

Cross-Protection Mechanisms Between Biotic and Abiotic Stresses in Plants

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Abstract

In order to investigate cross-protection mechanisms between stresses of different origins, greenhouse experiments were conducted to determine whether resistance levels to the fungal pathogen *P. capsici* were affected on wounded plants. To this purpose, tomato roots were wounded at 24h-intervals and allowed to age for up to 7 days before inoculation. Data from preliminary experiments indicate first (0–48 h old wounds) an increase in disease severity in wounded as compared to unwounded tomato plants infected with *P. capsici*. Then, as the wounds age, disease severity decreases to the point that plants wounded 3 days before inoculation are less susceptible than nonwounded plants. Here, with the use of tomato mutant lines, we suggest the involvement of ethylene (C₂H₄) and jasmonates (Ja) in the development of these responses towards *P. capsici* upon wounding of tomato plants.

Keywords: *Phytophthora capsici*; *Lycopersicum esculentum*; wound; cross-protection; induced resistance

INTRODUCTION

Converging data indicate the possible existence of a general adaptation syndrome (GAS) in which different types of stress evoke identical coping mechanism. In order to assess involvement of the hormones ethylene (C₂H₄) and jasmonates (Ja) in such phenomena, greenhouse experiments on mutant tomato plants were conducted to determine the progression of damping off caused by the fungal pathogen *Phytophthora capsici* on nonwounded and wounded plants, and to determine whether resistance or susceptibility are affected by the absence of these hormones. Data from preliminary experiments indicate an increase in disease severity in wounded as compared to unwounded infected tomato plants (Figure 1). However wound-induced susceptibility decreased as the wounds aged, resulting in significantly lower disease severity on plants with 3 to 5 days old wounds as compared to those inoculated at the time of wounding and to the undisturbed control itself (Figure 1 and data not shown). These findings are in good agreement with the report on root-wounded pepper plants infected by *P. capsici* by ADORADA *et al.* (2000).

MATERIALS AND METHODS

Two weeks old tomato plants were wounded 0, 3 and 7 days before inoculation (dbi), by trimming off the bottom 2 cm of the root system with a sterile blade. 15 plants per treatment were used in average. 20 000 zoospores of a very virulent strain of

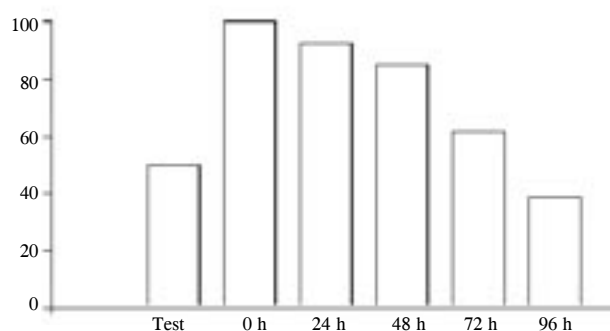


Figure 1. Disease severity (tomato dead plants in %) 14 days after *P. capsici* inoculation on nonwounded roots (Test), wounded at the moment of inoculation (0 h), 1 day before inoculation (dbi) (24 h), 2 dbi (48 h), 3 dbi (72 h), 4 dbi (96 h)

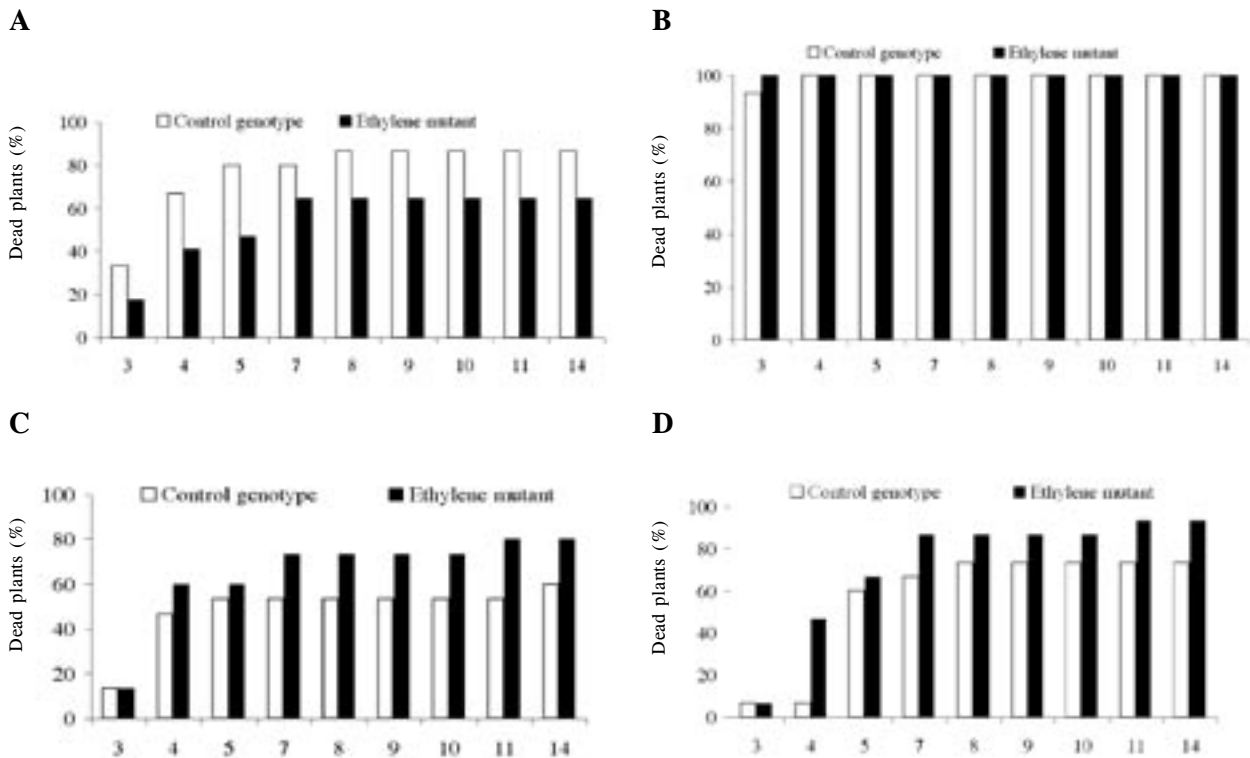


Figure 2. Mortality trend in differently pre-treated C_2H_4 mutated/control plants. A – nonwounded plants; B – wounded and inoculated at the same time; C – wounded 3 dbi; D – wounded 7 dbi

Phytophthora capsici (P4) were inoculated as spore suspension at the root crown of each plant. The same experiments were performed on the *Never ripe* (*Nr*, impaired in C_2H_4 perception; LANAHAN *et al.* 1994) and the *defenseless1* (*def1*, altered in the Ja signaling pathway; HOWE *et al.* 1996) mutant lines and corresponding control genotypes.

RESULTS

Looking at mortality trends in differently pre-treated C_2H_4 mutants and control plants we observed:

- mutant shows lower mortality than its control genotype (best evident 4/5 days post inoculation [dpi]) in nonwounded plants (Figure 2A);
- plants wounded and inoculated at the same time show equally increased susceptibility independently of the genotype (Figure 2B);
- C_2H_4 mutant shows higher mortality than its control genotype when wounded both 3 (Figure 3C) and 7 days before inoculation (Figure 2D) (best evident 4 days post inoculation, Figure 3).

The effect of different pre-treatments on disease symptoms at four days post inoculation are compared in Figure 3.

Mortality trends in differently pre-treated Ja mutants and control plants resulted as follows:

- Ja mutant shows lower mortality than its control genotype (best evident 4 dpi) in nonwounded plants (Figure 4A);
- Both *def1* and control plants wounded and inoculated at the same time are more susceptible to infection (Figure 4B);

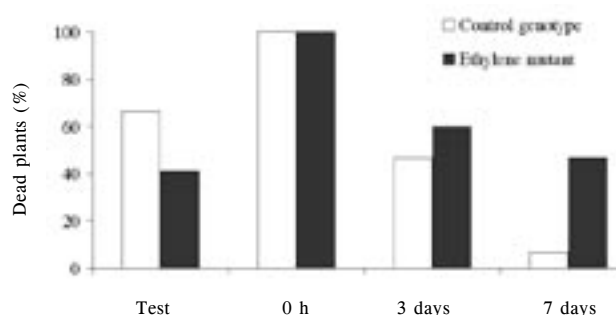


Figure 3. Effect of different root treatments on damping off symptoms. Disease severity (mortality in %) 4 days post inoculation on nonwounded plants (Test), plants wounded at the time of inoculation (0 h), 3 dbi (3 days), 7 dbi (7 days)

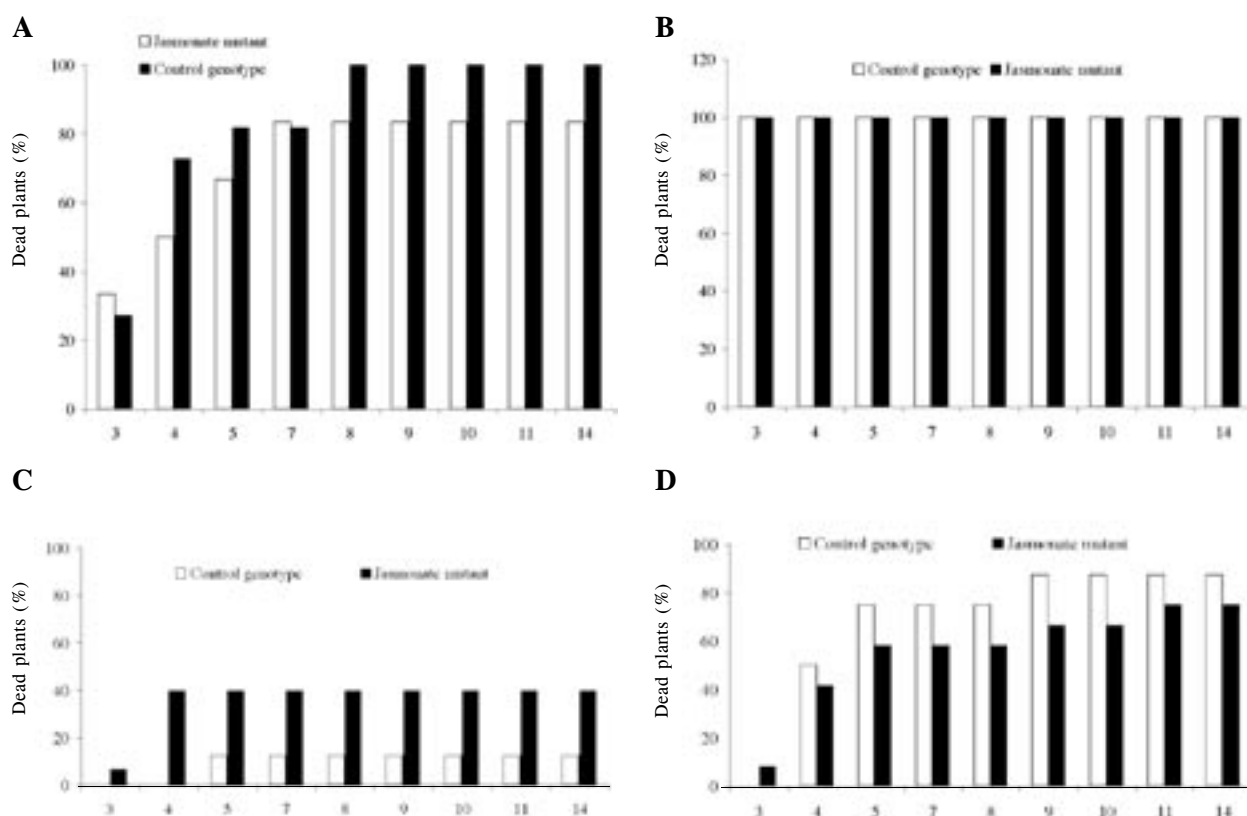


Figure 4. Mortality trend in differently pre-treated Ja mutated/control plants. A – nonwounded plants; B – wounded and inoculated at the same time; C – wounded 3 dbi; D – wounded 7 dbi

- Ja mutant shows higher mortality than its control genotype when wounded 3 days before inoculation (best evident 4 dpi, Figure 5) (Figure 4C);
- as the age of wounds increases (7 days before inoculation) Ja mutant seem perform better than its control genotype (Figure 4D), as in nonwounded plants (Figure 4A).

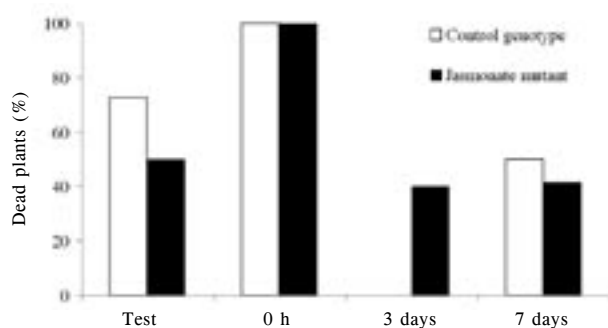


Figure 5. Effect of different root treatments on damping off symptoms. Disease severity (mortality [%]) 4 days post inoculation (dpi) on nonwounded root (Test), wounded at the moment of inoculation (0 h), 3 dbi (3 days), 7 dbi (7 days)

The effect of different pre-treatments on disease symptoms at four days post inoculation are compared in Figure 5.

DISCUSSION

Many of the chemical and structural barriers associated with the expression of resistance to pathogens are also induced by mechanical injury. Our experimental results suggest the involvement of C_2H_4 and Ja in de-

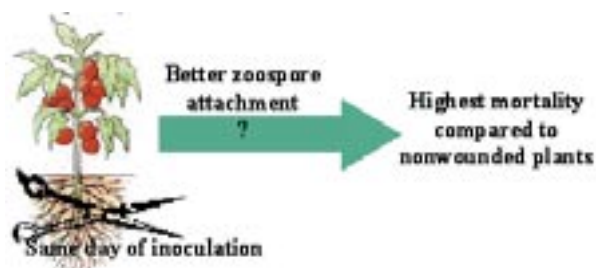


Figure 6. Wounding at the moment of inoculation increases susceptibility towards *P. capsici*, perhaps due to increased attachment of the zoospores to the roots, as suggested by ADORADA *et al.* (2000)

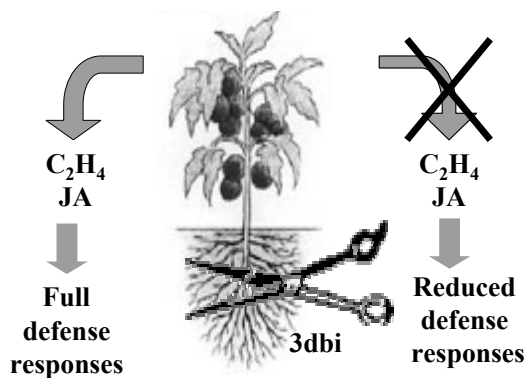


Figure 7. Susceptibility decreases with wound ageing and C_2H_4 and Ja are positively involved in this process, as suggested by the behaviour of the mutant tomato lines *nr* and *defl* in our model

velopment of these responses toward *P. capsici* upon wounding of tomato plants.

Wounding at the moment of inoculation increases susceptibility towards *P. capsici* both in mutant plants and in their controls (Figures 2B–4B), perhaps due to increased attachment of the zoospores to the roots, as suggested by ADORADA *et al.* (2000) (Figure 6). We are going to verify this hypothesis. Moreover we cannot exclude that the lack of difference between the C_2H_4 or Ja mutants and their control genotype when wounded and inoculated at the same time is due to the masking effect of the highly increased susceptibility.

However, susceptibility decreases with wound ageing, until plants become more resistant to *P. capsici* as compared to nonwounded controls. C_2H_4 and Ja are positively involved in this process, as suggested by the behaviour of the mutant tomato lines *nr* and *defl* in our model (Figures 2A,C,D–4A,C). This is best evident 3 dpi. We are going to verify if the correlation between reduced disease severity and peroxidase activity reported for pepper plants (ADORADA *et al.* 2000) is to some extent altered in our C_2H_4 and Ja mutant lines (Figure 7).

C_2H_4 and Ja seem to influence disease severity with mostly overlapping timing, although differences are evident at the latest time point of wounding (7 dbi; Figures 3D–4D). In this case, Ja-mediated defence seems not to be effective anymore.

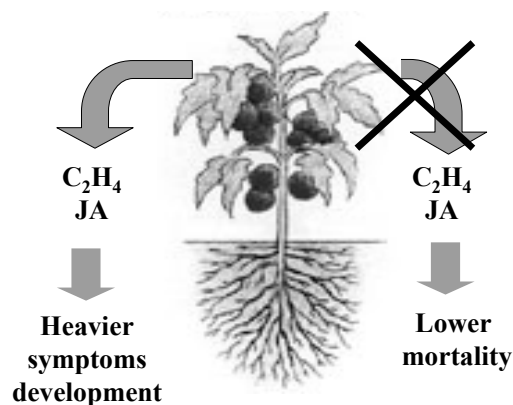


Figure 8. C_2H_4 and Ja seem to favour symptoms development in nonwounded, inoculated plants

On the other hand, C_2H_4 and Ja seem to favour symptoms development in nonwounded, inoculated plants (Figure 8).

This is, to our knowledge, the first work in which the role of ethylene and jasmonates is investigated in a model of wound induced cross-protection to pathogens. Further experiments need to be conducted to elucidate these mechanisms who could have important application for future work aimed at engineering plants with improved responses to biotic and abiotic stresses.

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