Physiological and Molecular Plant Pathology 93 (2016) 67–74

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

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

Investigating interactions of salicylic acid and jasmonic acid signaling pathways in monocots wheat

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ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
5 January 2016
Accepted 7 January 2016
Available online 8 January 2016

Keywords:
Wheat
Salicylic acid
Jasmonic acid
Cross talk
Interaction

ABSTRACT

Upon pathogen or insect attack, plants respond with production of a specific blend of the alarm signals salicylic acid (SA) and jasmonic acid (JA), which are recognized as key players in the regulation of the signaling pathways involved. SA and JA responsive genes and SA/JA cross talk were well characterized in dicotyledonous species, but little is known in monocotyledonous plants. Using qRT-PCR, the expression profiles of SA and JA responsive genes were investigated after SA and JA treatments in monocots wheat. The results showed that *Glu2* and *PR-2* responded almost exclusively to SA, *PR-3* and *LOX2* responded positively to methyljasmonate (MeJA) treatment, while *Lipase* and *PR-1.1* were induced in response to treatment with SA or MeJA. Furthermore, either by pathogen infection or exogenous application of hormones can activate the antagonistic effect between SA and JA in wheat, which has been well elucidated in dicotyledonous species. The outcomes of SA-JA interactions could be affected by the relative concentration of each hormone. This study shed light on marker genes that can represent SA and JA pathways in wheat and provided some clues for better understanding their interactions in monocot.

1. Introduction

When plants were subjected to attack by different types of pathogens or herbivorous insects, specific defense response was activated. Plant hormone SA, JA and ethylene (ET) mediated signaling pathway is considered to play a key role in the defense reaction [1]. Although there are exceptions, in general, plants protect themselves against infection by biotrophic pathogens through activation of SA mediated induced defenses, whereas JA and ET mediated defenses are mainly triggered upon infection by necrotrophic pathogens and herbivorous insects [2–4]. Over the past years, many key SA or JA/ET responsive genes have been identified in *Arabidopsis*, such as a variety of pathogenesis related (PR) genes. Although the expression pattern of PR genes may exhibit difference in different plant species, it can be stated that in most dicotyledonous species, including tomato and *Arabidopsis*, *PR-1*, *PR-2* and *PR-5*, which are systemic acquired resistance (SAR) marker genes, are induced by exogenous SA or its analogues of 2,6-dichloro-isonicotinic acid (INA) and benzothiadiazole (BTH), and the resistance to biotrophic pathogens infection is enhanced correspondingly [5,6]. JA and ET co-regulate the expression of *PR-3*, *PR-4* and *PR-12* (*PDF1.2*), which encode antimicrobial peptides [7,8]. The accumulation of JA in *Arabidopsis* can also strongly induced the expression of *LOX2* and *VSP2* [9]. Moreover, JA is a key signal in the SA-independent induced systemic resistance elicited by rhizosphere biocontrol bacteria [10,11].

There commonly exist either mutually antagonistic or synergistic effect between signaling pathways, resulting in negative or positive function outcomes. Hence, cross talk between induced defense-signaling pathways, which can enable the plant to reduce energy loss and enhance the ability against specific pathogen infection, is crucial to the final control results in plant. In recent years, the interaction between SA and JA signaling pathway has been extensively studied. Most reports indicate a mutually antagonistic interaction between SA- and JA-dependent signaling. That is, either pathogen infection or exogenous application of SA to activate SA signaling pathway, can strongly supress the expression of JA responsive genes, such as *PDF1.2*, *LOX2* and *VSP2* [12,13]. As a result of negative cross talk between SA and JA, activation of the SA response should render a plant more susceptible to attackers that are resisted via JA-dependent defenses and vice versa. For example,

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http://dx.doi.org/10.1016/j.pmpp.2016.01.002
0885-5765/

necrotrophic pathogen *Botrytis cinerea* produces an exopolysaccharide, which acts as an elicitor of the SA pathway. In turn, the SA pathway antagonizes the JA signaling pathway, thereby allowing the fungus to develop its disease in tomato (*Solanum lycopersicum*) [14]. Furthermore, SA-mediated antagonistic effect on JA-responsive genes expression is conserved among *Arabidopsis* accessions and may be modulated by SA-mediated redox changes [2]. Although most reports indicate a mutually antagonistic interaction between SA- and JA-dependent signaling, synergistic interactions have been described as well in *Arabidopsis* and tomato [15–17]. For example, treatment of a low concentration of SA and JA (10–100 μM) can more effectively enhance the JA/ET genes expression than JA treated alone, but continuous increase of SA concentration will lead to antagonism, which shows that there was a transient synergistic between SA and JA signaling when both signals were applied at low doses for short durations [16]. Thus, in the interaction between plant and multiple aggressors, the outcomes of JA-SA interactions may depend on the relative concentration of SA and JA, the phase of interaction and the sequence of hormone treatment.

Despite the existence of SAR is still not precisely defined in monocotyledonous plants, pathogens or chemical inducer treatment can also activate resistance response [18–21]. In wheat, the expression of CC-NB-LRR type gene *TmMla1* functions in pathogen-induced immune responses [22]. Another wheat gene, *WCI*, regulates disease resistance induced by chemical SAR inducer BTH [23]. There are two different types of *PR-1* homologous gene in wheat, namely *PR-1.1* and *PR-1.2*. Interestingly, these two genes isolated from wheat are both induced by pathogens, but not by SA or other SAR inducers [24]. However, MeJA treatment can simultaneously induce the expression of *PR-1.1*, *PR-1.2*, *chitinase 1* (*Chi1 1*), *chitinase 3* (*Chi3*), *chitinase 4* (*Chi4*), *beta-1,3-glucanase-1* (*Glu1*) and *lipase*, while *β-(1,3;1,4)- glucanase-2* (*Glu2*) was specificly induced by SA [25]. In monocot rice, JA treatment induced the expression of *PR-1*, *-2*, *-3*, *-5* and *-9* [26,27]. These results indicate that there is some difference in SA or JA response between monocots and dicots.

In our previous study, we found that SA and JA might be related to wheat scab resistance through systematic analysis of the proteome of young spikes of wheat after infection [28]. In order to determine the marker genes that can represent SA and JA pathways and the cross talk of these two pathways in wheat, here, the expression profile of SA and JA responsive marker genes after SA and JA treatment was examined in detail, and the interaction between SA and JA pathways was also studied in wheat plants challenged with biotrophic and necrotrophic pathogens respectively. Our data suggested that, in wheat, the existence of greater subtlety in SA and JA interactions than simple antagonism that could provide wheat with a powerful regulatory potential in response to pathogen and insect attack.

2. Materials and methods

2.1. Plant materials and chemicals

Seeds of wheat (*Triticum aestivum* L. cv. Yangmai 18) were germinated in culture utensil with moistened filter paper, and grown at room temperature for 2 days. Then the rooted normal green plants were transplanted into flower pots (8–10 seedlings/pot) and grown for a week in the incubators under the temperature adjusted at 25/18 °C (daytime/night), illumination time of 16 h/d and illumination intensity of 150 μmol m^{-2} s^{-1}. All chemicals were purchased from Sigma Pharmaceuticals.

2.2. Chemical treatments of plants

Chemical induction treatments were performed by spraying one-week-old soil-grown wheat seedlings with different concentrations of SA (10, 100, 250, 500, 1000 μM) and JA (2, 10, 50, 100, 250 μM) respectively. Each pot was treated with 50 ml SA or JA solution, and tissues were collected 24 h after spraying. Surfactant Tween 20 (0.1%) was added during the treatment. When assaying for the time-course expression of marker genes, seedlings were treated with 500 μM SA and 10 μM JA respectively, and were sampled at the time of 3, 6, 12, 24, 36 h. When examining SA + JA effects on gene transcription in wheat, seedlings were treated with 500 μM SA and increasing concentrations of JA, or 10 μM JA and increasing concentrations of SA for 24 h respectively. The chemical compounds were dissolved in 0.1% ethanol and Tween 20 mixture treated seedlings were used as control. After treatment, approximately 1 g of leaf tissue was harvested from each treatment for RNA extraction. Each treatment repeated three times.

2.3. Fungus inoculation

Wheat seedlings inoculated with *Blumeria graminis* f. sp. *tritici* and *B. cinerea* were conducted as described in Xiang et al. [29]. Inoculated leaf tissues were harvested at 6, 12, 24, 36 h following the inoculation. Or alternatively, *B. graminis*- and *B. cinerea*-inoculated wheat seedlings were pretreated with 10 μM JA and 500 μM SA, respectively, and harvested at the corresponding time points. Tissues from seedlings without treatment were used as the control.

2.4. Endogenous SA and JA measurement

SA and JA measurements were carried out using HPLC-ESI-MS/MS as stated in Ding et al. [28]. For HPLC-ESI-MS/MS, approximately 0.2 g homogenized sample from *B. graminis* and *B. cinerea*-challenged wheat was extracted with 0.5 mL of 1-propanol/H_2O/concentrated HCl (2:1:0.002, v/v/v). The standard curves for SA and JA quantification were generated using a series of SA and JA (Sigma) dilutions. These experiments were all performed with two biological replicates and each sample was measured three times.

2.5. RNA extraction and reverse transcription

RNA extracted from young spikes and wheat seedlings and reverse transcription were performed according to Ding et al. [28]. RNA was quantified with a spectrometer (Ultrospec2100 pro, Amersham Pharmacia, England).

2.6. Expression assays

The template was calibrated through RT-PCR amplification of the wheat *β-tubulin* gene. Semi-quantitative RT-PCR (sqRT-PCR) reaction was performed in a total volume of 25 μl, containing 6 ng cDNA, 2.5 pmol of each primer, 2.5 nmol of each dNTP, 18.6 nmol MgCl2, 0.5 U of rTaq DNA polymerase (Takara, Japan) and 2.5 μl of 10 × PCR buffer. The thermal cycle parameters were 94 °C for 3 min; 22–30 cycles of 94 °C for 15 s, 58 °C for 25 s, 72 °C for 30 s; and a final extension of 72 °C for 5 min. PCR products were resolved on 1.5% agarose gels and visualized under UV light after staining with ethidium bromide.

Quantitative real-time RT-PCR (qRT-PCR) reaction was performed in a total volume of 20 μl, containing 3 ng cDNA, 250 M of each primer and 10 μl of 2 × iQ SYBR Green Supermix (Bio-Rad) on an iCycler iQ fluorescence real time PCR (Bio-Rad). The Q-PCR setting was 1 min at 95 °C, followed by 40 cycles of 94 °C 10 s, 60 °C 20 s and 72 °C 30 s. The relative expression level was normalized