Plant Physiology and Biochemistry 49 (2011) 545–556

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Auxin: A major player in the shoot-to-root regulation of root Fe-stress physiological responses to Fe deficiency in cucumber plants

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ARTICLE INFO

Article history:
Received 29 October 2010
Accepted 17 February 2011
Available online 26 February 2011

Keywords:
ABA
Auxin inhibitors
Cucumber
Fe(III)-chelate reductase activity
Gene expression
IAA
Iron deficiency

ABSTRACT

The aim of this study was to investigate the effects of IAA and ABA in the shoot-to-root regulation of the expression of the main Fe-stress physiological root responses in cucumber plants subjected to shoot Fe functional deficiency. Changes in the expression of the genes *CsFRO1, CsIRT1, CsHA1* and *CsHA2* (coding for Fe(III)-chelate reductase (FCR), the Fe(II) transporter and H^+-ATPase, respectively) and in the enzyme activity of FCR and the acidification capacity were measured. We studied first the ability of exogenous applications of IAA and ABA to induce these Fe-stress root responses in plants grown in Fe-sufficient conditions. The results showed that IAA was able to activate these responses at the transcriptional and functional levels, whereas the results with ABA were less conclusive. Thereafter, we explored the role of IAA in plants with or without shoot Fe functional deficiency in the presence of two types of IAA inhibitors, affecting either IAA polar transport (TIBA) or IAA functionality (PCIB). The results showed that IAA is involved in the regulation at the transcriptional and functional levels of both Fe root acquisition (FCR, Fe(II) transport) and rhizosphere acidification (H^+-ATPase), although through different, and probably complementary, mechanisms. These results suggest that IAA is involved in the shoot-to-root regulation of the expression of Fe-stress physiological root responses.

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1. Introduction

Bienfait et al. [1] showed that the main Strategy I root responses to Fe deficiency were expressed independently of the presence of the shoot. However, further studies with whole plants (mainly split-root based experiments), have shown that the main regulation of the expression of the Fe-stress root responses does depend on the shoot [2–8]. One or several biochemical consequences of Fe-starvation in the leaf would produce one (or several) still unknown signal(s), which then regulate the activation of Fe-stress physiological and morphological responses in the roots. We have reported previously [8] that these unknown shoot biochemical events, able to activate the Fe-stress root responses, are not directly related to the total leaf Fe concentration, but to the ability of the plant to optimize the functional utilization of Fe in the leaf.

All these physiological events linked to Fe leaf functional availability and utilization must be connected through molecular messengers able to activate and/or regulate the expression of Fe-stress Strategy I root responses, including physiological (rhizosphere acidification (H^+-ATPase activity), Fe(III) chelate reductase (FCR) activity, root release of phenolics and reductants, and Fe(II) transport into the root cells via the Iron Regulated Transporter (IRT)), as well as morphological ones (subapical swelling, root hair and transfer cell formation [1,9–13]). Plant hormones are clear candidates to play these roles [14]. In a recent study, we have investigated the time-course changes in the concentrations of the main plant hormones in shoots and roots of cucumber plants

Abbreviations: ABA, abscisic acid; BPDS, bathophenanthroline disulfonate; D-ABA, [$^2H_6$] (+)-*cis*, *trans*-abscisic acid; D-IAA, [$^2H_5$] indol-3-acetic acid; DDTC, sodium diethyldithiocarbamate; DMSO, dimethylsulfoxide; FCR, Fe(III)-chelate reductase activity; Fe(III)-EDTA, Fe(III)-ethylenediamine tetraacetic acid; Fe(III)-HBED, Fe(III)-*N*, *N′* di-ortho hydroxibenzyl ethylenediamine diacetate; *FRO*, ferric reduction oxidase gene; *HA1,2*, plasma membrane H^+-ATPase genes; HPLC-ESI-MS/MS, high performance liquid chromatography electrospray mass spectrometry; IAA, indole-3-acetic acid; IRT, iron-regulated transporter; MRM, multiple reaction monitoring; NO, nitric oxide; PCIB, anti-auxin p-chloro-phenoxyisobutyric acid; PPFD, photosynthetic photon flux density; RT-PCR, real time-polymerase chain reaction; SCF^{TIR1}, Skp1-Cullin-F-Box complex, transport inhibitor response 1; TIBA, auxin polar transport inhibitor 2,3,5-triiodobenzoic acid; TIR, transport inhibitor response.

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doi:10.1016/j.plaphy.2011.02.018

subjected to shoot Fe functional deficiency, in relation to the root expression and activity changes of FCR, H^+-ATPase and Fe(II) transport [15]. This study showed that all these parameters were well synchronized, with a significant, but transient, increase in the concentration of indole-3-acetic acid (IAA), nitric oxide (NO), abscisic acid (ABA) and ethylene in roots, and a very significant increase in IAA concentration in shoots [15]. Some studies have reported that auxin is involved in the induction of Fe deficiency stress responses (including acidification, subapical root hair and transfer cell formation) [12,19–23], that exogenous auxin is able to increase FCR activity in several plant species [6,24,25], and also that there is a potential auxin-NO inter-connexion in the regulation of FCR in *Arabidopsis* [26].

Ethylene has been shown to have a role in the activation of the gene expression and physiological functionality of several components of the Strategy I response (FCR, H^+-ATPase, IRT) in roots of plants grown under shoot Fe functional deficiency [16]. Recently, the same group has also described a possible inter-connexion between ethylene and NO in the regulatory network governing the activation of Fe-stress physiological responses in roots [17]. In general, these ethylene-mediated actions were Fe-sensitive and blocked or attenuated under Fe-sufficient conditions [17]. Concerning the role of NO in the regulation of the expression of the Fe-stress physiological responses under Fe deficiency, Lamattina and co-workers reported results similar to those of ethylene [18], and, as in the case of ethylene, the NO-mediated action did not occur in Fe-sufficient conditions. These results clearly show that both ethylene and NO must be involved in some way in the shoot-to-root regulatory cascade that is activated when a functional Fe deficiency occurs in shoots. However, the nature of the signal(s), or signal network, which is activated in the shoots of Fe-deficient plants, remains unsolved.

Our working hypothesis is that the signal network activated in the shoot under Fe functional deficient conditions could involve phytoregulator(s) different from NO and ethylene, which could in turn activate a molecular-signal cascade involving ethylene, NO and other possible effectors in the root. This shoot-originated promoting signal would be activated under conditions of shoot Fe functional deficiency. This would explain why the effects of ethylene and NO are Fe-sensitive, because in the presence of shoot active Fe the shoot promoting signal would be inactive. This shoot promoting signal should be able, when applied exogenously, to activate Fe-stress root responses even under conditions of Fe sufficiency. This signal (or signal network) might involve ethylene and NO in its mechanism of action, but it should include additional, but essential, biochemical events. If the regulatory signal acts only through ethylene and/or NO-dependent pathways, the exogenous addition of these phytoregulators should activate Fe-stress root responses even under Fe-sufficient conditions, a fact that does not occur.

Our experimental design includes two consecutive steps: (i) To study the ability of exogenous IAA and ABA to activate Fe-stress physiological root responses at the transcriptional and enzyme activity levels under conditions of Fe sufficiency; and (ii) To study the effect of specific inhibitors of the synthesis and function of phytoregulator(s) found capable to activate the main Fe-stress root responses, using Fe deficiency conditions.

2. Results

2.1. Changes in root Fe-stress responses and IAA and ABA concentrations under Fe-deficient conditions

Six hours after imposing Fe deprivation, Fe-starved plants show an increase in FCR activity, and large increases in this parameter were also found at 24 and 48 h when compared to the controls (Fig. 1A). The expression of three genes associated to Fe-deficiency root responses (H^+-ATPase: *CsHA1*; Fe reduction: *CsFRO1*; and Fe uptake: *CsIRT1*) was up-regulated at all sampling times (6, 24 and 48 h; Fig. 1B–E). However, *CsHA2* (coding for a less active isoform of H^+-ATPase), was not affected or slightly down-regulated (Fig. 1C). Regarding IAA concentration in shoots (Fig. 1F), there was a significant increase in Fe-starved plants 6 h after Fe deprivation, and the same effect was observed in roots 6 and 24 h from the beginning of the treatments (Fig. 1G). ABA concentration in shoots and roots of Fe-starved plants decreased when compared to the controls at 6 h, and a decrease was also observed in shoots 48 h after imposing Fe deficiency (Fig. 1H–I).

2.2. Could IAA and ABA activate the root Fe-stress responses even under Fe sufficiency growth conditions?

In order to investigate the IAA and ABA role on Fe-stress root responses, we studied the effect of both hormones under Fe-sufficiency conditions. According to our hypothesis, the main signal from the aerial part involved in the activation of Fe-stress root responses would also induce the typical Strategy I responses even under Fe-sufficiency conditions.

First, we applied ABA in the nutrient solution of Fe-sufficient plants during 24 h, and measured the root FCR activity, the expression of several genes related to Strategy I responses in roots and the concentrations of IAA and ABA in shoots and roots (Fig. 2). The FCR activity increased at 6 and 24 h after adding ABA to the nutrient solution (Fig. 2A), although the maximum increase was lower than that found in the case of Fe-starved plants (Fig. 1A). Twenty-four h after ABA was removed from the nutrient solution the FCR activity decreased (48 h data point in Fig. 2A). The expression of *CsHA1*, *CsHA2*, *CsFRO1* and *CsIRT1* was generally down-regulated in Fe-sufficient plants supplied with ABA when compared with the control Fe-sufficient plants without ABA supply, with the exception of *CsFRO1* and *CsIRT1*, which increased somewhat 24 h after ABA treatment (Fig. 2B–E). Twenty-four h after ABA was removed from the nutrient solution, the expression of all four genes decreased markedly (48 h data points in Fig. 2B–F). On the other hand, the ABA treatment decreased the IAA concentration in all root samples (Fig. 2G), whereas the IAA concentration in shoots was unchanged by the treatment (Fig. 2F). The concentration of ABA increased markedly in both roots and shoots (Fig. 2H–I).

Then, we applied IAA in the nutrient solution of Fe-sufficient and Fe-deficient plants during 24 h and measured the root FCR activity and the expression of several genes related to Strategy I responses in roots (Fig. 3A–E). The root FCR activity increased significantly 24 h after adding IAA in both Fe-treatments. However, 24 h after the IAA was removed from the nutrient solution (48 h data point in Fig. 3A), the FCR activity was similar to that of the controls (Fig. 3A). The expression of *CsHA1*, *CsFRO1* and *CsIRT1* in Fe-sufficiency conditions was generally up-regulated by IAA, specially at 24 h (Fig. 3B,D,E). Under Fe-deficiency conditions, the up-regulation was only significant for *CsHA1* at 24 h and 48 h (the latter 24 h after removal of IAA) (Fig. 3B). The concentrations of IAA and ABA in shoots and roots were measured only in Fe-sufficient plants (Fig. 3F–I). The treatment with IAA caused an increase in root IAA concentration after 6 and 24 h (Fig. 3G), whereas no changes were observed in IAA shoot concentrations (Fig. 3F). On the other hand, the IAA treatment increased root ABA concentrations significantly in roots after 6 h, to decrease afterwards down to control levels (Fig. 3I). However, the ABA concentration in shoots of IAA-supplied plants increased significantly when compared with the controls at 6 and 24 h (Fig. 3H).

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