Jurnal Teknologi, 39(C) Dis. 2003: 9–16

THE ROLE OF JASMONATE IN PLANT PATHOGEN INTERACTIONS IN *ARABIDOPSIS THALIANA*

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Abstract. The system of plant defence against fungi is complex. It encompasses several cross-talking pathways that respond to various signals. Methyl jasmonate, a plant growth regulator is one of the various signals that stimulate a defence response. In order to comprehend the involvement of jasmonate in the defence of *Arabidopsis* against *Erysiphe cruciferarum*, a mutant that is defective in jasmonate was used (*fad3-2fad7-2fad8*). This mutant is susceptible to *E.cruciferarum* infection, which results in powdery mildew. The wild-type plants were resistant towards powdery mildew. Mutant plants sprayed with methyl jasmonate received substantial protection against *E.cruciferarum* infections. Disease incidence in mutants were reduced to levels observed in the wild-type which is ~17%. The *coiI* mutant however was not protected through treatment with methyl jasmonate. This therefore indicates that methyl jasmonate induces the plants resistance through the activation of the plants defence system and not as an antifungal agent. One of the genes activated in the defence response is the *AtVSP* gene. The transcript of *AtVSP* in wild-type plants was induced when infected by *E.cruciferarum*. Transcri pt levels in mutants were comparable to the constitutive levels observed in the controls. Therefore it is apparent that jasmonate-signalling is essential in protection against *E.cruciferarum* infections in *Arabidopsis*.

Keywords: Pathogens, jasmonate, defence, powdery mildew

Abstrak. Sistem pertahanan tumbuhan terhadap serangan patogen kulat melibatkan tapakjalan pengisyaratan yang kompleks. Sistem pengisyaratan yang kompleks ini melibatkan beberapa tapakjalan bersilang yang bertindak terhadap sumber isyarat yang berbeza. Salah satu molekul isyarat yang terlibat dalam pengisyaratan tumbuhan adalah metil jasmonat. Metil jasmonat adalah sejenis bahan kimia yang terlibat dalam pengawalaturan pertumbuhan. Untuk memahami peranan yang dimainkan oleh jasmonat dalam pertahanan *Arabidopsis* terhadap serangan kulat *Erysiphe cruciferarum*, sejenis mutan yang tidak berupaya menumpukkkan jasmonat (*fad3-2fad7-2fad8*) telah digunakan. Mutan ini adalah rentan terhadap penyakit kulapok berdebu (powdery mildew) yang dihasilkan oleh kulat tersebut. Tumbuhan jenis liar tidak menunjukkan kesan penyakit. Penyemburan tumbuhan mutan dengan metil jasmonat dapat memberikan perlindungan yang nyata terhadap serangan kulat tersebut dan membawa insiden penyakit kepada tahap yang diperhatikan dalam tumbuhan liar iaitu kira-kira 17% insiden penyakit. Rawatan yang sama tidak memberikan perlindungan pada mutan *coiI* yang tidak peka terhadap jasmonat. Ini menunjukkan bahawa penyemburan dengan metil jasmonat menghasilkan perlindungan melalui pengaktifan sistem pertahan tumbuhan dan bukan melalui tindakan sebagai agen antikulat. Salah satu gen yang diaktifkan dalam sistem pertahanan tumbuhan adalah *AtVSP*. Transkrip *AtVSP* diaruh oleh infeksi *E.cruciferarum* dalam tumbuhan jenis liar tetapi bukan

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dalam tumbuhan mutan. Tahap penghasilan transkrip dalam mutan menyerupai tahap penghasilan konstitutif transkrip dalam tumbuhan kawalan. Hasil yang diperolehi daripada kajian ini menunjukkan bahawa jasmonat diperlukan dalam pertahanan terhadap jangkitan *E.cruciferarum* dalam *Arabidopsis*.

Kata kekunci: Patogen, jasmonat, pertahanan, kulapok berdebu

1.0 INTRODUCTION

Plants have defence mechanisms that are activated in the event of infection by pathogenic microorganisms such as fungi, bacteria and viruses. Generally the plant-pathogen interaction elicits a local and systemic hypersensitive response that is controlled by a complex molecular mechanism that is triggered by signal molecules [1]. Amongst the signal molecules involved in activating the defence response are salicylic acid, methyl jasmonate, ethylene, hydrogen peroxide and superoxide radicals [2].

Certain fungal elicitors such as chitosan and oligogalacturonidase activate genes that are involved in the wound response [3][4]. The activation of wound response genes by fungal elicitors indicates that there may be some cross-talking between the pathways that react to fungal infection and the pathways that react to wounding in plants. Both pathways use jasmonate as the effector of the defence response [5][6]. The interpretation of jasmonates involvement in the plant-pathogen interaction is further complicated by the presence of other alternative defence pathways that do not require the involvement of jasmonate. Certain jasmonate-activated genes may also activate parallel pathways that do not require jasmonate as an elicitor of the response [2][7].

Until now the importance of jasmonate in plant-pathogen interaction has not been studied. Therefore the importance of this pathway in the plant defence against pathogens remains obscure. In this research we used two mutants (*fad3-2fad7-2fad8* and *coiI*) that are defective in the jasmonate synthesis to show that jasmonate-signalling is important in the protection against the pathogenic fungi *Erysiphe cruciferarum*. The mode by which *fad3-2fad7-2fad8* is defective in jasmonate-signalling has been detailed in [9]. The *coiI* mutant was generated in Dr John Turner's laboratory. The *coiI* mutant is resistant to the phytotoxin coranatine, is male sterile and is insensitive to methyl jasmonate. These mutants are however resistant to bacterial pathogens[8].

2.0 MATERIALS AND METHODS

2.1 Plant Material and Growth Conditions

Seeds of wild-type *Arabidopsis thaliana* ecotype Columbia and seeds from the F_2 population segregating for *coiI* mutants were obtained from our laboratory. The *coiI* mutant was mutated using ethylmetanosulphonate and later selected in Murashige and Skoog (MS) plates supplemented with 10g L^{-1} Sucrose, vitamins and 1μM coranatine. Seedling were then transferred to MS plates for 3-days before being transferred once again to MS coranatine (1 μM) plates. Coranatine is a phytotoxin

produced by *Pseudomonas syringae* pv Glya 4180. Wild-type seeds were germinated in MS medium. All seedlings were grown in white light (150 $\mu Em^{-2}s^{-1}$) with a 16-h-day/ 8-h-night photoperiod at 22°C. The method of generation of the coiI and the *fad3-2fad7-2fad8* mutants is presented elsewhere [8][9]. All seedling were then transferred and grown in peat-based compost (sterile) in a controlled environment with 18-h-day (white light 150 $\mu Em^{-2}s^{-1}$)/ 6-h-night photoperiod at 22°C.

2.2 Preparation of *E.cruciferarum* spores for Inoculation

E.cruciferarum cultures were maintained on Potato Dextrose Agar (PDA) slants and plates. Live cultures were maintained on squash plants. 5 mL of sterile distilled water with 10 ppm Tween 20 was used to harvest spores from the *E.cruciferarum* stock on PDA plates. The spores were counted using the *Improved Neubauer Chamber Haemasitometer*. A 10^7spore inoculum was prepared for inoculation on *Arabidopsis* plants.

2.3 Inoculation on *Arabidopsis* Wild-type and Mutant Seedlings

Mutant and wild-type seedlings were washed with sterile distilled water. The leaves were allowed to dry before 5 µl of spore suspension was inoculated onto the leave. The inoculated plants were maintained in the growth environment mentioned in 2.1. Once the plants began to show signs of powdery mildew infections they were ready for treatment with jasmonate.

2.4 Methyl Jasmonate Treatment of Plants

Both wild-type and mutants plants were sprayed with sterile distilled water containing 50 µM methyl jasmonate and 10 ppm Tween 20 at an four day interval. Plants were sprayed until the leaves were saturated with methyl jasmonate solution. In the test conducted with higher concentrations of jasmonate, the solutions were prepared and administered in the same manner. Control plants were sprayed with distilled water containing 10 ppm Tween 20.

2.5 RNA Extraction and Analyses

Tissue was harvested from infected *coiI*, *fad3-2fad7-2fad8* and wild-type plants two weeks post inoculation. The tissue was snap frozen and total RNA was extracted from the seedlings of mutant and wild-type plants according to method as in [14]. RNA was denatured in 40% (V/V) formamide, 18% (V/V) formaldehyde at 65°C for 15 minutes, separated in a 1.4% agarose/formaldehyde gel and transferred onto a nylon membrane [15]. RNA blots were hybridized to a 330bp cDNA fragment corresponding to the

end of the *Arabidopsis* 27kDa *AtVSP*. After hybridization, membranes were washed three times for 15 min in 2% (W/V) SSC, 0.1% (W/V) SDS at 65°C [15].

3.0 RESULTS AND DISCUSSION

3.1 The Mutant Plants are Susceptible to *E.cruciferarum* Infections

Our observation of the *coiI* and *fad3-2fad7-2fad8* mutants in the green house showed that these plants easily succumbed to powdery mildew caused by a fungal infection. The fungus was later identified as *E.cruciferarum* through DNA sequence analysis. The sequence analysis was conducted by a commercial laboratory on DNA obtained from the fungus. The wild-type *Arabidopsis* that were placed in the same green house did not show any signs of the disease even when placed in a planter together with infected mutant plants. The susceptibility of the *fad3-2fad7-2fad8* and the *coiI* mutant to the disease was assumed to be due to a defective jasmonate-signalling mechanism [2][9][12].

3.2 Jasmonate is Essential in Plant Defence

In order to study the mechanism of jasmonate-signalling in plant-pathogen interactions, the mutant and wild-type plants were inoculated with spores of *E.cruciferarum*. Two weeks post-inoculation, almost 90% of the control *coiI* and *fad3-2fad7-2fad8* were infected (Figure 1). The wild-type population however had only 5% of its population infected. The *fad3-2fad7-2fad8* mutants that were sprayed with 50 µM methyl jasmonate solution showed a reduction in the number of seedling dead or severely infected. The percentage of diseased or dead *fad3-2fad7-2fad8* was reduced to

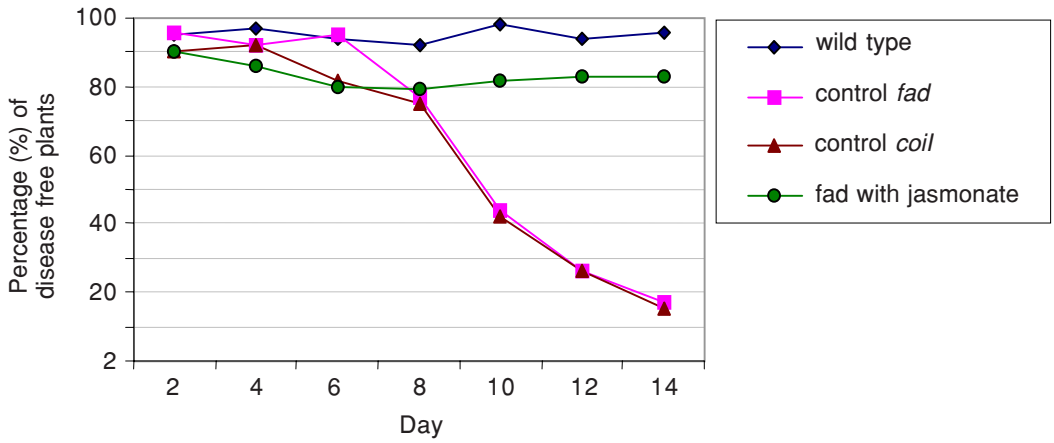

Figure 1 Effect of jasmonate on *Arabidopsis*

~17% (Figure 1). Though the *fad3-2fad7-2fad8* mutants are incapable of producing linolenic acid, a precursor of jasmonate, the defence mechanism in these plants can be activated through the exogenous application of the methyl jasmonate [10]. The spraying of the *coiI* mutant plants with 50 µM methyl jasmonate however did not reduce the rate of mortality in the plants. This is due to the jasmonate-signalling being completely blocked in the *coiI* mutants [11].

Taking into account the results obtained so far, we conclude that the wild-type *Arabidopsis* plants are resistant to infections by *E.cruciferarum*. This shows that a functional jasmonate-signalling is required to provide protection against this pathogenic fungus. A defect in the signaling will result in disease and/or death of host.

3.3 Increase of Jasmonate Concentration Had no Effect on the Protection Afforded to the Mutants

Methyl jasmonate (50 µM) treated *fad3-2fad7-2fad8* mutants exhibited reduction in the incidence of disease and death by approximately 70% (from 90% to 17% after treatment). The impressive reduction in the disease incidence upon methyl jasmonate application prodded us to study if an increase in the methyl jasmonate concentration would reduce the prevalence of this disease. Therefore this experiment was repeated using three different methyl jasmonate concentrations of 100, 150 and 200 µM. The results obtained however showed that an increase in the concentration did not significantly alter the disease incidence. The percentage of diseased and/or dead mutant plants were between 12 to 17% (Figure 2). Methyl jasmonate remained ineffective against the *coiI* mutants even at very high concentrations (1 M) (Figure 3).

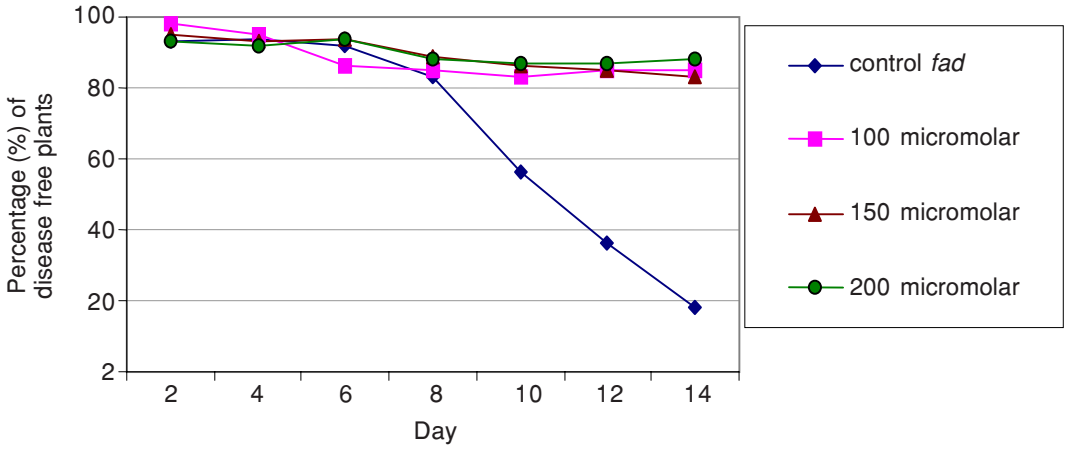

Figure 2 Effect of jasmonate concentration on the *fad3-2fad7-3fad8* mutant

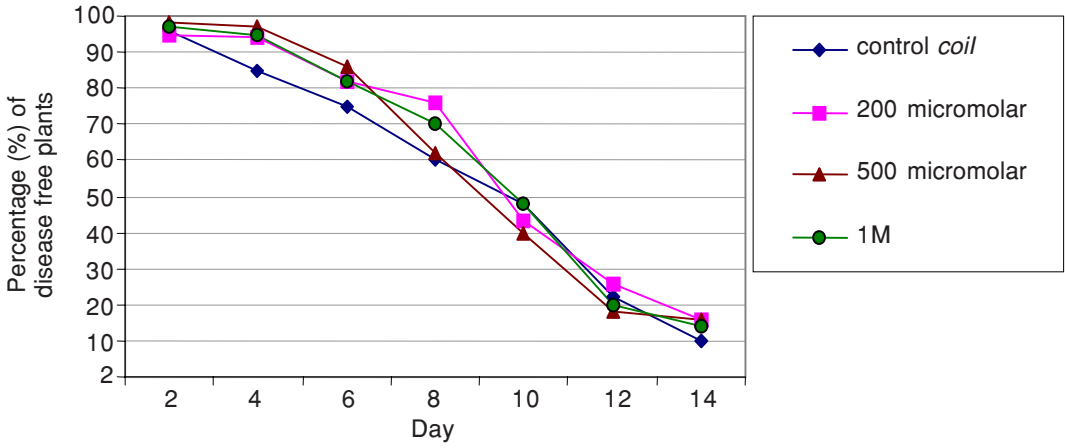

Figure 3 Effect of jasmonate on the *coiI* mutant

3.4 *E.cruciferarum* Induces the Expression of *AtVSP* Gene in Wild-type *Arabidopsis*

The *AtVSP* gene is activated in response to infection. The *AtVSP* gene encodes a vegetative storage protein that is produced during injury, infestation by insects and nematode and during microbial infections [12][13]. The involvement of this gene in the above activities has caused this gene to be classified as a defence related gene. In our work, a comparison between the expression levels of this gene in wild-type and mutant plants showed an increase in the transcript levels of the gene in wild-type plants post inoculation. There was no increase in transcript levels within the mutant plants following infection (Figure 4). This shows that both the mutants are defective or the non-existence of jasmonate-signalling pathway in both mutants [10][12].

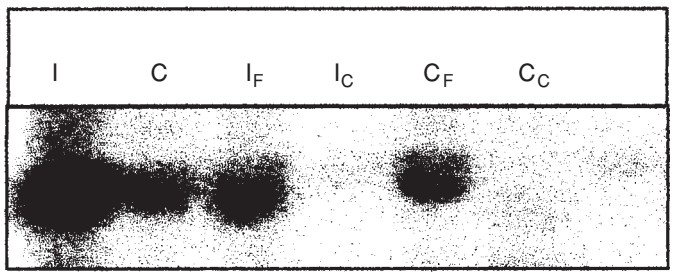

Figure 4 Comparison of *AtVSPA* expression in wild type and mutant plants. The transri ption level in *E.cruciferarum* inoculated (I) and uninoculated (C) plants are shown in this Northern Blot. Transcription levels were analysed 96 hours post inoculation. Subscript F is for the *fad3-2fad7-2fad8* and subscript C is for the *coi1* mutant.

4.0 CONCLUSION

The results showed the involvement of the jasmonate-signalling in the defence against *E.cruciferarum* infections. Wild-type *Arabidopsis* with intact and functional jasmonate-signalling appeared to be resistant to powdery mildew caused by *E.cruciferarum*. This was however not true for the mutant lines as they exhibited susceptibility to infections by the fungus. Though the mutants were defective in their jasmonate-signalling, the experimentation conducted showed that it was possible to rescue the *fad3-2fad7-2fad8* mutant from powdery mildew through exogenous application of methyl jasmonate. This was not possible with the *coiI* mutant as the jasmonate-signalling was non-existent in these plants. Increasing the methyl jasmonate concentration administered showed no significant reduction in the disease incidence in mutant *Arabidopsis*. Since the mutant plants were defective in their jasmonate-signalling they were not able to activate jasmonate-responsive genes such as *AtVSP*. The transcript levels in the mutants remained unchanged upon infection, while the levels within the wild-type plants increased significantly post inoculation.

5.0 ACKNOWLEDGEMENT

I would like to thank Dr John Browse for his kind donation of the *fad3-2fad7-2fad8* mutants seeds. This project was supported by Universiti Kebangsaan Malaysia and a BBSRC grant awarded to Dr John Gordon Turner, School of Biological Sciences, University of East Anglia.

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