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The effect of silicon on the uptake and translocation of arsenic in tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Measuring tomato seed germination on a medium containing either arsenite or arsenate showed that the presence of 0.5 mM $NaH_2AsO_4{\cdot}7H_2O$ reduced germination by between 20% and 40%, depending on cultivar. The inhibitory effect was mitigated by the addition of $CaSiO_3$. However, the presence of both forms of As had a drastic negative effect on seedling shoot elongation, which was not mitigated by the presence of $CaSiO_3$. In a subsequent soil-based pot trial, damage due to the presence of As was visible by 15 days after the initiation of the treatment, and the provision of $CaSiO_3$ was significantly ameliorative; again, the severity of the effects was cultivar-dependent. Analysis of the accumulation and distribution of As showed that some of the cultivars are As excluders, and others accumulators. As was taken up by the latter cultivars whether or not $CaSiO_3$ supplementation was provided. The extent of As entry into the fruit varied from cultivar to cultivar, but never rose above the safety threshold. A survey of stress response-associated genes showed that LeGR was strongly up-regulated by exposure to As.

1. Introduction

The metalloid element Arsenic (As) is toxic to most life forms; in humans, its ingestion has been associated with a whole series of pathologies (Tondel et al., 1999; Brown et al., 1989), leading to its classification as a carcinogenic agent (Goyer, 1995). The element is present in natural systems in both organic (as mono or dimethylarsonic acid (MMA, DMA)) and inorganic (arsenate and arsenite) form. The majority of the As present in the earth's crust is tied up in minerals containing Cd, Pb, Ag, Au, Sb, P, W and Mo (O'day, 2006), and its presence is most frequently associated with pedogenesis and volcanism; occurrences of antropic As pollution have been largely ascribed to coal combustion, mining, certain industrial processes and agricultural activity; the latter reflects its presence in certain pesticides.

An excessive soil content of As is prejudicial to plant growth. Its availability is dependent on a range of chemical and physical factors (Bissen and Frimmel, 2003). An important topic in the context of food safety is to understand how plants uptake, transport, metabolize and tolerate As (Ali et al., 2009). In terrestrial plants, the capacity to take up As appears to be quite species-specific (Baroni et al., 2004). Most of the As encountered by the root is in the inorganic form, and our current understanding of how it is taken up and translocated (and the interaction between silicon (Si) and As uptake) is represented in Fig. 1. The strategy adopted by tomato plants is mainly avoidance (Carbonell-Barrachina et al., 1997).

Si, the second most abundant element in the earth's crust (Exley, 1998), occurs within soils mainly in the form of inert quartz or crystalline silicate; however, monosilicic acids are soluble and thus available to plants and microbes (Balakhnina et al., 2012). Absorbed Si is beneficial for plant growth, largely because of its role in combating biotic and abiotic stress (Balakhnina et al., 2012; Ma and Yamaji, 2006; Ma, 2004). Its effect on biotic stress resistance relies on the formation of a sub-cuticular double layer (Ma and Yamaji, 2006). The contribution of Si to abiotic stress tolerance is thought to reflect not just its capacity to reduce transpirative water loss

Abbreviations: As III, arsenite; As V, arsenate; AFLP, amplified fragment length polymorphisms; cv., cultivar; DMA, dimethylarsinic acid; D.W., dry weight; exp, expressed sequence; F.W., fresh weight; GDA, genetic data analysis; GSH, glutathione; GSSH, reduced glutathione; GST, glutathione S transferase; HG-AAS, atomic absorption spectroscopy with hydride generation; HSP 90-1, heat shock Protein 90-1 gene; LeGR, lycopersicum esculetum glutathione reductase gene; MMA, monomethylarsonic acid; MS, Murashige and Skoog medium; NIP, oduline 26-like intrinsic proteins; PCs, phytochelatins; PCS, phytochelatins synthase; Phyt1200, phytochelatins synthase gene; PIC, polymorphic information content; RADP, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphisms; ROS, reactive oxygen species; RTqPCR, reverse transcription quantitative polymerase chain reaction; SEM/EDX, scanning electron microscope with X-ray detector; Si, silicon; SNP, single nucleotide polymorphisms; SSR, simple sequence repeats; TAE, *tris*-acetate EDTA; T_a, annealing temperature; T_m, melting temperature; UPGMA, unweighted pair group method using arithmetic average; XIP, X intrinsic proteins.

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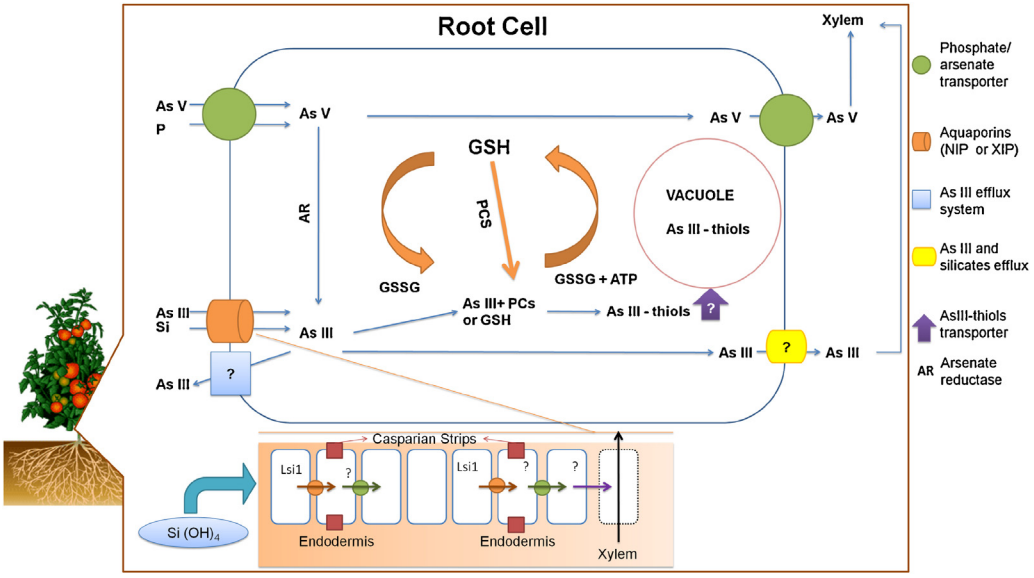

Fig. 1. A schematic illustration of the mechanism of As uptake and translocation, adapted from Ali et al. (2009) and Ma et al. (2008). Arsenate enters the root via phosphate transporters where it behaves as a P toxic analogue (Ali et al., 2009). Most of the arsenate taken up is rapidly reduced to arsenite by the action of arsenate reductase (AR) using glutathione (GSH) as a reductant. In most plant species, arsenite is chelated by phytochelatins (PCs) and deposited in the root cell vacuoles (Salt et al., 2008; Ali et al., 2009). Arsenite is taken up via the same channels as Si (Ma et al., 2008).

through the cuticle, but also its restriction over the uptake of toxic minerals due to Si deposition in the root, its ability to immobilize certain toxic metals via chelation, and its enhancement of stem strength (Ma and Yamaji, 2006); furthermore, the involvement of Si in certain enzyme complexes promotes and protects photosynthesis (Toresano-Sánchez et al., 2012). The concentration of Si in plant tissue ranges from 0.1% to 10% expressed on a dry weight basis (Ma, 2004). Si uptake and transport systems vary from species to species and three distinct Si uptake models have been proposed: active, passive and rejective (Mitani and Ma, 2005).

Tomato is a major horticultural crop in both Europe and the US, but its fruit can be compromised by As contamination (Burlò et al., 1999). Feeding of Si to tomato crops has been used to alleviate drought and salinity stress (Toresano-Sánchez et al., 2012), but to date no attempt has been made to correlate Si supplementation with As uptake and its translocation to the aerial part of the plant. Here, we show that Si treatment can indeed influence As uptake in tomato. In addition, we demonstrate that tomato cultivars differ from one another with respect to their capacity to take up and translocate As, whether or not the plants are supplied with soluble Si.

2. Materials and methods

Eight commercial processing tomato cultivars were used in the experiments, namely Aragon, Axel, Frigio, Gladis, Podium, Rapidus, Ruphus and Wally-Red; six of these produce round and two plum fruits (Fig. 5). The seed was provided by ESASEM s.p.a., Casaleone, Verona, Italy. The cultivars are all subject to plant breeders' rights, and pedigree information is not publicly available.

2.1. Germination and seedling growth on culture plates

Five seeds of each cultivar were rinsed in deionized water to remove any fungicidal coating, surface-sterilized by immersion in 5% (v/v) sodium hypochlorite and plated on Murashige-Skoog medium (Duchefa Biochemie, Haarlem, The Netherlands) containing 10 g l^{-1} sucrose (AppliChem GmbH, Darmstadt, Germany) and 0.8% (w/v) agar (AppliChem GmbH, Darmstadt, Germany). The medium was supplemented with either 0.2 mM or 0.5 mM of $NaAsO_2$ or $NaH_2AsO_4{\cdot}7H_2O$ (Sigma-Aldrich, St. Louis, MO, USA), with and without further supplementation with 0.025 mM $CaSiO_3$ (Sigma-Aldrich, St. Louis, MO, USA). Other experiments involved non-treated substrate or substrate with added Si. Three replicates were performed per treatment per cultivar. The plates were housed in an incubator (Innova 4230, New Brunswick Scientific, Edison, New Jersey, USA) held at 25 °C in the dark, and germination was scored after 48 h. The seedlings were then provided with 16 h per day of 300 μmol m^{-2} s^{-1} light (supplied by metal halide lamps) for two weeks, and shoot length was monitored every two days.

2.2. Pot trials

2.2.1. Experiment # 1

In an initial series of pot trials, 31 pots were filled with garden soil (Gebr. Brill Substrate GmbH and Co. KG, Germany) in each of which a single plant was grown. The soil composition was white peat (40%), black peat (20%) and wood fibre (20%), pH ca. 6.0, range nitrogen content 180–300 mg l^{-1}, mean phosphorus content 190–310 mg l^{-1} and mean potassium content 240–400 mg l^{-1}. The plants were watered as necessary. After three months, either 5 mg l^{-1} of $NaAsO_2$ or $NaH_2AsO_4{\cdot}7H_2O$ was added, either with or without 2 mg l^{-1} of $CaSiO_3$. Control treatments involved either no additive or the provision of only $CaSiO_3$. The experiment comprised three replicates per treatment per cultivar. The temperature was maintained at 25 °C, the relative humidity at 50%, and the photoperiod at 16 h, with the light provided by metal halide lamps supplying a photon flux density of 300 μmol m^{-2} s^{-1}. Two weeks after the addition of As and Si, the plants were harvested, washed with deionized water and separated into root, stem and leaf material. The distribution of As and Si in the various tissues was evaluated by SEM/EDX: a scanning electron microscope (Jeol 6400, Osaka, Japan) combined with an energy dispersive X-ray analyser (SEM/EDX) and LINK ISIS software (Oxford Instruments, Oxford, UK). Methods adopted for sample preparation, the operating parameters and the acquisition of dot maps followed Marmiroli et al. (2011). The concentration of As in the root, stem and leaf tissue was measured by hydride generation atomic absorption spectrometry (HG-AAS)