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RESEARCH ARTICLE - BIOLOGICAL SCIENCES

GC–MS Analysis of Cell Wall-Bound Phenolic Compounds and Lignin Quantification in Date Palm Cultivars that are Resistant or Susceptible to *Fusarium oxysporum* f. sp. *albedinis*

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Abstract This study aimed to collect data about the interactions that occur between the date palm (*Phoenix dactylifera* L.) and *Fusarium oxysporum* f. sp. *albedinis* (*F.o.a*), the causal agent of bayoud disease. Alkaline hydrolysis was carried out using the root parietal residue of three cultivars of date palm, among which two are susceptible (Deglet Nour (DN) and Tggaza (TG)) and one is resistant (Takerbucht (TK)) to bayoud disease. Gas chromatography coupled with mass spectrometry (GC–MS) analysis revealed that *p*-hydroxybenzoic acid is the major phenolic compound of cell wall-bound phenolics. In uninfected palm groves, the resistant date palm cultivar contained a high level of *p*-hydroxybenzoic acid; however, in palm groves infested with *F.o.a*, a significant decrease in *p*-hydroxybenzoic acid was observed. In the roots of susceptible cultivars with bayoud symptoms, we noted a qualitative and quantitative increase in phenolic compounds, with a remarkable increase in *p*-hydroxybenzoic acid content during the infection of susceptible cultivars. We investigated lignin content in roots. An increase in total lignin content was observed in both cultivars collected from palm groves infested by *F.o.a*, but more accumulated in the roots of the resistant cultivar than in those of the susceptible cultivars. Our findings indicate that *p*-hydroxybenzoic acid plays an important role in date palm defense mechanisms against *F.o.a*. However, its accumulation in susceptible cultivars as a response to pathogens did not block the progression of the parasite and thus was not an effective mode of resistance. Susceptible cultivars used phenolic compounds from the benzoic series for their defense, while resistant cultivars used lignification that reinforced the cell wall.

Keywords Date palm · *Fusarium oxysporum* f. sp. *albedinis* · GC–MS analysis · *p*-hydroxybenzoic acid · Lignin

1 Introduction

Date palm (*Phoenix dactylifera* L.) is the main fruit crop in arid areas. In all Saharan oases, the date palm constitutes the main source of food. These plants are linchpin of economic and, hence, social and cultural life in the Arab region. Unfortunately, this plant is threatened by several diseases; bayoud, a vascular fusariosis caused by *Fusarium oxysporum* f. sp.*albedinis* (*F.o.a*.), is the most serious affliction. It has already destroyed millions of trees in Morocco and Southwest Algeria [1] and is a serious threat Mauritanian palm groves [2].

Numerous works reported on cytological, physiological [3] and biochemical aspects [4–8] of the interactions between the host and the parasite, but little is known about plant defense mechanisms. In Algeria, one resistant cultivar (TK) exists. However, the mechanisms involved have not yet been explained and are under investigation. Research with Moroccan varieties has demonstrated the presence of molecular markers of resistance, such as caffeoyl shikimic acid [9] and other phytoalexins [10].

We tried to identify the underlying resistance mechanism by studying the secondary metabolites of Algerian cultivars through analysis of cell wall phenolic compounds using a GC–MS (gas chromatography coupled with mass spectrometry) technique.

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Cell walls are a natural physical barrier against invaders. However, *F.o.a.* possesses an arsenal of enzymes that lyse cell wall components. This cell wall degradation allows penetration into date palm cells, allowing the pathogen to reach xylem vessels, where it develops. *F.o.a.* very often causes damage followed by the death of sensitive cultivars but not the resistant variety.

It has been shown that the cell wall is reinforced by lignification in response to parasite attack [5–8]. Attack also leads to the induction of the synthesis of phenolic compounds [4], which accumulate to higher levels in the resistant variety.

Cell wall-bound phenolics can act directly as molecules of defense or can be used as precursors of lignins [11] and/or of suberin [12]. The accumulation of low-weight molecules, such as benzoic acids and other phenolic compounds, constitutes an initial response to infection [13]. Benzoic and cinnamic acids are the phenolic compounds associated with cell walls and play a significant role in the mechanisms of defense during parasite attack [14].

In the present work, we analyzed lignins and phenolic compounds associated with the cell walls in the roots of three cultivars of date palm, two of which are susceptible (Deglet Nour (DN) and Tggaza (TG)) and one of which is resistant (Takerbucht (TK)) to bayoud disease.

Fig. 1 Date palm tree, the palms carrying the symptoms of bayoud

2 Materials and Methods

2.1 Plant Material

Roots (2–5 mm in diameter) were collected from the plants of two cultivars (DN and TG, susceptible) that were either uninfected or infected by *F.o.a.* (the pathogen invades the plant through the roots, producing foliar withering and leading to death of the date palm) [15].The latter samples were collected in palm groves infected by *F.o.a* (Fig. 1).

Roots of the resistant cultivar were also collected from two types of palm groves: uninfected or infected by *F.o.a.* For each cultivar, roots were taken from six palm trees, rinsed, dried on filter paper, labeled and stored in liquid nitrogen until use.

Roots of TK and TG cultivars were collected from Adrar (southwestern Algeria) in 2011 and those of the DN cultivar, not present in that grove, were collected from Metlili (Ghardaia, southeastern Algeria) in the same period.

2.2 Extraction of Cell Wall-Bound Phenolics

The extraction of cell wall phenolic compounds was performed according to the method described by Parr et al. (1996) [16]. Roots were finely crushed at 4 °C in 80% methanol (500 µL for 100 mg of dry material); the mixture is left to stand under continuous stirring for 20 min at 4 °C. The homogenate was centrifuged for 10 min at 10,000×*g*. Residues containing cell walls were successively subjected to methanol (50%), distilled water, sodium dodecylsulfate (SDS; 0.5% (w/v)), NaCl (1 M), methanol (100%), acetone and diethyl ether and then vortexed at vigorous speed for 15 min at 4 °C. These washes were performed to optimize alkaline hydrolysis by elimination of any molecules adsorbed on the cell wall. The mixture was centrifuged for 10 min at 10,000×*g* to collect the pellets.

Dried residues were subjected to alkaline hydrolysis using 2 M NaOH and left to stand for 24 h in the dark. These mild saponification conditions allowed the selective release of cell wall phenolic compounds.

Saponified extracts were neutralized using 2N HCl and extracted three times with ethyl acetate. The ethyl acetate fraction was reduced to dryness, suspended in 0.5 mL of aqueous methanol (2:1, v/v) and kept at −20 °C until the next analysis.

2.2.1 TMS Derivatization of Phenolic Acids

Derivatization of all samples was performed using BSA (N,O-bis[trimethylsilyl]acetamide), which is the most suitable reagent for derivatizing phenols when used with N,N-dimethylformamide (DMF) [17,18].

The cell wall-bound phenolic extracts previously obtained were evaporated to dryness under reduced pressure and resuspended in 500 µL of aqueous dimethylformamide (sily-

lation grade) and transferred into polypropylene (PP) vials. Twenty microliters of N,O-*bis*[trimethylsilyl]acetamide (Sigma-Aldrich) was added, and the obtained mixture was incubated at 60 °C for 30 min in a furnace. The silylated samples were shaken for 30 s and immediately analyzed using GC–MS.

2.2.2 GC–MS Instrumentation and Conditions

The analysis of derivatized samples was performed using an Agilent GC-6890 gas chromatograph and 5973 quadrupole mass spectrometer equipped with an HP-5 MS capillary column (30 m, 0.25 mm, 0.25 lm film thickness). The injector was set at 250 °C, and the oven temperature program was 60 °C for the first 2 min, ramped at 6 °C/min to 250 °C and maintained for 20 min. Helium was the carrier gas (1 mL/min). Ionization of the sample components was performed in EI mode (70 eV). Positive fragment ions were analyzed over a 50–500 m/z mass range in the SCAN mode. All control of GC and MS parameters and analysis of data were performed using a microcomputer (Vectra 487/333VI Hewlett-Packard), equipped with HPCHEM software for acquisition, recording and chromatographic and spectrometric data processing. Volatile compounds were detected and identified by comparing the mass spectrum (m/z ratio) we obtained with those of the National Institute of Standards and Technology Library (Nist2002). Each detected compound is characterized by its retention time (RT), its distribution area and its recognition percentage (%), as defined by Nist2002. The chromatograms were traced with OriginPro 8.0 software.

2.3 Lignin Determination

To prepare the cell wall residue (CWR), dry root material underwent successive extractions with ethanol–hexane–water (2:2:1, v/v), ethanol–water (4:1, v/v), ethanol–water (1:1, v/v) and water, followed by vacuum filtration. The obtained CWR was dried under vacuum and then stored until use. Lignin and lignin-like phenolic polymers were assayed quantitatively by derivatization with thioglycolic acid [19] from CWR from date palm roots. Ten milligrams of dried CWR was treated with 1 mL of 0.5 M NaOH. The tubes were incubated at 96 °C for 1 h to hydrolyze the cell wall-bound phenolics. The mixture was neutralized with 0.25 mL of 2 M HCl, and the residue was collected by centrifugation at 12,000 × *g* for 10 min. One milliliter of 2 M HCl and 0.1 mL of thioglycolic acid were added to the residue. The tubes were placed in a boiling water bath for 4 h at 96 °C and shaken to hydrate the CWR. The tubes were cooled and centrifuged at 12,000 × *g* for 10 min at room temperature. Following centrifugation, the precipitate was washed once with 1 mL of H_2O, resuspended in 1 mL of 0.5 M NaOH and shaken at 25 °C for 18 h to extract the lignin thioglycolate. The samples were centrifuged at 12,000 × *g* for 10 min. Then, 0.2 mL of concentrated HCl was added to the supernatant and the lignin thioglycolic acid was allowed to precipitate at 4 °C for 4 h. The precipitated lignin thioglycolic acid was finally collected by centrifugation at 12,000 × *g* for 10 min, the orange–brown precipitates were dissolved in 1 mL of 0.5 M NaOH, and the lignin contents were determined spectrophotometrically at 280 nm (UV–Vis spectrophotometer, SHIMADZU).

2.4 Statistical Analysis

All measurements were taken at least in 5 replicate, and values were averaged. Results are given as means ± standard deviation (SD). The Student's *t*-test was used to test for statistical differences between the parameters investigated in the different plants (roots of susceptible cultivars and those resistant). The levels of significance with $*p < 0.05$ were considered statistically significant, and $**p < 0.01$ was considered highly significant according to the resulting random effects analysis.

3 Results

3.1 GC–MS Analysis of Cell Wall-Bound Phenolic Compounds

GC–MS analysis, which occurred after TMSA derivatization of root extracts of the three date palm cultivars, showed a significant abundance of cell wall phenolic compounds, as well as the presence of fatty acids and aliphatic hydrocarbons (Table 1). Sixteen phenolic compounds associated with the cell walls were identified (Table 2) and divided into four polyphenolic classes (Fig. 2): simple phenolics and benzoquinones, which all have the C6 structure (Fig. 2a), hydroxybenzoic acids (C6-C1) (Fig. 2b), hydroxycinnamic acids (phenylpropanoids and coumarins) (C6-C3) (Fig. 2c) and flavones (C6-C3-C6) (Fig. 2d).

Hydroxybenzoic acids were the most dominant; they represent 10 of the 16 compounds detected and were also better represented in all cultivars. The most abundant were the butylic, methylic and propylic esters of *p*-hydroxybenzoic acid (commonly called parabens), and the methoxylated (vanillic acid and vanillin) and dimethoxylated derivatives (syringic acid and syringaldehyde) of hydroxybenzoic acid. The *p*-hydroxybenzoic acid isomer was the most abundant compound; it was present in the roots of both resistant and susceptible cultivars (Table 2).

In the cell wall of healthy DN, the susceptible cultivar, phenolic acids and their derivatives represent approximately 50.39% of total phenolics with only three phenolic compounds, *p*-hydroxybenzoic acid, the major compound, rep-

Table 1 Total components (%) detected by GC–MS from root extracts of resistant and susceptible cultivars of date palm

Components	Relative area %					
	DNna	DNa	TGna	TGa	TKnis	TKis
Phenolic compounds	56.80	89.38	37.66	67.21	93.61	24.98
Fatty acids and aliphatic hydrocarbons	28.14	8.27	50.59	29.27	3	50.44
Unidentified	10.49	0.51	7.91	2.88	2.05	21.23
Total components	95.43	98.16	96.6	99.36	98.66	96.65

DNna Deglet Nour non-affected, *DNa* Deglet Nour affected, *TGna* Tggaza non-affected, *TGa* Tggaza affected, *TKnis* Takerbucht uninfected soil, *TKis* Takerbucht in infected soil

Table 2 Phenolic compounds (%) detected by GC–MS from root extracts of resistant and susceptible cultivars of date palm

No.	Compounds identified	RT (min)	Relative area (%)						
			m/z (major fragment ions)	DNna	DNa	TGna	TGa	TKnis	TKis
1	Phenol	7.36	94, 66, 73, 95	–	2.70	–	–	0.31	–
2	Benzoic acid	13.27	179, 105, 135, 120, 77	–	2.11	–	0.5	0.64	–
3	2-Methoxy-4-vinylphenol (*p*-vinylguaiacol)	14.96	150,135, 107, 77	–	1.10	–	2.07	0.59	–
4	Benzaldehyde, 4-Hydroxy-3-methoxy- (Vanillin)	16.42	151, 109, 123, 81, 167	–	–	–	0.78	0.43	–
5	Methylparaben	17.62	121, 152, 93, 65		–	10.66	1	2.17	–
6	Benzoic acid, 4-methoxy- (*para*-anisic acid)	18.69	135, 152, 77	–	–	0.34	0.19	–	0.44
7	Benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester	18.85	151, 182, 123, 167	–	–	1.12	–	0.69	–
8	Phenol, 2,3-Dimethyl 5-methoxy-	19.19	151, 123, 138, 77	0.62	0.67	–	3.94	0.67	0.44
9	Propylparaben	20.38	121, 138, 180, 93, 65	0.62	–	–	0.99	1	–
10	4-Hydroxybenzoic acid (*para*-hydroxybenzoic acid)	21.35	267, 223, 193, 73, 121	48.15	82.63	23.22	55.19	85.67	23.45
11	Benzaldehyde 4-hydroxy-3,5-dimethoxy- (syringic acid)	21.61	355, 121, 182, 138, 73	–	–	–	–	0.44	0.41
12	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	23.62	212, 181, 197	–	–	0.83	–	0.54	0.24
13	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	24.77	208, 177, 145	–	–	1.49	0.5	1.04	–
14	Dimethylchrysin (flavones, 5,7-Dimethoxy-)	29.44	139, 282, 121, 267	–	–	–	0.57	–	–
15	Butylparaben	32.74	121, 138, 55, 69, 93	–	0.17	–	–	0.13	–
16	Coumarin-6-ol, 3,4-dihydro-4,4-dimethyl-7-nitro-	33.14	237, 255, 207, 195	–	–	–	1.43	0.5	–

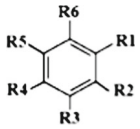
Underlined masses are the abundant ones

RT (min) retention time, *DNna* Deglet Nour non-affected, *DNa* Deglet Nour affected, *TGna* Tggaza non-affected, *TGa* Tggaza affected, *TKnis* Takerbucht uninfected soil, *TKis* Takerbucht in infected soil

resenting 48.15% of total phenolics, butylparaben (1.62%) and 2,3-dimethyl-5-methoxy phenol. This minor compound represents only 0.62% of the total identified compounds (Fig. 3a).

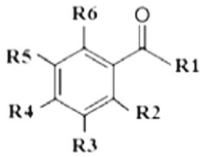
In TG, phenolic acids comprise approximately 37.66%. Their phenolic profile was more diverse, with six identified compounds; the major compound was *p*-hydroxybenzoic acid, which represented 23.66%. The other identified com-

a



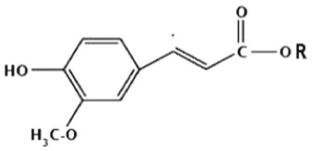
1 : R1=R2=R3=R4=R5=R6=H
3 : R1=R3=R4= H, R2= C_2H_5, R5=OH, R6=OCH_3
8 : R1=OH, R2=R3=CH_3, R4=R6=H, R5=OCH_3

b



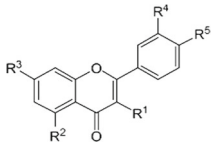
2 : R1=OH, R2=R3=R4=R5=R6=H
4 : R1=R2= R5=R6=H, R3= OCH_3, R4=OH
5 : R1= OCH_3, R2=R3= R5=R6=H, R4=OH
6 : R1=OH, R2=R3= R5=R6=H, R4= OCH_3
7 : R1=OC_2H_5, R2=R3= R6=H, R4=OH, R5=OCH_3
9 : R1=OC_3H_7, R2=R3= R5=R6=H, R4=OH
10 : R1= R4=OH, R2=R3=R5=R6=H
11 : R1=R2= R6=H, R3= R5=OCH_3, R4=OH
12 : R1=$NHNH_2$, R2= R6=H, R3= R5=OC H_3,R4=OH
15 : R1=OC_4H_9, R2=R3= R5=R6=H, R4=OH

c



13 : R=CH_3

d



14 : R1=R4=R5=H, R2=R3= OCH_3

Fig. 2 Chemical structure of phenolic compounds detected in cell walls root extracts from three date palm cultivars. **a** Simple phenols, **b** benzoic acid derivatives, **c** cinnamic acid derivative, **d** flavone derivative (No.: denote the number of the compounds according to Table 1)

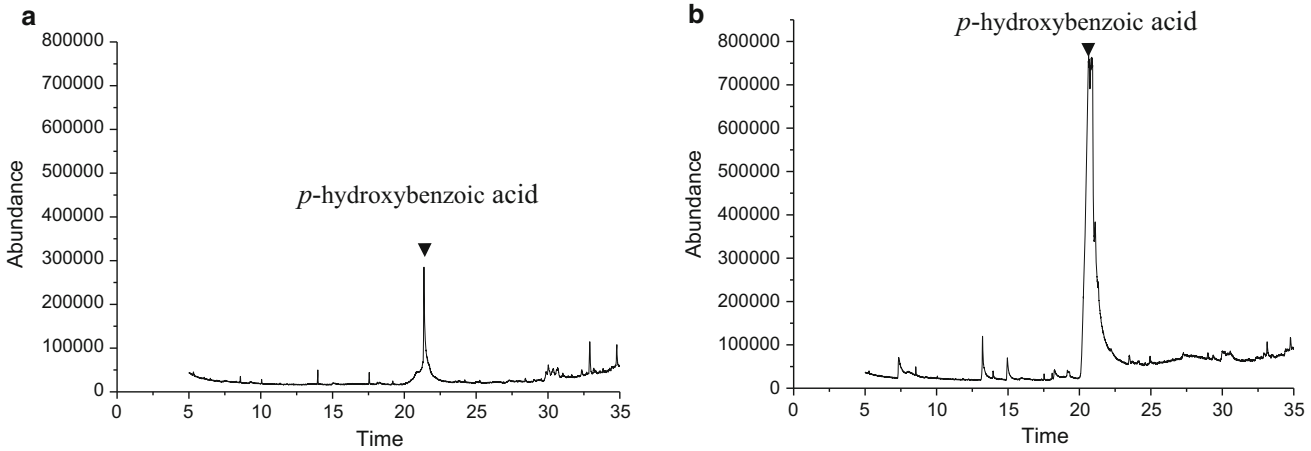


Fig. 3 Total ion chromatogram obtained by GC–MS analysis of cell wall-bound phenolic compounds extracted from date palm roots of healthy (**a**) and affected (**b**) susceptible cultivar DN

pounds were benzoic acid 4-methoxy-(*p*-anisic acid) (0.34%), benzoic acid 4-hydroxy-3,5-dimethoxy-, hydrazide (hydrazide of syringic acid) (0.83%), benzoic acid 4-hydroxy-3-methoxy-, ethyl ester (vanillic acid, ethyl ester) (1.12%), 2-propenoic acid 3-(4-hydroxy-3-methoxyphenyl)-methyl ester (ferulic acid, methyl ester) (1.49%) and methylparaben (10%) (Fig. 4a).

The cell wall of the roots of the resistant variety is characterized by a particular abundance of phenolic compounds (94.82%) and a diverse profile (14 identified compounds) when compared to that of the susceptible cultivars. In addition, seven other compounds were detected in the resistant cultivar, including 2-methoxy-4-vinylphenol, 4-hydroxy-3-methoxy-benzaldehyde (vanillin), 2,3-dimethyl 5-methoxyphenol, propylparaben, 4-hydroxy-3,5-dimethoxy-benzaldehyde (syringic acid), butylparaben and 3,4-dihydro-4,4-dimethyl-7-nitro-coumarin-6-ol. The majority compound was *p*-hydroxybenzoic acid, which represented 85.67% of the total cell wall phenolic compounds. The chromatographic profiles of the phenolic extracts of roots revealed a clear dif-

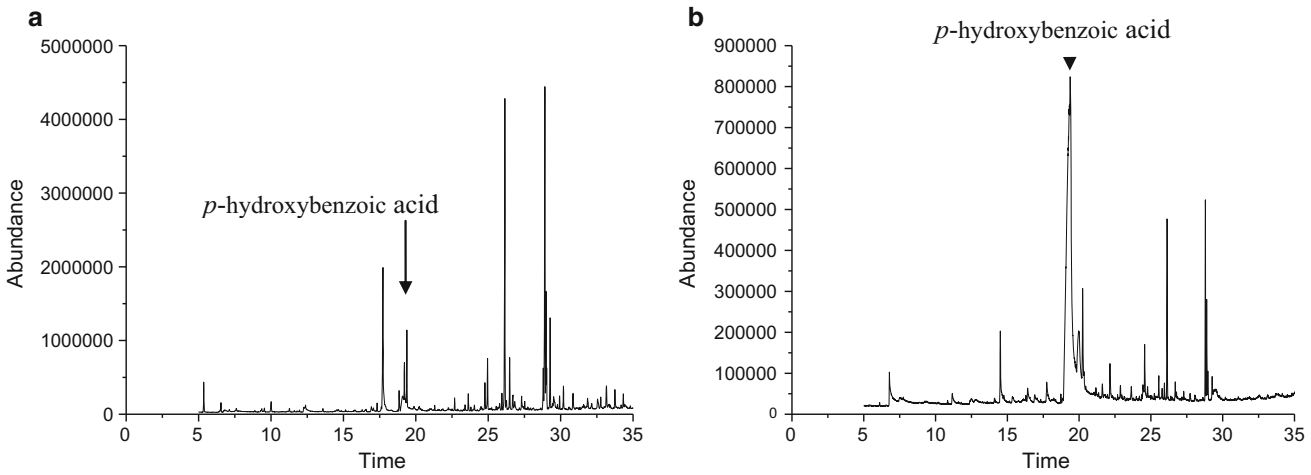


Fig. 4 Total ion chromatogram obtained by GC–MS analysis of cell wall-bound phenolic compounds extracted from roots of healthy (**a**) and affected (**b**) TG susceptible date palm cultivar

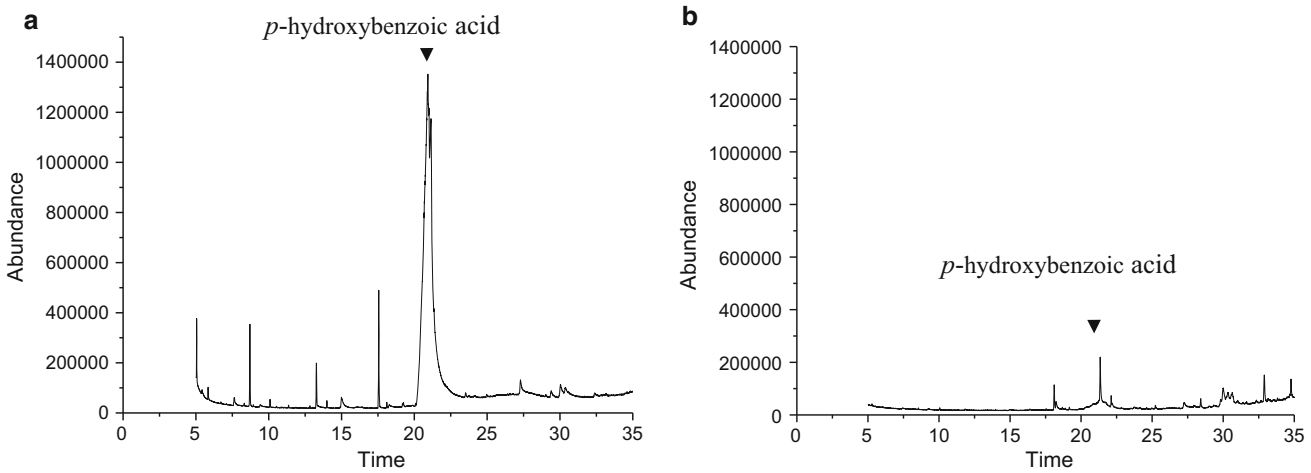


Fig. 5 Total ion chromatogram obtained by GC–MS analysis of cell wall-bound phenolic compounds extracted from roots of date palm resistant cultivar (TK) collected in palm groves free of *F.o.a*. (**a**) and palm groves infested by *F.o.a* (**b**)

ference between the cell wall of healthy susceptible cultivars and that of the resistant cultivar (Fig. 5a).

In roots of DN infected by *F.o.a*., we noted not only a diversification of the phenolic profile, with the appearance of four molecules that were not detected in healthy plants (benzoic acid, 2-methoxy-4-vinylphenol, butylparaben acid and phenol) and a particular increase (1.7-fold) in the *p*-hydroxybenzoic acid proportion, which reached 82.63% (Fig. 3b).

In susceptible TG roots infected by *F.o.a*., we also noted an increase in the number of detected compounds; the new substances were benzoic acid, 2-methoxy-4-vinylphenol, benzaldehyde 4-hydroxy-3-methoxy-, 2,3-dimethyl 5-methoxyphenol, propylparaben, dimethylchrysin (flavones, 5,7-dimethoxy-) and coumarin-6-ol, 3,4-dihydro-4,4-dimethyl-7-nitro-. Nevertheless, as in DN, a strong increase in the *p*-hydroxybenzoic acid proportion (2.33 times) was noted; its percentage reached 55.19% (Fig. 4b).

In contrast, in the resistant cultivar TK, the proportion of *p*-hydroxybenzoic acid decreased; it was estimated to compose 85.67% in the roots of cultivars collected from uninfected palm groves and decreased to 23.45% in fields infested with *F.o.a*., a 3.5-fold decrease. A decrease in the number of compounds was also noticed; 14 were detected in ground roots that represented the only unscathed samples from five fields infested with the pathogen (Fig. 5b).

3.2 Quantification of Lignins

We quantitatively assayed the accumulation of lignins and phenolic polymers using derivatization with thioglycolic acid from CWR of date palm root tissue. UV spectra of solutions

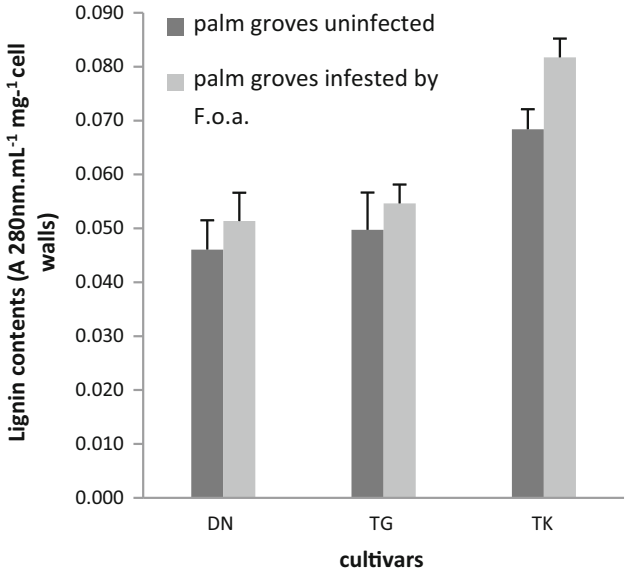


Fig. 6 Lignin contents in date palm roots of susceptible (DN, TG) and resistant (TK) cultivars from palm groves uninfected and infested by *F.o.a.* Means ± SE, $n = 5$

containing lignin thioglycolate (LGTA) from 10 mg CWR in 0.5 M NaOH were obtained from uninfected and infected roots, and the absorbance was measured at the characteristic λ_{max} of 280 nm. As shown in Fig. 6, lignin content was approximately equal for all cultivars in uninfected palm groves. In infested palm groves, lignin content increased in all analyzed cultivars. However, the maximum levels accumulated in the roots of resistant cultivars, not the sensitive cultivars.

4 Discussion

A comparison of the phenolic profiles of the susceptible cultivars DN and TG to that of the resistant cultivar TK reveals that the root cell walls of this last variety are richer in phenolic compounds, both qualitatively and quantitatively. We think that this characteristic is implied in the resistance. Indeed, it is known that phenolic acids play a very significant role in the reinforcement of cell walls. The solubilization of phenolic compounds in cell walls can modify their mechanical properties by decreasing their extensibility [20–22]; consequently, the wall is less biodegradable [20]. Therefore, the abundance of phenols in the cell wall makes polysaccharides less sensitive to the cell wall-degrading enzymes of pathogens [23].

In the susceptible cultivars with bayoud symptoms, we noted a qualitative and quantitative increase in phenolic compounds related to the root cell wall. The level of *p*-hydroxybenzoic acid content remarkably increased in DN and TG. Infection of the susceptible cultivars by *F.o.a.* caused an overproduction of this molecule and a diversification of the phenolic profiles. Thus, infection by *F.o.a.* likely stimulates the production of defense compounds, which are constitutively present in the resistant cultivar.

For this reason, it can be expected that changes in the phenolic compound metabolism induced by a pathological process are related to changes in hydroxybenzoic acid content. It has been demonstrated in many plants that the interaction between the pathogen and host plant frequently results in the accumulation of various hydroxybenzoic acids [24,25]. Numerous studies have highlighted the major role of *p*-hydroxybenzoic acid in defense mechanisms [26]. Significant accumulation of *p*-hydroxybenzoic acid and its derivatives in two susceptible Algerian cultivars was observed as a response to the presence of pathogens.

Some studies [27] have shown that the experimental inoculation of date palm roots by *F.o.a.* induced the accumulation of this molecule in the walls of the resistant Moroccan cultivar "Bousthami Noir." In cellular carrot cultures, *p*−hydroxybenzoic acid accumulated in the cell walls after elicitation by *Pythium aphanidermatum* [28]. *P*-hydroxybenzoic acid was synthesized in the cytosol and accumulated in the wall during pathogen attack [29]. Its accumulation is associated with antimicrobial activity [30], and it has an antifungal role [31]. Thomas-Barberan and Clifford [32] consider this molecule as antioxidant. Salicylic acid, which is an analog of *p*-hydroxybenzoic acid, is implicated in the defense response as a signal molecule that has been confirmed in many species [33]. Aldehydic derivatives of *p*-hydroxybenzoic acid present *in vitro* antifungal action against *Botrytis cinerea, Penicillium digitatum, Sclerotinia sclerotiorum, Fusarium oxysporum* and *Alternaria* sp., respective pathogenic agents of vine, orange tree, lettuce, cucumber and artichoke [34]. Compared to other hydroxybenzoic acids, *p*-hydroxybenzoic acid gives the clearest response to pathogens. It is possible that the predominant formation of *p*-hydroxybenzoic acid rather than other hydroxybenzoic acids as a cell response to pathogens is related to the salient features of phenolic compound biosynthesis because *p*-hydroxybenzoic acid is one of the first products of this biosynthesis [35]. Specifically, *p*-hydroxybenzoic acid differs from all other phenolic compounds because from the early stages of protective response, its content represents the most significant index of the fungus–plant interaction. This point of view is supported by the results obtained in the present experiments (Table 2).

The resistant cultivar had an opposite reaction, which we previously described in the sensitive cultivars. We observed a significant decrease in *p*-hydroxybenzoic acid in the roots of trees in the infected palm groves, but lignin content increased. Figure 3 clearly shows that lignin content increased in all studied cultivars. However, the maximum content accumulated in the resistant cultivar roots compared to the susceptible cultivars.

Lignin results from the polymerization of the monomer units *p*-coumaryl, coniferyl and sinapyl alcohols to give *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units in various ratios [34]. The abundance of these monolignol residues in lignin varies, and the defense lignin synthesized at infection differs in composition, adding an antimicrobial function to the structure. Lignin is a polymer that forms a structural barrier in root tissue [35]. Finally, the *p*-hydroxybenzoic acid present in abundance in the cell walls of the resistant cultivar likely lends the cell walls a mechanical constitutive resistance. It could also exert a chemical defense through its antifungal activity; however, its accumulation in the sensitive cultivars as a response to pathogen did not lead to blockage of the parasite progression or to an effective resistance.

In conclusion, this study provides more information about the involvement of aromatic secondary metabolism in the cell wall of Algerian date palm cultivars against *F.o.a*. In addition, it clearly indicates that much more work is required to unravel the complex interplay between the poorly defined "soluble" and "cell wall" compartments and to elucidate the actual mechanisms of pathogen defense at the biochemical level.

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