that carried the leaf surface topography. The sticky tape was examined under an optical microscope (Nikon Eclipse 80i), and the number of stomata per leaf area was calculated.

Water flux and flow rates through the root system

Root water flux (Jr, water-uptake) was measured in four-week-old sorghum plants, grown in 4-L pots with mineral sand (Negev Minerals), on the second lysimeters-array located in Rehovot. This time the plants were grown under semi-controlled temperature conditions (20–28 °C day and 12–16 °C night), natural day length and maximal light intensity of approximately 1000 µmol photons m^{-2} s^{-1}. Soil probes (GS3 5TE, Decagon Devices, USA) were used to measure momentary soil water content. Jr was calculated according to the measured changes in soil water content as described in detail by Halperin et al. (2017), normalized to root dry weight (see the following paragraph).

At the end of the growth period, stems of the same sorghum plants were cut with a razor blade three centimeters above the ground, and the stumps were sleeved with a silicone tube forming an airtight seal. Sleeves were connected to a vacuum pump adjusted to 80 kPa. Sap was collected for 2–4 h using a liquid trap, and its final volume was measured. Roots were dug, washed thoroughly from the sand, and dried at 60 °C. Root water flow rate was calculated as volume sap per time pumped, and normalized to the dry weight of the roots.

Statistical analysis

t-test or one-way ANOVA analysis followed by the Tukey–Kramer honestly significant difference (HSD) test were used to compare means. All statistical analyses were conducted using JMP statistical software version 7.0.2 (SAS Institute, USA). Statistical significance was assumed at $P \leq 0.05$, unless indicated otherwise. Error bars represent standard errors.

Results

As a baseline, we tested variation in major mineral uptake, including Si, between the genotypes grown under benign conditions in pots in the greenhouse (Table 1). Mature flowering plants differ significantly in the boron and Si content. Both silicic acid and boric acid have affinity to the rice Lsi1 (Mitani et al. 2008). Thus, the reduction in B and Si intake is expected with knockdown of *sbLsi1*.

Table 1 Shoot mineral content in flowering BTx623 wild type (wt) and *sblsi1* mutant plants (defective in Si absorption). Reported are averages and standard deviation. Ppm—parts per million

	B (ppm)	Ca (ppm)	K (ppm)	P (ppm)	Si (ppm)
Sblsi1	5 ± 1	2,683 ± 1,602	21,629 ± 7,433	5,141 ± 1,255	70 ± 33
wt	7 ± 2	2,004 ± 1,967	20,449 ± 11,275	4,589 ± 1,769	1,211 ± 388
P (t-test)	0.01	0.36	0.76	0.39	9×10^{-10}

We further compared grain yields of plants grown in the field. *Sblsi1* mutant plants that were grown in a protected field under a net house showed no reduction in grain yield as compared to the wt plants. Nonetheless, when grown in an open field, the mutant plants produced about 2/3 total grain weight per plant that of the wt (Table 2). The reduction in grain yield may have stemmed from random mild stresses that had stronger effects in the mutated plants. To test the influence of stress, we applied a 3-week water arrest. In comparison to wt, mutant plants produced a 1/3 the grains and 3/4 the straw weight yields. Under drought stress, the average grain weight was reduced by 40% (Table 2).

Momentary gas-exchange in greenhouse plants

In order to identify physiological processes that were most sensitive to the mutation, we grew plants under protection and applied drought stress. Under these conditions, we could assure no random stress was affecting the plants. Indeed, pot-grown plants of both genotypes showed similar momentary photosynthesis parameters, as measured by a portable photosynthesis and fluorescence measurement system (LICOR 6400) (Fig. 1A-C). However, after exposure to drought for 13 days, significantly higher transpiration, stomatal conductance and photosynthesis rates were measured in the stressed wt compared to *sblsi1* mutant plants (Fig. 1A-C). Interestingly, the leaf water content was similar between the genotypes (Fig. 1D). We also measured similar stomatal densities of 70 ± 3 stomata per millimeter square in wt, vs. 66 ± 5 in the *sblsi1* mutant (Table 3).

Whole plant transpiration

To measure the evapotranspiration (ET) along the day we used a lysimeter system consisting of large pots of 75 L located in a greenhouse. Three-week-old wt and *sblsi1* mutant sorghum plants were transplanted into sand-filled pots of the lysimeter system, and were well irrigated for four weeks. No differences were observed in the ET between the genotypes (Fig. 2A). Starting on day 28, the irrigation was stopped for two weeks. We found that the average daily ET rate of the wt plants was higher than that of the *sblsi1* mutants starting four days after the irrigation arrest, for five days (Fig. 2A, inset). During the last four days of the drought, ET

Table 2 Yield components of wt and *sblsi1* mutant sorghum grown in the field. Plants were grown in soil under a net house (protected field) or outside (open field). Plants grown in an open field were also treated by a 3-week water arrest. Averages and standard deviations are reported. Letters represent statistical significant differences at 5% level by Tukey–Kramer honestly significant difference

Treatment	Genotype	Grain yield (gr per plant)	Straw yield (gr)	1000-sSeed weight (gr)
Irrigated Protected field	wt	78 ± 25 A		
	sblsi1	76 ± 18 A		
Irrigated Open field	wt	61 ± 8 AB	206 ± 44 A	32 ± 3 A
	sblsi1	39 ± 8 C	199 ± 7 A	27 ± 4 B
3-week drought Open field	wt	47 ± 13 BC	172 ± 38 A	26 ± 4 B
	sblsi1	17 ± 10 D	127 ± 26 B	17 ± 4 C

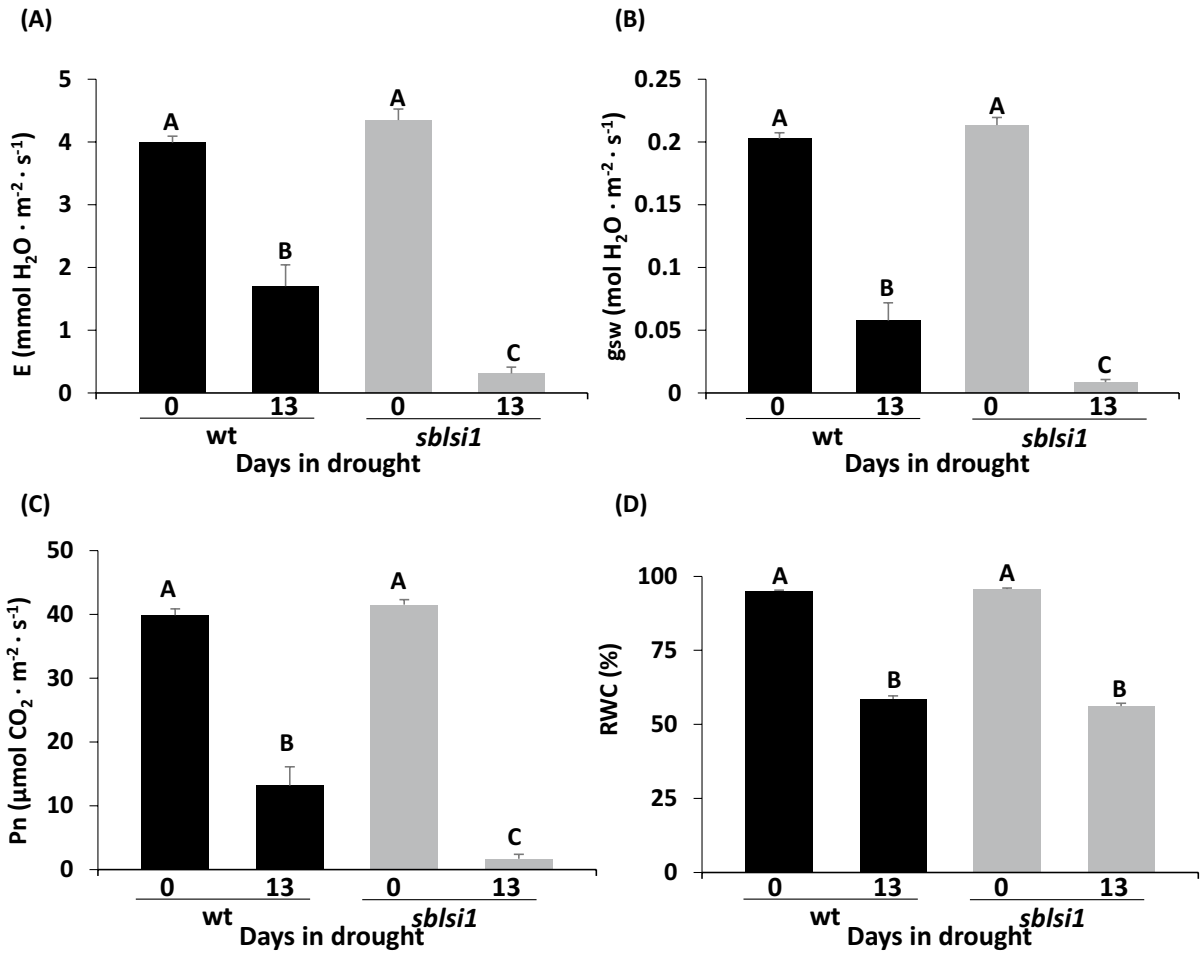

Fig. 1 Momentary photosynthesis parameters in wt and *sblsi1* sorghum mutant grown in a greenhouse. Plants were measured before (0) and after (13) 13 days without irrigation. Before the water arrest, no variations were detected between the genotypes. After the drought period, significant reductions in transpiration rate (E) (**A**), stomatal conductance (gsw) (**B**), and photosynthesis rate (Pn) (**C**) were observed in the *sblsi1* mutant as compared to the wt plants ($n=10$ $P\leq 0.05$). The relative water content (RWC) was measured but no difference between wt and *sblsi1* mutant before and after drought was observed (**D**) $n=5$, $P\leq 0.05$. All data are reported as means ± standard errors. Different letters indicate significant differences between treatments

Table 3 Morphological traits of 1-month-old BTx623 wild type (wt) and *sblsi1* mutant plants (defective in Si absorption). Stomata were counted in the abaxial surface of mature leaves. Root length and number of lateral roots were measured in two weeks old seedlings grown hydroponically. Reported are averages and standard errors

	Stomatal density (# per leaf mm^2)	Root length (cm)	Lateral roots (# per plant)	Root dry weight (watered) (gr per plant)	Root dry weight (water stressed) (gr per plant)
Sblsi1	66 ± 5	15 ± 2	32 ± 3	19 ± 2	12.4 ± 0.6
wt	70 ± 3	14 ± 1	32 ± 3	22 ± 1	12.5 ± 0.8
P (t-test)	n.s	n.s	n.s	n.s	n.s

rates were equal between the genotypes. On day 42, irrigation was resumed. Both genotypes recovered their ET rates within 3 days. Higher ET rate of the wt plants implied that they kept their stomata open for longer periods than the *sblsi1* mutants, which indicated a higher potential photosynthesis rate in the wt leaves. This probably led to the higher dry weight of the wt plants at the end of this experiment (Fig. 2B). We also found that after drought, the wt fresh weight was significantly higher than that of the *sblsi1* mutant (Fig. 2C). The similar trends of the dry and fresh weights suggested that the water content of two genotypes was similar.

We repeated these experiments in a more sensitive lysimeter system using smaller 4 L pots. The average daily weight of wt and *sblsi1* mutant plants were simultaneously followed during a well-irrigated period and through 21 days of continuous drought. In agreement with the previous results, we found no significant differences between the weight gain of wt and *sblsi1* mutant plants when they were well watered. When irrigation was stopped, weight loss was faster in the wt as compared to the *sblsi1* mutant plants (Fig. S1). The weight losses were normalized to plant weight and leaf area (Halperin et al. 2017), and reflected transpiration rates (E). To eliminate differences in water available to roots, we plotted the

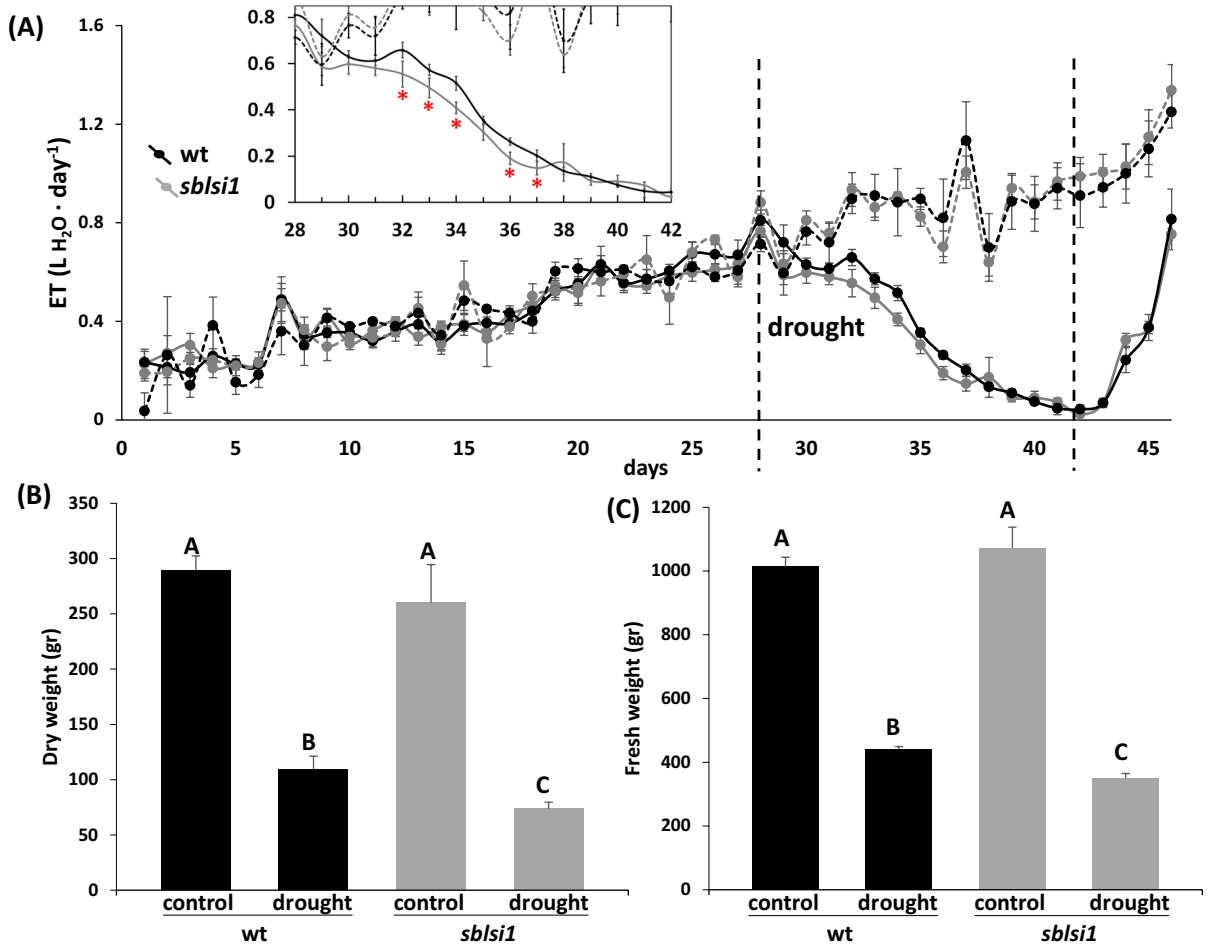

Fig. 2 Whole-plant weight-loss under irrigation and during drought stress. Evapo-transpiration (ET) was measured by a lysimetric system over 45 days (**A**). The plants were submitted to drought from day 28 to day 42. The evapotranspiration of wt plants was significantly higher under drought, from day 32 to day 37 (inset, asterisks). At the end of the experiment (day 45) the dry (**B**) and fresh (**C**) weight of the plants were measured. While in the control plants no significant differences were observed, under drought treatment, the dry and fresh weights were significantly higher in the wt as compared to the *sblsi1* mutant plants. Reported are average plant weights calculated from 16 plants, each 4 grown together in one pot. $P \leq 0.05$

transpiration and stomatal conductance during similar soil relative volumetric water content (VWC) (Fig. 3).

When the soil VWC was high (80%), the daily transpiration rate and canopy stomatal conductance (gsc) of the two genotypes were similar (Fig. 3A, D). When irrigation was stopped and the soil VWC was reduced to 40%, the transpiration and stomatal conductance of the plants were also reduced. However, the transpiration of the wt plants was significantly higher than that of the *sblsi1* plants (Fig. 3B). Even at soil VWC of 35%, transpiration of the wt plants was significantly higher than that of *sblsi1* plants during most of the day (Fig. 3C). Similar trends were recorded for the stomatal conductance (Fig. 3E, F). Higher stomatal conductance at similar leaf water contents in the wt plants implied that the drought stressed wt plants absorbed more water from the soil than *sblsi1* plants. Indeed, the soil in wt pots contained less water at the end of the drought period (27% in wt vs. 35% in *sblsi1* mutant), explained by increased water intake by wt roots, compared to the *sblsi1* mutant.

Characterization of roots

In order to study the root role in the plant water-relations we compared root morphology, water flux, flow rate, and biomass between the wt and the *sblsi1* mutant. Root architecture was estimated in seedlings of wt and *sblsi1* mutant sorghum plants that were grown for two weeks hydroponically. Root lengths, measured 14 ± 1 cm for wt, and 15 ± 2 for *sblsi1* mutant, were not different. Similarly, the number of lateral roots was 32 ± 3 for both genotypes (Table 3). Roots of plants grown hydroponically for six weeks showed no visible differences in morphology, however, no quantitative assessments were made.

To measure differences in the root size under water stress, we grew plants in sand on a lysimeter system, and followed the soil water content using a soil moisture sensor. The plants were subjected to a rather fast and strong drought because the sand maximal water content was less than 20%. Both wt and *sblsi1* mutant pots lost on average 8% of their weight over four days in a similar rate (Fig. 4A). Transpiration and root water-uptake rates were similar between the genotypes, and reduced with soil water content (Fig. 4B, C). Root dry weights were also similar between the genotypes and treatments, and equaled 22 ± 1 and 19 ± 2 g per the watered wt and *sblsi1* mutant plants, and 12.5 ± 0.8 and 12.4 ± 0.6 g per the stressed wt and *sblsi1* mutant plants, respectively (Table 3). Root water flow rate

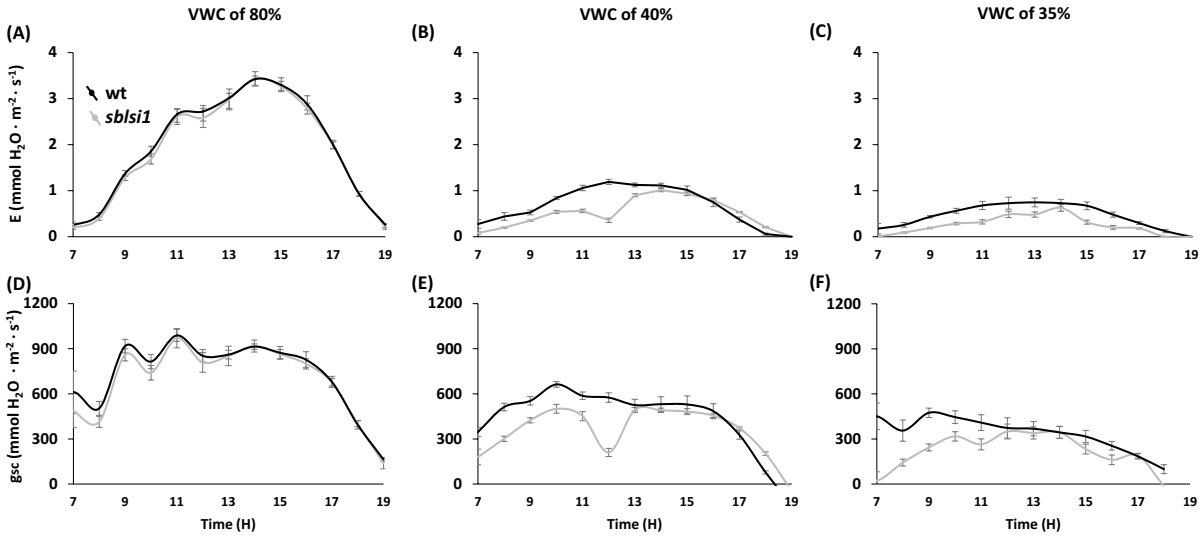

Fig. 3 Whole-plant transpiration and stomatal conductance at three soil volumetric water contents (VWC). Average ± standard errors of whole-plant daily transpiration (E) of wt and *sblsi1* mutant plants at VWC of 80% (**A**), 40% (**B**) and 35% (**C**). Average ± standard errors of whole-plant daily stomatal conductance (gsc) of wt and mutant sorghum plants at VWC of 80% (**D**), 40% (**E**), and 35% (**F**). Under drought, the wt plants were able to maintain higher transpiration and conductance of stomata for a longer period than *sblsi1* mutant plants. $n = 10$, $P \leq 0.05$

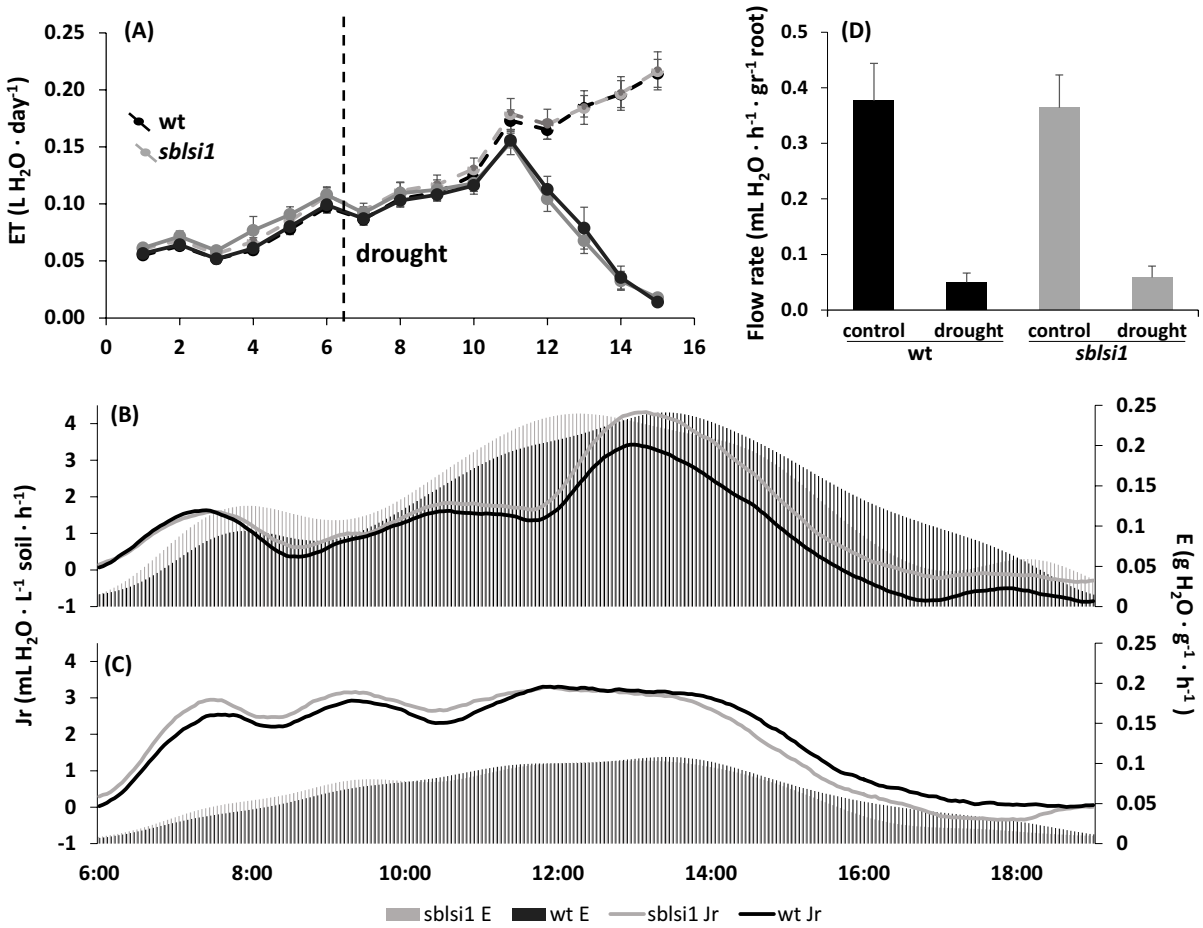

Fig. 4 Root hydraulic conductance. (**A**) Mean daily evapotranspiration (ET) of wt and *sblsi1* mutant plants was measured throughout the experiment. Irrigation of drought-treated plants was stopped at day 6. No significant differences were found between the wt and *sblsi1* mutant plants. Bars represent standard error calculated for eight plants. (**B**, **C**) Mean root water flux (Jr, bars) and whole plant transpiration normalized to plant's weight (E, line) of wt and *sblsi1* mutant plants during two representative days under normal irrigation conditions (**B**), and after five days with no irrigation (**C**). The data represents means of eight plants. (**D**) Mean water flow rate through the root system of wt and *sblsi1* mutant plants under control conditions and after five days drought treatment. No significant differences were found between the wt and *sblsi1* mutant plants. Bars represent standard error calculated for 3–4 plants

was measured by cutting the shoot and connecting the remaining stem to a pump that imitated transpiration pressure (Sade et al. 2010). Roots subjected to drought were watered, and immediately pumped. We found that the stressed roots hydraulic conductance was 6–8 times lower than non-stressed roots (Fig. 4D). Under the similar imitated transpiration pressure, no difference was found between the water flow rate in wt and *sblsi1* mutant roots.

Discussion

This work shows that under benign conditions, there is no yield reduction in the sorghum mutant defective in Si uptake. This is in agreement with published literature on the effects of Si under stress, but not in non-stressed plants (Cooke and Leishman 2016). Our findings fit nicely with the genetic results in wheat, showing that no genes are up or down regulated by

Si treatment alone (Chain et al. 2009). The sorghum genotype carrying a knockout mutation in the silicon transporter Lsi1 contained about 15 times less silica than wt plants grown in soil. We detected no structural variations in the low silicon mutant plants: the plants were erect, similar in height to the wt, had similar root structure, root water conductance, and similar stomata density. These observations are in accord with the physiological measurements, and the reported similarities between rice wt and *lsi1* mutant (Ma et al. 2002, 2006).

Nonetheless, under drought stress the wt plants maintained higher rates of photosynthesis and yields, possibly resulting from higher rates of transpiration and stomatal conductance, in comparison to the *sblsi1* mutant plants (Table 2, Figs. 1, 2). The density of the stomata was similar between the genotypes (Table 3), leaving stomatal opening as the reasonable cause for the difference in transpiration. Either larger fraction of stomata was open simultaneously, or each of the apertures opened to a larger degree in leaves of the wt as compared to the *sblsi1* mutants. Our results suggest that the averaged stomatal aperture was larger in the wt as compared to the *sblsi1* mutant plants only under drought stress. Direct measurements of stomata apertures will support this model.

Based on the following reasoning, our results indicate that the mutation in silicon uptake affected the sorghum plants on a molecular level rather than structurally. (1) Root morphology was similar between the genotypes and thus cannot explain the more effective drying of soil by wt plants. (2) Root hydraulic conductance and leaf water content were similar between the genotypes in watered as well as drought stressed plants. This infers that water transport from the root to the shoot was not affected by the mutation and lack of silica deposition, rejecting structural changes in the xylem. In addition, previous publications report that Si application results in both increased and reduced transpiration, depending on the species tested (Agarie et al. 1999; Gao et al. 2006; Gong and Chen 2012). Specifically, application of Si to sorghum seedlings under osmotic stress causes increased transpiration (Hattori et al. 2007), in agreement with our observations. Thus, the effect cannot be related to a simple change in the cell wall elasticity and the movement of guard cells as a result of silica deposition (Kollist et al. 2014). Therefore, the mutation in Si uptake possibly inferred changes on a molecular level, leading to early closure of stomata and onset of drought physiology by either increased stress signaling or reaction. Similarly, we showed a delayed onset of senescence in Si-treated versus low Si sorghum leaves of the *sblsi1* mutant. There, the Si treatment to the *sblsi1* leaves caused an enhancement of cytokinin biosynthesis (Markovich et al. 2017).

Conclusions

Utilizing a genetic manipulation to reduce silicon intake, we showed that under drought stress the *sblsi1* mutant plants close their stomata already at soil water content of 35%, in relation to the wild type plants, which are able to dry the soil to 27% water content and utilize the additional soil water to produce more biomass and grains. Our results suggest that the mutation affects molecular mechanisms rather than structures of tissues. However, further experiments should test whether pleiotrophic effects of the *sblsi1* mutation rather than a simple effect of the Si itself modulated the reaction of the plants to drought stress.

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Authors' contributions OM conceived and conducted the experiments and analyzed the data, NZ measured root hydraulic conductance and analyzed the data, BN supported the second lysimetric experiment and its analysis, YZ supported the data analysis, SB supported the experimental work, ABG supervised the first lysimetric experiment and its analysis, RE conceived the scientific question and supervised the experimental and analytical work. All authors commented on the data interpretation, and reviewed and approved the manuscript.

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Code availability Not relevant.

Declarations

Conflicts of interest/Competing interests None.

References

Agarie S, Uchida H, Agata W, Kaufman PB (1999) Effects of silicon on stomatal blue-light response in rice (*Oryza sativa* L.). Plant Prod Sci-Tokyo- 2:232–234

Ben-Gal A, Shani U (2002) A highly conductive drainage extension to control the lower boundary condition of lysimeters. Plant Soil 239:9–17. https://doi.org/10.1023/A:1014942024573

Chain F, Côté-Beaulieu C, Belzile F et al (2009) A comprehensive transcriptomic analysis of the effect of silicon on wheat plants under control and pathogen stress conditions. Mol Plant Microbe Interact 22:1323–1330

Cooke J, Leishman MR (2016) Consistent alleviation of abiotic stress with silicon addition: a meta-analysis. Funct Ecol 30:1340–1357. https://doi.org/10.1111/1365-2435.12713

Coskun D, Deshmukh R, Sonah H et al (2019) The controversies of silicon's role in plant biology. New Phytol 221:67–85. https://doi.org/10.1111/nph.15343

Deshmukh R, Bélanger RR (2016) Molecular evolution of aquaporins and silicon influx in plants. Funct Ecol 30:1277–1285. https://doi.org/10.1111/1365-2435.12570

Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci USA 91:11–17. PMC42876

Epstein E (1999) Silicon. Annu Rev Plant Physiol Plant Mol Biol 50(641–664):15012222

Gao X, Zou C, Wang L, Zhang F (2006) Silicon decreases transpiration rate and conductance from stomata of maize plants. J Plant Nutr 29:1637–1647. https://doi.org/10.1080/01904160600851494

Gong H, Chen K (2012) The regulatory role of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat leaves in field drought conditions. Acta Physiol Plant 34:1589–1594. https://doi.org/10.1007/s11738-012-0954-6

Gong H, Chen K, Chen G et al (2003) Effects of Silicon on Growth of Wheat Under Drought. J Plant Nutr 26:1055–1063. https://doi.org/10.1081/PLN-120020075

Halperin O, Gebremedhin A, Wallach R, Moshelion M (2017) High-throughput physiological phenotyping and screening system for the characterization of plant–environment interactions. Plant J 89:839–850. https://doi.org/10.1111/tpj.13425

Hattori T, Inanaga S, Araki H et al (2005) Application of silicon enhanced drought tolerance in *Sorghum bicolor*. Physiol Plant 123:459–466. https://doi.org/10.1111/j.1399-3054.2005.00481.x

Hattori T, Sonobe K, Inanaga S et al (2007) Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. Environ Exp Bot 60:177–182. https://doi.org/10.1016/j.envexpbot.2006.10.004

Hodson MJ, White PJ, Mead A, Broadley MR (2005) Phylogenetic variation in the silicon composition of plants. Ann Bot 96:1027–1046. https://doi.org/10.1093/aob/mci255

Kollist H, Nuhkat M, Roelfsema MRG (2014) Closing gaps: linking elements that control stomatal movement. New Phytol n/a-n/a. https://doi.org/10.1111/nph.12832

Lazarovitch N, Ben-Gal A, Shani U (2006) An automated rotating lysimeter system for greenhouse evapotranspiration studies. Vadose Zone J 5:801–804. https://doi.org/10.2136/vzj2005.0137

Liang Y, Sun W, Zhu YG, Christie P (2007) Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. Environ Pollut 147:422–428. S0269–7491(06)00385-X

Ma JF, Tamai K, Ichii M, Wu GF (2002) A rice mutant defective in Si uptake. Plant Physiol 130:2111–2117. https://doi.org/10.1104/pp.010348

Ma JF, Tamai K, Yamaji N, et al (2006) A silicon transporter in rice. Nature 440:688–691. nature04590

Ma JF, Yamaji N (2015) A cooperative system of silicon transport in plants. Trends Plant Sci 20:435–442. https://doi.org/10.1016/j.tplants.2015.04.007

Markovich O, Kumar S, Cohen D et al (2019) Silicification in leaves of sorghum mutant with low silicon accumulation. SILICON 11:2385–2391. https://doi.org/10.1007/s12633-015-9348-x

Markovich O, Steiner E, Kouřil Š et al (2017) Silicon promotes cytokinin biosynthesis and delays senescence in Arabidopsis and Sorghum. Plant Cell Environ 40:1189–1196. https://doi.org/10.1111/pce.12913

Mitani N, Yamaji N, Ma J (2008) Characterization of substrate specificity of a rice silicon transporter, Lsi1. Pflüg Arch Eur J Physiol 456:679–686. https://doi.org/10.1007/s00424-007-0408-y

Sade N, Gebretsadik M, Seligmann R et al (2010) The role of tobacco Aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. Plant Physiol 152:245–254. https://doi.org/10.1104/pp.109.145854

Savant NK, Korndörfer GH, Datnoff LE, Snyder GH (1999) Silicon nutrition and sugarcane production: a review. J Plant Nutr 22:1853–1903

Schaller J, Cramer A, Carminati A, Zarebanadkouki M (2020) Biogenic amorphous silica as main driver for plant available water in soils. Sci Rep 10:2424. https://doi.org/10.1038/s41598-020-59437-x

Shi Y, Zhang Y, Han W, et al (2016) Silicon enhances water stress tolerance by improving root hydraulic conductance in *Solanum lycopersicum* L. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00196

Van Bockhaven J, Spíchal L, Novák O et al (2015) Silicon induces resistance to the brown spot fungus Cochliobolus miyabeanus by preventing the pathogen from hijacking the rice ethylene pathway. New Phytol 206:761–773. https://doi.org/10.1111/nph.13270

Verma KK, Song X-P, Lin B et al (2021) Silicon induced drought tolerance in crop plants: physiological adaptation strategies. SILICON. https://doi.org/10.1007/s12633-021-01071-x

Vivancos J, Labbé C, Menzies JG, Bélanger RR (2015) Silicon-mediated resistance of Arabidopsis against powdery mildew involves mechanisms other than the salicylic acid (SA)-dependent defence pathway. Mol Plant Pathol 16:572–582. https://doi.org/10.1111/mpp.12213

Zhu Y, Gong H (2014) Beneficial effects of silicon on salt and drought tolerance in plants. Agron Sustain Dev 34:455–472. https://doi.org/10.1007/s13593-013-0194-1