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Insights into the Morphological and Molecular Characteristics of *Oidium neolycopersici*: A Study from Syria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Powdery mildew poses a global challenge to tomato greenhouse production. In Syria, identification and characterization of the specific fungal species responsible for this disease remain relatively limited. The present study aimed to identify four local isolates of *O. neolycopersici* (M8, M10, G12, and R12). The morphological features of the four isolates were similar to those of *O. neolycopersici* based on symptoms, light, and scanning electron microscopy (SEM) observations. The analyses of the internal transcribed spacer (ITS) region confirmed that the four Syrian isolates belong to *O. neolycopersici* and presented 99–100% sequence similarity with the many other isolates registered in GenBank on tomatoes. Nucleotide sequences and translated nucleotides into amino acids for isolates M8 and M10 were found to be 100% identical, as were isolates G12 and R12. The two groups of isolates differed in only one nucleotide position within the ITS region and in six amino acids. Phylogenetic analysis revealed that the Syrian isolates could be classified into the same cluster group as the Netherlands *O. neolycopersici* isolate (VPRI20724). To our knowledge, this is the first well-founded report on the protected tomato powdery mildew, *O. neolycopersici*, in Syria.

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide [1,2]. It is susceptible to various fungal diseases such as wilt, grey muold, blights, and powdery mildew [3]. In particular, powdery mildew poses a significant threat, as it is prevalent in both protected and field cultivation environments, leading to yield losses ranging from 10% to 90% [4,5]. *Leveillula taurica* [Lév.] G. Arnaud was recognized as the most prevalent powdery mildew pathogen affecting tomato plants in all over the world including Syria [6-10]. It spreads in all dry regions across the Mediterranean and tropical countries [11-13]. In addition, *Oidium neolycopersici* has emerged as a significant powdery mildew pathogen on tomatoes and various other hosts, such as eggplant, potatoes, and tobacco [14,15]. Over the past two decades, it has spread extensively worldwide, according to numerous reports confirming its presence on tomatoes in multiple countries, including North America [16], Venezuela and Mexico [17,18], China and Turkey [19,20,21], Croatia [22], Korea [23], Serbia [24], Africa and Iran [25,26], Syria [10], and Kazakhstan [23]. Consequently, it has become a destructive disease that affects tomatoes worldwide, resulting in substantial economic losses [16,28-33]. Another species of Oidium, *O. lycopersici* (Cooke and Massee), was reported on tomatoes in only Australia [34], Connecticut, and California in the United States of America [18,58]. *O. neolycopersici* can be distinguished by white powdery spots covering the upper side and occasionally the lower side of the leaflets, petioles, stems, and calyx of the fruit [37,14,38,39]. In addition to lobed appressoria on the external hypha and single conidia, at high relative humidity, *O. neolycopersici* forms pseudochains of 2-3 conidia and rarely 6 or more. Nonomura [40] observed 4-8 conidial pseudochains on the conidiophores of wild tomatoes. In contrast, *O. lycopersici* forms conidia in chains, with powdery spots on both sides of the tomato leaves and simple, unlobed appressoria. Because of the absence of the sexual stage in some Oidium species, morphological identification becomes more difficult and insufficient [41]. Therefore, molecular characterization has become an urgent necessity to confirm morphological identification, and accurate identification of powdery mildew species as a crucial condition for selecting effective control methods. The internal transcribed spacer ITS region is the preferred barcode for fungal classification and has been widely used for molecular identification and phylogenetic studies of powdery mildew [42,43,44,45]. To our knowledge, there are no previous studies of tomato powdery mildew *O. neolycopersici* characterization in Syria. Alio [10] reported mild-to-severe powdery mildew infection in tomatoes during two consecutive seasons (2019 and 2021) in several regions of Tartous governorate along the Syrian coast. *O. neolycopersici* was determined to be one of the causative agents based only on apparent field symptoms and microscopic examination. Thus, the present study aimed to confirm the identity of this species by characterizing four local isolates M8, M10, G12, and R12 according to morphological and molecular characteristics.

2. MATERIALS AND METHODS

2.1 Morphological Characteristics

2.1.1 *O. neolycopersici* isolates

Four isolates (M8, M10, G12, and R12) were obtained during a field survey conducted in the 2019 and 2021 seasons from three regions of Tartous governorate in Syria [10]. Specifically, M8 was collected from Adimeh region (35°9′4″N 35°55′41″E), M10 from Al-Kharab region (35°4′30″N 35°53′53″E), and G12 and R12 from Maten Abo Rya region (35°02'20.4"N 35°56'04.4"E). Three successive cycles of inoculation were performed to obtain isolates that exhibited consistent symptoms and morphological features. Inoculation was performed on a tomato cultivar (Mandalon) at the growth stage of third true leaf under greenhouse conditions (26 ± 4°C). Fresh infection leaf spots were harvested, placed in 100 ml of sterile distilled water, and shaken well to produce a conidial inoculum of 3 × 104 spores/ml [46]. The inoculum was sprayed onto the upper sides of plant leaves, and then the inoculated plants were covered with plastic bags for 24 hours. Subsequently, symptoms of infection were monitored for each isolate 7–10 days after inoculation.

2.1.2 Microscopic examination

Light Microscopy: After 9–10 days post-inoculation, fresh infected leaves were examined under light microscopy to determine the

morphological features of the asexual stage. Morphological traits and morphometric measurements were observed under a light microscope equipped with software (OPTIKA PROVIEW, Italy), all measurements were expressed in micrometers (µm). The cuticle with fungal growth was stripped off according to the method described by Moreira [17], using a colorless base nail varnish applied to the powdery spots. After drying for 10 minutes, a transparent adhesive tape was pasted over the enameled leaf surface. Gentle pressure was applied between the fingers, and the tape was carefully peeled off to remove the cuticle with attached fungal structures. The tape containing the cuticle was then inverted as a coverslip onto a microscope slide covered with lactophenol/aniline blue for the examination of hyphae, conidiophores, conidia, and haustoria. Conidial germination and appressorium formation were examined according to the methods described by Cook and Braun [47] by holding fresh infected tomato leaves approximately 2 cm above the inner surface of a plastic Petri dish lid and tapping sharply to release conidia onto it. The lid was then placed over its base, which contained paper tissue moistened with water, and incubated for 24–48 h at room temperature (20–25°C).

2.1.3 Scanning electron microscopy SEM

Fresh infected leaves from each isolate were examined by scanning electron microscopy (FEI Quanta200) at Albath University, Homs, Syria, using Environmental Scanning Electron Microscopy (ESEM) mode to allow true observation of the specimens in their natural state without any coating [5]. Infected tomato leaves from each isolate were air-dried and sent to the Technical University of Dresden, Institute of Botany, Germany, for examination by electron microscopy (JEOL JSM-75OOF) in order to recognize wrinkling patterns on the surface of conidia, following the method described by Cook [48]. The specimens were mounted with double-sided clear adhesive tape on an aluminum stub and gold-coated without cryoscopy before being transferred to the scanning electron microscopy (SEM).

2.2 Molecular Characterization

2.2.1 DNA extraction, PCR amplification, and sequencing of rDNA ITS sequences

DNA extraction from the four isolates M8, M10, G12, and R12 was conducted from the asexual stage following the CTAB method described by Liu [49]. The ITS region of rDNA was amplified using the ITS1, ITS2, ITS3, and ITS4 primer sets [50] Table 1, which were obtained from Metabion Company, Germany.

The primer pair ITS1-ITS2 was used for PCR1 (the first round of PCR) and ITS3-ITS4 for PCR2 (the second round of PCR). The first round of PCR was performed according to the following protocol: preheating at 94°C for 2 min, 30 cycles of denaturation (94°C, 30s), annealing (62°C, 30s), and extension (72°C, 30s), followed by a final extension cycle of 7 min at 72°C. The subsequent nested PCR was initiated by mixing 1 µl of the first round amplification with 49 µl of the previously described first round amplification mixture using the nested primers at a concentration of 0.2 µM and cycled under the same protocol. After electrophoresis of the amplified DNA on a 1.5% agarose gel, specific DNA bands corresponding to the target ITS region were carefully excised from the gel. The PCR products were then purified using the NucleuSpin®Gel and PCR Clean-up Kit (Machery-Nagel, Düren, Germany) following the manufacturer's instructions. Genetic sequences and alignments of nucleotides and translated nucleotides into amino acids were analyzed by the Macrogen Europe sequencing service (Amsterdam, Netherlands) and read using PhyDE® (Phylogenetic Data Editor) version 0.9971. The four isolates were deposited in GenBank under accession numbers (OM921389-OM921390-OM921392-OM921393). A homology search using BLAST was performed at the National Center for Biotechnology Information (NCBI). A comparation in the nucleotide sequence of 5.8S rDNA ITS regions and the translated nucleotides into amino acids btween the isolates were defined.

2.2.2 Phylogenetic analysis

Evolutionary analysis was conducted using the Maximum Likelihood (ML) method with the Kimura 2-parameter model [51], along with 1000 bootstrap replicates to assess the robustness of the inferred phylogeny. The initial tree (s) for the heuristic search were constructed using the Neighbor-Joining (NJ) method based on pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The topology with the highest log-likelihood value was selected as the best-fitting phylogenetic tree. Phylogenetic analysis was performed using MEGA11 [52] and included a dataset consisting

Table 1. Primer sequences used in this study

PCR	Primer	Primer sequences 5´- 3´
1	ITS1	F: TCCGTAGGTGAACCTGCGG
	ITS2	F: GCTGCGTTCTTCATCGATGC
2	ITS3	R: GCATCCATGAAGAACGCAGC
	ITS4	R: TCCTCCGCTTATTGATATGC

Table 2. Sample ID, country of origin, and database accession numbers of powdery mildew rDNA ITS sequences used for phylogenetic analysis

Species	host	Sample ID	Country of origin	Accession numbers	Source of ITS sequence data
O. neolycopersici	*S. lycopersicum*	ET1	France	AF229019	Kiss et al., [51]
O. neolycopersici	*S. lycopersicum*	VPRI20724	Netherlands	AF229015	
O. neolycopersici	*S. lycopersicum*	809-11	Serbia	JQ619840	Stevanovic et al., 2012
O. neolycopersici	*S. lycopersicum*	H1	China	JQ972700	Li et al., 2012 (Unpublished)
O. neolycopersici	*S. lycopersicum*	HUSL-05	China	MH137258	Wu, H. 2018 (Unpublished)
Erysiphe neolycopersici	*S. lycopersicum*	MUMH66	Japan	AB032483	Kiss et al., [37]
E. neolycopersici	*S. lycopersicum*	MUMH775	Japan	AB034722	
E. neolycopersici	*S. lycopersicum*	AAPB	India	MF991288	Rana et al., 2017 (Unpublished)
E. neolycopersici	*S. lycopersicum*	FQ-3	China	MG171168	Zhou and Wang, 2017(Unpublished)
E. neolycopersici	*S. lycopersicum*	KTP-03	Japan	LC663220	Nonomura et al., 2021(Unpublished)
E. aquilegiae	*Clematis terniflora*	MUMH98	Japan	AB015929	Takamatsu et al., [24]
E. macleayae	*Macleaya cordata*	TPU-1873	Japan	AB016048	
E. juglandis	*Pterocarpa rhoifolia*	TPU1745	Japan	AB015928	
O. neolycopersici	*S. lycopersicum*	M10	Syria	OM921389	This study
O. neolycopersici	*S. lycopersicum*	M8	Syria	OM921390	
O. neolycopersici	*S. lycopersicum*	R12	Syria	OM921393	
O. neolycopersici	*S. lycopersicum*	G12	Syria	OM921392	

of 17 sequences. This dataset comprised 14 sequences of tomato powdery mildew, including four local isolates and three sequences from other host plants. All sequences were obtained from the NCBI database (Table 2). Multiple sequence alignments of the ITS gene were performed using a dataset consisting of the extracted and existing isolate sequences in the library. *Erysiphe juglandis* (accession number: AB015928) was used as the outgroup sequence [37].

3. RESULTS AND DİSCUSSİON

3.1 Morphological Characterization

3.1.1 Inoculation symptoms

All four isolates exhibited identical inoculation symptoms. It appeared as dense, irregular white spots on the upper surface of the leaves, stems, branches, petioles, and calyxes (Fig. 1). Rarely, powdery spots appeared on the lower surface of the R12 isolate. As the infection progressed, the affected tissues started to turn yellow and the white spots gradually turned brown.

3.1.2 Microscopic observations

The isolates displayed distinct characteristics upon microscopic examination. They are distinguished by a translucent superficial hyphe with a width of 4.2–8.7 µm. Short, slightly lobed, opposite, and double appressoria were observed (Fig. 2D, Fig. 3 B).

Spherical haustoria were formed in the epidermal cells (Fig. 2 C) with a diameter of 10.5–13.2 µm. The Pseudoidium conidiophores were straight and semi-rectal, bent at their base in some isolates, with lengths of 54–118 µm (Table 3).

The foot cell was cylindrical, slightly tortuous, and may have been wide in the middle and narrow or slightly retracted at the base in some isolates with dimensions (28–65 × 6–13 µm). Almost all isolates had 1-2 cells above the foot cell. 1-2 and 3 conidia are present on the conidiophores of (M8, M10, G12) and (R12), respectively (Fig. 3 D, E, and F). These conidia were oval or cylindrical (24–48 × 10–26 µm) with an angular, rectangular pattern of wrinkles on the outer wall and no fibrosin bodies (Fig. 3 C). Germination tube emergence sites on conidia were mostly lateral, subterminal, and terminal (Fig. 4).

3.2 Molecular Characterization

3.2.1 ITS sequence analysis

The complete sequences of the Syrian isolates (M8, M10, G12, and R12) were obtained after reading and assembling the sequences resulting from PCR2 and PCR1. The length of the studied ITS region ranged between 576 and 600 bp. The homology search using BLAST showed a high similarity to *O. neolycopersici*, with a query coverage reaching 100% and more than 99% identity to many isolates recorded in the GenBank database on tomatoes (Table 4).

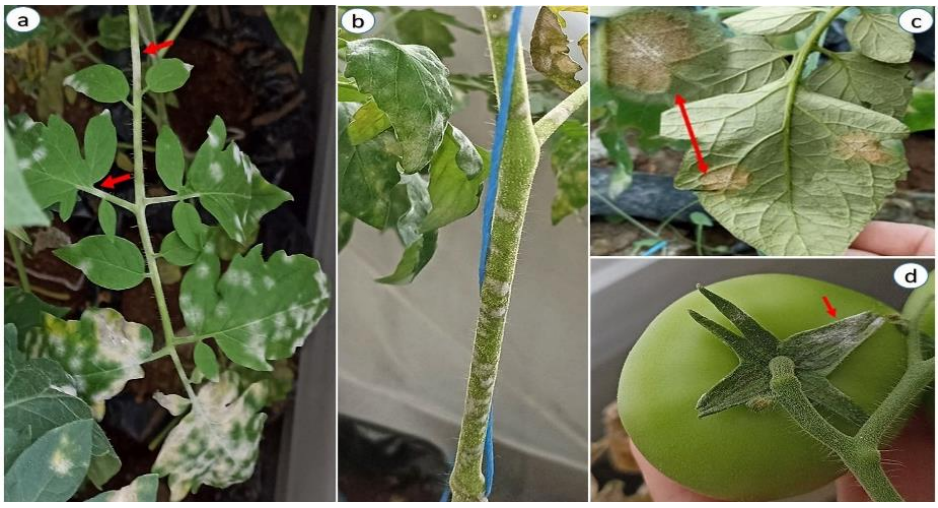

Fig. 1. a. Powdery spots of *O. neolycopersici* on the upper side of a Mandalon cultivar leaves, branches and petioles b. on stem c. on the lower side of the leaf d. on calyx

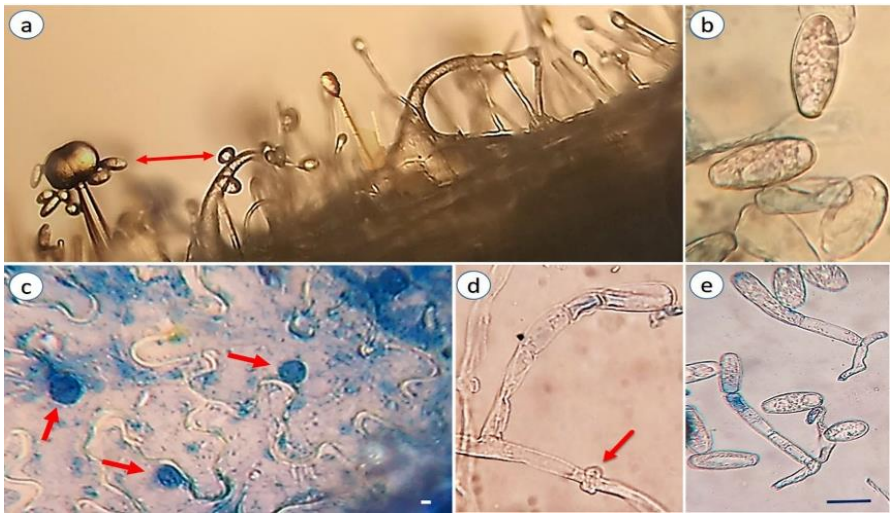

Fig. 2. a. Conidiophores with single conidia on the leaf petiole and conidia on trichomes (200x Mag) b. conidia with vacuoles and no fibrosin bodies (400x Mag) c. haustoria in epidermal cells on the upper side of the leaf (scale bar: 5 µm) d. e. conidiophores from fresh specimens (scale bar: 10µm), lobed appressoria (arrow)

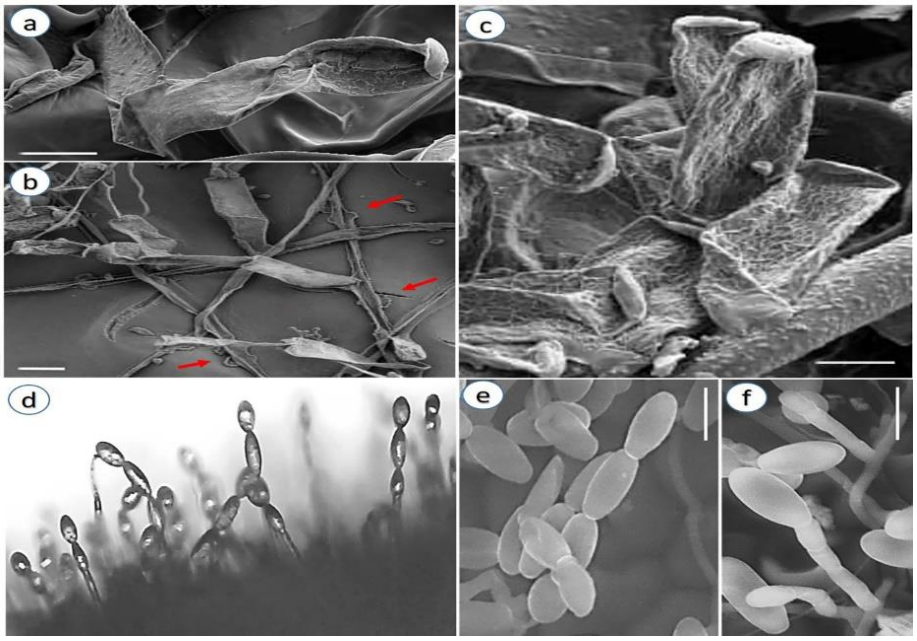

Fig. 3. a. Scanning electron micrographs of conidiophores from herbarium specimens (scale bar: 10 µm) b. lobed appressorium on the hypha (arrows) (scale bar: 20 µm) c. conidia with an angular, rectangular pattern of wrinkling on the outer wall (scale bar: 20 µm) d. conidiophores with 2-3 conidia of R12 isolate (200X Mag) e. scanning electron micrographs of the conidia series (scale bar: 20 µm) f. conidiophore with single conidia (M8, M10, G12) (scale bar: 50 µm)

By comparing the nucleotide sequence alignments of the rDNA ITS region and translated nucleotides into amino acids of the four isolates, it was found that the isolates (M8 and M10) were 100% identical, as were the isolates (G12 and R12). Furthermore, the two groups of isolates showed a high level of similarity of 99.83% and 99.50% in nucleotide sequences and amino acids, respectively. The variability among them was observed at only one nucleotide position, where position 573 showed an A instead of a T, and in only six amino acids (Fig. 5) (Table 5).

Table 3. Biometric measurements of four Syrian *O. neolycopersici* isolates

Feature	Isolate			
µm	**M10**	**M8**	**R12**	**G12**
Conidia	(26- 37)[a] × (15- 20)[b]	(26- 42) × (14-18)	(30- 48) × (15-26)	(24- 44) × (10-16)
Mean ± SD	30.2± 3.3 × 16 ± 1.9	34.5± 2.9 × 16.2 ± 1.5	39.4 ± 2.4 × 20.8 ± 1.2	33.8 ± 3.5 × 16.2 ± 1.2
Conidiophore	(59.6- 118.3)[a]	(73.2- 118.6)	(78.8- 113)	(54.7- 113.8)
Mean ± SD	88.5 ± 12.3	87.5 ± 10.2	85.4 ± 8.8	82.3 ± 11.7
Foot cell	(34- 65][a] × (7-12][b]	(33- 62) × (7-13)	(30- 55) × (6-10)	(28- 52) × (6-10)
Mean ± SD	39.6 ± 5.3 × 9.6 ± 1.9	37.5 ± 5.5 × 9.5 ± 1.9	33.6 ± 4.5 × 7.4 ± 1.5	33.5 ± 4.3 × 7.4 ± 1.5

- *a. range (min–max) for length; b. range (min–max) for width; SD. standard deviation. (100 replicates were measured for each feature)*

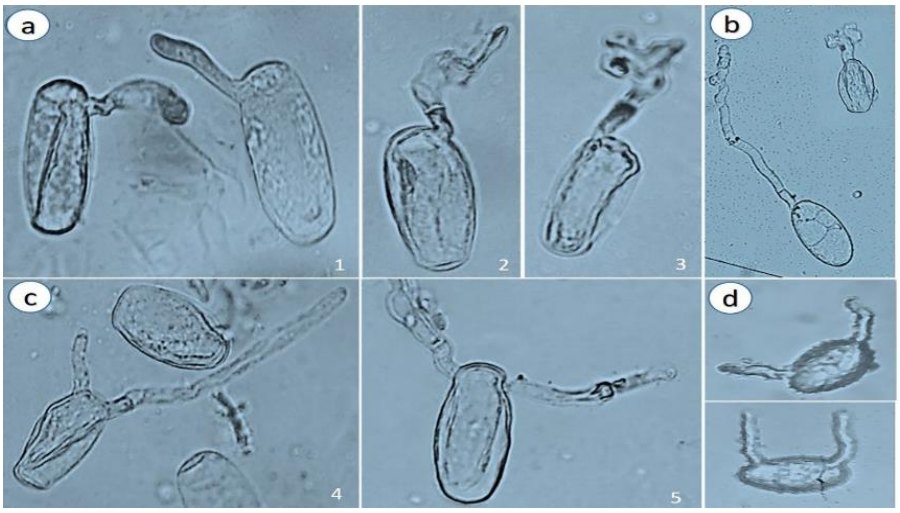

Fig. 4. Germinated conidia (400X Mag) a.1.2. lateral; 3. subterminal; and b. terminal germination tube with appressorium (arrow). c.4.5.d. two germination tube for each conidium

Table 4. Homology search results for local isolates of tomato powdery mildew *O. neolycopersici* using BLAST search with NCBI

Species	Isolate/strain	Identity present%	Query coverage%	Local isolates
O. neolycopersici	H1	**99.84**	100	R12, G12
O. neolycopersici	HUSL-05	**99.84**	100	
E. neolycopersici	KTP-03	**99.83**	100	
O. neolycopersici	VPRI20724	**99.83**	100	M10, M8
O. neolycopersici	ET1	**99.82**	100	
E. neolycopersici	MUMH775	**99.82**	94	
E. neolycopersici	MUMH66	**99.66**	99	

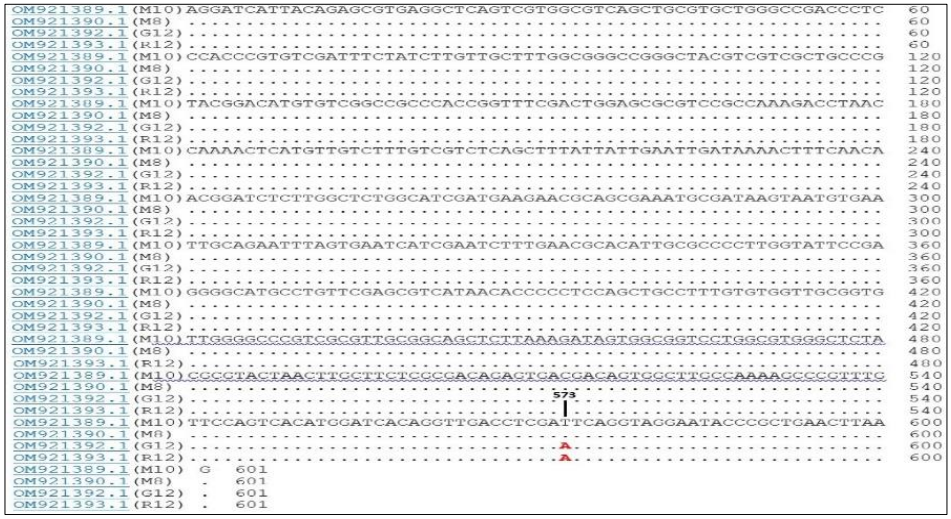

Fig. 5. Nucleotide sequence alignment of the amplified 601-bp bands of the RNA polymerase gene among four Syrian *O. neolycopersici* isolates (M10, M8, G12, R12)

3.2.2 Phylogenetic analysis

The phylogenetic tree (Fig. 6) revealed two main clades: Clade I consists of a group comprising *Oidium/Erysiphe neolycopersici* isolates infecting tomatoes, including the four studied Syrian isolates (M8, M10, G12, and R12); Clade II includes sequences from *Erysiphe aquilegiae* isolate (MUMH98) and *E. macleayae* isolate (TPU-1873).

Table 5. Sequence alignment of translated nucleotides into amino acids among the Syrian *O. neolycopersici* isolates (M10, M8, G12, R12), the differences are in yellow

Isolate code and NCBI number	Amino acids sequences
M10 (OM921389) M8 (OM921390)	RIITEREAQSWRQLRAGPTLPPVSISILLLWRAGLRRRCPYGHVSAAHRFRLERVRQRPNQNSCCLCRLSFIIELIKLSTTDLLALASMKN AAKCDK*CELQNLVNHRIFERTLRPLVFRGACLFERHNTPSSCLCVVAVLGPVALRQLLKIVAVLAWALRVLTCFSRQSDDSGLPKARL FQSHGSQVDLDSGRNTR*T*LSSAGIPT*IEVNL*SM*LEQTGFWQATVVTLSREAS*YA*SPRQDRHYL*ELPQRDGPQHRNHTKAAGG GVMTLEQACPSEYQGAQCAFKDSMIH*ILQFTLLIAFRCVLHRCQSQEIRC*KFYQFNNKAETTKTT*VLVRSLADALQSKPVGGRHMSV RAATT*PGPPKQQDRNRHGWEGRPSTQLTPRLSLTLCNDP*VQRVFLPESRSTCDPCDWNKRAFGKPLSSLCREKQVSTRRAHART ATIFKSCRNATGPNTATTQRQLEGVL*RSNRHAPRNTKGRNVRSKIR*FTKFCNSHYLSHFAAFFIDARAKRSVVESFINSIIKLRRQRQH EFWLGLWRTRSSRNRWAADTCPYGQRRRSPARQSNKIEIDTGGRVGPGSLQSVRLSRGVSCVLGRPSHPCRFLSCCFGGPGYVVA ARTDMCRPPTGFDWSASAKDLTKTHVVFVVSALLLN**NFQQRISWLWHR*RTQRNAISNVNCRI**IIESLNAHCAPWYSEGHACSSVI TPPPAAFVWLLYAY*LASRDRVTTVACQKPVCSSHMDHRLTSIQVGIPAELKQPHKGSWRGCYDARTGMPLGIPRGAMCVQRFDDSL NSAIHITYRISLRSSSMPEPRDPLLKVLSIQ**S*DDKDNMSFG*VFGGRAPVETGGRPTHVRTGSDDVARPAKATR*KSTRVGGSAQHA ADATTEPHAL**SLKFSGYSYLNRGQPVIHVTGTNGLLASHCRHSVARSKLVRVEPTPGDHYRA*GSVVASAACWADPPTRVDFYLVAL AGRATSSLPVRTCVGRPPVSTGARPPKT*PKlmlslssqlYY*IDKTFNNGSLGSGIDEERSEMR*VM*IAEFSESSNL*THIAPLGIPRGMPV RAS*HPLQLPLCGCGVGARRVAAALKDSGGPGVGSTRTNLLLATE*RQWLAKSPFVPVTWITG*PRFR*EYPLNL
G12 (OM921392) R12 (OM921393)	RIITEREAQSWRQLRAGPTLPPVSISILLLWRAGLRRRCPYGHVSAAHRFRLERVRQRPNQNSCCLCRLSFIIELIKLSTTDLLALASMKN AAKCDK*CELQNLVNHRIFERTLRPLVFRGACLFERHNTPSSCLCVVAVLGPVALRQLLKIVAVLAWALRVLTCFSRQSDDSGLPKARL FQSHGSQVDLESGRNTR*T*LSSAGIPT*FEVNL*SM*LEQTGFWQATVVTLSREAS*YA*SPRQDRHYL*ELPQRDGPQHRNHTKAAG GGVMTLEQACPSEYQGAQCAFKDSMIH*ILQFTLLIAFRCVLHRCQSQEIRC*KFYQFNNKAETTKTT*VLVRSLADALQSKPVGGRHM SVRAATT*PGPPKQQDRNRHGWEGRPSTQLTPRLSLTLCNDP*VQRVFLPDSRSTCDPCDWNKRAFGKPLSSLCREKQVSTRRAHA RTATIFKSCRNATGPNTATTQRQLEGVL*RSNRHAPRNTKGRNVRSKIR*FTKFCNSHYLSHFAAFFIDARAKRSVVESFINSIIKLRRQR QHEFWLGLWRTRSSRNRWAADTCPYGQRRRSPARQSNKIEIDTGGRVGPGSLQSVRLSRGVSCVLGRPSHPCRFLSCCFGGPGYV VAARTDMCRPPTGFDWSASAKDLTKTHVVFVVSALLLN**NFQQRISWLWHR*RTQRNAISNVNCRI**IIESLNAHCAPWYSEGHACSS VITPPPAAFVWLLYAY*LASRDRVTTVACQKPVCSSHMDHRLTSNQVGIPAELKQPHKGSWRGCYDARTGMPLGIPRGAMCVQRFDD SLNSAIHITYRISLRSSSMPEPRDPLLKVLSIQ**S*DDKDNMSFG*VFGGRAPVETGGRPTHVRTGSDDVARPAKATR*KSTRVGGSAQ HAADATTEPHAL**SKFSGYSYLNIRGQPVIHVTGTNGLLASHCRHSVARSKLVRVEPTPGDHYRA*GSVVASAACWADPPTRVDFYLV ALAGRATSSLPVRTCVGRPPVSTGARPPKT*PKlmlslssqlYY*IDKTFNNGSLGSGIDEERSEMR*VM*IAEFSESSNL*THIAPLGIPRGM PVRAS*HPLQLPLCGCGVGARRVAAALKDSGGPGVGSTRTNLLLATE*RQWLAKSPFVPVTWITG*PRIR*EYPLNL

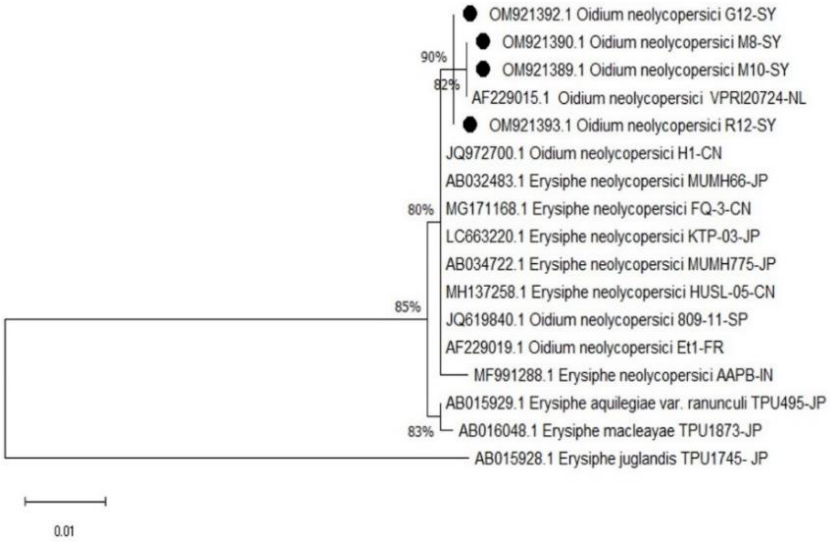

Fig. 6. Phylogenetic tree based on the nucleotide sequence of the rDNA internal transcribed spacer regions ITS for 17 powdery mildew taxa of the *Pseudoidium* subgenus. The tree with the highest log likelihood is shown. Numbers at nodes represent bootstraping Coverages higher than 50% based on 1000 replications

The main objective of the study was to confirm the identity of *O. neolycopersici* in Syria by determining the morphological characteristics of local isolates and linking them molecularly. This research is considered important because greenhouses in Syria provide an essential source of tomatoes throughout the year, and any disease threat to the plant may affect the amount of production. Furthermore, the precise identification of pathogens plays an important role in determining appropriate control strategies. The present study revealed that the symptoms and morphological characteristics of the local isolates (M8, M10, G12, and R12) were similar to those documented in previous studies [37,53]. A notable difference between isolates R12 and (M8, M10, and G12) was in the quantity of conidia on the conidiophores. Despite this variation, the homology search showed that they were 99.83% identical. This was corroborated by Nonomura [40], who found that the ITS sequences of the multiple conidial phenotype isolates, KTP-03 and KTP-04, were identical to those of the single conidial phenotype. Moreover, the nucleotide sequences of 5.8S rDNA and both ITS regions of R12 and G12 were 99.84% identical to Chinese isolates of *O. neolycopersici* (H1 and HUSL-0)], as were isolates M10 and M8 to the French isolate ET1. The phylogenetic tree has been divided into two clades due to the differences between isolates in the host plant and reproduction units employed in the ITS. The present phylogenetic analysis showed that the Syrian isolates could be classified into the same cluster group as the Netherlands isolate (VPRI20724). Based on these findings, the four Syrian isolates M8, M10, G12, and R12 are related to *O. neolycopersici.* The ITS sequences of powdery mildew samples obtained from the same host plant and from nearby locations were found to be identical by Hirata [54,55] and Kiss [56]; however, despite the small number of isolates and the close proximity of the locations from which they were collected, we were able to distinguish differences between the two groups of isolates G12, R12, and M10, M8 in six amino acids and one nucleotide position within their ITS region. This does, to some extent, reflect genetic diversity between the species isolates. Jankovics [57] found genotype diversity among *O. neolycopersici* isolates collected from different geographical locations using AFLP analysis, even though the ITS sequences of the samples under investigation seemed to be identical. This limitation of ITS sequences in fully describing genetic diversity has also been mentioned by Kovács [58] and Lücking [59], who emphasized that using more sensitive molecular analysis techniques will be necessary to assess genetic variation comprehensively [60-65].

4. CONCLUSION

ITS analysis corroborated the morphological examination of the Syrian isolates, confirming their identification as *O. neolycopersici*. To the

best of our knowledge, the present study provides the first contribution to the morphological and molecular identification of *O. neolycopersici* isolates from Syria. Although *O. neolycopersici* had previously been recorded in only three locations of the areas surveyed on the Syrian coast, its presence must be taken into consideration as it is able to spread very easily and in a relatively short time. Further comprehensive research is essential to enhance our understanding of the host range, physiology, epidemiology, and control of this pathogen, as well as the development of resistant tomato varieties. Employing advanced molecular techniques with a larger number of isolates covering different regions in Syria will be crucial for exploring the genetic diversity of this species.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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