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The Signalling Pathway Involved 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 accumulation, 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. The incidence of disease in methyl jasmonate treated mutants was reduced to levels observed in the wildtype. The *coi1* 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 in wildtype plants was induced when infected by *E.cruciferarum*. Transcript 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 of *Arabidopsis*.

Key words: Pathogen, jasmonate, defence, powdery mildew

Introduction

Plants have defence mechanisms that are activated in the event of infection by fungi and other microorganisms. Generally the plant-pathogen interaction elicits a local and systemic hypersensitive response controlled by a complex molecular mechanism triggered by signal molecules, (Creelman and Mullet, 1997). Amongst the signal molecules involved in activating the defence response are salicylic acid, methyl jasmonate, ethylene, hydrogen peroxide and superoxide radicals (Vijayan *et al.*, 1998).

Certain fungal elicitors, such as chitosan and oligogalacturonidase activate genes that are involved in the wound response (Farmer, 1992; Doares *et al.*, 1995). The activation of wound response genes by fungal elicitors indicates that there may be some cross-talking between the pathways that react to fungal infections and pathways that react to wounding in plants. Both these pathways use jasmonate as the effector of the defence response (Koiwa, 1997; Somssich and Halhbrock, 1998). The interpretation of the involvement of jasmonate in the plant-pathogen interaction is further complicated by the presence of other alternative defence pathways that does not require the involvement of jasmonate. Certain genes that are activated by jasmonate may also be activated by parallel pathways that do not require jasmonate as an elicitor of the response (Vijayan *et al.*, 1998, Epple *et al.*, 1995).

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 that are defective in the jasmonate synthesis to show that jasmonate-signalling is important in the protection against the patogenic fungi *Erysiphe cruciferarum*.

Materials and Methods

Mutant *Arabidopsis thaliana ecotype Columbia* plants were produced using ethylmetanosulphonate. Methods of screening for *fad3-2fad7-2fad8* and *coil* are presented elsewhere (Feys *et al.*, 1994, McConn and Browse, 1996). The wildtype and mutant plants were inoculated with 10^7 spores of *E.cruciferarum* in sterile distilled water containing 10ppm Tween20. The control plants were inoculated with 10ppm Tween20 in sterile water to examine the effect of jasmonate on the defence system in plants infected with *E.cruciferarum*, the plants were sprayed with sterile water containing 50μm methyl jasmonate and 10ppm Tween20. The controls were sprayed with 10ppm Tween20 in sterile water. The transcriptional activity of *AtVSP* gene in control and infected plants were examined using the Nothern assays.

Results and Discussion

The mutant plants are susceptible to *E.cruciferarum* infections: Our observations of the *coil* and *fad3-2fad7-2fad8* mutants in the green house showed that these plants were easily succumbed to powdery mildew caused by a fungal infection. The fungus was later identified as *E.cruciferarum* through DNA sequence analysis. The wildtype *Arabidopsis* that were placed in the same greenhouse did not show any sign of the disease even when placed in a planter together with infected mutant plants. The susceptibility of the *fad3-2fad7-2fad8* and the *coil* mutant to the disease was assumed to be due to a defective jasmonate-signalling mechanism.

Jasmonate is essential in plant defence: In order to study the mechanism of jasmonate-signalling in plant-pathogen interactions, the mutant and wildtype plants were inoculated with spores of *E.cruciferarum*. Two weeks post inoculation almost 90% of the *coil* and *fad3-2fad7-2fad8* were infected (Fig. 1). The wildtype population however had only 5% of its population infected. The *fad3-2fad7-2fad8* mutants that were sprayed with 50μM methyl jasmonate solution showed a reduction in the number of seedlings dead or severely infected. The percentage of diseased or dead *fad3-2fad7-2fad8* was reduced to ~17% (Fig. 1). The spraying of the *coil* mutant plants with 50μM methyl jasmonate however did not reduce the rate of mortality in the plants. Though the *fad3-2fad7-*

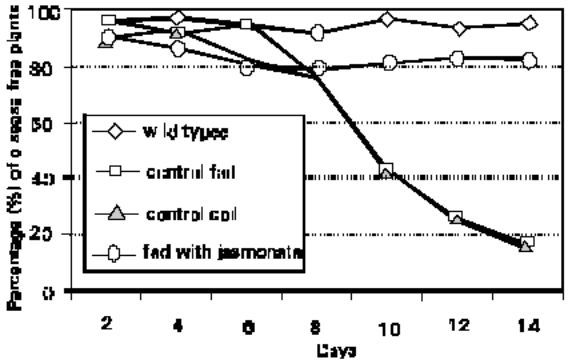

Fig. 1: Effect of Jasmonate on Arabidopsis

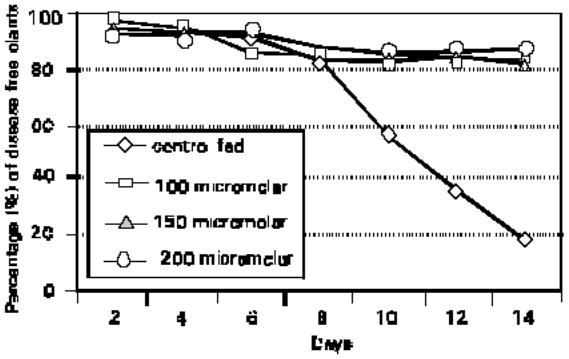

Fig. 2: Effect of Jasmonate concentration on the fad3-2fad7-3fad8 mutant

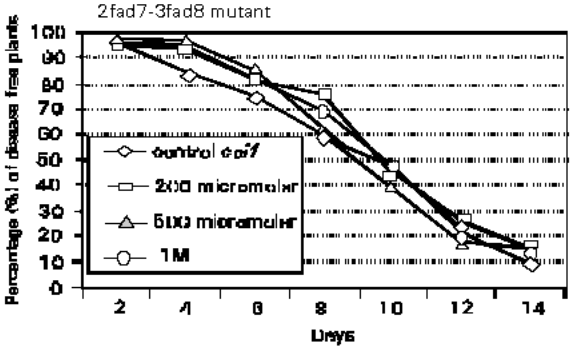

Fig. 3: Effect of Jasmonate on the *coil* mutant

2fadB 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 methyl jasmonate, (McConn *et al.*, 1997). This is however not possible in the *coil* mutants that have their jasmonate-signalling completely blocked (Rojo *et al.*, 1997).

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

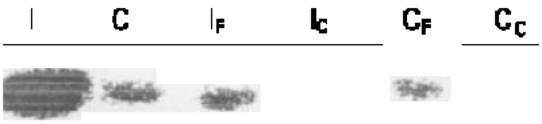

Fig. 4: Comparison of *AtVSPA* expression in wild type and mutant plants. The transcription level in *E. cruciferaum* inoculated (I) and uninoculated © plants are shownj in this Northern Blot. Transcription levels were analysed 96 hours post inoculation. Subscript F is for the fad3-2fad7-2fad8 and subscript C is for the *coil* mutant

Increase of jasmonate concentrations had no effect on the protection afforded to the mutants: The administration of 50μM methyl jasmonate reduced the incidence of disease and death in the *fad3-2fad7-2fadB* mutants 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 the experiment was repeated using three different methyl jasmonate concentrations of 100, 150 and 200μM. The results obtained however showed that an increase in the concentration did not significantly alter the disease incidence. The percentage of diseased and dead mutant plants were between 12 to 17% (Fig. 2). Methyl jasmonate remained ineffective against the *coil* mutants even at very high concentrations (1M) (Fig. 3).

E.cruciferarum* induces the expression of *AtVSP* gene in wildtype *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 (Benedetti *et al.*, 1995; Blechert *et al.*, 1995). The involvement of this gene in the activities above, 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 wildtype and mutant plants showed an increase in the transcript levels of the gene in wildtype plant post inoculation. There was no increase in transcript levels within the mutant plant following the infection (Fig. 4). This shows that both the mutants are defective in *AtVSP* gene activation. This is largely due to the presence of a defective or the nonexistence of the jasmonate-signalling pathway in both mutants (M. McConn *et al.*, 1997, Benedetti *et al.*, 1995).

The results presented here show the involvement of the jasmonate-signalling in the defence against *E.cruciferarum* infections. Wildtype *Arabidopsis* with intact and functional jasmonate-signalling appeared to be resistant to powdery mildew caused by *E.cruciferarum*. This was however not the case with both the mutant lines. Though both mutants were defective in their jasmonate-signalling, it was however

possible to rescue the *fad3-2fad7-2fad8* from powdery mildew through the exogenous application of methyl jasmonate. This was not possible with the *coil* 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 the jasmonate -responsive genes such as *AtVSP*. The transcript levels in the mutants remained unchanged upon infection , while the levels within the wildtype plants increased significantly post inoculation.

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