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# The role of silicon in metabolic acclimation of rice plants challenged with arsenic

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ABSTRACT

Silicon (Si) plays key roles in alleviating various abiotic stresses, including arsenic (As) toxicity, via physiological mechanisms that remain poorly understood. Here, we combined photosynthetic measurements with analyses of central metabolism and gene expression to explore the consequences of As-related alterations on primary metabolism and examined whether these consequences could be affected by the application of Si to rice (*Oryza sativa* L.) plants challenged with As but supplemented with Si. The negative effects of As on photosynthesis and carbohydrate status were largely reversed by Si. However, no major metabolic reprogramming was observed, as denoted by minor, if any, significant changes in (i) the activities of a range of enzymes associated with C metabolism; (ii) the levels of a wide range of organic acids and amino acids; and (iii) the pools of NAD(P)H/NAD(P)$^+$ and the redox states of ascorbate and glutathione. Arsenic toxicity was apparently unrelated to oxidative stress. We suggested that the search for As-tolerant plants under real field conditions should not focus solely on oxidative stress, and hence the focus on photosynthesis might be of higher significance. In conclusion, we identified Si nutrition as a central player that restricts photosynthetic impairment in As-treated plants, in addition to limiting As uptake via modulation of the expression of genes with prime importance in As uptake and translocation.



## 1. Introduction

Arsenic (As) is a non-essential metalloid that is highly toxic to most plants and animals (Takahashi et al., 2004; Zhao et al., 2010). Inorganic As is the prevalent form present in the environment and exists as As(V) (arsenate), which is the predominant form in aerated soils, and as As(III) (arsenite), which prevails in reducing environments such as paddy soils (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 proteins, a subclass of the water channel aquaporins (Panda et al., 2010). Among these proteins, some members are silicon (Si) transporters that are able to load As(III) into the xylem or secrete As(III) outside of the roots (Zhao et al., 2010). Within the cell, As(III) can be detoxified via a reaction with phytochelatins, and the resulting As(III)–phytochelatin complex is ultimately sequestered within vacuoles (Briat, 2010). The transport of that complex across the vacuolar tonoplast is believed to be

*Abbreviations:* *A*, net $CO_2$ assimilation rate; *ABC*, ATP binding cassette C; AGPase, ADP-glucose pyrophosphorylase; As, arsenic; As(III), arsenite; As(V), arsenate; AsA, ascorbate; ATP-PFK, ATP-dependent phosphofructokinase; Cd, cadmium; DHA, dehydroascorbate; ETR, apparent electron transport rate; $g_s$, stomatal conductance; GSH, reduced glutathione; GSSG, oxidised glutathione; Gt, genotype; $F_m$', light-adapted maximum fluorescence; $F_s$, steady-state fluorescence yield; *lsi1*, low-silicon 1; NAD-GAPDH, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase; NAD-MDH, NAD-dependent malate dehydrogenase; NADP-IDH, NADP-dependent isocitrate dehydrogenase; NSC, non-structural carbohydrate; PGK, phosphoglycerate kinase; PPFD, photosynthetic photon flux density; qRT-PCR, quantitative real-time PCR; RuBisCo, ribulose-1,5-bisphosphate carboxylase/oxygenase; Si, silicon; SPS, sucrose-phosphate synthase; Susy, sucrose synthase; TCA, tricarboxylic acids cycle; TPI, triose phosphate isomerase; WT, wild-type; $\Phi_{PSII}$, actual quantum yield of PSII electron transport.

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mediated by a C-type ATP-binding cassette (ABC) transporter (Song et al., 2010, 2014), which may therefore be of paramount importance for As tolerance in plants (Briat, 2010).

The underlying mechanisms through which As affects animals have been extensively elucidated; however, our current knowledge of how As affects plants under environmentally relevant conditions is still relatively limited (Finnegan and Chen, 2012; Kumar et al., 2015). Cumulative evidence suggests that As may inhibit root extension and proliferation, and upon translocation to the shoots, can induce oxidative stress. This effect is coupled with concomitant decreases in photosynthetic rates; as a consequence, growth and biomass accumulation are impaired and death may eventually occur (Finnegan and Chen, 2012; Tripathi et al., 2012; Kumar et al., 2015). As(III) toxicity is thought to be the result of As(III) reacting with the sulphydryl groups of proteins, thus interfering with enzymes and tissue protein functions (Delnomdedieu et al., 1993). Nevertheless, the effects of As on primary carbon metabolism in plants remain poorly understood, although the transcriptional profiles of genes encoding proteins in carbon metabolism seem to be largely unaltered both in *Arabidopsis thaliana* and rice (Finnegan and Chen, 2012; and references therein). Notably, most of the studies on the effects of As on plants have been centred on short-term exposure (*e.g.* Requejo and Tena, 2005; Kaur et al., 2012), which hampers plant acclimation responses, in conjunction with As levels that are usually much higher than those measured in most soils (*e.g.* Requejo and Tena, 2005; Srivastava et al., 2011; Hoffmann and Schenk, 2011; Marmiroli et al., 2014). In addition, the majority of experiments dealing with As have been performed in growth chambers with low irradiance (typically in the range of 100–350 μmol photons $m^{-2}$ $s^{-1}$ (*e.g.* Requejo and Tena, 2005; Shri et al., 2009; Srivastava et al., 2011; Kaur et al., 2012; Tripathi et al., 2012, 2013). Taken together, these conditions are far from those encountered by plants under real field conditions, and therefore, the key results of many reports on As toxicity have limited practical importance and as such should ultimately be cautiously considered. Furthermore, some negative effects of As on physiological traits, such as photosynthetic quantum yield and RuBisCo (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity, may well be short-term stress responses triggered upon moving a plant from an As-free to an As-treated environment (Finnegan and Chen, 2012).

Silicon has not been recognised as an essential element for plant growth, particularly because there is no compelling evidence showing its involvement in plant metabolism (Ma et al., 2011). In any case, Si has been proven to be beneficial for improving the growth and production of a range of crop species, particularly when they are under some form of imposed stress (Epstein, 2009), including toxicity associated with metals and metalloids (Wu et al., 2013; Adrees et al., 2015; Keller et al., 2015; Meharg and Meharg, 2015). The ameliorative effect of Si on plants suffering from abiotic stresses is believed to chiefly occur via counteraction of oxidative stress (Liang et al., 2007), although the understanding of the physiological mechanisms underlying the ability of Si to increase plant stress resistance remains largely elusive to date.

In rice roots, Si 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 sap (Ma et al., 2006). In the xylem, Si is present in the form of monosilicic acid and is unloaded by Lsi6, a homologue of Lsi1 in rice (Yamaji et al., 2008). However, in contrast to Lsi1 and Lsi2, Lsi6 is also expressed in the leaf sheaths and leaf blades in addition to the root tips of rice (Ma et al., 2011). In this species, both Si and As(III) are believed to compete for the same transport pathway via Lsi1 and Lsi2 (Ma et al., 2008), which could explain the substantial decreases in As accumulation in rice shoots and grains when Si is applied to the root environment (Bogdan and Schenk, 2008; Li et al., 2009).

The effects of Si on the uptake and translocation of As have received some attention in recent years (Ma et al., 2008; Zhao et al., 2010; Marmiroli et al., 2014). To our knowledge, however, only two studies (both on rice) have been undertaken, from a physiological perspective, to explore the mechanisms by which Si could alleviate As toxicity in plants. Tripathi et al. (2013) demonstrated that Si could mediate As(III) tolerance by lowering As uptake and improving the antioxidant defence system, whereas Sanglard et al. (2014) showed that As(III) limited carbon fixation by chiefly impairing leaf conductance at the stomatal and mesophyll levels and indicated that these As-related impairments could be largely reversed by Si. Regardless of how carbon fixation is constrained by As, the anticipated carbon shortage in As-treated plants is herein hypothesised to impact plant homeostasis with concordant alterations in central metabolism.

In this study, we explore the consequences of As-related decreases in photosynthetic rates on central metabolism, and determine whether these consequences could be affected by Si application in rice. To reach this goal, we use integrative approaches combining gas-exchange measurements, analyses of major metabolites, activities of a range of enzymes and the expression of some genes encoding for key enzymes of the central metabolism of rice plants (a wild-type (WT) cultivar and its *lsi1* mutant defective in Si uptake) challenged with As and amended with Si. Furthermore we investigate whether As and Si can affect the expression of genes associated with Si and/or As uptake and transport in both the roots and leaves of rice plants.

## 2. Materials and methods

### 2.1. Plant materials, growth conditions and experimental design

The experiments were carried out in Viçosa (20°45′S, 42°54′W, 650 m altitude) in southeastern Brazil from May to June 2013. Rice plants from cv. 'Oochikara' and its low-silicon 1 (*lsi1*) mutant (see Ma et al. (2006) for further details) were hydroponically grown in plastic pots (5 L volume) in a greenhouse with controlled air temperature (30/25 ± 2 °C (day/night) and naturally fluctuating photosynthetic photon flux density (PPFD). The maximum PPFD values inside the greenhouse (approx. 75% of light transmittance) were *c.* 1650 μmol photons $m^{-2}$ $s^{-1}$ as measured at midday. Si was supplied (0 or 2 mM) over the entire course of the experiment as monosilicic acid (which was prepared by passing potassium silicate through cation-exchange resin). Forty-five days after transplanting, As was applied (0 or 25 μM) in the form of $NaAsO_2$; sampling and measurements were performed at 10 days after the As additions. The Si and As(III) concentrations used here were chosen based on environmental relevance (Takahashi et al., 2004) as well as on previous studies in which Si concentration resulted in improved growth of rice plants (*e.g.* Dallagnol et al., 2011), whereas the As(III) concentration produced remarkable decreases in photosynthetic rates (particularly in the WT plants) without killing the plants (Sanglard et al., 2014). Further details of the applied methodology (*e.g.* seed germination, composition of the nutrient solutions, pH control, application of Si and As treatments, etc.) have been described by Dallagnol et al. (2011) and Sanglard et al. (2014).

The experiment had a completely randomised design, with six plants in individual pots (48 in total) per treatment combination serving as conditional replicates. The pots were randomised periodically to minimise any variation among treatments.

### 2.2. Si and As concentrations

The youngest fully expanded leaves and the bulk root system were collected, and their Si concentrations were determined