Morphological and Molecular Characterization of Two Isolates of *Leveillula taurica* Causing Powdery Mildew on Protected Tomatoes in Tartous Coastal Region, Syria

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Abstract

Alio, N., S. Al-Maghribi and N. Mualla. 2024. Morphological and Molecular Characterization of Two Isolates of *Leveillula taurica* Causing Powdery Mildew on Protected Tomatoes in Tartous Coastal Region, Syria. Arab Journal of Plant Protection, 42(4): 436-442. https://doi.org/10.22268/AJPP-001275

Two isolates were characterized as *Leveillula taurica* based on morphological features and sequencing of ITS regions. The typical symptoms appeared on Mandalon cultivar as bright yellow spots on the upper side of the leaves, and whitish powdery sporulation on the corresponding lower surface, with no cleistothecia formation. Light and electron microscopy showed a lanceolate primary and cylindrical secondary conidia on pseudoidium conidiphores, and other morphological features were similar to those of *L. taurica.* The ribosomal DNA internal transcribed spacer (ITS) sequence confirmed the morphological characterization. The results of sequence homology for the two isolates using BLAST showed similarity to *L. taurica* species, with a query coverage reached 100% and more than 99% identity with many isolates registered in the GenBank which infect tomato and other hosts. To the best of our knowledge, this is the first molecular characterization of *L. taurica* on protected tomato in the Syrian coast.

Keywords: Tomato, powdery mildew, *Leveillula taurica*, ITS.

Introduction

Tomato, *Solanum lycopersicum* is one of the most widely cultivated and economically significant vegetable crops globally. However, their cultivation, particularly in protected environments, is often plagued by various diseases that reduce yield and fruit quality. Among these pathogens, powdery mildew caused by *Leveillula taurica* stands out as a major concern for tomato growers in the Tartous region. Globally the species *Leveillula tauricta* (Lev.) G. Arn., is the main causal agent of powdery mildew on tomato, either in protected agriculture or in the open field cultivation (Awad *et al*., 2004; Forster, 1989; Hoseinkhaniha *et al*., 2012). In addition, it affects pepper, eggplant, cotton and more than 1000 plant species including cultivated crops, herbs and trees (Palti, 1988). This species may cause severe outbreaks on tomatoes and reduce yield and quality (Aegerter *et al*., 2016; Aydın & Göre, 2010; Jones & Thomson, 1987; Mendieta *et al*., 2020; Paçe *et al*., 2016). *L. taurica* has been recorded since the seventies in all dry areas around the Mediterranean and tropical countries (Spencer, 1978), then spread all over the world. All studies so far indicated that it occurs only in the conidial phase (*Oidiopsis taurica* (Lév.) E.S. Salmon) on solanaceae crops, but it is still mentioned under the name of its sexual stage, *L. taurica*, even when studies did not report the formation of cleistothecia that represent the sexual stage (Palti, 1988). In light of the scarcity or absence of cleistothecia, the description of this species is based on the morphological characteristics of the asexual phase. But, when dried herbarium materials are used some characters are not accessible such as conidium germination; exact measurement and description of conidia; conidiophores and appressoria (Khodaparast, 2016). Accordingly, molecular characterization is a solution, which has become a global trend for characterizing fungi particularly in powdery mildew species due to the absence of the sexual phase for many of its species, and not rely only on the differences in their morphological characteristics according to the plant host and environmental circumstances. Khodaparast *et al.* (2001) indicated that *L. taurica* is a complex species composed of several biological species, and in 2012 he found that the sequence of *L. taurica* varied from one host to another. In addition, DNA data showed different lineages among specimens which were hardly distinguishable by morphology. Therefore, molecular characterization has become a necessity for more precision. The internal transcribed spacer (ITS) region has been proposed as the standard DNA barcode for fungi and has been widely used in DNA barcoding analyses (Wang *et al*., 2014). ITS technology helps detect small amounts of DNA, and it can be used for dried specimens so there is no worry about spreading diseases when these samples are sent to other countries (Cunnington *et al*., 2003). *L. taurica* is the most widespread species on protected tomatoes in many regions of Tartous governorate in Syria and throughout the growing season (Alio *et al*., 2023).

Therefore, the aim of this study was to characterize two local isolates of this species based on the morphological and molecular characteristics, and compared them with global isolates.

Materials and Methods

L. taurica isolates

Two local isolates (B7 and B12) were collected through a field survey conducted during the 2020/2021growing season.

https://doi.org/10.22268/AJPP-001275

The isolate B7 was collected from Maten Abo Rya region, and isolate B12 from Huraysun, along the Tartous coastal line, Syria (Alio *et al*., 2023). The isolates were maintained on the tomato cultivar Mandalon under greenhouse conditions. Inoculation was done on the tomato third true leaf, and following three transfer cycles, at least ten fresh infection spots were harvested and placed in 100 ml sterile distilled water and shaken well to produce a conidial inoculum 4×10^4 conidia/ml, and sprayed onto the adxial surfaces of plant leaves. Inoculated plants were covered for 24 h with plastic bags.

For light microscopy studies, fresh fungal colonies were stripped off by adhesive tape according to Moreira *et al*. (2014), mounted in cotton lactophenol blue solution. Scanning electron microscope (SEM) (FEI Quanta 200 in Albath University, Homs, Syria) has been used for scanning fresh infected samples.

DNA extraction and PCR amplification and sequencing of the rDNA ITS

The extraction was made from herbarium specimens by using a modified CTAB method (Kahl, 2001), according to the steps followed by (Liu *et al*., 2015). The entire internal transcribed spacer region (ITS) of rDNA was amplified using the primers ITS1/ITS2/ITS3/ITS4 (White *et al*., 1990) (Figure 1) manufactured by Metabion Company, Germany. For DNA replication, the DNA extract was diluted with distilled water to reach the appropriate concentration for PCR1 and PCR2 (10 ng/µl). A PCR (Flexi Gene, Germany) was used to conduct the reaction with a volume of 50 µl per sample, including 50 ng/µl of DNA and a buffer solution consisting of 10 mM Tris-HCL, pH 8.3, 50 mM KCL, 1.5 mM $MgCl_2$, 0.01% Gelatine, dNTP-nucleotide mix (0.2 mM), 0.4 µl of each of the two primers (Forward and Reverse) (20 pmol), and a unit of the enzyme Taq polymerase manufactured by the Qiagen company, Germany. The PCR amplification was performed with the following thermocycles: an initial denaturation step at 94°C for 2 min, followed by 30 cycles consisting of a denaturation step of 30 s at 94°C, primer annealing for 30 s at 62°C, and extension for 30 s at 72°C. The final extension step was performed at 72°C for 7 min. The products of the PCR reaction were separated on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide in TBE electrophoresis solution and using a horizontal electrophoresis apparatus (Apelex-France), under 80 V for two hrs and then visualized under UV light using (CAMAG Reprostar3 –Switzerland). PCR products were purified using the NucleuSpin®Gel and PCR Clean-up Kit (Machery-Nagel, Duren, Germany) according to the steps laid down by the manufacturer. Genetic sequences were analyzed by Macrogen Europe sequencing service (Amsterdam, Netherlands), and read using PhyDE®-Phylogenetic Data Editor version 0.9971, and deposited in GenBank. Alignments of ITS sequences obtained from GenBank were made.

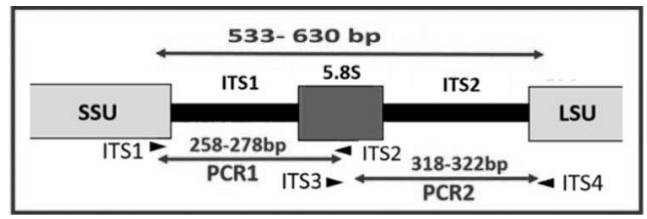

Figure 1. Four primer pairs used for polymerase chain reaction (PCR) amplification of 5.8S rDNA and internal transcribed spacer. The primer pair ITS1-ITS2 was used for PCR1 (first round of PCR), and ITS3-ITS4 for PCR2 (second round of PCR).

Phylogenetic analysis

Evolutionary analysis was performed using the Maximum Likelihood method with the Kimura 2-parameter model (Kimura, 1980), along with 1000 bootstrap replicates. The initial tree(s) for the heuristic search were constructed using the Neighbor-Joining method, based on pairwise distances estimated via the Maximum Composite Likelihood (MCL) approach. The topology with the highest log likelihood value was selected. Phylogenetic analysis was conducted using MEGA11 (Tamura *et al*., 2021) for a total of 8 sequences of the *L. taurica*, which included 4 sequences of tomato powdery mildew (including the two local isolates) and 4 on other host plants, all obtained from the NCBI database (Table 1). Multiple sequence alignments of the ITS gene were performed for the extracted and existing isolate sequences in the library, with *L. taurica* from *Artemisia annua* (accession numbers: AB044384) used as an out-group sequence (Khodaparast *et al*., 2001). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 763 positions in the final dataset. Evolutionary analyses were conducted in MEGA11. Local isolates are marked with a black circle.

Table 1. Sample ID, countries of origin and database accession numbers of *L. taurica* rDNA ITS sequences used for phylogenetic analysis.

Accession numbers	Sample ID	Plant Host	Country of origin	Source of the ITS sequence data
MT370494.1	TARI_PM-3	Tomato	Taiwan	Lin *et al*., 2022
MT370495.1	TARI_PM-4	Tomato	Taiwan	Lin *et al*., 2022
MT509564.1	O	Pepper	Iraq	Al-Hamada *et al*., 2022
OL314781.1	ASU50	Pepper	Egypt	Unpublished
AM498634.1	IRAN 11691F	*Polianthes tuberosus*	Iran	Khodaparast *et al*., 2007
MW242832.1	MKUBK-SOh11	Spinach	Türkiye	Soylu *et al*., 2021
OM921011.1	B7	Tomato	Syria	This study
OM921010.1	B12	Tomato	Syria	This study

Results and Discussion

Symptoms produced

On the Mandalon tomato cultivar, the powdery mildew isolates developed the following characteristic symptoms: bright yellow spots on the upper side of the leaves, accompanied by a distinct whitish powdery sporulation on the corresponding lower leaf surface (Figure 2). As the infection advanced, these initially yellow spots gradually transformed into brown spots.

Microscopic observations

The two *L. taurica* isolates formed transparent divided superficial mycelium, semi-straight into confluent, branched on the leaf surface. It was fixed by adhesion bodies that are branched, forked or lobed in the shape of a double coral (Figures 3 and 4). The width of hyphae cells was 5-8 µm. Internal hypha surrounded the mesophyll cells. Conidiophores were of the pseudoidium type, arised from superficial hypha and stomata, but the majority of conidiophores came out of the stomata in groups (2-6), mostly 4, (63-275×4-10 µm), and was branched in the B7 isolate. The foot cell was straight, elongated, 38-115×5-7 µm long, 2-3 cells above the foot cell and under the secondary conidium. The lanceolate primary and cylindrical elongated secondary conidia dimensions ranged between 43-69×10-22 and 38-67×10-20 µm, respectively. The conidia had a rough, wavy surface, especially the primary one. Small thorn-like protrusions around the tips of conidiophores appeared in the B7 isolate (Figure 3). The morphological features of isolates corresponded to those of *L . taurica* (Lin *et al*., 2022; Palti, 1988). Cleistothecia were not observed. The biometric dimensions of the two isolates were close to each other (Table 2).

Table 2. Biometric dimensions of morphological structures of two local isolates of *L. taurica*.

	Feature (µm)			
Isolate	Conidiophore Mean±SD	Foot cell Mean±SD	Primary conidia Mean±SD	Secondary conidia Mean±SD
B7	66-275*×5-10**	58-115×5-7	47-69×10-22	39-67×10-20
	168.1±12.5×7.2±1.7	87.7±6.4×5.1±1.3	59.4±5.2×14.8±1.3	50.8±5.2×13.8±1.5
B12	63-250×4-9	38-89×5-6	43-67×10-20	38-60×10-19
	158.2±11.2×5.7±1.4	64.4±5.3×4.8±1.1	54.2±5.7×13.2±1.8	48.7±4.8×12.5±1.2

*= the range (min–max) for length, **= the range (min–max) for width, SD= standard deviation. (100 replicates for each feature were measured).

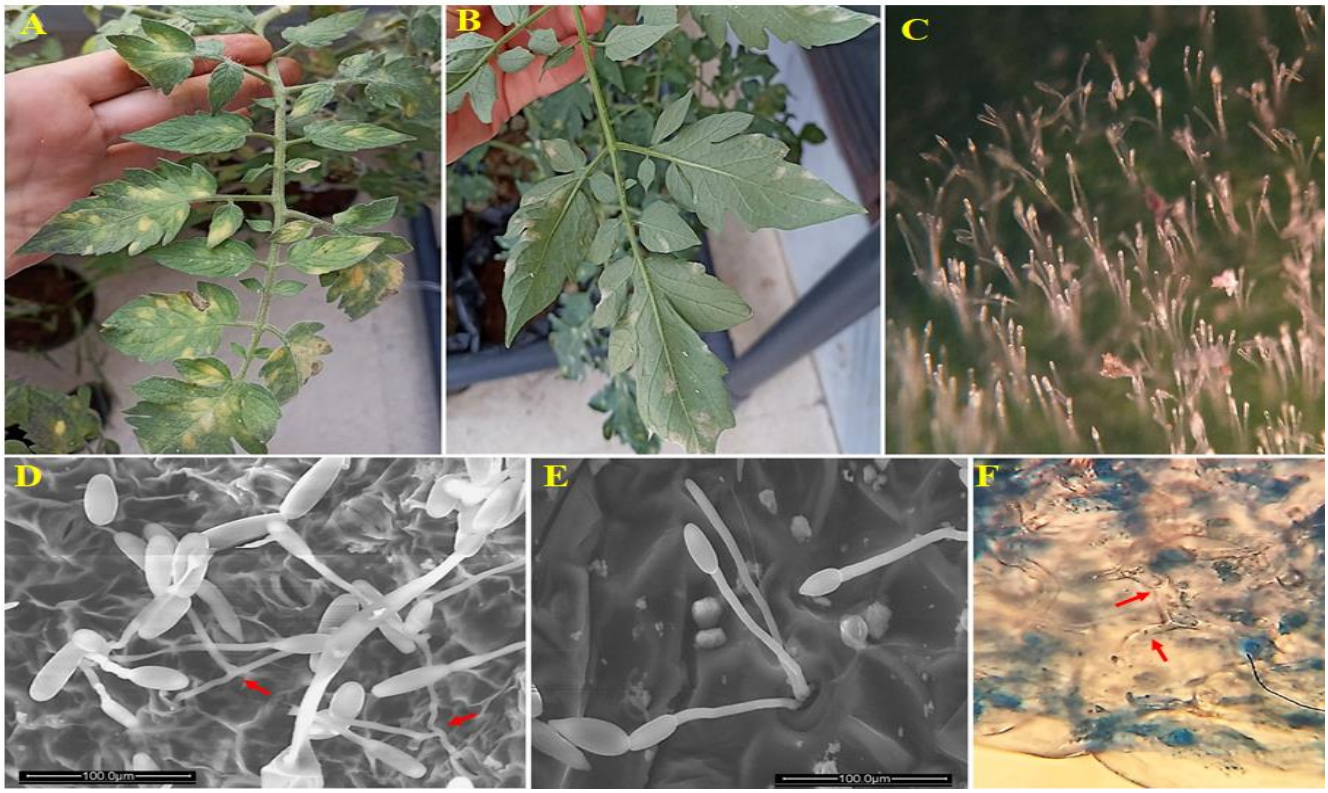

Figure 2. A & B= Symptomes of *L. taurica* on upper and lower leaf surface of Mandalon cultivar leaf, C= Conidiophores emerging from stomata on the lower leaf surface (200x magnification), D= Scanning electron micrograph of conidiophores arrising from superficial hypha (arrows) and stomata (E), F= Internal hypha (arrows) (600x magnification).

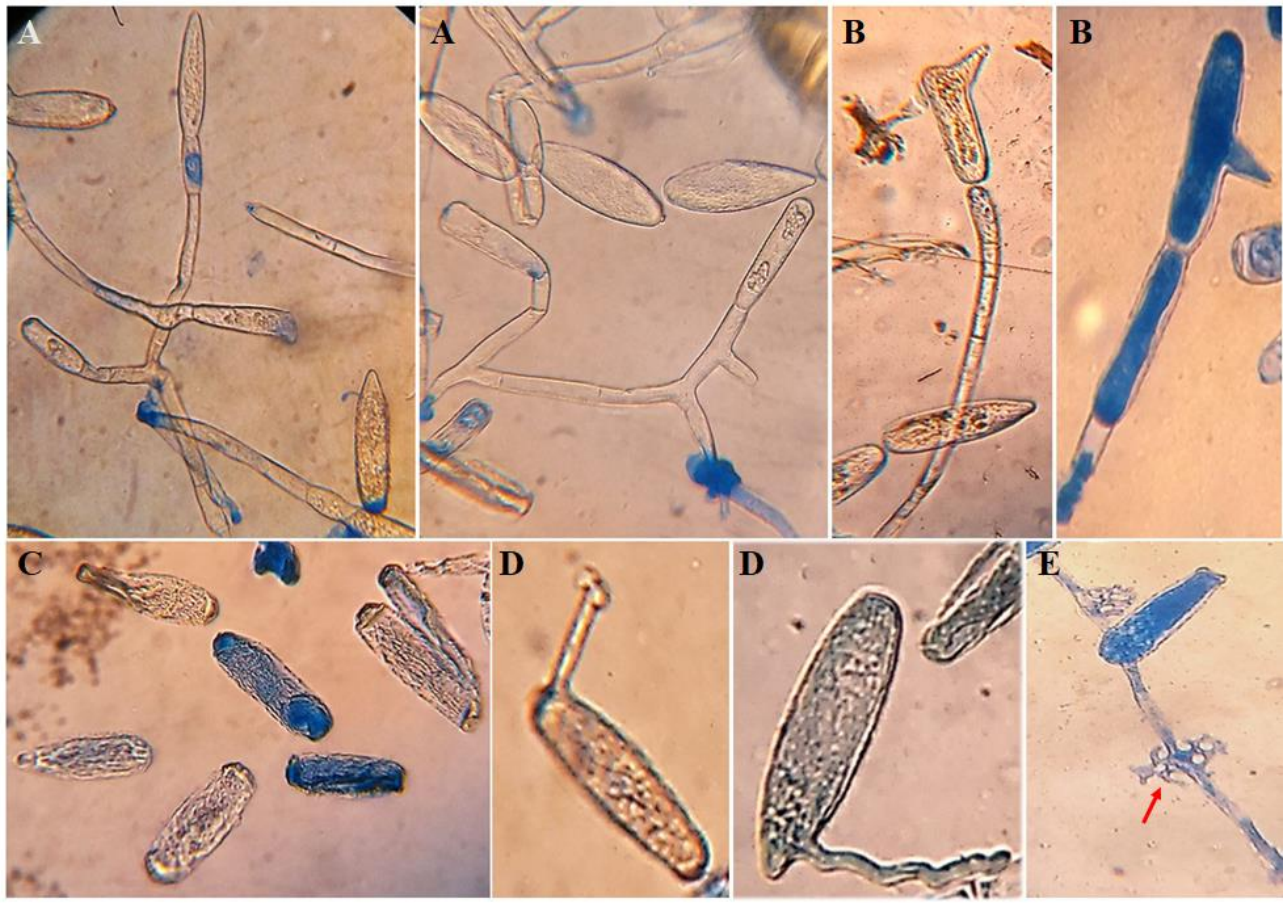

Figure 3. Morphological characteristics of B7 isolate: A= Conidiophores (400x), B= Conidiophores with thorn-like protrusions, C= Rough, wavy surface of conidia (400X), D= Germinated conidia patterns according to Cook & Braun (2009) (600X), F= Adhesion bodies on superficial hypha (arrow).

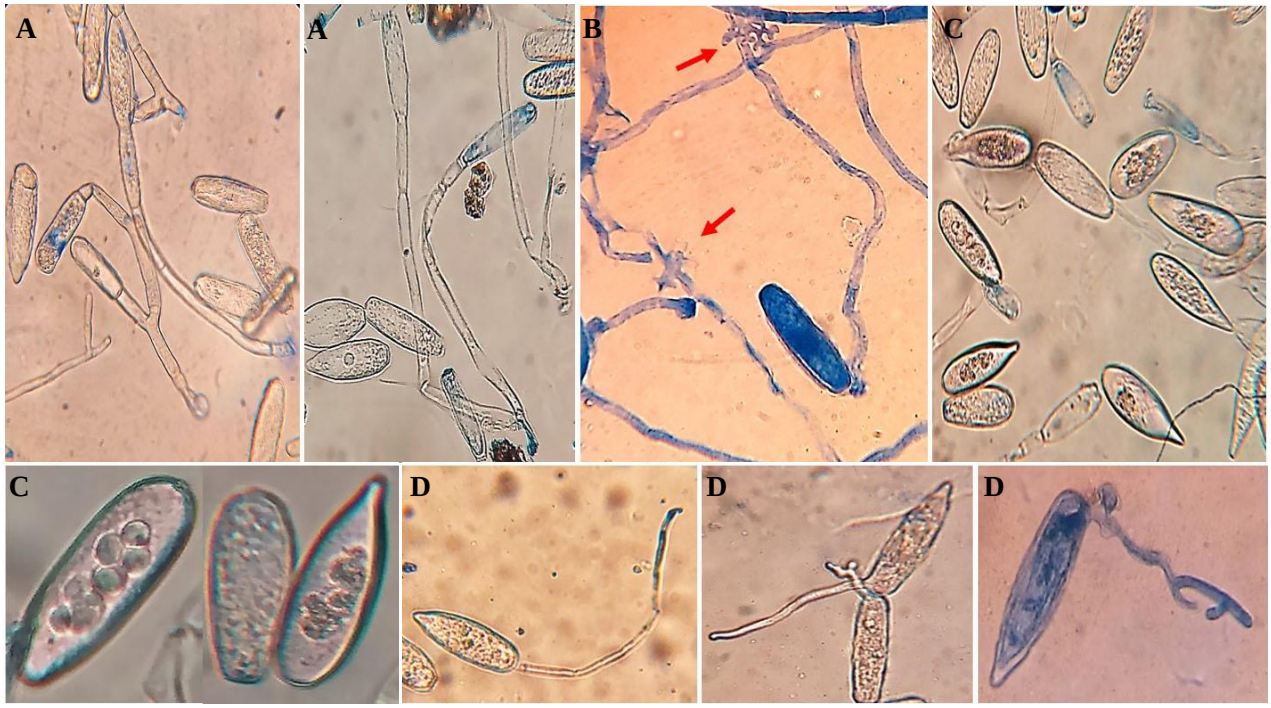

Figure 4. Morphological characteristics of B12 isolate: A= Conidiophores (400X), B= Germinated conidia with adhesion bodies on superficial hypha (arrows), C= Conidia, D= Germinated conidia patterns (600X).

Phylogenetic analysis

The ITS region of the two Syrian isolates (B12 and B7) were sequenced and deposited in the GenBank with accession numbers OM921011 and OM921010. Sequence homology for the isolates using BLAST search showed similarity to *L. taurica* with query coverage reached 100% with more than 99% identity to many isolates recorded in the Gene Bank on tomato and other hosts. (Table 3). The homology between the two local isolates was 99.62% with 100% query coverage.

B7 and B12 isolates were almost identical (Figure 5), and the variability in nucleotide sequence among them was found in only two positions. Position 212 is a A instead of a G, and position 215 is G instead of a A, and sequence analysis showed a similarity of 99.62% between them. The constructed phylogenetic tree (Figure 6) showed two main clades, Clade I included two local Syrian isolates of *L. taurica* on tomato and this result confirmed the morphological characterization that showed no significance differences between the two isolates; Clade II included the global isolates on tomato and other hosts, and this result is consistent with what has been reported by Khodaparast *et al*. (2001) who found that the sequence of *L. taurica* varied from one host to another. 34 isolates were included in 6 clades and four basal taxa.

To the best of our knowledge, this study represents the first molecular characterization of *L. taurica* isolates on protected tomato along the Syrian coast. In the near future, it is imperative to extend our investigation to cover isolates from various regions across Syria, not only on tomatoes but also on other host plants. Employing cutting-edge molecular techniques will be essential to explore the species genetic diversity, considering its documented high intraspecific gene sequence variability, as previously highlighted by Khodaparast *et al*. (2012).

Acknowledgment

The authors thank Dr. Hafez Mahfoud, Institute of Botany, Technical University of Dresden, Germany, for his cooperation in implementing this study.

Table 3. Homology search results for B7 and B12 isolates of *L. taurica* using BLAST search with NCBI.

Accession numbers	Isolate/strain	Host	Origin	Homology (%)	Query coverage (%)
MT370494.1	TARI_PM-3	Tomato	Taiwan	99.44	100
MT370495.1	TARI_PM-4	Tomato	Taiwan	99.44	100
OL314781.1	ASU50	Pepper	Egypt	99.44	100
MT125857.1	LtCAPS-Aus	Pepper	Australia	99.44	100
MW242832.1	MKUBK-SOh11	Pepper	Turkey	99.44	100
MT125856.1	LtCAPS-HU	Pepper	Hungary	99.44	100
AM498634.1	IRAN 11686F	Onion	Iran	98.87	100
MT509564.1	O	Pepper	Iraq	98.50	100

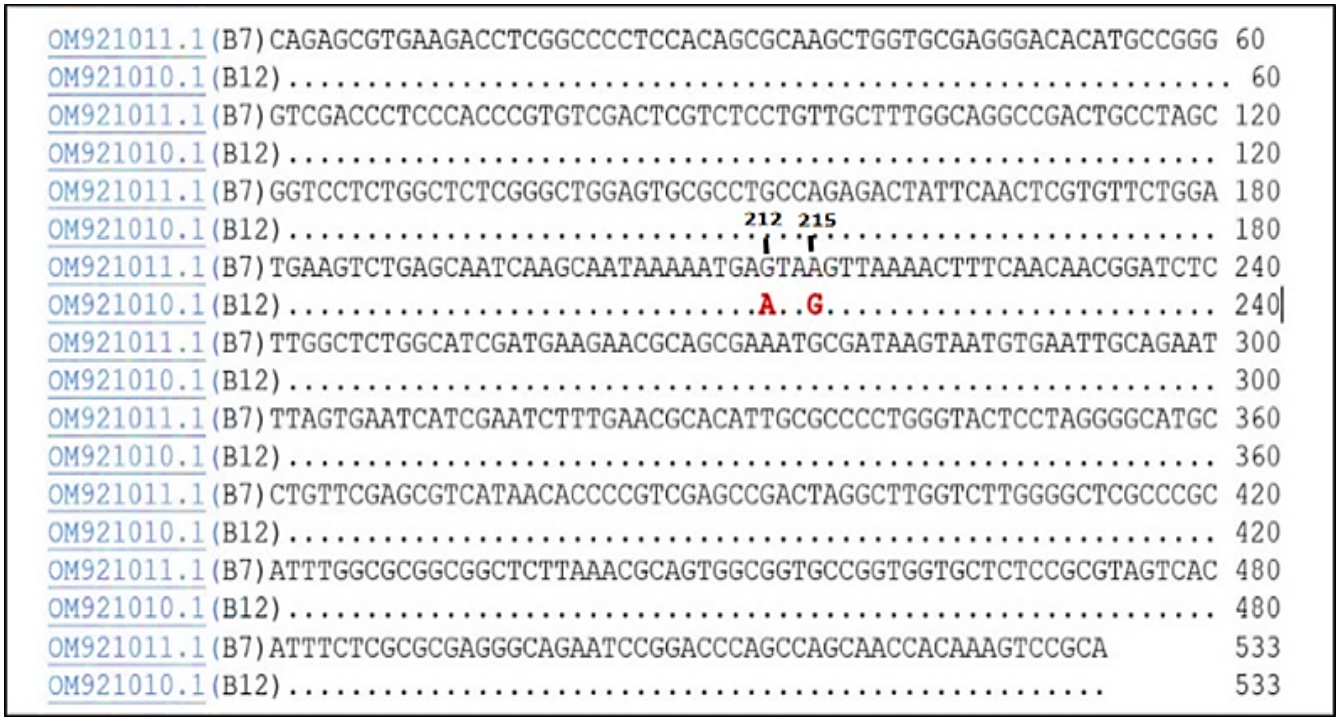

Figure 5. Variability in nucleotide sequence of 5.8S rDNA ITS regions of B7 and B12 isolates of *L. taurica*.

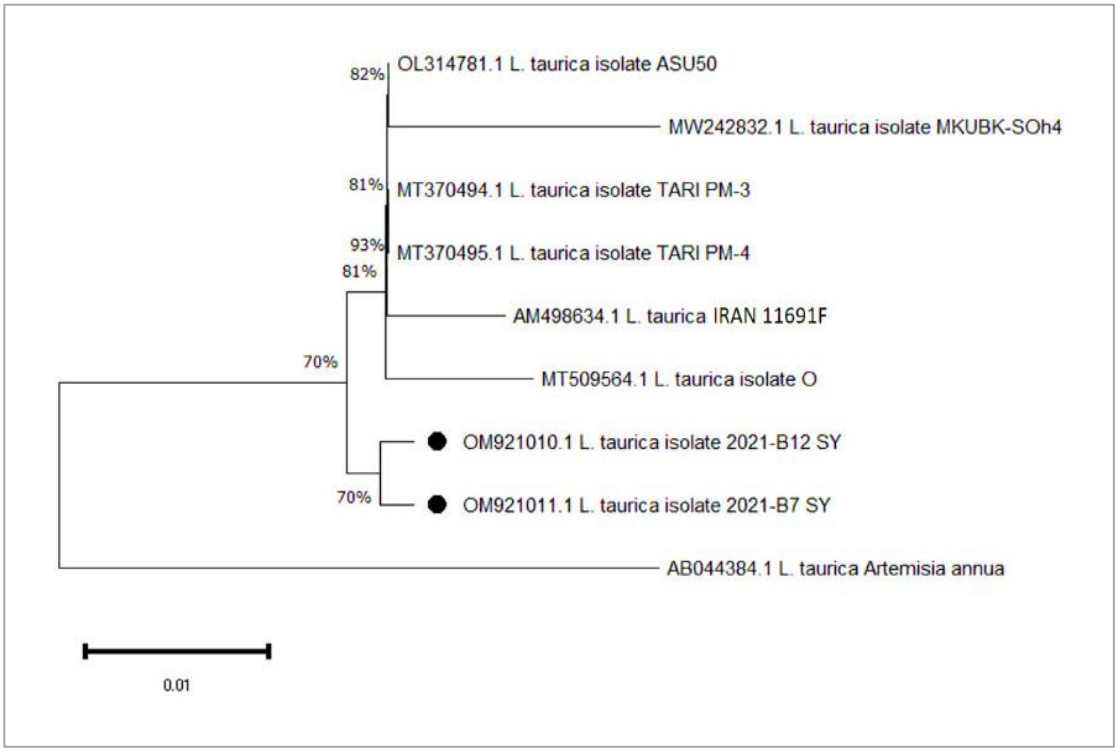

Figure 6. Phylogenetic tree based on the nucleotide sequence of the rDNA internal transcribed spacer regions (ITS) for 9 powdery mildew taxa of *L. taurica*. The tree with the highest log likelihood (-1302.67) is shown. The percentages of replicate trees in which the associated taxa clustered together (bootstrap coverage higher than 50% based on 1000 replications) is shown next to the branches.

الملخص

عليو، نهى، صباح المغربي ونزار معلا. 2024. التوصيف الشكلي والجزيئي لعزلتين من فطر *Leveillula taurica* المسبب للبياض الدقيقي على البندورة/الطماطم المحمية في ساحل طرطوس، سورية. مجلة وقاية النبات العربية، 42(4):436–442. https://doi.org/10.22268/AJPP-001275

وصفت عزلتان تابعتان للنوع *Leveillula taurica* بالاعتماد على الصفات الشكلية وبيانات تسلسل ITS. حيث ظهرت الأعراض النموذجية للإصابة على البندورة/الطماطم صنف مندلون على شكل بقع صفراء باهتة على السطح العلوي للأوراق قابلها نموات دقيقية على السطح السفلي، ولم تلاحظ الثمار الزقية المغلقة. أظهر الفحص بالمجهر الضوئي والإلكتروني تشكل أبواغ كونيدية أولية رمحية وثانوية أسطوانية على حامل كونيدي كاذب، وقد كانت باقي الصفات مشابهة للنوع *L. taurica*. أكد الفاصل الداخلي المنسوخ (ITS) للحمض النووي الريبي منزوع الأوكسجين DNA صحة التوصيف الشكلي للنوع. كما أظهرت نتائج البحث التماثلي للعزلتين باستخدام BLAST انتمائهما للنوع *L. taurica*، حيث وصلت نسبة التماثل مع تسلسل عزلات الفطر في بنك الجينات إلى 100% وأكثر من 99% نسبة تشابه مع العديد من العزلات المسجلة في بنك الجينات على البندورة/الطماطم وعوائل أخرى. ويعدّ هذا البحث التوصيف الجزيئي الأول للنوع *L. taurica* على البندورة/الطماطم المحمية في الساحل السوري.

كلمات مفتاحية: البندورة/الطماطم، البياض الدقيقي، *Leveillula taurica*، ITS.

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Received: September 11, 2023; Accepted: November 13, 2023

تاريخ الاستلام: 2023/9/11؛ تاريخ الموافقة على النشر: 2023/11/13