Plant Physiology and Biochemistry 49 (2011) 530–536

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Suppression of Fe deficiency gene expression by jasmonate

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ARTICLE INFO

Article history:
Received 15 October 2010
Accepted 26 January 2011
Available online 3 February 2011

Keywords:
Jasmonate
Iron uptake
IRT1
FRO2
FIT
Split root

ABSTRACT

Fe deficiency genes are regulated in response to external supply of Fe as well as internal plant signals. Internal plant signals include plant hormones and systemic signals which coordinate shoot physiological requirements for Fe with local availability of Fe in roots. Induction of *IRT1* and *FRO2* gene expression can be used to monitor the Fe deficiency status of plant roots. Here, we investigated the role of jasmonate in the regulation of Fe deficiency responses and in the split root system. We found that jasmonate suppressed expression levels of *IRT1* and *FRO2* but not their inducibility in response to Fe deficiency. Analysis of the jasmonate-resistant mutant *jar1-1* and pharmacological application of the lipoxygenase inhibitor ibuprofene supported an inhibitory effect of this plant hormone. Inhibition of *IRT1* and *FRO2* gene expression by jasmonate did not require the functional regulator FIT. By performing split root analyses we found that systemic down-regulation of Fe deficiency responses by Fe sufficiency of the shoot was not compromised by ibuprofene and in the jasmonate-insensitive mutant *coi1-1*. Therefore, we conclude that jasmonate acts as an inhibitor in fine-tuning Fe deficiency responses but that it is not involved in the systemic down-regulation of Fe deficiency responses in the root.

1. Introduction

Plant roots are nearly constantly exposed to nutrient salts in their environment. Yet, the uptake profiles for nutrients vary diurnally and in response to various physiological and developmental stimuli. Regulation of nutrient uptake is necessary not only to ensure minimum uptake of the essential nutrients but also to avoid excessive uptake followed by potentially toxic effects. Plants sense the availability of nutrients in their local environment and their physiological diurnal and developmental needs for these nutrients [1].

Uptake of Fe can be used as a model system to study the role of plant internal signals related to nutrient regulation to investigate the underlying regulatory mechanisms. The Fe status of a wild type plant is reflected by the expression levels of marker genes. For example, increased expression of *IRT1* and *FRO2* in the root compared to a control condition indicates Fe deficiency [2–4]. Mutant studies showed that these two genes encode structural core components for Fe acquisition from the soil following the Fe reduction-based strategy I. *FRO2* encodes the root plasmamembrane-bound ferric chelate reductase [3], while *IRT1* codes for the divalent metal transporter for Fe uptake [5–7]. *FRO2* and *IRT1* were often found co-regulated [8]. Their expression is controlled by a bHLH transcription factor named FIT [9]. In the absence of a functional FIT protein the expression of *FRO2* and *IRT1* is far lower than in wild type and if not supplemented with Fe *fit* mutants develop a lethal leaf chlorosis [10–12]. Ethylene and nitric oxide positively affect expression of *IRT1* and *FRO2* suggesting that these two signals increase the sensitivity of plants for Fe uptake [13–16]. Cytokinins on the other hand cause a down-regulation of the two genes [17]. Hormonal influence on Fe acquisition gene expression may serve to coordinate physiology and stress responses with necessary adaptations for altered root growth and uptake of Fe [18–21]. Systemic signals controlling Fe uptake have been physiologically identified but their nature is not known. For instance, grafting of constitutive mutant Fe-deficient shoots to wild type roots can override any local Fe sufficiency sensing in the root and promote constitutive induction of Fe acquisition responses [22,23]. On the other hand, Fe-sufficient shoots may block Fe uptake in Fe-deficient parts of split roots [4,8,18,19].

Jasmonates are oxylipin-based plant hormones originating from poly-unsaturated fatty acids that act in response to developmental or environmental stimuli [24]. Environmental cues for activating the jasmonate signalling pathway include wounding, insect attack or UV light and as such jasmonate belongs to the so-called stress hormones. Jasmonate has an interesting property in that it is a systemically acting mobile plant hormone [25]. Progress has been made in identifying the jasmonate signalling pathway by thorough

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doi:10.1016/j.plaphy.2011.01.025

analysis of JA resistant and insensitive mutants [26]. Jasmonates are perceived intracellularly by a jasmonate receptor belonging to the F-box protein family that upon binding to the plant hormone targets a repressor of the jasmonate response pathway for degradation [27–29]. The activated jasmonate form most efficiently bound by the jasmonate receptor was found to be an Ile-conjugated derivative of jasmonic acid (JA-Ile, namely (+)-7-*iso*-JA-Ile) [30,31]. Isoleucine conjugation to jasmonate is catalyzed by an enzyme encoded by the *JAR1* gene [32,33].

Here, we tested whether the plant hormone jasmonate had any effect on the regulation of Fe uptake responses, and whether jasmonate might be a candidate for a systemic signal involved in Fe deficiency regulation.

2. Results

2.1. Gene expression analysis of Fe deficiency genes in response to jasmonate treatment

In search for mobile plant signalling compounds that influence the regulation of Fe acquisition and may represent candidates as systemic signals we tested the effect of jasmonate on the regulation of the *IRT1* promoter using transgenic *pIRT1*::*GUS* plants [7]. We exposed two week-old Arabidopsis seedlings for 3 days to + or − Fe in the presence of 0 or 100 μM jasmonate, respectively. We found that in the absence of jasmonate GUS activity was induced four times in the root upon − Fe treatment (Fig. 1). Upon jasmonate treatment absolute GUS activity levels were lower at both + and − Fe conditions compared to the non-jasmonate- treated controls (Fig. 1). However, despite of generally lower GUS activity levels the *IRT1* promoter was still induced by − Fe in the presence of jasmonate (Fig. 1).

In a further experiment we confirmed these results by reverse transcription-qPCR gene expression analysis. We exposed 6 day-old wild type Arabidopsis seedlings grown at + or − Fe for 6 h to 0 or 100 μM methyl-jasmonate, respectively, and analyzed the expression of the Fe acquisition genes. We found that *FIT* was induced about 2.5fold in the root under − Fe conditions compared to the + Fe control (Fig. 2B), while *FRO2* and *IRT1* were up-regulated seven- and eightfold (Fig. 2A). Upon methyl-jasmonate treatment, absolute expression levels of *FIT*, *IRT1* and *FRO2* were reduced to 40% at − Fe, respectively, compared to the untreated controls (Fig. 2A, B). However, *FIT*, *FRO2* and *IRT1* were still up-regulated by − Fe in the presence of methyl-jasmonate. As a control for successful methyl-jasmonate action, expression of the jasmonate response gene *PDF1.2* [34,35] was analyzed and found to be markedly induced in the methyl-jasmonate-treated samples compared to the controls (data not shown). Therefore, jasmonate treatment was effective in our experiments.

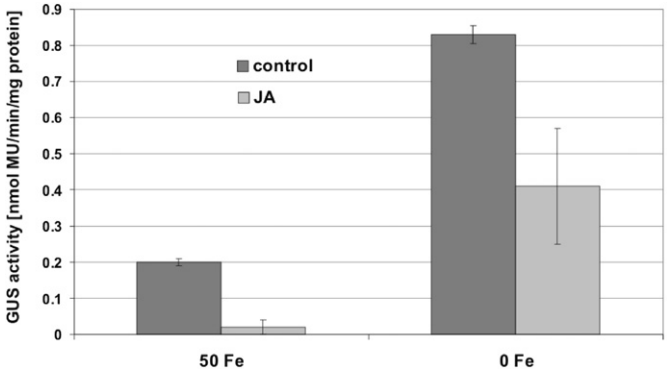

Fig. 1. *IRT1* promoter regulation in response to jasmonate. Regulation of p*IRT1* was determined by quantitative GUS activity measurements. Two week-old p*IRT1*::*GUS* plants were exposed for three days to + (50 Fe) and − Fe (0 Fe), in the presence of 100 μM jasmonate (JA) or its absence (control); roots were harvested for analysis.

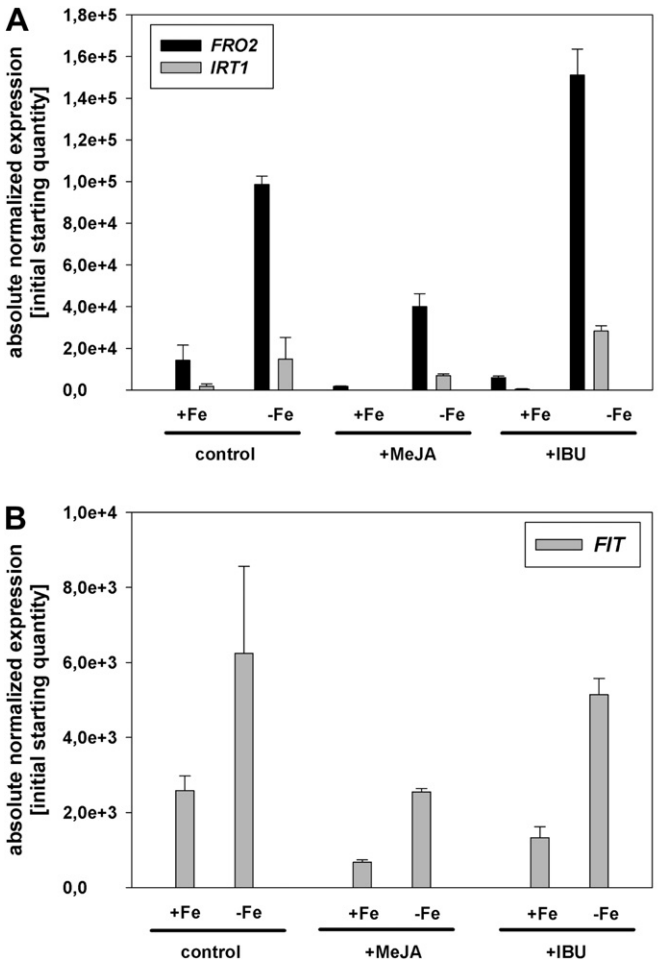

Fig. 2. Analysis of gene expression in response to Fe supply, methyl jasmonate and ibuprofene. A, *FRO2* and *IRT1* expression; B, *FIT* expression; reverse transcription-qPCR analysis in six day-old seedlings grown under + or − Fe and exposed for 6 h to 100 μM methyl-jasmonate (MeJA), 10 μM ibuprofene (IBU) or none of them (control); $n = 2$, SD were calculated for two biological replicates.

In conclusion, our results demonstrate that external supply of methyl-jasmonate suppressed absolute expression levels of Fe deficiency marker genes in the root but did not inhibit inducibility of these genes by − Fe.

To obtain further confirmation for the inhibitory effect of jasmonate on Fe deficiency gene expression, plants were treated with 10 μM ibuprofene. Ibuprofene is a known inhibitor of lipoxygenase activity, and such an enzymatic activity is needed during jasmonate biosynthesis. For this reason, ibuprofene is commonly used to assess jasmonate function in plants [36,37]. We found that application of ibuprofene at − Fe resulted in comparable expression levels of *FIT* (Fig. 2B), but 1.5 and 1.9fold increased expression levels of *FRO2* and *IRT1*, respectively, compared to the untreated controls. Thus, the ibuprofene results supported an inhibitory action of jasmonate.

2.2. Gene expression analysis of Fe deficiency marker genes in the jasmonate-resistant mutant jar1-1

JAR1 encodes the jasmonate-amino acid conjugate synthase that transforms jasmonate into the active jasmonate-Ile form