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Resistance of Olive Tree to *Spilocaea oleagina* is Mediated by the Synthesis of Phenolic Compounds

AMAL ZINE EL AABIDINE, YVES BAISSAC†, ABDELMAJID MOUKHLI‡, CHRISTIAN JAY-ALLEMAND†, BOUCHAIB KHADARI¶ AND CHERKAOUI EL MODAFAR[1]

Laboratory of Biotechnology Valorisation and Protection of Agro-Resources, Faculty of Sciences and Technology Gueliz, University of Cadi Ayyad, F-40 000 Marrakech, Morocco
†Laboratory of Biochemistry and Plant Physiology, University of Montpellier II, F-34095 Montpellier Cedex 05, France
‡UR Plant Genetic Improvement, National Institute of Agronomic Research, F- 40 000 Marrakech, Morocco
¶INRA, UMR 1098, Développement et Amélioration des Plantes (DAP), Campus CIRAD TA A 96/03, Av. Agropolis, 34398 Montpellier Cedex 5, France
[1]Corresponding author's e-mail: elmodafar@fstg-marrakech.ac.ma; elmodafar@ucam.ac.ma

ABSTRACT

To understand the resistance of the olive tree to the leaf-spot disease caused by *Spilocaea oleagina*, the constitutive and postinfectional synthesis phenolic compounds of the leaves were analyzed by HPLC in 110 genotypes F1 (susceptible cultivar "*Picholine marocaine*" x resistant cultivar "*Picholine du Languedoc*") presenting of the differential behaviours to this disease (highly resistant, resistant, intermediate, susceptible & highly susceptible genotypes). The HPLC analysis distinguished 15 majors phenolic compounds according to their chromatographic and spectral characteristics into five phenolic families (hydroxycinnamic derivatives, flavonoids, verbascoside derivatives, tyrosol derivatives, oleuropein derivatives). No qualitative difference was observed between cultivars. Principal components analysis (PCA) highlighted three multifactorial components distinguishing the various genotypes according to their behaviour to the disease. These components were determined by the postinfectional contents of oleuropein and rutin and by the constitutive contents of tyrosol and its derivatives. The tyrosol and its derivatives were associated with constitutive resistance, whereas the oleuropein and rutin were associated with induced resistance. These results suggest that the activity ratio of the enzymes implied in various biosynthesis ways of these phenolic compounds and/or the expression rate of the corresponding genes would be at the origin of the resistance degree of olive tree to S. *oleagina*. © 2010 Friends Science Publishers

Key Words: Olive tree; *Spilocaea oleagina*; Resistance; Multifactorial phenolics; Morocco

INTRODUCTION

The olive tree is widely cultivated as major fruit species in Morocco constituting more 50% of the surface of the fruit trees) (Loussert & Brousse, 1978). It has a major socio-economical rule since it contributes to the maintenance of the rural populations (Boulouha *et al.,* 1992). The "*Picholine marocaine*" is the most dominant cultivar; more than 98% of the olive growing orchards are planted by this cultivar (Boulouha *et al.,* 1992). Despite of its adaptation to the Moroccan orchards, this cultivar is susceptible to the principal fungal diseases particularly to the leaf-spot disease caused by *Spilocaea oleagina,* which represents the most widespread fungal disease of olive tree in the world (Anton & Laborda, 1989). This disease is caused by *S. olegina*, specific biotrophe pathogen of olive tree presenting a sub-cuticular development (Gonzalez-Lamothe *et al.,* 2002). The disease appears by circular tasks on the leaves and the fruits leading to their fall and the general weakening of the olive tree (Sanchez *et al.,* 1998). The chemical treatment by fungicides containing copper (Cu) appears rarely effective, because of the appearance of resistant pathogen races to Cu and of the disturbance of the plant metabolism following Cu accumulation in the soil (Obanor, 2008). The genetic resistance represents currently the only effective mean to stop this disease (Anton & Laborda, 1989).

In order to introduce resistance to *S. oleagina* in the Moroccan cultivar, a cross made in 1993 by the National Institute of Agronomic Research of Marrakech between the susceptible cultivar "*Picholine marocaine*" and the resistant cultivar "*Picholine du Languedoc*", cultivar of French origin. The reliability of the hybrid population were verified using 35 microsatellite loci, which revealed the presence of only two illegitimate descendants among 220 analyzed (Charafi *et al.,* 2007). The evaluation of the responses of the descendants to *S. oleagina* showed a great variability of the behaviour, which varied from the absolute susceptibility until to the total resistance (Zine El Aabidine *et al.,* 2009). This variability in their resistance degree corresponded to a

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complete segregation, which offered a useful tool for research of QTL linked to *S. oleagina* resistance in olive tree (Charafi, 2007 & 2009; Zine El Aabidine *et al.,* 2008) and for the characterization of the defense mechanisms implied in this resistance.

The phenolic compounds constitute the molecules often implied in plant defence to pathogens and associated with the plant host resistance (El Modafar & El Boustani, 2005). A preliminary work undertaken on 11 cultivars of olive tree presenting differential behaviours to *S. oleagina* showed that the resistance was related to multifactorial components determined by the constitutive phenolic compounds. This work which makes continuation and supplements previous work (Rahioui *et al.,* 2009), aims to establish a potential correlation between the constitutive and the postinfectional phenolic compounds contents in 110 genotypes F1 (Picholine marocaine x Picholine du Languedoc) and the degree resistance of olive tree to *S. oleagina*.

MATERIALS AND METHODS

Plant and fungal materials and inoculation: The study was carried out with olive tree genotypes F1 issued from a crossing between a susceptible cultivar "*Picholine marocaine*" and a resistant cultivar "*Picholine du Languedoc*". These genotypes were planted in station of National Institute of Agronomic Research of Marrakech since 1993. Leaves of 110 genotypes presenting a differential behaviours to *S. oleagina* (highly resistant, resistant, intermediate, susceptible & highly susceptible genotypes) (Table I) were collected in February, period preceding the attack by the leaf-spot disease. The study related to constitutive and post-infectional phenolic compounds.

The inoculation was carried out by deposit of 10 µL droplets of conidial suspension of S. *oleagina* (titrated to 10^5 conida. mL^{-1}) on the upper surface of the leaves maintained in survival in glass Petri dishes containing Whatman filter paper according to the technique previously described (El Modafar *et al.*, 1995). The control plants were treated in the same way by replacing the suspension of spores by sterile distilled water. The leaves were incubated in the cold room at 15°C and the darkness for 3 days then in the culture room at 18°C under light (16 h/8 h; day/night period) intensity of 240 µmol m^{-2} s^{-1}. After five days of incubation, the leaves were freeze-dried. The results represent the means of five repetitions (20 leaves per repetition).

Extraction of phenolic compounds: Extraction of phenolic compounds was carried out as previously described (El Modafar *et al.,* 1996; Rahioui *et al.,* 2009). The freeze-dried leaves (25 mg) are crushed in 1.5 mL of methanol-water (4: 1, v/v) added with 10^{-4} M of D-glucoronique lactone acid to prevent the hydrolysis of the glucosidic fraction and 10^{-4} M of 5-méthoxyflavone used like internal standard. The extraction is carried out in a vat ultrasound for 20 min at 20°C. The extract was then centrifuged at 5000x*g* for 15 min and the supernatant obtained constitutes the phenolic extract.

Identification of phenolic compounds: The phenolic compounds were characterized according to the techniques previously described (El Modafar *et al.,* 1993; El Modafar & El Boustani, 2001) by high-performance liquid chromatography (HPLC). Their *Rf* values were determined in thin-layer chromatography (TLC) in various solvents and their colour in the presence of the mixture of ferricyanide potassium-ferric chloride revealing the phenolic molecules and their UV fluorescence (254 nm & 366 nm) in the presence of the ammonia vapor (Ribereau-Gayon, 1968) The Benedikt reagent distinguished monophenols/orthodiphenols (Reznik & Egger, 1961), the *p*-toluene sulphonic acid highlighted the radical secoiridoide (Weiffering, 1966) and the Neu reagent revealed the flavonoids and the caffeic acid derivatives (Brasseur & Angenot, 1986). Each spot characterized in TLC was then isolated for determinate the peak corresponding in HPLC and to provide additional information for the identification (retention time, absorption spectrum). The phenolic moiety was determined after enzymatic hydrolysis (β-glycosidase, phosphate buffer, pH 6.8, for 4 h), acid hydrolysis (HCl 2 N for 1 h at 100°C) and alkaline hydrolysis (El Modafar *et al.,* 2000b; El Modafar & El Boustani, 2001). The phenolic compounds identification was supplemented by co-chromatography in TLC, co-injection in HPLC and by comparing their chromatographic and spectral characteristics to phenolic standards (Sigma-Aldrich chimie S.a.r.l., St-Quentin Fallavier, French).

Quantification of phenolic compounds: Phenolic contents were determined by HPLC according to the technique previously described (El Modafar *et al*., 1996; El Modafar *et al*., 2000). The HPLC (Waters 600 E with a photodiode bar detector Waters 2996) was performed on Spherisorb C18 column (250 x 4 mm, 5µm) and µBondapack C18 Waters Guard PAK precolumn. Samples were eluted with a solvent consisting of acetonitrile and water acidified by acetic acid following a gradient of 5-37% of acetonitrile within 40 min with 1 mL min^{-1} flow rate. The phenolic compounds contents was expressed in equivalent 5-methoxyflavone, phenolic compound used like internal standard.

Statistical analysis: One way variance analysis was made according to a random model (variation between cultivars) was carried out using the Statitcf software. The differences between the means were determined by the Duncan's range ($P < 0.05$). The principal components analysis is carried out using the software Statistica (version 6).

RESULTS

Characterization of phenolic compounds of olive tree leaves: The analysis of the olive tree leaves phenolic extract by HPLC showed the presence of 15 majors phenolic compounds (Fig. 1) distinguished in five phenolic families

according to their chromatographic and spectral characteristics (flavonoïds, tyrosol derivatives, verbascoside derivatives, oleuropein derivatives & hydroxycinnamic derivatives). The flavonoïds were represented by five compounds such as rutin, luteolin-7-glucoside, apigenin-7-glucoside and two flavonol monoglucoside not completely identified. The tyrosol derivatives were represented by four compounds such as tyrosol, hydroxytyrosol and two tyrosol derivatives not completely identified. The verbascoside derivatives were represented by two compounds (verbascoside & a verbascoside derivative not completely identified). The oleuropein derivatives were represented by two compounds (oleuropein & oleuropein derivatives not completely identified). The hydroxycinnamic derivatives were represented by only one compound identified as chlorogenic acid.

The versbacoside, luteolin-7-glucoside, the oleuropein and a flavonol heteroside not completely identified (monoglucoside flavonol 2) represented the major phenolic compounds of olive tree leaves.

Relationship between phenolic compounds and resistance to the leaf-spot disease: Qualitatively all the characterized phenolic compounds were present in each of the 110 studied genotypes, no qualitative difference was detected. However the quantitative analysis showed a great variability of the constitutive of phenolic compounds contents in the various genotypes but without enabling establishing clear relations between the phenolic compounds contents taken individually and the resistance degree of the different genotypes to *S. oleagina* (Table II). In the same way, important quantitative differences noticed in post-infectional phenolic contents did not allow distinguish various genotypes according to their behaviour to the leaf-spot disease (Table III).

Given that no relation could be established between the amount of phenolics compound and the degree of resistance to *S. oleagina*; thus in the goal to establish a multifactoriel relation, we examined the distribution of the different behavior classes of disease resistance in comparison with the totality of the phenolic considerate as variables across a principal components analysis (PCA). The PCA was based on the constitutive contents of phenolic compounds, which identified two main axes explaining 79.21% of the total variability including 48.55% for the first axis and 30.66% for the second axis (Fig. 2). The first axis was determined on the positive side by the contents of luteolin-7-glucoside and flavonol monoglucoside 2 and on the negative side by the contents of the oleuropein and the apigenin-7-glucoside. The second axis was determined on the positive side by the contents of chlorogenic acid and apigenin-7-glucoside and on the negative side by tyrosol derivatives (tyrosol, hydroxytyrosol & two tyrosol derivatives). This analysis allowed differentiating three groups without establishing relations therefore between the constitutive contents of phenols and the degree of resistance of the different classes of resistance to *S. oleagina.*

Table I: Behaviour of the studied olive-tree genotypes to *S. oleagina*

Degree of resistance	Attack index*	Number of genotypes
Highly susceptible	6-10	10
Susceptible	5-6	9
Intermediate	1-5	81
Resistant	0-1	9
Highly resistant	0	10

* 0: absence of attack; 10: severe attack

Table II: Constitutive phenolic compounds contents (µg/g DW) in olive tree leaves of highly susceptible (HS), susceptible (S), intermediate (I), resistant (R) and highly resistant (HR) genotypes to *S. oleagina*. For each phenolic compound, the values followed by a common letter do not differ significantly at *P* = 0.05 according to Duncan's range test

Phenolic compounds	HS	S	I	R	HR
Hydroxytyrosol	37.02 b	31.54 a	38.23 b	40.92 b	41.38 b
Tyrosol derivative 1	18.93 b	15.83 a	19.01 b	21.19 b	19.20 b
Tyrosol	32.07 b	21.73 a	53.38 c	47.65 c	52.84 c
Tyrosol derivative 2	18.15 a	25.72 a	37.27 b	47.83 c	34.92 c
Chlorogenic acid	38.86 a	35.33 a	32.30 a	34.33 a	32.09 a
Verbascoside derivative	57.53 bc	33.37 a	50.68 bc	41.68 b	54.94 bc
Rutin	123.57 b	92.64 a	94.40 a	91.09 a	139.77 b
Versbacoside	106.52 b	75.96 a	92.12 b	96.87 b	78.97 a
Luteolin-7-glucoside	341.83 b	305.33 a	355.57 b	293.23 a	354.24 b
Flavonol monoglucoside 1	88.32 ab	84.89 ab	82.13 ab	116.28 b	66.21 a
Apigenin-7-glucoside	127.87 a	146.18 ab	122.34 a	178.67 b	114.23 a
Flavonol monoglucoside 2	868.69 b	552.56 a	759.56 b	569.00 a	883.63 b
Oleuropein	372.52 a	404.19 ab	361.62 a	470.31 b	348.49 a
Oleuropein derivative	57.92 a	61.14 a	69.55 a	101.28 b	53.49 a
Apigenin	12.11 a	49.45 c	21.32 b	31.09 bc	14.76 a

Table III: Postinfectional phenolic compounds contents (µg/g DW) of olive tree leaves in highly susceptible (HS), susceptible (S), intermediate (I), resistant (R) and highly resistant (HR) genotypes to *S. oleagina*. For each phenolic compound, the values followed by a common letter do not differ significantly at *P* = 0.05 according to Duncan's range test

Phenolic compounds	HS	S	I	R	HR
Hydroxytyrosol	41.71 a	37.36 a	38.55 a	39.96 a	37.90 a
Tyrosol derivative 1	20.14 a	18.01 a	20.10 a	21.11 a	20.17 a
Tyrosol	34.32 a	35.46 a	36.35 a	67.24 c	45.00 b
Tyrosol derivative 2	17.50 a	22.22 a	31.31 b	38.29 b	41.24 c
Chlorogenic acid	46.49 c	30.23 b	37.20 c	29.28 ab	21.84 a
Verbascoside derivative	63.72 c	49.51 b	50.59 b	39.98 a	39.31 a
Rutin	57.23 a	84.99 c	83.61 c	66.45 b	96.03 c
Versbacoside	101.57 b	93.93 ab	99.91 ab	81.36 a	71.34 a
Luteolin-7-glucoside	357.83 b	351.70 b	338.23 b	294.65 a	299.07 a
Flavonol monoglucoside 1	91.92 b	85.81 b	90.16 b	88.43 b	62.42 a
Apigenin-7-glucoside	137.11 b	120.29 b	126.91 b	154.45 c	93.80 a
Flavonol monoglucoside 2	843.67 c	684.76 b	658.66 b	516.81 a	630.44 b
Oleuropein	278.94 a	455.50 c	351.96 b	445.66 c	324.73 b
Oleuropein derivative	62.13 a	66.99 a	70.27 ab	83.23 c	73.69 b
Apigenin	14.65 a	31.66 c	29.59 c	27.50 bc	23.32 b

Fig. 1: HPLC Chromatogram of phenolic compounds of olive tree leaves

1.-Hydroxytyrosol, 2- Dérivé du tyrosol 1, 3- Tyrosol, 4- Dérivé du tyrosol 2, 5- Acide chlorogénique, 6- Dérivé de la verbascoside, 7- Rutine, 8- Versbacoside, 9- Lutéoline-7-glucoside, 10- Flavonol monoglucoside 1, 11- Apigénine-7-glucoside, 12- Flavonol monoglucoside 2, 13- Oleuropéine, 14- Dérivé de l'oleuropéine, 15- Apigénine, STD - 5-methoxyflavone (internal standard)

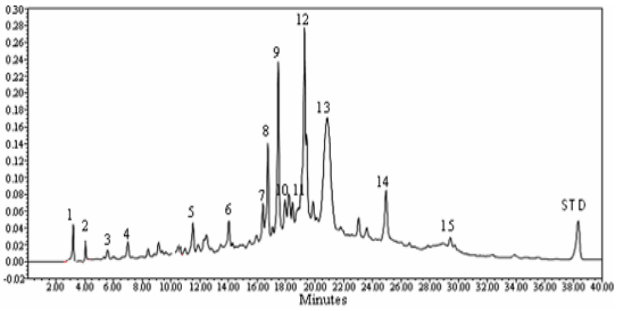
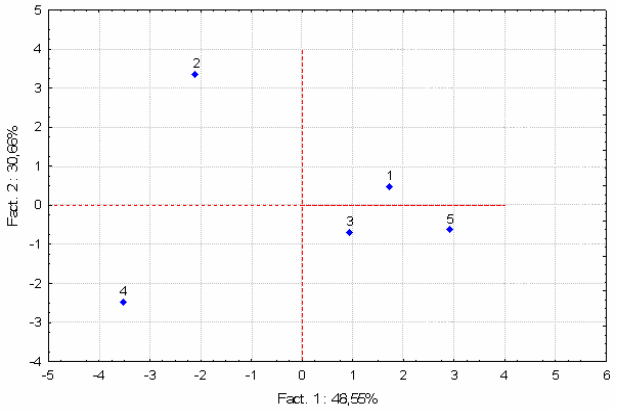


Fig. 2: Principal components analysis distribution of highly susceptible (1), susceptible (2), intermediate (3), resistant (4) and highly resistant (5) olive-tree genotypes to *S. oleagina* according to their constitutive phenolic compounds contents



Next we sought postinfectional multifactorial components by carrying out a second analysis principal component through the post-infectional contents of phenolic compounds (Fig. 3). This analysis distinguishes two main axes explaining 79.60% of the total variability including 53.87% for the first axis and 25.73% for the second axis. The first axis, determined on the positive side by the contents of five compounds (chlorogenic acid, luteoline-7-glucoside, apigenin-7-glucoside 2, verbascoside & its derivative) and on the negative side by four compounds (oleuropein & its derivative, tyrosol & its derivative 2), distinguished the genotypes according to their resistance degree to *S. oleagina*. Thus, the resistant and highly resistant genotypes were clearly distinguished from the highly susceptible genotypes by the contents of chlorogenic acid contents, luteolin-7-glucoside, flavonol monoglucoside 2 and of verbascoside and its derivative. The intermediate and susceptible genotypes present intermediate contents.

Fig. 3: Principal Components Analysis distribution of highly susceptible (1), susceptible (2), intermediate (3), resistant (4) and highly resistant (5) olive-tree genotypes to *S. oleagina* according to their postinfectional phenolic compounds contents

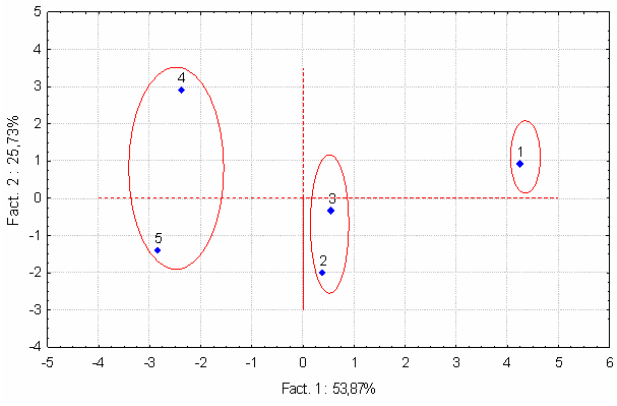
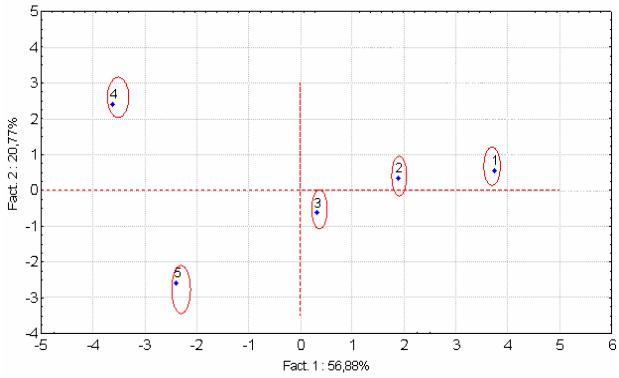


Fig. 4: Principal components analysis distribution of highly susceptible (1), susceptible (2), intermediate (3), resistant (4) and highly resistant (5) olive-tree genotypes to *S. oleagina* according to their contents of constitutive (hydroxytyrosol, tyrosol, tyrosol derivative 1, tyrosol derivative 2, luteolin-7-glucoside) and postinfectional (tyrosol, tyrosol derivative 2, chlorogenic acid, verbascoside derivative, rutin, versbacoside, luteolin-7-glucoside, apigenin-7-glucoside, flavonol monoglucoside 2, oleuropein, oleuropein derivative) discriminator phenolic compounds



The second axis, determined on the side positive by the post-infectional contents of apigenin-7-glucoside and on the negative side by the postinfectional contents of rutin, distinguishes the resistant genotypes and the highly resistant genotypes. Highly resistant genotypes present higher contents of rutin and lower contents of apigenin-7-glucoside.

The distribution of the genotypes by the postinfectional contents of phenolic compounds did not make it possible to distinguish between the susceptible genotypes and the intermediate genotypes (Fig. 3).

Nevertheless, the PCA established by the constitutive contents (Fig. 2) distinguished between the susceptible genotypes from the intermediate ones. This distinction related to higher constitutive contents of tyrosol derivatives (four derivatives) and of luteolin-7-glucoside in the intermediate genotypes.

A third analysis principal component through the constitutive (hydroxytyrosol, tyrosol, tyrosol derivative 1, tyrosol derivative 2, luteolin-7-glucoside) and postinfectional (tyrosol, tyrosol derivative 2, chlorogenic acid, verbascoside derivative, rutin, versbacoside, luteolin-7-glucoside, apigenin-7-glucoside, flavonol monoglucoside 2, oleuropein, oleuropein derivative) discriminating phenolic compounds (Fig. 4) confirmed these results and released three multifactorial components having the most effect in the distinction of the genotypes studied according to their behaviour to *S. oleagina*. The first component, determined by the postinfectional contents of tyrosol and its derivative 2 and by the oleuropein and its derivative, clearly distinguished highly resistant and resistant genotypes as compared to others. Highly susceptible genotypes contain the lowest contents. The second component, determined by the postinfectional contents of rutin, distinguishes the resistant genotypes and the highly resistant genotypes. The latter contain the highest contents. The third component, determined by the constitutive contents of tyrosol derivatives (four derivatives), distinguishes the intermediate genotypes from the susceptible and highly susceptible genotypes. The intermediate genotypes contain the highest contents.

DISCUSSION

The HPLC analysis of phenolic extracts of olive tree leaves highlights 15 major phenolic compounds belonging to five phenolic families (hydroxycinnamic derivatives, flavonoids, verbascoside derivatives, tyrosol derivatives, oleuropein derivatives). The phenolic extract of the olive tree leaves is dominated by seven major phenolic compounds identified to verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, oleuropein and two flavonol monoglucosides. Several works reported the abundance of these phenolic compounds in the olive tree leaves, particularly the luteolin-7-glucoside, the verbascoside and the oleuropein (Ryan & Robards, 1998; Silva *et al.,* 2006; El-Hassani *et al*., 2009; Rahioui *et al.,* 2009).

The various constitutive and postinfectional phenolic compounds characterized were highlighted in the entire genotypes studied; no qualitative difference was observed between the highly resistant, the resistant, the intermediately, the susceptible and the highly susceptible genotypes. However the PCA highlighted three multifactorial components distinguishing the various genotypes according to their behaviour to the disease.

On the basis of these multifactorial phenolic components, four metabolic relations can be deduced:

- A negative relation between the postinfectional contents of tyrosol derivatives and of verbascoside knowing that the verbascoside is an ester of the hydroxytyrosol and cafeic acid (Perrin, 1992).
- A negative relation between the postinfectional contents of oleuropein and of verbascoside knowing that the oleuropein is an ester of the hydroxytyrosol and of the glycosylated elenolic acid (Perrin, 1992).
- A negative relation between the postinfectional contents of rutin (quercetin-3-rutin) and apigenin. Quercetin and apigenin are formed starting from the same compound, the naringenin (Jeonyoung *et al.,* 2009).
- The biosynthesis of the tyrosol and its derivatives starting from tyrosin was more important in the intermediate genotypes than in the susceptible genotypes.

It is evident from the above that resistance degree of olive tree to *S. oleagina* was related positively to tyrosol derivatives, oleuropein and rutin contents and negatively to verbascoside and apigenin contents. Thus the resistance degree seems to be positively dependent with the metabolic process of three possible principal biosynthesis ways:

- The hydroxytyrosol was transformed more into oleuropein in the resistant and highly resistant genotypes, whereas it was transformed more into verbascoside in the highly susceptible genotypes.
- The naringenin was transformed more into rutin in the highly resistant genotypes, whereas it was transformed more into apigenin in the resistant genotypes. The naringenin undergoes an oxidation by a flavone synthase leading to the the apiginine formation, whereas it undergoes a hydroxylation by a oxoglutarate dioxygenase and a flavanone hydroxylase leading to the formation of the dihydroquercetin, which is transformed into quercetin *via* a flavonol synthase then into rutin *via* a flavonol glucosyltransferase (Winkel, 2008; Jeonyoung *et al.,* 2009).
- The biosynthesis of the tyrosol and its derivatives starting from tyrosin is more important in the intermediate genotypes than in the susceptible genotypes. Four enzymes are implied in the transformation of tyrosin into tyrosol: tyrosine décarboxylase, monoamine oxydase, réductase and tyrosol-glycotransférase (Landtag *et al.,* 2002).

The post-infectional contents of oleuropein and of rutin and the constitutive contents of tyrosol and its derivatives seemed to constitute factors of resistance. The activity ratio of the enzymes implied in the various biosynthesis ways of these determining phenolic compounds and/or the expression rate of corresponding genes would be at the origin of the resistance degree of olive tree to *S. oleagina*. The implication of the phenolic compounds in the plant resistance is largely reported (El Modafar & El Boustani, 2005) and their mode of action in defense to the pathogen micro-organisms is very variable, and can go from a direct antimicrobial effect until the modulation and the induction of the mechanisms of defense (El Modafar & El Boustani, 2005). The phenolic compounds are also precursors of the lignin whose role in

defence is largely verified (Vance *et al.,* 1980; El Modafar & El Boustani, 2000, 2001).

In olive tree, several studies showed that the oleuropein, the tyrosol, the hydroxytyrosol and the rutin displays a fongitoxic effect (Baidez *et al.,* 2007; Pereira *et al.,* 2007). The oleuropein glucoside seems to be implied in the defense of the olive tree by inhibiting the pectinases of *S. oleagina* (Graniti, 1993) and by constituting a precursor of phytoalexins (Uccella, 2000), extremely toxic molecules induced in response to pathogen infections (El Modafar *et al.,* 1995, 1999). The oleuropein aglycone, non-glucosidic form of the oleuropein, which is more hydrophobic, expresses an antioxydant activity (Galli *et al.,* 1999) and a great cytotoxicity (Babich & Visioli, 2003). These properties could confer to the oleuropein aglycone an important role in the hypersensitive reaction frequently associated to leaves defense in response to the pathogen aggressions (Lam *et al.,* 2001). The contents of orthodiphenols of the olive tree, particularly the oleuropein and the rutin were implied in the tissue browning resulting from their oxidation (Roussos & Pontikis, 2001). The oxidation of these orthodiphenols, the preferential substrates of the polyphenol oxidase (Mayer & Harel, 1979), give orthoquinones and melanins (El Modafar & El Boustani, 2005), very toxic products to the pathogen micro-organisms (El Modafar *et al.,* 2000a), which appear in the form of brown pigments during the hypersensitive reaction (El Modafar & El Boustani, 2005). In addition the studies of the interaction olive tree-*Verticillium dahliae* suggest a strong implication of the phenolic compounds in the olive tree defense particularly, oleuropein and tyrosol (Baidez *et al.,* 2007).

In conclusion, resistance of olive tree to *S. oleagina* was related to multifactorial phenolic components suggesting a polygenic resistance. The tyrosol and its derivatives were in relation to constitutive resistance, whereas the oleuropein and rutin were in relation to induced resistance. This work will have to be confirmed by in-depth studies of the various biosynthesis ways of discriminator phenolic compounds.

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