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BRIEF COMMUNICATION

Suppression of phenylalanine ammonia-lyase activity elicited in date palm by *Fusarium oxysporum* f. sp. *albedinis* hyphal wall elicitor

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Abstract

The inoculation of the seedling roots of the resistant (Bousthami Noir) and susceptible (Jihel) date palm (*Phoenix dactylifera*) cultivars by *Fusarium oxysporum* f. sp. *albedinis* induced an increase in phenylalanine ammonia-lyase (PAL) activity. The response of the PAL activity in the resistant cultivar was faster and higher than in the susceptible one. However, the elicitation of the seedlings with the hyphal wall elicitor (HWE) of the pathogen induced identical PAL activity in both cultivars. In the resistant cultivar, the the PAL activity elicited with the HWE was not influenced by the addition of the fungal culture filtrate (FCF) whereas it was suppressed in the susceptible cultivar. This FCF suppressor effect was dose-dependent, not influenced by sodium periodate, whereas it was strongly reduced by the heat (121 °C for 45 min) and pronase E. These results show that differential induction of the defence mechanisms in both cultivars was not related to differences in the induction of the PAL activity, but to the suppression of its elicitation in the susceptible cultivar.

Additional key words: *Phoenix dactylifera*, resistant and susceptible cultivars.

The infection of date palm by *Fusarium oxysporum* f. sp. *albedinis* (*Foa*) was accompanied by several host defence mechanisms such as the induction of biosynthesis of phytoalexins (El Modafar *et al.* 1999), the accumulation of the fungitoxic cafeoylshikimic acid (Ziouti *et al.* 1996, El Modafar *et al.* 2000a), the insolubilisation of phenolic acids in cell wall (El Modafar *et al.* 2000b, El Modafar and El Boustani 2001), and the intensification of the lignification (El Modafar *et al.* 2000b, El Modafar and El Boustani 2000). The induction of these defence mechanisms is always earlier and stronger in resistant cv. Bousthami Noir (BSTN) than in the susceptible cv. Jihel (JHL). These differences were related to a difference in the PAL activity (El Modafar *et al.* 2001). The changes in PAL activity after infection in BSTN roots were faster and higher than that in JHL. However, the elicitation of the date palm roots by a hyphal wall elicitor (HWE) of *Foa* induced an identical PAL response in the resistant and susceptible cultivars (El Modafar *et al.* 2001). This differential induction of the PAL activity in response to the infection by *Foa* and to the elicitation by the HWE could be related to a suppression of the PAL induction in the susceptible cultivar as it was shown in other host-parasite interactions (Shiraishi *et al.* 1991, Lu and Higgins 1993, Yamada *et al.* 1996, Seki *et al.* 1999, Shiraishi *et al.* 2001). The suppression of the PAL induction was generally related to extracellular suppressors in the fungal culture filtrate or in the germination fluid of spores (Yamada *et al.* 1994, Wada *et al.* 1995, Amano *et al.* 1997, Seki *et al.* 1999). The objective of this work is to seek the culture filtrate suppressor effect on the PAL activity elicited by *Foa* hyphal wall elicitor in date palm.

The seedlings (3-week-old) of date palm (*Phoenix dactylifera* L.) resistant cultivar Bousthami Noir (BSTN) and susceptible cultivar Jihel (JHL) were used. The inoculum was a conidial suspension of strain 133 of *Foa* which is known for its virulence (Sedra and Besri 1994).

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Abbreviations: *Foa* - *Fusarium oxysporum* f. sp. *albedinis*; FCF - fungal culture filtrate; HWE - hyphal wall elicitor; PAL - phenylalanine ammonia-lyase.
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The conidial suspension (4×10^6 spores cm^{-3}) was prepared in sterile distilled water from a thallus cultivated on potato dextrose agar (PDA) medium for 5 d at 25 °C.

The liquid culture medium of Czapek was inoculated with 20 cm^3 of a conidial suspension (El Modafar *et al*. 2000a). The cultures were then incubated at 25 °C, in the dark, under agitation (125 rpm). After 16 d, mycelium and culture filtrate were separated by vacuum filtration (0.22 µm). The fungal culture filtrate (FCF) was freeze-dried and homogenized in sterile distilled water (pH 5.2) to prepare variable concentrations (5 to 25 mg cm^{-3}). The mycelial mass obtained was washed three times with distilled water then suspended in distilled water. The mixture was centrifuged at 13 000 *g* for 30 min. The pellet obtained was dried (40 °C overnight) and ground in a mortar. The powder obtained was resuspended in distilled water (pH 5.2), autoclaved at 121 °C for 45 min and centrifuged at 1 000 *g* for 20 min. The supernatant represented hyphal wall elicitor (HWE) (El Modafar *et al*. 2001). The concentration of the HWE used in the elicitation tests was 40 mg of mycelium per cm^3 of distilled water (El Modafar *et al*. 2001).

The treatment of the FCF by the sodium periodate and by the protease (Pronase E, Type XIV) was carried out according to the technique previously described (El Modafar *et al*. 2001). The FCF (100 cm^3) was incubated with 100 cm^3 of 50 mM sodium periodate in the dark at room temperature overnight. The samples were then dialysed against distilled water for 24 h, freeze-dried and homogenized in distilled water (pH 5.2). For each 1 cm^3 of FCF, 20 mm^3 of protease (70 units cm^{-3} in distilled water, pH 5.2) was added. The mixture was incubated in a water bath at 37 °C under agitation overnight. The mixture was then heated in a 100 °C water bath for 5 min followed by centrifugation at 1 000 *g* for 30 min. The supernatant obtained was added to the culture medium of the seedlings to test its effects on elicitation of PAL activity.

The seedlings were placed into 6-cm^3 tubes containing 3 cm^3 of a mineral solution (10 g dm^{-3} of KNO_3, 5 g dm^{-3} of KH_2PO_4, 2.5 g dm^{-3} of $MgSO_4$, 0.02 g dm^{-3} of $FeCl_3$, pH 5.2) and 1 cm^3 of the HWE (40 mg cm^{-3}) in presence or not of the FCF (treated or not treated by protease or periodate). Control plants were treated with sterile distilled water. The seedlings were then placed in a culture room at 25 °C. After 48 h, the roots were taken, freeze-dried and then stored under vacuum. At each time and for each test, a sampling of 10 seedlings per cultivar and three repetitions were carried out.

The phenylalanine ammonia-lyase activity was determined according to the technique previously described (El Modafar *et al*. 2001). The roots of the seedlings were ground in 100 mM potassium borate buffer, pH 8.8, with 14 mM 2-mercaptoethanol. The homogenate was then centrifuged at 13 000 *g* for 30 min and the supernatant constitutes the enzymatic extract. The reaction mixture consisted of 100 mm^3 of enzymatic extract, 1 cm^3 of 100 mM potassium borate buffer, pH 8.8, and 200 mm^3 of 100 mM L-phenylalanine. After incubation at 40 °C for 60 min, the reaction was stopped by addition of 250 mm^3 of 5 M HCl. The cinnamic acid formed was extracted two times by 2 cm^3 of diethyl ether. After evaporation of the ether, the residue was resuspended in 500 mm^3 of methanol and absorbance was determined at 290 nm. In the same way, a curve standard was carried out with the cinnamic acid under the same experimental conditions.

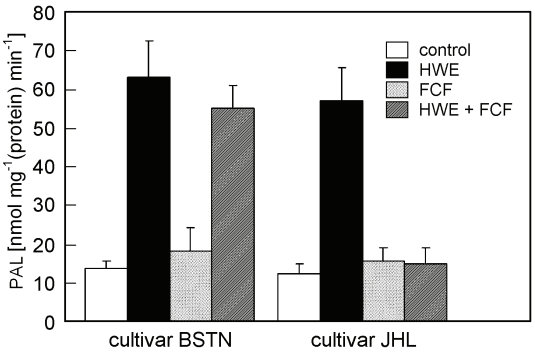

Fig. 1. Effect of fungal culture filtrate (FCF) on the date palm PAL activity elicited by hyphal wall elicitor (HWE) of *F. oxysporum* f. sp. *albedinis*. The enzyme activity was measured 48 h after the treatments. HWE and FCF were used at the final concentrations of 10 mg cm^{-3} and 20 µg cm^{-3}, respectively.

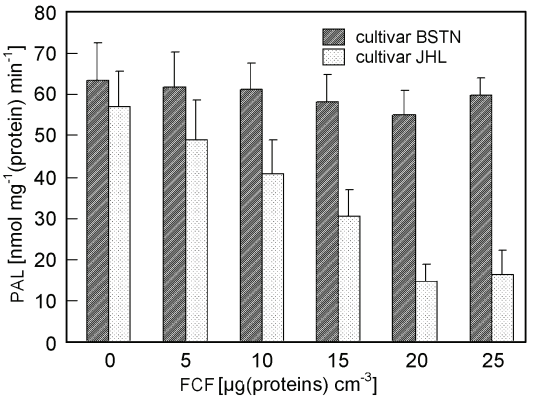

Fig. 2. Dose-dependent effects of fungal culture filtrate (FCF) on the date palm PAL activity elicited by hyphal wall elicitor (HWE) of *F. oxysporum* f. sp. *albedinis*. The enzyme activity was measured 48 h after the treatments. HWE was used at the final concentration of 10 mg cm^{-3}.

The treatment of the roots of date palm seedlings by the HWE of *Foa* stimulated PAL activity similarly in both cultivars studied (Fig. 1); the PAL activity was approximately 4.6 times higher than that of the control. The treatment of the date palm roots with the FCF did not have a significant effect on PAL activity in both cultivars. The addition of the FCF to the HWE was accompanied by differential responses of PAL activity in resistant and susceptible cultivars (Fig. 1). In the resistant cultivar, the FCF had not a significant effect on the induction of PAL

activity elicited by HWE whatever the concentration of FCF used (Fig. 2). However, in the susceptible cultivar, the HWE elicitor effect was strongly inhibited in the presence of FCF (Fig. 1). This inhibition was concentration-dependent (Fig. 2). The PAL activity induced by HWE was completely suppressed by protein concentrations higher than 15 µg cm^{-3} (Fig. 2). The inhibiting effect of FCF on PAL activity elicited by HWE was not influenced significantly by the treatment with sodium periodate (Table 1). However, this inhibiting effect was strongly reduced with by the pronase E treatment or heat treatment (121 °C for 45 min).

Table 1. Effect of autoclaving and treatments with periodate and pronase E of fungal culture filtrate (FCF) on the PAL activity in roots of susceptible cultivars elicited by hyphal wall elicitor (HWE) of *F. oxysporum* f. sp. *albedinis*. The enzyme activity was measured 48 h after the treatments. HWE and FCF were used at the final concentrations of 10 mg cm^{-3} and 20 µg cm^{-3}, respectively. FCF-pronase E - fungal culture filtrate treated with pronase E, FCF-periodate - fungal culture filtrate treated with periodate. Mean ± SE.

Treatments	PAL activity [nmol mg^{-1}(protein) min^{-1}]
Control	12.36 ± 2.40
HWE	57.03 ± 8.59
HWE + FCF	14.78 ± 4.13
HWE + FCF-autoclaved	47.45 ± 10.56
HWE + FCF-pronase E	43.58 ± 11.86
HWE + FCF-periodate	23.88 ± 5.58

Our previous studies showed that the inoculation of the roots of resistant (BSTN) and susceptible (JHL) date palm cultivars by *Foa* induced an increase in activity of PAL (El Modafar *et al*. 2001). The increase response in PAL activity in BSTN was faster and higher than that in the JHL. However, the elicitation by the HWE of *Foa* induced an identical PAL response in the resistant and susceptible cultivars (El Modafar *et al*. 2001). Our data show that the FCF did not have an effect on the induction of the PAL activity elicited by HWE in the resistant cultivar. However, the FCF suppress the elicitation of the PAL activity induced by HWE in the susceptible cultivar. This suppressing effect of FCF was concentration dependent. This suppression of the elicitation of PAL, reported in other host-parasite interactions was related to pathogen suppressors which inhibited the PAL gene expression (Shiraishi *et al*. 1991, Yamada *et al*. 1996, Seki *et al*. 1999, Shiraishi *et al*. 1999). The suppressors are generally soluble sugars (Shiraishi *et al*. 1991, Hahn *et al*. 1993, Lu and Higgings 1993), glycoproteins (Shiraishi *et al*. 2001) or proteinaceous compounds (Yamada *et al*. 1996) synthesized constitutively by the fungal pathogens and present in the fungal culture filtrate and the germination fluid of spores (Yamada *et al*. 1994, Wada *et al*. 1995, Amano *et al*. 1997, Seki *et al*. 1999). In other host-parasite interactions, the toxins can suppress the expression of host defence mechanisms (Vidhyasekaran *et al*. 1992, Vurro and Ellis 1997). In the case of the date palm-*Foa* interaction, the suppression of the elicitation of the PAL activity in susceptible cultivar seems to be related to a proteinaceous soluble suppressor present in the fungal culture filtrate and in the germination fluid of spores of *Foa* (data not shown). Indeed, the treatment of the FCF by a protease (pronase E) or by heat temperature (121 °C for 45 min) inhibited the elicitor effect of the PAL activity whereas the treatment by the periodate had not a significant effect.

As in other host-parasite interactions (Shiraishi *et al*. 1991, 1999, Yamada *et al*. 1996), the suppression of the PAL elicitation in date palm-*Foa* interaction constitutes a determining factor of the host behaviour (susceptibility or resistance). The PAL represents the key enzyme of the phenylpropanoid metabolism leading to the biosynthesis of phytoalexins, precursors of lignin, and fungitoxic phenolic compounds (Ebel *et al*. 1984, Jones 1984, Hahlbrock and Scheel 1989, Nicholson and Hammerschmidt 1992, Weisshaar and Jenkins 1998, Repka *et al*. 2004, El Modafar and El Boustani 2005). The inoculation of date palm by *Foa* is accompanied by the induction of phytoalexins biosynthesis (El Modafar *et al*. 1999), the insolubilisation of phenolic acids in cell wall (El Modafar *et al*. 2000b, El Modafar and El Boustani 2001), the increase of the fungitoxic cafeoylshikimic acid accumulation (Ziouti *et al*. 1996, El Modafar *et al*. 2000a), and the intensification of the lignification (El Modafar *et al*. 2000b, El Modafar and El Boustani 2000). The induction of these defence mechanisms is always early and intense in the resistant cultivar whereas it is late and weak in the susceptible cultivar. This differential induction of the defence mechanisms in both cultivars to *Foa* does not seem to be related to the degree of PAL elicitation (El Modafar *et al*. 2001), but seems to be related to the suppression of the PAL gene expression by pathogen proteinaceous suppressor(s). In the susceptible cultivar, the PAL gene expression would be suppressed by the parasite, whereas in the resistant cultivar, the suppression would be absent or substantially reduced. The PAL suppression by *Foa* seems to constitute a primary factor determining date palm susceptibility to *Foa*. These results suggest that the specificity determinism of the date palm-*Foa* interaction was not explained by a difference of the defence mechanisms elicitation, but by a difference in the degree of their suppression. The early suppressor production by the pathogen, in particular by the spores in germination, may predispose the plants to pathogen invasion before the penetration. The characterization of the PAL induction suppressor constitutes an essential prospect on which future work should concentrate.

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