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Research Article

Molecular Identification and Pathogenesis of *Xanthomonas arboricola* pv. *juglandis* Isolates from Walnut in Azad Jammu and Kashmir, Pakistan

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ABSTRACT

Xanthomonas arboricola pv. *juglandis* causes bacterial blight of walnut, a highly destructive disease of walnut (*Juglans regia*) in the temperate climates of Pakistan. Although the geographical distribution and biochemical characterization of the pathogen in Azad Jammu and Kashmir (AJK) have been reported previously, its molecular characterization and pathogenicity on local cultivars remain largely unexplored. In the present study, we provided the first comprehensive molecular characterization and phylogenetic analysis of 20 *X. arboricola* pv. *juglandis* isolates recovered from infected walnut trees across multiple locations in AJK, along with one reference isolate from the University of Punjab, Lahore. The bacterial isolates were cultured, and genomic DNA was extracted. The 16S ribosomal RNA (rRNA) gene was amplified using universal bacterial primers, sequenced, and analyzed alongside reference sequences retrieved from GenBank. Phylogenetic analysis was performed using the maximum likelihood method with appropriate outgroup sequences. The 16S rRNA sequences exhibited high nucleotide identity (98.5-100%) with previously reported sequences of *X. arboricola* pv. *juglandis*, confirming the species and pathovar identity of the isolates. Phylogenetic analysis revealed two major genetically distinct clades among the studied isolates, indicating both evolutionary divergence and close relatedness to reference strains. Pathogenicity assays on walnut seedlings demonstrated that all isolates were virulent, producing disease severity scores ranging from 1.0 to 3.5, indicating moderate to high pathogenic potential. These molecular and pathogenicity findings enhance the understanding of the epidemiology of walnut bacterial blight in AJK and are expected to support the development of region-specific disease management strategies and breeding programs for sustainable walnut production.

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Introduction

Walnut (*Juglans regia* L.) is a highly valued nut tree cultivated worldwide and in Pakistan, particularly in the temperate regions of the country, including Azad Jammu and Kashmir (AJK) and Khyber Pakhtunkhwa. It serves as an important source of food, nutrition, and income for local communities. However, walnut productivity in these regions

is constrained by several biotic and abiotic factors. Among the biotic stresses, walnut bacterial blight caused by *Xanthomonas arboricola* pv. *juglandis* is considered one of the most destructive diseases affecting walnut production (Moragrega and Llorente, 2023). This Gram-negative, rod-shaped bacterium produces characteristic necrotic lesions on leaves, twigs, flowers, and fruits, leading to fruit drop,

defoliation, and substantial yield losses. Severe infections may reduce fruit marketability, cause premature fruit abscission, and, in rare cases, result in tree death (Schaad et al., 2001; Teviotdale, 2002).

The disease was first reported in California and has subsequently spread to major walnut-growing regions of Europe, Asia, and other parts of the world (Lamichhane, 2014). The pathogen overwinters epiphytically on buds and twigs and becomes active during spring and early summer when environmental conditions, particularly moderate temperatures and prolonged leaf wetness, favor its growth and dissemination, resulting in secondary infections (Kałużna et al., 2021). Our previous geospatial investigations revealed the widespread occurrence of walnut bacterial blight across several districts of AJK, identifying the disease as a major constraint to walnut production in the region. However, that study primarily relied on morphological and biochemical characteristics for pathogen identification.

Molecular identification methods are considered more reliable and precise than conventional identification approaches. These methods enable accurate species and pathovar determination, reveal genetic diversity and population structure, facilitate phylogenetic analysis, and assist in the detection of virulence-associated markers (Essakhi et al., 2015). Among molecular markers, the 16S rRNA gene has been widely recognized as a standard tool for bacterial identification and classification because of its highly conserved nature at the species level and variable regions at higher taxonomic levels (Weisburg et al., 1991). Sequencing of the 16S rRNA gene provides reliable taxonomic assignment of bacterial isolates and offers insights into their evolutionary relationships (Tamura et al., 2013).

Therefore, the present study was conducted to perform the first molecular characterization of *X. arboricola* pv. *juglandis* isolates collected from AJK using 16S rRNA gene sequencing and phylogenetic analysis. We hypothesized that molecular characterization would confirm the identity of the biochemically characterized isolates and provide insights into their phylogenetic relationships with strains reported worldwide. Specifically, the objectives of this study were to isolate high-quality genomic DNA from selected bacterial isolates, amplify and sequence the 16S rRNA gene, perform phylogenetic reconstruction using closely related reference sequences, and validate the molecular findings through *in vivo* pathogenicity tests. The outcomes of this study will provide a molecular basis for understanding the epidemiology of walnut bacterial blight in AJK and contribute to the development of effective disease management strategies.

Materials and Methods

Bacterial isolate selection and culture

A total of 20 isolates of *X. arboricola* pv. *juglandis* were selected from the culture collection established through an extensive geospatial survey of walnut bacterial blight conducted in AJK, Pakistan, during 2023-2024. The isolates were recovered from symptomatic twigs, fruits, and leaves of walnut trees collected from orchards located in four districts, namely Muzaffarabad, Jhelum Valley, Poonch, and Bagh. In addition, a reference isolate (XAJ-T) was obtained from the University of the Punjab, Lahore, and used as a tester strain for comparative analyses.

All isolates were maintained as pure cultures on nutrient agar (NA) medium at 4°C. Prior to molecular characterization, bacterial isolates were revived by streaking onto fresh NA plates and incubating at 25°C for 48 h. Colony morphology characteristic of *Xanthomonas* spp., including yellow, smooth, and mucoid colonies, was examined and recorded before further molecular analyses.

DNA extraction and amplification of the 16S rRNA gene

Genomic DNA was extracted from each bacterial isolate using the GeneJET Genomic DNA Purification Kit (K0721, Thermo Scientific, USA) according to the manufacturer's instructions. The 16S rRNA gene was amplified using the universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), following the protocol described by Weisburg et al. (1991).

Polymerase chain reaction (PCR) was performed in a final reaction volume of 25 µL containing approximately 25 ng genomic DNA template, 1× PCR buffer supplemented with MgCl₂, 0.2 mM dNTPs, 0.5 µM of each primer, and 1 U of *Taq* DNA polymerase. PCR amplification was carried out in a thermal cycler under the following conditions: initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 90 sec; with a final extension step at 72°C for 7 min.

Amplified PCR products were separated on 1% agarose gel electrophoresis stained with ethidium bromide to confirm the presence of the expected amplicon size of approximately 1,500 bp. The PCR products were subsequently purified using a PCR purification kit and quantified prior to sequencing.

Sequence analysis and phylogenetic characterization

Purified PCR amplicons were submitted to Microgen (Korea) for bidirectional sequencing. Forward and reverse sequence reads were assembled into consensus sequences after trimming low-quality regions and removing ambiguous nucleotides. The obtained consensus sequences were compared with sequences available in the GenBank database

using the Basic Local Alignment Search Tool (BLAST) to confirm species identity.

Phylogenetic relationships among the isolates were inferred using the maximum likelihood method implemented in MEGA version 6.0 software. Sequence alignment and evolutionary analyses were performed to determine the genetic relatedness among the collected isolates and reference strains.

Pathogenicity assessment

To validate molecular identification and evaluate the virulence of the bacterial isolates, pathogenicity assays were conducted on healthy walnut seedlings. Three-month-old seedlings of walnut cultivar ‘Chandler’ were grown under greenhouse conditions maintained at $25 \pm 2^\circ\text{C}$ and $70 \pm 5\%$ relative humidity.

Each bacterial isolate was cultured in nutrient broth for 24 h, and the bacterial suspension was adjusted to approximately 1

$\times 10^8$ CFU mL^{-1} . The seedlings were spray-inoculated until runoff with the prepared bacterial suspensions. Five seedlings were inoculated per isolate, whereas two seedlings sprayed with sterile distilled water served as negative controls.

Following inoculation, the seedlings were maintained in high-humidity chambers (95-98% RH) for 48 h to facilitate infection. Thereafter, the plastic covers were removed, and plants were monitored daily for symptom development. Disease severity was assessed based on lesion development and the percentage of infected leaf area using a 0-4 disease rating scale (Table 1). The incubation period, symptom initiation time, and rate of disease progression were also recorded.

At the conclusion of the experiment, symptomatic tissues were collected for bacterial re-isolation to fulfill Koch’s postulates and confirm the pathogenicity of the inoculated isolates.

Table 1. Disease rating scale used for pathogenicity testing of *X. arboricola* pv. *juglandis* on walnut seedlings.

Disease Rating	Symptoms
0	No symptoms
1	Water-soaked lesions only (< 5% leaf area affected)
2	Water-soaked lesions progressing to necrotic spots (5-15% leaf area affected)
3	Extensive necrotic lesions (15-40% leaf area affected)
4	Severe necrosis with leaf defoliation (> 40% leaf area affected)

Results

Isolate initial characterization

A total of twenty isolates of *X. arboricola* pv. *juglandis* were collected during the 2023-2024 growing season from infected walnut tissues in AJK, along with one reference isolate obtained from Punjab University, Lahore (Table 2). Field samples were collected from orchards in the districts of Muzaffarabad (n = 8), Jhelum (n = 6), Poonch (n = 4), and Bagh (n = 2). Infected plant tissues included leaves, fruits, and twigs from diseased walnut trees.

All isolates exhibited typical morphological characteristics of *X. arboricola* pv. *juglandis*, producing yellow, mucoid colonies on nutrient agar and yeast dextrose calcium carbonate (YDC) media.

DNA quality and PCR amplification

The quality of genomic DNA extracted from all twenty-one isolates was sufficiently high for downstream analyses, as indicated by A260/A280 ratios consistently ranging from 1.8 to 2.0. PCR amplification using universal 16S rRNA gene primers yielded a single, clear amplicon of approximately 1500 bp for each isolate. No amplification was observed in the negative controls (without template DNA), confirming the absence of contamination and ruling out the formation of primer-dimers or non-specific products (Figure 1a and 1b). The purified PCR products were quantified prior to sequencing (Khan et al., 2000).

16S rRNA sequencing and species identification

High-quality consensus sequences of approximately 1,500 bp were successfully obtained for all twenty isolates. BLAST analysis against the GenBank database revealed that all sequences showed highest similarity with reference sequences of *X. arboricola* pv. *juglandis*. Nucleotide identity ranged from 98.5% to 100%, with three isolates exhibiting 98.5-99% identity and seventeen isolates showing 99-100% identity. Comparisons with other *X. arboricola* pathovars revealed substantially lower identity values, while no non-*Xanthomonas* taxa exhibited $\geq 97\%$ identity, thereby ruling out contamination (Bradbury, 1986). The maximum likelihood phylogenetic analysis clearly resolved the phylogenetic placement of the isolates within the *X. arboricola* pv. *juglandis* clade (Figure 2).

The phylogenetic analysis grouped the studied isolates into two major clades. Clade I comprised isolates XAJ-2, XAJ-14, XAJ-3, XAJ-5, XAJ-12, XAJ-7, XAJ-10, XAJ-4, XAJ-9, XAJ-11, and XAJ-13. These isolates clustered closely together, indicating a high degree of genetic similarity and suggesting a common evolutionary origin. Within this clade, certain isolates, particularly XAJ-3 and XAJ-5, as well as XAJ-4 and XAJ-9, exhibited very close phylogenetic relationships. The bootstrap values supporting this clade indicated moderate to strong support, confirming the robustness of the inferred grouping among these isolates.

Table 2. Bacterial isolates of *X. arboricola* pv. *juglandis* collected from different localities during 2023-2024.

Sr. No.	Location/Source	Host Tissue	Isolation Code
1	Muzaffarabad Orchard-1	Walnut Leaf	XAJ-1
2	Muzaffarabad Orchard-1	Walnut Fruit	XAJ-2
3	Muzaffarabad Orchard-2	Walnut Leaf	XAJ-3
4	Muzaffarabad Orchard-2	Walnut Twig	XAJ-4
5	Muzaffarabad Orchard-3	Walnut Leaf	XAJ-5
6	Muzaffarabad Orchard-3	Walnut Fruit	XAJ-6
7	Muzaffarabad Orchard-4	Walnut Leaf	XAJ-7
8	Muzaffarabad Orchard-4	Walnut Twig	XAJ-8
9	Jhelum Orchard-1	Walnut Leaf	XAJ-9
10	Jhelum Orchard-2	Walnut Fruit	XAJ-10
11	Jhelum Orchard-3	Walnut Leaf	XAJ-11
12	Jhelum Orchard-3	Walnut Twig	XAJ-12
13	Jhelum Orchard-4	Walnut Fruit	XAJ-13
14	Jhelum Orchard-4	Walnut Leaf	XAJ-14
15	Poonch Orchard-1	Walnut Leaf	XAJ-15
16	Poonch Orchard-2	Walnut Fruit	XAJ-16
17	Poonch Orchard-2	Walnut Twig	XAJ-17
18	Poonch Orchard-3	Walnut Leaf	XAJ-18
19	Bagh Orchard-1	Walnut Fruit	XAJ-19
20	Bagh Orchard-2	Walnut Leaf	XAJ-20
21	Punjab University Lahore (Tester)	Culture Collection	XAJ-T

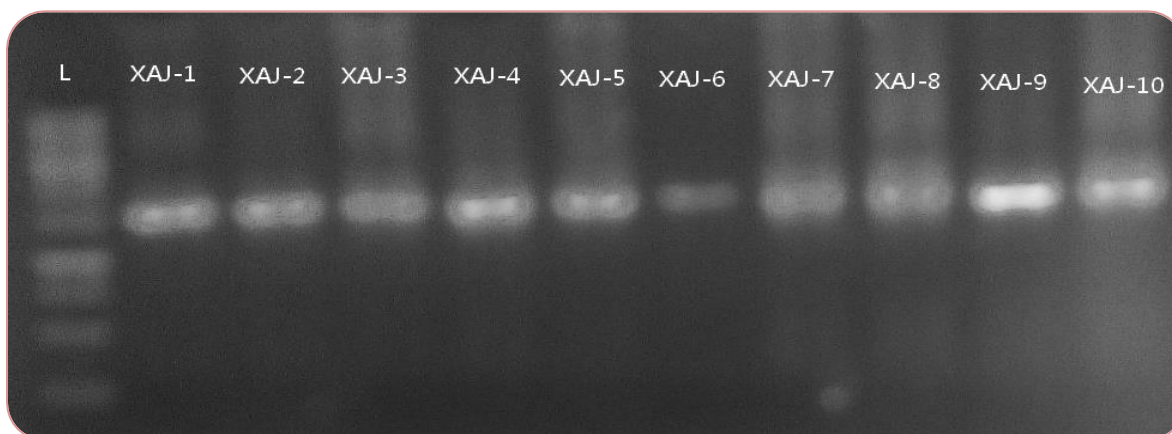


Figure 1(a). PCR amplification showing ~1500 bp fragments using 16S rRNA primers.

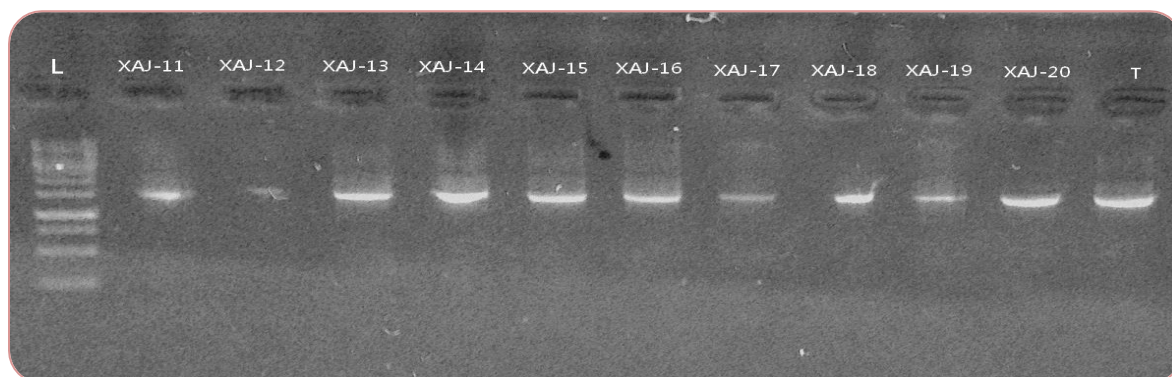


Figure 1(b). PCR products showing approximately 1500 bp fragments amplified using 16S rRNA primers.

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nucleotide identity with previously published *X. arboricola* pv. *juglandis* sequences in GenBank, exceeding the 97% threshold commonly used for species-level classification (Stackebrandt and Goebel, 1994). This molecular consistency, in agreement with earlier biochemical and morphological characterization, further confirms *X. arboricola* pv. *juglandis* as the causal agent of walnut blight in AJK (Kałużna et al., 2021).

Three isolates showed slightly lower sequence similarity (98.5-99%), yet were still confidently identified as *X. arboricola* pv. *juglandis*. This identification was supported by multiple lines of evidence, including BLAST analysis, robust clustering within phylogenetic trees with high bootstrap support, characteristic phenotypic traits of *Xanthomonas*, and successful pathogenicity on walnut. Such minor sequence variation is common within bacterial species and has been previously reported in studies of *X. arboricola* from different geographical regions (Essakhi et al., 2015; Assis et al., 2021).

The very low genetic diversity observed among the twenty AJK field isolates is particularly noteworthy. This pattern is consistent with global studies reporting limited sequence variability in *X. arboricola* pv. *juglandis* populations despite wide geographic distribution (Lamichhane, 2014). The absence of district-wise sequence variation suggests either a single introduction event in AJK or continued homogenization through the movement of infected plant material and agricultural tools. In contrast, other *Xanthomonas* pathovars show greater genetic variability, suggesting that *X. arboricola* may have undergone founder effects during its global dissemination (Kałużna et al., 2021).

An important finding of this study is that, despite near-identical 16S rRNA sequences, pathogenicity scores varied considerably among isolates (1.0-3.5), indicating the presence of strain-level differences not captured by this molecular marker. Similar observations have been reported for other bacterial pathogens (Cowan et al., 1993). Future studies employing whole-genome sequencing or targeted PCR assays for virulence-associated genes (e.g., type III secretion system components or toxin-related genes) may help elucidate the genetic basis of these differences. Such understandings could facilitate the identification of potentially hypervirulent strains requiring priority management (Sundin et al., 2004).

All twenty-one isolates were pathogenic on walnut seedlings, producing symptoms within 5-8 days post-inoculation. The observed lesion development, beginning as water-soaked spots and progressing to necrosis, closely matched field symptoms. Successful re-isolation of the

pathogen from infected tissues confirmed Koch's postulates (Nutter et al., 2006). The comparable virulence of the reference isolate from Punjab University and the AJK field isolates supports its use as a standard strain for controlled experiments and cultivar evaluation. This standardization could enhance inter-institutional research consistency and facilitate screening of resistant walnut genotypes.

The molecular findings of this study have important implications for disease management in AJK. The confirmed dominance of *X. arboricola* pv. *juglandis* supports the implementation of pathogen-specific control strategies, including timely bactericide applications during infection periods (Teviotdale, 2002). The observed genetic homogeneity suggests that resistant cultivars developed elsewhere may also be effective in AJK, enabling the deployment of existing resistant germplasm rather than region-specific breeding programs, which are time-consuming and costly (Lamichhane, 2014).

Furthermore, the availability of molecular sequence data facilitates the development of rapid diagnostic tools, such as PCR-based assays, which could replace labor-intensive culture-based identification methods. At the research level, these molecular references provide a foundation for future studies on local adaptive evolution and host-pathogen interactions under AJK-specific agro-ecological conditions (Khan et al., 2000).

Although 16S rRNA sequencing is effective for species-level identification, it has limited resolution for distinguishing virulence variation or fine-scale strain diversity. The observed discrepancy between genetic uniformity and phenotypic variability in pathogenicity highlights the need for higher-resolution genotyping approaches. Although the present study included 20 field isolates, future research should expand sampling to neighboring walnut-producing regions, including Khyber Pakhtunkhwa and other areas, to better understand broader population structure and potential regional differentiation (Essakhi et al., 2015). Moreover, serological and historical surveys of orchards may help determine whether the observed homogeneity reflects a recent introduction or long-term endemic establishment (Moragrega and Llorente, 2023).

Conclusion

This study provides the first comprehensive molecular characterization of *Xanthomonas arboricola* pv. *juglandis* in Azad Jammu and Kashmir, confirming it as the causal agent of walnut bacterial blight in the region. Analysis of 20 field isolates and one reference isolate based on 16S rRNA gene sequencing revealed 98.5-100% sequence

similarity with previously reported strains, with all isolates clustering strongly within *X. arboricola* pv. *juglandis* in phylogenetic analysis.

The low genetic diversity observed among isolates suggests either a recent introduction or a genetically homogenized pathogen population without clear geographic structuring. Pathogenicity assays confirmed that all isolates were virulent, with disease severity scores ranging from 1.0 to 3.5, and successful re-isolation of the pathogen fulfilled Koch's postulates.

These findings provide a molecular foundation for region-specific disease management strategies in AJK, support the development of rapid diagnostic tools, and facilitate the selection of resistant walnut cultivars. The availability of local molecular reference sequences also opens avenues for future research on population dynamics, virulence mechanisms, and host-pathogen interactions. Overall, this study enhances understanding of walnut blight epidemiology in AJK and provides valuable insights for improving sustainable walnut production in the region.

Author's contributions

BM and RA conceived and designed the study. BM, RA, and MTK conducted field surveys and collected diseased walnut samples from Azad Jammu and Kashmir, Pakistan. BM and UM carried out the isolation, molecular identification, and pathogenicity assessment of *Xanthomonas arboricola* pv. *juglandis* isolates, performed statistical and data analyses, and prepared the original draft of the manuscript. MAI and MTK provided critical scientific guidance, constructive feedback, and valuable suggestions to improve the scientific quality, coherence, and clarity of the manuscript. RA supervised the overall research activities and ensured adherence to scientific rigor throughout the study. All authors critically reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work, ensuring accuracy, transparency, and integrity in the reporting of results.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

Policy Addressed

The present study supports the National Food Security Policy of Pakistan, Pakistan Vision 2025, and FAO-led Integrated Pest Management strategies by providing molecular epidemiological insights into walnut bacterial blight. The findings are expected to strengthen disease surveillance, improve pathogen diagnostics, and support sustainable walnut production and plant health management in the temperate regions of Pakistan.

References

- Assis, R.A., Varani, A.M., Sagawa, C.H., Patané, J.S., Setubal, J.C., Uceda-Campos, G., Dandekar, A.M., 2021. A comparative genomic analysis of *Xanthomonas arboricola* pv. *juglandis* strains reveal hallmarks of mobile genetic elements in the adaptation and accelerated evolution of virulence. *Genomics* 113, 2513-2525.
- Bradbury, J.F., 1986. Guide to plant pathogenic bacteria. CAB International, Farnham Royal, Slough, UK, pp. xviii-332.
- Cowan, S.T., 1993. Cowan and Steel's manual for the identification of medical bacteria. Cambridge University Press, Cambridge, UK.
- Essakhi, S., Cesbron, S., Fischer-Le Saux, M., Bonneau, S., Jacques, M.A., Manceau, C., 2015. Phylogenetic and variable-number tandem-repeat analyses identify nonpathogenic *Xanthomonas arboricola* lineages lacking the canonical type III secretion system. *Applied and Environmental Microbiology* 81, 5395-5410.
- Kałużna, M., Fischer-Le Saux, M., Pothier, J.F., Jacques, M.A., Obradović, A., Tavares, F., Stefani, E., 2021. *Xanthomonas arboricola* pv. *juglandis* and pv. *corylina*: Brothers or distant relatives? Genetic clues, epidemiology, and insights for disease management. *Molecular Plant Pathology* 22, 1481-1499.
- Khan, A.A., Furuya, N., Matsumoto, M., Matsuyama, N., 2000. Differentiation of phytopathogenic *Pseudomonas* and *Xanthomonas* pathovars and strains by PCR analysis for DNA topoisomerase genes.
- Lamichhane, J.R., 2014. *Xanthomonas arboricola* diseases of stone fruit, almond, and walnut trees: progress toward understanding and management. *Plant Disease* 98, 1600-1610.
- Moragrega, C., Llorente, I., 2023. Effects of leaf wetness duration, temperature, and host phenological stage on infection of walnut by *Xanthomonas arboricola* pv. *juglandis*. *Plants* 12, 2800.

- Nutter Jr, F.W., Esker, P.D., Netto, R.A.C., 2006. Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data. *European Journal of Plant Pathology* 115, 95-103.
- Schaad, N.W., Jones, J.B., Chun, W., 2001. Laboratory guide for the identification of plant pathogenic bacteria. APS Press, St. Paul, Minnesota, USA.
- Stackebrandt, E., Goebel, B.M., 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology* 44, 846-849.
- Sundin, G.W., Mayfield, C.T., Zhao, Y., Gunasekera, T.S., Foster, G.L., Ullrich, M.S., 2004. Complete nucleotide sequence and analysis of pPSR1 (72,601 bp), a pPT23A-family plasmid from *Pseudomonas syringae* pv. *syringae* A2. *Molecular Genetics and Genomics* 270, 462-476.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725-2729.
- Teviotdale, B.L., Michailides, T.J., Pscheidt, J.W. (Eds.), 2002. Compendium of nut crop diseases in temperate zones. APS Press, St. Paul, Minnesota, USA.
- Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173, 697-703.

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