Journal of Plant Physiology 167 (2010) 1592–1597

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Interactions between iron and titanium metabolism in spinach: A chlorophyll fluorescence study in hydropony

Petr Cigler[a], Julie Olejnickova[b,c], Martin Hruby[d,*], Ladislav Csefalvay[b,c], Jiri Peterka[e], Stanislav Kuzel[e]

[a] Gilead Sciences and IOCB Research Center, Institute of Organic Chemistry and Biochemistry, AS CR, v.v.i., Flemingovo n. 2, 166 10 Prague 6, Czech Republic
[b] Institute of Systems Biology and Ecology AS CR, v.v.i., Zamek 136, 373 33 Nove Hrady, Czech Republic
[c] Institute of Physical Biology USB, Zamek 136, 373 33 Nove Hrady, Czech Republic
[d] Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovsky Sq. 2, 162 06 Prague 6, Czech Republic
[e] University of South Bohemia, Faculty of Agriculture, Department of Applied Plant Biotechnologies, Studentska 13, 370 05 Ceske Budejovice, Czech Republic

ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form 1 June 2010
Accepted 3 June 2010

Keywords:
Chlorophyll fluorescence
Iron
Photosynthetic apparatus
Spinach
Titanium

ABSTRACT

One of the elements showing strong beneficial effect on plants at low concentrations and toxic effects at higher concentrations is titanium (Ti). We investigated the interconnection between the Fe uptake and the Ti intoxication in model experiment on Fe-deficient spinach (*Spinacia oleracea*) plants to help to elucidate the mechanism of the biological activity of titanium in plants. The two different Ti (0 and 20 mg L^{-1}) and two different Fe (0 and 1.35 mg L^{-1}) concentrations in hydroponic medium were used in all four possible combinations. We compared chemical analysis of Ti and Fe in roots and shoots with the changes of the *in vivo* chlorophyll fluorescence. Although Fe and Ti concentration found in shoots of Ti-non-treated Fe-deficient plants was comparable with that in Ti-treated Fe-deficient plants, the soluble form of Ti present in the growth media had a negative effect on photosynthetic activity monitored by chlorophyll fluorescence measurements. The presence of Fe in growth medium significantly decreased the Ti concentration in shoots and increased the photosynthetic activity. Here, we propose that Ti affect components of electron transport chain containing Fe in their structure (particularly photosystem I) and decrease the photosystem II efficiency.

Introduction

According the biological effects on living organisms, the elements could be divided into three groups (Pais, 1992):

1. *Essential elements* that improve the organism's health status, the organism cannot grow and healthily develop itself in their absence (deficiency could be observed) – N, Mg, B, etc.
2. *Beneficial elements* that improve the organism's health status at low concentrations, but the organism can grow and healthily develop in their absence (deficiency does not exist) – La, Ti, Ga, etc.
3. Some elements that are *toxic only* – Hg, Tl, etc.

In contrast to the essential and toxic elements, effects of the beneficial elements on plants as well as mechanisms of their action are much less explored. One of these typical elements, possessing strong beneficial impact on plants in very low concentrations and toxic effects at higher concentrations, is titanium (Ti). Its influence on plants was studied during last almost 100 years (Pais, 1983). The attention was focused on agricultural and beneficial use of Ti, because it significantly improves health status of the plants and increases crop yields at very low concentrations, which are non-toxic for animals and humans (Pais, 1983; Carvajal and Alcaraz, 1998). However, much less is known about its phytotoxicity and progression of phytopathologic effects.

First systematical research of dose–response relationship was done on mustard, pea and alfalfa grown on soil (Nemec and Kas, 1923). They observed that at certain "optimal" Ti concentration the growth and development of the plants was intensified, the chlorophyll content was increased (the plants became greener), the leaf area was increased and also the ripening was accelerated. However, at higher dose of Ti the effect was rather opposite, which is also a finding of later hydroponic experiments (Hruby et al., 2002).

This concentration dependent "switch" of physiological behavior is also known for several other elements (e.g. Zn, Se, Pb) (Calabrese and Baldwin, 2003). It is usually a result of complicated systemic reactions that lead to an extraneously observable effect called "hormesis" (Hruby et al., 2002). In the case of Ti it was hypothesized that the benefit and the phytotoxicity could be

Abbreviations: Chl-F, chlorophyll fluorescence emission; HL, high light; LEDs, light-emitting diodes; LL, low light; PSII, photosystem II.

* Corresponding author. Tel.: +420 296 809 230; fax: +420 296 809 410.
E-mail address: mhruby@centrum.cz (M. Hruby).

0176-1617/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2010.06.021

connected in one complex mechanism involving replacement of some essential elements from their binding sites (Kuzel et al., 2003; Hruby et al., 2002).

Since soluble Ti(IV) has high affinity to ligands containing oxygen, we hypothesize that Ti can substitute iron (Fe) and magnesium (Mg) from their binding sites (Hruby et al., 2002). In this way, Ti could cause an apparent Fe deficiency resulting in the significant changes in Fe uptake *via* roots. The influence of Ti on the Fe uptake and its metabolism including the increase of the content of photosynthetic pigments was described in several works (Simon et al., 1988; Carvajal and Alcaraz, 1995, 1998) and the theory based on premise of low redox potential of Ti(IV)/Ti(III) couple influence on the activity of metals in chloroplast and cytoplasm was formulated (Carvajal and Alcaraz, 1995, 1998).

The influence of Ti on the Fe uptake and the consecutive Fe deficiency could induce different biochemical and activity changes in the photosynthetic apparatus. Among other effects, Fe deficiency decreases the content of light-harvesting pigments, carotenoids being less sensitive than chlorophylls (Abadía and Abadía, 1993; Morales et al., 2000), and the antenna disconnection in photosystem II (PSII) (Morales et al., 2001; Moseley et al., 2002). Moreover, Fe deficient leaves show lower actual PSII efficiency (Φ_{II}; Genty et al., 1989) and decrease in the proportion of open, oxidized PSII reaction centers (Larbi et al., 2006).

Since PSII activity is particularly sensitive to stress, we decided to use chlorophyll fluorescence emission (Chl-F) as a suitable, non-invasive tool to determine the photosynthetic activity in Ti treated plants *in vivo*. Chl-F is produced in plant photosynthetic tissues after absorption of light energy as one of the two de-excitation pathways that competes with the photochemical energy conversion leading to CO_2 assimilation; the other pathway is a heat loss (Maxwell and Johnson, 2000). Coupling between the photochemistry and Chl-F is the strongest in PSII, because at room temperature Chl-F originates almost exclusively from population of the first excited singlet state of chlorophyll *a* molecules in PSII complexes (Govindjee, 1995). Thus, the competition between Chl-F and photosynthesis makes the Chl-F ideal non-invasive reporter of the photosynthetic activity in plant tissue. Moreover, Chl-F dynamic changes of previously dark adapted plant in actinic light (Chl-F transient or Kautsky effect, Kautsky and Hirsch, 1931; Maxwell and Johnson, 2000) were shown to carry information that can be used for early detection of abiotic stress (Mallick and Mohn, 2003; Lichtenthaher and Babani, 2004). Fe deficiency has been reported to induce marked changes in the shape of the Chl-F induction curve (Belkhodja et al., 1998; Morales et al., 2001; Moseley et al., 2002; Larbi et al., 2006). However, no paper was found to deal with the alternation of Chl-F due to the Ti intoxication.

In this work, we investigate the interconnection between Fe uptake and Ti intoxication in model experiment on Fe-deficient spinach plants. Ti has thus been previously shown to interfere with the Fe metabolism and it is likely that this is one of the key issues of its beneficial effects on plants. Since disturbances in Fe nutritions leads to dramatical and sensitive changes in Ch-F, following Ch-F under various Fe and Ti nutrition conditions should give us better insight into the biological effects of Ti, which are of great interest in agriculture. To best of our knowledge, this is for the first time the progress of the effects of Ti on the photosynthetic apparatus are studied in detail by *in vivo* fluorescence methods.

Materials and methods

Plant cultivation and treatment

Spinach (*Spinacia oleracea* L.) plants were grown hydroponically in a full Hoagland nutrient medium (according to Hruby et al., 2002) in greenhouse for 21 days. Four plants were taken as "non-stressed" controls and were grown in full Hoagland nutrient solution until the end of the experiment. In the case of the remaining plants, the medium was replaced by Fe-deficient Hoagland medium, in which the plants were cultivated for further seven days and the Fe-deficiency was induced.

After this preparatory phase the experiment started. Fe-deficient medium was again replaced by four types of modified Hoagland medium containing different concentrations of Fe [as ethylenediaminetetraacetic acid iron(III) sodium salt] and Ti [as ascorbate; ascorbate does not possess any biological effects on plants in relevant concentrations (Hruby et al., 2002; Pais, 1983), so ascorbate control was omitted]:

(1) Fe0Ti0 (Fe = 0 mg L^{-1}, Ti = 0 mg L^{-1}) – prolonged Fe-deficiency;
(2) Fe1Ti0 (Fe = 1.35 mg L^{-1}, Ti = 0 mg L^{-1}) – recovery from the Fe-deficiency;
(3) Fe1Ti1 (Fe = 1.35 mg L^{-1}, Ti = 20 mg L^{-1}) – induced competition between Fe and Ti;
(4) Fe0Ti1 (Fe = 0 mg L^{-1}, Ti = 20 mg L^{-1}) – sole effect of Ti.

The concentration 1.35 mg L^{-1} Fe is adequate to 24.2 μmol L^{-1} and the concentration 20 mg L^{-1} Ti is adequate to 418 μmol L^{-1}. There were four replicates per experimental treatment. The whole experiment took nine days.

Chemical analysis

Plants were harvested at the end of the experiment, washed with water from contamination by nutrient solution and the concentrations of Fe and Ti in dry mass of both shoots and roots were analyzed by atomic absorption spectrophotometry after wet microwave mineralization.

Chlorophyll fluorescence (Chl-F) measurements

Chl-F was measured using the commercial kinetic chlorophyll fluorescence instrument FluorCam (P.S. Instruments, Ltd., Brno, Czech Republic; www.psi.cz), described by Nedbal et al. (2000). Chl-F from a leaf was excited by two panels of light-emitting diodes (LEDs) ($\lambda_{max} \approx 635$ nm) that generate measuring light flashes and actinic light. Brief intense saturating light white light pulses [1500 μmol (photons) m^{-2} s^{-1}, 1.6 s] were generated by a 250 W halogen lamp. Chl-F transients were captured by a CCD (charged coupled device) camera in series of images at 12-bit resolution in 512 × 512 pixels, taking 50 images per second.

Spinach plants were dark-adapted for 30 min before each measurement. All measurements were done on attached fully expanded leaves. First, leaves of six "non-stressed" spinach plants cultivated in full Hoagland medium were measured at day 21 of the preparatory experimental phase to determine the Chl-F response of healthy non-stressed plants. Then, the leaves were measured again after 7 days of Fe deficiency, at the start point of the experiment. Dynamics of Ti and/or Fe effect was measured at 1st, 3rd, 6th and 9th day of the experiment (calculated from the nutrient replacement). Here, we measured three leaves from each treated plant, always identical, leaves during the whole experiment.

In order to evaluate contrast between the treated plants, a complex actinic light protocol with two light intensities [50 μmol (photons) m^{-2} s^{-1} – low light (LL), 200 μmol (photons) m^{-2} s^{-1} – high light (HL)] was employed. First, the levels of minimum (F_0) and maximum (F_M) Chl-F yields in dark-adapted leaves were determined followed by a short dark relaxation (16 s). Then, the leaf was exposed to LL for 100 s and the corresponding Chl-F induction was recorded. Two saturation pulses [at 2 and 90 s after switching on the actinic light] were