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Phenolic compounds in date palm cultivars sensitive and resistant to *Fusarium oxysporum*

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Abstract

In date palm (*Phoenix dactylifera* L.) leaves, the main compounds of the phenolic pool were quercetin and isorhamnetin heterosides, (+)-catechin and (-)-epicatechin. Although previously observed only in date palm fruits, 5-caffeoylshikimic acid (dactylifric acid) and its positional isomers (3-caffeoylshikimic acid and 4-caffeoylshikimic acid) were detected also in the leaves and roots. Quantitative, but not qualitative, differences between cultivars resistant and susceptible to *Fusarium oxysporum* f. sp. *albedinis* during growth period were observed.

Additional key words: caffeoylshikimic acids, flavonoids, *Phoenix dactylifera*.

Introduction

Date palm (*Phoenix dactylifera*) has been detrimentally affected by Bayoud disease caused by the soil fungus *Fusarium oxysporum* f. sp. *albedinis* (Foa) (Foex and Vayssiere 1919). Although there have been detailed studies on the fungus and host symptoms, only few studies have focused on characterizing date palm resistance (Assef *et al.* 1986, Assef 1987, Baaziz and Saaidi 1988, Baaziz 1989). Consequently, biochemical markers should be developed to detect plants obtained through controlled cross-breeding of genotypes showing high resistance and date quality.

The present investigation was carried out within this overall framework. Phenolic compounds were studied since they are often involved in plant resistance to pathogenic microorganisms (Misagui 1982, Harborne 1989, Nicholson and

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Hammerschmidt 1992). The first part of this study was aimed at identifying the main compounds in the roots (through which the fungus colonizes the plant) and leaves (site where symptoms appear) in resistant and susceptible cultivars.

Materials and methods

Plants: The study was carried out with material from 4 adult date palm (*Phoenix dactylifera* L.) cultivars grown in the experimental palm orchard at Zagora (southern Morocco): cv. BSTN and cv. IKL are resistant and cv. BFG and cv. JHL are susceptible to the disease (Saaidi 1992).

The roots (3 to 5 mm diameter) and median leaflets of palm leaves from the middle crown were analyzed for phenolic compounds. Four samples were taken during the year, corresponding to different physiological stages: stage 1 (during April, beginning of vegetative activity), stage 2 (during July, peak vegetative activity), stage 3 (early October, ripening of dates) and stage 4 (late October, after fruit harvest).

Analysis of phenolic compounds: Extraction and purification of phenolic compounds were carried out as previously described (Ziouti *et al.* 1992, El Modafar *et al.* 1993). They were separated by TLC on a cellulose stationary phase using several solvent systems: butanol:acetic acid:water (4:1:5, upper phase), 15 % acetic acid, butyl acetate:acetic acid:water (4:1:5, upper phase), butanol:2 % ammonia (1:1, upper phase) and benzene:acetic acid:water (6:7:3, upper phase).

The phenolic compounds were separated and identified by HPLC (*Varian 5000*). It was performed on a C18 column (250 × 5 mm) and samples were eluted in a solvent consisting of acetonitril and bidistilled water (pH 2.6) following a gradient of 4 - 40 % acetonitril within 40 min with a 1 cm^3 min^{-1} flow rate. Detection was carried out at 280 nm for flavans, 320 nm for hydroxycinnamic derivatives and 350 nm for flavonols and flavones.

Several other identification criteria were also used to characterize the separated phenolic compounds: Rf in the different solvent systems, use of specific developing reagents: vanilline-chlorhydric acid for revelation of flavans (Ribereau-Gayon 1972), *Benedikt reagent* for distinction of monophenols/*o*-diphenols (Reznik and Egger 1961) and *Neu reagent* (Brasseur and Angenot 1986). Identification was completed by absorption spectrum obtained by HPLC equipped with photodiode array detector (*Waters 990*) and by comparing spectral and chromatographic characteristics with commercial standards (*Extrasynthèse*). The phenolic moiety was determined after alkaline hydrolysis (2M NaOH under nitrogen atmosphere for 3 h), acid hydrolysis (2M HCl for 1 h at 100 °C) and enzymatic hydrolysis (β-glucosidase, phosphate buffer, pH 6.8, for 4 h).

Total phenols were quantified as described by Marigo (1973). They were expressed in mg tannic acid per g dry matter. The different phenols were assayed by HPLC under the previously described conditions (Ziouti *et al.* 1992). Flavonols were expressed as rutin, hydroxycinnamic derivatives as caffeic acid and flavans as

(+)-catechin equivalents. For each stage and cultivar, all data correspond to means of 3 replicates with samples of 5 date palms.

Results

Identification of the main phenolic compounds in the leaves and roots: Phenolic compounds detected in the leaves belonged to three important families: flavans, flavonol and flavone heterosides and hydroxycinnamic derivatives.

Two flavan-3-ols, (+)-catechin (peak 1) and (-)-epicatechin (peak 2) were identified in the leaves (Fig. 1*A*). We also detected other compounds with similar characteristics but these were not identified. They were likely combined forms of proanthocyanidins. In the roots, the level of flavans was very low (2 to 5 % of total phenolic compounds).

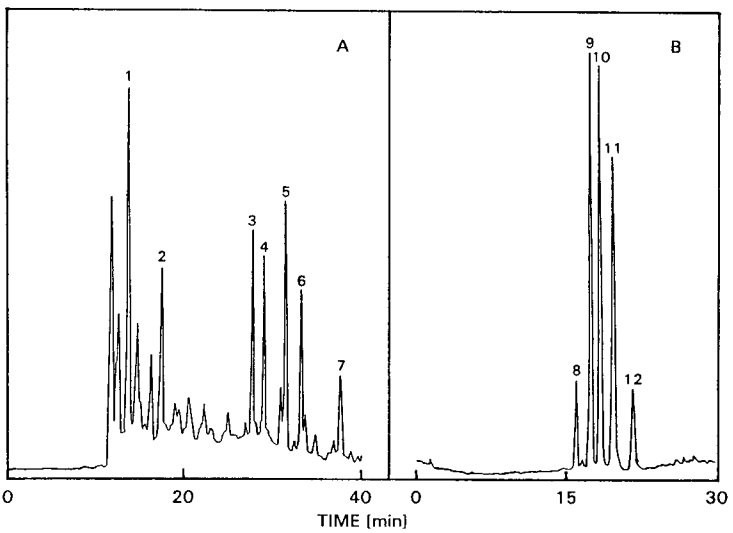

Fig. 1*A*. HPLC chromatogram of date palm leaf extract. The detection was carried out at 280 nm. Identification of the main peaks: *1* - (+)-catechin, *2* - (-)-epicatechin, *3* - quercetin-3-rhamnoglucoside (rutin), *4* - quercetin-3-galactoside, *5* - isorhamnetin-3-rhamnoglucoside, *6* - isorhamnetin heteroside, *7* - isorhamnetin-3-galactoside.

Fig. 1*B*. HPLC chromatogram of a date palm root extract. The detection was carried out at 320 nm. Identification of peaks: *8* - *cis* 3-caffeoylshikimic acid, *9* - *trans* 3-caffeoylshikimic acid, *10* - *trans* 4-caffeoylshikimic acid, *11* - *trans* 5-caffeoylshikimic acid, *12* - *cis* 5-caffeoylshikimic acid.

The five main peaks (3, 4, 5, 6 and 7), showing maximum absorption at 350 nm (Fig. 1*A*), corresponded to two quercetin heterosides (quercetin-3-rhamnoglucoside or rutin at peak 3 and quercetin-3-galactoside at peak 4) and three isorhamnetin heterosides (isorhamnetin-3-rhamnoglucoside at peak 5, isorhamnetin-3-galactoside at peak 7; peak 6 was not fully identified). These were the five main compounds in the leaves, accounting for 80 % of all compounds detected at 350 nm. Other peaks probably corresponded to flavone derivatives (including luteolin glycosides) and to

C-glycosylflavones, as revealed by acid hydrolysis. Flavonol and flavone heterosides were not detected in the roots.

Hydroxycinnamic derivatives, mostly caffeic esters, were the main components detected in the roots (Fig. 1*B*), while they represented only 3 - 4 % of total phenolic compounds in the leaves. These hydroxycinnamic derivatives were identified as 5-caffeoylshikimic acid (dactylifric acid) and its positional isomers (3-caffeoylshikimic acid and 4-caffeoylshikimic acid) by comparing UV spectrum and chromatographic characteristics (Rf and UV fluorescence in TLC, retention times in HPLC) with caffeoylshikimic acids isolated from date where they were very abundant (Maier and Metzler 1965) or synthesized by an *in vitro* enzymatic procedure (Lotfy *et al.* 1992).

Comparison of cultivars: A comparative study of date palm roots and leaves during an annual growth period revealed no qualitative differences between the cultivars studied. However, some quantitative differences were noted particularly in the roots.

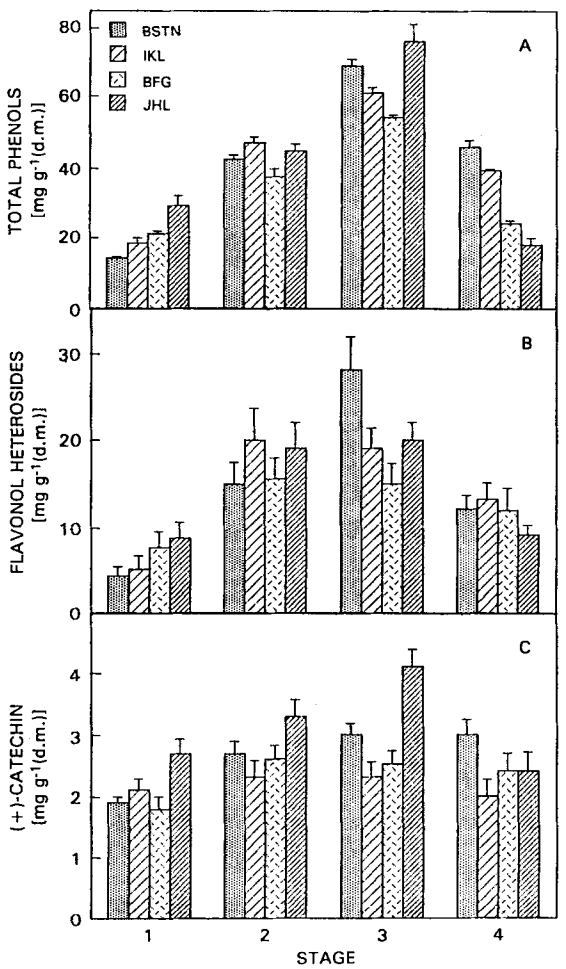

Fig. 2. Changes in total phenol content, (*A*), flavonol heterosides, (*B*), and (+)-catechin, (*C*) during an annual cycle in date palm leaves.

The overall patterns for total phenols (Fig. 2*A*), flavonols (Fig. 2*B*) and flavans (Fig. 2*C*) in the leaves were almost identical in the four cultivars, with peaks in early October (stage 3) during the fruit ripening period. Moreover, between-cultivar differences were not always significant or constant during the growth period.

In the roots, caffeoylshikimic acids were generally absent during stages 1 and 2, but the significant differences between cultivars were observed in levels of these phenolic compounds in stage 3 and to a lesser extent in stage 4 (Table 1). In stage 3, there was observed higher accumulation in the resistant cultivar roots [72 and 272 $\mu g\ g^{-1}$(d.m.) for BSTN and IKL, respectively] than in roots of sensitive cultivars [only 10 and 17 $\mu g\ g^{-1}$(d.m.) for BFG and JHL, respectively]. This difference was not maintained in the stage 4: the level of accumulated caffeoylshikimic acids was higher in sensitive cultivar BFG [84 $\mu g\ g^{-1}$(d.m.)] than in resistant cultivar IKL [only 34 $\mu g\ g^{-1}$(d.m.)].

Table 1. Variations in caffeoylshikimic acids (include all 3 positional isomers) in date palm roots during the growth period [$\mu g\ g^{-1}$(d.m.)].

Stage	1	2	3	4
cv. BSTN	-	-	71.9 ± 6.8	106.0 ± 2 5
cv. IKL	-	-	271.7 ± 10.2	33.5 ± 3.5
cv. BFG	-	-	9.9 ± 2 0	83.6 ± 3.1
cv. JHL	-	-	17.1 ± 0.8	30.3 ± 3.2

Discussion

Only few studies have investigated phenolic compounds in the leaves and fruit of date palms (Maier and Metzler 1965, Williams *et al.* 1973, Ouafi *et al.* 1988, Lorente and Ferreres 1988). However, to our knowledge, the phenolic composition of the roots has never been assessed.

Flavonoids were found to be the main phenolic compounds in the leaves. They were chiefly flavonol heterosides (quercetin and isorhamnetin heterosides), with some luteolin derivatives. These three aglycones were previously detected by acid hydrolysis in the leaves of some other date palm cultivars (Ouafi *et al.* 1988). The same authors reported high levels of C-glycosylflavones, although we found only trace amounts in our cultivars. Flavans were also part of the phenolic composition of the leaves, the main ones identified were (+)-catechin and (-)-epicatechin. It is quite likely that more complex forms of flavans were present (proanthocyanidins), as previously reported (Ouafi *et al.* 1988, Ziouti *et al.* 1992). The flavonol, flavone and flavan levels in the leaves were not significantly different in the 4 cultivars.

The presence of hydroxycinnamic esters in date palm leaves and roots has not yet been published. In our cultivars, their content in leaves was very low, although they represented most of the phenolic pool in the roots. They were identified as caffeoylshikimic esters, whose have been already characterized in dates (Maier and

Metzler 1965). The levels of these root phenolic compounds indicated some between-cultivar differences. The presence of the esters in the roots is of particular interest for their potential fungitoxic activity. In fact, the caffeic derivatives are known to be toxic to microorganisms and could be involved in plant defense mechanisms (Bell 1981, Friend 1981, Harborne 1989) particularly after infection when they form the oxidized products (quinones) with the high fungitoxic character (Beckman 1987, De Cleen 1988, Harborne 1989).

The present results do not permit a valid discussion on the overall role of phenolic compounds, more specifically caffeoylshikimic acids, in date palm resistance because of the limited number of investigated cultivars. However, the difference in the accumulation of these compounds in the roots of resistant and sensitive cultivars during the growth period (particularly at the stage 3) might be important for further study of the sensitivity stage of date palm. The studies should be extended to a wide range of genotypes with different degrees of resistance. Future work concerning the effect of infection on the phenolic composition of the roots and the effects of these compounds on growth and development of *Fusarium oxysporum* f. sp. *albenidis* could define their possible role in date palm resistance to this disease.

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