**Effect of salicylic acid on phenolic compounds related to date palm resistance to *Fusarium oxysporum* f. sp. *albedinis***

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**Summary.** Salicylic acid (SA) plays a key role in establishing resistance to pathogens in many plants. To study the possible involvement of SA in the resistance of date palm (*Phoenix dactylifera* L.) to *Fusarium oxysporum* f. sp. *albedinis* (FOA), we investigated levels of phenolic compounds, known as indicators of resistance in the date palm/ *Fusarium* pathosystem. After treatment with SA the content of root soluble phenolics in *F. oxysporum* inoculated date palm seedlings was about 4 times higher in cv. Bousthami noir and 6 times higher in cv. Jihel than that in untreated plants showing disease symptoms. The largest increase was at a SA concentration of 50 µM. SA treatment also enhanced the content of cell wall phenolics. In addition, inoculation of SA-treated roots of date palm with FOA (strain ZAG) resulted in a greater number of plants showing only limited hypersensitive reaction-like necrotic lesions. In contrast, SA-untreated plants normally showed spreading necrosis in response to fungus inoculation.

**Key words:** bayoud, defence mechanisms, *Phoenix dactylifera*, *Fusarium* wilt, phytoalexins.

**Introduction**

Plants have evolved numerous mechanisms to defend themselves against pathogens. Some of these mechanisms are constitutive, while others are induced in response to pathogen attack. These induced defence mechanisms are activated at infection site, and manifest themselves in part as necrotic lesions (hypersensitive response, HR). Induced resistance also occurs in the distal uninfect ed parts of infected plants, leading to systemic acquired resistance (SAR) (Goodman and Novacky, 1994). Salicylic acid (SA) has been recognised as an important signal molecule indicating that plant defence has been activated against pathogens (Enyedi *et al*., 1992; Gaffney *et al*., 1993). SAR and local resistance are generally accompanied by higher levels of endogenous SA (Malamy and Klessig, 1992; Dorey *et al*., 1997). SAR can also be induced by exogenous application of SA (Kessmann *et al*., 1994; Lawton *et al*., 1996). In tobacco, the same defence genes that were expressed upon infection with tobacco mosaic virus were also activated by treatment with SA (Ward *et al*., 1991). More recent results have shown that transgenic tobacco plants expressing bacterial salicylate hydroxylase, which metabolises SA, are unable to induce SAR, and even show enhanced susceptibility to pathogens (Gaffney *et al*., 1993). At the same time phenol metabolism is activated in plants reacting to pathogens (Ellard-Ivery and Douglas, 1996). This is due not only to the mechanical role that phenolics play in cell walls, but also to their anti-
fungal properties (Harborne, 1991). New phenolics are synthetised in date palms (*Phoenix dactylifera* L.) following infection with *Fusarium oxysporum* f. sp. *albedinis* (FOA), the agent causing bayoud disease (El Hadrami *et al*., 1997). However, little is known about the effect of SA on phenol metabolism in relation to the resistance of date palm to FOA.

The aim of this work was to determine the effect of SA treatment on phenol metabolism in relation to date palm resistance to FOA.

**Materials and methods**

**Fungal and plant material**

*Fusarium oxysporum* f. sp. *albedinis* conidia were harvested from infected date palm leaves brought from Zagora in the south of Morocco and germinated on malt-agar at 25°C in darkness. The inoculum was a conidial suspension of the ZAG strain known for its aggressiveness (El Idrissi Tourane *et al*., 1995). The conidial suspension was adjusted to $6 \times 10^6$ spores ml$^{-1}$. Three-month-old date palm seedlings were obtained from seeds produced by mother cultivars highly susceptible to FOA wilt (bayoud). They were grown in pots containing sand (75%) and peat (25%) in a greenhouse at 30°C, under a 16 h light/8 h darkness regime. The seedling roots of two date palm cultivars, susceptible Jihel (JHL) and resistant Bousthami noir (BSTN), were infected by injecting 10 µl of conidial suspension into the roots.

**Effect of SA treatment on the content of soluble phenolics**

In two separate experiments, the roots of three-month-old date palm seedlings of both cultivars were soaked in SA at various concentrations (1 mM, 0.2 mM, 0.05 mM). Control plants were treated with sterile distilled water. For the soluble phenolic contents, 30 date palm seedlings were obtained from seeds produced by mother cultivars highly susceptible to FOA wilt (bayoud). They were grown in pots containing sand (75%) and peat (25%) in a greenhouse at 30°C, under a 16 h light/8 h darkness regime. The seedling roots of two date palm cultivars, susceptible Jihel (JHL) and resistant Bousthami noir (BSTN), were infected by injecting 10 µl of conidial suspension into the roots.

One hundred seedlings of each cultivar were treated with SA (50 µM) by soaking their roots in SA solution for 10 days before inoculation with one of the conidial suspensions. The control plants were inoculated but not treated with SA. The survival of seedlings was recorded one month after inoculation. The results were the mean of two repetitions with 50 plants for each repetition. The phenolic content of the seedling roots was also determined.

**Extraction and quantification of soluble phenolic compounds**

Levels of soluble phenolic compounds were determined according to Ziouti *et al*., (1992). Root tissues (400 mg from 5 plants), taken from around the infection sites, were homogenised in an ice bath with 2 ml (80%) methanol. The homogenate was centrifuged three times at 7000 g for 3 min; supernatants were recuperated each time. One hundred microlitres of the supernatant was added to Folin-Ciocalteu reagent and sodium carbonate. The mixture was incubated at 40°C for 30 min and the blue colour was read at 760 nm. The content of soluble phenolic compounds was determined for the experiments described above; it was expressed in mg-equivalents of catechin per g of f wt.

**Extraction and quantification of cell wall phenolic compounds**

The residue of tissue after soluble phenol extraction was washed three times with methanol and then incubated in the presence of 6N HCl at 100°C for 2 h. The cell wall phenolic compounds were extracted three times with diethyl ether. After evaporation of the diethyl ether, the phenolics were resuspended in 200 µl methanol. The cell wall phenolic content was determined as described for soluble phenolics.

**Chromatographic analysis by TLC and HPLC**

TLC was conducted on microcrystalline cellulose plates in the upper phase of BAW (butanol-acetic acid-water, 4:1:5 [v:v:v]). UV was used for phenolic detection with and without NH$_3$ vapour.

Phenolic compounds were separated and identified by HPLC (El Hadrami *et al*., 1997) with a Waters 600 E liquid chromatograph (Waters Millenium, En Yvelines Cedex, France) equipped with a Waters 990 photodiode array detector.
Results

Effect of SA treatment on the survival of date palm seedlings

SA treatment enhanced the resistance of date palm to FOA. Without SA treatments, diseased control plants showed progressing wet necrosis of the roots earlier in the susceptible JHL cv. (4 days) than in the resistant BSTN cv. (8 days). One month after inoculation, 51% of the plants of the susceptible cultivar and 21% of the resistant cultivar had completely collapsed. In contrast, one month after SA treatment 24% of plants of cv. JHL and 9% of plants of cv. BSTN had died from Fusarium wilt (Table 1). SA-treated plants without disease symptoms generally developed limited necrotic lesions, which reflected a delay in the colonisation of the host plant by the pathogen.

Effect of SA on soluble phenolic compounds

The relation between SA and soluble phenolic compounds following SA treatments at 50 µM, 200 µM and 1 mM was studied in the roots of the date palm cv. JHL and BSTN. For cv. JHL (Fig. 1), treatment with a low SA concentration (50 µM) enhanced the content of soluble phenols with two peaks, 0.62 and 0.65 mg per g of f wt, respectively 3 and 12 days after treatment. A higher SA concentration (1 mM) decreased phenol content, to 0.24 and 0.2 mg per g of f wt at 3 and 12 days after treatment respectively. With cv. BSTN (Fig. 2), no significant differences were detected between SA-treated plants and the controls. However, higher SA concentrations (200 µM and 1 mM) slightly inhibited the levels of soluble phenolics.

Table 1. Effect of salicylic acid (50 µM) treatment on the survival of seedlings of two cultivars of date palm, one month after inoculation with Fusarium oxysporum f. sp. albedinis. Values are the mean of two repetitions, 50 plants per repetition. The results obtained were significantly different. SE ≤ 5%.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Survival (%)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Jihel</td>
<td>49</td>
</tr>
<tr>
<td>Bousthami noir</td>
<td>80</td>
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Fig. 1. Time-course of changes in the content of free phenolics in roots of date palm cv. Jihel in response to different SA concentrations. Each bar is the mean of two replicates root extracts.
Effect of SA treatment on the soluble phenols content in date palm roots inoculated with *F. oxysporum* f. sp. *albedinis*

There was a relationship between the level of soluble phenolic compounds in the roots of both cultivars and the survival rate of SA-treated and untreated plants. The level of soluble phenolics in roots was much higher in SA-treated plants than in symptomless plants infected with FOA (Fig. 3). Indeed, the content of soluble phenolics in infected roots of SA-treated cultivar JHL showing no symptoms was 3.5 mg g\(^{-1}\) f wt. This was about 2 times higher than that found in untreated infected plants without disease symptoms and 6 times higher than that in untreated and inoculated plants with disease symptoms.

For date palm cv. BSTN, the content of root soluble phenolics in infected SA-treated plants was 2.5 mg g\(^{-1}\) f wt. For infected plants showing no symptoms (Fig. 3), this was about 2 times higher than that found in untreated infected plants without symptoms and 4 times higher than that found in untreated inoculated plants with symptoms.

Establishment of resistance was associated with the formation of HR-like lesions around the point of inoculation of FOA. In contrast, inoculated untreated plants showed spreading necrosis.

Inoculation of date palm roots stimulated the phenol metabolism of symptomless infected plants of both cultivars; this metabolism was greater when SA treatment was given, and was more evident for susceptible JHL than for resistant BSTN cultivar.

**SA treatment (0.05 mM) and cell wall phenolics in date palm roots inoculated with *F. oxysporum* f. sp. *albedinis***

The content of cell wall phenolic compounds was greater in the resistant than in the susceptible cultivar. This content showed little increase in either cultivar after inoculation with FOA. Furthermore, the level of cell wall phenolics induced by SA did not significantly differ from that in the untreated controls (Fig. 4).

**Characterisation of soluble phenolic compounds in SA-treated date palm roots after inoculation with *F. oxysporum* f. sp. *albedinis***

Analysis by TLC and HPLC showed a relationship between resistance to FOA and the level of new soluble phenolic compounds. The control roots
Fig. 3. Effect of SA treatment (50 µM) on soluble phenolic contents in roots of date palm cv. Jihel (JHL) and cv. Bousthami noir (BSTN) one month after inoculation with *Fusarium oxysporum* f. sp. *albedinis*. UU (uninoculated plants); UIR (untreated and inoculated plants without disease symptoms); UIS (untreated and inoculated plants with disease symptoms); TUU (treated and uninoculated plants); TIR (treated and inoculated plants without disease symptoms); TIS (treated and inoculated plants with symptoms). Each bar is the mean of two replicate root extracts. Sol-phe, soluble phenolics.

Fig. 4. Effect of SA treatment (50 µM) on cell wall phenolics in roots of date palm cv. Bousthami noir (BSTN) and cv. Jihel (JHL) one month after inoculation with *Fusarium oxysporum* f. sp. *albedinis*. UU (untreated and uninoculated plants); UIR (untreated and inoculated plants without disease symptoms); UIS (untreated and inoculated plants with disease symptoms); TU (treated and uninoculated plants); TIS (treated and inoculated plants with symptoms); TIR (treated and inoculated plants without disease symptoms). Each bar is the mean of two replicate root extracts. Wall-phe, cell wall phenolics.
had higher levels of caffeoylshikimic acids (Fig. 5, left). When infected, the phenolic profiles of the roots changed completely. Several new non-constitutive compounds now accumulated. They were hydroxycinnamic derivatives, revealed at 320 nm, and mainly represented in this experiment by a $p$-coumaric derivative (pic 1) and a sinapic derivative (pic 2) (Fig. 5, right). The phenolics that accumulated in infected plants and those in infected SA-treated plants were the same, and they were associated with the establishment of resistance.

Fig. 5. Typical HPLC profiles obtained with three wave length (280 nm, 320 nm and 350 nm) of phenolics in root extracts of date palm before (left) and after (right) inoculation with Fusarium oxysporum f. sp. albedinis. 1: $p$-coumaric derivative; 2, 3: caffeoylshikimic acids, I: sinapoyl-amide; 4, 5, 6, 7 and 8: other hydroxycinnamic derivatives.

Discussion
SA treatment increased the resistance of seedlings to Fusarium wilt. This is in agreement with other reports showing that the treatment of plants with SA can provide a significant degree of protection against pathogens (Métraux et al., 1990; Kauss et al., 1992; Raskin, 1992; Gaffney et al., 1993; Van Kan et al., 1995). This study showed that the lower susceptibility of date palm to FOA that was induced by SA was associated with the formation of HR-like lesions around the point of infection. Normally, hypersensitive lesions occur during an incompatible interaction. However, it has been reported that plants in a state of SAR resist to virulent fungal pathogens with single-cell HR (Kogel et al., 1994). Inoculation of SA-treated date palm roots with aggressive FOA strain ZAG caused a higher number of plants with such HR-like necrotic lesions. The HR is associated with cell death and
restricts fungal colonisation by synthesis of chemical barriers against pathogen spread (Hammond-Kosack et al., 1996; Hammerschmidt, 1999). As well as delaying the development of disease, SA also affected the accumulation of phenolic compounds which are themselves likewise associated with plant resistance (Nicholson and Hammerschmidt, 1992; Métiaux and Raskin, 1993; El Hadrami et al., 1997; Mansfield, 1999). At higher SA concentration (1 mM), the level of soluble phenolics in date palm roots decreased; this reduction was already reported by Dixon and Paiva (1995). SA may inhibit the activity of PAL, a key enzyme in the synthesis of phenolic compounds (Nicholson and Hammerschmidt, 1992). At low concentrations, SA stimulated the accumulation of soluble phenolic compounds in date palm roots. It has been suggested that SA inhibits catalase activity, leading to increased levels of $H_2O_2$ (Chen et al., 1993), which in turn induces PAL gene expression (Desikan et al., 1998) and synthesis of phenolic compounds (Dorey et al., 1997). The study also showed that SA treatment greatly increased the content of soluble phenolics in inoculated date palm root compared with uninoculated plants. This is in agreement with Kauss et al. (1992), who reported that SA did not directly induce the expression of resistance genes, but rather increased the sensitivity of cells to subsequent elicitor treatment. In cucumber cotyledons, it was also found that SA increased the ability of a tissue to trigger a burst of $H_2O_2$ in response to subsequent elicitor treatment (Kauss and Jeblick, 1995; Fauth et al., 1996). In addition, it was shown that levels of soluble phenolics were higher in the symptomless parts of the susceptible SA-treated cv. JHL than in the resistant BSTN. These higher levels are likely to be associated with the establishment of resistance since phenolic compounds have been found to have anti-fungal properties in several host-parasite interactions (Harborne, 1991). Moreover, treatment with SA slightly increased the content of cell wall phenolics. The accumulation of cell wall phenolics clearly distinguished the cultivars tested being faster and more intense in the resistant cultivar than in the susceptible one. Such an accumulation is often associated with plant resistance (Niemann et al., 1991; Ikegawa et al., 1996). The insolubilisation of phenolic compounds in the cell walls constitutes a mechanical defence barrier protecting the wall against cell wall-degrading enzymes (Matern and Grimmig, 1993). In addition, we found that in both the susceptible and the resistant cultivar tested, infected SA-treated roots showed the same soluble phenolics as those that occur in untreated symptomless plants. These phenolics were different from those expressed by uninoculated plants. In summary, the results indicate that SA protects date palm from Fusarium wilt and suggest that SA induces date palm resistance by enhancing induced chemical defences.

Acknowledgements

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