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Aparna S. Balan[1*], Antonio Giovino[2], Tiziano Caruso[1], Annalisa Marchese[1]

[1]*Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Palermo, Italy.*

[2] *Council for Agricultural Research and Economics (CREA)—Research Centre for Plant Protection and Certification (CREA-DC), Palermo, Italy.*

Email : *aparna.sheelabalan@unipa.it*

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Highlight

Omics-based approaches reveal regulatory networks underlying olive immune responses, identify critical knowledge gaps and propose a systems-level roadmap for functional validation and development of resistant cultivars.

Abstract

Olive (*Olea europaea*) is a perennial crop of major economic, cultural, and ecological importance in the Mediterranean basin and worldwide, yet its productivity and sustainability are increasingly constrained by diverse pathogens, including fungi, bacteria, and viruses. The primary objective of this review is to critically evaluate how recent omics advances have improved our understanding of olive immune regulation under biotic stress, and to clarify why an integrative systems-level multi-omics perspective is essential for developing durable disease management strategies. By synthesizing genomic, transcriptomic, and emerging proteomic, microbiomics and metabolomic studies, we show that olive defense relies on tightly coordinated transcriptional reprogramming, hormone crosstalk, metabolic remodelling, and structural barriers, within a multilayered immune framework, supported by expanding genomic resources and resistance-associated loci in cultivated and wild germplasm. However, the review also identifies major limitations that currently hinder progress, including a predominant transcript-centric focus, limited proteomic, microbiomics and metabolomic depth, insufficient attention to post-translational and epigenomic regulation, underdeveloped pan-genomic frameworks, and scarce functional validation using genome-editing approaches. We conclude that integrating multi-layered omics with functional genomics and pan-

genomics is crucial for translating molecular insights into resilient cultivars and sustainable disease management, and we outline key priority directions for future research and breeding applications.

Keywords : RNA-Seq, pathways, epigenomics, metatranscriptomics, transcriptomics, metabolomics

1. Introduction

Olive (*Olea europaea* L.) is one of the oldest cultivated tree crops and is now grown worldwide due to its adaptability to diverse agro-climatic conditions (Julca et al., 2020). Beyond its economic value for olive oil and table production, the olive plays a crucial ecological role in semi-arid environments, contributing to soil conservation and sustainable agroecosystems. Moreover, the persistence of centennial and even millennial olive trees highlights the remarkable longevity, genetic resilience, and adaptive capacity of this species under prolonged environmental and biotic pressures (Marchese et al., 2023a).

Despite this environmental resilience, olive cultivation is increasingly threatened by fungal and bacterial pathogens that compromise productivity, oil quality, and orchard longevity. Major diseases include peacock spot caused by *Spilocaea oleaginea*, Verticillium wilt caused by *Verticillium dahliae*, olive knot induced by *Pseudomonas savastanoi*, anthracnose associated with *Colletotrichum* spp., and Olive Quick Decline Syndrome linked to *Xylella fastidiosa* (*Xf*) subsp. *pauca* (Saponari et al., 2013; Luvisi et al., 2017b; Keykhasaber et al., 2018; Talhinhas et al., 2018; Martinelli et al., 2019; Licciardello et al., 2023; Marchese et al., 2023b). These pathogens differ in epidemiology and infection strategy, ranging from foliar necrotrophs to vascular colonizers, yet collectively threaten the sustainability of olive production under changing climatic conditions. Consequently, omics-based approaches are increasingly used to unravel host-pathogen interactions, identify resistance mechanisms, and accelerate the development of disease-resilient cultivars.

Like other higher plants, olive relies on a multilayered immune system in which physical and chemical barriers (cuticle, cell wall, antimicrobial secondary metabolites) form the first line of defense. In this context, resistance refers to the ability to restrict pathogen establishment and/or proliferation, whereas tolerance describes the capacity to maintain physiological and metabolic functions despite infection (Pagán and García-Arenal, 2018). Pathogen recognition activates pattern-triggered immunity (PTI) via pattern recognition receptors (PRR), inducing rapid reactive oxygen species (ROS) production, signaling cascades, and defense gene expression; when effectors suppress PTI, effector-triggered immunity (ETI) can be activated through intracellular resistance proteins, leading to stronger localized responses (Castañeda-Ojeda et al., 2017; Moretti et al., 2021). These responses are regulated by hormonal networks involving salicylic acid, jasmonic acid, ethylene, and abscisic acid, together with transcriptional and post-transcriptional

regulators that fine-tune defense gene expression (Gómez-Lama Cabanás et al., 2015; Gharbi et al., 2017; Cardoni et al., 2022; Marchese et al., 2023b). Overall, olive resistance is predominantly quantitative and cultivar-dependent, reflecting coordinated structural, metabolic, and regulatory adjustments rather than single major resistance genes.

Climate change further exacerbates these challenges by influencing pathogen distribution, host susceptibility, and disease severity (Montes-Osuna and Mercado-Blanco, 2020; Morelli et al., 2021; Poveda and Baptista, 2021). The perennial nature and long lifespan of olive trees complicate disease management, as infections may persist and spread within orchards over extended periods. Traditional control measures often show limited efficacy and raise environmental concerns, reinforcing the need for genetically based, durable resistance strategies (Basile et al., 2023; Lantero et al., 2023).

In recent years, advances in genomics, transcriptomics, and other omics technologies have generated valuable resources for dissecting olive-pathogen interactions. High-quality genome assemblies, resequencing datasets, and RNA sequencing studies have identified defense-associated pathways involving hormone signaling, phenylpropanoid metabolism, oxidative stress regulation, and vascular remodelling. Nevertheless, most studies remain largely descriptive and transcriptome-centered, with limited integration of proteomic, metabolomic, and phenotypic data into predictive frameworks.

Emerging research on olive highlights the growing use of integrated omics to better understand complex stress responses. Multi-omics approaches have been applied to salt stress in olive, showing how transcriptomic, proteomic, and metabolomic integration provides a more complete view of adaptation mechanisms (Claros et al., 2025). Similarly, omics studies in table olives have used data integration to characterize microbial and biochemical dynamics (Randazzo et al., 2017). However, integrative omics approaches in olive biotic stress research remain limited, highlighting a significant gap. This review addresses this by synthesizing current omics-based knowledge of olive defense responses to major pathogens, critically evaluating existing limitations, and outlining strategies to translate molecular insights into practical applications for resistance breeding and sustainable olive production.

2. Overview of Plant Immune Responses in Olive

Unlike annual model plants, where single resistance genes often determine host-pathogen compatibility, olive defense is a multilayered and dynamic system. It integrates constitutive structural barriers, inducible immune signaling, and extensive metabolic reprogramming. Olive tissues exhibit strong baseline defenses, including lignin- and phenolic-rich cell walls and antimicrobial secondary metabolites such as oleuropein derivatives, which together create physical and biochemical barriers limiting pathogen penetration and colonization (Fig.1 A). These constitutive traits are particularly important against vascular pathogens like *V. dahliae* and root-associated oomycetes, where resistant cultivars maintain tissue integrity and vascular functionality during infection (Luvisi et al., 2017a; Saponari et al., 2019; Mascuñano et al., 2025). High basal

levels of phenolic metabolites from the phenylpropanoid pathway, including oleuropein, hydroxytyrosol derivatives, and flavonoids, further contribute to antimicrobial activity and redox homeostasis. Complementing these structural defenses, olive mounts inducible immune responses upon pathogen perception, conceptually framed as PTI and ETI. Contrary to many annual model species, olive relies heavily on constitutive defenses operating over long temporal scales, reflecting its perennial growth habit and prolonged exposure to diverse biotic challenges. Taken as a whole, the evidence supports the notion that olive defense relies predominantly on sustained PTI-associated signalling and metabolic adaptation, with comparatively limited experimental evidence supporting canonical ETI, highlighting the importance of integrated, multilayered defense strategies in this long-lived woody species (Fig.1). This emerging view is consistent with the predominance of quantitative resistance observed across olive pathosystems, where disease outcomes appear to depend on the cumulative contribution of multiple defense mechanisms acting in concert.

2.1. Pattern Recognition and Early Immune Signaling

The first layer of inducible plant immunity is based on the recognition of pathogen-associated molecular patterns by plasma membrane-localized PRR, including receptor-like kinases (RLKs) and receptor-like proteins (RLPs), which activate PTI and restrict pathogen establishment (Fig. 1B; Movahedi et al., 2022); however, in olive, evidence for these components is currently derived mainly from transcriptomic analyses, and their functional roles in PTI have not yet been experimentally validated. Consequently, current models of pathogen perception in olive rely primarily on comparative genomics and expression profiling, highlighting a substantial gap between candidate receptor identification and functional validation. Several RLKs and RLPs have been identified in olive as putative components of PTI, although their functions are largely inferred from homology and expression data. LRR receptor-like serine/threonine kinases homologous to canonical PRRs such as FLAGELLIN SENSING 2 (*FLS2*), which recognizes flg22, and ELONGATION FACTOR TU RECEPTOR (*EFR*), which recognizes EF-Tu, have been detected in olive genomic and transcriptomic datasets, with an *FLS2*-like gene showing sequence similarity and pathogen-induced expression, suggesting conserved PAMP perception mechanisms (Estudillo et al., 2025). In addition, multiple LRR-RLKs, G-type lectin S-RLKs, and LysM- and malectin-like kinases are differentially expressed or accumulated during interactions with pathogens such as *S. oleaginea* and *V. dahliae* (Giampetruzzi et al., 2016; Marchese et al., 2023b). RLPs are also present in the olive genome, but none of these RLK/RLP candidates has yet been functionally validated as a PRR through ligand-binding or reverse genetics, leaving PRR-mediated perception in olive largely based on indirect evidence (La Notte et al., 2024; Fig. 1 B).

As a key second messenger in downstream defense responses, cytosolic Ca^{2+} influx is rapidly triggered by PTI activation, representing one of the earliest signaling events following PRR activation. In olive roots infected with *V. dahliae*, transcriptomic analyses show early induction of Ca^{2+}-related genes, including calmodulins, calcium-dependent protein kinases (*CDPKs*), and Ca^{2+} transporters such as Ca^{2+} ATPases and Ca^{2+} exchangers, indicating a rapid and coordinated calcium

response. This Ca^{2+} burst is decoded by Ca^{2+} sensors, which translate the signal into phosphorylation cascades and transcriptional activation of defense genes, positioning Ca^{2+} signaling as a central hub linking pathogen perception to immune responses in olive (Gómez Lama Cabanás et al., 2015). Whether specific calcium signatures contribute to pathogen-specific defense outputs remains unknown, representing an important area for future investigations.

Following Ca^{2+}-mediated signaling, *O. europaea* activates an early oxidative burst against pathogens such as *Xf*, *V. dahliae*, *Colletotrichum* spp., and *S. oleaginea*, marked by rapid ROS (mainly H_2O_2) accumulation with pathogen- and genotype-dependent kinetics. This response involves induction of NADPH oxidases (e.g., *RbohD-1*) and protein kinases, and in resistant cultivars higher ROS levels support a signaling rather than merely toxic role (Novelli et al., 2019). *Colletotrichum* spp. elicit a fast, strong oxidative burst prior to symptom onset, whereas *V. dahliae* induces a delayed but sustained ROS response accompanied by increased antioxidant enzymes such as superoxide dismutase and glutathione peroxidases, reflecting tight ROS homeostasis (Jiménez-Ruiz et al., 2017; Conti Taguali et al., 2025a). Transcriptomic data from *S. oleaginea* infection also indicate early induction of oxidative stress-related genes, including ankyrin repeat proteins, reinforcing ROS as central signaling mediators. Overall, ROS dynamics integrate with salicylic acid and jasmonic acid pathways to regulate defense gene expression and systemic acquired resistance, shaping pathogen outcomes in olive (López-Moral et al., 2022). The ability to maintain ROS homeostasis may represent a critical determinant of quantitative resistance, since excessive ROS accumulation may cause cellular damage, whereas controlled ROS production supports defense signaling and immune activation.

Genomic annotation in *O. europaea* has identified MAP kinase genes, including a *MAPK15*-like sequence in var. *sylvestris* containing the conserved TDY motif. In cv. Koroneiki infected with *S. oleaginea*, transcriptomic data show co-expression of serine/threonine receptor kinases and downstream transcription factors (WRKY, MYB, AP2/ERF), consistent with activation of a putative MAPK cascade downstream of PRR signaling (Marchese et al., 2023b; Fig. 1B). More broadly, pathogen infection induces RLKs, cell wall-associated kinases, MAPK components, and hormone-responsive transcription factors in olive (Giampetruzzi et al., 2016; Jiménez-Ruiz et al., 2017; Estudillo et al., 2025), supporting a functional PTI-like signaling framework. However, the complete MAPKKK → MAPKK → MAPK cascade and its direct targets have not been biochemically validated in olive, so this pathway remains inferred rather than experimentally confirmed.

2.2. Hormonal Regulation of Olive Defense Responses

Defense responses of olive against vascular pathogens and herbivores involve the coordinated action of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling pathways, each playing with distinct temporal roles (Fig. 1C). During *V. dahliae* infection, SA accumulates rapidly and induces PR genes (*PR1*, *PR2*, and *PR5*) together with transcription factors such as WRKY5. Resistant cultivars show stronger and earlier activation, which correlate with reduced disease

severity (Gharbi et al., 2016; Leyva-Pérez et al., 2018; Cardoni et al., 2022; López-Moral et al., 2022). As infection progresses, the levels of JA and its bioactive conjugate, JA-Ile increase, particularly in elicited plants, reflecting activation of JA-dependent defenses linked to systemic resistance and secondary metabolite production (López-Moral et al., 2022). Herbivory by *Bactrocera oleae* further stimulates this defence network through a rapid ethylene burst, alterations in 12-oxo-phytodienoic acid (OPDA) and other jasmonate intermediates, and modulation of defense-related transcription factors, highlighting the contribution of both ET- and JA signaling pathways (Alagna et al., 2016). Overall, olive defense follows a phased hormonal pattern characterised by early SA dominance during pathogen establishment and subsequent activation of JA/ET-dependent pathways during disease progression (Fig.1 C). Although SA-, JA-, and ET-mediated defense responses are increasingly recognized in olive, how their interactions regulate cultivar-dependent resistance remains largely unresolved.

2.3. Metabolic and Structural Defense Outputs

Immune activation in olive triggers targeted metabolic and structural reprogramming (Fig. 1D). Infection by *Alternaria alternata* induces the expression of genes involved in flavonoid and phenylpropanoid biosynthesis, resulting in the accumulation of defensive secondary metabolites (Wang et al., 2024c). Likewise, resistant cultivars challenged with *S. oleaginea* show increased expression of pathogenesis-related proteins and resistance gene analogs, reflecting a substantial transcriptional remodeling of defense pathways (Marchese et al., 2023b). Herbivory by *B. oleae* induces hormone signaling, oxidative stress responses, and secondary metabolite biosynthesis, thereby linking immune perception to downstream metabolic and structural responses (Grasso et al., 2017). These responses are coordinated by hormonal-regulated networks regulating ROS production, phenylpropanoid metabolism, and cell wall reinforcement. Structural defenses, including lignification and phenolic accumulation, contribute to restricting pathogen colonization and vascular spread. Omics evidence for activation of lignin biosynthetic pathways is supported by experimental demonstrations of pathogen-induced lignification and vascular reinforcement in olive tissues (Gharbi et al., 2017; Jiménez-Ruiz et al., 2017; Novelli et al., 2019; Cardoni et al., 2022), supporting the model depicted in Fig. 1D.

2.4. Effector-Triggered Immunity

Effector-triggered immunity (Fig.1 E) in *O. europaea* is proposed to rely on NLR receptors capable of recognizing pathogen effectors and activating strong defense responses, such as hypersensitive cell death and systemic immune signaling (Giampetruzzi et al., 2016; Estudillo et al., 2025). Genomic data indicate an expanded repertoire of NLR-like genes, supporting the existence of a putative ETI system, however, direct avirulence (Avr)-resistance (R) gene interactions remain largely unverified.

In bacterial infections, *P. savastanoi* pv. *savastanoi* effectors (e.g., HopAZ1, HopBL1) appear to suppress or evade ETI, while in oomycetes *Phytophthora oleae* secretes RxLR, CRN, elicitins, and necrosis-inducing proteins that are typically ETI-activating in other plants, though no receptor

recognition has been demonstrated in olive (Buonaurio et al., 2015; Rapicavoli et al., 2018 a, b; Uceda-Campos et al., 2022; Conti Taguali et al., 2025b). Similarly, although the *V. dahliae* effector Ave1 triggers Ve1-mediated ETI in other species, neither functional recognition nor corresponding olive R genes have been identified despite the presence of Ave1 homologs. Overall, the ETI model in Fig.1 E represents a conserved conceptual framework rather than a fully validated system in olive, reflecting limitations of a non-model woody species where genetic tools are scarce and transcriptomic studies have mainly identified candidate effectors rather than confirmed Avr-R interactions (De Jonge et al., 2012; Estudillo et al., 2025).

3. Role of Multi-Layered Omics

Most olive studies have relied on single-omics approaches; however, a shift toward multi-omics is necessary because plant defense and tolerance mechanisms involve complex interactions among genes, metabolites, and microbial communities that cannot be captured by a single layer. Integrating genomic, transcriptomic, proteomic, and metabolomic data enables a more complete understanding of coordinated defense mechanisms (Table 1), including hormone signaling and metabolic reprogramming, and supports improved disease management strategies. In this context, epigenetic regulation may also play a role in stress-induced gene expression, although it remains unexplored in olive.

3.1. Genomic Resources for Olive Defense Studies

Genomic resources provide the essential foundation for understanding the molecular basis of biotic stress responses in olive, aiding the identification of genetic determinants underlying resistance, tolerance, and adaptation. Whole-genome scanning has expanded this knowledge by revealing extensive genomic variation across Mediterranean germplasm and identifying candidate genes associated with key traits (Bazakos et al., 2023), providing a framework to connect genetic diversity with stress responses. In parallel, olive exhibits a distinctive genome structure shaped by the expansion of tandem repeats rather than typical transposable element dynamics, suggesting high genomic plasticity and regulatory complexity (Mascagni et al., 2022). Together, these advances support omics approaches to dissect defense mechanisms and improve olive resilience to biotic stresses.

3.1.1. Olive Genomes

Advances in genomic resources have transformed the study of disease resistance in olive, providing a foundation for understanding the genetic basis of host defence. Sequencing and assembly of the olive genome have generated high-quality reference maps that enable the identification of resistance-associated loci and candidate genes. These genomic frameworks complement transcriptomic analyses by facilitating the discovery of resistance gene analogs (RGAs) and quantitative trait loci (QTLs), while genome-wide association studies (GWAS) and linkage mapping are increasingly used to link genetic variation with pathogen resistance. Collectively, these resources offer powerful tools for breeding programs, allowing marker-assisted

and genomic selection in a species with long juvenile phases and complex reproductive biology, and enabling comparative analyses that explore diversity among cultivars and wild relatives to understand the evolutionary dynamics of defence-related traits.

The growing availability of *Olea* genome assemblies (Table 2) has provided an essential foundation for dissecting the genetic basis of disease resistance. Early draft genomes, such as the cv. 'Farga', offered scaffold-level references anchored to linkage groups, enabling the initial identification of RGAs and candidate loci (Cruz et al., 2016). Concurrently, the draft assembly of *O. europaea* subsp. *sylvestris* regarded as the wild ancestor of cultivated olive, provided a partially anchored genome that facilitated initial comparative and domestication studies (Unver et al., 2017). The scaffold-level PacBio assembly of cv. 'Picual', further improved through Illumina-based polishing, increased sequence continuity relative to short-read drafts and enhanced the characterization of repetitive regions and gene families, including those implicated in stress responses (Jiménez-Ruiz et al., 2020). Subsequent integration of long-read sequencing technologies with Hi-C scaffolding substantially enhanced genome contiguity, as exemplified by the chromosome-level assembly of cv. Arbequina, which resolved sequences into 23 pseudochromosomes and significantly improved structural annotation and gene model accuracy (Rao et al., 2021).

The recent gapless genome assembly of the tolerant cultivar 'Leccino' represents a major advance in olive genomics by improving the resolution of complex regions, including resistance gene clusters and regulatory elements, thereby supporting studies on quantitative resistance and genomic selection against pathogens such as *Xf* (Lv et al., 2024). In parallel, highly complete PacBio HiFi-based assemblies of cultivars such as 'Leccino' and 'Frantoio' further enhanced genomic resources for comparative genomics, GWAS, and marker-assisted identification of pathogen resistance loci in olive (Sebastiani et al., 2025).

QTL analysis is a valuable approach for dissecting complex disease resistance traits by linking phenotypic variation with molecular markers to identify loci underlying quantitative resistance (Young, 1996). Although resistance-focused QTL studies in *O. europaea* are still limited, studies in *Malus domestica* and *Prunus dulcis* demonstrate its potential (Le et al., 2010; Wöhner et al., 2014; Calle et al., 2025). In olive, QTL analyses have mainly targeted agronomic traits such as flowering, oil composition, frost tolerance, and vigor (Ben Sadok et al., 2013; Kaya et al., 2024; Çetin et al., 2025; Granata et al., 2025; Lamoumni et al., 2025). Candidate-gene studies also identified SNPs and InDels in defense-related genes (*TLP1*, *PFN2*, *DRR2*, *COMT*) associated with *V. dahliae* responses (Serrano et al., 2020). However, markers for resistance to major pathogens such as *Xf* and *Colletotrichum* spp. are still lacking, emphasizing the need for dense SNP genotyping, standardized phenotyping, and genomic selection strategies.

3.1.2. R-gene Analogs (RGAs) and Resistance Repertoires in Olive

High-quality genome sequences and transcriptomic resources in *O. europaea* have enabled in silico annotation of immune-related gene families, including NLR-like sequences and RLK

families, which are frequently differentially expressed under stress and are implicated in pathogen perception and defense activation (Lagiotis et al., 2024). Although no functional R-genes have yet been experimentally validated against major pathogens such as *V. dahliae*, *Xf*, or *Colletotrichum* spp., the presence of annotated receptor and NLR gene families provides a foundation for future studies (Parvez et al., 2025). Integrating RGA annotation with expression profiling, GWAS, and pan-genomic analyses may help identify loci underlying quantitative disease resistance in olive.

3.2. Transcriptomic Approaches

Traditional plant pathology and conventional molecular methods, such as microscopy, culture-based pathogen identification, targeted gene expression analyses (e.g., qPCR), and biochemical assays, have long been foundational in studying olive diseases by revealing visible symptoms, pathogen presence, and specific host responses (Campos et al., 2019; Gharsallah et al., 2020; Conti Taguali et al., 2025a). However, traditional approaches often focus on single pathogens or predefined host genes, providing only partial insight into the complex molecular interactions occurring during infection. In contrast, transcriptomic technologies enable unbiased genome-wide analysis of gene expression in both host and pathogen simultaneously, offering a broader and more precise understanding of disease processes at cellular and tissue-specific levels that are difficult to capture using conventional methods (Jiménez-Ruiz et al., 2019).

The identification of differentially expressed genes in RNA sequencing (RNA-Seq) studies is particularly valuable for gaining an understanding of complex molecular pathways, such as those involved in pathogen recognition and defence activation. Comparative transcriptomic studies of *O. europaea* infected with *V. dahliae* revealed cultivar-dependent responses, with the tolerant cultivar 'Frantoio' showed stronger expression of defense-related genes, including *BAK1*, *NHL1*, dirigent-like proteins, and ROS-associated pathways, whereas the susceptible cultivar 'Picual' exhibited higher expression of fungal virulence- and signaling-related genes (Leyva-Pérez et al., 2018; Jiménez-Ruiz et al., 2019). Similarly, dual RNA-Seq analyses of *Xf* infected olive tissues identified strong in *planta* overexpression of bacterial bacteriocin-related genes, including *cvaC-1*, *cvaC-2*, and Blp family class II bacteriocins (Amoia et al., 2025), implicated in bacterial competition, colonization, and host interaction.

Collectively, these findings demonstrate that transcriptomics not only identifies candidate defense genes but also captures the temporal and regulatory complexity of olive–pathogen interactions. Importantly, the choice of transcriptomic platform critically determines the depth, sensitivity, and interpretative resolution of these analyses.

Building on RNA-Seq studies of infection-induced gene expression, higher-resolution approaches such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) could further resolve host responses at single-cell and spatial levels, particularly in spatially structured infections like *Xf* colonization of xylem or *S. oleaginea* infection of leaf mesophyll. However, these technologies have not yet been applied to olive-pathogen systems, representing a key

methodological gap and a promising avenue to uncover cell-type-specific mechanisms of quantitative resistance.

3.2.1. Microarrays

Microarrays are hybridization-based tools that use predefined probes to detect known transcripts; while limited to previously characterized genes, they remain useful for expression analysis in resource-limited settings due to their lower cost and minimal computational demands.

In *O. europaea*, a custom microarray comprising ~60,000 probes derived from 454-based unigene assemblies and implemented on a CustomArray platform was employed to profile transcriptional responses in drupes challenged by *B. oleae*, allowing a direct comparison between tolerant and susceptible cultivars. Differences in transcriptional profiles between cultivars were associated with less than half the number of genes in the infestation response of the tolerant genotype alone, yet a majority of these genes were up-regulated, supporting the idea that differences in tolerance between the two varieties are driven more by the targeted induction of key genes rather than by widespread changes in gene expression (Grasso et al., 2017). However, microarrays were not widely adopted for pathogen-response studies in olive, largely because early research was constrained by incomplete genomic resources, which hindered reliable probe design. Furthermore, interactions with pathogens such as *Xf* or *V. dahliae* require detection of novel transcripts and low-abundance genes, tasks for which microarrays are inherently limited due to restricted dynamic range and cross-hybridization issues. As genomic resources improved, RNA-Seq rapidly became the preferred platform for unbiased, high-resolution transcriptome profiling (Rai et al., 2018).

3.2.2. RNA-Seq

Unlike microarrays, RNA-Seq does not rely on predefined probes but directly sequences cDNA fragments, enabling detection of rare transcripts, alternative splice variants, and non-coding RNAs (Sekhon et al., 2013). Consequently, most recent studies on olive-pathogen interactions have adopted RNA-seq.

RNA-Seq has been central to understanding olive tolerance to *Xf* subsp. *pauca*. Giampetruzzi et al. (2016) showed extensive transcriptional reprogramming involving receptor-like kinases, hormone signaling, and cell wall modification genes, while Arnholdt-Schmitt et al. (2024) linked tolerance to acetaldehyde production, cyanide-resistant respiration, and regulation of the alternative oxidase (AOX) pathway, highlighting mitochondrial redox balance. Structural defenses also contribute, with increased lignin deposition and elevated quinic acid, along with upregulation of cinnamoyl-CoA reductase and polyphenol oxidase in the tolerant cultivar ‘Leccino’ (Sabella et al., 2018). Field studies further confirmed heritable tolerance traits in ‘Leccino’ progeny under high infection pressure (La Notte et al., 2024), while pathogen-side transcriptomics revealed bacterial induction of bacteriocins during infection, indicating host-pathogen transcriptional interplay (Amoia et al., 2025). Collectively, olive tolerance to *Xf* reflects integrated regulation of

immune signaling, vascular reinforcement, redox homeostasis, and mitochondrial respiratory plasticity.

Integrative transcriptomic and protein network analyses indicate that tolerance to *Xf* in *O. europaea* involves coordinated regulation of receptor-like kinases such as *BAK1*, MAPK cascades, WRKY transcription factors, lipid remodeling enzymes, and carbohydrate metabolism pathways (Balan et al., 2025). Tolerant cultivars exhibit moderated and sustained transcriptional responses associated with vascular homeostasis and metabolic balance. Similarly, RNA-Seq studies of *V. dahliae* identified early activation of pathogen perception, MAPK signaling, phenylpropanoid metabolism, and oxidative stress pathways (Jiménez-Ruiz et al., 2017), while tolerant cv. 'Frantoio' displayed stronger lignification and jasmonate/ethylene signaling responses (Leyva-Pérez et al., 2018). Transcriptomic plasticity also varied with cultivar susceptibility (Jiménez-Ruiz et al., 2019). In *S. oleaginea* interactions, resistant genotypes showed enhanced induction of pathogen-recognition, phenylpropanoid/flavonoid biosynthesis, and ROS detoxification pathways (Marchese et al., 2023b).

3.2.3. Metatranscriptomics

Building on bulk RNA-Seq, metatranscriptomics enables simultaneous profiling of gene expression from the host and associated microbial communities. Reanalysis of RNA-Seq data from *O. europaea* roots infected with *V. dahliae* revealed a temporal shift from predominantly host-derived transcripts to a diverse microbial transcript pool, reflecting increasing activity of fungi, bacteria, and protists during infection. These findings demonstrated that Verticillium wilt is not merely a binary host-pathogen interaction but a dynamic polymicrobial process shaped by rhizosphere community activity, highlighting the value of metatranscriptomics for resolving ecological complexity inaccessible to single-organism approaches (Martí et al., 2020).

3.3. Metabolomics

Metabolomics provides a direct functional readout of plant defense activation by capturing changes in primary and secondary metabolites during pathogen challenge. In olive, metabolomic investigations of biotic stress responses are limited but are beginning to reveal infection-associated biochemical reprogramming, particularly in fruit tissues affected by anthracnose.

In olive, resistance to *V. dahliae* is primarily associated with constitutive root metabolite profiles rather than extensive induced changes. Tolerant cultivars display higher basal levels of secoiridoids, particularly oleuropein derivatives and elenolic acid glucosides, while susceptible ones accumulate compounds such as verbascoside and methoxypinoresinol glucoside. These phenolic and triterpenic metabolites contribute to defense through antifungal and antioxidant activities, limiting pathogen establishment at the root level. Notably, infection triggers only minor and transient metabolic shifts, indicating that resistance relies mainly on preformed chemical defenses and rapid early responses, rather than large-scale metabolomic reprogramming (Cardoni et al., 2023).

Recent metabolomics studies of *O. europaea*-*Colletotrichum* interactions reveal coordinated host and pathogen metabolic responses during infection. In infected olive drupes, reductions in chlorophyll a, chlorophyll b, and carotenoids indicate impaired photosynthesis and pigment degradation, while increased hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and altered soluble sugar levels reflect oxidative stress, lipid peroxidation, and shifts in primary metabolism (Conti Taguali et al., 2025a). Complementary metabolomic profiling of *Colletotrichum acutatum*, *C. gloeosporioides*, *C. godetiae*, and *C. karsti* identified 45 fungal metabolites, including fatty acids, phenolics, pyrones, sterols, and terpenoid-related compounds (Riolo et al., 2023). Infection was associated with accumulation of polyketide-derived toxins such as pyrenocine B, which increased during disease progression and peaked at late infection stages. Together, these findings indicate that olive anthracnose involves both host metabolic disruption and pathogen-derived secondary metabolite production that collectively shape disease progression.

Metabolomic studies of *Xf* infected olive plants have shown accumulation of sugars such as mannitol and sucrose, along with shifts in organic acids, reflecting adjustments in primary metabolism to cope with stress (Jlilat et al., 2021). Secondary metabolites, including oleuropein and several flavonoids, are often altered, indicating modulation of defense-related pathways. Stress-associated compounds such as pyridoxine, abscisic acid, and certain fatty acids are also increased, suggesting the activation of stress and defense response pathways (Di Masi et al., 2022). Comparisons between tolerant and susceptible cultivars in responses to *Xf* infection show differences in flavonoid, phenolic, and sugar metabolism, highlighting cultivar-specific responses and the influence of both genetic background and experimental treatments on host metabolomic profiles (Girelli et al., 2021).

Across olive-pathogen interactions, phenolic and primary metabolism are consistently involved, but responses differ by pathogen lifestyle. In *V. dahliae*, resistance relies mainly on constitutive secoiridoids (e.g., oleuropein derivatives) with limited metabolic reprogramming. In contrast, *Colletotrichum* infection induces strong oxidative stress, pigment degradation, and sugar metabolism shifts, alongside fungal secondary metabolites linked to disease progression. *Xf* also triggers broad changes in sugars, organic acids, and phenolics (including oleuropein and flavonoids) with cultivar-dependent variation. Overall, olive defense ranges from preformed chemical resistance to extensive inducible metabolic reprogramming, depending on the pathogen.

3.4. Proteomics

Proteomic investigations of olive responses to biotic stress remain comparatively underdeveloped relative to transcriptomic studies, yet they are uniquely positioned to bridge the gap between gene expression and functional phenotype. Unlike transcript abundance, protein accumulation, post-translational modification, and subcellular redistribution more directly reflect the biochemical state of immune signaling networks and metabolic fluxes. One of the earliest proteomic studies in olive focused on herbivory, particularly infestation by the olive fruit fly, a major constraint on drupe quality and yield. Using two-dimensional electrophoresis coupled with nanoLC-ESI-LIT-MS/MS,

Corrado et al. (2012) identified proteins involved in stress signaling, phytohormone crosstalk, transcriptional regulation, redox balance, and primary carbon metabolism, revealing that olive fruits undergo extensive proteome remodelling in response to biotic challenge. These findings underscore that olive defense extends beyond canonical immune pathways and involves coordinated metabolic and signaling reprogramming. Methodological advances in protein extraction and mass spectrometry optimization (Capriotti et al., 2013) have since improved analytical robustness, enabling higher-resolution profiling of olive tissues rich in phenolics and lipids. However, direct proteomic characterization of olive responses to major vascular pathogens such as *V. dahliae* and *Xf* remains largely unexplored. This represents a critical gap, particularly given that vascular pathogens perturb xylem integrity, systemic signaling, and long-distance metabolic coordination, processes that are not fully captured by transcriptomic data alone. Despite extensive transcriptomic characterization of olive immunity, the scarcity of large-scale proteomic datasets limits the connection between gene expression and functional resistance Given that protein activity is extensively shaped by phosphorylation, redox regulation, and subcellular dynamics, advanced proteomics approaches will be crucial for the identification of molecular regulators, connecting immune signaling with resistance in olive. Integrating quantitative, phospho-, redox-, and spatial resolved vascular proteomics, therefore, represents a key priority for future research.

3.5. Microbiomics

Although olive defense has been studied primarily from a host-centric perspective, olive trees function as holobionts whose health, stress resilience, and productivity are strongly shaped by associated microbial communities. Consequently, stress responses should be viewed at the whole-system level, integrating host physiology with microbiome dynamics. Olive-associated microbiota influence disease outcomes through competitive interactions, network dynamics, and biocontrol functions, while biotic stresses can disrupt these consortia. Conversely, specific microbial taxa can enhance host defense and resilience, highlighting the potential of microbiome-informed strategies for sustainable disease management (Cardoni & Mercado-Blanco, 2023).

Xf infection responses in olive are strongly associated with differences in endophytic microbiome composition across genotypes, indicating that tolerance is not solely genotype-driven but closely linked to specific microbial assemblages. Tolerant or asymptomatic cultivars harbor a more structured endosphere community enriched in bacterial taxa such as *Pseudomonas*, *Sphingomonas*, *Methylobacterium*, and *Bacillus/Paenibacillus*, widely recognized for plant growth promotion and pathogen antagonism. These genotypes also show enrichment of fungal endophytes such as *Cladosporium* and *Alternaria*, whereas susceptible plants exhibit more dysbiotic fungal profiles dominated by stress-associated assemblages (Vergine et al., 2024).

Olive resistance or reduced disease severity associated with *V. dahliae* is linked to coordinated host-microbiome interactions within the holobiont, integrating plant immune regulation and belowground microbial network structure. Resistant cultivars exhibit upregulation of genes

involved in immune signaling and defense activation, including transcription factors (MYB, bHLH), receptor-like kinases, ethylene-responsive factors, and pathogenesis-related proteins such as β-1,3-glucanases and thaumatin-like proteins, along with genes related to oxidative stress and secondary metabolism. These host responses are closely associated with a structured root microbiome enriched in beneficial taxa, particularly Proteobacteria and Actinobacteria, and keystone genera such as *Actinophytocola*, *Kibdelosporangium*, and *Nocardia*, which correlate with defense gene expression and negatively associate with *Verticillium* spp., suggesting a role in pathogen suppression. In contrast, susceptible cultivars display more disrupted microbial networks, lower resilience, and incorporation of *V. dahliae* into the community, often accompanied by positively connected fungal hubs (Fernández-González et al., 2020; Fernández-González et al., 2025).

Collectively, these findings emphasize that olive defense depends on interactions between host genetic responses and associated microbial communities. Integrating microbiomics with host-centered approaches is therefore essential for understanding disease outcomes and developing resilient, sustainable disease management strategies in olive.

4. Discussion

Given that fully integrated multi-omics studies in olive are still scarce, it is essential to consider a set of strongly interconnected works that collectively represent a stepwise multi-omics exploration of the system. Cardoni et al. (2022) analyzed root defense responses to Verticillium Wilt using targeted gene expression and physiological assays, which was complemented by Cardoni et al. (2023) through metabolomics profiling of phenolic and triterpenic compounds linked to resistance. These findings were then synthesized by Cardoni and Mercado-Blanco (2023) into a broader framework integrating transcriptomics, metabolomics, and microbiomics to conceptualize olive resilience. Similarly, Fernández-González et al. (2020) showed cultivar-dependent differences in belowground microbial networks and keystone taxa, followed by the Fernández-González et al. (2021) study, which integrated endophytic microbiome data with host transcriptomic responses. The Fernández-González et al. (2025) study further consolidated these approaches by jointly identifying key host genes and microbial taxa associated with tolerance, moving toward a unified holobiont-level interpretation of disease resistance.

Plant immunity in olive is a multilayered system that broadly follows the canonical PTI-ETI framework established in *Arabidopsis thaliana*, but with key differences in functional validation. At the basal level, olive relies more heavily than *Arabidopsis* on constitutive defenses, including lignified cell walls and phenolic-rich compounds such as oleuropein derivatives, which function as preformed structural and antimicrobial barriers against pathogens like *V. dahliae* (Jones and Dangl, 2006; Luvisi et al., 2017a; Saponari et al., 2019; Marchese et al., 2023b). In both olive and *Arabidopsis*, PTI is initiated by receptor-like kinases (RLK); however, while receptors such as FLS2 and EFR are functionally validated in *Arabidopsis* (Zipfel et al., 2004; Boller and Felix, 2009), RLKs in olive are mainly inferred from genomic and transcriptomic analyses without direct

biochemical validation (Giampetruzzi et al., 2016; Estudillo et al., 2025). Early signaling is broadly conserved, including Ca^{2+} influx mediated by calcium-dependent protein kinases and ROS bursts generated by NADPH oxidases, which are well-established PTI outputs in *Arabidopsis* (Torres et al., 2006; Boudsocq et al., 2010) and similarly observed in olive-pathogen interactions (Gómez-Lama Cabanás et al., 2015; Novelli et al., 2019). Downstream signaling shows similar conceptual conservation but differing levels of mechanistic resolution. In *Arabidopsis*, MAPK cascades such as MPK3/MPK6 are well characterized and directly link PRR activation to transcriptional reprogramming (Asai et al., 2002), whereas in olive only MAPK-associated genes and transcription factors (WRKY, MYB, AP2/ERF) have been inferred from expression studies without full pathway validation (Giampetruzzi et al., 2016; Marchese et al., 2023b). Hormonal regulation is also conserved, with salicylic acid mediating early defense against biotrophic pathogens and jasmonic acid/ethylene coordinating later or necrotroph-associated responses in both systems (Pieterse et al., 2012; Gharbi et al., 2017; Li et al., 2019; López-Moral et al., 2022). At the output level, both species activate phenylpropanoid metabolism and structural reinforcement; however, olive relies more heavily on constitutive and long-term metabolic defenses consistent with its perennial woody habit (Luvisi et al., 2017a; Cardoni et al., 2023), whereas *Arabidopsis* defense responses are predominantly inducible (Glazebrook, 2005). Finally, ETI is well established in *Arabidopsis* through NLR-mediated recognition of pathogen effectors (Jones and Dangl, 2006; Kourelis and van der Hoorn, 2018), while olive possesses NLR-like genes but lacks experimentally confirmed Avr-R interactions, making ETI largely conceptual rather than functionally validated (Giampetruzzi et al., 2016; Estudillo et al., 2025).

Progress in deciphering olive defense responses remains constrained by the gap between large-scale molecular discoveries and their experimental validation. This limitation largely arises from biological and technical challenges associated with *O. europaea*, including its slow growth, recalcitrance to stable transformation, and the lack of standardized genetic tools. In particular, genetic manipulation in olive is hindered by low transformation efficiency, strong genotype dependence, limited regeneration capacity, and difficulties associated with in vitro culture and plant recovery, collectively constraining the development of reliable transgenic systems (Palomo-Ríos et al., 2021). To overcome these limitations, heterologous and surrogate systems are widely used, enabling functional characterization of candidate genes in tractable model plants such as *Arabidopsis*. This approach has proven effective in biotic stress research, as demonstrated by the heterologous expression of cotton NBS-LRR genes conferring *Verticillium* resistance (Li et al., 2018) and rice WRKY transcription factors enhancing pathogen defense in *Arabidopsis* (Jing et al., 2009). In parallel, emerging functional genomics tools are expanding the experimental repertoire for woody perennial crops. Virus-induced gene silencing (VIGS) provides a rapid, transformation-independent approach for gene function analysis, as shown in citrus where silencing of *CsLOB1* reduced citrus canker susceptibility (Wang et al., 2024a). Similarly, CRISPR/Cas-mediated genome editing has enabled targeted mutagenesis in poplar, citrus, and grapevine for genes involved in development, stress responses, and pathogen resistance (Dong et al., 2024; Ren et al., 2024; Wang et al., 2024b). Together, these approaches help overcome long

generation times and transformation recalcitrance in woody perennials and offer promising avenues for functional validation in olive immunity research.

5. Directions for Future Research

Although omics approaches have substantially advanced our understanding of olive responses to biotic stress, significant conceptual, technical, and translational gaps still remain. Current knowledge is still largely transcript-centric, providing only a partial view of the complex regulatory networks underlying immunity. Future progress will therefore depend on moving beyond single-omics studies toward truly integrative, systems-level multi-omics approaches that combine transcriptomic, proteomic, metabolomic, microbiomics, epigenomic, and genomic data.

A major bottleneck in olive immunity research is the scarcity of functional validation. Candidate R-genes, RLKs, transcription factors, and defense-associated metabolites have been identified, yet causal relationships remain largely untested. Future priorities include optimizing CRISPR/Cas genome editing in olive, developing heterologous and surrogate systems and VIGS-like platforms adapted to woody species, and targeting susceptibility genes to achieve durable, broad-spectrum disease resistance in olive.

Recent advances in genome sequencing have generated multiple whole-genome assemblies for olive cultivars, providing the foundation for an olive pangenome. However, a comprehensive olive pangenome has not yet been established. Future efforts should focus on constructing a high-quality olive pan-genome that includes cultivars and wild relatives to capture structural and presence-absence variation relevant to disease resistance.

In addition, epigenetic regulation of olive immunity remains largely unexplored, particularly DNA methylation, chromatin accessibility, and potential transgenerational immune memory, which may contribute to durable disease resistance. Similarly, microbiome studies are still limited and should be expanded through integrated metagenomic, metatranscriptomic, and metaproteomic approaches to identify active microbial functions and validate keystone host–microbiome interactions. Finally, integrating multi-omics data with systems biology and machine learning will support predictive models of resistance, while direct translation into breeding through marker-assisted selection, genomic selection, and pyramiding of quantitative resistance loci remains a key priority.

Overall, omics-driven research indicates that olive immunity is governed by interconnected transcriptional, hormonal, metabolic, physiological and microbiome-associated process rather than by single resistance genes. Although substantial progress has been achieved through genomics and transcriptomics, huge gaps remain in functional validation and in the integration of molecular information across biological scales. Therefore, future advances will depend on the combination of multi-omics datasets, functional genomics, systems biology, and predictive modelling, supported by AI, to transform descriptive knowledge into mechanistic understanding to accelerate the development of durable disease resistance in olive.

Conflict of interest

The authors declare no conflict of interest.

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Table 1 : A detailed summary of omics studies addressing olive defence mechanisms against biotic stress, including the type of omics applied, the associated biotic stress agents, and the important molecular, biochemical, or physiological outcomes related to plant resistance.

Publication	Omics Category	Biotic stress agents	Outcomes
Estudillo et al., 2025	Genomics	*Spilocaea oleaginea*	• Identified multiple olive genes homologous to apple scab resistance (Rvi) genes, suggesting that olives may possess similar genetic mechanisms for disease resistance.
Serrano et al., 2020	Genomics	*Verticillium dahliae*	• Identified nucleotide diversity in candidate resistance genes that are potentially involved in olive defence against *V. dahliae*. • TLP1 (thaumatin-like protein) and PFN2 were highlighted for their potential roles in pathogen

			recognition and disease resistance responses.
Cardoni et al., 2023	Metabolomics	*Verticillium dahliae*	• Showed that tolerant cultivars have higher basal levels of secoiridoids (e.g., oleuropein-related compounds), whereas susceptible cultivars accumulate compounds such as verbascoside and lignan derivatives. • Demonstrated that infection induces only minor and transient metabolic changes, indicating that olive tolerance is primarily driven by preformed phenolic and triterpenic defenses rather than extensive inducible metabolomic reprogramming.
Conti Taguali et al., 2025a	Metabolomics	*Colletotrichum spp.*	• Olive response to *Colletotrichum* infection is strongly cultivar-dependent, with 'Leccino' being the most tolerant and 'Ottobratica' the most susceptible. • Infection triggered distinct physiological and biochemical changes, including oxidative stress and metabolic shifts.
Di Masi et al., 2022	Metabolomics	*Xylella fastidiosa*	• Modulation of phenolic and secondary metabolites, together with shifts in oxidative stress-related and lipid compounds, reflects coordinated antimicrobial, ROS-driven, and membrane-related defence responses in olive.
Girelli et al., 2021	Metabolomics	*Xylella fastidiosa*	• Tolerant and susceptible olive cultivars show distinct metabolomic responses to *Xf* infection, with tolerant cultivars exhibiting increased accumulation of phenolic and secondary metabolites linked to antimicrobial defence and resistance. • Metabolomic changes involve stress-related pathways, including

			oxidative stress and lipid metabolism, can serve as indicators of defence status and tolerance for identifying resistant cultivars.
Jlilat et al., 2021	Metabolomics	*Xylella fastidiosa*	• An enhanced accumulation of phenolic compounds, together with alterations in oxidative stress-related metabolites and lipid and secondary metabolism, reflects integrated antimicrobial, ROS-driven, and structural defence responses to infection.
Riolo et al., 2023	Metabolomics	*Colletotrichum*	• The study identified 45 secondary metabolites produced by four *Colletotrichum* species during infection of olive, including fatty acids, phenolics, pyrones, sterols, and terpenes. • It showed that metabolite accumulation increases during infection, peaking at later stages and correlating with disease severity.
Cardoni and Mercado-Blanco (2023)	Microbiomics	*Verticillium dahliae, Xylella fastidiosa, Pseudomonas savastanoi, Colletotrichum, Spilocaea oleaginea, Phytophthora, Meloidogyne, Pratylenchus, Bactrocera oleae, Prays oleae*	• Olive stress tolerance depends on the holobiont system, where plant performance under biotic stress is shaped by interactions between the host, root traits, and associated microbiome, rather than plant genetics alone. • Disease suppression is linked to beneficial microbiome recruitment, where microbes such as *Pseudomonas* and *Paenibacillus* spp. help suppress pathogens and are influenced by cultivar-dependent root traits and constitutive chemical defenses.
Fernández-González et al., 2020	Microbiomics	*Verticillium dahliae*	• 'Frantoio' (tolerant) and 'Picual' (susceptible) showed similar baseline microbial diversity but differed in composition, with 'Frantoio' enriched in beneficial taxa and maintaining greater

			microbiome stability after *V. dahliae* infection, whereas 'Picual' exhibited stronger microbial disruption. • Infection also induced more connected and stable microbial networks in 'Frantoio', while 'Picual' showed fragmented networks and stronger shifts in arbuscular mycorrhizal fungal distribution.
Fernández-González et al., 2025	Microbiomics & Transcriptomics	*Verticillium dahliae*	• Found enrichment of defense- and metabolism-related biological processes in tolerant cultivars, including carbohydrate metabolism, amino acid transport, transcription regulation, oxidoreductase activity, and immune response pathways, indicating coordinated metabolic–defense reprogramming. • Identified of keystone microbial taxa associated with tolerance, particularly bacterial genera such as *Actinophytocola*, *Kibdelosporangium*, and *Nocardia*, which were significantly enriched in tolerant olive genotypes.
Vergine et al., 2024	Microbiomics	*Xylella fastidiosa*	• Asymptomatic or low-symptomatic olive genotypes exhibited a distinct endophytic microbiome compared to highly symptomatic plants under the same *Xf* pressure, indicating strong genotype-driven selection of microbial communities. • Tolerant or low-symptomatic olive genotypes were enriched in beneficial bacterial genera such as *Burkholderia*, *Bacillus*, *Sphingomonas*, and *Hymenobacter*, which are commonly associated with plant

			growth promotion and pathogen suppression.
Corrado et al., 2012	Proteomics	*Bactrocera oleae*	• Olive fruits respond to *B. oleae* infestation through complex molecular changes, including activation of stress responses, phytohormone signaling, and defense-related pathways. • Identified specific defense-related genes and proteins, such as chitinases and trypsin inhibitors.
Wang et al., 2024a	Transcriptomics and Metabolomics	*Alternaria alternata*	• Flavonoid biosynthesis genes, including chalcone synthase (*CHS*), cinnamate 4-hydroxylase (*C4H*), and flavanone 3-hydroxylase (*F3H*), were strongly upregulated in resistant olive cultivars, indicating their direct role in defence against *A. alternata*.
Amoia et al., 2025	Transcriptomics	*Xylella fastidiosa*	• The bacteriocin gene cvaC-1 is highly overexpressed during infection, making it a reliable molecular marker for detecting active growth of *Xf* in olive. • Variation in cvaC-1/cvaC-2 ratio across strains indicates strain-specific infection dynamics.
Arnholdt-Schmitt et al., 2024	Transcriptomics	*Xylella fastidiosa*	• Tolerance to *Xf* is linked to metabolic adjustments, particularly enhanced aerobic fermentation, phenolic metabolism, and cyanide-mediated regulation of respiration. • Increased expression of the alternative oxidase (*AOX*) gene family as a key marker of resilience.
Balan et al., 2025	Transcriptomics	*Xylella fastidiosa*	• Immune regulators (BAK1, WRKYs) form interconnected defense hubs which can be the targets for broad-spectrum resistance in plants including olive. • Plant defense against *Xf* involves hormonal signaling (JA, SA, ABA)

			and metabolic pathways that enhance stress response and antimicrobial compound production.
Giampetruzzi et al., 2016	Transcriptomics	*Xylella fastidiosa*	• *Xf* infection triggers distinct transcriptomic responses in olive cultivars, with the tolerant 'Leccino' activating stronger defense mechanisms than the susceptible 'Ogliarola salentina'. • Showed enhanced expression of receptor-like kinases and cell wall-related genes in 'Leccino' contributes to its lower pathogen levels and intrinsic tolerance.
Gómez-Lama Cabanás et al., 2015	Transcriptomics	*Verticillium dahliae*	• *V. dahliae* induces extensive systemic transcriptomic changes in the tolerant olive cultivar 'Frantoio', involving many genes related to defense responses. • Identified specific genes, such as *GRAS1* and *DRR2*, whose expression patterns are associated with tolerance.
Grasso et al., 2017	Transcriptomics	*Bactrocera oleae*	• Tolerant olive cultivar shows widespread activation of genes involved in oxidative stress responses, hormone signaling, and secondary metabolism. • Genes affecting cellular structure and primary metabolism are differentially regulated as a result of the induced defense.
Jiménez-Ruiz et al., 2019	Transcriptomics	*Verticillium dahliae*	• Pathogen activated genes related to virulence, pathogenicity, niche adaptation, and microsclerotia development mainly in 'Picual', while these genes remain largely unexpressed in 'Frantoio'.
Jiménez-Ruiz et al., 2017	Transcriptomics	*Verticillium dahliae*	• Coordinated transcriptomic changes happened during infection,

			with genes related to proteolysis, protein folding, and biosynthesis. • Revealed a temporal defense pattern in which reactive oxygen species (ROS) responses are activated first in the pathogen and later in the olive roots.
La Notte et al., 2024	Transcriptomics	*Xylella fastidiosa*	• Identified large-scale transcriptomic changes in resistant genotypes, highlighting key pathways like photosynthesis, cell wall, and metabolism.
Leyva-Pérez et al., 2018	Transcriptomics	*Verticillium dahliae*	• Identified key defense-related genes, including those involved in ROS response, lignification, and pathogen recognition, that are differentially expressed and likely contribute to the tolerant phenotype. • Genes related to pathogen recognition (e.g., *BAK1*, *NHL1*) and defense signaling are more effectively regulated in the tolerant cultivar.
Marchese et al., 2023b	Transcriptomics	*Spilocaea oleaginea*	• Cultivar 'Koroneiki' mounts a strong and early defense response against *S. oleaginea*, characterized by extensive hormonal crosstalk involving ABA, ethylene, and jasmonate. • Results highlight enhanced cell wall biosynthesis and remodeling, including lignin formation.
Mascuñano et al., 2025	Transcriptomics	*Verticillium dahliae*	• Resistance to *V. dahliae* in wild olive clone AC18 is associated with constitutively high lignin levels, which act as a pre-formed physical defence barrier. • Highlights the role of cell wall strengthening and structural defense mechanisms as a key factor in resistance to Verticillium wilt.

Martí et al., 2020	Metatranscriptomics	*Verticillium dahliae*	• Infection triggered microbiome-driven defence modulation, where shifts in microbial gene expression influence the overall defence outcome. • Coexistence of biotrophic and necrotrophic microorganisms requires the plant to coordinate multiple defence pathways, likely involving both salicylic acid- and jasmonate-related responses.

Table 2 : Summary of major *Olea europaea* genome assemblies and their assembly characteristics.

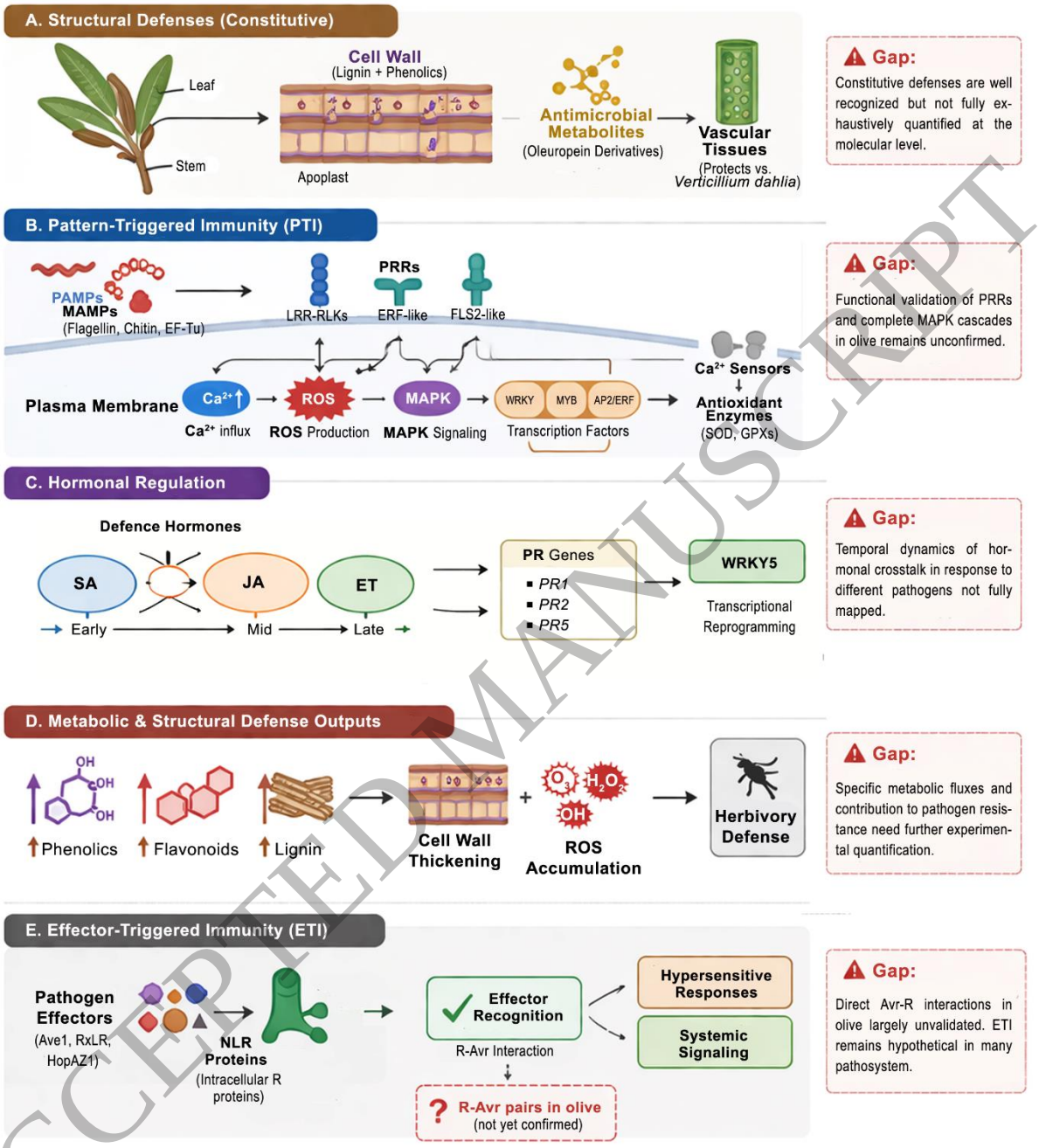
Assembly / Name	Assembly Level	Approx. Size	Reference
O. europaea var. *europaea* cv. Farga (Oe9)	Chromosome-anchored draft	~1.32 Gb	Cruz et al., 2016
O. europaea subsp. *sylvestris* (Oe451_v1)	Draft assembly, partially anchored	~1.48 Gb	Unver et al., 2017
O. europaea cv. 'Picual' (Oleur061)	Draft assembly - scaffold-level (PacBio + Illumina reads)	~1.68 Gb	Jiménez-Ruiz et al., 2020
O. europaea cv. Arbequina (Oe_Rao)	Chromosome-level (Hi-C)	~1.30 Gb	Rao et al., 2021
O. europaea cv. (telomere-to-telomere; T2T) 'Leccino'	Chromosome-level gap-free T2Tassembly	~1.28 Gb	Lv et al. 2024
O. europaea cv. Leccino (2025 PacBio)	Contig level (HiFi)	~1.43 Gb	Sebastiani et al., 2025
O. europaea cv. Frantoio (2025 PacBio)	Contig level (HiFi)	~1.18 Gb	Sebastiani et al., 2025

Figure Legend

Fig.1 : Multilayered defence strategies in olive (*O. europaea*) depicting structural, molecular, hormonal, and metabolic defenses against pathogens, including pattern-triggered immunity (PTI)

and effector-triggered immunity (ETI) with highlighted knowledge gaps indicating areas where mechanisms remain incompletely characterized or experimentally unvalidated. A. Structural Defenses (Constitutive): Lignin- and phenolic-enriched cell walls, along with oleuropein derivatives in the apoplast, provide basal protection against pathogens such as *V. dahliae*. B. Pattern-Triggered Immunity (PTI): Plasma membrane receptors detect PAMPs, activating Ca^{2+} influx, ROS production, MAPK signaling, and transcription factors (WRKY, MYB, AP2/ERF). C. Hormonal Regulation: SA (Salicylic Acid), JA (Jasmonic Acid), and ET (Ethylene) coordinate defense gene expression (*PR1, PR2, PR5*) and transcription factors (WRKY5) over time. D. Metabolic & Structural Defense Outputs: Accumulation of phenolics, flavonoids, lignin, ROS (Reactive Oxygen Species), and cell wall thickening enhances defense, including protection against herbivory. E. Effector-Triggered Immunity (ETI): NLR receptors recognize pathogen effectors (Ave1, RxLR, HopAZ1), triggering hypersensitive response and systemic signaling, with some interactions remaining unconfirmed.

ALT TEXT: Schematic overview of multilayered defense mechanisms in olive (*Olea europaea*) against pathogens. Constitutive structural barriers include lignified cell walls and phenolic compounds. Pattern-triggered immunity (PTI) is activated through plasma membrane receptors that recognize pathogen-associated molecular patterns, leading to calcium signaling, reactive oxygen species production, MAPK cascades, and activation of WRKY, MYB, and AP2/ERF transcription factors. Salicylic acid, jasmonic acid, and ethylene coordinate downstream defense responses, including expression of pathogenesis-related genes. Defense outputs include phenolic accumulation, flavonoid biosynthesis, lignification, reactive oxygen species production, and cell wall reinforcement. Effector-triggered immunity (ETI) is mediated by NLR receptors that recognize pathogen effectors and induce hypersensitive responses and systemic signaling. Knowledge gaps are highlighted for immune components and interactions that remain incompletely characterized or experimentally unvalidated in olive.



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