Determination of Silica in Wheat Leaves with ATR-FTIR-Chemometrics

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Title

Abstract

Keywords

Introduction

The presence of silicon (Si) in plants contributes to the response of stress-relief mechanisms for environmental events such as drought and pathogen attack. Because of the importance of crops such as wheat, barley, rice, and other grasses that accumulate Si, the understanding of the relationship between this element and plant science is the focus of numerous scientific efforts. Additionally, a new scientific quest is emerging for the study of Si accumulation in grasses: the huge amounts of agricultural waste that these crops generate have to be used in circular economies, but its full potential is hindered by Si presence in the residues.

Silicon is a major soil constituent, therefore plants invariably grow in Si-rich-environments [2]. In soil, Si is available in the form of silicic acid solutions. Some plants accumulate so much silicic acid that Si precipitates as silica, e.g., phytoliths. The accumulation occurs mainly in root endodermis, epidermal cells of both leaf and inflorescence bracts [3], as well as awns and leaf macrohairs [4].

The molecular forms of Si that are transported through the xylem are known: only monomers and dimers of silicic acid have been found in the shoots of wheat and rice and no organo-silicon structures have be en identified [5]. Remarkably, the hemicellulose callose may be templating Si deposition in horsetails (Equisetum) [6], and may also play a role in Si deposition of algae and plants in general [7]. Si accumulation seems to be an ancient mechanism because it is present in early divergent plant lineages, and was lost in the course of evolution as silica is less present in later-evolving plants [8]. In ancient silica - accumulators such as Equisetum species, silica may play a major structural role [9]. This structural role was taken later in evolution by lignin. As a general tendency, later-evolving plants have more lignin and less silica [8]. Lignin may fulfill a stronger mechanical role because, unlike silica, it can crosslink with cell wall polysaccharides [9].

However, the presence of Si and lignin is not mutually exclusively, and they relationship (if any) unknown. For example, in rice Si deposition starts when the plant cell synthesize and accumulate lignin in the secondary wall [10,11], while Si surplus is positively correlated with lignin content and mechanical strength 12[. In Brachypodium, Si surplus induces changes in lignin composition [4].

Silicon quantification. . . .

Methods

Greenhouse experiments

Greenhouse experiment to test Si interactions with lignin A greenhouse experiment was set up to compare the effects of Si deficiency and surplus in both total lignin content, lignin monomeric composition, as well as its crosslinking with cell wall polysaccharides, specifically with arabinoxylans –the major hemicelluloses in grass cell walls 13 . The experimental design was inspired by an article published in 1999 14. In that experiment, four wheat plants were grown in a 100 ml tank containing 0.5 mM Na2SiO3. Si was absorbed by the plants until it was depleted in ca. 80 days. Another set of plants was grown in a Si free environment with the same conditions; as there were not stressors, the Si-free plants grew normally. At day 80, the mature Si-free plants were transferred

into a solution containing 0.5 mM Na2SiO3. The mature plants absorbed the same amount of Si in three days, as did plants grown in solutions to which Si had been added during their whole life. The experiment was set-up both in a greenhouse and in a controlled growing chamber yielding the same result: plants deprived from Si avidly absorbed the element at maturity, when all lignin was in place. We wondered if there was any difference in lignin content, quantity, and quality between these treatments. Thus, in our experimental design we included the same treatments: (1) A group grown for 90 days in a solution containing 1.5 mM Na2SiO3; this group was called Si+; (2) a group (Si++) grown without Si until day 85, then 1.5 mM Na2SiO3 added to the tank: (3) a control group without silicon (Si-). Nutrient solution: The rest of the nutrient solution was prepared according to a Standard Operational Procedure of the greenhouse at the University of Copenhagen in Rolighedsvej. In this procedure, commercial fertilizers are mixed in three different 250 L tanks with the formulations in Table 1. Then, an automatized pumping system mixes different ratios of the solutions in the tanks. The solutions are mixed with tap water which contained Si levels of 0.24±0.08 mM SiO2. For the purpose of the experiment, ca.150 L of this nutrient solution was used weekly. The Si content was measured in every occasion with the method described below. Then 1.5 mM Na2SiO3 was added to 50 or 100 L of solution (depending on the time line) from a 30 mM stock solution diluted in dm-water, while the same amount of dm-water was added to the controls. The measured Si content after dilution was between 1.5±0.16 mM. At the end of the week another Si sampling was performed, and the concentrations was always above 0.6 mM, which ensure that there was always Si surplus. pH was adjusted to 6 using HCl or NaOH solutions. Table 1. Nutrient solution

Silicon measurements

Dissolved silica was measured by heteropoly blue method using the Hach protocol 8186 and a DR/1900 spectrophotometer (HACH Company, Loveland, USA) as described by the manufacturer. A Si standard solution of 1.0 mg/L also from Hach (cat 110649) was used for calibration. Polypropylene volumetric flasks were used for dilution of samples to avoid Si contamination. Plant production: Wheat seeds (JB Asano) were surface sterilized as follows. The seeds were immersed in 2.7% sodium hypochlorite solution (v/v) and a drop of Tween 80 for 30 minutes with constant stirring, then rinsed with MilliQ water five times (10 min each). The seeds were vernalized in the dark at 5°C in MilliQ water for 4 days. Thereafter, the seeds were placed in Si-free oasis horticubes (Smithers-Oasis, Kent, Ohio). The cubes were soaked in 1/10 strength nutrient solution (adjusted to pH 5.5) and excess water drained. Each seed was placed directly into a wet cube. Once seeds started rooted and started to actively grow (4-7 days), they were put it in the cold room for a vernalization period of 50 days. After vernalization the plants were cultured using the RainForest 72 aeroponic culture system (GHE, Fleurance, France), and placed in a greenhouse, mean temperature 20°C, under 9h/15h light (80–100uEm-2s-1)/dark regime. (Figure 1).

Elemental analysis

Multi-elemental analyses of the solid samples was performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES). A sample (10–50 mg) was mixed with 500–2500 uL 70% HNO3, 250–1000 uL 15% H2O2 and 40–200 uL 49% HF, and then digested in a pressurized microwave oven for 10 minutes with a starting pressure of 40 bar and a temperature of 240°C. After digestion, samples were diluted to a final 3.5% acid concentration with MilliQ water before measurement on an ICP-OES (Model Optima 5300 DV, PerkinElmer) equipped with a HF-resistant sample introduction kit. For quantification, an external 10-point calibration standard P/N 4400-132565 and P/N 4400-ICP-MSCS (CPI International, Amsterdam) was used. A certified reference material (CRM) NCS 73013 Spinach leaf was analyzed together with the samples to evaluate the accuracy and precision of the analysis.

Spectral Acquisition

The FTIR spectra of wheat samples were colected using a ### infrared spectrometer (brand and model). Each spectrum was acquired (. . .) over the range of 4000 to 400 cm^{-1} ## Spectra pre processing method

Selection of Range of Interest and Baseline correction

The selection of the range of interest for infrared spectra was made taking into account that silicon bands appear (Frost 2006)

Results and Discussion

Conclusion

Acknowledgements

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Supporting Materials