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# Effect of jasmonic acid on the induction of polyphenoloxidase and peroxidase activities in relation to date palm resistance against *Fusarium oxysporum* f. sp. *albedinis*

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ABSTRACT

Bayoud, caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa), is the most damaging disease of date palm in Morocco. In the present study we have investigated the effect of jasmonic acid (JA) on two defence-related enzymes, namely peroxidases (POX) and polyphenoloxidases (PPO) in date palm seedlings root. Our data show that exogenous application of JA at a concentration of 50 μM increased the activity of both enzymes. The increase of POX activity in the presence of JA was much more important than that observed following infection with the pathogen. As compared to untreated plants, PPO activity was 2.2 and 1.3 times higher in BSTN and JHL cultivars respectively. In addition, PAGE analysis revealed increased band intensity of the major constitutive isoforms of POX and PPO in both JA-treated and Foa-treated seedlings. Close examination of symptomatic and asymptomatic plants showed that root tissues of symptomatic plants were massively colonized by Foa. Also, disease development in these plants appeared to involve a marked degradation of the host cell walls early during the process of pathogen invasion. In contrast, the presence of Foa in asymptomatic plants induced limited necrotic lesions (hypersensitive-reaction like lesions) that were probably involved in reducing the progression of the pathogen. Together, our findings indicate that JA is capable of enhancing date palm root resistance to infection by Foa *via* the activation of defence-related enzymes such as PPO and POX.



## 1. Introduction

The main disease of date palm in Morocco is a vascular fusariosis (Bayoud) caused by *Fusarium oxysporum* f. sp. *albedinis* (Foa). Although many strategies for controlling this Fusarium wilt have been introduced [1–4], considerable losses still occur. A promising approach for minimizing the severity of diseases is based on the induction of systemic resistance using localized pre-treatment with elicitors in order to enhance resistance to pathogen infection [5]. Jasmonic acid (JA) is a natural phytohormone involved in many processes during plant development [6]. It has also been shown that JA is involved in the signaling pathway that mediates defence responses to abiotic and biotic stress including defence against pathogens and insects [6,7]. JA has been demonstrated to upregulate the expression of defence-related genes as well as the accumulation of defence-related proteins and metabolites [8–10]. In barley and tomato, Bücking et al. [11] reported that exogenous application of JA leads to the expression of JA-inducible genes indicating that the perception is completed by JA signal transduction.

The perception of elicitors by plants involves the expression of a coordinated series of biochemical changes leading to the induction of defence responses. These include the accumulation of active oxygen species; the synthesis of pathogenesis-related proteins and phytoalexins, and the activation of various defence-related enzymes [12]. Among these, peroxidases (POX) are oxido-reductase enzymes that participate to cell wall reinforcement thereby limiting fungal penetration [13]. Polyphenoloxidases (PPO) are copper metaloproteins that contribute to plant cell defence by catalyzing the oxidation of phenolics and their conversion into antimicrobial quinones. These compounds are highly reactive, modifying and cross-linking a variety of cellular constituents [14]. In date palm, we have previously shown that these enzymes are involved in resistance of date plam plants to infection by Foa [2–4,15].

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The aim of this study was to determine the effects of pre-treatment of date palm seedlings with JA on the development of Bayoud disease and to assess its involvement in the induction of defence-related enzymes. To this end, we examined the effect of JA on peroxidase and polyphenoloxidase activities as well as on their isoenzyme patterns in the roots of two date palm cultivars, namely BSTN, (resistant to Foa) and JHL (susceptible to Foa). We focused on these enzymes based on previous studies in which they were shown to be involved in resistance to Foa [2–4,15].

## 2. Materials and methods

### 2.1. Plant and fungal materials and inoculation technique

The aggressive (ZAG) *F. oxysporum* f. sp. *albedinis* isolate was isolated from naturally-diseased date palm tissues originating from Zagora, Morocco. The aggressiveness of this isolate was previously and regularly tested on seedlings of resistant and susceptible cultivars [16]. Fungal culture was routinely conducted in darkness on Potato Dextrose Agar (PDA) medium at 25 ± 2 °C.

Date palm seedlings obtained from seeds of two cultivars "Bousthami noir" (BSTN, resistant) and "Jihel" (JHL, susceptible) were used in this study. They were cultivated in plastic containers filled with a mixture of sterile sand and peat in greenhouse under 16 h light regime and 60–70% relative humidity at 25 ± 2 °C.

Seedlings were inoculated at the 2–3 leaves stage (4–6 months old) by micro-injecting into roots 10 μl of conidial suspension ($10^6$ spores/ml) of Foa isolate ZAG. For JA treatment, roots were injected with 10 μl of jasmonic acid solution prepared at a final concentration of 50 μM. JA was dissolved in methanol and completed with distilled water to obtain the concentration used. Control plants were inoculated with the same solution as above without JA. The seedlings were incubated in the same conditions as for culture and sampled at various times for further study.

### 2.2. Tissue processing for light and electron microscopy

These processes were carried out as previously described by Verdeil et al. [17].

#### 2.2.1. Light microscopy

Tissues were fixed for 48 h using 10% paraformaldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were dehydrated through a graded alcohol series (50°, 70° and 95°) and impregnated in methyl methacrylate; each sample was embedded in polymethylacrylate, LKB Historesin (Leica Rueil-Malmaison, France). Polymerization was performed at 37 °C for 24 h.

The 3 μm thick sections were obtained using a microtome (Historange, LKB) and were double-stained with Periodic Acid-Schiff (PAS) reagent, combined with protein-specific naphtol blue-black (NBB) [18]. PAS stains starch reserves and cell walls in pink and NBB specifically stains soluble or reserve proteins in dark blue.

#### 2.2.2. Electron microscopy

Root fragments (≈1 $mm^3$) were fixed by immersion in 3% (v/v) glutaraldehyde in 0.1 M pH 7.2 cacodylate buffer for 12 h at room temperature. After washing in cacodylate buffer three times (1 h each), samples were post fixed for 2 h at room temperature in darkness in 1% OsO4 prepared in cacodylate buffer. The samples were dehydrated through a graded ethanol series (70°, 95° and 2 times 100°) for 20 min each; following by infiltration and embedding in Spurr's low viscosity epoxy resin [19].

Ultra thin sections (80–100 nm) were obtained using a Reichert-ultracut S microtome with a diamond knife. They were contrasted with 2% uranyl acetate for 30 min, followed by lead citrate for 30 min in darkness for direct examination in transmission electron microscope.

### 2.3. Enzymes extraction and activity assays

Seedling roots (200 mg F.W.) were homogenized in 1 ml of Tris–maleate buffer pH 6.5 (0.1 M) containing Triton x-100 (0.1 g/l). After centrifugation at 10 000 *g* for 15 min, the supernatant was used for enzymatic activities determination. Peroxidase (POX) activity was assayed by measuring the oxidation of guaiacol at 470 nm. Twenty microliters of enzyme extract was added to 2 ml of reaction mixture consisting of a solution of 0.1 M Tris–maleate buffer (pH 6.5), 25 mM guaiacol and 25 mM $H_2O_2$. Polyphenoloxidase activity was determined by measuring oxidation of 0.2 M catechol at 410 nm in 0.1 M sodium phosphate buffer (pH 6). Enzymatic activities were expressed as enzymatic unit $g^{-1}$ FW.

Electrophoretic separation of POX and PPO isoenzymes was performed by SDS-PAGE using a vertical mini-gel electrophoresis unit as described by Baaziz [20] and Jaiti [21]. The resolving gel was 10% and the stacking gel 5% (w/v). The electrode buffer was Tris (0.025 M)–glycine (0.129 M)-SDS (0.1%) pH 8.3. Each gel slot was loaded with 12 μg (POX) and 30 μg (PPO) proteins samples. Electrophoresis was performed at a constant voltage of 100 V.

For POX staining, the gel was incubated for 15 min in 100 ml of 0.1 M sodium acetate buffer pH 5 containing 0.1 g of benzidine and 0.1 ml 10% hydrogen peroxide.

Staining mixture for PPO consisted of 100 ml of 0.1 M sodium acetate buffer pH 5 containing 0.1 g of DL-1,3-dihydroxy phenylalanine and 0.1 g of catechol. Gels were incubated with the substrates for 30 min in dark until dark bands appeared.

## 3. Results

Inoculation of date palm seedlings with Foa led to disease symptoms that could be observed after 6–8 days of incubation. Symptomatic seedlings showed diffused wet necrosis in root tissues while asymptomatic plants developed a limited necrotic lesion around the inoculation site. In both cultivars (BSTN and JHL), seedlings treated with JA developed as in the case of Foa treatment a limited necrotic lesion around the application site after the first week of incubation. These lesions were similar to those observed in Foa resistant seedlings.

### 3.1. Histology of infection

In the case of susceptible seedlings of the JHL cultivar, observations of cross-sections of root samples inoculated with Foa, showed that all cortex tissues were colonized after one week (Fig. 1B and C). Close examination of infected root ultrastructure showed that the colonization was accompanied by a marked wall modification including primary wall alteration and middle lamella dissolution (Fig. 2B) as compared to the preserved cell-wall architecture in untreated roots (Fig. 2A). The host cells underwent a complete degradation; but the pathogen hyphae did not exhibit any apparent disorganization (Fig. 2C). The observed disruption of root cells coincided with the occurrence of macroscopically visible symptoms (e.g. an important softening of root tissues) leading to plant death.

In resistant seedlings of cultivar BSTN, cortical parenchyma cells exhibited marked structural modifications including the formation of multitextured wall appositions and intracellular plugging (Fig. 2D). Some parenchyma cells both in the cortex and in vascular stele were characterized by a coating of secondary walls with a band of electron-opaque material that forms osmiophilic droplets and apposition of amorphous material (Fig. 2E and F). Another host

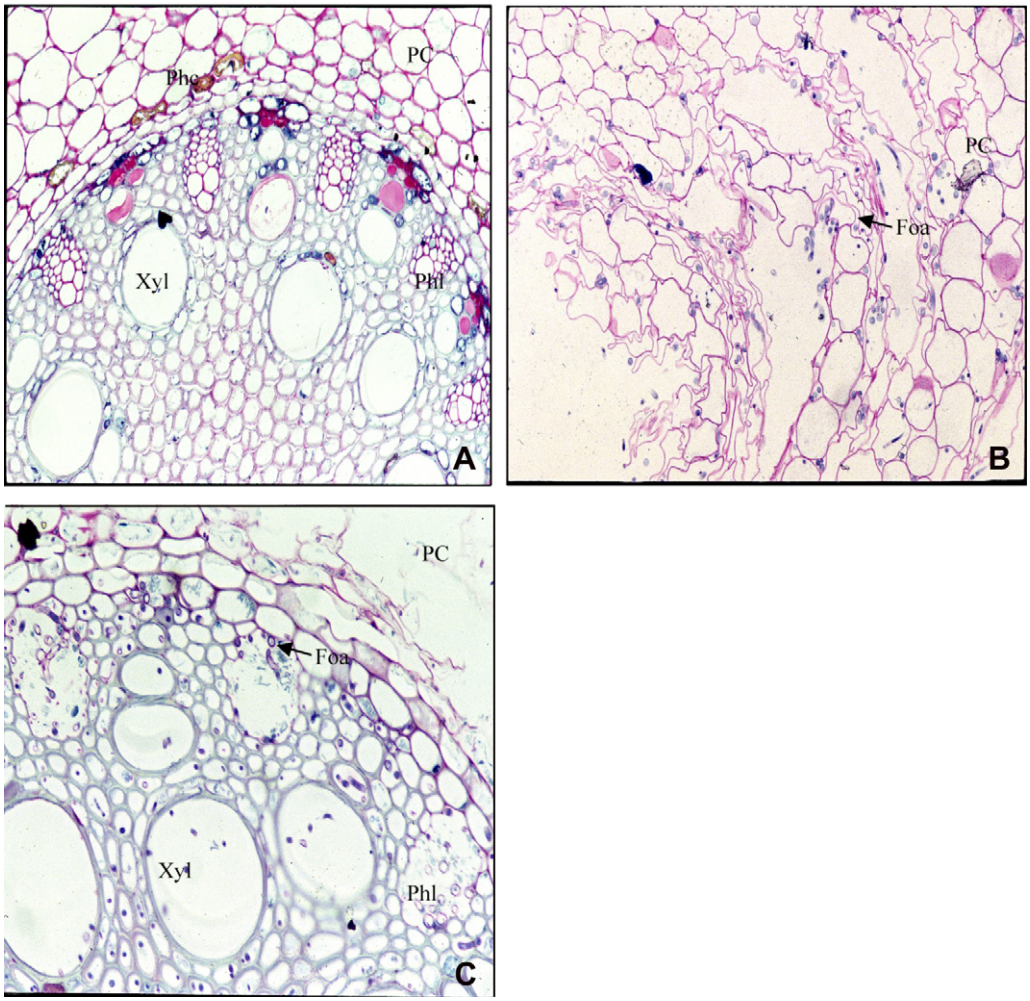


**Fig. 1.** Light micrographs of samples from date palm root tissues infected by *Fusarium oxysporum* f. sp. *albedinis* (Foa). A: control roots (20×). B: 8 days after inoculation, with the fungus; note that all parts of the root have been invaded. Plasmolysis is visible throughout the cortex (20×). C: General view of a root 2 weeks after inoculation, the cortical tissues and the phloem were hydrolyzed, xylem was more resistant to degradation (40×). Xyl: xylem vessels, Phl: Phloem tubes, PC: parencymatous cells, Phc: Phenolic-storing cells.

reaction pertained to the presence of phenolic-storing cells that were observed in 2–3 parenchyma cells layers adjacent to vascular stele and in vascular parenchyma (Fig. 2G).

### 3.2. Effect of JA treatment on disease resistance

Exogenous application of JA enhanced resistance of date palm to Foa. Indeed, in the absence of JA treatment, 30% of the seedlings of the resistant cultivar BSTN and 72% of the susceptible JHL died four weeks after Foa inoculation. In contrast, pre-treatment with JA three weeks before Foa infection, led to a reduction of plant mortality by 17 and 40% in cultivars BSTN and JHL, respectively. Treatment of date palm seedlings by JA at a concentration of 50 μM led to the mortality of a 5–6% of seedlings (Table 1).

### 3.3. Enzyme activities

Enzyme assay experiments showed that treatment of date palm seedlings with either Foa or JA increased POX activity. The activity of POX reached a maximal value 48 days after treatment with both agents. At this time, no significant difference was observed between the two tested cultivars. In the cultivar BSTN, the POX activity value was 2532 ± 79 U/g FW in JA-treated seedlings, 2233 ± 99 U/g FW in Foa-infected seedlings and only 1108 ± 63 U/g FW in untreated seedlings.

SDS-PAGE analysis of POX isoforms confirmed these results and revealed that the POX patterns in BSTN and JHL cultivars were similar. These patterns present the expression of a relevant number of isoenzymes ranging from 0.052 to 0.812 Rm value (Fig. 3). In asymptomatic Foa-infected or JA-treated plants, POX isoenzymes with Rm values ranging from 0.416 to 0.562 accumulated significantly. In addition, in the same samples the POX pattern showed a low induction of three other isoenzymes with Rm value 0.125, 0.145 and 0.208 as compared to untreated samples (Fig. 3). In plants showing disease symptoms, activity stain of POX isoenzymes declined until a complete loss of POX activity in plants softening roots (Fig. 3).

PPO activity increased following Foa infection or JA treatment. Fig. 4 shows that JA treatment hastened the rise in PPO activity from the 12th days in both cultivars, whereas Foa infection did not enhance PPO activity until days 24. At that moment, in Foa-infected roots, the PPO activity was 2.2 and 1.3 times higher than in untreated roots of cultivars BSTN and JHL, respectively.

Electrophoretic analysis and staining of PPO activity of BSTN and JHL roots samples revealed the same pattern with isoenzymes ranging from 0.06 to 0.8 Rm values (Fig. 5). Neither Foa infection nor JA treatment induced qualitative differences as compared to

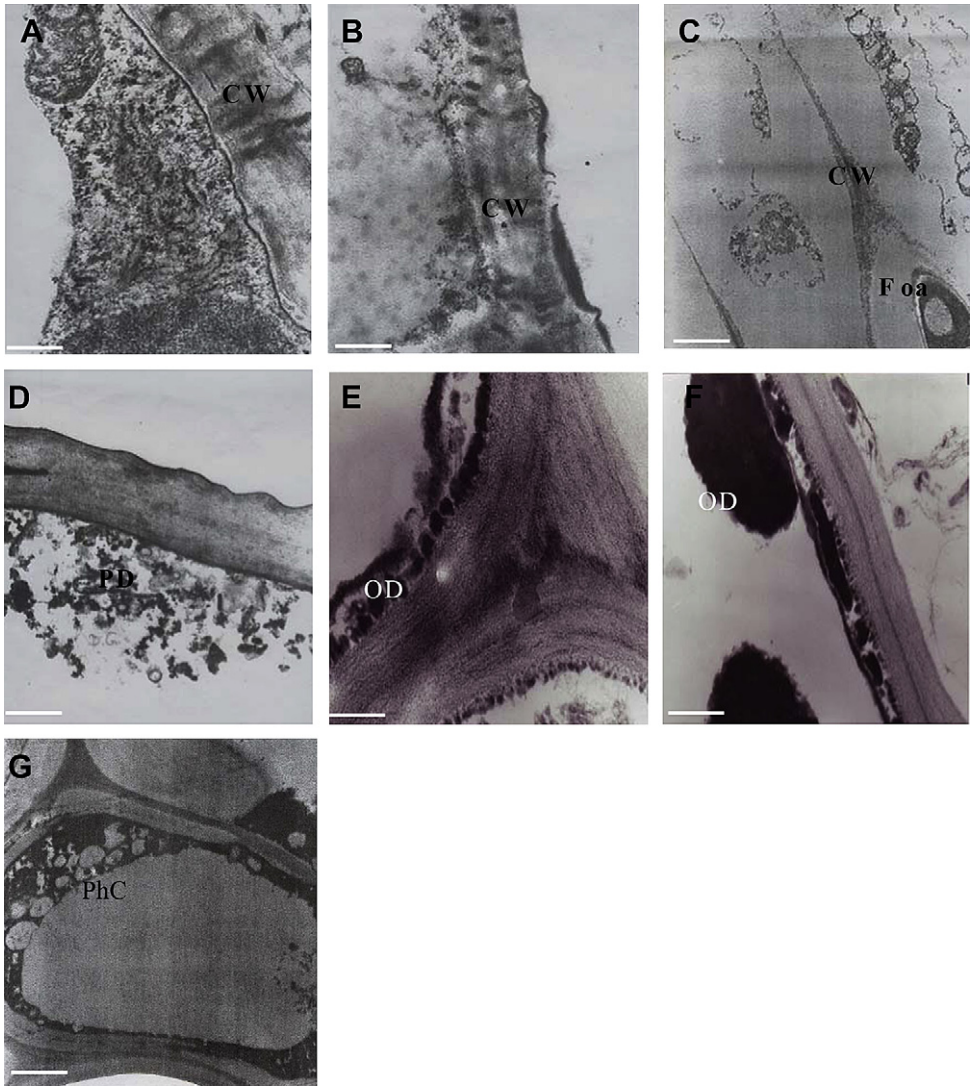


**Fig. 2.** Transmission electron micrographs of samples from date palm root tissues infected by *Fusarium oxysporum* f. sp. *albedinis* (Foa). A: Preserved cell wall (CW) in control (non-infected plants). B: Root tissues colonization is associated with primary wall alteration and middle lamella dissolution. C: Complete disruption of the cell wall as well as cytoplasm alteration and presence of undamaged fungal hyphae. D: Polymorphic deposits (PD) in parenchyma cells. E–F: 2 weeks after inoculation, parenchyma cells in cortex show retraction of plasmalemma and accumulation of electron dense material along the tonoplast vessels, a coating material accumulates along the secondary walls extends to form osmiophilic droplets (OD). G: Phenolic-storing cell (PhC) observed 1 week after inoculation, around and in vascular stele of root. Scale bars represent 1 μm (G plate); 2 μm (C plate); 100 nm (E plate); 200 nm (A, B, F plates) and 500 nm (D plate).

untreated samples. However, they enhanced the activity of isoenzymes with Rm value 0.7 and 0.75 after 2 weeks of incubation. PPO activity staining decreased in all isoforms in the case of symptomatic plants (Fig. 5).

**Table 1**
The mortality of date palm seedlings from two cultivars Bousthami noir (BSTN, resistant) and Jihel (JHL, susceptible), after their treatment with water (control), jasmonic acid (JA, 50 μM), pathogen (Foa) or pre-treated three weeks with jasmonic acid and then challenged with pathogen.

| Treatment | Mortality (%) | |
|---|---|---|
| | BSTN | JHL |
| Control | 0 | 0 |
| JA-treated plants | 5 | 6 |
| Foa-infected plants | 30 | 72 |
| JA-treated plants challenged with Foa | 16.6 | 40 |

Values are the average of three independent replicates with 40 plants tested per replicate (SE $\leq$ 5%).

## 4. Discussion

The present study shows that JA treatment leads to the enhancement of date palm seedlings resistance to Bayoud disease. To our knowledge, this is the first report of beneficial effects of JA on date palm resistance. Similar enhanced disease resistance induced by JA was shown in melon seedlings against the soil-borne pathogens, *Didymella bryoniae*, *Sclerotinia sclerotiorum* and *F. oxysporum* f. sp. *melonis* [22]. It has also been shown that application of exogenous methyl jasmonate (MeJA) to *Arabidopsis thaliana* mutants deficient in the production of jasmonates enhanced their protection against root rot disease caused by *Pythium mastophorum* [23]. Similarly, application of MeJA to wheat (*Triticum aestivum* L.) seedlings after inoculation with *Tilletia laevis Kühn* induced the accumulation of transcripts encoding several defence-related proteins and reduced common bunt infection [24]. Treatment of tobacco transgenic plants, which had reduced content of monogalactosyl diacylglycerol (MGCG) with MeJA restored resistance to

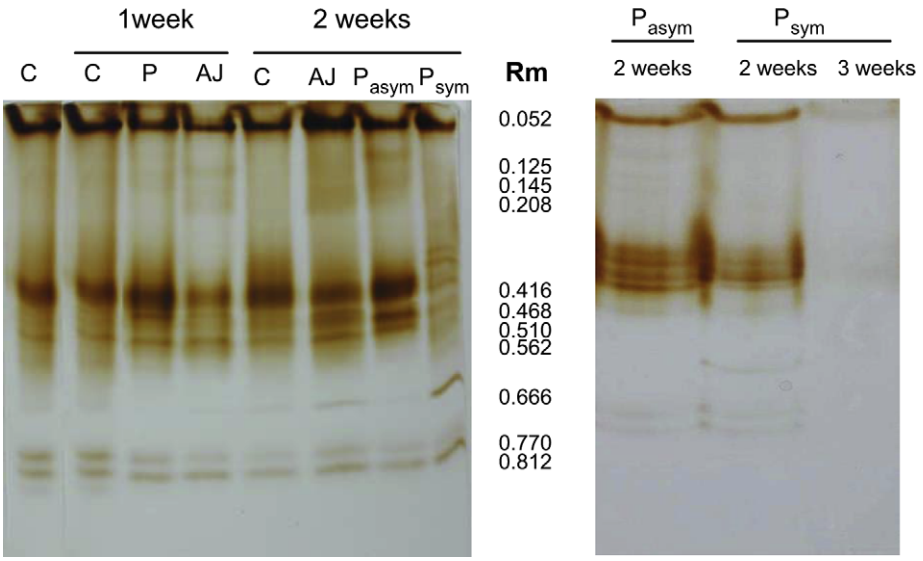


**Fig. 3.** Active staining of peroxidases in date palm roots. (C) Control, (AJ) JA-treated plants, (P) Plants infected by pathogen, with ($P_{asym}$) or without ($P_{sym}$) symptom disease, (Rm) relative electrophoretic mobility.

*Helicoverpa armigera* and expression of hydroperoxide lyase and proteinase inhibitor (PI-I and PI-II) suggesting that MGDG plays important roles as source of C18:3 and C16:3 in JA biosynthesis and JA-mediated defence responses to insect herbivores in tobacco [9]. In date palm, we have demonstrated that treatment of seedling with JA increased the content of $H_2O_2$ and enhanced lipid peroxidation, two defence responses that are involved in date palm resistance to Bayoud disease [2].

Jasmonates affect many physiological processes including those regulating resistance mechanisms [25]. The roles of these molecules in the activation of plant defence response are suggested by their ability when applied exogenously to enhance resistance to subsequent challenge with specific pathogen [8,22,23] and to activate genes encoding defence proteins [10]. A close relationship was found between resistance in date palm against Foa and the activation of POX and PPO enzymes. Furthermore, the resistance induced by JA on date palm seedlings is associated with increased POX and PPO activities.

In JA-treated and Foa-infected plants, a low accumulation of several new isoforms of POX were induced and the major constitutive isoperoxidases (Rm: 0.416–0.562) were found at their highest levels. These isoforms may play an important role in date palm resistance. The increase in POX activity has been observed in many plant-pathogenic fungal interactions [26–28] and during plant-microbe/elicitor interactions [29,30]. In date palm, El Hassni et al. [4,15] have shown that the enhancement of resistance by seedlings treatment with a hypoaggressive *F. oxysporum* isolate was correlated with an increase in POX activity. Also, we showed earlier that the effectiveness of arbuscular mycorrhizal fungi in protecting date palm seedlings against Bayoud disease was correlated with induction of POX activity [3]. The stimulation of POX activity can be obtained in resistant and susceptible plants to Foa. However, the difference is related to the precocity and the importance of response of these plants to the infection [2]. Acidic POX are often very active in date palm. Their role in protecting the plant against Bayoud is related to their cross-linking activities within the cell wall (formation of lignin, extension cross-link, dityrosine bonds) and create a highly toxic environment by massively producing reactive oxygen species (oxidative burst), which results in adverse growth conditions for the pathogen [13].

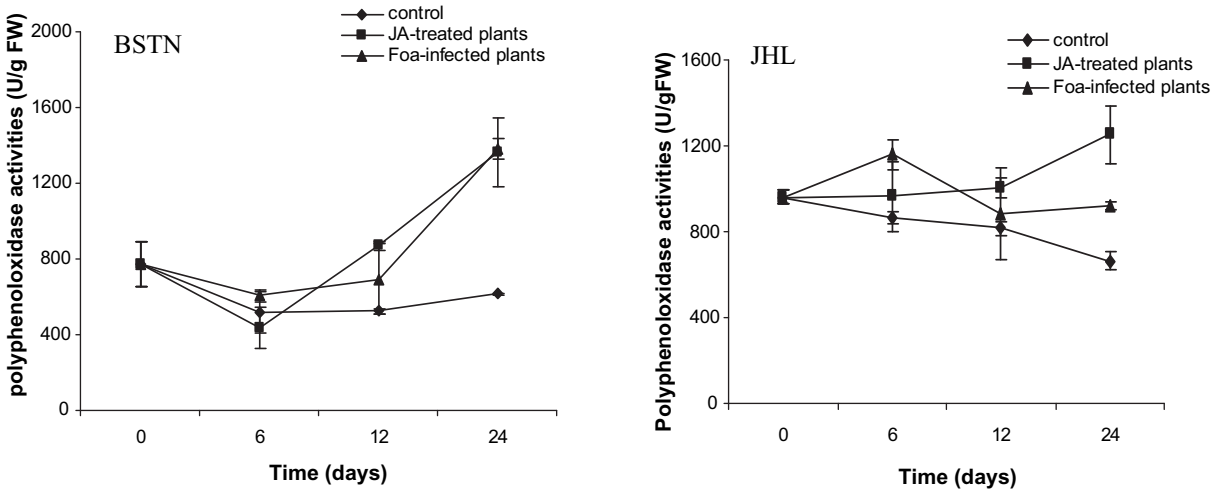


**Fig. 4.** Polyphenoloxidase activities in root seedlings of two cultivars of date palm, Bousthami noir (BSTN, resistant) and Jihel (JHL, susceptible), after their treatment with water (control), Jasmonic acid (JA, 50 μM) or Pathogen (Foa). Data are means of three independent experiments (mean ± SE).

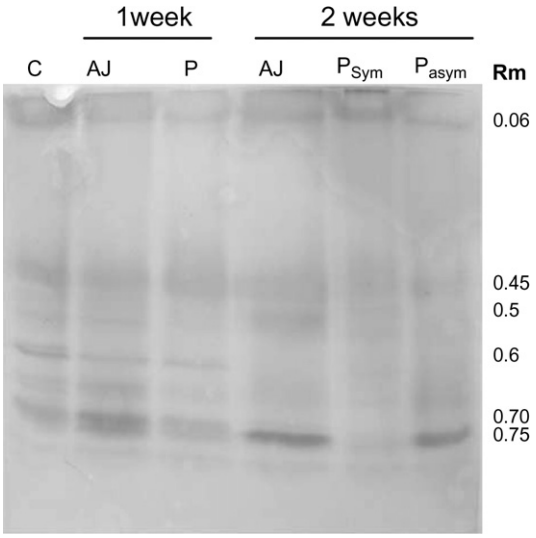


**Fig. 5.** Active staining of polyphenoloxidases in date palm roots. (C) Control, (AJ) JA-treated plants, (P) Plants infected by pathogen, with ($P_{asym}$) or without ($P_{sym}$) symptom disease, (Rm) relative electrophoretic mobility.

PPO activity increased in Foa-infected and JA-treated plants but earlier in the latest treatment. During the first days (before 12 days), there was no significant difference in PPO activity among treatments. This may be explained by the fact that PPO are compartmentalized in plant cells but as a result of pathogenic attack, membrane disruption may occur initiating the exposure of vacuole phenolics to lumenall PPO [31]. In Foa-infected roots of date palm, El Hassni et al. [4] have shown that PPO activity was present in the plastids of parenchyma cells or revealed as brown deposits within the cell walls and in the cytoplasm of browning tissues. Recently, Koussevitzky et al. [32] reported that MeJA increased markedly the ability of tomato plastids to import and process PPO precursors. PPO activity is reported to be induced by wounding and by pathogens in different plant species such as pepper [33], wheat [34] and tomato [35] and also by MeJA treatment [36].

POX and PPO contribute to the formation of defence barriers for reinforcing the cell structure [37]. In our pathosystem, we have shown that in symptomatic plants, which present the lowest enzyme activities, the disease development is associated with a degradation of the host cell walls. This degradation is a result of a loosening of the host cell-wall compounds, demonstrating that Foa secrete cell-wall degrading enzymes. In tomato, the infection of plants with *F. oxysporum* f. sp. *radicis-lycopersici* was accompanied by severe host cell wall alterations [38]. More recently, Kang and Buchenauer [39] reported that *Fusarium culmorum* causes a disintegration of cellulose, xylan and pectin in the host cell wall. During infection of onion with *Sclerotium cepivorum*, the production of polygalacturonases and pectinases was shown to be associated with cell wall degradation and cortex dissolving [40]. Similar results have also been reported in the compatible interaction between carnation callus and *F. oxysporum* [41].

In asymptomatic plants, showing the highest POX and PPO activities, the cell wall remained apparently well preserved. This observation suggests that the hydrolytic enzymes produced by the fungus are not sufficient to induce a full hydrolysis of the main wall compounds or because the cell walls were reinforced by other substances induced upon the pathogen attack. Support for this hypothesis is provided by the accumulation of osmiophilic electron dense substances in host cell wall and plugging cytoplasm. They were never observed in cell wall of susceptible plants. This typical host response was reported in many plant/fungal interactions such as tomato/*Pythium oligandurum* [42]; *A. thaliana*/*Pseudomonas syringae* [43]; carrot/*Phytium violae* [44], melon/*Podosphaera fusca* [45] and ginseng/*Fusarium equiseti* [46]. The nature of this material remains to be determined. Its reactivity to osmium tetraoxide indicates that these newly synthesized compounds are possibly of phenolic nature [44]. We have previously reported an increase in phenolic compounds in date palm roots especially the accumulation of non-constitutive hydroxycinnamic acid derivatives in response to infection by Foa [4,47–49].

To summarize, this study showed that JA is able to protect date palm seedlings against Bayoud disease through, at least in part, the induction of peroxidase and polyphenoloxidase activities. Further investigations on the transcriptional expression of the genes encoding these enzymes after elicitation with JA are in progress. A quantification of the endogenous jasmonic acid is also needed to clarify its implication in the signaling pathways during date palm resistance to Bayoud.

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## References

[1] Louvet J. Que devons nous faire pour lutter contre le Bayoud? In: Physiologie des arbres et Arbustes en zones arides et semi-arides. Paris, France: Groupe d'Etude de l'Arbre; 1991. p. 337–46.
[2] Jaiti F, Dihazi A, EL Hadrami A, El Hassni M, El Hadrami I. Effect of exogenous application of jasmonic acid on date palm defense reaction against *Fusarium oxysporum* f. sp. *albedinis*. Phytopathol Mediterr 2004;43:325–31.
[3] Jaiti F, Meddich A, El Hadrami I. Effectiveness of arbuscular mycorrhizal fungi in the protection of date palm (*Phoenix dactylifera* L.) against bayoud disease. Physiol Mol Plant Pathol 2007;71:166–73.
[4] El Hassni M, Jaiti F, Dihazi A, Ait Barka S, Daayf F, El Hadrami I. Enhancement of defence responses against bayoud disease by treatment of date palm seedling with a hypoaggressive *Fusarium oxysporum* isolates. J Phytopathol 2004;152:1–8.
[5] Hammerschmidt R, Métraux JP, Van Loon LC. Inducing resistance: a summary of papers at the first international symposium on induced resistance to plant diseases, Corfu, May 2000. Eur J Plant Pathol 2001;107:1–6.
[6] Creelman RA, Mullet JE. Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 1997;48:355–81.
[7] Howe GA, Browse J. New weapons and a rapid response against insect attack. Plant Physiol 2008;146:832–8.
[8] Ueeda M, Kubota, Nishi K. Contribution of jasmonic acid to resistance against Phytophthora blight in *Capsicum annuum* cv. SCM334. Physiol Mol Plant Pathol 2006;67:149–54.
[9] Wang J. Monogalactosyl diacylglycerol deficiency affects jasmonic acid biosynthesis and defense responses to insect herbivores in *Nicotiana tobacum*. Plant Sci 2008. doi:10.1016/j.plantsci.2008.11.003.
[10] Cheong JJ, Choi YD. Methyl jasmonate as a vital substance in plants. Trends Gene 2003;19:409–13.
[11] Bücking H, Förster H, Stenzel I, Miersch O, Hause B. Applied jasmonates accumulate extracellularly in tomato, but intracellularly in barley. FEBS Lett 2004;562:45–50.
[12] Nürnberger T, Lipka V. Non-host resistance in plants: new insights into an old phenomenon. Mol Plant Pathol 2005;6:335–45.
[13] Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep 2005;24:255–65.
[14] Mayer AM, Harel E. Phenoloxidases and their significance in fruit and vegetables. In: Fox PF, editor. Food enzymology, vol. 1. New York: Elsevier; 1991. p. 373–98.
[15] El Hassni M, Verdeil JL, El Hadrami I. A hypoaggressive *Fusarium oxysporum* isolate induces polyphenoloxidase activity in the date palm seedlings allowing their protection against the bayoud disease. Plant Pathol J 2005;4:96–102.
[16] El Idrissi Tourane A, Oihabi A, El Hadrami I. Effet des polyamines et des inhibiteurs de leur biosynthèse sur la croissance mycelienne de *Fusarium oxysporum* f. sp. *albedinis* agent causal du bayoud. Rev Res Amelior Prod Agr Milieu Aride 1995;7:241–7.
[17] Verdeil JL, Hocher V, Huet C, Grosdemange F, Escoute J, Ferriere N, et al. Ultrastructural changes in coconut calli associated with the acquisition of embryogenic competence. Ann Bot 2001;88:9–18.
[18] Fisher DB. Protein staining of ribboned epon sections for light microscopy. Histochimie 1968;16:92–6.
[19] Spurr R. A low viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct 1969;26:31–43.

[20] Baaziz M. Contribution à l'étude des peroxydases du palmier dattier, *Phoenix dactilyfera* L. relation avec la résistance de la plante à la maladie du bayoud, fusariose vasculaire à *Fusarium oxysporum* f. sp. a lbedidnis. Thèse de Doctorat d'Etat. Université Cadi Ayyad, Faculté des Sciences-Semlalia, Marrakech, Maroc, 1990. 173p.
[21] Jaiti F. Culture d'anthères et d'embryons immatures chez le blé dur et tender (*Triticum durum* L. et *Triticum eastivum* L.): essais de la levée de la récalcitrance des variétés et caractérisation biochimique des potentialités morphogènes des tissus. Thèse de troisième cycle. Université Cadi Ayyad, Faculté des Sciences Semlalia, Marrakech, Maroc, 1997. p 140.
[22] Buzi A, Chilost G, Magro P. Induction of resistance in melon seedlings against soil-borne fungal pathogens by gaseous treatment with methyl jasmonate and ethylene. J Phytopathol 2004;152:491–7.
[23] Vijayan P, Shockey J, André Lévesque A, Cook RJ, Browse J. A role for jasmonates in pathogen defense of *Arabidopsis*. Proc Natl Acad Sci USA 1998;95:7209–14.
[24] Lu Z-X, Gaudet D, Puchalski B, Despins T, Frick M, Laroche A. Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defence-gene expression. Physiol Mol Plant Pathol 2006;67:138–48.
[25] Preston CA, Laue G, Baldwin I. Methyl jasmonates is blowing in the wind, but can it act as a plant–plant airborne signal? Biochem Syst Ecol 2001;29: 1007–23.
[26] Kruger WM, Szabo LJ, Zeyen RJ. Transcription of the defense response genes chitinase IIb, PAL and peroxidase is induced by the barley powdery mildew fungus and is only indirectly modulated by R genes. Physiol Mol Plant Pathol 2003;63:167–78.
[27] Cooper C, Isaac S, Jones MG, Crowther T, Smith BM, Collin HA. Morphological and biochemical response of carrots to *Pythium violae*, causative agent of cavity spot. Physiol Mol Plant Pathol 2004;64:27–35.
[28] Silva MC, Guerra-Guimarães L, Loureiro A, Nicole MR. Involvement of peroxidases in the coffee resistance to orange rust (*Hemileia vastatrix*). Physiol Mol Plant Pathol 2008;72:29–38.
[29] Bargabus RL, Zidack NK, Sherwood JE, Jacobsen BJ. Characterization of systemic resistance in sugar beet elicited by a non-pathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. Physiol Mol Plant Pathol 2002;61:289–98.
[30] Baysal Ö, Soylu EM, Soylu S. Induction of defence-related enzymes and resistance by the plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis* ssp. *Michiganensis*. Plant Pathol 2003;52:747–53.
[31] Steffens JC, Harel E, Hunt MD. Polyphenol oxidase. Differential response of the polyphenol oxidase F promoter to injuries and wound signals. Plant Physiol 1994;115:409–18.
[32] Koussevitzky S, Neeman E, Harel E. Import of polyphenol oxidase by chloroplasts is enhanced by methyl jasmonate. Planta 2004;219:412–9.
[33] Juang W-J, Jin Y-L, Kim Y-C, Kim K-Y, Park R-D, Kim T-H. Inoculation of *Paenibacillus illinoisensis* alleviates root mortality, activates of lignification-related enzymes, and induction of the isozymes in pepper plant infected by *Phytophthora capsici*. Biol Control 2004;30:645–52.
[34] Mohammadi M, Kazemi H. Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Sci 2002;162:491–8.
[35] Li L, Steffens JC. overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta 2002;215:239–47.
[36] Constabell CP, Bergey DR, Ryan CA. Systemin activates synthesis of wound-inducible tomato leaf polyphenoloxidase via the octadecanoid defense signaling pathway. Proc Natl Acad Sci USA 1995;92:407–11.
[37] Avdiushko SA, Ye XS, Kuć J. Detection of several enzymatic activities in leaf prints cucumber plant. Physiol Mol Plant Pathol 1993;42:441–54.
[38] Benhamou N, Belanger RR. Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. Plant Physiol 1998;118: 1203–12.
[39] Kang Z, Buchenauer H. Ultrastructural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium culmorum*. J Phytopathol 2000;148:263–75.
[40] Metcalf DA, Wilson CR. Histology of *Sclerotium cepivorum* infection of onion roots and the infection process. Plant Pathol 1999;48:445–52.
[41] Trillas MI, Cotxarrera L, Casanova E, Cortadellas N. Ultrastructural changes and localization of chitin and callose in compatible and incompatible interactions between carnation callus and *Fusarium oxysporum*. Physiol Mol Plant Pathol 2000;56:107–16.
[42] Rey P, Benhamou N, Wulff F, Trilly Y. Interactions between tomato (*Lycopersicon esculentum*) root tissues and the mycoparasite *Pythium oligandrum*. Physiol Mol Plant Pathol 1998;53:105–22.
[43] Soylu S. Accumulation of cell-wall bound phenolic compounds and phytoalexin in *Arabidopsis thaliana* leaves following inoculation with pathovars of *Pseudomonas syringae*. Plant Sci 2006;170:942–52.
[44] Guérin L, Benhamou N, Rouxel F. Ultrastructural and cytochemical investigations of pathogen development and host reactions in susceptible and partially-resistant carrot roots infected by *Pythium violae*, the major causal agent for cavity spot. Eur J Plant Pathol 1998;104:653–65.
[45] Romero D, Rivera ME, Cazorla FM, Codina JC, Fernández-Ortuño D, et al. Comparative histochemical analyses of oxidative burst and cell wall reinforcement incompatible and incompatible melon–powdery mildew (*Podosphaera fusca*) interactions. J Plant Physiol 2008;165:1895–905.
[46] Goswami RS, Punja ZK. Molecular and biochemical characterization of defense responses in ginseng (*Panax quinquefolius*) roots challenged with *Fusarium equiseti*. Physiol Mol Plant Pathol 2008;72:10–20.
[47] El Hadrami I. Infections racinaires localisées et rôle des dérivés hydroxicinnamiques dans la résistance du palmier dattier (*Phoenix dactylifera* L.) au *Fusarium oxysporum aledinis*, agent causal du bayoud. Polyhenols Actualités 2002;22:19–26.
[48] Daayf F, El Bellaj M, El Hassni M, Jaiti F, El Hadrami I. Elicitation of soluble phenolics in date palm (*Phoenix dactylifera*) callus by *Fusarium oxysporum* f. sp. *albedinis* culture medium. Environ Exp Bot 2003;49:41–7.
[49] Jaiti F, Kassami M, Meddich A, El Hadrami I. Effect of arbuscular mycorrhization on the accumulation of hydroxycinnamic acid derivatives in date palm seedlings challenged with *Fusarium oxysporum* f. sp. *albedinis*. J Phytopathol 2008;156:641–6.