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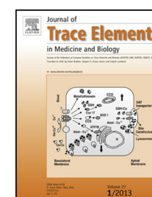

Callose-associated silica deposition in Arabidopsis

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ABSTRACT

The mechanism of biological silicification in plants remains to be elucidated. There are strong arguments supporting a role for the plant extracellular matrix and the β-1-3-glucan, callose, has been identified as a possible template for silica deposition in the common horsetail, *Equisetum arvense*. The model plant *Arabidopsis thaliana*, which is not known as a silica accumulator, can be engineered to produce mutants in which, following a pathogen-associated molecular pattern challenge, callose production in leaves is either induced (*35S:PMR4-GFP*) or not (*pmr4*). We have grown these mutants hydroponically in the presence of added silicon to test if the induction of callose results in greater silica deposition in the leaves. Callose induction was identified throughout leaf tissue of wild type Arabidopsis and the mutant *35S:PMR4-GFP* but not in the mutant *pmr4*. Similarly both wild type Arabidopsis and the mutant *35S:PMR4-GFP* showed extensive silicification of leaf tissue while the *pmr4* mutant deposited very little silica in its leaf tissues. Wild type Arabidopsis and the mutant *35S:PMR4-GFP* responded to a pathogen-like challenge by producing both callose and biogenic silica coincidently in their leaf tissues. Trichomes in particular showed both callose deposition and extensive silicification. The lack of both induced callose deposition and subsequent silicification in the *pmr4* mutant strongly suggested that the biochemistry of callose formation and deposition were allied to biological silicification in Arabidopsis.

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1. Introduction

All plants take up silicic acid ($Si(OH)_4$) via their roots and transport it throughout the tissues following water [1]. However, not all plants deposit $Si(OH)_4$ as biogenic silica to the same degree with some plants such as Equisetum (horsetails) being considered as silica accumulators with as much as 5% of their tissue dry weight being attributed to biological silicification [2]. The biochemical machinery which differentiates silica accumulators such as rice and horsetail from non-accumulators such as Arabidopsis remains to be understood and is the subject of a significant research effort. Of particular interest is the plant extracellular matrix as a factor in templating biogenic silica deposition [2] and we have identified the β-1-3-glucan callose as a biomolecule involved in silica deposition in horsetail [3]. We were able to show that not only does silica deposition in horsetail mirror callose deposition but also that in vitro callose could induce the formation of silica from an undersaturated solution of $Si(OH)_4$. To test a role for callose in biological silicification we obtained seeds of wild type Arabidopsis and two mutants with differing capabilities with respect to stress-induced callose formation [4]. The hypothesis being that under identical conditions of availability of $Si(OH)_4$ there would be significantly less silica deposition in the mutant engineered to resist callose induction (*pmr4*) than one engineered to show increased callose synthase activity (*35S:PMR4-GFP*).

2. Materials and methods

2.1. Hydroponic culture of Arabidopsis

Seeds of Arabidopsis (*Arabidopsis thaliana*) wild type (Columbia) and *pmr4* (allele 1) and *35S:PMR4-GFP* transgenic plants were kindly provided by CA Voigt [4]. Seeds were germinated in the dark on 1% agar contained within the lid of a punctured Eppendorf tube. The lids are placed in a tube rack which in turn is placed in a tank filled with ¼ strength MS medium. Two growth mediums were used, one with (+Si) and one without (−Si) added Si at 2 mM, at pH 5.80 ± 0.05. The latter medium (−Si) included an additional 8 mM Na^+ to account for Si addition as Na_4SiO_4. After 2 weeks, during which time roots have traversed agar plugs and entered the growth medium, the Eppendorf lids were placed on a polystyrene support floated on the appropriate growth medium (Fig. 1a). After a further

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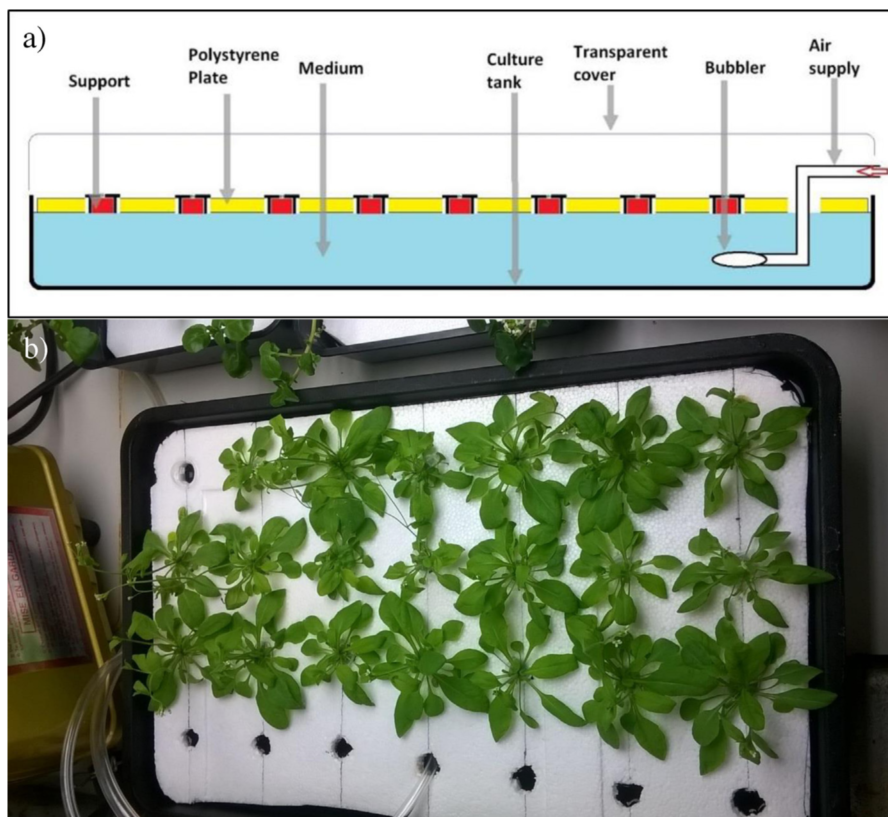

Fig. 1. a. Schematic of the experimental apparatus for hydroponic culture of Arabidopsis. b. Experimental set-up showing 5 week old Arabidopsis prior to harvesting.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3 weeks of a 14 h light/10 h dark cycle at 25 °C growth media were supplemented with 35 mg/L chitosan, an elicitor mimicking fungal infection, and known to induce the formation of callose [5], and grown for an additional 2 weeks. At this point all plants are harvested for examination of both presence of tissue callose and silica deposition.

2.2. Identification of callose in tissues

We used an established method for the identification of callose in plant tissue using aniline blue [6]. Briefly, leaves from plants from each group are detached and fixed and destained in a 1/3 acetic acid/ethanol solution until approximately transparent. Leaves are then washed for 30 min in 150 mM Na_2HPO_4 and then incubated for 2 h in 150 mM Na_2HPO_4 which included 0.01% aniline blue. Images of callose were obtained using an Olympus BX50 fitted with a BXFLA fluorescent attachment using a U-MWU filter cube (Ex: 333–385 nm; Em: 400–700 nm). A ColourView III digital camera (OSIS FireWire Camera 3.0 digitizer) was used to capture images in conjunction with CELL* Imaging software (Olympus Cell* family, Olympus Soft Imaging solutions GmbH 3.0). Callose was identified as distinct green fluorescence.

2.3. Digestion of plant tissue

Leaves from plants from each group were detached and dried to a constant weight in an incubator at 37 °C at which point 0.1 g of each were placed in acid-washed 20 mL PFA teflon© vessels. The samples were then digested in a 1:1 mixture of 15.8 M HNO_3 and 18.4 M H_2SO_4 using a Mars Xpress microwave oven (CEM Microwave Technology Ltd.). The acid digests were clear and, upon dilution with 8 mL of ultrapure water, were filtered and the residues washed several times with further volumes of ultrapure water. Filters were then placed in plastic Petri dishes in an incubator at 37 °C to achieve dryness over several days. Dry residues off each filter were then collected into Bijoux tubes and stored in a dry, sealed, Perspex cabinet.

2.4. PDMPO labelling of Arabidopsis-derived silica

We used an established method for the identification of biogenic silica in plant tissues [3]. Briefly, silica residues collected from filters were suspended in 20 mM PIPES at pH 7 and containing 0.125 μM 2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl)-methoxy) phenyl) oxazole (PDMPO; LysoSensor Yellow/Blue DND-160, 1 mM in DMSO). Suspensions were left for 24 h to allow the reaction between silica surfaces and PDMPO to achieve completion after which 50 μL samples were transferred to a cavity slide and viewed using an Olympus BX50 fitted with a BXFLA fluorescent attachment using a U-MWU filter cube (Ex: 333–385 nm; Em: 400–700 nm). A ColourView III digital camera (OSIS FireWire Camera 3.0 digitizer) was used to capture images in conjunction with CELL* Imaging software (Olympus Cell* family, Olympus Soft Imaging solutions GmbH 3.0).

3. Results and discussion

3.1. Germination and plant growth

There were no differences in germination and plant growth between those treatments which were (+Si) or were not (−Si) supplemented with silicon. However, wild type seeds germinated better than seeds of either of the transgenic plants. Similarly, wild type plants grew larger than either *pmr4* or *35S:PMR4-GFP* plants

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