Journal of Advances in Biology & Biotechnology
5(4): 1-9, 2016; Article no.JABB.23694
ISSN: 2394-1081

SCIENCEDOMAIN *international*
www.sciencedomain.org

Inductions of Defense Response in Olive Plants against *Verticillium dahliae* through Application of Salicylic Acid as Abiotic Inducer

Yaakoub Gharbi[1*], Emna Bouazizi[1] and Mohamed Ali Triki[1]

[1]Laboratory of Amelioration and Protection of Olive Genetic Resources, Olive Tree Institute, BP 1087, University of Sfax, Sfax 3064, Tunisia.

Authors' contributions

This work was carried out in collaboration between all authors. Author YG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EB and MAT managed the analyses of the study. Author MAT managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/23694
Editor(s):
(1) Joana Chiang, Department of Medical Laboratory Science and Biotechnology, China Medical University, Taiwan.
Reviewers:
(1) Ilias Faiza, Center University of Belhadj Bouchaib, Ain Temouchent, Algeria.
(2) Mervat Shamoon Sadak, National Research Centre, Cairo, Egypt.
(3) Mohammad Arif, Kansas State University, USA.
Complete Peer review History: http://sciencedomain.org/review-history/13523

Original Research Article

Received 16th December 2015
Accepted 4th January 2016
Published 3rd March 2016

ABSTRACT

Aims: To find suitable approach to control *V. dahliae*, we evaluated the potential of salicylic acid in the induction of systemic resistance in olive plants and determine whether acquired resistance is correlated with soluble proteins and polyphenol contents.
Place and Duration of Study: This work was performed in the Laboratory of Phytopathology at the Olive Tree Institute (Sfax, Tunisia) between July 2014 and June 2015.
Methodology: Olive plants were pre-treated with 10 mM salicylic acid and then inoculated with pathogenic *V. dahliae* isolate. Symptoms were monitored for three months and plant tissues samples were regularly analyzed for their soluble protein and polyphenol contents at 15, 30 and 45 days after inoculation. The effect of salicylic acid treatment on soluble proteins expression was evaluated using SDS-PAGE.
Results: Pre-treatment with salicylic acid resulting in a decrease of disease incidence from 91.96% to 29.33% after 45 days of pathogen inoculation. The maximum increase in soluble proteins

**Corresponding author: E-mail: yaakoub.gharbi@yahoo.com;*

content was recorded in salicylic acid pre-treated plants with 1.5-fold, 1.87-fold and 2.25-fold at 15, 30 and 45 days after pathogen inoculation as compared to control plants. Similarly, phenol content was also significantly higher in salicylic acid treated plants, representing 1.5-fold, 2.1-fold and 2.7-fold at 15, 30 and 45 days of inoculation as compared to the control plants. Correlation coefficient analyses revealed that there was negative correlation between disease severity, soluble protein and polyphenol contents after 15, 30 and 45 days of treatment. Protein profiling by SDS-PAGE revealed that salicylic acid induced the synthesis of new proteins. The genomic DNA integrity was confirmed by obtaining of unique RAPD banding patterns for treated and untreated plants.

Conclusion: Overall, a pre-treatment of olive plants by salicylic acid lead to low disease severity and suppress the fungus pathogenicity through overexpression of proteins and polyphenol compounds involved in plant defense.

Keywords: Olea europaea; soluble proteins; polyphenols; chemical induction; SDS-PAGE; RAPD.

1. INTRODUCTION

Olive (*Olea europaea*) is considered as one of the most important tree species cultivated throughout the Mediterranean basin due to its high nutritive value as well as its antioxidant and curative properties. In Tunisia, olive oil industry plays a central economic role with an annual currency income of 1.4 million US dollar [1]. However, olive crop is threatened by many pest and diseases that could notably affect the yield and quality of olive oil. Verticillium wilt of olive caused by the soil-borne fungi *Verticillium dahliae* is one of the most diseases affecting olive in Tunisia. This disease was first reported in southern Tunisia and then in other olive growing regions where it causes severe yield losses [2,3]. However, this wilt is difficult to manage and there are no efficient treatments that could limit its expansion. Nevertheless, the management of the disease can be performed through combination of cultural, chemical, biological control and use of resistant cultivars [4]. But most of the conventional chemical, biological and use of resistant cultivars tend towards the direct control of the pathogen by its elimination. Sometimes, these control measures raise problem due to development of resistant strains of the pathogen which may become very difficult to control. To overcome these problems, search for new approaches for managing the disease are explored. One of the best strategies developed to manage various diseases is induced resistance [5]. It has been found that pre-application of tomato seedling with biological agents such as endophytic bacteria, plant extracts, non-pathogenic races of pathogens and some inorganic chemical like phosphate salt, silicon provided the systemic induced resistance in various crops [6]. Biochemical and physiological changes associated with induction of resistance are due to the response to inducing agents which are in the form like phytoalexins, lignin [7], callose [8] and plant pathogenesis related proteins (PRP) [9]. Inducers also lead to formation of additional secondary xylem vessels in plant system [10]. These observations led to exploration in the present investigation.

Such question could be addressed by investigating the response of the extremely susceptible cultivar Chemlali treated with salicylic acid after infection *V. dahliae* infection. This will provide useful information about the reliability of induced systemic resistance in the control of VWO and thus contribute to design better disease management programs in this region. Therefore, the present study aimed to (i) Investigate the development of wilt symptoms and progression of disease severity in plants treated with salicylic acid (ii) estimate the polyphenols and soluble proteins contents before and after salicylic acid treatment and (iii) assess the effect of salicylic acid treatment on genomic DNA integrity.

2. MATERIALS AND METHODS

2.1 Pathogen Isolation and Identification

Stems and roots of olive plants with typical wilt symptoms were collected from different olive growing regions in Tunisia. The diseased tissue plants were immediately packed in polythene bags and transported to the laboratory for initial diagnosis. The samples were immediately stored at temperature ranging between 4 and 8℃ until subsequent analysis. The diseased plant's stem and root was removed from the polyethylene bags, rinsed twice with sterile distilled water to remove all soil particles. The surface of the tissue was then sterilized using 75% ethanol solution and air dried at room temperature. The diseased part of the stem or the root was then

cut into small pieces (0.5 cm) by a sterile scalpel. The obtained tissues pieces were transferred into Potato Dextrose Medium (PDA) and incubated for 7 days at 25℃. Colonies rising from plant tissues were transferred into new PDA medium for purification and fungal isolates were subjected to morphological and molecular identification as described by Gharbi et al. [3].

2.2 Plant Material

Two-years-old olive rooted cutting belong to the cultivar Chemlali were used in this study. This cultivar was previously assessed for resistance to VWO under controlled conditions using an artificial infection bioassay and was classified as extremely susceptible to this wilt. All cuttings were obtained from genetically authenticated mother plants from the Olive tree Institute (OTI). All plants were potted in plastic bags containing (50% peat; 50% sand) and placed in a greenhouse and irrigated weekly until subsequent use.

2.3 Salicylic Acid Solution Preparation and Plant Treatment

Salicylic acid solution was prepared immediately before application. Thus, salicylic acid (Sigma-Aldrich, Lyon, France) was applied at a final concentration of 10 mM. Salicylic acid solution was prepared by dissolving 0.13 g of the chemical inducer in 100 ml sterile distilled water. Conical flasks containing the mixture were placed under stirring condition until complete dissolution. Olive plants were treated two times before inoculation at 48 hours intervals. Application of salicylic acid was performed by spraying 20 ml on the both leaves sides.

2.4 Plant Inoculation and Disease Assessment

Pathogen inoculation was performed as previously described by Gharbi et al. [3]. The experiment was conducted in a greenhouse under controlled conditions (23℃±2℃; 16/8 h of light/dark period. The conidial suspension of each isolate was prepared from 10 days old cultures on PDA and adjusted to 10^6 conidia/ml. Plant roots were dipped for 1 h in the conidial suspension and then transplanted into new polyethylene pots containing a sterile substrate (peat: sand, 1:1 v/v). The experiment was arranged in a completely randomized block with three replicates and three control plants dipped in sterile distilled water (SDW). In order to ascertain the effect of salicylic acid on disease development, disease severity was assessed weekly, starting 15 days after inoculation. A scale from zero to four was used according to the percentage of affected plant tissue, in which, zero = healthy plant; one = ≤ 33% affected tissue; two = 34-66% affected tissue; three = 67-99%; four = dead plant). Estimation of the area under disease progress curve (AUDPC) was calculated as described previously by Rodriguez-Juardo et al. [11]. Statistical analysis of variance was performed using SPSS software to determine the variability among the isolates.

2.5 Biochemical Analysis Response of Olive Plants after Salicylic Acid Treatment

Biochemical changes in olive plant upon foliar spray with salicylic acid were monitored in order to determine the effect of this inducer agent on the contents of soluble protein and phenol in the plant. Olive leaves were collected before and after treatment at different time points. Thus, the changes in the content of soluble protein and phenol in leaves were estimated at 15, 30 and 45 days after inoculation by *V. dahliae*.

2.6 Soluble Protein Content

Olive leaves were collected from plants sprayed with salicylic acid. It was washed with distilled water several times and dried on blotter paper. A quantity of 1 g of each sample was cut into small pieces and ground in pestle and mortar using extraction buffer [50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 100 µM PMSF and 2% PVP (w/v)] as 1:5 (w/v). The suspension was centrifuged at 10,000 rpm for 30 min at 4℃. The supernatant was collected and used for quantification and profiling of protein.

2.7 Quantification of Soluble Proteins

The total proteins content was determined according to the protocol described by Bradford [12]. Briefly, 0.5 g of fresh leaves were placed in a grinding medium containing 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 100 µM phenylmethylsulfonyl fluoride (PMSF) and 2% (PVP) (w/v). Bovine serum albumin was used as a standard.

2.8 Polyphenols Extraction and Quantification

The accumulation of phenols in tomato seedlings was estimated following the procedure

developed by Sofo et al. [13] with little modification. Polyphenol compounds were extracted from leaves and roots using the Folin-Ciocalteu method. The total phenolic content was determined by measuring the absorbance of the mixture at 760 nm. A standard curve was prepared with different concentrations of gallic acid (GA) (Sigma, Lyon, France) ranging from one to 200 mg/L. The mean of three measures was calculated and the total phenolic content was expressed in mg of GA equivalents (GAE)/100 g of FW.

2.9 Correlation Coefficient and Regression equation

The biochemical analysis of olive leaves under salicylic acid treatment and disease incidence expressed as AUDPC values was performed to determine the level of correlation coefficients (r) between soluble protein and disease incidence as well as between total phenol and disease incidence. Simple regression equations were also developed for both the variables separately to define their relation with disease incidence.

2.10 Genomic DNA Extraction

Plant genomic DNA was extracted from stems and leaves of treated and control plants. Hundred mg of plant tissue were thoroughly ground in liquid nitrogen and suspended in 200 µl sterile distilled water. The total volume was then extracted by ZR Plant/Seed DNA mini prep D6020 Kit (Zymo Research, Irvine, CA, USA) as recommended by the manufacturer. The extracted DNA was re-suspended in 50 µl of elution buffer. DNA elution was quantified using a Nanodrop, ND-1000 spectrophotometer (Thermo scientific, Quebec, Canada). DNA samples were also analyzed in a 0.8% agarose gel to check their quality and finally stored at -20℃ until subsequent analysis.

2.11 RAPD Analysis

Eight primers of different size were tested and those resulting in visible, reproducible and well defined bands were selected (Table 1). PCR reactions were carried out in 25 µl final volumes, containing 50 ng of plant DNA, 2 mM $MgCl_2$, 200 µM of each dNTPs, 0.25 µM of each primer and 1 U of Taq DNA polymerase (Invitrogen, Toronto, Canada). Amplifications were carried out in a DNA Thermal Cycler (Biorad, Ontario, Canada) under the following conditions: initial denaturation cycle of 3 min at 95℃, followed by 34 cycles of 95℃ for 1 min, annealing for 1 min at 35℃ and extension for 2 min at 72℃, with a final extension period of 6 min at 72℃. Tubes containing all reaction components except for template DNA were included as controls. Amplification products were observed in ethidium bromide-stained 1.5% agarose gels, visualized under UV light, and photographed using a gel documentation system HP (Protein Simple, California, USA).

Table 1. List of RAPD primers used in this study

Primer name	Primer sequence
OPA-1	CAGGCCCTTC
OPA-3	AGTCAGCCAC
OPB-5	TGCGCCCTTC
OPB-7	GGTGACGCAG
OPC-17	TTCCCCCCAG
OPE-02	GGTGCGGGAA
OPH-02	TCGGACGTGA

3. RESULTS

The present study was performed to evaluate the potential of salicylic acid as systemic resistance inducer in olive against Verticillium wilt caused by *V. dahliae*. Biochemical changes in response to induction of resistance in olive plants after treatment by abiotic inducers were assessed. Results of the experiments are presented below.

3.1 Effect of Salicylic Acid Treatment on Development of Disease

The effect of pre-foliar spray of salicylic acid on olive plants revealed that there is a decrease in wilt incidence due to the pre-treatments under greenhouse condition (Fig. 1). Inoculated untreated plants belong to the susceptible olive cultivar, Chemlali showed 91,96% wilt incidence. By contrast, inoculated treated plants have shown a significantly reduced disease severity, which was around 29.33%. The minimum wilt incidence was recorded after 45 days were disease severity was reduced by 3.2-fold as compared to the inoculated untreated plants. The decrease in disease incidence might be the activity of the inorganic inducer, which act as systemic resistance activator in olive against *V. dahliae* and other pathogens.

3.2 Biochemical Changes Associated with Foliar Spray of Chemicals

To evaluate the biochemical changes associated with foliar spray, two-year old plants were sprayed with solutions of salicylic acid. The

pathogen was inoculated after 48 hours of foliar spray. The soluble protein and total phenol content were estimated at 15 days, 30 days, and 45 days of pathogen inoculation.

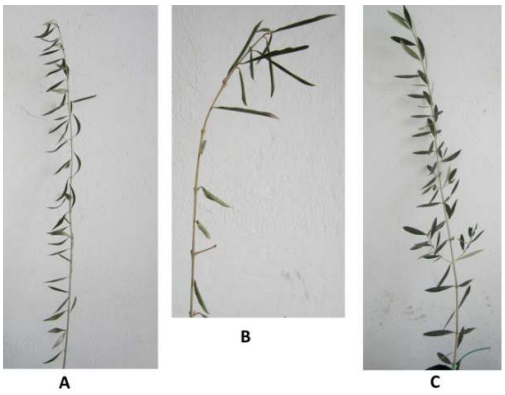

Fig. 1. Symptoms of Verticillium wilt recorded after 3 months of infection by *V. dahliae*: (A) inoculated and pre-treated salicylic acid plants; (B): Inoculated untreated plant; (C): Control uninoculated plants

3.3 Total Soluble Protein

Quantification of soluble proteins in salicylic acid treated plants and untreated ones revealed that the treatment significantly induce the accumulation of soluble proteins. Indeed, soluble protein contents in salicylic acid treated leaves were 43.25 mg/g, 54.23 mg/g and 65.25 mg/g of fresh leaves at 15, 30 and 45 days of pathogen inoculation which represent an increase of 1.5-fold, 1.87-fold and 2.25-fold as compared to the control plants (Fig. 2). In the untreated inoculated plants, soluble protein contents were 29.50 mg/g, 37.61 mg/g and 42.31 mg/g at 15, 30 and 45 days after pathogen inoculation, which represent an increase of 1.0-fold, 1.274-fold and 1.434-fold as compared to the control plants. Overall, obtained results revealed that salicylic treatment increased protein content to a maximum at 45 days of pathogen inoculation, thereafter; it was decreased gradually from 45 days. The increased protein content in treated plants might be responsible for defense response in plants.

3.4 Total Polyphenols Content

Quantification of total phenols in salicylic acid treated plants and untreated ones revealed that the treatment significantly induce the accumulation of soluble proteins. Indeed, soluble protein contents in salicylic acid treated leaves were 32.93 mg/g, 46.11 mg/g and 67.98 mg/g of fresh leaves at 15, 30 and 45 days of pathogen inoculation which represent an increase of 1.5-fold, 1.8-fold and 1.92-fold as compared to the control plants (Fig. 3). In the untreated inoculated plants, soluble protein contents were 21.95 mg/g, 25.62 mg/g and 35.41 mg/g at 15, 30 and 45 days after pathogen inoculation, which represent an increase of 1.05-fold, 1.22-fold and 1.68-fold as compared to the control plants. Overall, obtained results revealed that salicylic treatment increased protein content to a maximum at 30 days of pathogen inoculation, thereafter; it was decreased gradually from 45 days. The increased protein in treated plants might be responsible for defense response in plants.

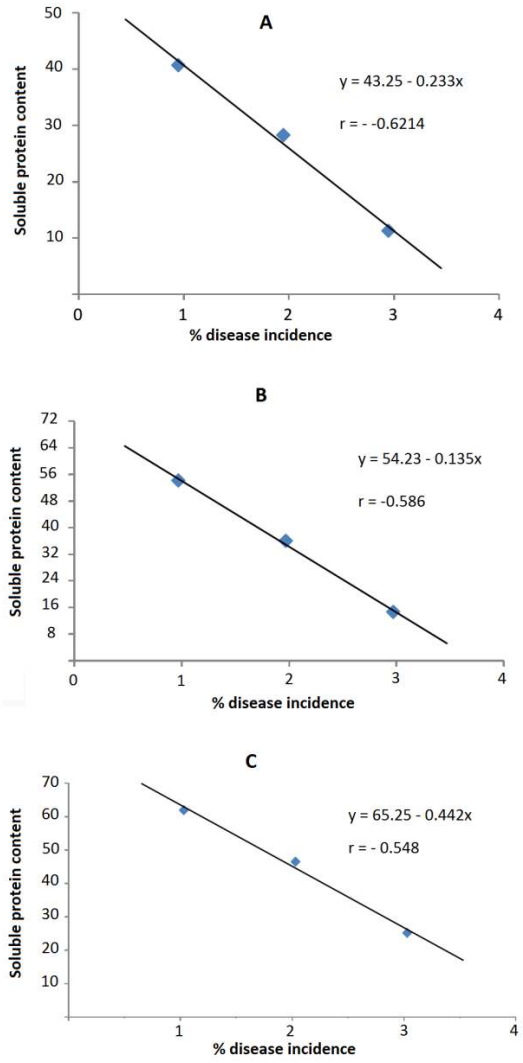

Fig. 2. Correlation between disease severity progress and the soluble protein contents at 15 days (A), 30 days (B) and 45 days (C) after inoculation by *V. dahliae*

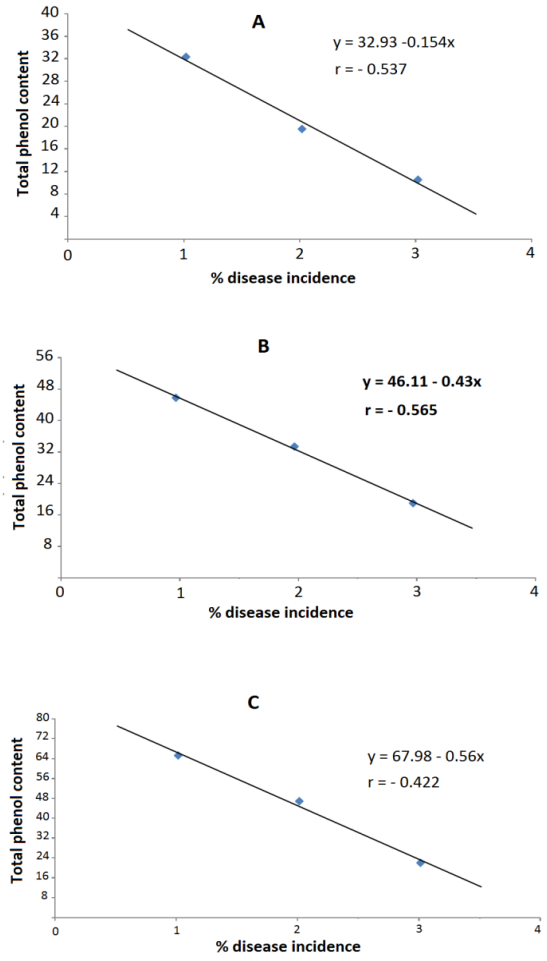

Fig. 3. Correlation between disease severity progress and the total polyphenol contents at 15 days (A), 30 days (B) and 45 days (C) after inoculation by *V. dahlia*

3.5 Genomic DNA Integrity

The olive genomic DNA has been purified from leaves and stems of olive plants treated with salicylic acid and the untreated ones. In the present study, eight RAPD primers are employed separately to evaluate the DNA integrity after treatment. Amplification results revealed the presence of 10 molecular markers per each sample (Fig. 4). Overall, a total of 35 molecular markers ranging between 100 and 1000 bp were identified using all the tested primers. These markers were all monomorphic and were well defined in all samples representing different time points of the experiment. Therefore, it may be concluded that salicylic acid treatment could induce the systemic resistance of olive plants without any DNA damage.

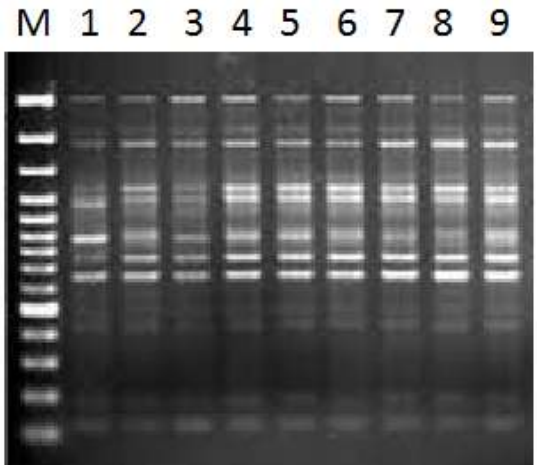

Fig. 4. Random amplified polymorphic markers (RAPD) patterns of *Olea europaea* obtained by OPA1 primer. (M: DNA ladder 1 kb; 1, 2 and 3: control plants at 15, 30 and 45 days; 4, 5 and 6: pre-treated inoculated plants at 15, 30 and 45 days; 7, 8 and 9: inoculated untreated plants at 15, 30 and 45 days after infection)

3.6 Protein Profiling

Protein profiling of soluble protein from fresh olive leaves was performed to determine whether any new protein was associated with resistance to *V. dahliae* in olive or not due to the treatment by the salicylic acid as inducers. SDS PAGE was used to define the banding patterns of proteins. The banding patterns of soluble proteins representing different time points of the treatment are shown in Fig. 5. The number of protein bands present in each treatment range from 6 to 13. The maximum number of bands is obtained from salicylic acid treated plants followed by untreated ones. The banding pattern of proteins revealed that some new proteins are synthesized in salicylic acid treated plants which were not found in the untreated ones. The occurrence of new protein bands might be due to the activities of abiotic inducers in plant which may also be key factor for defense mechanism in tomato against *V. dahliae.*

3.7 Correlation Coefficient and Regression Equation

The leaves treated with salicylic acid as inducer of systemic resistance showed decreased disease incidence with increased level of soluble protein. A negative correlation (r), -0.6214, -0.5867 and -0.5484 at 15, 30 and 45 days

respectively was found between disease incidence and soluble protein content. Similarly, diseased incidence decreased with increased level of total phenol content and there was also a negative correlation (r) -0.5370, -0.5656 and -0.4225 at 15, 30 and 45 days between the total phenol content and disease incidence. The corresponding simple regression equation also showed the negative correlation between total soluble protein and disease incidence as well as total phenol and disease incidence.

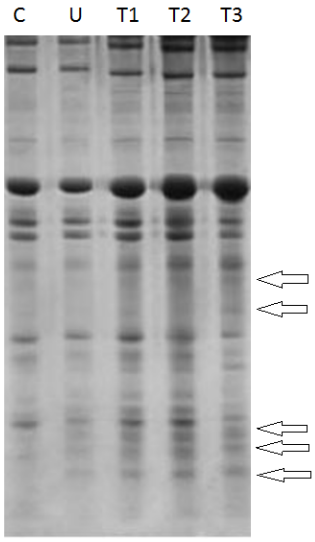

Fig. 5. Banding patterns of soluble proteins in different treatments (C: control plants; U: untreated inoculated plants; T1: pre-treated inoculated plants at 15 days; T2: pre-treated inoculated plants at 30 days; T3: pre-treated inoculated plants at 45 days)

4. DISCUSSION

Plant disease resistance can be defined as the ability of the plant to prevent or restrict the pathogen growth and multiplication. All plants, ever they are resistant or susceptible, react to pathogen attack by the induction of well-orchestrated resistance strategies. Early activation and amplification of the plant responses by the application of systemic resistance inducers could provide a biologically, environmentally and commercially valuable alternative to the existing pathogen control methods. Induced resistance prior to pathogen infection increase the level of some defense compounds and sensitize the plants to rapidly produce some compounds after infection and thereby, provide protection against the disease. This alternative was widely used to induce a systemic resistance in other corps. For instance, the molecule Ciba-Geigy was successfully applied in cucumber plants against *C. lagerarium* and was attributed to the major accumulations of protein including chitinase and chitinase mRNA [14]. Kinetin molecule was also applied to induce systemic resistance in wheat and has resulted in in an increase of soluble proteins in treated leaves compared to the untreated ones [15]. Induction of resistance in tomato seedlings against verticillium wilt of olive was also performed by combination of chitosan treatment and endophytic bacteria [16].

Polyphenols are well known as antifungal, antibacterial and antiviral compounds. The phytoalexins involved in disease resistance are classified as polyphenols compounds based on their chemical composition. In fact, polyphenols are involved in disease resistance in many defense pathways like the hypersensitive cell death or the lignification process of cell walls [17,18]. Higher polyphenols content were previously reported in other studies. For instance, high polyphenols content were recorded in soybean after inoculation with *H. carbonum* as compared to the control plants [19]. It has also been reported that rice plants exhibited high polyphenols contents after infection by *Helminthosporium oryzae* as compared to the control plants [20].

The negative correlation coefficient between soluble protein and polyphenol contents with disease incidence were also reported in rice against brown leaf spot and in wheat against spot blotch [21,22]. The profiling of soluble protein by SDS-PAGE revealed the qualitative and quantitative differences by comparing the pattern of soluble proteins among the different treatments. For instance, it has been reported that some new proteins were associated with resistance to *Bipolaris sorokiniana* after induction by total crude extracts of the fungal endophyte *Chaetomium globosum*. Indeed, some new proteins of 110 kDa, 105 kDa, 32 kDa, 35 kDa and 38 kDa resolved by SDS PAGE analysis were exclusively presents in treated plants while they are missing in the untreated ones [23]. In fact, it has been reported that PR-proteins are involved in the defense of plants against pathogens [24]. Induction of systemic resistance in tobacco after inoculation with *Pseudomonas tabaci* was followed by an increase in concentration of PR proteins [25]. A 23 kDa protein was detected

in leaves of tobacco which was previously immunized with TMV [26].

In another study aiming to decipher the role of *Trichoderma hamatum* in the induction of systemic defense of tomato plants, authors have identified 45 genes to be differentially expressed across the replicated treatments and 41 of these genes could be assigned to at least one of seven functional categories such as biotic or abiotic stress, as well as RNA, DNA and protein metabolism.

5. CONCLUSION

In conclusion, the results of the present studies confirm that the role of salicylic acid in plant disease resistance is complex and varies depending upon type of cultivars and plant-pathogen interactions. As the exact impact of salicylic acid on olive-*V. dahliae* interaction was not known, we have investigated the effect of salicylic acid on the basal resistance of olive to *V. dahliae*. Furthermore, we found that 10 mM salicylic acid gave the best effect to control VWO in greenhouse conditions. Based on both polyphenols and soluble proteins contents, we confirmed that salicylic acid played a positive role in the defense against *V. dahliae*. Results presented in this study provide support for the incorporation of salicylic acid as plant activator in management of fungal disease. These results will greatly contribute to improvement of plant resistance thorough genetic breeding and genetic manipulation by plant breeders.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. National Olive Office. Positionnement de la Tunisie sur le marché mondial d'Huile d'Olive; 2015. Available:http://www.onh.com.tn/index.php/fr/positionnement-de-la-tunisie-sur-le-marche-mondial-d-huile-d-olive (Accessed 20 November 2015)
2. Triki MA, Hassairi A, Mahjoub M. Premières observations de *Verticillium dahliae* sur olivier en Tunisie. EPPO Bull. 2006;36:69–71.
3. Gharbi Y, Triki MA, Trabelsi R, Fendri I, Daayf F, Gdoura R. Genetic structure of *Verticillium dahliae* isolates infecting olive tree in Tunisia using AFLP, Pathogenicity and PCR markers. Plant Pathol. 2015;64: 871-879.
4. Jiménez-Díaz RM, Cirulli M, Bubici G, Jiménez-Gasco MM, Antoniou PP. Verticillium wilt, a major threat to olive production: Current status and future prospects for its management. Plant Dis. 2012;96:304-329.
5. Mercado-Blanco J, Collado-Romero M, Parrilla-Araujo S, Rodríguez-Jurado D, Jiménez-Díaz RM. Quantitative monitoring of colonization of olive genotypes by *Verticillium dahliae* pathotypes with real-time polymerase chain reaction. Physiol. Mol. Plant. Pathol. 2003;63:91–105.
6. Fuchs JG, Moenne-Loccoz Y, Defago G. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. Plant Dis. 1997;81:492-496.
7. Brown SA. Lignin and tannin biosynthesis. In: Biochemistry of phenol compounds: Academic Press, London and New York; 1964.
8. Hinch JM, Clark AE. Callose formation in *Zea mays* as a response to infection with *Phytophthora cinnamorn*. Physiol Plant Pathol. 1982;21:113-124.
9. Van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria. Ann Rev of Phytopathol. 1998;36:453-483.
10. De Cal A, Garcia-Lepe R, Melgarejo P. Induced resistance by *Pencillium oxalicum* against *Fusarium oxysporum* f. sp. *lycopersici*; Histological studies of infected and induced tomato stems. Phytopathol. 2000;90:260-268.
11. Rodriguez-Jurado D, Blanco-Lopez MA, Rapoport HF, Jimenez-Diaz RM. Present status of verticillium wilt of olive in Andalusia (southern of Spain). EPPO Bull. 1993;23:513-516.
12. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Annals of Biochemistry. 1976;72:248-254.
13. Sofo A, Dichio B, Xiloyannis C, Masia A. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during re-watering in olive tree. Plant Sci. 2004;166:293-302.
14. Metraux JP, Streit L, Staub Th. A pathogenesis related protein in cucumber

is a chitinase. Physiol Mol Plant Pathol. 1988;33:1-9.

15. Kaul K, Sabharwal PS. Effects of sucrose and kinetin on growth and chlorophyll synthesis in tobacco tissue cultures. Plant Physiol. 1971;47(5):691-695.
16. Bonhamou N, Kloepper JW, Tuzun S. Induction of resistance against fusarium wilt of tomato by combination of chitosan with an endophytic bacterial strain: Ultra-structure and cytochemistry of the host response. Planta. 1998;204:153-168.
17. Nicholson RL, Hammerschmidt R. Phenolic compound and their role in disease resistance. Ann Rev Phytopathol. 1992;30:369-389.
18. Kumawat GL, Biswas SK, Srivastava SSL. Biochemical evidence of defense response in paddy induced by bio-agents against brown leaf spot pathogen. Indian Phytopathol. 2000;61.
19. Biehn WL, Kuc J, William. Accumulation of phenols in resistant plantfungi interaction. Phytopathol. 1968;58:1255-1260.
20. Vidhyasekaran P, Ramadoss N, Ranganathan K, Krishnasamy V. Increase in protein content of rice due to Helminthosporium infection (*Helminthosporium oryzae*). Indian Phytopathol. 1973;26:736-738.
21. Lai GK, Biswas SK, Rajik M. Antagonistic evaluation of *Trichoderma* spp. and their effect on seed germination and growth of paddy seedling. J Plant Dis Sci. 2010;5: 203-207.
22. Mishra VK, Biswas SK, Rajik M. Biochemical mechanism of resistance to Alternaria blight by different varieties of wheat. Int J Plant Pathol. 2010;2:72-80.
23. Biswas SK, Srivastava KD, Aggarwal R, Praveen S, Singh DV. Biochemical changes in wheat induced by *Chaetomium globosum* against spot blotch pathogen. Ind Phytopathol. 2003;56(4):374-379.
24. Antoniw JF, Ritter CE, Pierpoint WS, Van Loon LC. Comparison of three pathogenesis related proteins from plants of two cultivars of tobacco infected with *TMV*. J Gen Virol. 1980;47:79-87.
25. Tuzun S, Rao MN, Vogeli U, Scharde CH, Kuc J. Induction of systemic resistance to blue mold: Early induction and accumulation of β-1,3-glucanases, chitinases and other pathogenesis related proteins (PR-proteins) in immunized tobacco. Phytopathol. 1989;79:979-983.
26. Spiegel S, Gera A, Salomon RAWP, Harlap S, Loebenstein G. Recovery of an inhibitor of virus replication from the intercellular fluid of hypersensitive tobacco infected with *TMV* and from uninfected induced resistance tissue. Phytopathol. 1989;79:258-267.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13523