Novel *Arabidopsis* jasmonate-responsive mutants have variations in bacterial disease resistance

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Jasmonates (JAs) regulate a wide range of developmental processes in plants and play a central role in regulating plant defense against biotic stresses such as pathogen infections and insect attacks. A transgenic Arabidopsis line containing the JA inducible VSP1 promoter linked to the Luciferase (LUC) reporter gene was mutagenized using random T-DNA insertion tags. Previous genetic screening of these lines through LUC activity identified 12 Arabidopsis mutants with reduced (jas mutants) or enhanced (jae mutants) P_{VSP1}::Luciferase reporter gene expression upon JA treatment. Here we report phenotypic evaluation of these mutants in response to pathogen infection using Pseudomonas syringae pv. tomato DC 3000 strain. Out of 12 JA-related mutants, five (jas1, jas2, jas5, jas9 and jae1) showed increased susceptibility compared to the parental line, while one mutant (jas7) expressed significant resistance to the bacterial DC 3000 strain. Further molecular characterization of the selected resistant (jas7) and susceptible (jas1) mutants was conducted. Although both mutants were identified as jasmonate-signaling suppressors (jas) at the protein level, the LUC gene was constitutively expressed in jas7 at the mRNA level, while it was abolished in jas1. On the other hand, the endogenous VSP1 gene was constitutively expressed in jas1, but not in jas7 and the parental line. In addition, the expression of two pathogen responsive marker genes (PDF1.2 and THI2.1) were constitutively expressed in the disease resistant mutant (jas7), while expression in the disease susceptible mutant (jas1) was nearly undetectable before JA treatment. Genetic analysis of the jas1 mutant in an F_2 segregation population demonstrated that a single recessive gene in jas1 regulates its disease susceptibility.

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Introduction

Adverse environmental factors, such as pathogens and insects, are a continuous threat to land plants throughout their life cycles [1, 2]. These adverse environmental factors affect crop productivity for all crop species [3] and cause significant economic losses world-wide, especially in developing countries [4]. Biotic stresses such as fungal, bacterial and viral infections, and insect attack, caused 31-42 percent ($500 billion) economic losses worldwide [4].

To cope with the variable stresses, plants have evolved intricate mechanisms for perceiving external signals, allowing adequate and fine-tuned responses to environmental conditions. One of the mechanisms by which plants regulate their protective responses against both biotic and abiotic stresses is through plant phytohormones, such as jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA), ethylene (ET) and indole acidic acid (IAA). Of these, JA, SA and ABA are at center stage in governing the environmental-defense system in plants. JA and SA play a major role in plant biotic stress defense while ABA plays a critical role in plant abiotic stress defense. Cross-talk and established networks among all these hormones are the keys to plant defense systems [5, 6]. For example, JA also plays

important roles in plant defense against abiotic stress through a key regulator, JIN1/MYC2, which mediates crosstalk between biotic and abiotic stress response pathways via JA and ABA signaling [7-10].

JAs are fatty acid-derived signaling molecules that are terminal products of the octadecanoid pathway. JA synthesis is induced by a number of biotic and abiotic stresses, such as insect attack, pathogen infections, and drought [11]. JA induces the expression of wound and pathogen responsive genes including vegetative storage proteins [12] and thionins [13].

Mutant screening based on insensitivity to a JA analog, coronatine, identified the *COI1* gene that belongs to F-box proteins involved in protein degradation [14-17]. *COI1* complex directly targeted transcriptional repressors, such as JAZ proteins [18-21]. JAZ proteins are differentially expressed in response to various environmental conditions [18, 19, 21, 22]. Out of 12 JAZ genes, at least 6 are induced by DC3000 infection [21, 22] suggesting an essential role of the JA pathway in plant biotic defense. In addition to *COI1* and JAZ genes, other JA-insensitive mutants identified include *jar1* [23], *jin1* and *jin4* [24], and *jue1*, *jue2*, and *jue3* [25]. Among these, only *jar1* and *jin1*(myc2) have been identified at the molecular level [9, 26]. JA mutants exhibit varying susceptibility to different types of pathogens. For example, *coi1* is more resistant to bacterial pathogens [14, 27] but more susceptible to fungal pathogens *Alternaria brassicicola* and *Botrytis cinerea* [28]. Similarly, *jin1*/*myc2* mutants displayed reduced susceptibility to *P. syringae* pv. *tomato* DC3000 [29, 30]. On the other hand, *jar1* mutant showed no detectable effect on Arabidopsis susceptibility to *P. syringae* pv. *tomato* DC3000 [27, 29].

Mutants with constitutive or enhanced responses to JA include *cev1* [31], *cet1* to *9* [32], *cex1* [33], and *joe1/2* [25]. Among them, only *cev1* has been characterized at the molecular level and the mutation lies on the cellulose synthase gene, suggesting that the inhibition of cellulose synthesis activates JA- and ethylene-dependent stress responses [34]. *cev1* mutant was resistant to pathogen infections [31].

A critical issue in the improvement of crop stress tolerance is the lack of knowledge on the mechanisms used by plants to defend themselves against stresses. Therefore, understanding these mechanisms and identifying genetic components of the signaling pathways regulating stress defense responses in plants is essential. Identification of such new genetic components will enable scientists to develop new strategies for improving stress tolerance in crops. In spite of the importance of JA in mediating the response to stresses which include ozone exposure, wounding, insect attack, water deficit, and pathogens [35-38], current knowledge about the JA signaling pathway is limited. Several JA-related mutants have been identified and extensively studied [16, 23-25, 39], however, their utilization in crop improvement is not yet demonstrated. In addition, many components in JA signaling pathways are currently unknown, and mechanisms controlling JA-mediated plant defense have yet to be elucidated. Identifying these components is important for a comprehensive understanding of JA signaling and plant defense regulation, and may thus lead to the improvement of crop stress tolerance.

One of the JA-responsive genes is the vegetative storage protein gene (*VSP*) first identified in soybean [40]. VSPs were originally named based on their localization in vacuoles of paraveinal mesophyll cells and accumulation in leaves upon de-podding [40, 41]. They were presumed to act as a temporary storage of amino acids during growth inhibition due to lack of water [41]. *VSP* gene expression is induced by wounding, insect attack, water deficit and JA treatment [41]. The soybean *VSP* promoter is well characterized and the JA-, sugar- and phosphate-response domains are well defined [42]. Arabidopsis *VSP* was shown to be

regulated similarly to soybean *VSP* [43]. AtVSP expression is well characterized and suitable for dissection of signal transduction pathways as VSP gene expression is activated by mechanical wounding, insect feeding and JA treatment [43]. Though some components of JA signaling are known, there are many components in JA signaling that are poorly understood or unknown. Exploiting *VSP* as a target gene of JA will enable scientists to identify new components of JA signaling and elucidate the mechanisms governing JA-mediated plant defense.

Previously, we transformed an *AtVSP* promoter-reporter fusion construct into the *Arabidopsis WS4* ecotype using the firefly luciferase (LUC) reporter gene. Subsequent T-DNA mutagenesis generated 12 novel mutants that exhibited altered reporter gene expression upon JA treatment [44]. Further characterization of these mutants in response to different stresses and identification of the corresponding genes will provide deep insights into the involvement of the JA pathway in plant defense and may provide new strategies to improve stress tolerance in crops. The objectives of this study are to develop an *in vitro* pathogen inoculation system and use it to investigate the response of JA mutants to *P. syringae* strain DC 3000 infection. Molecular characterization was also performed for the resistant mutant *jas7* (see materials section) and the selected susceptible mutant *jas1* (see materials section). In addition, genetics on disease susceptibility in *jas1* was analyzed in an F_2 segregation population generated between *jas1* and Arabidopsis ecotype Col-0. A single recessive gene mutation was responsible for the susceptibility to DC 3000 in *jas1*. Further genetic studies and map-based cloning of the corresponding gene in *jas1* may lead to new strategies for improvement of crop disease resistance.

Materials and methods

Materials and growth conditions

Wild type Arabidopsis (ecotype Col-0 and/or WS4) and the parental line WS-LUC, together with 12 previously isolated JA-related mutants were grown in pots in a 14-hr-light/10-hr-dark growth chamber at temperature of 23^{o}C. Light intensity and humidity were controlled at approximately 130 µmol m^{-2} and 50% respectively. The 12 JA mutants analyzed in this study were named *jas1*-9 and *jae1-3*; *jas* mutants exhibited luciferase activities lower than the parental line, while *jae* mutants displayed enhanced luciferiase activity compared to the parental line.

For bacterial pathogen DC3000 growth and recovery, all cultures and recovered bacteria from the infected leaves were incubated on King's B (KB) medium at 30^{o}C with (bacteria preparation) or without (bacteria recovery) shaking.

In vitro pathogen inoculation system

An *in vitro* system for evaluating plant disease resistance was developed using wild-type ecotype Col-0. Leaves from 3-week old WS4 plants were inoculated with *P. syringae* pv. *tomato* strain DC 3000 (ATCC). DC 3000 bacteria were cultured overnight, diluted in a 1 to 10 ratio, and then grown at 30^{o}C until the OD 600 reached 0.6-0.9. 1 ml of the freshly cultured bacteria was centrifuged and the pellet was re-suspended in 1 ml $MgSO_4$ (10mM). The final concentration of the bacteria was adjusted to OD = 0.001 with $MgSO_4$. To improve pathogen attachment onto the leaf surfaces, the pathogen-$MgSO_4$ mixture was supplemented with 0.05% Silwet L-77. For *in vitro* inoculation, leaves from position 5 or 6 were detached from Col-0 plants and plated on water-agar medium. Leaves were inoculated with 5 µL of OD=0.001 DC 3000 mixture or a mock solution (10 mM $MgSO_4$ and 0.05% Silwet L-77) as a control. The inoculated and control leaves were covered and incubated at 23^{o}C 14/10 light dark cycle. Bacterial growth rate on leaves was quantified by rescuing bacterial cultures from inoculated leaves on day 0, 1, 2, 3, and 4. Leaves were disinfected with 70% ethanol then ground in

500 µl $MgSO_4$. Series of dilution were made depending on the incubation time and symptoms. 20 µl of the diluted sample was spread on solid KB medium containing 25 mg/L rifamycine and incubated at 30°C for 2 days. The number of bacterial colonies rescued from infected leaves was recorded and bacterial growth rates were calculated.

Evaluation of Disease Resistance in Mutants

To evaluate the mutants' responses to pathogens, leaves from 3-week old mutant plants were inoculated with *P. syringae* strain DC 3000 as described above. After 3 days incubation, photographs were taken to assess the disease symptoms. The bacterial growth rates on the leaves were quantified as described above on leaves 3 days post inoculation. The number of bacterial colonies rescued from inoculated leaves was recorded and bacterial growth rates were calculated. All experiments were biologically duplicated and the results were statistically analyzed using Student's t-test.

Total RNA Isolation and RT-PCR

To molecularly characterize JA mutants, leaves from three-week old plants from parental line and the selected mutants were treated with 0.1 mM MeJA or in the mock solution (control) for 24 hrs. Total RNAs were extracted using the Spectrum Plant Total RNA Kit (Sigma, St. Louis, MO, USA) following the manufacturer's instructions. Total RNAs were quantified by NanoDrop and quality assessed by agarose gel electrophoresis. RT-PCR reactions were performed using 2 µg of total RNA in a 20 µl reaction with a one step reverse transcriptase Superscript III (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions. The PCR reactions were performed with gene specific primers using 1 µl of the RT mixtures as template under the following conditions: 95°C, 3 min, then 30 cycles of amplification (95°C for 1 min, 60°C for 1 min, and 72°C for 1 min) and a final elongation period of 10 min at 72°C. Both LUC reporter gene (forward primer 5'-CTGCCTGCGTCAGATTCTCG-3', and reverse primer 5'-GAAGTTCACCGGCGTCAT-3') and endogenous *AtVSP1* gene (forward primer 5'-GTCGATGGATCCATGAAAATCCTCTCACTTTCAC-3' and reverse primer 5'-CGTGCGCTCGAGTTA AGAAGGTACGTAGTAGAGT-3') were examined for their expression and response to JA treatment. The expression of JA and pathogen responsive marker genes *Thi2.1* (forward primer 5'-GGTCATGGCACAAGTTCAAGTA-3' and reverse primer 5'-GGTGGGACTACATAGCTCTTGG-3'), *PDF1.2* (forward primer 5'-TCATGGCTAA GTTTGCTTCC-3' and reverse primer 5'-AATACACACGATTTAGCACC-3'), were also examined. Arabidopsis *actin 1* gene (forward primer 5'-ATGCTGGTATCCATGAAACCACCT-3' and reverse primer 5'-CCTGTGAACAATCGA TGGACCTGA-3') was used as loading control. PCR products were resolved by electrophoresis on 1% agarose gels and detected by UVP gel imagining system.

Results

Development of *in vitro* pathogen inoculation system

The *in planta* inoculation system, either by dip infection or leaf infiltration, has been widely used for plant disease resistance evaluation in Arabidopsis [21, 45]. We developed and validated an *in vitro* inoculation system for Arabidopsis to accelerate the disease resistance evaluation and screening process. Leaves excised from 3-week-old Col-0 plants were placed on water-agar medium and inoculated with *P. syringae* strain DC3000 or a mock solution as a control. Mock-inoculated leaves showed no signs of infection within a 4-day incubation period (Figure 1A) and no DC3000 bacteria were recovered (data not shown). However, DC 3000 inoculated leaves gradually developed infection symptoms within the 4-day period (Figure 1A). Bacteria recovery was performed 0, 1, 2, 3, and 4 DPI (Day Post Inoculation). Over the first 3 DPI, DC3000 bacteria grew significantly and the amount recovered increased steadily (Figure 1B). However, after 3 days of inoculation, bacterial

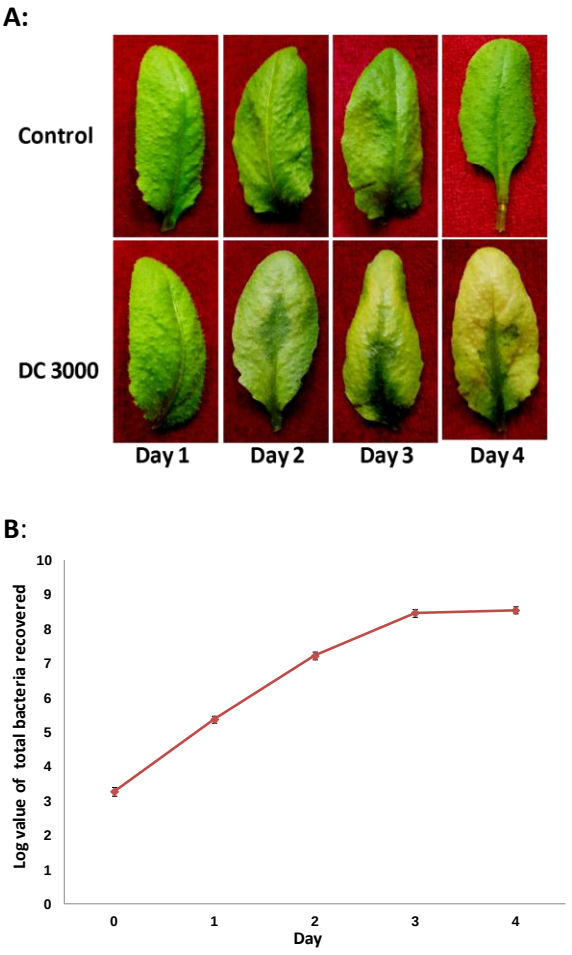

Figure 1. Development of *in vitro* pathogen inoculation and evaluation system in Arabidopsis. **A**. Disease symptoms developed during 4-day incubation period. Upper panel is representatives from control (inoculated with 5 µl of mock solution) experiment; lower panel are representative leaves that were inoculated with 5 µl DC 3000 bacteria at OD=0.001. **B**. Bacterial growth in Arabidopsis wild type Col-0 following *in vitro* inoculation. Data points represent the average of two replicates. Bars represent standard deviation (SD).

growth reached stationary stage, and the number of bacteria recovered did not change significantly between day 3 and day 4. This result is comparable to those found using the *in planta* leaf-infiltration inoculation system [45-47]. Therefore, the *in vitro* inoculation system was used in this study to examine the response of JA-related mutants to pathogen infections.

Evaluation of DC 3000 induced responses in mutants

Previously, we identified 12 novel JA-related mutants using P_{VSP}*::Luciferase* as the reporter [44]. Because JA signaling affects several responses to biotic factors, we wanted to determine whether the newly isolated JA mutants had altered responses to pathogen infections. We used *P. syringae* strain DC3000, to inoculate leaves from 3-week old plants grown in a growth chamber with 14/10 hour L/D cycle. As shown in Figure 2, considerably different responses were observed among JA related mutants. Of these, the *jas7* mutant showed strong resistance to DC3000 infection, whereas *jas1*, *jas2*, *jas5*, *jas*9 and *jae1* mutants displayed significantly increased susceptibility to pathogen infection. All other mutants showed no difference compared to the parental line WS-LUC.

The number of DC3000 colonies rescued from inoculated leaves was recorded to quantify bacterial growth rates as a measure of susceptibility to pathogen infection in JA mutants (Figure 3). Consistent with the observed phenotypes (Figure 2), the amount of bacteria recovered from inoculated *jas7* leaves was significantly less than the amount recovered from the parental line (Figure 3). On the other hand, significantly more bacteria were recovered from infected *jas1*, *jas2*, *jas5*, *jas9* and *jae1* leaves than that of wild-type. Taken together, we concluded that among all mutants tested, the mutant *jas7* was strongly resistant to pathogen infection, while mutant *jas1*, *jas2*, *jas5*, *jas9* and *jae1* were significantly susceptible to DC 3000 infection.

Alternation of pathogen responsive genes in disease resistant and sensitive mutants

The relationship between JA and disease resistance was further investigated by analyzing the expression of several JA and pathogen responsive marker genes in the *jas1* (susceptible) and *jas7* (resistant) mutants. We first examined the expression of the reporter gene luciferase in response to JA treatment. In the parental line WS-LUC, as predicted, LUC gene expression was considerably induced upon JA treatment (Figure 4). In contrast, LUC

Figure 2. Disease symptoms of all 12 novel JA-related mutants 3 days after inoculation with 5 µl of DC 3000 (OD=0.001). Upper panel showed *jas1-5* and lower panel showed *jas6-9* and *jae1-3*. At least two leaves were inoculated for each mutant. Photographs showed the representatives for each.

expression in *jas1* was completely inhibited, even after JA treatment, consistent with our previous findings at the luciferase protein level [44]. On the other hand, LUC gene expression was constitutive in the mutant *jas7*, regardless of JA treatment. Previously we identified *jas7* as JA-signal suppressive mutant at the protein level [44]. These contradictory results may implicate that post transcriptional regulation may be involved in LUC gene expression in *jas7*.

We then investigated the expression of Arabidopsis endogenous *VSP1* gene. Both parental line and the mutant *jas7* showed the expected expression patterns: low or no expression before JA treatment, and significant induction by JA treatment (Figure 4). Surprisingly, in *jas1*, *VSP1* was constitutively expressed. Lastly, we analyzed the expressions of two marker genes in plant defense responses, *PDF1.2* and *THI2.1*. The expression of these two genes in wild type Arabidopsis was both induced by JA and pathogen infection [48-49]. As shown in Figure 4, before JA treatment, both genes were slightly expressed in the parental line, but barely detectable in *jas1*. When treated with JA, these two genes were significantly induced in both the parental line and *jas1*, however no changes were observed in their expression in the resistant mutant *jas7*. The constitutive expression of *PDF1.2* and *THI2.1* may confer DC 3000 resistance to *jas7* mutants.

A single recessive allele regulates disease susceptibility in *jas1* mutant

To further genetically characterize the corresponding gene in *jas1* mutant, *jas1* was crossed to Arabidopsis ecotype Columbia (Col-0), and disease susceptible phenotypes of *jas1* mutants in the F_2 progeny were screened using *in vitro* inoculation system. Figure 5 showed representatives of inoculated leaves from individual F_2 plants. Segregation among F_2 individuals on disease susceptibility was observed. 47 of 159 F_2 progenies tested were susceptible to DC3000 infection while 112 showed resistance (or no difference with wild-type) phenotype (Table 1). Chi square test confirmed a single recessive allele controlling

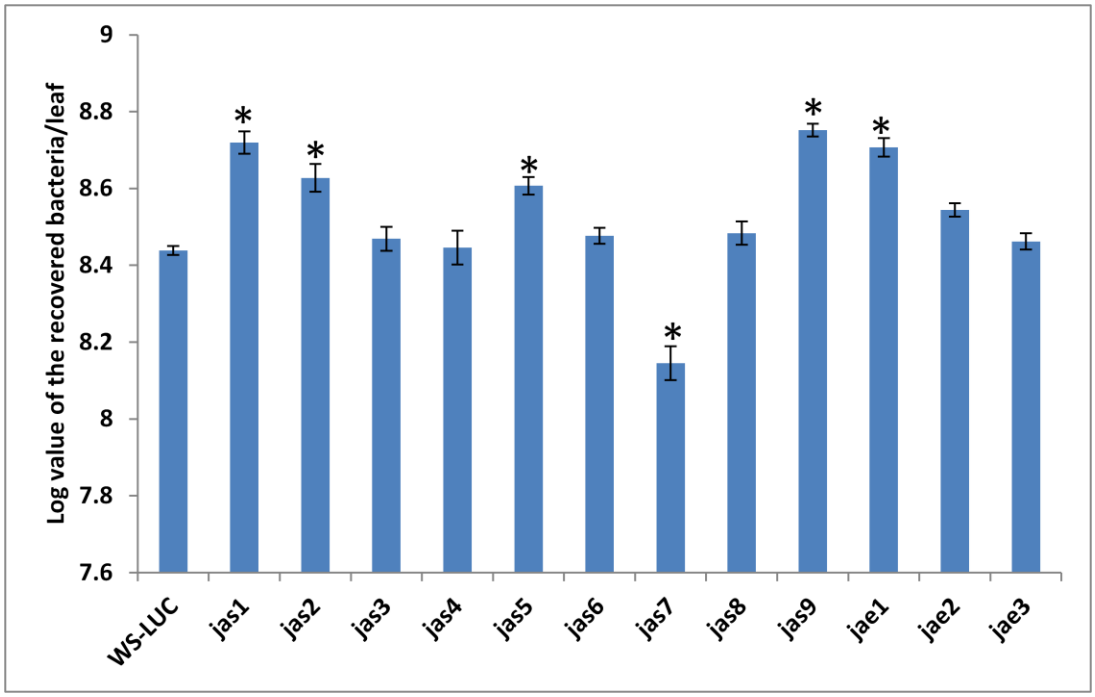

Figure 3. Recovery of bacterial growth in Arabidopsis JA-related mutants after 4 days *in vitro* inoculation with 5 µl DC 3000 (OD=0.001). Data represent the average of two replicates. * indicates significant difference with the parental line (WS-LUC) at P=0.05. Bars represent standard deviation of the means (SD).

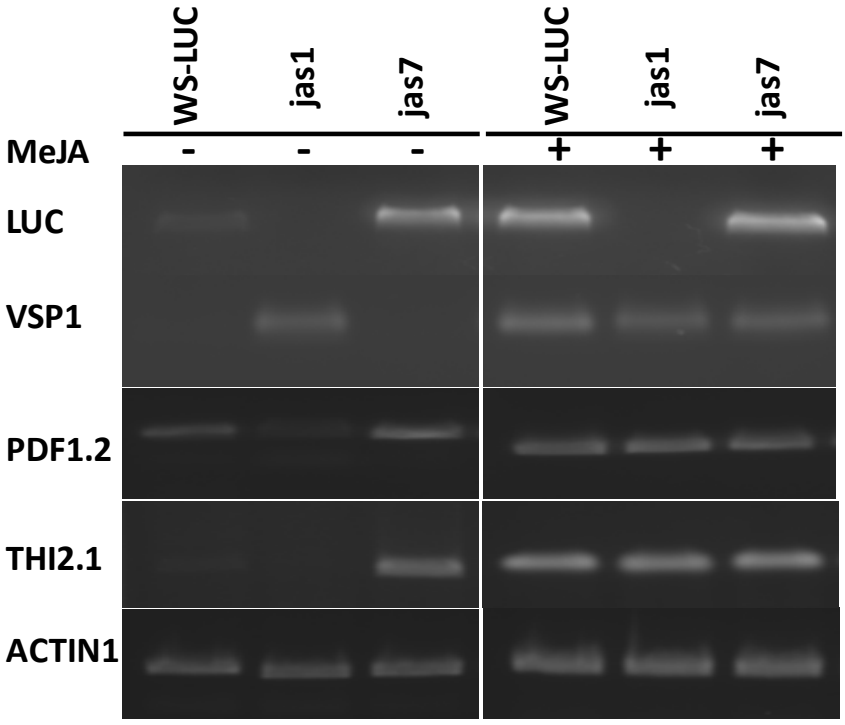

Figure 4. Molecular characterization of *jas1* (susceptible) and *jas7* (resistant) mutants. The parental plants, *jas1* and *jas7* mutants were treated with 0.1 mM JA or mock for 24 hours prior to total RNA isolation. Transcript levels for *LUC*, *VSP1*, *PDF1.2* and *THI2.1* were monitored by reverse transcription-polymerase chain reaction (RT-PCR). Arabidopsis *actin1* gene was used as an internal control.

Table 1. Chi square statistics for one gene control of disease resistance in F_2 population.

Phenotype	Observed	Expected
Resistant	112	119
Susceptible	47	40
Total	**159**	**159**
Chi-square for 3:1 ratio	**1.6368**	
P-*value*	**0.20<P<0.975**	

susceptibility to DC3000 infection in *jas1* mutant (Table 1).

Discussion

Pathogen infection is a major challenge for crop production and seed quality improvement. Identifying novel mutants related to disease resistance, and understanding their functions in defense against pathogens are essential to improve disease resistance in crops. Here, we developed an *in vitro* plant disease evaluation system in Arabidopsis, and used it to investigate disease resistance ability of novel JA-related mutants. *In planta* inoculation has been widely used for disease resistance evaluation [21, 45-47]; however, it is time consuming for inoculation and needs more space and facilities for the evaluation. To accelerate the mutant screening and map-based cloning processes for disease related mutants, a fast, accurate and easy screening protocol is needed. An *in vitro* inoculation system was evaluated in Arabidopsis in this study. Excised leaves were inoculated with bacterial DC 3000 pathogen on water-agar plates, and were incubated at 23^{o}C under 14/10 light-dark cycle. Leaves became symptomatic 3 DPI. The bacterial recovery rate was monitored from day 0 to day 4, and was similar to *in planta* inoculation recovery rates [21, 45-47]. Therefore, this *in vitro* inoculation system can be used for further disease investigation research in Arabidopsis and, with proper modification, can be extended to crop species.

With the *in vitro* inoculation system, incubation conditions can be easily synchronized so pathogens grow uniformly. This system is suitable for large scale mutant screening. In addition, since the same amount of pathogen was applied to all leaves, the symptoms will be due to the response to infection, and not on variations of initial inoculum levels. If bacterial recovery is desired for the experiment, the procedure is simplified because leaf areas do not have to be considered.

Although the roles of JA signaling pathway in plant stress responses have been widely studied [35-38], and several JA related mutants have been identified and extensively characterized for their disease resistance [16, 23-25, 39], current knowledge about the JA signaling pathway is still limited and many genetic components of the JA signaling pathway remain to be isolated. In order to understand and elucidate mechanisms controlling JA-mediated plant defense, we further investigated our newly isolated JA mutants on their bacterial pathogen (DC 3000) susceptibility using the *in vitro* inoculation system. Half of these mutants showed no difference in susceptibility to infection compared to the parental line (Figures 2 & 3). This is not surprising since the JA signaling pathway is not only involved in plant biotic and abiotic stress defense, but also

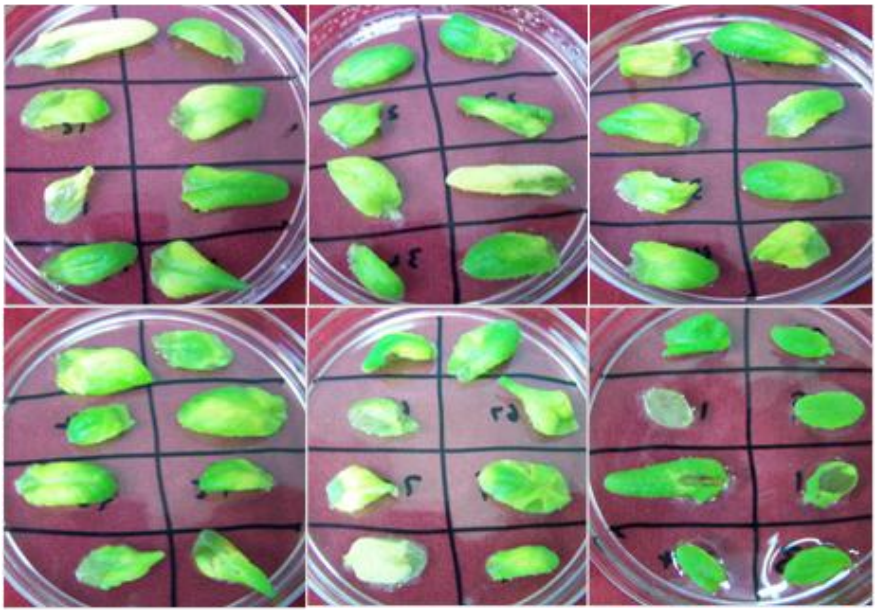

Figure 5. Segregation of disease susceptibility in an F_2 population between *jas1* and Arabidopsis ecotype Col-0. At least two leaves from each individual F_2s were detached from plants and in vitro inoculated with DC 3000 strain. Representative individuals were photographed after 4 days *in vitro* inoculation.

regulates plant developmental processes such as growth, and reproductive development [9, 14, 50, 51].

Of the 12 JA-related mutants studied, only *jas7* showed hyper resistance to DC 3000 strain, whereas 5 JA mutants (*jas1, jas2, jas5, jas9* and *jae1*) showed increased susceptibility to bacterial strain DC 3000 infection (Figures 2 and 3). Of these, *jae1* was defined as JA-signal enhancing mutant, and all *jas* mutants were classified as JA-signal suppressing mutants. The fact that both *jas* and *jae* mutants were susceptible to bacterial pathogen infection suggests that both positive and negative regulators of JA signaling are involved in JA-mediated plant defense. This is in agreement with previous reports demonstrating the roles of both positive regulators of JA signaling, such as WRKY33 [52] and ERF4 [53], and negative regulators of JA pathway, such as JAZ genes [21] in defense against biotic stresses. Further cloning and characterization of these newly isolated JA-related mutants will provide insight into how the JA pathway regulates plant defense.

All *jas* and *jae* mutants were isolated based on altered luciferase activity following JA treatment [44]. *jas* mutants displayed significantly lower LUC activity than the parental line. To further characterize the disease resistant mutant (*jas7*) and the selected disease susceptible mutant (*jas1*) at the molecular level, gene expression of LUC and endogenous *VSP1* was analyzed using RT-PCR. Contrary to luciferase protein activity level, the LUC gene was constitutively expressed in *jas7* regardless of JA treatment, while *VSP1* was induced by JA treatment. Furthermore, LUC gene was not detectable by RT-PCR in *jas1* mutants both before and after JA treatment (consistent with the LUC activity), however *VSP1* was constitutively expressed. Differences between gene expression and protein levels of *LUC* and *VSP1* in *jas1* and *jas7* mutants suggest that post transcriptional and/or translational regulation mechanisms are involved in

regulating functional expression of *LUC* and *VSP1* genes in these mutants.

Constitutive expression of *VSP1* gene (*cev1*) has led to constitutive activation of jasmonate pathways and enhanced resistance to fungal pathogens [31]. *cev1* was isolated through EMS mutagenesis of the P_{vsp1}*::luciferase* reporter transgenic line by monitoring the LUC activity without JA treatment. Although the VSP1 gene was constitutively expressed in *jas1*, the LUC activity was completely inhibited. In addition, *cev1* mutant displayed strong resistance to pathogen infection, whereas *jas1* showed increased susceptibility to pathogen infection. Thus, *jas1* and *cev1* belong to different class of JA related mutants.

It is of interest that *jas1* and *jas7* showed different responses to pathogen infection although they were defined as JA-signaling suppressors. In order to understand the molecular mechanisms regulating pathogen responses, we examined the expression of two pathogen responsive marker genes, *PDF1.2* and *THI2.1*. Both genes were induced in response to JA and pathogen infection in the parental lines in our study (Figure 4), consistent with previous studies in wild type Arabidopsis [48-49, 54]. However, the expression of these two marker genes in *jas1* and *jas7* mutants is different from that of the parental line. In *jas7*, the pathogen resistant mutant, both genes were constitutively expressed regardless of JA treatment. In *cev1* mutant, all of these marker genes were constitutively expressed and displayed enhanced resistance to pathogen infections [31]. Constitutive expression of these marker genes indicate that the mutant plants could always turn on their pathogen defense system. This could be the cause that leads to the enhanced resistance to pathogen infections. On the other hand, *jas1* mutant showed enhanced susceptibility to pathogen infection, even though *VSP1* gene was constitutively expressed. Furthermore, both *PDF1.2* and *THI2.1* expression was lower or undetectable in *jas1* before JA treatment compared to the parental line.

In an effort towards map-based cloning of the *jas1* gene, an F_2 mapping population between *jas1* and Arabidopsis wild type Col-0 was generated. The study indicated that a single recessive gene in *jas1* was associated with increased susceptibility. Further identification of the corresponding gene will provide new insights into the role of the JA pathway in plant defense and may lead to novel approaches to improve biotic stress tolerance in crop species.

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