

Silicon nutrition alleviates the negative impacts of arsenic on the photosynthetic apparatus of rice leaves: an analysis of the key limitations of photosynthesis

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Silicon (Si) plays important roles in alleviating various abiotic stresses. In rice (*Oryza sativa*), arsenic (As) is believed to share the Si transport pathway for entry into roots, and Si has been demonstrated to decrease As concentrations. However, the physiological mechanisms through which Si might alleviate As toxicity in plants remain poorly elucidated. We combined detailed gas exchange measurements with chlorophyll fluorescence analysis to examine the effects of Si nutrition on photosynthetic performance in rice plants [a wild-type (WT) cultivar and its *lsi1* mutant defective in Si uptake] challenged with As (arsenite). As treatment impaired carbon fixation (particularly in the WT genotype) that was unrelated to photochemical or biochemical limitations but, rather, was largely associated with decreased leaf conductance at the stomata and mesophyll levels. Indeed, regardless of the genotypes, in the plants challenged with As, photosynthetic rates correlated strongly with both stomatal ($r^2 = 0.90$) and mesophyll ($r^2 = 0.95$) conductances, and these conductances were, in turn, linearly correlated with each other. The As-related impairments to carbon fixation could be considerably reverted by Si in a time- and genotype-dependent manner. In conclusion, we identified Si nutrition as an important target in an attempt to not only decrease As concentrations but also to ameliorate the photosynthetic performance of rice plants challenged with As.

Introduction

Pollution with transition metals and metalloids is an increasing environmental problem, and arsenic (As), in particular, is highly toxic for all life forms, including plants (Takahashi et al. 2004, Zhao et al. 2010). Plants take up As(V) (arsenate), the predominant form of As in aerated soils; however, in reducing environments such as in paddy soils, As(III) (arsenite) predominates (Takahashi et al. 2004). The main As(V) uptake pathway in

plants occurs through phosphate transporters, whereas As(III) is believed to be taken up through the nodulin 26-like intrinsic aquaporin channels (Panda et al. 2010). As accumulation in plants negatively impacts their morphology and physiology by inhibiting root and shoot growth, decreasing photosynthetic activity, altering carbohydrate and amino acid metabolism and inducing oxidative stress (Hoffmann and Schenk 2011, Finnegan and Chen 2012, Tripathi et al. 2012).

Abbreviations – BL, biochemical limitations; Chl, chlorophyll; DAA, days after As addition; DW, dry weight; EDO, Exhaustive Dual Optimization; ML, mesophyll limitations; PPFD, photosynthetic photon flux density; PS, photosystem; SL, stomatal limitations; WT, wild-type.

Silicon (Si) is not considered an essential element for higher plants, although it has been proven to be beneficial for improving the growth and crop yields of some plant species, such as rice (Ma and Takahashi 2002). Studies addressing Si nutrition have extensively demonstrated the ability of Si to alleviate biotic and abiotic stresses in a wide variety of plant species (Epstein 2009, Keeping and Reynolds 2009), including toxicity associated with metals, such as aluminum (Liang et al. 2001), boron (Gunes et al. 2007), cadmium (Shi et al. 2010), copper (Nowakowski and Nowakowska 1997), iron (You-Qiang et al. 2012), manganese (Li et al. 2012) and zinc (Neumann and zurNieden 2001). The ameliorative effect of Si on plants suffering from abiotic stresses is believed to occur, to a large extent, through counteracting oxidative stress via modulating antioxidant enzymes (Liang et al. 2007).

In rice roots, two genes encoding Si transporters (*Lsi1* and *Lsi2*) have been identified to date (Ma et al. 2006, 2007). Silicon is transported via *Lsi1* and *Lsi2* from the root epidermis into the root steles and then travels to the shoot with the transpirational water flow via the xylem (Ma et al. 2006). These Si transporters are associated with the great ability of rice to actively take up Si and could explain the high Si levels observed in this species, which can reach values as high as 10% of the shoot dry weight (Ma and Takahashi 2002). Intriguingly, high levels of *Lsi1* and *Lsi2* expression in rice have been observed to not only lead to high Si accumulation but also enhance As accumulation in rice shoots and grains (Ma et al. 2008), suggesting that As shares the Si transport pathway for entry into rice root cells. Indeed, increasing the Si concentration in a soil solution was observed to lead to decreased As accumulation in rice shoots and grains (Bogdan and Schenk 2008, Guo et al. 2009, Li et al. 2009), which most likely occurred through competitive inhibition of As(III) transport via *Lsi1* and *Lsi2* (Zhao et al. 2010).

Despite the recent progress in understanding the mechanisms underlying the uptake and distribution of As and how they can be affected by Si (Ma et al. 2008, Zhao et al. 2010), the physiological mechanisms by which Si might alleviate As toxicity in plants has received no attention until the study by Tripathi et al. (2013). These authors demonstrated that Si could mediate As(III) tolerance in rice by lowering As uptake and improving the antioxidant defense system. However, how these responses may impact key physiological processes such as photosynthesis have yet to be fully elucidated. Here, we hypothesized that Si application could mitigate the toxic effects of As through improving photosynthetic performance, which is expected to be further facilitated via the competitive inhibition effect of Si on As(III) uptake as observed in rice

(Ma et al. 2008, Zhao et al. 2010). To test this hypothesis, we conducted an in-depth analysis of photosynthetic performance and disentangled the relative contributions of stomatal (LS), mesophyll (ML), photochemical and biochemical limitations (BL) of photosynthesis in rice plants challenged with As and amended with Si. This analysis was realized by comparing wild-type (WT) rice (cv. 'Oochikara') with its Si uptake-defective *Lsi1* mutant.

Materials and methods

Plant material, growth conditions and experimental design

The experiment was conducted in Viçosa (20°45'S, 42°54'W, 650 m altitude) in south-eastern Brazil from April through June 2013. Rice (*Oryza sativa* L.) plants from cv. 'Oochikara' and its low-silicon 1 (*Lsi1*) mutant (Ma et al. 2006) were grown in a greenhouse in plastic pots with 5 l of a nutrient solution containing 1.0 mM KNO₃, 0.25 mM NH₄H₂PO₄, 0.1 mM NH₄Cl, 0.5 mM MgSO₄, 1.0 mM Ca(NO₃), 0.30 μM CuSO₄, 0.33 μM ZnSO₄, 11.5 μM H₃BO₃, 3.5 μM MnCl₂, 0.1 μM (NH₄)₆Mo₇O₂, 25 μM FeSO₄ and 25 μM EDTA bisodic, under semi-controlled conditions [maximum photosynthetic photon flux density (PPFD) inside the greenhouse was approximately 1500 μmol photons m⁻² s⁻¹ with an air temperature of 30 ± 2°C]. Si was supplied over the entire course of the experiment as monosilicic acid, which was prepared by passing potassium silicate through cation-exchange resin (Amberlite IR-120B, H⁺ form; Sigma-Aldrich, São Paulo, Brazil). The applied methodology has been detailed elsewhere (Dallagnol et al. 2011). Forty-five days after transplanting, As was applied in the form of NaAsO₂.

In a preliminary experiment, we tested the effects of two Si levels (0 or 2 mM) combined with four As levels (0, 15, 25 or 50 μM) on photosynthetic gas exchange parameters using the two above mentioned rice genotypes. The analyses were repeated at 5, 9 or 13 days after As addition (DAA). Regardless of the genotype, most plants grown with 50 μM As did not survive. The majority of the results obtained with 15 μM As differed only slightly from those obtained with 25 μM As; similarly, most results obtained with 9 DAA differed minimally when compared with those obtained with 13 DAA. Therefore, we performed detailed photosynthetic measurements using two As levels (0 or 25 μM) combined with two Si levels (0 or 2 mM) at 5 and 13 DAA for simplicity. Our Si treatment concentration was chosen based on environmental relevance (Takahashi et al. 2004) as well as on previous studies in which such a concentration resulted

in improved growth for the rice plants (Dallagnol et al. 2011). Furthermore, we chose to use 25 μM As based on the above described preliminary trial [remarkable decreases in A (particularly in the WT plants) without killing the plants].

The experiment was performed using a completely randomized design, with six plants in individual pots per treatment serving as conditional replicates. The pots (96 in total) were randomized periodically to minimize any variation among treatments.

Si and As concentrations

The youngest fully expanded leaves and the bulk root system were collected, and the Si concentrations in these tissues were determined colorimetrically according to Dallagnol et al. (2011). To quantify As, plant tissues were oven-dried under hot air at 40°C until a constant weight was reached. The tissues were then digested using a mixture of HNO_3 and H_2O_2 (3:1 by vol.), after which As was quantitatively analyzed using an inductively coupled plasma emission spectrometer (Optima3300 DV; Perkin Elmer, Bethesda, MD). Further details have been described elsewhere (Rofkar et al. 2007).

Gas exchange and chlorophyll a fluorescence measurements

The leaf gas exchange parameters were determined simultaneously via conducting measurements of chlorophyll a (Chl a) fluorescence using two cross-calibrated portable open-flow gas exchange systems (LI-6400XT; LI-COR Inc., Lincoln, NE) equipped with integrated fluorescence chamber heads (LI-6400-40; LI-COR Inc.). The net CO_2 assimilation rate (A) and stomatal conductance to water vapor [which was subsequently converted into stomatal conductance to CO_2 (g_s)] were measured on attached, fully expanded leaves from 10:00 to 13:00 h (solar time), which is when A was at its maximum, under artificial PPFD, i.e. 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf level and 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air. All measurements were performed by fixing the block temperature at 25°C, and the vapor pressure deficit was maintained at approximately 1.0 kPa while the amount of blue light was set to 10% of PPFD to optimize the stomatal aperture.

Previously dark-adapted (30 min) leaf tissues were illuminated with weak modulated measuring beams (0.03 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to obtain the initial fluorescence (F_0). Saturating white light pulses of 8000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were applied for 0.8 s to ensure maximum fluorescence emissions (F_m), from which the variable-to-maximum

Chl fluorescence ratio, $F_v/F_m = [(F_m - F_0)/F_m]$, was calculated. In light-adapted leaves, the steady-state fluorescence yield (F_s) was measured after registering the gas exchange parameters. Then, a saturating white light pulse (8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 0.8 s) was applied to achieve the light-adapted maximum fluorescence (F_m'). The actinic light was then turned off, and far-red illumination was applied (2 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to measure the light-adapted initial fluorescence (F_0'). Using the values of these parameters, the photochemical quenching coefficient (q_p) and the capture efficiency of excitation energy by open photosystem (PS) II reaction centers (F_v'/F_m') were estimated (Logan et al. 2007). The actual PSII photochemical efficiency (ϕ_{PSII}) was determined as $\phi_{\text{PSII}} = (F_m' - F_s)/F_m'$ following the procedures of Genty et al. (1989). The electron transport rate (J) was then calculated from the equation $J = \phi_{\text{PSII}} \beta \alpha \text{PPFD}$, where α is leaf absorbance, and β reflects the partitioning of absorbed quanta between PS II and PS I. The product of β and α was determined according to Valentini et al. (1995) from the relationship between ϕ_{PSII} and ϕ_{CO_2} obtained by varying the light intensity under non-photorespiratory conditions.

The mitochondrial respiration rate in the light (R_l) was determined according to Martins et al. (2013) as the value that forces the intercept of the plot A vs $C_i - C_c$ (chloroplastic CO_2 concentration) to be zero using points in the C_i range strictly limited by Rubisco ($C_i < 300 \mu\text{mol mol}^{-1}$ air).

The partitioning of electrons between photosynthesis (J_c) and photorespiration (J_o) was obtained as described elsewhere (Valentini et al. 1995, Long and Bernacchi 2003) using the following equations:

$$J_c = 1/3 [J + 8 (A + R_l)] \quad (1)$$

$$J_o = 2/3 [J - 4 (A + R_l)] \quad (2)$$

Four to six A/C_i curves were obtained from different plants per treatment. These curves were initiated at an ambient CO_2 concentration (C_a) of 400 $\mu\text{mol mol}^{-1}$ under a saturating PPFD of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Once a steady state was reached, C_a was gradually decreased to 50 $\mu\text{mol mol}^{-1}$ air. Upon completion of the measurements at low C_a , C_a was returned to 400 $\mu\text{mol mol}^{-1}$ air to restore the original A. Next, C_a was increased stepwise to 2000 $\mu\text{mol mol}^{-1}$ air. A/C_i curves consisted of 13 different C_a values. Corrections for the leakage of CO_2 into and out of the leaf chamber of the LI-6400 were applied to all gas exchange data as described by Rodeghiero et al. (2007).

Estimations of mesophyll conductance to CO₂ (g_m), the maximum rate of carboxylation (V_{cmax}), the maximum rate of carboxylation limited by electron transport (J_{max}) and quantitative analysis of limitations of photosynthesis.

C_c was estimated according to Harley et al. (1992) as follows:

$$C_c = (\Gamma^* (J + 8 (A + R_i)) / (J - 4 (A + R_i))) \quad (3)$$

where J and A were obtained from the gas exchange and Chl fluorescence measurements conducted under saturating light; R_i was estimated as described above; and Γ* is the chloroplastic CO₂ photocompensation point in the absence of mitochondrial respiration (the conservative value of Γ* for rice was obtained from Li et al. 2009).

The CO₂ conductance from intercellular airspaces to the sites of CO₂ fixation in the stroma of chloroplasts, termed mesophyll conductance (g_m), was estimated as the slope of the A vs C_i – C_c relationship as

$$A = g_m (C_i - C_c) \quad (4)$$

so the estimated g_m is an averaged value over the points used in the relationship (C_i < 300 μmol mol⁻¹ air).

As all of the available methods for estimating g_m rely on models that include several assumptions as well as technical limitations and sources of error that need to be considered to obtain reliable estimates of this parameter (Pons et al. 2009), g_m was also estimated using an alternative approach, the Exhaustive Dual Optimization (EDO) curve-fitting technique of Gu et al. (2010). For this purpose, data from A/C_i curves were uploaded to the Oak Ridge National Laboratory (USA) website (www.leafweb.ornl.gov), which uses EDO to parameterize A/C_i curves based on the Farquhar-von Caemmerer-Berry model (Gu et al. 2010). Averaging combined Harley-derived g_m values resulted in a significant relationship (r² = 0.71, P < 0.05) with g_m values estimated using the EDO approach (Fig. S1). On the basis of such a relationship, all of the g_m values reported below were obtained using the Harley et al. (1992) technique. Additionally, we also observed a significant correlation (r² = 0.70, P < 0.01) between the R_i used in our study and the R_i given as an output of the EDO approach (data not shown) and thus corroborated the validity of the respiration value used in the g_m estimates.

From the A – C_c curves, V_{cmax} and J_{max} were calculated by fitting the mechanistic model of CO₂ assimilation proposed by Farquhar et al. (1980) using the C_c-based temperature dependence of kinetic parameters of Rubisco (K_c and K_o) (Bernacchi et al. 2002). The

curve-fitting procedures have been detailed elsewhere (Detmann et al. 2012). Later, the photosynthetic parameters V_{cmax}, J_{max} and g_m were normalized to 25°C using the temperature response equations from Sharkey et al. (2007).

The overall photosynthetic limitations were partitioned into their functional components (LS, ML and BL) using the values of A, g_s, g_m, V_{cmax}, Γ*, C_c and K_m = K_c (1 + O/K_o) based on the approach proposed by Grassi and Magnani (2005).

$$LS = \frac{\left(\frac{g_{tot}}{g_s} \times \frac{\partial A}{\partial C_c} \right)}{\left(g_{tot} + \frac{\partial A}{\partial C_c} \right)} \quad (5)$$

$$LM = \frac{\left(\frac{g_{tot}}{g_m} \times \frac{\partial A}{\partial C_c} \right)}{\left(g_{tot} + \frac{\partial A}{\partial C_c} \right)} \quad (6)$$

$$LB = \frac{g_{tot}}{\left(g_{tot} + \frac{\partial A}{\partial C_c} \right)} \quad (7)$$

where g_s is the stomatal conductance to CO₂, g_m is the mesophyll diffusion conductance and g_{tot} is the total conductance to CO₂ from ambient air to chloroplasts (g_{tot} = 1/[(1/g_s) + (1/g_m)]). ∂A/∂C_c was calculated as:

$$\frac{\partial A}{\partial C_c} = \frac{[V_{cmax} (\Gamma^* + K_m)]}{(C_c + K_m)^2} \quad (8)$$

Statistical analysis

The data obtained from the experiment were analyzed using a completely randomized design following a 2 × 2 × 2 factorial scheme (two genotypes × two Si levels × two As levels) with six replicates. The data were subjected to an analysis of variance (three-way ANOVA with all main factors evaluated as fixed factors) performed using the MIXED procedure of SAS (version 9.1) and an α = 0.05. When any interaction was significant, the MIXED Slice statement was used to interpret the dependency effect between the factors. Regression analyses were used to examine the relationships among the variables.

Results

Si addition increases Si levels and decreases As levels

Both Si and As levels were higher at 13 than at 5 DAA in both leaves (Fig. 1) and roots (Fig. S2). As would be expected, Si addition remarkably increased the leaf Si concentration (Fig. 1A, B). Among +Si plants, the Si concentrations were significantly higher in WT [17.5 g kg⁻¹

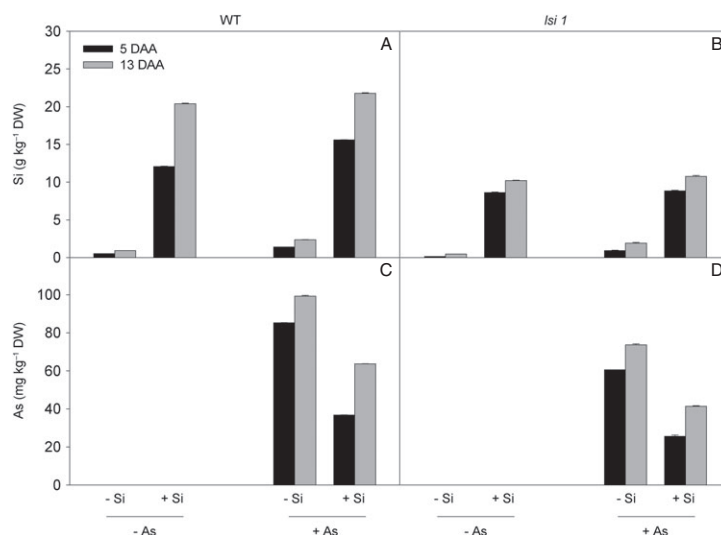


Fig. 1. The effects of silicon, Si (0 or 2 mM: –Si or +Si, respectively), and arsenic, As (0 or 25 μ M: –As and +As, respectively), on the leaf concentrations of Si (A and B) and As (C and D) of the two rice genotypes [cv. ‘Oochikara’ (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solutions. The measurements were performed at 5 or 13 days after As addition (DAA). $n = 6 \pm \text{SE}$. Arsenic was not detected in –As plants.

Table 1. The results (significance: ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) of ANOVA for the effects of silicon (Si), arsenic (As) and genotype (Gt) and their interactions are presented for the tested concentrations of Si and As, net CO₂ assimilation rate (A), stomatal conductance (g_s), mesophyll conductance (g_m), maximum rate of carboxylation limited by electron transport (J_{max}), maximum rate of carboxylation (V_{cmax}), ratio of electron transport rate devoted to oxygenation/carboxylation (J_o/J_c), stomatal limitations (SL), mesophyll limitations (ML), biochemical limitations (BL), variable-to-maximum chlorophyll fluorescence ratio (F_v/F_m), efficiency of the capture of excitation energy by open photosystem II reaction centers (F_v'/F_m') and photochemical quenching coefficient (q_p). The results are presented in the form of x/y, i.e. at 5 or 13 days after As addition. When a single result is presented, it represents the same significance for both days.

Parameters	Si	As	Gt	Si $\times$ As	Si $\times$ Gt	As $\times$ Gt	Si $\times$ As $\times$ Gt
Si	***	***	***	***	***	***/**	***/**
As	***	***	***	***	***	***	***
A	ns/**	***	*	ns/**	ns	*/***	ns
g_s	*	***	*/ns	ns	ns	*/ns	ns
g_m	ns/*	ns/**	**/ns	ns/**	ns	ns/**	ns
J_{max}	ns	ns/**	ns/*	ns	ns	ns/*	ns
V_{cmax}	ns	ns	ns/*	ns	ns	ns/**	ns
J_o/J_c	ns	ns/**	*/ns	ns/**	ns/**	ns	ns
SL	ns	**/**	ns	ns	ns	*/ns	ns
ML	ns	ns/**	**/ns	ns/**	ns/**	ns	ns/*
BL	ns/*	ns/**	***/*	ns/**	ns/*	*/ns	ns
F_v/F_m	*	**/**	*/ns	**/*	ns	ns	**
F_v'/F_m'	ns	ns	ns	ns	ns	ns	ns
q_p	ns	ns/**	ns	ns/**	ns	ns	ns

DW (dry weight) on average; Fig. 1A] than in *lsi1* mutant plants (9.6 g kg^{–1} DW on average; Fig. 1B). Curiously, Si levels were higher (19% on average) in +As plants compared with –As individuals (significant Si $\times$ As interaction; Table 1). This genotype-dependent pattern (significant Si $\times$ As $\times$ genotype interaction; Table 1) is likely a result of growth inhibition associated with As [visual observation; also see Yu et al. (2012)] and thus concentrating Si levels in plant tissues. Quite similar results were observed in roots (Fig. S2), although the Si

contents were lower in the root than in the leaf tissues, particularly in +Si individuals.

We were unable to detect As in plants that were not treated with this metalloid. Regardless of the sampling date and genotype, As levels decreased significantly (48% in leaves and 12% in roots, on average) in +Si plants compared with their –Si counterparts (Fig. 1C, D, Table 1). Within a given Si treatment, the As levels were lower in the mutant than in the WT plants (30% on average), where a significant Si $\times$ As $\times$ genotype

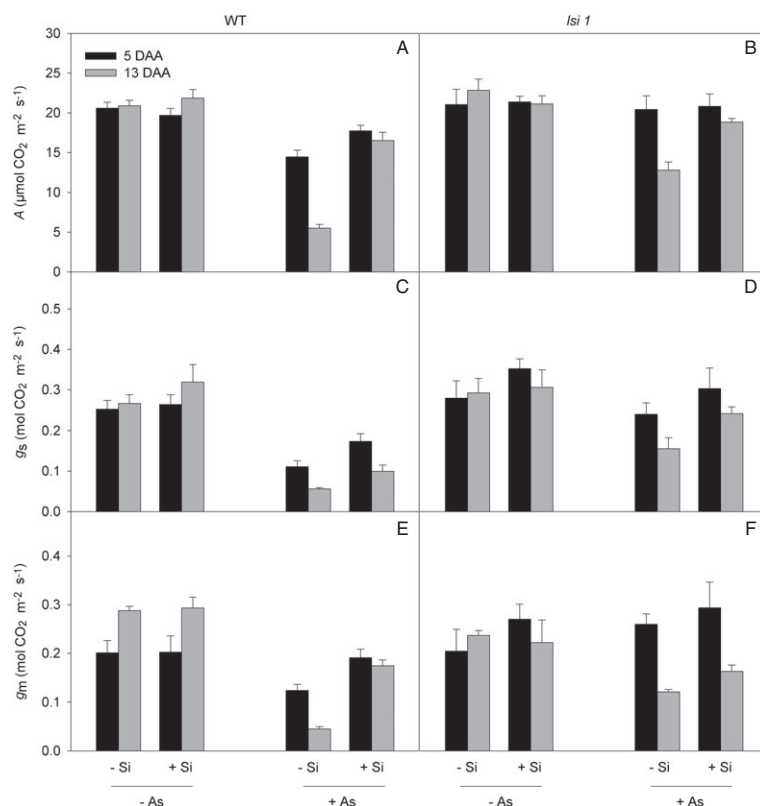


Fig. 2. The effects of silicon, Si (0 or 2 mM: -Si or +Si, respectively), and arsenic, As (0 or 25 μM: -As and +As, respectively), on the net CO₂ assimilation rate, A (A and B), stomatal conductance to CO₂, g_s (C and D) and mesophyll conductance to CO₂, g_m (E and F) in the two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solutions. The measurements were performed at 5 or 13 days after As addition (DAA). For A and g_s, the data are the means of two independent experiments (n = 12 ± SE); for g_m, n = 4–6 ± SE.

interaction was detected. In sharp contrast to the situation observed for Si, As levels were remarkably higher in the roots (200% on average) than in the leaves (Figs 1C, D and S2).

The negative effects of As on photosynthetic rates may be reversed by Si in a genotype- and time-dependent manner

The Si supply did not affect A in plants not treated with As. Overall, As treatment decreased A, particularly in -Si plants (significant Si × As interaction) (Fig. 2A, B, Table 1). These decreases were more pronounced at 13 than at 5 DAA, especially in WT plants (significant As × genotype interaction) (Table 1), which displayed remarkably lower A values than *lsi1* plants (Fig. 2A, B). Most importantly, the negative effects of As on A were partially reversed by the addition of Si to WT plants, as noted at 13 DAA; such effects were only slight (5 DAA) or even non-existent (13 DAA) in *lsi1* plants (significant As × genotype interaction) (Fig. 2A, B, Table 1).

The As-induced decreases in photosynthetic rates were mirrored by changes in both g_s and g_m

Changes in A were accompanied by changes in both g_s (Fig. 2C, D) and g_m (Fig. 2E, F) that were also time- and genotype-dependent, i.e. the decreases in g_s and g_m induced by As were more pronounced at 13 than at 5 DAA (although not significant for g_m at 5 DAA; Table 1), more in WT plants than their *lsi1* counterparts, and such decreases could, to a larger extent, be reversed by the addition of Si (Fig. 2C, D). In fact, in the plants challenged with As, A correlated logarithmically with both g_s (r² = 0.90, P < 0.001) and g_m (r² = 0.95, P < 0.001), and g_s and g_m were, in turn, linearly correlated with each other (r² = 0.86, P < 0.01) (Fig. 3).

The V_{cmax} was unresponsive to the treatments, whereas the J_{max} and J_o/J_c ratio were altered by As in a time- and genotype-dependent manner

Regardless of the genotypes, V_{cmax} was unresponsive to Si and As (and their interactions), although a significant As × genotype interaction was observed at

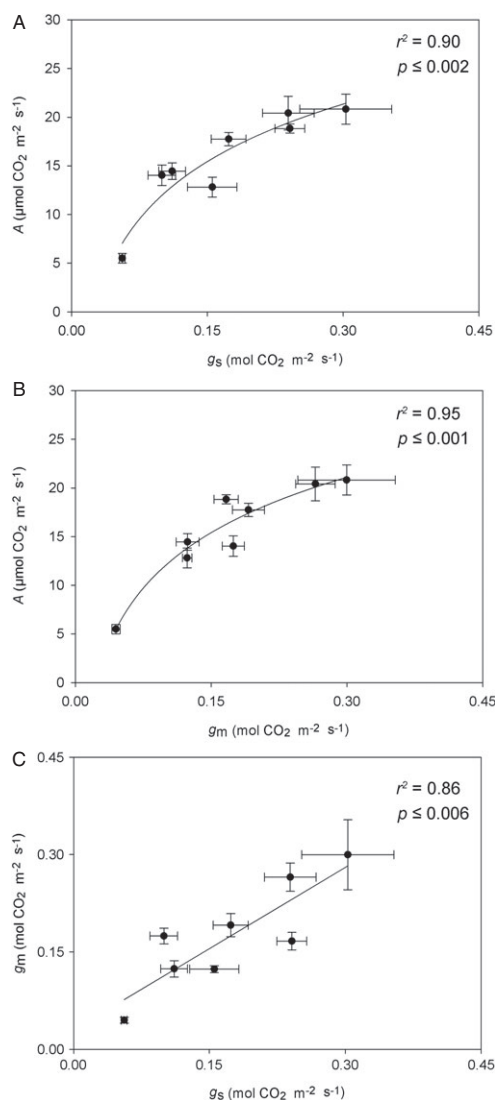


Fig. 3. The relationships among the net CO_2 assimilation rate (A), stomatal conductance to CO_2 (g_s) and mesophyll conductance to CO_2 : (A) A and g_s , (B) A and g_m ; (C) g_s and g_m . $n = 8 \pm \text{SE}$.

13 DAA (Fig. 4A, B, Table 1). In contrast, J_{max} (Fig. 4C, D) decreased in the presence of As but only in WT plants at 13 DAA (significant As $\times$ genotype interaction; Table 1). Notably, Si addition did not observably restore J_{max} under As stress (not significant Si $\times$ As interaction; Table 1). Whereas some decreases in J_c were evident in both WT and *lsi1* plants at 13 DAA, J_o changed minimally, if at all, irrespective of the Si and As supplies (data not shown). Taken together, these changes significantly increased the J_o/J_c ratio (Fig. 4E, F), particularly in $-\text{Si}$ WT plants treated with As at 13 DAA (significant Si $\times$ As and As $\times$ genotype interactions), suggesting that a relative increase in electron flow to photorespiration is expected to occur in these plants.

Arsenite impacts the photosynthetic performance by limiting CO_2 diffusion

Under control ($-\text{As}$) conditions, approximately 20, 30 and 50% of the total limitations to photosynthesis were observed for SL, ML and BL, respectively, regardless of the genotype (Fig. 5). The overall components of the photosynthetic limitations changed across the $+\text{As}$ treatments in different ways. The SL (Fig. 5A, B) increased (at both 5 and 13 DAA) in $+\text{As}$ plants independent of Si and the genotype (not significant Si $\times$ As and As $\times$ genotype interactions; Table 1). The ML (Fig. 5C, D) changed in plants fed with As (only at 13 DAA) in a complex manner, as noted by the increased ML in $-\text{Si}$ WT plants and in *lsi1* individuals regardless of the Si supply (significant Si $\times$ As, Si $\times$ genotype and Si $\times$ As $\times$ genotype interactions; Table 1). In contrast, BL (Fig. 5E, F) decreased in response to As at 13 DAA independent of the genotype (not significant As $\times$ genotype interaction; Table 1), where a significant Si $\times$ As interaction was detected. Notably, in $-\text{Si}$ WT plants fed As, which displayed the lowest A values recorded at 13 DAA (Fig. 2A), the SL and ML concomitantly accounted for approximately 80% of the total limitation to photosynthesis.

Photochemical events are minimally affected by Si and As levels

The F_v/F_m ratio, which represents the maximum PSII photochemical efficiency, decreased slightly upon As addition (Fig. 6A, B). This effect was mostly observed in $-\text{Si}$ plants independent of the genotype. The q_p (Fig. 6C, D) was affected by As, where a significant As $\times$ Si interaction was detected as observed at 13 DAA (Table 1). However, F_v'/F_m' (Fig. 6E, F) remained virtually unchanged regardless of the treatments (Table 1). Both the F_v/F_m ratio and q_p varied minimally across the treatments; therefore, the photochemical factors are unlikely to account for the differences observed in A.

Discussion

Both the Si and As concentrations in leaf tissues were manipulated by changing the supply of these elements to the culture solution and by using the low-Si rice mutant *lsi1*, which is less able to take up both Si and As than WT (Ma et al. 2008). This approach revealed new insights into the physiological mechanisms through which Si might alleviate As toxicity on the photosynthetic process of rice. We demonstrated that As decreased the leaf conductance at the stomata and mesophyll levels, the most prominently affected photosynthesis-related parameters. We provided compelling evidence demonstrating that Si can, to a large extent, revert the negative

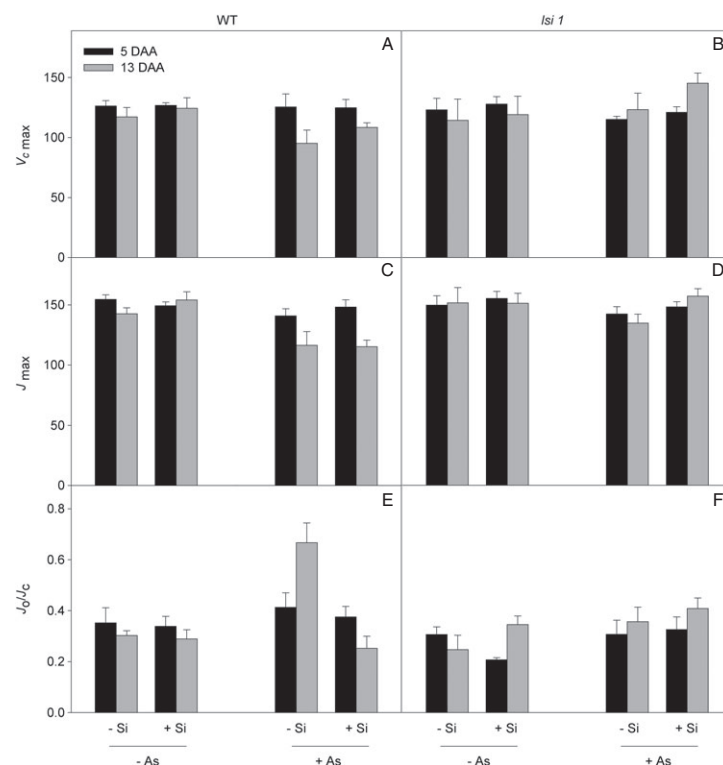


Fig. 4. The effects of silicon, Si (0 or 2 mM: -Si or +Si, respectively), and arsenic, As (0 or 25 μ M: -As and +As, respectively), on the maximum rate of carboxylation, $V_{c\max}$ (A and B), maximum rate of carboxylation limited by electron transport, $J_{\max}$ (C and D) and the ratio of electron transport rate devoted to oxygenation/carboxylation, J_o/J_c (E and F), in the two rice genotypes [cv. 'Oochikara' (WT) and the *lsi1* mutant defective for Si uptake] grown in nutrient solutions. The measurements were performed at 5 or 13 days after As addition (DAA). $n = 4-6 \pm$ SE.

impacts of As on photosynthetic performance in a time- and genotype-dependent manner. We therefore identified Si nutrition as a central player in preserving, at least partially, the photosynthetic activity in As-treated rice plants, which agreed well with our working hypothesis.

As previously demonstrated (Detmann et al. 2012), Si did not affect A in rice plants during the vegetative growth stage under unstressed (-As) conditions. In contrast, we demonstrated that As strongly impaired A in a time- and genotype-dependent manner (Fig. 2, Table 1). When the As concentration increased in plant tissues upon As addition, its toxic effects on A would be expected to increase accordingly over time. By decreasing the As levels in rice plants (Fig. 1), as reported elsewhere (Ma et al. 2008, Zhao et al. 2010), Si reverted most of the toxic effects of As, particularly in the *lsi1* mutant, which displayed the lowest As concentrations recorded. Indeed, in both WT and *lsi1* plants challenged with As, A was unaltered from 5 to 13 DAA in the presence of Si despite remarkable increases in As levels in this period combined with long-term (13 days) As exposure. Taken together, these data indicate that there was an acclimation of the biochemical reactions

involved in CO_2 fixation to As stress, which was mediated by an uncharacterized role of Si. We recently suggested that Si likely plays an unidentified role in rice metabolism, even under unstressed conditions (Detmann et al. 2013).

We obtained compelling evidence that demonstrated that the effects of As on A were largely associated with diffusive constraints. In fact, As appeared to primarily affect A through reductions in g_s that were exacerbated over time. This result is in sharp contrast with a recent report that demonstrated that decreases in A were accompanied by invariant g_s in *Hydrilla verticillata* treated with As (Srivastava et al. 2013). In addition, the diffusive limitations imposed by the mesophyll are also likely to have played an important role in constraining A as suggested by the substantial reductions in g_m in the plants challenged with As. Indeed, the close association between g_m and g_s (Fig. 3) suggests that an intrinsic co-regulation of these conductances (Flexas et al. 2008, 2012) is maintained under As stress conditions, which was also observed in sugar beets grown with excess zinc (Sagardoy et al. 2010). To our knowledge, this is the first report of a direct effect of As on g_m , but the mechanism

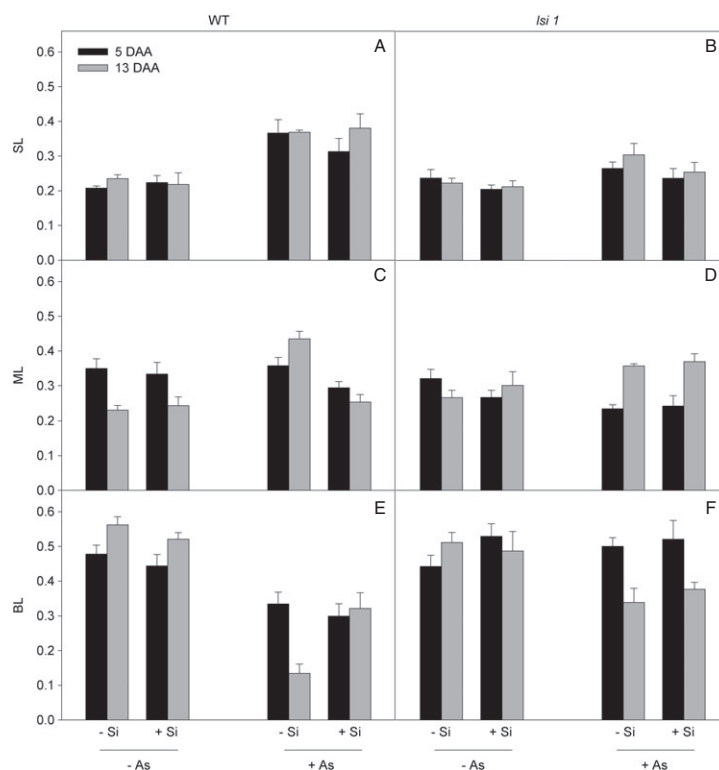


Fig. 5. The effects of silicon, Si (0 or 2 mM: -Si or +Si, respectively), and arsenic, As (0 or 25 μ M: -As and +As, respectively) on the functional components of the overall photosynthetic limitations: stomatal limitations, SL (A and B), mesophyll limitations, ML (C and D) and biochemical limitations, BL (E and F), in the two rice genotypes [cv. 'Oochikara' (WT) and the *Isi1* mutant defective for Si uptake] grown in nutrient solutions. The measurements were performed at 5 or 13 days after As addition (DAA). $n = 4-6 \pm \text{SE}$.

underlying this relationship is not immediately evident. Hoffmann and Schenk (2011) recently suggested that symptoms of As stress in rice plants were associated with increasing As(III) binding on the outer side of the plasmalemma to proteins such as aquaporins; if so, the observed decreases in g_m might be linked to impaired aquaporin activity, as these proteins are an important component governing g_m (Tholen and Zhu 2011, Flexas et al. 2012). In contrast, Si may increase g_m (Detmann et al. 2012) coupled with increased aquaporin activity (Lavinsky and DaMatta, unpublished results) in rice leaves. These assumptions might provide a mechanistic link that may, at least partially, explain the ameliorative effects of Si on A via preservation of g_m in rice plants challenged with As.

Overall, we demonstrated that BL (and photochemical limitations) should be ruled out as a prime constraint to explain the decreases in A induced by As. First, the relatively high J_{max} values observed, even in the treatments in which A was severely impaired, suggest that the synthesis of both ATP and NADPH required to fuel carbon fixation reactions was largely uncompromised in As-treated plants, in contrast to what has

been proposed elsewhere (Finnegan and Chen 2012). Second, the lack of treatment effect on V_{cmax} implies that the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) capacity to fix CO_2 was preserved. This result somewhat contrasts with data obtained in As-treated rice plants by Ahsan et al. (2010), who observed a down-regulation of the Rubisco large subunit and the chloroplast 29 kDa ribonucleoproteins.

Given that carbon fixation, which generally represents the main sink for absorbed light in chloroplasts, was found to be depressed, especially in -Si WT plants, adjustment of light capture, use and dissipation is required to provide photoprotection to the photosynthetic apparatus. Here, we demonstrated that Chl a fluorescence parameters varied minimally, if at all, which suggests that light capture and use also differed minimally across the treatments. Indeed, because both F_v/F_m and F_v'/F_m' (a useful proxy for thermal dissipation; Logan et al. 2007) were relatively unresponsive to the imposed stress, modification/inactivation of the PSII reaction center and photoprotection by xanthophylls engaged in sustained thermal energy dissipation may have been unlikely (Logan et al. 2007) in this current

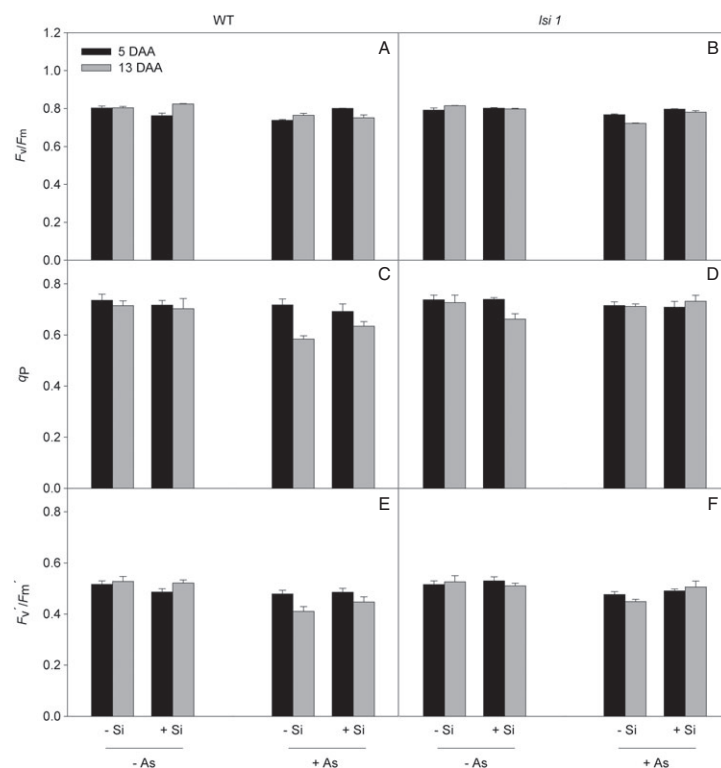


Fig. 6. The effects of silicon, Si (0 or 2 mM: -Si or +Si, respectively), and arsenic, As (0 or 25 μ M: -As and +As, respectively), on the variable-to-maximum chlorophyll fluorescence ratio, F_v/F_m (A and B), photochemical quenching coefficient, q_p (C and D) and efficiency of the capture of excitation energy by open PSII reaction centers, F_v'/F_m' (E and F), in the two rice genotypes [cv. 'Oochikara' (WT) and the *Isi 1* mutant defective for Si uptake] grown in nutrient solutions. The measurements were performed at 5 or 13 days after As addition (DAA). The data are the means of two independent experiments ($n = 12 \pm \text{SE}$).

study. Given that the J_o/J_c ratio tended to increase under As stress conditions, we contend that photorespiration should have acted as a key pathway for dissipating excess energy. Other alternative pathways, such as the cyclic flow of electrons within PSII or the Mehler-peroxidase reaction, (Logan et al. 2006; Foyer and Shigeoka 2011) could also have played a role in dissipating the excess reducing power under elevated As.

In conclusion, we demonstrated that As remarkably decreased A by fundamentally impairing the diffusion of CO_2 at the stomata and mesophyll levels with no apparent effect on the photosynthetic photochemistry and biochemistry. The ameliorative effects of Si on A observed in rice plants challenged with As were initially largely related to the decreased leaf As concentrations in the plants amended with Si. Nonetheless, the preservation of the photosynthetic biochemistry in +Si plants despite the increased As levels also suggests that Si likely has an uncharacterized function in rice physiology, which has recently been suggested (Detmann et al. 2013). In summary, we identified Si nutrition as an important target in an attempt to not only decrease As

concentrations but also to improve the photosynthetic performance of rice plants challenged with As in a time- and genotype-dependent manner.

Author contributions

L. M. V. P. S., A. O. L. and F. M. D. have conceived and designed the experiments. L. M. V. P. S., S. C. V. M., K. C. D., P. E. M. S., A. O. L. and M. M. S. have performed the experiments. L. M. V. P. S., S. C. V. M., E. D., W. L. A. and F. M. D. have analyzed the data. L. M. V. P. S., W. L. A. and F. M. D. wrote the paper.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. The relationship of mesophyll conductance (g_m) values estimated using two independent methods.

Fig. S2. The silicon and arsenic root concentrations of the two rice genotypes.