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Relationship between cell wall susceptibility to cellulases and pectinases of *Fusarium oxysporum* and susceptibility of date palm cultivars

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Abstract

Fusarium oxysporum f. sp. *albedinis*, the bayoud disease agent of date palm, grows on a mineral medium containing the cell walls of date palm roots as a sole carbohydrate source. The growth and development of pathogen under these conditions was related to the production of extracellular cell wall-degrading enzymes (CWDE): cellulases, polygalacturonases, polygalacturonate transeliminases, and pectinmethylesterases. The mycelial growth and the sporulation of pathogen were higher in the presence of cell walls of susceptible cultivars (BFG, JHL, BSK) than in the presence of those of resistant cultivars (IKL, SLY, BSTN). After 8 d of fungal culture, the activity of CWDE was equal whatever is the origin of the cell walls (resistant or susceptible cultivars). After 16 d of culture, the activity of these CWDE was higher when the parasite was cultivated on the cell walls of the susceptible cultivars than on those of the resistant cultivars. A positive correlation was observed between CWDE activities and the growth and the sporulation of *F. oxysporum* after 16 d of culture. These results clearly show a relation between the susceptibility/resistance of the cell walls of the roots of the date palm to the parasitic CWDE and the susceptibility/resistance of the cultivars.

Additional key words: cell wall-degrading enzymes, *Phoenix dactylifera*, resistant cultivars.

Introduction

Fusarium oxysporum f. sp. *albedinis* is a vascular pathogen responsible for the bayoud disease of date palm. The pathogen penetrates by the roots and causes foliar withering and then the death of the tree. Our previous studies on the interaction host-parasite highlighted the intervention of phytoalexins (El Modafar *et al.* 1999) and soluble phenolic compounds particularly the cafeoylshikimic acid (Ziouti *et al.* 1996, El Modafar *et al.* 2000) in the resistance of the date palm to *F. oxysporum*. However, the implication of these metabolites in the strategy of date palm defense does not explain all aspects of the host resistance to pathogen (Ziouti *et al.* 1996, El Modafar *et al.* 1999), suggesting the existence of other mechanisms of host defense. The plant cell wall constitute the site of various plant defense mechanisms. It limits the penetration and progression of pathogens (Ride 1983, Clérivet and El Modafar 1994, Rioux and Biggs 1994, Beckman 1987) and the action of pathogenic extracellular cell wall-degrading enzymes (CWDE) (Byrde and Archer 1979, Beckman 1987, Mendgen *et al.* 1996).

This work aims *1*) to check if *F. oxysporum* can grow on cell walls of date palm roots as a sole carbohydrate source, *2*) to show if there is a difference in the pathogen growth and development when the cell walls come from susceptible or resistant cultivars, and *3*) to show if there is a relationship between the cell wall susceptibility to *F. oxysporum* cellulolytic and pectinolytic enzymes and susceptibility of date palm cultivars to this pathogen.

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Abbreviations: CWDE - cell wall-degrading enzymes, CWR - cell wall residue, PG - polygalacturonase, PGTE - polygalacturonate transeliminase, PME - pectinmethylesterase.

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Materials and methods

Plants and cell wall preparation: The roots (3 to 5 mm in diameter) were taken from three susceptible (BFG, JHL, BSK) and three resistant (IKL, SLY, BSTN) date palm (*Phoenix dactylifera* L.) cultivars (Saaidi 1992) coming from the palm plantation of Zagora (Southern of Morocco). For each cultivar, the roots were taken from 7 trees. The roots were rinsed, dried between filter paper then crushed in the presence of liquid nitrogen. Soluble compounds of the roots were removed by successive extractions by several solvents: ethanol-hexane-water (2:2:1, v/v/v), ethanol-water (4:1, v/v), ethanol-water (1:1, v/v) and finally water (El Modafar *et al.* 1999). Final cell wall residue (CWR) obtained after vacuum filtration was freeze-dried and then stored under vacuum.

***Fusarium* culture:** The culture medium used was the liquid medium of Richard (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 6.2) containing 2 g of CWR as a sole carbohydrate source. This medium was distributed in 50 cm^3 Erlenmeyer flasks (30 cm^3 per flask), and then inoculated with 1 cm^3 of a conidial suspension (4×10^6 spores cm^{-3}) of strain 133 of *F. oxysporum* f. sp. *albedinis* which is known for its virulence (Sedra and Besri 1994). The conidial suspension was prepared in sterile distilled water from a thallus cultivated on PDA medium (potato dextrose agar) for 5 d at 25 °C. The cultures were then incubated at 25 °C under agitation (2.01 rps). The study related to the CWR of 7 palm trees per cultivar and for the CWR of each palm tree, three repetitions were carried out.

Fungal growth and sporulation: After 4, 8, 12 and 16 d of culture, the rate of sporulation was expressed of numbers of spores cm^{-3}. The mycelial growth was evaluated according to dry mass of mycelium after dehydration of the fungal biomass at 70 °C during 24 h (El Modafar *et al.* 1993).

CWDE activities: The activities of cellulases, polygalacturonases (PG), polygalacturonate transeliminases (PGTE) and pectinmethylesterases (PME) was evaluated after 8 and 16 d of fungal culture, in the culture filtrate obtained by filtration on millipore membrane (0.22 µm, *Millipore S.A.*, Molsheim, France). The culture filtrate was incubated in a specific reactional medium for each enzyme as described previously (El Modafar *et al.* 2000). The enzymatic activity of the different enzymes was expressed in U mg^{-1}(proteins) s^{-1}. For cellulases, PG and PGTE, an enzymatic unit corresponds to the increase in absorbance of 1.66×10^{-3} units s^{-1}, for PME, to the decrease in pH of 1.66×10^{-4} units s^{-1}.

Results

Fungal growth and sporulation of *F. oxysporum*: The results obtained show that *F. oxysporum* grows on a mineral medium containing the cell walls of date palm roots as a sole carbohydrate source (Fig. 1). The growth and the development of *F. oxysporum* were higher in the presence of cell walls of susceptible cultivars (BFG, JHL, BSK) than in the presence of those of resistant cultivars (IKL, SLY, BSTN). In the presence of cell walls of susceptible cultivars, the mycelial growth was 2.6 to 3.1 mg (d.m.) of mycelium (according to the cultivars) after 4 d of culture and 27.2 to 35.8 mg after 16 d (Fig. 1*A*). However, in the presence of cell walls of resistant cultivars, the mycelial growth was only 0.5 to 0.7 mg after 4 d and 8 to 13.5 mg after 16 d. The fungal sporulation (conidiogenesis) was higher on cell walls of the susceptible cultivars than on those of the resistant cultivars (Fig. 1*B*). After 16 d of culture, the sporulation was on average 4.3 times higher when the pathogen was cultivated on the cell walls of the susceptible cultivars than on those of the resistant cultivars.

Production of CWDE by *F. oxysporum* f. sp. *albedinis*: In order to explain the differential growth of *F. oxysporum* on the cell walls of the resistant and susceptible cultivars, we studied the production of CWDE. The results obtained show that *F. oxysporum* produces in the medium cellulases and pectinases (PG, PGTE and PME) in the presence of cell walls of the date palm roots (Table 1). After 8 d of fungal culture, the activities of the different CWDE were equal in all the studied cultivars. The activities of these CWDE remained constant between 8th and 16th day of culture in the presence of cell walls of resistant cultivars (IKL, SLY, BSTN) whereas it increases in the presence of cell walls of susceptible cultivars (BFG, JHL, BSK).

Whatever was the origin of the cell walls present in the medium, the PG represented the most active CWDE secreted by *F. oxysporum* into the extracellular medium. The activity of PME was weakest and that of PGTE and cellulases was intermediate.

Relationship between fungal growth and production of CWDE: After 8 d of culture, the growth and sporulation of *F. oxysporum* f. sp. *albedinis* were not in relation with the CWDE production. However, after 16 d of culture, a positive correlation was observed between the CWDE production by *F. oxysporum* and its growth and sporulation (Fig. 2).

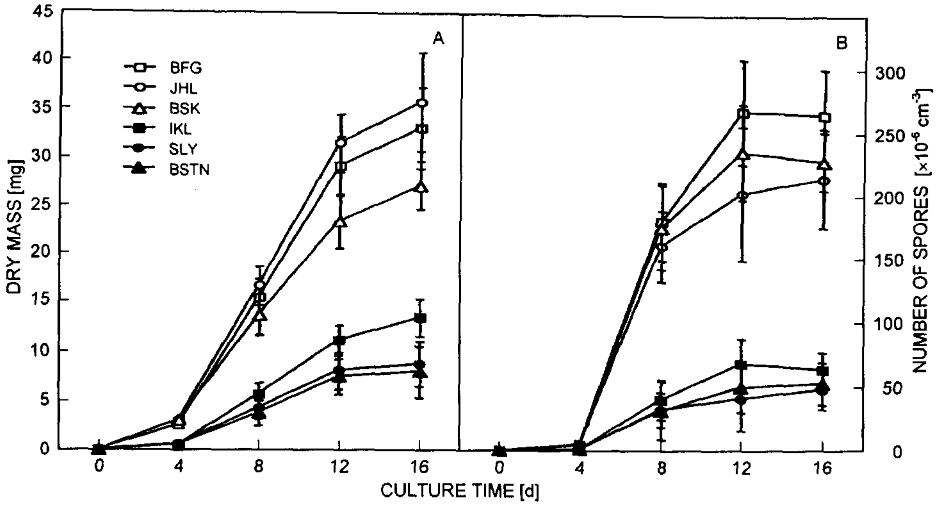

Fig. 1. Mycelial growth (*A*) and production of spores (*B*) by *F. oxysporum* f. sp. *albedinis* cultivated in the presence of date palm cell wall roots of susceptible (BFG, JHL, BSK) and resistant (IKL, SLY, BSTN) cultivars. Means ± SE, n = 7.

Table 1. Activity of the cell wall-degrading enzymes produced by *F. oxysporum* f. sp. *albedinis* [U mg (protein) s^{-1}] cultivated in the presence of date palm cell wall roots of susceptible (BFG, JHL, BSK) and resistant (IKL, SLY, BSTN) cultivars after 8 and 16 d of culture. Means ± SE, *n* = 7.

Cultivar	Cellulase 8 d	16 d	PG 8 d	16 d	PGTE 8 d	16 d	PME 8 d	16 d
BFG	5.34 ± 1.63	18.20 ± 3.80	11.42 ± 4.42	36.52 ± 6.32	9.52 ± 4.18	28.23 ± 6.55	1.12 ± 0.06	3.67 ± 1.1
JHL	4.12 ± 1.71	16.08 ± 4.25	9.18 ± 3.85	28.80 ± 7.24	8.14 ± 2.66	24.10 ± 4.24	1.06 ± 0.48	4.25 ± 1.4
BSK	6.22 ± 1.92	15.68 ± 4.65	9.53 ± 4.50	30.55 ± 6.55	10.15 ± 3.02	24.35 ± 3.84	0.83 ± 0.42	3.30 ± 1.4
IKL	6.55 ± 2.19	5.35 ± 2.39	9.29 ± 4.03	8.53 ± 2.51	7.01 ± 2.85	5.37 ± 2.27	0.98 ± 0.36	0.75 ± 0.2
SLY	5.27 ± 1.76	4.91 ± 1.84	11.08 ± 3.92	10.58 ± 3.33	8.63 ± 3.24	7.80 ± 2.01	0.86 ± 0.31	0.70 ± 0.3
BSTN	4.50 ± 1.26	3.56 ± 1.06	8.77 ± 2.98	7.82 ± 2.12	8.22 ± 2 60	7.04 ± 1.50	0.59 ± 0.28	0.34 ± 0.1

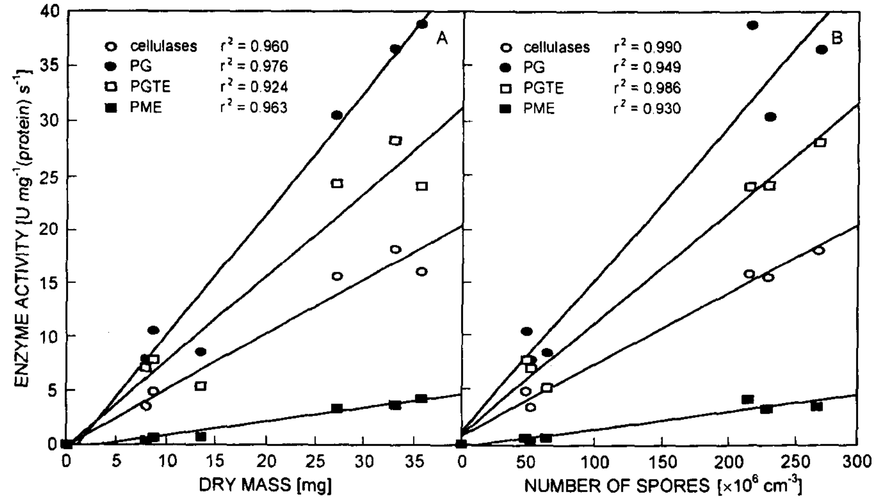

Fig. 2. Relationship between production of cell wall-degrading enzyme and mycelial growth (*A*) or production of spores (*B*) by *F. oxysporum* f. sp. *albedinis* cultivated in the presence of cell wall roots of date palm roots after 16 d of culture.

Discussion

This work shows for the first time that *F. oxysporum* f. sp. *albedinis* was able to develop on a mineral medium containing the cell walls of date palm roots as a sole carbohydrate source. The growth of pathogen under these conditions was related to the production of extracellular cellulases and pectinases (PG, PGTE, PME). The cellulases and the PG were already reported at *F. oxysporum* (Dubost *et al.* 1970, Bounaga 1975) whereas the PGTE and PME were never reported in our knowledge at *F. oxysporum* f. sp. *albedinis*.

Our results shows a relation between the susceptibility of cell walls of the date palm roots to *F. oxysporum* CWDE and the susceptibility of cultivars to this pathogen. This differential susceptibility of cell walls of the resistant and susceptible cultivars to *F. oxysporum* CWDE could be explained by the involvement of constitutive mechanisms of defense in cell walls of the resistant cultivars. Two defense mechanisms could be considered. The first mechanism intervenes in the 8 first days of culture to limit the CWDE action on the cell walls of resistant cultivars. Thus, although the activities of the pectinases (PG, PGTE, PME) and the cellulases by *F. oxysporum* were similar to the 8th day whatever is the origin of cell walls, the fungal growth and sporulation were higher when the fungi was cultivated on cell walls of the susceptible cultivars than on those of the resistant cultivars. The second mechanism intervenes after 8 d of culture to inhibit the production of the CWDE of *F. oxysporum*. This could explain the weak enzymes activities observed at the 16th day in the presence of cell walls of the resistant cultivars compared to that observed with cell walls of the susceptible cultivars.

The resistance of the date palm to *F. oxysporum* can be dependent, at least partly, on the involvement of constitutive mechanisms of defense of the cell wall which limit the action of pectinases and cellulases in the first stages of pathogenesis and inhibit their production in more advanced stages. The difference in susceptibility of the cell walls of the resistant and susceptible cultivars to the pectinolytic and cellulolytic enzymes could then be related to a quantitative or qualitative difference on the level of lignin (Vance 1980, Asada and Matsumoto 1987, Nicholson and Hammerschmidt 1992, Rioux and Biggs 1994), cell wall-bound phenolic compounds (Matern and Grimmig 1993, Ikegawa *et al.* 1996), calcium (Cooper and Rankin 1978, Beckman 1987) and/or hydroxyproline-rich proteins (Esquerré-Tugayé *et al.* 1979, Mellon and Helgeson 1982) which increase the mechanical properties of the cell wall limiting the action of these fungal CWDE. The inhibition of the biosynthesis and activity of the CWDE could also be related to the intervention of cell wall-bound phenolic compounds (Friend 1981, Eraso and Harteley 1990, Ikegawa *et al.* 1996), of cinnamylic alcohols implied in the structure of lignin (Vance 1981, Southerton and Deverall 1990, Daurade-Le Vagueresse and Bounias 1992), of calcium (Corden 1965, Jauneau *et al.* 1994), and/or of CWDE-inhibiting proteins (Albersheim and Anderson 1971, Devoto *et al.* 1998, Glinka and Protsenko 1998).

In addition, the identical production rate of CWDE by *F. oxysporum* in the presence of the cell walls of all cultivars in the first 8 d of culture could explain the identical behaviour of the resistant and susceptible date palm cultivars during the parasite penetration in the roots (Oihabi 1984). However, at advanced stages of the culture of *F. oxysporum* (16 d), a positive correlation exists between the activities of the cellulases and the pectinases (PG, PGTE, PME) and the growth and the sporulation of *F. oxysporum*. This phenomenon observed *in vitro* with isolated cell walls could thus appear *in planta*. Indeed, in the case of the vascular diseases, the difference in behaviour of the resistant and susceptible genotypes generally appears on the level of the capacity of limitation of the fungal dissemination in the plant in order to prevent the parasite abundantly developing and producing toxins responsible for the typical symptoms of disease (Beckman 1987). Thus, the horizontal dissemination of the vascular parasites was assured primarily by the mycelial growth and the vertical dissemination by the spores which were involved remotely by the sap current and constitute secondary foci after their germination (Beckman 1987, Clérivet and El Modafar 1994). The pectinolytic and cellulolytic enzymes excreted by plant pathogen in the host tissue were involved indirectly in the growth and the dissemination of the parasite (Beckman 1987, Annis and Goodwin 1997). These extracellular CWDE were indeed responsible for the decomposition of constitutive polymers of the cellular structures in nutrients essential for the fungal growth and the sporulation of plant pathogens (Annis and Goodwin 1997). This could explain the positive relation observed between the production of pectinases and cellulases by *F. oxysporum* and the fungal growth and the sporulation of pathogen after 16 d of culture.

The resistance of the date palm to the *F. oxysporum* f. sp. *albedinis* would be depend, at least partly, on mechanisms of defense in cell wall. In the first stages of pathogenesis, mechanical mechanisms limit the action of the cellulases and pectinases (PG, PGTE, PME) of pathogen on the host cell walls. At more advanced stages, chemical mechanisms inhibit the biosynthesis of these CWDE. These mechanisms could be lignin, cell wall-bound phenolic compounds, calcium, hydroxyproline-rich glycoproteins, and/or CWDE-inhibiting proteins.

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