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Phenotyping and expression profile of clones of the olive cultivar Leccino for the resistance to *Xylella fastidiosa*

Raied Abou Kubaa [a], Annalisa Giampetruzzi [a,*], Carmine Del Grosso [a], Serafina Serena Amoia [a], Giovanni Caruso [c], Susanna Bartolini [d], Giuseppe Altamura [e], Antony Surano [a,b], Pasquale Saldarelli [a,**]

[a] Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche (IPSP CNR), Bari, Italy
[b] Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA), Università degli Studi di Bari, Bari, Italy
[c] Dipartimento di Scienze Agrarie, Alimentari, Agro-Ambientali, Università di Pisa, Pisa, Italy
[d] Istituto di Produzioni Vegetali, Scuola Superiore Sant'Anna, Pisa, Italy
[e] Centro Ricerche e Sperimentazioni in Agricoltura «Basile Caramia», Locorotondo, Bari, Italy

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ABSTRACT

The severe epidemic of *Xylella fastidiosa* subspecies *pauca* ST53 in olive in Apulia, southern Italy, prompted to evaluate the adoption of resistant germplasm as a possible strategy of coexistence with the bacterium. To date, resistance traits have been identified in four olive cultivars and, among them, Leccino reacts to infections with limited desiccation and a better physiological response, while hosting low populations of the bacterium. In this study, seven Leccino clones were experimentally inoculated in replicates with the Apulian isolate of *X. fastidiosa* subsp. *pauca* and compared over time for the physiological and phenotypic response. At sixteen months after inoculation, the percentages of infected plants ranged from 57.1 % to 100 % among the clones, whose severity of desiccation was variable, causing in four of them 16 %–40 % of dead plants. Measurements over time of stomata conductance and stem water potential indicate that all clones perform better than the susceptible cultivar Cellina di Nardò, although showing a different clone adaptation to the drought stress imposed by the bacterium. Forty-eight hours post-inoculation clones differently perceived the bacterium, responding with the expression of genes related to plant immunity, cell wall remodeling or secondary metabolism. The work addresses the study of Leccino resistance to *X. fastidiosa* subsp. *pauca* with different approaches contributing to the knowledge of the mechanisms of resistance and identifying possible molecular targets useful for the screening of olive germplasm and the exploitation of assisted breeding strategies.

1. Introduction

Isolates of *Xylella fastidiosa* subspecies *pauca* ST53 were detected in olives in 2013 in Salento, Apulia (southern Italy), causing the Olive Quick Decline Syndrome (OQDS), a disease characterized by severe desiccations of susceptible local cultivars such as Cellina di Nardò and Ogliarola salentina [1,2]. The occurrence of large populations of the insect vector *Philaenus spumarius,* and the extension of the initial focus, caused the rapid spread of the bacterium in an epidemic form and the death of centennial trees having a great landscape importance [3–5]. This quarantine Gammaproteobacterium, belonging to the family *Lysobacteraceae* [6], is the agent of several severe diseases in economically important crops, such as grapevine and citrus, as well as species of forestry interest [7–9].

From the time of its first discovery in Italy, intense research programs have allowed to deeply characterize the bacterial genotype associated with OQDS [2,10] and its interactions with olives [11]. Notably, phenomena of bacterial resistance have been documented in cultivated olive varieties [12], with the cultivar Leccino being the most extensively studied, as the one displaying the most consistent resistant phenotype

* Corresponding author.
** Corresponding author.
E-mail addresses: raedsir@hotmail.com (R. Abou Kubaa), annalisa.giampetruzzi@cnr.it (A. Giampetruzzi), carmine.delgrosso@cnr.it (C. Del Grosso), serafinaserena.amoia@cnr.it (S.S. Amoia), giovanni.caruso@for.unipi.it (G. Caruso), susanna.bartolini@sssup.it (S. Bartolini), giuseppealtamura@crsfa.it (G. Altamura), suranoantony@gmail.com (A. Surano), pasquale.saldarelli@cnr.it (P. Saldarelli).

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under conditions of experimental and natural infection [12–14]. Research efforts to study resistance mechanisms have followed different routes, with the aims to describe the mechanism(s) and to identify resistant genes to be exploited in breeding activities in olive, to extend the currently available limited number of resistant cultivars (http://www.emergenzaxylella.it/portal/portale_gestione_agricoltura/Documenti/normRegionale/PortalXylellaNormativaRegionaleIstanceWindow?IDNEWS=788&action=e&windowstate=normal&mode=view&ACTION_NEWS=DETAIL). The outputs of these studies indicate that Leccino resistance is due to genetic features, a resilient microbiome and a xylem anatomy that limit the spread of the bacterium [11,15–17]. Indeed, initial gene expression studies showed that the cultivar Leccino response to *X. fastidiosa* subsp. *pauca* involves cell wall receptors likewise Leucine Rich Repeat Receptor-like kinases (LRR-RLK), previously associated to the response to *Xylella* infections in citrus [18] and in grapevine [19], whose orthologous genes are present in grapevine for the Pierce's disease (PD) resistant locus 1 (PDR1) [20,21]. A resilient microbial community represents another host component contributing to counteract bacterial infections, since minor alterations of the microbiome were detected in the resistant *vs* the susceptible cultivars [22,23].

Recently, the attention has been focused on the physiology and anatomy of olive cultivars in relation to *X. fastidiosa* subsp. *pauca* infections. Like in grapevine [24], the occurrence of vessels of small diameters has been invoked [25,26] as responsible of the cultivar Leccino resistance, allowing this cultivar to be more prone to compartmentalize the bacterium in the colonized vessels and less subject to embolism phenomena. Recent X-ray computed tomography-based studies have better characterized Leccino xylem geometry, showing that the distribution of vessel diameters, rather than the stem-average vessel diameters, plays a role in resistance [27]. The authors suggest that the cultivar Leccino has a lower proportion of larger vessels making this cultivar less susceptible to air embolism than the susceptible cultivar Ogliarola salentina. These anatomical features directly correlate with the physiological responses, since water conductivity in infected hosts is affected by the bacterial biofilm formation and the degradative activity by the cell wall degrading enzymes. As already observed in the PD-affected grapevines [28], Pit Membranes (PM) of the susceptible cultivar Cellina di Nardò appear more degraded than those of the cultivar Leccino during *X. fastidiosa* subsp. *pauca* infection [17]. Moreover, it seems that the cultivar Leccino is able to entrap the bacterium in a callose-like matrix [17]. Such complex mechanisms impairing the xylem hydraulics of susceptible plants, can be measured by the changes of the physiological parameters such as stomata conductance and stem water potential. It was showed that infected cultivars Leccino and FS17 suffer to a lower extent the water stress induced by the bacterial multiplication and colonization, than the cultivar Cellina di Nardò and that these physiological parameters can be exploited as early markers for the screening of olive genotypes for *X. fastidiosa* subsp. *pauca* resistance [16].

This wealth of studies has been recently corroborated by the demonstration that the cultivar Leccino resistant traits can be transmitted to the cross-bred progenies, as shown by analyzing the open-pollinated seedlings surviving the OQDS epidemics in the affected area of the Apulia region [14]. To further advance the knowledge about the defense mechanisms acting in the Leccino cultivar, and to widen the number of olive cultivars resistant to *X. fastidiosa* subsp. *pauca*, in this work it was explored the *X. fastidiosa* subsp. *pauca* -response of seven clones identified in the frame of a selection program within the olive cultivar. These Leccino clones have been selected for desirable agronomical characteristics such as a low tendency to alternate bearing, consistent fruiting, good fruit size, and environmental adaptability [29,30].

In trials carried out under greenhouse conditions, the seven clones showed different responses upon experimental infections as highlighted by the analysis of transcriptome profiles and through the assessments of *X. fastidiosa* subsp. *pauca* infection and measurements of the xylem water conductivity.

2. Materials and methods

2.1. Plant material and growing conditions

The study was carried out from January 2021 to October 2023 (Fig. 1B) in the greenhouse and laboratory facilities located at the CNR-IPSP/University of Bari. The behavior of seven clones of the olive cultivar Leccino (*Olea europaea* L.), developed as part of a joint breeding program between the two University institutions of Pisa (Dipartimento di Scienze Agrarie, Alimentari, Agro-Ambientali, and Istituto di Produzioni Vegetali, Scuola Superiore Sant'Anna), were evaluated. Clones L1.1, L1.3, L1.6, L1.10, and L1.13 are registered in the Italian National Register of Fruit Plant Varieties of the Ministry of Agriculture (Fig. 1A). Two more registered clones, namely L1.7 and L1.15, were also included in the study. To simplify the greenhouse manipulation of the plants, the seven clones were identified by color identifiers (Fig. 1A). Self-rooted plants were grown in 3.4 lt pots in a peat substrate with a pH in H_2O of 6.5, and an electrical conductivity of 0.4 dS/m. They were maintained under insect proof greenhouse conditions at a constant temperature of 25 °C and a relative humidity of 65 % for 24 months after the *X. fastidiosa* subsp. *pauca* inoculation. Measurements of stomatal conductance and stem water potential were made at field capacity, corresponding to a soil water potential (Ψsoil) of $-0.02 < \Psi soil < -0.03$ MPa.

2.2. X. fastidiosa subsp. pauca inoculation and detection

The trial was performed on 6 to 9 plants *per* clone (Fig. 1A) which were indicated by different IDs: AR; VE; BL; RO; RA; Bi; Vi. In detail, 6 BL, 8 AR, VE, Bi, RO and Vi, and 9 RA olives were used for the trial. Two plants *per* clone were inoculated only with Phosphate Buffer Saline (PBS 10 mM pH 7.2) (in normal typing in Fig. 1A), while the remaining olives were inoculated with *X. fastidiosa* subsp. *pauca* (in bold typing in Fig. 1A). Six control olives of the cultivars Leccino and Cellina di Nardò [16] were included in the trial for comparison, of which three were inoculated and three were used as mock non-inoculated control, respectively indicated in bold and normal typing in Fig. 1A. Experimental inoculations were made by the "pin prick" method [2] using as inoculum a 10^9 cells/ml suspension of *X. fastidiosa* subsp. *pauca* strain De Donno (DD; CFBP 8402) scraped from an 8-10-days-old agar culture in PBS. A 10 μl drop of water-suspended cells was pipetted on young twigs and immediately punctured with a sterile needle to allow bacterial entrance. Three to five twigs *per* plant were inoculated. Pin prick inoculations were carried out on the same plants on January 02, 2021, December 03, 2021 and 07/15/2021 (Fig. 1B). Inoculations of the Leccino clones were further performed on the same plants by top-grafting on the apex of the plant shoots harvested from field-grown infected trees of the cultivar Ogliarola salentina on 04/15/2021. Graft inoculation was repeated on 09/23/2021 for plants where the first graft did not survive. Overall, the Leccino clones were inoculated three times by the "pin prick" method and two times by grafting (Fig. 1B), while control Leccino and Cellina di Nardò cultivars only once by "pin prick". All control (mock) olives were only inoculated with PBS by needle.

2.3. Measurements of stem water potential and stomatal conductance

A total of six plants were used for these assessments: four *X. fastidiosa* subsp. *pauca*-infected and two healthy control plants were used for each Leccino clone, while three infected and three non-infected plants of the Leccino and Cellina di Nardo were used as control for comparison. Stem water potential (Ψstem) was assessed using the Scholander-type pressure chamber Soil Moisture 3000 (Soil Moisture Corp., Goleta, CA, USA). Starting from four months after the last inoculation, five sequential measurements were performed (Fig. 1B) on 01/31, 02/16, 03/03, 03/16

A

Clones ID:	L1.10	L1.15	L1.13	L1.7	L1.1	L1.3	L1.6	Control cvs.	
RNAseq ID:	AR	VE	Bi	RO	BL	RA	Vi	cv. Leccino	cv. Cellina
Plants for RNAseq	AR1	VE1	Bi1	RO1	BL1	RA1	Vi1	LC1	CL1
	AR2	VE2	Bi2	RO2	BL2	RA2	Vi2	LC2	CL2
	AR3	VE3	Bi3	RO3	BL3	RA3	Vi3	LC3	CL3
	AR4	VE4	Bi4	RO-4	BL4	RA4	Vi4	LC4	CL4
Plants for RNAseq	AR5	VE5	Bi5	RO5	BL5	RA5	Vi5	LC5	CL5
	AR6	VE6	Bi6	RO6	BL6	RA6	Vi6	LC6	CL6
	AR7	VE7	Bi7	RO7		RA7	Vi7		
	AR8	VE8	Bi8	RO8		RA8	Vi8		
						RA9			

B

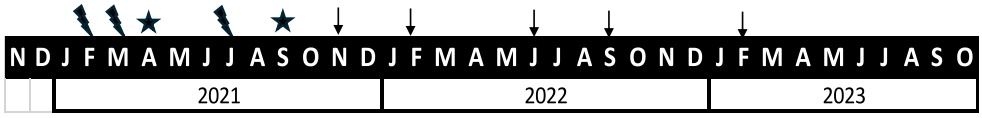


Fig. 1. Experimental design of the trial. A) The seven Leccino clones are indicated by different colours and identifiers, and the plant replicates by numbers. Leccino and Cellina di Nardò are control plants for comparison. Bold labelled plants were experimentally inoculated with *Xylella fastidiosa* subsp. *pauca*, while mock inoculated control plants are indicated by normal typing. B) Schedule of the trial; thunders indicate experimental (needle) inoculations with *in vitro* grown *X. fastidiosa* subsp. *pauca*, while stars indicate inoculation with *X. fastidiosa* subsp. *pauca*-infected shoots and arrows the date of qPCR detection of *X. fastidiosa* subsp. *pauca*. Leccino and Cellina cultivars control olives were only inoculated once by needle.

and 04/16/2022. Ψstem was measured on two non-transpiring leaves *per* plant, covered with aluminum foil 1 h before the measurements, so that leaf water potential equilibrates the stem water potential [31]. Before the Ψstem measurement, the aluminum foil was removed and the leaf was deprived of half of the leaf blade to facilitate its placement inside the sample holder [16]. The Ψstem values of two leaves from each plant were averaged and used to calculate the Differential stem water potential (ΔΨstem) as follows:

$$\Delta\Psi\text{stem} = H\Psi\text{stem} - I\Psi\text{stem}$$

Where HΨstem and IΨstem are the average of the stem water potential of healthy (H) and infected (I) plants from the same clone, respectively. The calculation was performed using absolute (positive) values of Ψstem in bar.

Measurements of stomatal conductance (g_s) of the Leccino clones and control olives were taken using a portable porometer (LI-600, LI-COR, Lincoln, Nebraska 68504, USA). Starting from 4 months after the last inoculation, seven sequential measurements were performed for the clones (Fig. 1B) in 2022 on January 31, February 16, March 3 and 16, April 6, June 6, August 9, while only five for the control olives. Values from each plant were the average of the readings from four different leaves. Measurements were carried out at field capacity achieved by irrigation. The differential stomatal conductance (Δg_s) was calculated between healthy and *X. fastidiosa* subsp. *pauca*-infected olives (*X. fastidiosa* subsp. *pauca* positive to *X. fastidiosa* subsp. *pauca* detection by qPCR analysis) according to the formula:

$$\Delta g_s = Hg_s\text{-}Ig_s$$

Where Hg_s and Ig_s are the average of the stomatal conductance of healthy (H) and infected (I) plants, respectively. Raw data measurements of Ψstem and g_s observed are reported in the Supplementary Table 1.

2.4. RNASeq library preparation and sequencing

RNASeq analysis was carried out on three inoculated (1, 2, 3 in Fig. 1A) and mock inoculated (5, 6 in Fig. 1A) plants of each Leccino clone and on the inoculated and mock inoculated olives of the control cultivars Leccino and Cellina di Nardò. After 48 h from the first inoculation of the clones with *X. fastidiosa* subsp. *pauca*, total RNAs were extracted from 1 g of xylem tissues of twigs, including the *X. fastidiosa* subsp. *pauca* (Xfp olives) or PBS (mock) inoculation points. The bark was removed from the twigs using a scalpel, and the xylem tissues were powdered in liquid nitrogen and immediately stored at −80 °C until RNA extraction, which was performed according to Ref. [11]. RNA concentrations were quantified using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, USA). The quality and integrity of the RNAs were assessed by 1.2 % agarose gel visualization.

Library preparation and high throughput sequencing were outsourced to Macrogen B.V. (The Netherlands). Briefly, cDNA was generated from the total RNAs using Illumina's Stranded mRNA Sample Prep Kit, followed by library preparation according to Illumina's guidelines for the TruSeq Stranded mRNA LT sample prep kit. Amplified products were then sequenced using an Illumina Novaseq platform (Illumina, San Diego, USA) on a paired-end 100 bp run. FastQC tool was used for RNA-Seq data processing to assess the quality and quantity of the raw reads. Paired-end reads were aligned by Tophat 2 tool [32] on the genome of *O. europaea* subsp. *europaea* cv. Leccino, recently assembled and released [33]. Raw reads count data for each mapping genome were determined using SeqMonk software version 1.48.1 (https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/) and the resulting abundance data were analyzed for a first exploratory analysis by PCAexplorer R package [34], to cluster the samples according to the mock-inoculated/*X. fastidiosa* subsp. *pauca*-inoculated condition. Genes with less than 1 counts summed up across all samples were excluded from further analysis. Principal component analysis (PCA) was carried out based on the normalized gene expressions (variance-stabilizing transformation (VST) from the RNA-seq datasets with samples clustered according to condition (mock-inoculated/*X. fastidiosa* subsp. *pauca*-inoculated) and olive clone (AR, VE, Vi, RO, RA, BL, Bi, cv. Leccino, cv. Cellina).

2.5. Differential gene expression analysis

DESeq2 package version 1.36.0 [35] was used to identify differentially expressed genes (DEGs) considering 69,398 genes identified in the cv. Leccino genome [33]. Differential gene expression analysis was performed by pairwise comparisons between the cluster of libraries of inoculated (1, 2, 3) *vs* mock-inoculated samples (5, 6) of each Leccino clone, AR, VE, Vi, RO, RA, BL, Bi, and the Leccino and Cellina control cultivars (Fig. 1A). DEGs were considered significantly differentially expressed when the log2 fold change was greater than +2 or less than −2, with an adjusted p-value of less than 0.05 and a Benjamini-Hochberg false discovery rate (FDR) of less than 0.01.

2.6. Gene functional annotation and categorization

Genes identified in the cv. Leccino genome [33] were functionally annotated by BLASTN/X searching for gene sequences (69,398) against the nr (NCBI), including protein functions related to the cv. Farga [36] and *O. europaea* var. *sylvestris* [37].

For functional categorization, annotated genes (cDNA sequences) were mapped to MapMan classification using the online tool Mercator4 v2.0 [38].

MapMan software is used for the functional analysis of plant genes, enabling the integration and visualization of the functions of DEGs in regulatory pathways [39]. DEGs with FDR $\leq$0.05 were analyzed using MapMan software version 3.7 and the mapping file generated by Mercator4. Significant under- and over-represented biochemical pathways categories (BINs) were identified by the Wilcoxon rank-sum test with FDR adjustment, according to Ref. [40] and $P \leq 0.05$ was used as the significance threshold.

2.7. Statistical analysis

Data from all physiological measurements were evaluated for normality using the Shapiro-Wilk test and subjected to analysis of variance (ANOVA) using Past 4 [41] and GraphPad Prism 9 (GraphPad Software, San Diego, CA) software. When ANOVA results were significant, the means of the treatments were compared using Tukey's pairwise comparison test ($P \leq 0.05$). The same software was used to construct the graphical representation of the data.

3. Results

3.1. Periodical assessment of the infections and symptoms

The seven Leccino clones were subjected to three sequential *X. fastidiosa* subsp. *pauca* inoculations by the "pin prick" method followed by grafting of infected shoots to maximize the success of the infections (Fig. 1B). The percentage of viable grafts ranged between 17 % and 50 %, likely because of the use of infected shoots. Periodic monitoring of the pathogen presence was carried out using qPCR detection of *X. fastidiosa* subsp. *pauca*. At 16 months post-inoculation (mpi), the seven Leccino clones showed diverse prevalence rates (*i.e.*, number of *X. fastidiosa* subsp. *pauca* positive plants out of the total inoculated), ranging from 57.1 % to 100 % (Fig. 2A). The lowest percentage of prevalence (33.3 %) was observed in the cultivar Leccino control olives, which were, however, only once inoculated by pin prick, similarly to the Cellina di Nardò which, conversely, showed a 66.7 % of prevalence. At the end of the trial, 24 mpi, the prevalence of the surviving olive clones (*i.e.* a few plants died during the trial) ranged between 71.4 and 100 % (Fig. 2B). Indeed, symptoms of desiccation were variable among the clones. Severe symptoms were observed on olives of clone BL, of which three out of four *X. fastidiosa* subsp. *pauca*-inoculated plants get infected (75 % prevalence in Fig. 2A) and all these three (100 % in Fig. 2B) were dead at 19 mpi, like the susceptible cultivar Cellina di Nardò. Variable percentages (16,6 %–40 %) of dead plants were observed in clones Vi, RO, VE and Bi out of the total *X. fastidiosa* subsp. *pauca* infected plants, while all *X. fastidiosa* subsp. *pauca* infected plants of clones AR and RA survived up to the end of the trial. None of the *X. fastidiosa* subsp. *pauca* infected olives of the control Leccino died (data not shown). In summary, although repeatedly inoculated, not all olive clones get infected at 24 mpi, as was also observed with the Leccino control, which was inoculated only once. Clone BL exhibited the worst disease progression, as all infected olive trees ultimately died. Some deaths also occurred in RO, Bi, VE and Vi. As expected, all the Cellina di Nardò control olives died, even after a single inoculation, while all Leccino control olives survived at the end of the trial.

3.2. RNASeq analysis of early gene expression

Olive tissues for RNASeq were sampled at 48 h post inoculation (hpi) using portions of twigs that included the *X. fastidiosa* subsp. *pauca*- or mock-inoculation points. Three *X. fastidiosa* subsp. *pauca*-inoculated and two mock samples were sequenced. Similar numbers of reads were sequenced from all the samples, ranging from 84,736,440 to

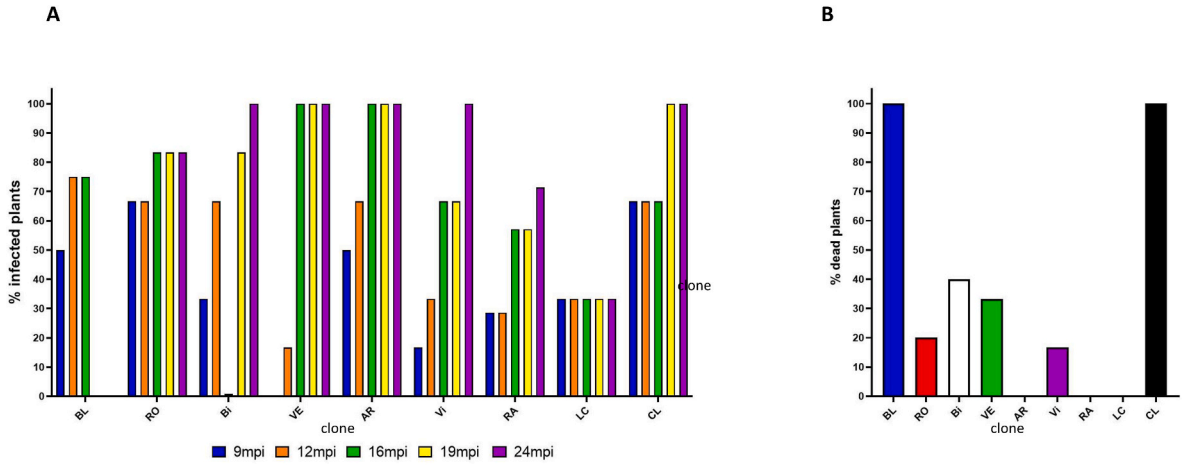


Fig. 2. Progress of the infections. Graph A) reports the percentages of plants of each Leccino clone found *Xylella fastidiosa* subsp. *pauca*-infected by qPCR at 9,12,16,19 and 24 months post inoculation (mpi) together with the control cultivars Leccino (LC) and Cellina di Nardò (CL), out of the total inoculated plants. Graph B) indicates the percentage of plants of each genotype which were dead at 19 mpi out of the total which get infected.

138,617,694 raw reads and 47,281,441 to 103,255,121 mapped reads (Supplementary Table 2).

PCA analysis using VST-transformed data clearly separated the samples according to the cultivar (Fig. 3A), with Cellina di Nardò cultivar being very distant from all the Leccino clones, which were, however, dispersed along the PC2. Excluding Cellina di Nardò cultivar from the graph, further illustrates such dispersal, with Leccino clones likely reflecting the cultivar's biodiversity (Fig. 3B). Clones AR and RO cluster very close to the cultivar Leccino control (LC), showing no clear distinction between *X. fastidiosa* subsp. *pauca*- and mock-inoculated tissues (Fig. 3B). In contrast, VE and Vi exhibited a significantly different genetic basis as they grouped far from the Leccino control and all other genotypes. Genotypes BL and RA showed an intermediate clustering between the above groups. A clear distinction between *X. fastidiosa* subsp. *pauca*-inoculated and mock olives was observed among plants of genotypes BL, Bi and RA (Fig. 3B).

The gene expressions of the seven Leccino clones were differently altered 48h after the *X. fastidiosa* subsp. *pauca* inoculation (Fig. 4). Indeed, the numbers of Differentially Expressed Genes (DEGs) between *X. fastidiosa* subsp. *pauca*- and mock-inoculated olives of the same clone ranged from 52 for AR to 6824 for Bi. Significant changes in gene expression were observed for BL (6522 DEGs) and, in the order, Vi (3100 DEGs) and RA (1,912) (Fig. 4). A modest number of DEGs were altered for RO (244 DEGs) and VE (58 DEGs). In comparison, cultivar Leccino control plants had 499 DEGs. The majority of DEGs had a fold change in the range of +2 to −2, but a high number of DEGs exceeding this range (up- and down-regulated) were observed for genotypes BL, RA, Bi and Vi (Fig. 4). There was a partial agreement between the 48h DEG response of the clones and the outcome of symptoms at 19 mpi. Indeed, clones BL and Bi, showing the highest numbers of DEGs, had 100 % and 40 % of plants dead respectively, while the same did not occur for genotypes VE and RO, which had a very low number of DEGs.

3.3. Molecular responses in X. fastidiosa subsp. Pauca-inoculated olive trees

The differential gene expression analysis described two opposite responses of the Leccino clones to *X. fastidiosa* subsp. *pauca* inoculation: a group of clones (AR, VE and RO) did not significantly respond to the presence of *X. fastidiosa* subsp. *pauca* at 48 hpi (*i.e.* low numbers of DEGs), while the bacterium clearly altered the gene expression of clones BL, Bi, RA and Vi. Because of this, to identify the pathways involved in these responses, the DEGs were analyzed using Mapman software. The MapMan analysis was carried out on gene expression data of these latter four clones, considering transcripts with the range −2 >fold change > +2 and false discovery rate (FDR) < E−05. Moreover, to better describe the effects of gene expression alterations the attention was focused on the most altered functional pathways, particularly those relevant to defense responses. MapMan overview of metabolism and external stimuli pathways highlighted the modulated expression of genes related to the secondary metabolism, specifically belonging to the terpenoid and phenylpropanoid biosynthesis, cell wall, phytormones and biotic stress (Fig. 5), in all four clones as below detailed.

3.4. Deregulation of terpenoid and phenylpropanoid biosynthesis of olive clones in response to X. fastidiosa subsp. pauca infection

The metabolic reprogramming of terpenoid and phenylpropanoid biosynthesis in the BL, Bi, Vi, RA olive clones revealed distinct patterns of transcriptional deregulation, highlighting potential clone-specific defense mechanisms and metabolic specializations (Supplementary file 1). Among the DEGs annotated in "Terpenoid Biosynthesis" pathway, several transcript coding for mono-/sesquiterpene synthases were markedly upregulated in BL and RA. Specifically, the germacrene D synthase transcripts gwhteuuu045523.1, gwhteuuu045528.1, gwhteuuu045530.1, and gwhteuuu045535.1 were strongly induced in both BL and RA (log_2FCs ranging from 4.52 to 5.76), whereas some of these isoforms were simultaneously downregulated in Bi (e.g., gwhteuuu045530.1, $log_2FC = -4.42$). This opposite regulation may reflect divergent strategies in volatile terpenoid production between clones. Notably, gwhteuuu046492.1, encoding a (3S,6E)-nerolidol synthase, showed strong upregulation in both BL and RA ($log_2FC = 5.55$ and 5.97, respectively), further supporting enhanced sesquiterpene synthesis in these clones. Conversely, transcripts for alpha-farnesene synthase (gwhteuuu050249.1, gwhteuuu050252.1, gwhteuuu050255.1) were consistently downregulated in Bi (log_2FCs ≈ −5.5), indicating clone-specific suppression of certain volatiles.

The triterpenoid pathway also showed transcriptional regulation. In BL, gwhteuuu009118.1 (encoding beta-amyrin synthase) was upregulated ($log_2FC = 4.09$), while gwhteuuu029441.1 (encoding dammarenediol II synthase) was downregulated ($log_2FC = -3.30$), suggesting metabolic channeling toward specific triterpenes. The squalene epoxidase gwhteuuu013897.1 was strongly repressed in Bi ($log_2FC = -5.26$), potentially constraining sterol or triterpene biosynthesis in this clone.

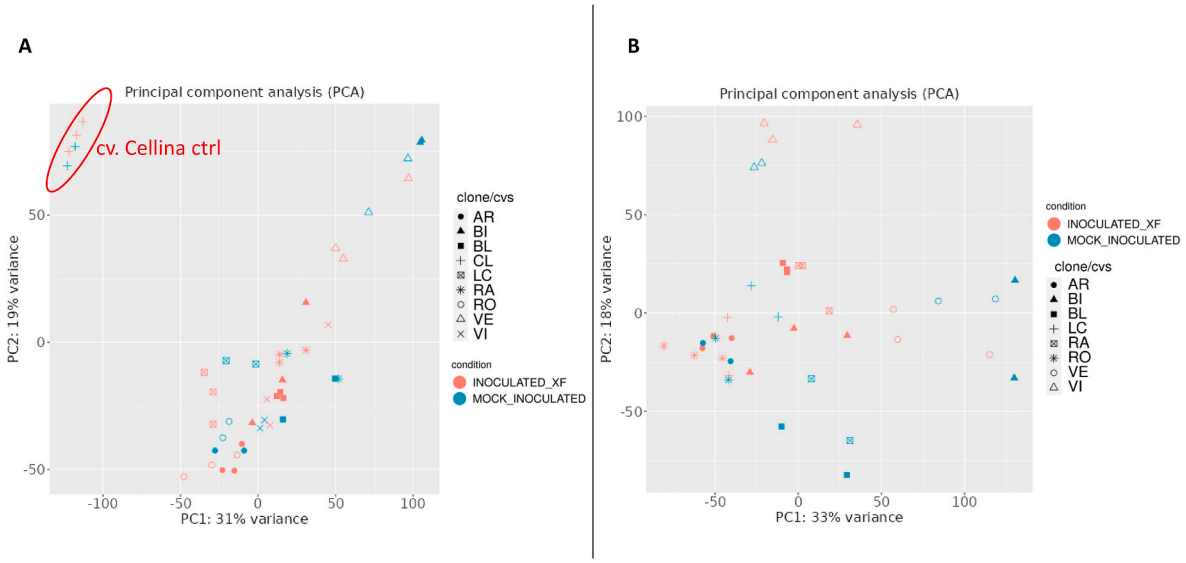
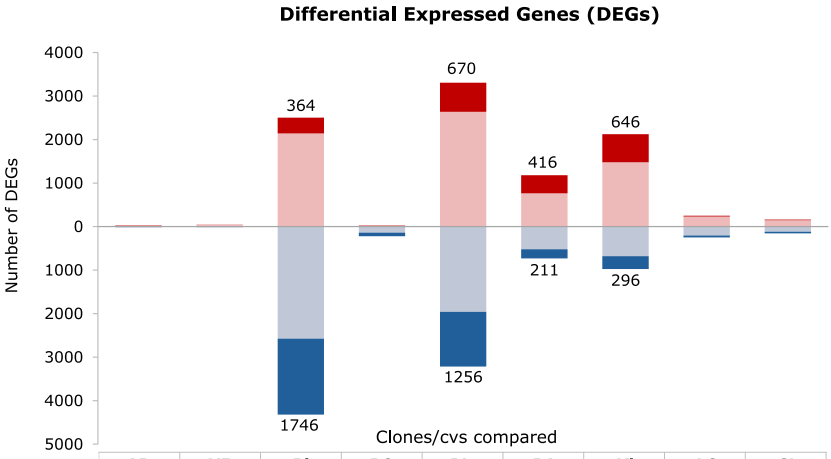


Fig. 3. Principal component analysis. Principal component analysis of VST-transformed data from RNASeq analysis of the olive samples with (A) or withouth (B) Cellina di Nardò. Infected (red) and mock inoculated (blue) samples of each clone (symbol) are indicated. LECC: Leccino and CELL: Cellina di Nardò control olives.



	AR	VE	Bi	RO	BL	RA	Vi	LC	CL
upreg (>+2 fold change)	9	3	364	14	670	416	646	18	15
upreg (<+2 and >0 fold change)	23	41	2140	9	2637	765	1479	232	147
downreg (<0 and >-2 fold change)	17	13	2574	139	1959	520	679	204	116
downreg (<-2 fold change)	3	1	1746	82	1256	211	296	45	39
all DEGs (pvalue <0.01)	52	58	**6824**	244	**6522**	**1912**	**3100**	499	317

Fig. 4. Differential expression. Differentially Expressed Genes (DEGs) at 48 hpi of the seven Leccino clones and the control Leccino (LC) and Cellina di Nardò (CL). The graph and table of data below the graph report the numbers of up- and downregulated DEGs distincted by respective fold changes in the 0 to +2 and 0 to −2 (red and blue colours) and >+2 and <-2 (dark red and dark blue colours).

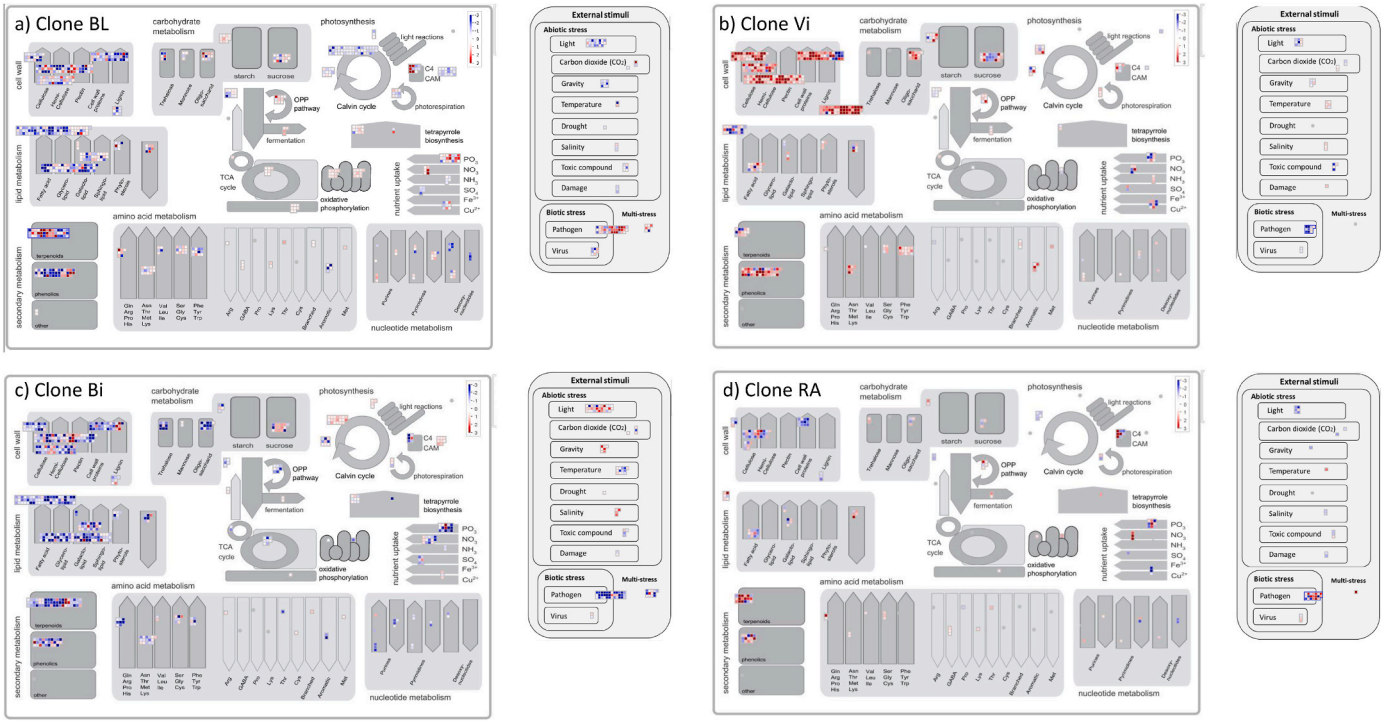


Fig. 5. MapMan analysis. MapMan overview of deregulated metabolic processes in four olive clones in response to infection. MapMan pathway diagrams depicting the distribution of differentially expressed transcripts in (a) Clone BL, (b) Clone Vi, (c) Clone Bi, and (d) Clone RA. Each panel shows major metabolic and regulatory processes, with individual bins shaded according to $\log_2$ fold change values (red for upregulated transcripts, blue for downregulated transcripts). Categories include cell wall organization, lipid metabolism, amino acid metabolism, secondary metabolism, carbohydrate metabolism, photosynthesis, nutrient uptake, and responses to external stimuli (abiotic and biotic stresses). The color scale represents the magnitude and direction of transcriptomic changes, highlighting clone-specific transcriptional reprogramming during infection.

The "Phenylpropanoid biosynthetic" pathway also displayed differential modulation. Two phenylalanine ammonia-lyase (PAL) transcripts were upregulated in Vi (gwhteuuu022319.1, $\log_2$FC = 2.01; gwhteuuu032341.1, $\log_2$FC = 3.14), consistent with activation of this key branch-point enzyme. Additionally, the E3 ubiquitin ligase adaptor targeting PAL, KFB-PAL (gwhteuuu059633.1), was induced in Vi

($log_2FC = 3.61$), which may contribute to PAL turnover and dynamic regulation. Downstream in this pathway, two 4-coumarate-CoA ligase (4CL) genes (gwhteuuu011133.1, gwhteuuu056228.1) were upregulated in Vi ($log_2FCs = 2.32$ and 2.09, respectively), suggesting active lignin or flavonoid biosynthesis. In contrast, chalcone synthase (CHS) transcripts (gwhteuuu019103.1 and gwhteuuu020670.1) were sharply downregulated in BL ($log_2FCs = -5.22$ and -3.62), pointing to suppression of early flavonoid biosynthesis in this clone.

These results highlight clone-specific regulatory patterns in terpenoid and phenylpropanoid biosynthesis. BL and RA display transcriptional activation of sesquiterpene synthases and triterpene biosynthetic genes, potentially enhancing defense-related volatile and non-volatile metabolites. In contrast, Bi shows a generalized suppression of isoprenoid and sterol biosynthesis. Vi, instead, appears to activate the phenylpropanoid pathway, possibly leading to increased lignin and flavonoid production as a defense strategy. This transcriptional divergence underscores the metabolic plasticity of olive clones in response to external stimuli.

3.5. External stimuli

The expression of genes related to the response to external stimuli showed that several key transcripts were significantly deregulated in the four olive clones, reflecting complex adaptations to environmental cues (Supplementary file 1).

Bi displayed strong downregulation of protein kinases involved in effector-triggered immunity (ETI), notably RIPK homologs (gwhteuuu058749.1, $log_2FCs = -6.49$; gwhteuuu060454.1, $log_2FCs = -2.95$; and gwhteuuu061240.1, $log_2FCs = -3.69$), which may indicate suppression of this immune branch. Additionally, negative regulation was seen in the NDR1 regulatory protein (gwhteuuu045775.1, $log_2FCs = -2.72$) and systemic acquired resistance (SAR)-related transcription factors within the CBP60/SARD family, including gwhteuuu004003.1 ($log_2FCs = -4.36$), gwhteuuu007990.1 ($log_2FCs = -3.90$), and gwhteuuu046602.1 ($log_2FCs = -4.82$). Such widespread repression suggests a dampened systemic immune response in Bi.

Conversely, BL showed upregulation of key defense enzymes and regulators. The polygalacturonase-inhibiting protein PGIP (gwhteuuu028737.1) was induced ($log_2FCs = 2.80$), alongside acidic (gwhteuuu035719.1, $log_2FCs = 3.34$) and basic (gwhteuuu042933.1, $log_2FCs = 2.43$) chitinases, highlighting an active defense against pathogens known to produce pectin-degrading enzyme polygalacturonase (PG) [42]. Moreover, BL displayed increased expression of WRKY33 transcription factors involved in plant immunity, such as gwhteuuu024409.1 ($log_2FCs = 2.58$) and gwhteuuu069376.1 ($log_2FCs = 3.20$), a WRKY33-activating protein *(SIB), which further underscores a robust local immune activation. RA also showed induction of defensin peptides, including PDF2 (gwhteuuu041297.1, $log_2FCs = 2.42$), suggesting activation of antimicrobial defenses.

Additional stress-related transcriptional changes were observed. In BL, the cold acclimation factor ICE1/2 (gwhteuuu060626.1) was downregulated ($log_2FCs = -4.24$), as was the heat response transcription factor HsfA3 (gwhteuuu022415.1, $log_2FCs = -2.22$), indicating modulation of temperature stress responses. Symbiosis-related genes were also affected; for instance, RAM2, a glycerol-3-phosphate acyl transferase essential for mycorrhizal lipid transfer (gwhteuuu025S19.1), was strongly repressed in Bi ($log_2FCs = -4.80$), possibly reflecting a trade-off between defense and symbiotic interactions.

Taken together, these expression patterns reveal clone-specific tuning of abiotic stress sensing, and immune signaling pathways, which likely contribute to the differential stress response observed in these olive genotypes.

3.6. Deregulation of cell wall-related genes in inoculated olive clones

Cell wall dynamics play a pivotal role in plant immunity, functioning both as a first line of physical defense and as a platform for pathogen recognition and signaling. In olive, transcriptome analysis mainly across three clones (BL, Bi, Vi) reveals strikingly divergent transcriptional programs affecting cell wall-related genes, reflecting distinct defense strategies against *X. fastidiosa* subsp. *pauca* (Supplementary file 1). These differences are particularly evident in the regulation of cellulose and hemicellulose metabolism, pectin modification and degradation, lignin and cutin/suberin biosynthesis. Among the "Cellulose Biosynthesis and Remodeling" category, key determinants of wall integrity and flexibility, cellulose metabolism displayed contrasting regulation across clones. Particularly, in clone BL and clone Bi, the gene encoding a class-B endo-1,4-β-glucanase (gwhteuuu012086.1) was strongly repressed ($log_2FC = -6.92$ in BL; -4.23 in Bi), indicating reduced cellulose remodeling suggesting a strategy of wall stiffening to impede pathogen penetration. In sharp contrast, clone Vi showed a robust induction of cellulose biosynthesis genes. Two CesA family transcripts (gwhteuuu010130.1 and gwhteuuu042113.1) and one CSC regulatory protein (gwhteuuu029041.1) were all upregulated ($log_2FC \sim 4$), accompanied by the COBRA-like gene (gwhteuuu026937.1, $log_2FC = 4.81$), which enhances cellulose–hemicellulose interactions. This pattern suggests active wall reinforcement as a frontline defense in clone Vi.

Among transcripts belonging to "Hemicellulose Biosynthesis and Remodeling" category, in clone Bi two mannan synthase transcripts (gwhteuuu019788.1 and gwhteuuu038094.1; $log_2FC \sim -4.5$) were downregulated, pointing to inhibit matrix remodeling and further rigidification of the wall. In contrast, clone Vi showed marked upregulation of xylan biosynthesis genes, including IRX9 xylosyltransferase (gwhteuuu035083.1, $log_2FC = 4.40$) and xylan O-acetyltransferase (gwhteuuu051700.1, $log_2FC = 4.18$). These modifications likely contribute to strengthened secondary walls, a classical defense strategy against vascular pathogens.

Regarding "Pectin Degradation and Remodeling" processes known to be essential for cell wall loosening and sealing during pathogen invasion [43], clone BL showed a clear suppression of pectin-modifying enzymes, including pectate lyase (gwhteuuu039234.1, $log_2FC = -6.80$) and two pectin methylesterase inhibitors (gwhteuuu032134.1, $log_2FC = -7.79$; gwhteuuu057928.1, $log_2FC = -5.29$). This repression suggests reduced wall porosity and a shift toward wall sealing, limiting pathogen diffusion. Whereas pectin-related regulation was not prominent in clone Vi, indicating that pectin remodeling might not be a primary axis of defense in this genotype.

Analysis of differentially expressed genes assigned to the "Cell wall organization, cell wall proteins" category revealed substantial transcriptional reprogramming of hydroxyproline-rich glycoprotein (HRGP) activities, particularly among arabinogalactan-proteins (AGPs) and extensins. Several transcripts encoding fasciclin-like AGPs were strongly upregulated in clone Vi, including gwhteuuu021306.1 ($log_2FC = 4.17$), gwhteuuu036506.1 ($log_2FC = 3.56$), and gwhteuuu060205.1 ($log_2FC = 3.15$), highlighting a pronounced activation of cell wall reinforcement pathways. Similarly, gwhteuuu034019.1 ($log_2FC = 3.30$) and the extensin gwhteuuu045313.1 ($log_2FC = 3.33$) showed significant induction, suggesting enhanced cross-linking and structural fortification of the cell wall matrix during infection. In contrast, marked downregulation was observed for specific AGP isoforms such as gwhteuuu014231.1 ($log_2FC = -4.18$) in clone Bi and the xylogen-type AGP gwhteuuu008828.1 ($log_2FC = -3.47$) in clone BL, indicating selective suppression of certain glycoprotein subtypes. This differential regulation may reflect a strategic remodeling of the cell wall proteome, prioritizing reinforcement while limiting components that could be exploited by pathogen-derived cell wall-degrading enzymes.

"Cutin and Suberin Biosynthesis" category harbored the highest number of deregulated transcripts, comprising 39 out of the 176 cell wall-related transcripts. Concerning this functional category, clone BL exhibited massive repression of genes involved in cuticle biosynthesis, including Omega-hydroxy fatty acid dehydrogenase

(gwhteuuu060533.1, $log_2FC = -5.86$), Fatty acyl-CoA reductase (gwhteuuu008968.1, $log_2FC = -7.65$), CYP77B fatty acid epoxygenase (gwhteuuu046403.1, $log_2FC = -5.42$), several cutin synthases (e.g., gwhteuuu033624.1, $log_2FC = -8.65$). This widespread repression suggests a weakened surface barrier, potentially reflecting a trade-off between defense and metabolic costs under pathogen pressure. Clone Bi mirrored this trend, though to a lesser extent (e.g., gwhteuuu028012.1, cutin synthase; $log_2FC = -4.34$), indicating a partial suppression of surface polymer biosynthesis. Clone Vi, by contrast, did not show significant suppression in this pathway, implying maintenance or enhancement of cuticular defenses.

Regarding the "Lignin monolignol biosynthesis and polimerization" category which includes transcripts involved in lignin deposition, enhancing mechanical strength and pathogen resistance, especially in the xylem [44], clone Vi displayed an extraordinary upregulation of laccase genes, with log_2FC values up to +10.16 (gwhteuuu041159.1). Other laccases (e.g., gwhteuuu053953.1, $log_2FC = 5,923894405$ gwhteuuu053851.1, $log_2FC = 4,577479362$) showed consistent activation, reinforcing the notion of intensive lignification as a major defense feature in this clone. No comparable activation was detected in clones BL or Bi, further underscoring the distinct defensive emphasis in Vi toward cell wall fortification and vascular integrity.

3.7. Differential expression of genes involved in phytohormone action in four olive clones

3.7.1. Abscisic acid (ABA)

The biosynthetic gene 9-cis-epoxycarotenoid dioxygenase, NCED (gwhteuuu038770.1) was upregulated in Bi ($log_2FC = 3.91$), suggesting increased ABA biosynthesis in this clone. In Vi, a cytoplasm-localized receptor phosphatase (ABI1/ABI2, gwhteuuu061189.1) showed upregulation ($log_2FC = 3.13$), while a suite of transcriptional repressors in the AITR family (gwhteuuu003009.1, 004198.1, 057305.1) also exhibited induction ($log_2FC > 2.38–3.00$), indicating active ABA signaling repression. Conversely, EAU (gwhteuuu021202.1) was sharply downregulated in BL ($log_2FC = -5.88$), which may reflect attenuation of ABA signal transduction in this clone. Notably, ABA hydroxylases (CYP707A, gwhteuuu006958.1, 006983.1) were significantly upregulated in both BL and RA ($log_2FC > 2.28–3.55$), consistent with enhanced ABA catabolism (Supplementary file 1).

3.7.2. Ethylene

A striking induction of ACC synthase (gwhteuuu016339.1, $log_2FC = 10.99$) and ACC oxidase (gwhteuuu039457.1, $log_2FC = 3.70$) in BL, together with similar responses in RA ($log_2FC = 8.59$ and 4.07, respectively), indicate a robust activation of ethylene biosynthesis in these clones. The modulator ARGOS (gwhteuuu031446.1) also showed high induction in both clones ($log_2FC = 5.99$ and 5.48), reinforcing enhanced ethylene signal modulation (Supplementary file 1).

3.7.3. Jasmonic acid (JA)

JA biosynthetic genes were dramatically downregulated in Bi, including DAD1, LOX, AOS, OPR3, and OPR2 with log_2FC values ranging from −2.04 to −7.62, indicating repression of JA production. The JA receptor component COI (gwhteuuu011613.1) was strongly downregulated in Bi ($log_2FC = -5.81$), which may limit JA perception. Moreover, several genes, involved in jasmonic acid degradation and coding for wound-inducible cytochromes P450 (CYP94C and CYP94B) and for JOX/JAO, were downregulated in Bi clone with log_2FC values ranging from −4.09 to −6.99. (Supplementary file 1).

Two genes gwhteuuu043407.1, gwhteuuu043409.1 were also highly downregulated in clone Bi ($log_2FC = -6.50$; −5.57, respectively). Both code for jasmonate O-methyltransferase-like (JMT) a plant enzyme that catalyzes the methylation of jasmonic acid (JA) to produce methyl jasmonate (MeJA) known as volatile plant compound that acts as a signal molecule in plant defense responses and also plays a role in lateral root promotion [45,46].

In contrast gwhteuuu003024.1 gene coding for CYP94B and gwhteuuu034686.1 coding for a jasmonic acid oxidase (JOX/JAO), are highly upregulated in clone BL (log2FC = 9.08; 5,57, respectively) and RA (log2FC = 9.11; 5,40) (Supplementary file 1).

3.8. Physiological responses in X. fastidiosa subsp. Pauca-inoculated olives

The study of the physiological response highlights different behaviors among the Leccino clones upon *X. fastidiosa* subsp. *pauca* infections. In particular, the stem water potentials (ΔΨstem) of BL, Bi, AR and Vi genotypes are almost like those of the cultivar Leccino control plants and are significantly more negative than that of RA (Fig. 6A). The ΔΨstem of genotypes RO, VE and RA are almost close to 0. The ΔΨstem of cultivar Cellina di Nardò has the more negative value which is significantly different from those measured in all genotypes and cultivar Leccino control (Fig. 6A). These findings indicate that *X. fastidiosa* subsp. *pauca* infections have limited effects on plant water transport since differences between the ΔΨstem of mock-inoculated and *X. fastidiosa* subsp. *pauca*-infected olive clones are limited. However, all the Leccino clones have significantly lower ΔΨstem than those of cultivar Cellina di Nardò, whose very negative value denotes the high suffering of this genotype when *X. fastidiosa* subsp. *pauca*-infected, consistent with the high susceptibility of this cultivar.

The stomatal conductance (Δg_s) of the clone AR is the highest measured and, in particular, higher than those of BL, Bi and Vi, despite having a similar ΔΨstem. This behavior likely indicates that, at the same ΔΨstem, the genotype AR more severely closes the stomata than the other genotypes (Fig. 6B). Although not significantly different from the cultivar Leccino control, clones BL, Bi and Vi have Δg_s values that were more variable throughout the five measurement times. Indeed, the Leccino control exhibited a more consistent behavior during the experiment. RA and VE showed the lowest Δg_s values, while RO had an intermediate condition. All the cultivar Leccino genotypes except AR had significantly lower Δg_s mean values than cultivar Cellina di Nardò, indicating that this cultivar reacts to the water stress imposed by infections with a longer time of stomata closure.

4. Discussion

The identification of genotypes resistant to *X. fastidiosa* is among the most promising strategies to control infections caused by this bacterium in perennial crops. This research aims at the identification of genetic traits responsible of resistance in olive trees. In grapevine, such traits are present in *Vitis arizonica*, from which they have been introgressed in the susceptible *V. vinifera* genome background [20,21]. In the recently discovered olive/*X. fastidiosa* subsp. *pauca* pathosystem, the screening of the germplasm of this species has identified four resistant cultivars: Leccino, FS17, Lecciana and Leccio del Corno. It was therefore useful to assess the *X. fastidiosa* subsp. *pauca* response of additional Leccino clones to provide farmers with more options for olive cultivation, also considering a recent work [14], which consolidates the existence of traits of resistance in this cultivar.

In this greenhouse trial, seven Leccino clones were subject to *X. fastidiosa* subsp. *pauca* infection, involving repeated inoculations and grafting of infected shoots within a limited timeframe, which caused the complete desiccation of plants in some clones. These conditions imposed an unnatural and continuous inoculation pressure with the aim of maximizing the chances of identifying clones with a robust resistance.

The RNASeq analysis indicates that 48h post-inoculation olive tissues respond to the presence of *X. fastidiosa* subsp. *pauca*, although clones react with diverse degrees of gene expression. Two genotypes (BL and Bi) showed the highest number of genes differentially expressed and the most severe symptoms. However, the nature of the *X. fastidiosa* subsp. *pauca* perception as external stimulus, was different between the

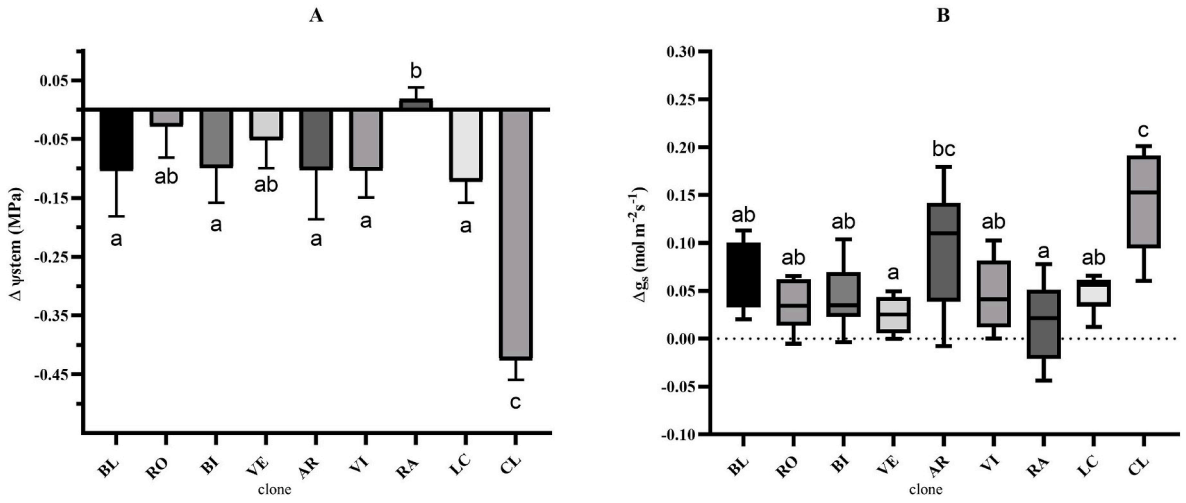


Fig. 6. Physiological measurements. Differences of stem water potential (A) and of stomatal conductance (B) among uninoculated (mock) and *Xylella fastidiosa* subsp. *pauca* inoculated olives of the same clones. Column and error bars (A) represent the mean and standard deviation from five ΔΨstem sequential measurement. The Box plot shows data distribution of seven Δgs sequential measurements where boxes represent the interquartile range (IQR), the line the median, and the whiskers the min to max values. Different letters indicate statistically significant differences ($P < 0.05$) according to the Tukey's pairwise test.

two clones. BL, which was severely affected (with 3 out of 4 inoculated plants desiccating), showed a defense response with the overexpression of a WRKY33-dependent plant immunity, a mechanism known to be induced in plant defense against necrotrophic pathogens. Specifically, a sigma factor binding protein (SIB), functioning as WRKY33 activators [47], was upregulated in the *X. fastidiosa* subsp. *pauca*-infected clone BL. Interestingly, this reaction has been also reported in the early response of Ponkan mandarin to *X. fastidiosa*, where the bacterium was recognized as a necrotrophic pathogen [18]. In addition, defense related genes, as a polygalacturonase-inhibiting protein and chitinases are also upregulated. Conversely, systemic acquired resistance was severely compromised in clone Bi where regulatory proteins and ETI-associated protein kinases were inhibited.

Further indications of an active plant immunity response include the stimulation of pathways devoted to producing antimicrobial compounds by the secondary metabolism pathways, belonging to the phenolics and terpenoids classes [48]. Clone Bi and BL showed an opposite regulation of terpenoids while a clear inhibition of the flavonoid biosynthesis occurred in clone BL. Notably a Dirigent 206 protein was upregulated in clone BL, whose role in stress response is related to lignans and lignin biosynthesis, important antimicrobial compounds [49]. The other two clones have a limited involvement of this pathway consisting, for RA and Vi, in the upregulation of genes devoted to terpenoid and phenylpropanoid biosynthesis, respectively.

A common trait linking the olive response of BL, Bi and Vi plants to *X. fastidiosa* subsp. *pauca* was the involvement of genes regulating cell wall organization, confirming previous findings [11]. Being at the forefront of infection, the plant's cell wall serves as the first barrier in its interaction with *X. fastidiosa* subsp. *pauca*, dynamically responding to the bacterium's aggressiveness. An altered expression was observed in genes responsible of cell wall remodeling, (*i.e.* endolyase, glucanase, pectate lyase), and cell wall relaxation (*i.e.* expansin). Although using different pathways the three clones pursue the same objective: to reinforce the cell wall, which consists in an inhibition of the degradation processes in clones BL and Bi and in the biosynthesis of the cellulose in clone Vi. The interpretation of this differential expression of genes related to the cell wall organization relies on the rerouting of gene expression from the growth to the defense. Another explanation of the cell wall remodeling likely depends on the surveillance mechanisms exerted by the cell wall, whose change of mechanical characteristics may promote resistance or susceptibility to pathogens [50]. This was particularly evident in the upregulation of laccases in Vi and RA clones, which are involved in cell wall lignification and plant defense responses to pathogens [51]. Evidence of the alteration of the abscisic acid pathway, observed in the four clones, was already reported in olive [11], as well as in grapevine [8,52] where it was explained as a perception of *X. fastidiosa* as a drought stress. Ethylene biosynthesis, stimulated in clones BL and RA, was already documented in Pierce's disease, due to its role in the production of tyloses which contribute to the hydraulic failure of the xylem [53]. The findings reported here indicate that a *X. fastidiosa* subsp. *pauca* induced biosynthesis occurs as early as 48 h after the inoculation. Finally, the jasmonic acid pathway, a plant hormone, having a preeminent role in stress response [54] was strongly repressed in both BL and Bi, the two clones having the worst outcome upon infection.

The study confirms the potential of physiological measurements as tools to screen olive germplasm in the analysis of the response to *X. fastidiosa* subsp. *pauca* infections [16]. Overall, the xylem conductivity of all Leccino clones upon infection was better than that of the cultivar Cellina di Nardò. Indeed, the more negative ΔΨstem values and lower Δg_s values observed in the cultivar Cellina di Nardò highlight its susceptibility and stronger stress response of infected plants. The experimental approach, however, evidenced a different adaptation to the stress of the genotypes. Among clones, RA, RO and, successively VE, performed best, as their ΔΨstem and Δg_s values were nearly zero, suggesting a hydraulic state of *X. fastidiosa* subsp. *pauca*-infected olives which was like that of mock-inoculated plants. All genotypes, except AR, exhibited similar behaviour; however, over time and with repeated inoculations, the BL genotype succumbed to the infection. The genotype AR was different, as its Δg_s was more similar to that of cultivar Cellina di Nardò, while its ΔΨstem remained uncompromised. It can be interpreted as a "more parsimonious" behavior as this genotype closes its stomata more quickly and effectively, even under relatively mild ΔΨstem values.

5. Conclusion

Revamping the olive cultivation in the *X. fastidiosa* subsp. *pauca* epidemic area cannot disregard the introduction of new resistant germplasm, to expand the available number of cultivars. Although all seven clones analyzed belong to the known resistant cultivar Leccino, they represent significant biodiversity within the cultivar, offering new agronomic opportunities for farmers. While this study focuses on Leccino clones, expanding resistance screening to lesser-known olive

varieties or wild relatives could uncover additional resistant germplasm, as demonstrated in recent surveys of wild grapevine relatives for Pierce's Disease resistance [20]. Understanding the mechanisms of resistance allows the identification of molecular targets that can be exploited in assisted breeding technologies. This knowledge is further enriched by studying the physiological response of infected plants, which complements simple symptoms inspections. However, the present work requires further "in field" confirmation to verify the retention of resistance under natural infection conditions.

CRediT authorship contribution statement

Raied Abou Kubaa: Methodology, Investigation, Formal analysis. **Annalisa Giampetruzzi:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Carmine Del Grosso:** Methodology, Investigation, Conceptualization. **Serafina Serena Amoia:** Methodology, Investigation, Formal analysis. **Giovanni Caruso:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation. **Susanna Bartolini:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis. **Giuseppe Altamura:** Investigation. **Antony Surano:** Methodology, Investigation, Formal analysis. **Pasquale Saldarelli:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pasquale Saldarelli reports financial support, article publishing charges, and equipment, drugs, or supplies were provided by Puglia Region. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2025.103018.

Data availability

All transcriptomic sequencing data presented in this study have been submitted to the NCBI BioProject under the accession number PRJNA1194796.

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