Differential Susceptibility of Olive Cultivars to Olive Knot Disease and Possible Involvement of Phenolic Compounds in Disease Tolerance

Imen Mougou[1,2*] and Ali Rhouma[2]

[1] *High Institute of Agronomy of Chott Mariem, 4042 Sousse, University of Sousse, Tunisia.*

[2] *Protection of Cultivated Plants and Environment Research Unit, Olive Tree Institute, Cité Mahrajéne BP208 Tunis, Tunisia.*

*E-mail: imenmougou@gmail.com

Received: 26/11/2021
Revised: 16/05/2022
Accepted: 25/05/2022

Abstract

Purpose: Olive knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi* is among the most serious bacterial disease affecting olives in many olive growing countries. This study aimed to evaluate the susceptibility of olive trees cultivars towards *Pseudomonas savastanoi* pv. *savastanoi*, and to verify the involvement of polyphenols in disease resistance or tolerance.

Method: The susceptibility of five olive cultivars against four strains of *Pseudomonas savastanoi* pv. *savastanoi* were evaluated by stem inoculation. The content of phenolic compounds was determined in different studied cultivars from shoots and Knots.

Results: Evaluated Tunisian strains AW9 and AW8 showed a similar and intermediate virulence in each cultivar, TEK appeared the less virulent. While the Spanish strain IVIA 1628 was the most virulent. Our results revealed that cv. Zarrazi was very susceptible to the disease. Cultivars Arbequina and Chetoui appeared susceptible, cv. Chemlali exhibited an intermediate infection level. While cv. Oueslati appeared tolerant to the disease. Polyphenolic content from shoots increased significantly according to the strain's virulence. For cvs. Zarrazi and Oueslati the polyphenol content of shoots inoculated with the most virulent strain IVIA1628 were (33.77 and 28, 69 mg GAE g^{-1} Ms), respectively. However, knot-polyphenol content increased significantly according to the virulence of the strains and cultivars susceptibility. The highest knot- polyphenol content value was recorded in the tolerant cultivar Oueslati inoculated with the most virulent strain IVIA 1628 (80.63 mg GAE g^{-1} Ms) while, cv. Zarrazi showed the lowest value with (30.16 GAE g^{-1} Ms). A negative correlation was observed between the polyphenol content in knot and the average of knot weight.

Conclusion: The hight value of polyphenols found in small knots formed on cv. Oueslati suggest the implication of these compounds in the defence mechanism against the bacterial disease.

Keywords: *Olea europaea*, Phenolic compounds, *Pseudomonas savastanoi* pv. *savastanoi*, Tolerance, Virulence.

Introduction

Olives (*Olea europaea* L.) are an important oil crop cultivated over large areas in many Mediterranean countries (Lumaret et al., 2004). Today, several

phytopathogenic microorganisms affected olive trees (Abuamsha et al. 2013) among them *Pseudomonas savastanoi* pv. *savastanoi* (Psv) a Gram-negative and fluorescent plant-pathogenic bacterium, which causes Olive knot disease (OK) (Gardan et al., 1992). In Tunisia, the OK occurs in the frost and hail regions (North and Central of the country), causing wounds to stems (Ouzari et al., 2008; Mougou and Boughalleb-M'hamdi, 2016 a).

The symptoms of infected trees include hypertrophy formation on the stems, branches occasionally on fruits and leaves (Kunkel and Harper, 2018). OK disease can cause severe damages and negatively affects the vegetative growth, fruit size, yield and oil quality (Mata et al. 2009). The Ok disease can reduce the productivity (Teviotdale, 1994).

The damages can directly affect the flowering and fruit development and indirectly lead to the weakening of immature branches, as well as an off-flavour of the fruit taste (Young, 2004). Two phytohormones which are the indole acetic acid (IAA) and cytokinins (CK) (Moreno et al., 2008) as well as type III secretion system (T3SS) biosynthesis, which is encoded by the hrp-hrc gene clusters (sisto et al., 2004) were involved in knot development. In fact, the IAA and CK production induced knot development by interfering with endogenous signals for rapid proliferation at the infection site (Surico et al. 1985).

Various studies confirm the importance of CK and IAA for perfect bacterial virulence. Furthermore, mutant stains without CK and IAA production are largely less symptomatic and cause only leaves necrosis and swelling on stems (Iacobellis et al., 1994).

The management of OK disease involves two strategies: sanitation pruning which is time consuming, expensive and difficult to achieve in high density plantings, and the application of copper bactericides (Salman et al., 2020) which could lead to serious environmental problems, human health damage and also the selection of copper-resistant bacteria (Brent and Hollomon, 1998; Marques et al., 2009; Mougou and Boughalleb-M'hamdi, 2018; Mougou, 2022).

In fact, to contain the disease and improve both the quality and quantity of fruit and oil yields, the most effective method of disease control was the selection of resistant or tolerant cultivars (Penyalver et al., 2006, Ramos et al. 2012; Mougou and Boughalleb-M'hamdi, 2016 b). According to Civantos (1999) the peak of OK disease depends on cultivars susceptibility.

To our best of knowledge, a few information is available about the susceptibility of olive cultivars to Psv (Lamichhane and Varvaro 2013; Quesada et al., 2010). Moreover, none of the cultivars were immune to the disease, but just few of them are considered tolerant to the OK (Godena et al., 2012).

It is well known that part of the defence mechanisms of the *Olea europaea* against the phytopathogenic microorganisms is provided by seco-biophenols, responsible for resistance to infection through phytoalexin biosynthesis (Bianco et al., 1999), and their local accumulation in the infection sites, by inducing cellular wall modification, enzymatic protection, or hypersensitivity reactions (Uccella, 2001). The olive tree shows high resistance to phytopathogenic bacteria (Cayuela et al., 2006).

This study was undertaken to provide information about the control of OK disease in Tunisia

with existing cultivars. The aims of this research were to study the susceptibility of olive trees cultivars towards Psv under greenhouse condition, and to verify the involvement of phenolic compounds (PC) in disease resistance or tolerance.

Material and Methods

Bacterial strains

Four strains of Psv were used to evaluate susceptibility (strain AW9, stain AW8, strain 1628 and stain TEK). IVIA 1628 strain was isolated from cultivar (cv.) Cornicabra and obtained from the Centro de Protección Vegetal y Biotecnología, IVIA, Valencia (Spain) while, AW9 and AW8 were supplied by the Labaratory of Improvement and protection of olive genetic resources, Olive Tree Institute, Tunis, Tunisia and isolated from cv. Chemlali grown in Ouedna (south-eastern Tunisia), and finally TEK isolated from cv. Chetoui grown in Tebourba (Tunis), Tunisia.

Identification of bacterial strain TEK was performed by using biochemical tests (LOPAT) according to Lelliott et al. (1966). The *iaaL* gene was amplified by PCR according to Penyalver *et al*. (2000) and the iaaL-derived primers directed the amplification of a 454 bp fragment.

16S rRNA gene sequences (Weishburg et al., 1992) were compared with the GenBank database by using the Basic Alignment Search Tool (BLAST). The similarity of the 16S rDNA sequences of TEK strain was more than 98% identical to the corresponding gene sequences of Psv present in the databases (accession number HM 190226.1). Strain pathogenicity (TEK) was confirmed on 2-year-old olive (cv. Arbequina), inoculated with a 10^8 CFU ml^{-1} bacterial suspension. The strain TEK developed typical knots, compared to the reference Psv strain IVIA 1628 (unpublished data).

Plant material

The olive plants (Two years-old) were obtained from a Tunisian nursery. After inoculation, plants were maintained in the greenhouse in individual plastic bag (dimension 1L) filled with a substrate composed of peat and sand and watered each three days. Five cultivars were used to evaluate susceptibility: Oueslati, Chemlali, Chetoui, Zarrazi and Arbequina. We selected the foreign cultivar Arbequina because Tunisian farmers strongly preferred this cultivar in super-intensive plantings.

Plant Inoculation

Olive plants were wounded at three sites on the stem. Each wound site was inoculated with10 µL of bacterial suspension at 10^8 CFU/ml. Non-inoculated plants treated with sterile distilled water were used as control plants. Ten plants were used (five wounds per plant per strain). After inoculation plants were kept in a greenhouse for 2 months and the average knot fresh weight *per* plant was determined by weighting the stem overgrowths.

The susceptibility of olive cultivars and the virulence of the strains were evaluated according to the following parameters: Percentage of wounds developing knots, Weight of the knots, and polyphenol content.

Determination of Phenolic compounds content

The content of phenolic compounds has been determined in investigated dry extracts of shoots of olive plants inoculated by (strain AW9, stain AW8, strain 1628 and stain TEK) and knot developed after inoculated by (strain AW9 and strain 1628) for all tested cultivars.

One hundred mg of powder were mixed with 10 ml of methanol. After 20 h of agitation at room temperature, the mixture was centrifuged for 10 min at 2,000 rpm. After that the

extract was stored at 4°C in the dark. The PC was determined spectrophotometrically by the method of Folin - Ciocalteu (Singleton et al., 1999).

A five hundred µl of the extract was diluted at 1:10 with methanol and mixed with 2.5 ml of Folin (10 %) to which two ml of NaCO3 (7.5 %) were added. After 90 min of incubation, the absorbance was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at a wavelength of 760 nm. The standard used in this work was gallic acid, prepared at concentrations ranging from 5 to 100 mg l–1. The PC was expressed as mg of equivalent of gallic acid per gram of dry matter (mg GAE g^{-1} Ms). The entire experiment was repeated three times.

Data analysis

Statistical analysis was performed using SPSS version 20.0 statistical software. The data were subjected to a two-way analysis of variance (ANOVA) and the comparison of means were pursued through the Duncan's multiple range test at the 5% (P = 0.05) level of significance.

Dendrogram relating to the study of cultivars susceptibility was constructed by using MVSP (Multivariate Statistical Package Plus ver. 3.12e) software with UPGMA algorithm through the Gower General Similarity Coefficient method (Gower, 1971).

Results and discussion

Strain virulence

The pathogenicity of the strains on the two-year-old olive cultivars produced characteristic knot symptoms (aerial knot) with variable sizes. Difference in virulence among Psv strains was demonstrated to study the cultivar susceptibility.

Following to stem inoculation of all tested cultivars, three groups of virulence were registered: group 1 represented by TEK which appeared the less virulent and developed knot weighting 0.43 g; group 2 by AW9 and AW8, with a weight of knots values of 0.60g and 0.61g, respectively, and finally group 3 by IVIA 1628 strain with weight of knots value of 0.85g (Table1).

The Tunisian AW9 and AW8 strains which were isolated from the same orchard and region showed similar and intermediate virulence in each cultivar, while TEK strain which is isolated from the region of Tebourba appeared the less virulent. On the contrary, the Spanish strain IVIA 1628 was the most virulent. We can conclude that the virulence of the strains is affected by the collection site. Indeed, Sisto et al., (2007), reported by using the AFLP method that Psv strains were regrouped according to the geographical site of isolation.

Cultivar susceptibility

Percentage of wounds developing knots

All inoculated sites of the tested cultivars developed knots with the strains IVIA1628, AW8 and AW9 except for the less virulent strain TEK. Therefore, this parameter cannot be considered as a discriminating criterion to classify the cultivars according to their degree of susceptibility towards the disease.

Average weight of knots

Knot weight was evaluated 60 days after inoculation. The ANOVA of the average weight

of knots in all cultivars inoculated with Psv strains (IVIA 1628, AW9, AW8 and TEK) showed a significant effect of cultivar (P< 0.05), strains (P<0.05) and interaction between cultivars x strains (P< 0.05). The results of stem inoculation of the cultivars with tested strains showed different levels of infection and a wide range of susceptibility according to the strains virulence and to olive cultivars susceptibility. The average weight of knots increased according to the strains virulence. The healthy olive trees treated with sterile distilled water as a control did not show any symptoms.

For the most virulent strain IVIA1628, the value was 1.11g (cv. Zarrazi) (Figure 1) which is considered as the highest knot weight and 0.76g (cv. Oueslati). For the two strains (AW9 and AW8) the values, ranged between (0.92g and 0.9g) (cv. Zarrazi), respectively. The lowest knot weight was observed in cv. Oueslati (Table1).

Figure 1. Knot symptoms induced by IVIA 1628, AW9, AW8 and TEK strains on cv. Zarrazi

Table 1. The average of the fresh knot weight for all tested cultivars

Cultivars	Average fresh knot weight (g)[a]					
	IVIA 1628	AW9	AW8	TEK	Mean	P-value [d]
Zarrazi	1.119 ± 0.209a [b] A [c]	0.927 ±0.058bA	0.902 ± 0.025bA	0.756 ± 0.031cA	0.926	<0.01
Chetoui	0.928 ± 0.076aB	0.740 ± 0.019bB	0.721 ± 0.037bB	0.518 ± 0.025cB	0.727	<0.01
Arbequina	0.902 ± 0.107aB	0.721 ± 0.028bB	0.710 ± 0.032bB	0.499 ± 0.038cB	0.708	<0.01
Chemlali	0.767 ± 0.065aC	0.705 ± 0.044bC	0.694 ± 0.022bC	0.385 ± 0.033cC	0.638	<0.01
Oueslati	0.543 ± 0.032aD	0 ± 0abD	0 ± 0bD	0 ± 0bD	0.136	<0.01
Mean	0.852	0.618	0.605	0.431	nd	nd
P-value	<0.01	<0.01	<0.01	<0.01	nd	nd

[a]Average fresh knot weight (g). Duncan's Multiple Range Test, values followed by different letters are significantly different at $P \leq 0.05$. [b]Duncan's Multiple Range Test is for comparison of the fresh knot weight means among all tested cultivars for the same strain. Small letters are for means of comparison in the same row. [c]Duncan's Multiple Range Test is for comparison of fresh knot weight means among strains for the same cultivar. Capital letters are for means comparison in the same column. [d]Probabilities associated with individual F tests. Each value is expressed as mean ± standard errors. nd: not determine.

Intermediate values of knot weight were recorded in cvs. Chetoui and Arbequina inoculated by IVIA1628 (0.92g and 0.9g), respectively and AW9 (0.74g and 0.72g), respectively. The average of knot weight in cv. Chemlali inoculated by strain AW9 and IVIA1628 was ranged between (0.7g and 0.76g), respectively (Table1).

Hierarchical analysis of five olive cultivars using UPGMA method showed four distinct clusters. The first cluster are represented by cv. Zarrazi which is highly susceptible to the disease. The second cluster included both cvs. Arbequina and Chetoui which are considered susceptible to the disease. The third cluster are represented by cv. Chemlali which is considered as intermediate sensitivity. The last cluster are represented by cv. Oueslati tolerant to the disease (Figure 2).

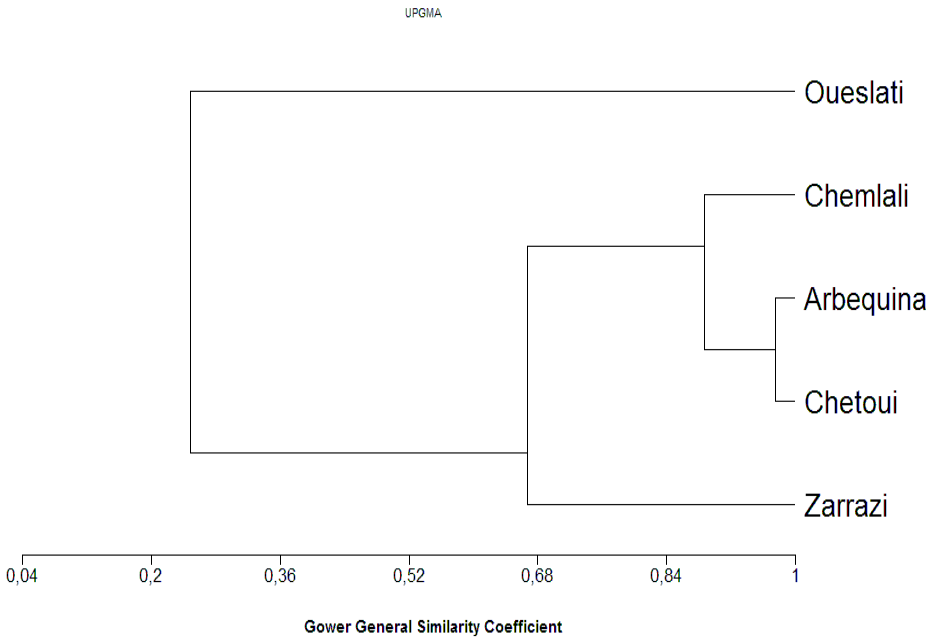

Figure 2. UPGMA dendrogram (based on Gower's coefficient of similarity) revealing cultivars susceptibility

Little information is available about cultivars susceptibility to OK disease. Based in our results different levels of infection and a wide range of susceptibility were observed according to the strains virulence and to olive cultivars susceptibility. The results revealed that some genotype display a low susceptibility against Psv infection such as cultivar Oueslati. However, Zarrazi was very susceptible to the disease. Cultivars Arbequina and Chetoui appeared susceptible, cv. Chemlali exhibited an intermediate infection level. While, cv. Oueslati appeared tolerant to the disease.

These results are in agreement with previous investigation reported by Penyalver et al. (2006). These authors showed that cv. Chemlali was not very susceptible to OK disease, nevertheless, cv. Arbequina display a high susceptibility to Psv. Moreover, the same authors reported that none of the cultivars were immune to the disease. Penyalver et al., (2006) reported that the susceptibility of olive cultivars depends on the bacterial strain virulence.

According to Loussert and Brousse (1978), the Tunisian cultivar Oueslati cultivated in the Djebel Ouslet seemed to be resistant to the disease. Our results indicated that cv. Oueslati was tolerant to Psv.

In Tunisia, no knots were obtained with cv. Chemlali when cultivated in the region of Sfax, while when planted in the center of Tunisia (Djebel Ouslet), this cultivar were quickly covered with knots (Loussert and Brousse, 1978). This demonstrated the involvement of the environmental factor and the climatic conditions on cultivar behaviour towards the pathogen (Godena et al., 2012).

Polyphenol content

We analyzed the PC in healthy and infested organs, to verify if the olive phenolic compounds were involved in disease resistance or tolerance.

Effect of strain virulence on shoots-polyphenol content

In the healthy shoots of cv. Oueslati tolerant to the disease, the value of PC was 25 mg GAE g^{-1} Ms, while in cv. Zarrazi susceptible to the disease, the value was 26. 63 mg GAE g^{-1} Ms).

For cvs. Zarrazi, Oueslati and Chetoui, the PC of shoots inoculated with the most virulent strain IVIA1628 were significantly higher (33.77, 28.69 and 44.58 mg GAE g^{-1} Ms), respectively than those inoculated with the less virulent strain TEK (30.36, 25.80 and 35.16 mg GAE g^{-1} Ms), respectively. Indeed, cv. Oueslati was tolerant to the disease, the PC was lower.

Our results in Table 2 revealed that after inoculation, shoots-polyphenol content increased significantly according to the strains virulence. Nevertheless, cv. Oueslati was tolerant to the disease, the PC in the shoots was lower than the amount obtained in the susceptible cultivars such as cvs. Zarrazi and Chetoui. No correlation was detected between shoots-polyphenol content and cultivar susceptibility to the disease.

Table 2. Polyphenol content of healthy and inoculated shoots by strains (IVIA 1628, AW9, AW8 and TEK)

Cultivars	Healthy shoots	Polyphenol content (mg GAE g^{-1} Ms)[a]					
		IVIA 1628	AW9	AW8	TEK	Mean	P-value[c]
Zarrazi	26.25 a±0.2[b]	33.77d±0.4	32.19 c±0.3	31.55c±0.9	30.36b±0.2	30.82	<0.01
Chetoui	28.63a±0.4	44.58d±0.4	38.77c±0.5	37.66c±0.3	35.16b±0.8	36.96	<0.01
Chemlali	19.055a±0.12	44.63d±0.9	36.97c±0.4	36.166c±0.2	28.027b±0.2	32.97	<0.01
Arbequina	30.61a±0.4	44.88d±0.9	36.55c±0.5	30.83c±0.1	34.66b±0.08	35.51	<0.01
Oueslati	25 a±0.3	28.69d±0.2	27.083c±0.2	26.83c±0.5	25.80b±0.5	27.23	<0.01

[a]Polyphenol content. Duncan's Multiple Range Test, values followed by different letters are significantly different at $P \leq 0.05$. [b]Duncan's Multiple Range Test is for comparison of Polyphenol content means among strains and healthy shoots for the same cultivar. [c]Probabilities associated with individual F tests. Small letters are for comparison of means in the same row. Each value is expressed as mean ± standard error a point at the end of the sentence.

In fact, as a response to the infection by the bacterium, the polyphenols are secreted by organs to stop the invasion of the phytopathogenic bacteria. This phenomenon was observed in the case of a hypersensitive reaction of tobacco leaves after the penetration of the pathogen in leaves tissues (Sisto et al., 2004; Hopkins, 1995; Willis et al., 1991).

The accumulation of polyphenols has been considered as a cause of resistance to pathogens in general (Kusumoto and Suzuki, 2003). Indeed, some studies about the interaction of olive plants with *Verticillium dahliae* suggest a strong involvement of phenolic compounds in the defence of the olive trees in particular, oleuropein and tyrosol (Baidez et al., 2007).

El Modafar and El Boustani (2005) demonstrated that phenolic compounds are implied in plant defence against microorganisms and associated with host resistance in plants.

Effect of strain virulence on knot- polyphenol content

For cvs. Zarrazi and Chetoui inoculated by IVIA1628 the knot-polyphenol content were

significantly higher (30.16 and 55.63 mg GAE g^{-1} Ms), respectively than those inoculated with the less virulent strain AW9 (20 and 46.83 mg GAE g^{-1} Ms), respectively in contrast to healthy shoots. The highest PC compared to all tested cultivars was recorded in cv. Oueslati inoculated with strain IVIA 1628 (80.63 mg GAE g^{-1} Ms) (Figure 3).

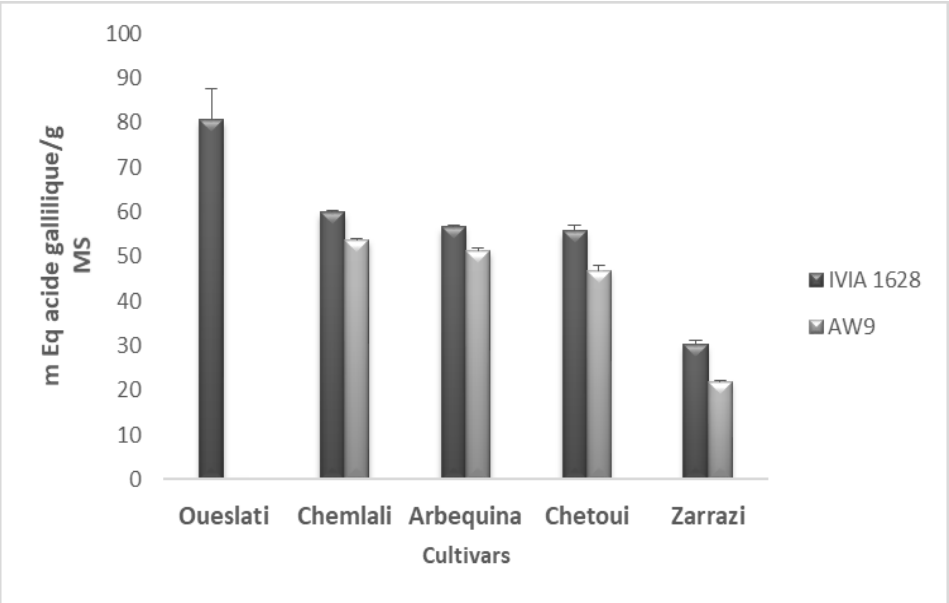

Figure 3. Knot- phenolic contents obtained after inoculation with IVIA1628 and AW9 strains in different studied cultivars

The highest knot- polyphenol content was recorded with the most virulent strain IVIA 1628. We observed also a high knot- polyphenol content in the tolerant cultivar Oueslati. Our results showed that after infestation knot- polyphenol content increased significantly according to the virulence of the strains and cultivars susceptibility. Indeed, a negative correlation was detected between the knot-polyphenol content and the average weight of knot (Figure 4).

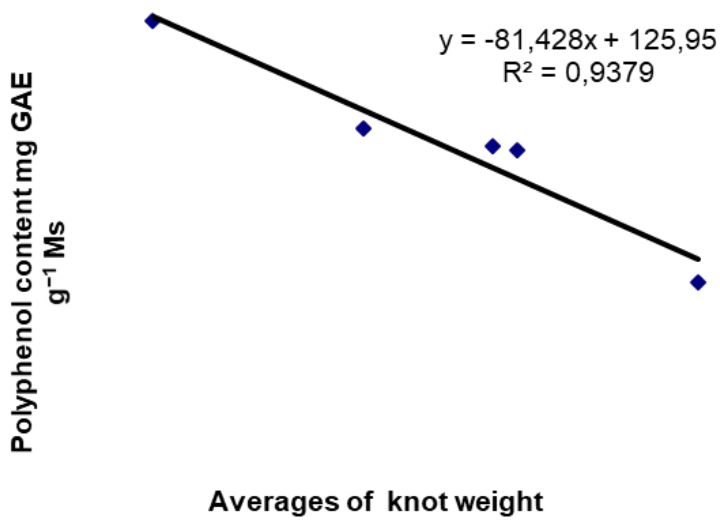

Figure 4. Correlation between polyphenol content and average knot weight

In reality, El Modafar and El Boustani (2005) reported the involvement of phenolic compounds in plant resistance. The mode of action of phenolic compounds in the defense of pathogenic microorganisms was widely variable and can range from a direct antimicrobial effect to the modulation and induction of defense mechanisms (El Modafar and El Boustani, 2005). Moreover, several authors have detected the antibacterial activity of olive tree phenolics (Tassou et al., 1995).

The difference in PC in shoots and knots is explained by the fact that shoots were collected from various parts more or less distant from the infection site. In fact, results indicated that the highest PC was found in the knot. These results agree with those previously reported by Cayuela et al., (2006) and Roussos et al., (2002) which demonstrated a very high amounts of knot-phenolic compounds rich in verbascoside and revealed that phenolic compounds were involved in the defence mechanism against the disease. In fact, more investigations about the presence of other phenolic compounds is required to explain the tolerance of the cv. Oueslati to the disease.

Conclusion

Indeed, the use of bactericidal compounds may lead to serious damage in relation to the environment, human health and promotes the selection of copper resistant bacteria. Thus, the most effective method of disease control was the selection of resistant or tolerant cultivars to the disease. Our results showed that the local cv. Oueslati was interestingly found tolerant to and therefore could be used to replant olive orchards mainly in the humid and hail regions where the environmental conditions are favourable to OK disease. The inclusion of cv. Oueslati in breeding programmes for resistance to the disease can be a great solution for the control of Psv.

On the other hand, the results showed that after infestation knot- polyphenol content increased significantly according to the virulence of the strains and cultivars susceptibility, highlighting the possible involvement of phenolic compounds in the defence mechanism against the OK disease.

Acknowledgments

This research was supported by the Tunisian Olive Institute. The authors are grateful to the review editor and the anonymous reviewers for their helpful comments and suggestions to improve the clarity of the research article.

References

Abuamsha, R, Abueid, M, Hajjeh , H, and Salman, M (2013) Evaluation of the incidence and severity of olive leaf spot caused by *Spilocaea oleagina* in different olive cultivars in Palestine. *J. Agric Environ Int Dev* 107(2): 201–212.

Baidez, AG, Gomez, P, Del Rio, JA, and Ortuno, A (2007) Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. Role of phenolic compounds in plant defense mechanism. *J Agric Food Chem* 55(9): 3373–3377.

Bianco, A D, Muzzalupo, I, Piperno, A, Romeo, G and Uccella, N (1999) Bioactive derivatives of oleuropein from olive fruits. *J. Agric Food Chem* 47: 3531-3534.

Brent, K J and Hollomon, D W (1998) Fungicide resistance: the assessment of risk

FRAC Monograph. Global Crop Protection Federation, Brussels.

Cayuela, JA, Rada, M, Rios, JJ, Albi, T and Guinda, A (2006) Changes in Phenolic Composition Induced by *Pseudomonas savastanoi* pv. *savastanoi* Infection in Olive Tree: Presence of Large Amounts of Verbascoside in Nodules of Tuberculosis Disease. *J. Agric Food Chem* 54(15): 5363–5368.

Civantos López-Villalta, M (1999) Olive Pest and Disease Management. IOOC publications, Madrid.

El Modafar, C and El Boustani, E (2005) The role of phenolics in plant defense mechanisms. In: Regnault-Roger, C, Philogène, BJR and Vincent, C (eds.) Biopesticides of Plant Origin, Intercept, Andover. Springer-Verlag, UK, pp. 157–172.

Gardan, L, Bollet, C, Abu, Ghorrah, M, Grimont, Fand Grimont, PAD (1992) DNA relatedness among the pathovar strains of *Pseudomonas syringae* subsp. *savastanoi* Janse (1982) and proposal of *Pseudomonas savastanoi* sp. nov. *Int J Syst Bacteriol* 42(4): 606–612.

Godena, S, Dminic, I and Dermic, E (2012) Differential susceptibility of olive varieties to olive knot disease in Istria. *J Cent Eur Agric* 13(1): 85-94.

Gower C (1971) A genral coefficient of similarity and some of its properties. *Biometrics* 27: 857–871.

Hopkins, WD (1995) Introduction to plant physiology. John Wiley and Sons Inc, New York, pp.1-560.

Iacobellis, NS, Lo, Cantore, P, Capasso, F and Senatore, F (2005) Antibacterial Activity of Cuminum cyminum L. and Carum carvi L. Essential Oils. *J Agric Food Chem* 53(1): 57-61.

Iacobellis, NS, Sisto, A, Surico, G, Evidente, Aand DiMao, E (1994) Pathogenicity of *Pseudomonas syringae* subsp. *savastanoi* mutants detective in phytohormone production. *J. Phytopathol* 140 (3): 238–48.

Kunkel, B N and Harper, C P (2018) The roles of auxin during interactions between bacterial plant pathogens and their hosts. *J. Exp Bot* 69 (2): 245–254.

Kusumoto, D and Suzuki, K (2003) Spatial distribution and time-course of polyphenol accumulation as a defense response induced by wounding in the phloem of Chamaecyparis obtuse. *New Phytologist* 195(1): 167–173.

Lamichhane, RJ and Varvaro, L (2013) Epiphytic *Pseudomonas savastanoi* pv. *savastanoi* can infect and cause olive knot disease on *Olea europaea* subsp. *cuspidata. Australas Plant Pathol* 42: 219–225.

Lelliott, RA, Billing, E and Hayward, AC (1966) A determinative scheme for the fluorescent plant pathogenic *Pseudomonads. J. Appl Bacteriol* 29 (3): 470-489.

Loussert R, Brousse G (1978) L'olivier; techniques agricoles et productions méditerranéennes. G.P. Maisonneuve et Larose, Paris. pp. 1-447.

Lumaret, R, Ouazzani, N, Michaud, H, Vivier, G, Deguilloux, MF and Di Giusto, F (2004)

Allozyme variation of oleaster populations (wild olive tree) (*Olea europaea* L.) in the Mediterranean Basin. *Heredity* 92: 343–351.

Marques, E, Uesugi, Ch and Ferreira, MASV (2009) Sensitivity to copper in *Xanthomonas campestris* pv. *viticola. Trop Plant Pathol* 34(6): 406–411.

Matas IM, Pérez-Martínez I, Quesada JM, Rodriguez-Hervá JJ, Penyalver R , Ramos C (2009) *Pseudomonas savastanoi* pv. *savastanoi* contains two iaaL paralogs, one of which exhibits a variable number of a trinucleotide (TAC) tandem repeat. *Appl Environ Microbiol* 75(4): 1030– 1035.

Mazen, S, Greenhut, R, Preece, J, Ferguson, L and Kluepfel, D (2020) Field evaluation of olive (*Olea europaea*) genotypes for resistance to *Pseudomonas savastanoi* pv. *savastanoi*. *J. Plant Pathol* 102: 663–670.

Mougou, I and Boughalleb-M'hamdi, N (2016a) Differential susceptibility of citrus cultivars toward blast and black pit in Tunisia caused by *Pseudomonas syringae* pv. *syringae*. *Eur J. Biotechnol. Biosci* 4(8):17-24.

Mougou, I and Boughalleb-M'hamdi, N (2018) Biocontrol of *Pseudomonas syringae* pv. *syringae* affecting citrus orchards in Tunisia by using indigenous *Bacillus* spp. and garlic extract. *Egypt J. Biol Pest Control* 28: 60.

Mougou, I (2022) Citrus blast and black pit disease: A review. DYSONA - *Life Science* 3(1): 1-6.

Mougou, I, and Boughalleb-M'hamdi, N (2016b) Detection, Survival, and Source of Inoculum of *Pseudomonas syringe* pv. *syringae* from Weeds and Plant Debris in Relation to Epidemiology of Bacterial Citrus Blast and Black Pit in Tunisia. *Br Microbiol Res J* 16(5): 1-10.

Ouzari, H, Khsairi, A, Raddadi, N, Jaoua, L, Hassen, A, Zarrouk, M, Daffonchio, D and Boudabous, A (2008) Diversity of auxin-producing bacteria associated to *Pseudomonas savastanoi*-induced olive knots. *J. Basic Microbiol*: 48(5): 370–377.

Penyalver, R, Garcıa, A, Ferrer, A, Bertolini, E and Lopez, MM (2000) Detection of *Pseudomonas savastanoi* pv. *savastanoi* in olive plants by enrichment and PCR. *Appl Environ Microbiol* 66 (6): 2673– 2677.

Penyalver, R, Garcia, A, Ferrer, A, Bertolini, E, Quesada, JM, Salcedo, C I, et al (2006) Factors affecting *Pseudomonas savastanoi* pv. *savastanoi* plant inoculations and their use for evaluation of olive cultivar susceptibility. *Phytopathol* 96(3): 313–319.

Quesada, JM, Penyalver, R, Perez-Panades, J, Salcedo, CI, Carbonell, EA and Lopez, MM (2010) Dissemination of *Pseudomonas savastanoi* pv. *savastanoi* populations and subsequent appearance of olive knot disease. Plant Pathol 59(2): 262–269.

Ramos, C, Matas, IM, Bardaji, L, Aragón, I M and Murillo J (2012) *Pseudomonas savastanoi* pv. *savastanoi*: some like it knot. *Mol. Plant Pathol* 13(9): 998–1009.

Rodríguez-Moreno L, Barceló-Muñoz A, and Ramos C (2008) In vitro analysis of the interaction of *Pseudomonas savastanoi* pvs. *Phytopathol* 98(7): 815–822.

Rousso, P A, Pontikis, C A and Tsantili E (2002) Root promoting compounds detected in

olive knot extract as response to infection by the bacterium *Pseudomonas savastanoi* pv. *savastanoi*. *Plant Sci*163 (3): 533-541.

Singleton, V L, Orthofer, R and Lamuela-Raventos, R M (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteau reagent. *Meth Enzymol* 299: 152–178.

Sisto, A, Cipriani, , MG, Tegli, S, Cerboneschi, M, Stea, G and Santilli, E (2007) Genetic characterization by fluorescent AFLP of *Pseudomonas savastanoi* pv. *savastanoi* strains isolated from different host species. *Plant Pathol*: 56(3) 366–372.

Sisto, A, Cipriani, MG and Morea, M (2004) Knot formation caused by *Pseudomonas syringae* subsp. *savastanoi* on olive plants is hrp-dependent. *Phytopathol* 94(5): 484–489.

Surico, G, Iacobellis, NS and Sisto, A (1985) Studies on the role of indole-3-acetic acid and cytokinins in the formation of knots on olive and oleander plants by *Pseudomonas syringae* pv. *savastanoi*. *Physiol Plant Pathol* 26: 309-320.

Tassou, C C and Nychas, GJ (1995) Inhibition of Salmonella enteridis by oleuropein in broth and in a model food system. *Lett Appl Microbiol* 20(2): 120-124.

Teviotdale BL (1994) Diseases of olive In: Ferguson, L, Sibbett, GS and Martin GC(eds.) Olive production manual, University of California Publication, USA, pp. 1-107.

Uccella, N (2001) Olive biophenols: biomolecular characterization, distribution and phytoalexin histochemical localization in the drupes. *Trends Food Sci. Technol* 11 (9-10): 315-327.

Weisburg, W, Barns, SM, Pelletier, DA and Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*, 173 (2): 697–703.

Willis, DK, Rich J J, and Hrabak, EM (1991) hrp genes of phytopathogenic bacteria. *Mol. Plant-Microbe Interact* 4:132-138.

Young, J M (2004) Olive knot and its pathogen. *Australas Plant Pathol* 33 (1): 33–39.

اختلاف حساسية اصناف الزيتون لمرض سل الزيتون وامكانية ارتباط المكونات الفينولية في مقاومة المرض

ايمان موقو[1,2]* وعلي رحومة[2]

[1] المعهد العالي للعلوم الفلاحية بشط مريم، 4042، جامعة سوسة، تونس.

[2] وحدة البحوث لحماية النباتات والبيئة، معهد الزيتونة، حي المهرجان ص. ب. عدد 208 تونس، تونس.

* البريد الإلكتروني: imenmougou@gmail.com

المُستَخلَص

تاريخ استلام البحث: 2021/11/26
تاريخ تعديل البحث: 2022/05/16
تاريخ قبول البحث: 2022/05/25

الهدف: مرض سل الزيتون الناجم عن *Pseudomonas savastanoi* pv. *savastanoi* من اخطر الامراض البكتيرية التي تصيب الزيتون في العديد من دول زراعة أشجار الزيتون. تهدف هذه الدراسة إلى تقييم حساسية اصناف الزيتون لبكتيريا *Pseudomonas savastanoi* pv. *savastanoi* والتثبت من دور البوليفينول في تحمل او مقاومة المرض.

الطريقة: تم تقييم حساسية خمسة اصناف من الزيتون ضد أربع سلالات من نوع *Pseudomonas savastanoi* pv. *savastanoi* عن طريق تلقيح الجذع كما تم تحديد محتوى المركبات الفينولية لدي الاصناف المدروسة على مستوي الاغصان والاورام.

النتائج: اظهرت الاختبارات لدراسة ضراوة السلالات ان السلالتين التونسيتين (AW8, AW9) لديهما نفس المستوى من الضراوة، اما السلالة (TEK) فهي الاقل ضراوة. بينما السلالة الاسبانية فهي الاكثر ضراوة. بينت النتائج ان صنف الزرازي فائق الحساسية، صنف الشتوي حساس، صنف الشملالي متوسط الحساسية، بينما صنف الوسلاتي فهو مقاوم للمرض. ارتفع محتوى المركبات الفينولية بالأغصان بشكل ملحوظ حسب ضراوة السلالات. بلغ محتوى المركبات الفينولية بالنسبة لصنفين الزرازي والوسلاتي في مستوى الاغصان الملقحة بالسلالة الاكثر ضراوة (IVIA 1628) حوالي (33.77mg GAE g^{-1} Ms و28.69) على التوالي. ارتفع محتوي المركبات الفينولية بالاورام بشكل ملحوظ حسب ضراوة السلالات وحساسية الاصناف. تم تسجيل أعلى قيمة لمحتوي المركبات الفينولية في مستوى الاورام لدى صنف الوسلاتي الملقح بالسلالة الاكثر ضراوة (IVIA 1628) بحوالي (80.66 mg GAE g^{-1} Ms)، بينما اظهر صنف الزرازي أدنى قيمة بحوالي (30.16mg GAE g^{-1} Ms). تم تسجيل ارتباط سالب بين محتوى المركبات الفينولية على مستوى الاورام ومتوسط وزن الاورام.

الاستنتاج: اظهرت النتائج ان القيمة العالية لمادة البوليفينول الموجودة في الاورام الصغيرة لدى صنف الوسلاتي تشير إلى ارتباط هذه المركبات في مقاومة المرض.

الكلمات المفتاحية: الضراوة، التسامح، *Pseudomonas savastanoi* pv. *savastanoi*، مركبات البوليفينول، *Olea europaea*.