Plant Physiology and Biochemistry 108 (2016) 132–138

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Silicon addition to soybean (*Glycine max* L.) plants alleviate zinc deficiency

M[a] Blanca Pascual, Virginia Echevarria, M[a] José Gonzalo, Lourdes Hernández-Apaolaza*

Agricultural Chemistry and Bromatology Department, Francisco Tomas y Valiente Street, n 7, Autonoma University 28049, Madrid, Spain

ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form
6 July 2016
Accepted 9 July 2016
Available online 11 July 2016

Keywords:
Apoplast
Silicon
Soybean
Zinc deficiency
Zn–Si interaction

ABSTRACT

It is well established the beneficial role of silicon (Si) in alleviating abiotic stress. However, it remains poorly understood the mechanisms of the Si-mediated protection against metal deficiency, especially the zinc (Zn) one. Recently, it has been proposed that Si may act by an interaction with this biometal in the root apoplast contributing to its movement through the plant, as in the case of Fe deficiency. In the present work, the effect of initial or continuous Si doses in soybean Zn deficient plants has been studied. For that purpose, plants grown in hydroponic culture were treated with different Si doses (0.0, 0.5 and 1.0 mM) under Zn limiting conditions. SPAD index in leaves, several growth parameters, mineral content in the whole plant and the formation of Zn pools in roots were determined. An initial addition of 0.5 mM of Si to the nutrient solution led to an enhancement of plants growth, Zn and Si content in leaves, and a higher storage of Zn in the root apoplast. The results suggest that this treatment enhanced Zn accumulation on roots and its movement to shoots when needed, mitigating Zn deficiency symptoms.

1. Introduction

Zinc (Zn) deficiency cause important disturbances in growth and development of in plants due to the large diversity of essential cellular functions and metabolic pathways directly influenced (Cakmak, 2000). Severe symptoms such as intervenial chlorosis in leaves, reddish-brown or bronze tints, epinasty, internode shortening, inward curling of leaf lamina and reductions in leaf size have been associated to Zn deficiency (Marschner, 1995).

Although silicon (Si) is the second most abundant element in the earth's crust, it is not still considered an essential element for higher plants. However, it has been largely known its beneficial effects alleviating various biotic (diseases, pests) and abiotic (salt and metal toxicity, high temperature, drought, freezing) stresses in many plant species (Epstein, 1999; Maksimovic et al., 2012; Romero et al., 2011; Song et al., 2011; Wu et al., 2013; Zhu and Gong, 2014). Moreover, in the past years there has been a rapid progress in the elucidation of how Si mediates under plant metal deficiency (Bityutskii et al., 2014; Gonzalo et al., 2013; Hernández-Apaolaza, 2014; Mehrabanjoubani et al., 2015; Pavlovic et al., 2013). However, despite the information available, the mechanisms of Si-mediated alleviation of nutrient deficiency in crops remain poorly understood.

There are several evidences that Zn distribution in plant changed by the Si addition in hydroponic and soil experiments (Bityutskii et al., 2014; Gu et al., 2012) and that both elements presented a similar location in plants (Gu et al., 2012). By using a fractionation technique, it has been shown that the cell wall bound fraction of Zn in roots, stems, sheaths and leaves of rice seedlings increased after Si addition (Gu et al., 2012). Moreover, in Fe plaque, Zn could be adsorbed (Chen et al., 1980) and remobilized when needed. Root Zn deposits could be used under Zn-deficient conditions through the activation of a Zn-deficiency mechanism (Hernández-Apaolaza, 2014). As for Fe (Pavlovic et al., 2013), the Zn apoplastic pools in the roots could be more mobile under deficiency conditions when Si was added, contributing to its better distribution inside plant (Hernández-Apaolaza, 2014). Recently, Bityutskii et al. (2014) studied the effect of Si on Zn deficiency in acidic condition (nutrient solution pH 6.0) by growing cucumber seedlings without Zn and two Si doses (0 and 1.5 mM) in hydroponic culture. Even though no significant changes were observed in roots or leaves' Zn and chlorophyll concentrations due to Si addition, Si partially diminished leaves necrotic spots. The reutilization of root apoplastic Fe via phenolics induced by Si nutrition did not appear under Zn deficiency and authors concluded that there were no

* Corresponding author.
E-mail addresses: bla.pascual@estudiante.uam.es (M.B. Pascual), virginia.echevarria@uam.es (V. Echevarria), mariajose.gonzalo@uam.es (M.J. Gonzalo), lourdes.hernandez@uam.es (L. Hernández-Apaolaza).

http://dx.doi.org/10.1016/j.plaphy.2016.07.008
0981-9428/

evidences of Si alleviation of Zn-deficiency symptoms, but as plants were grown without Zn in the nutrient solution, the possibility of Zn pools formation was suppressed, therefore their remobilization was not possible.

The aim of this work was to investigate the effect of Si addition on Zn deficient symptoms mitigation and the distribution of both elements, Zn and Si, in soybean seedlings under Zn limited conditions. In order to accomplish this objective different Si doses were applied to soybean plants grown in a nutrient solution with a deficient Zn content. The Zn deposits in apoplast were determined and its remobilization from root to shoot was also investigated.

2. Materials and methods

2.1. Plant material and growing conditions

Soybean seeds (*Glycine max* L. cv. Klaxon) were germinated on filter paper moistened with distilled water for one week in the dark at 28 °C. Thereafter, uniformly sized seeds were grown in aerated 1/5 strength nutrient solution for a week. Then, they were transferred to 2 L plastic buckets containing full-strength nutrient solution in which 0.2 g of solid $CaCO_3$ were added per bucket. The composition of the nutrient solution was: macronutrients (mM) 1.0 $Ca(NO_3)_2$, 0.9 KNO_3, 0.3 $MgSO_4$, and 0.1 KH_2PO_4; micronutrient (μM) solution to avoid metal precipitations 35 NaCl, 10 H_3BO_3, 0.05 Na_2MoO_4, 50 Fe (III)-HBED, 10 Zn-EDTA, 2.5 $MnSO_4$, 1.0 $CuSO_4$, 1.0 $CoSO_4$, 1.0 $NiCl_2$, and 115.5 $EDTANa_2$ and 0.1 mM HEPES, 0.05 mM KOH. The solution was continuously aerated and renewed weekly. The pH was adjusted to 7.5 with NaOH 1.0 M or HCl 1.0 M. When necessary, the buckets were refilled with distilled water. Soybean plants were submitted to different Si concentrations in presence/absence of Zn depending on the experiments.

For the preparation of the Fe chelate solution, HBED (N,N'-bis(2-hydroxybencyl) ethilendiamine-N,N'diacetic acid, Strem Chemicals) was dissolved in sufficient NaOH (1/3 M ratio) and then complexed with $FeCl_3 \cdot 6H_2O$ (Merck). After adjusting to 7.0 its pH, the solution was left to stand overnight to allow excess Fe to precipitate. Finally, the solution was filtered through 0.45 μm Millipore membrane and made up to volume. The Zn was supplied as Zn-EDTA, which was prepared by mixing at 1/1 M ratio $Zn(NO_3)_2 \cdot 6H_2O$ (98%. Sigma-Aldrich) and ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich).

Plants were grown under controlled conditions in a Dycometal-type CCK growth chamber provided with fluorescent and sodium vapor lamps with a 350 μE m^{-2} s^{-1} light intensity, photoperiod 16/8 h, temperature (day/night) 30/20 °C and 50/70% relative humidity.

2.2. Experimental design

2.2.1. Effect of Si in the formation and remobilization of the apoplast Zn pool

Soybean plants, grown as mentioned above, were cultivated with a full strength nutrient solution for two weeks. Three different Si doses were added: 0.0, 0.5 and 1.0 mM as $Na_2SiO_3 \cdot 5H_2O$ (Suprapur, Merk). Then, Zn supply was eliminated from all the nutrient solutions except for the plants of the control treatment and the Si was maintained in half of the plants (continuous silicon supply) for four more days. Table 1 gathers the six different treatments that were carried out.

2.2.2. Long term Si effect on Zn deficient soybean plants

The design of this second experiment was similar as the one just mentioned except for its duration. To ascertain the theory arose from the results of the first experiment, soybean plants where cultivated, instead of four days, for three weeks after the Zn removal from the nutrient solution. Therefore, this hydroponic experiment lasted a total of five weeks.

Table 1
Silicon and Zinc treatments (mM and μM, respectively) regarding to the days after treatments application (DAT).

Si (mM)		Zn (μM)		
0–14 DAT	14–18 DAT	0–14 DAT	14–18 DAT	Abbreviation
0.0	0.0	0	0	Zn 0 Si (0.0–0.0)
0.0	0.0	10	10	Zn10 Si(0.0–0.0)
0.5	0.0	10	0	Zn 0 Si(0.5–0.0)
0.5	0.5	10	0	Zn 0 Si(0.5–0.5)
1.0	0.0	10	0	Zn 0 Si(1.0–0.0)
1.0	1.0	10	0	Zn 0 Si(1.0–1.0)

2.3. Measurements

In the first experiment described, the root apoplastic accumulation of Zn was tested after Zn removal from the nutrient solution. The method used was the one proposed by Rengel (1999) in which intact roots were washed twice in a mixture of distilled water and ice for 5 min each, and then with a solution containing 2 mM $CaCl_2$ and 1 mM $LaCl_3$ for 10 min in continue stirring. This last washed solution was filtrated, made up to 50 ml and Zn concentration was assessed by atomic absorption spectrophotometer (AAs, Perkin-Elmer Analyst 800). In addition, the washed roots were oven dried, and Zn content was measured after a microwave (CEM Corporation MARS 240/50; Matthews, NC, USA) digestion following the method described by Zuluaga et al. (2011) to determine the total amount of Zn in roots and its distribution. For that, 500 mg of dry material were loaded into Teflon vessels with 8 ml HNO_3 at 65%, 2 ml H_2O_2 at 30% and 1 ml HF at 40% (all of them Suprapure reagent grade, Merck Millipore). After the digestion the samples were made up to 50 ml volume with distilled water and Zn concentration was determined by AAs.

During the whole long term experiment, the SPAD index was monitored every three days using a digital chlorophyll meter SPAD (Soil and Plant Analyzer Development) model 502 (Minolta Co., Osaka, Japan).

Samples of leaves, stems and roots of three plants per treatment were taken at 0(M0), 7 (M1), 14(M2) and 21 (M3) days before the Zn removal of the nutrition solution and fresh weight (FW) was determined. Immediately after the sampling, plants were washed with four different solutions: Tween 80 at 0.1%, 0.1 M of HCl (Suprapure, Merck Millipore), water and distilled water. Then, the biometric parameters dry weight (DW), stems length (SL), roots length (RL), and node number (NN) were measured. In addition, a sample of all the nutrient solutions was taken before every change for their pH measurement and nutrient analysis.

Fe, Cu, Mn, and Zn concentrations were determined in leaves, stems and roots following the previously described microwave method.

Silicon was determined by digesting 20 mg of ground plant materials in 1.0 ml of the mixture of 1 M HCl and 2.3 M HF (v/v = 1/2) and shaken overnight. The samples were centrifuged at 10 g for 10 min, and 0.5 ml of the supernatant was added to plastic tubes. Afterwards, 3.0 ml of 20% glacial acetic acid and 1.0 ml of 54 $g.L^{-1}$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ were added and incubated for 5 min, followed by addition of 0.5 ml of 20 $g.L^{-1}$ tartaric acid and 2.0 ml reductant. Thirty minutes after the reductant addition, the sample absorbance was measured at 650 nm with a spectrophotometer (Jasco V-650). The reductant was a mixture of solution A: 80 $g.L^{-1}$ Na_2SO_3 + 16 $g.L^{-1}$ 1-amino-2-naphthol-4-sulfoacid and solution B: