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ORIGINAL PAPER

Molecular and functional characterization of tomatinase secreted by isolates of *Fusarium oxysporum* f.sp. *lycopersici*, the causal of vascular wilt of tomato

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ABSTRACT

Tomato wilt is caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), a soilborne pathogen causing global economic losses. FOL aggressive isolates produce tomatinase to counteract tomato defense. The study aimed to identify and analyze the tomatinase gene in three FOL isolates with varying aggressiveness. Genetic analysis was employed using a polymerase chain reaction-based method, and the sequences were compared with several tomatinase sequences. Post-translational functional analysis predicted phosphorylation sites for the three tomatinase sequences using NetPhos3.1 server. Results showed that FO-19, the most aggressive isolate, had the highest tomatinase activity. Tomatinase activity was found to be correlated with phosphorylation, which may be quite important in the aggression of FOL isolates. The study highlights the importance of understanding the FOL-tomato interaction in developing effective strategies to control tomato vascular wilt.

Keywords: *Fusarium oxysporum*, tomatinase, aggressiveness, tomato vascular wilt

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INTRODUCTION

Wilt disease incited by the significant soil inhabiting ascomycete *Fusarium oxysporum* Schlectend.: Fr, comprises different species, varieties and forms. *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is the tomato pathogen which leads to important economic losses in different vegetables (Srinivas *et al.*, 2019). FOL invades the plant through root injury or natural openings leading to significant disruption to water and nutrient transport. Vascular darkening refers to blockage of xylem vessels which is a prominent symptom of Fusarium wilt. Young leaves developing vein clearing and leaf epinasty, abided by stunting, yellowing, gradual wilting of leaves and stem, defoliation, and eventually death are typical disease signs (Di-Pietro *et al.*, 2003). Based on host specificity, two symptomologically definite forms of the pathogen can cause the disease:*F. oxysporum* f.sp. *lycopersici* W.C. Snyder & H.N. Hans. which cause a vascular wilt and *F. oxysporum* f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker which cause crown and root rot (Manzo *et al.*, 2016). The fungus is known to generate spores capable of enduring in the soil for extended periods, posing a long-term threat to tomato cultivation (Nirmaladevi *et al.*, 2016). Once plants are infected with the fungus, it is impossible to eradicate disease with treatment and should be immediately discarded (Claeys and Inzé, 2013). Unfortunately, nightshade vegetables (e.g. tomatoes, peppers, and eggplants) cannot be grown in contaminated areas for at least one year (Wu *et al.*, 2022). Preventative measures to inhibit disease introduction are the most effective options.

A devastating tool in tomato plants' defense system, against FOL, is the production of α-tomatine. This steroidal glycoalkaloid saponin has been found in other Solanaceous plants acting as a chemical

barrier against microorganisms. The interaction between plants and pathogens is mediated by this fungitoxic agent. This major phytoanticipin is accumulated in all tissues of the healthy plants in its physiologically active form, with particularly high levels in leaves and immature fruits (Cataldi *et al.,* 2005). The ability of this secondary metabolite to lyse cells was linked to adherence to sterols in membranes of invading microorganism (Duffey and Stout, 1996). Microorganisms gradually acquire strategies to circumvent resistance because host resistance systems put pathogens under selection pressure. Tolerance to tomatine is based on two main mechanisms: modifications in their membrane composition or production of tomatinases, a specific tomatine-detoxifying extracellular enzyme (Lairini and Ruiz-Rubio, 1998).

Fusaric acid, secondary metabolite produced by the fungi, is a key factor contributing to the virulence of FOL (Singh *et al.,* 2017), while the production of tomatinase is required for full virulence (Pareja-Jaime *et al.* 2008; Nirmaladevi *et al.,* 2016). Tomatinase, as a prerequisite for successful infection of FOL, can break down α-tomatine into tomatidine and β-lycotetraose (Lairini and Ruiz-Rubio, 1998). These breakdown products are less toxic to most tomato pathogens (Sandrock and VanEtten, 1998). They also have an inhibitory impact on tomato defense signaling during infection, demonstrating the coexistence of detoxification and suppression (Alba *et al.,* 2011). However, this specific monomer of 50kDa is used as indicator for virulence in tomato plant in transcription level (Lairini *et al.,* 1996; Pareja-Jaime *et al.,* 2008 and Abdallah *et al.,* 2010).

Fungi engage post-translational changes as a crucial and useful tactic for environmental adaptation. Phosphorylation of proteins is a vital regulating event that affects fungal virulence, adaptability, and resistance to a variety of internal and external environmental stimuli (Dóczi and Bögre, 2018). Reversible and dynamic post-translational modifications can change the function of proteins by modifying protein conformation, subcellular localization, and protein-protein interactions. These modifications involve the addition or removal of phosphoryl groups by kinases and phosphatases, respectively (Vitrac *et al.,* 2019). Recent developments in the understanding the pathogenicity of FOL have demonstrated the role of phosphatase in the invasive growth and virulence of the fungus (Nunez-Rodriguez *et al.,* 2020).

The current study aimed to characterise isolate and characterize the tomatinase gene, to understand to what extent post-translational protein modifications by phosphorylation may be involved in the enzyme activity and virulence of the studied isolates of FOL pathogen.

MATERIALS AND METHODS

Fungal isolates and growth conditions

Three pathogenic isolates of FOL (FO-5, FO-14 and FO-19) that were utilized in the current study, were previously isolated from different Solanaceae hosts (Shahda *et al.,* 2015). All isolates were previously submitted to NCBI GenBank under Accession Numbers (KP898394, KP898403, KP898405, KP898408). Fungal isolates were kept on Potato Dextrose Agar medium (PDA) slant at 4°C or as spores in 20% glycerol under -20°C for further usage.

DNA extraction

FOL isolates subjected to DNA extraction were grown separately in Petri dishes containing PDA media supplemented with 200 mg/L chloramphenicol. All Petri dishes were incubated at a temperature of 28°C for 10 days. According to Edel *et al.* (2001), for every Petri plate containing a fungal isolate, 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.5; 50 mM EDTA and 3 % SDS) was added. Then the scraped mycelium was transferred into an Eppendorf tube and vortexed. After 10 min of incubation at 65°C, centrifugation takes place at 12000 x g for 10 min at 4°C. Subsequently transferring the supernatants to fresh microtubes, one volume of isopropanol and 0.5 volume of 3 M sodium acetate were

added to precipitate the DNA. Three gentle inversions of the microtubes were followed by a 15 min of centrifugation (12000 x g) at 4°C. After discarding the supernatant, 300 µl of ice-cold ethanol (70 %) was used to rinse the particle. Another 5 min of centrifugation, 12000 x g at 4°C, was used to eliminate the ethanol. The air-dried DNA pellet was dissolved in 100 µl of TE buffer (10 mM Tris-HCl; pH 8.0) and 1mM EDTA and the, stored at 4°C until use.
tree stability.

Prediction of Phosphorylation Sites

The NetPhos 3.1 server, which is accessible to the public at (http://www.cbs.dtu.dk/services/NetPhos/), was used to predict phosphorylation sites. Using ensembles of neural networks, the

Table 1: Primers used for PCR amplification of the tomatinase gene.

Primer code	Primer pair (5'→3')	Product
Tom 1_left **Tom 1_right**	ATG GAT CGA TGG GAT GTT GT CGT AGA GTT CTT GGC GCT TT	**199 bp**
Tom 2_left **Tom 2_right**	AAC ATC ACA GCC AAC GAC AC TGT GCA TCA TCG AGG TAG GA	**200 bp**
Tom 3_F **Tom3_R**	CCA AGA ACT CTA CGA GAT GG TTG GGA TAG TAG GTC TCG TT	**339 bp**

PCR amplification and DNA sequencing of tomatinase

The tomatinase gene of FOL isolates were amplified, using three specific primer pairs listed in Table (1). Primers were designed in Primer3 (http://bioinfo.ut.ee/primer3) using consensus sequences of *FoTom1* (EMBL accession no. AJ012668) retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

The PCR mixture included: 5 µl of 10x PCR buffer, 1 µl of each primer (100 pmol), 1.25 µl of dNTPs (10 mM), 1 µl of template DNA, 0.5 µl of Taq polymerase (2.5 U), and nuclease-free water to a final volume of 50 µl. PCR conditions were: 2 min. at 94°C, followed by 30 cycles of 30 seconds each at 94°C, 2 min at 57°C, and 2 min at 72°C, with a final extension of 5 min at 72°C.

The PCR products were separated on a 1.5% agarose gel for 140 min at 80 V and 400 mA before being visualized with ethidium bromide under UV light. Following gel purification, the amplified tomatinase genes were sequenced by Sigma Egypt using a Sanger ABI 3730 xl sequencer. The sequences were analyzed in the NCBI database, and a phylogenetic tree was drawn using the neighbor-joining method in ClustalW. A 1000 resampling bootstrap analysis was used to figure out NetPhos 3.1 service generates neural network predictions for the phosphorylation sites of serine, threonine, and tyrosine found in eukaryotic proteins. The tomatinase-deduced protein sequences from isolates FO-5, FO-14, and FO-19 are predicted.

Protein Extraction and Profiling of *F. oxysporum* Isolates by SDS-PAGE

Three 5 mm plugs of each fungal isolate were aseptically transferred to 50 ml the potato dextrose broth in 250 ml flasks and incubated under static conditions at room temperature. After 15 days of growing, the fungal mycelium was filtered and washed several times with distilled water then, the mycelial pellet was ready for protein extraction. One milliliter of the extraction buffer sodium phosphate (0.1 M - pH 7.0) was added to one gramme of freshly cleaned mycelia under 4°C. Centrifugation of the homogenate for 20 min at 10,000 x g to separate the supernatant, which was then utilised for SDS-PAGE. Protein concentrations of FOL samples have been measured using the Bradford method with bovine serum albumin as a standard (Bradford, 1976). Absorbance at 595 nm was recorded after 5 min at room temperature. A standard curve was used to

determine protein concentrations. Each sample (100 µg of protein) was mixed with 10 µl of sample buffer, boiled for 4 minutes, and then incubated at 4°C for 30 minutes. The samples were loaded into polyacrylamide gel wells, stained with Coomassie brilliant blue R-250, and analyzed using SDS-PAGE.

Tomatinase activity assays

Fungal isolates (2.5×10^6 microconidia.ml^{-1}) were cultivated in CA medium (10 g casamino acids per liter, 10 mM ammonium sulphate, and 0.5 g of yeast nitrogen base per litre) for 2 days on an orbital shaker (120 x g) at 28^{o}C. To achieve a final concentration of 20 g.ml^{-1}, α-tomatine was aseptically added to the culture in 50 mM sodium acetate buffer (pH 4.5) and incubated for continued to 48 hours. Control flasks held α-tomatine-free buffer. After the incubation period, the culture was filtered through sterile cheesecloth to get the filtrate, which was deployed directly as a crude extract for determining the activity of tomatinase which was assessed spectrophotometrically at 525 nm using the dinitrosalicylic acid (DNS) reagent to determine of reducing sugars (Miller, 1959). The filtrate has been mixed with 10 mM α-tomatine and 20 mM sodium acetate (pH 5.5), 0and the mixture was incubated at 37°C for 30 min. Following incubation, 3,5-DNS was added to the reaction mixture which was then boiled for 5 min. Finally, the volume was adjusted to one millilitre of water (Pareja-Jaim *et al.* 2008).

RESULTS

Detection of tomatinase genes

Isolation and amplification of tomatinase gene from three isolates of FOL were carried out using PCR primers listed in Table (1). The amplified DNA fragment was 199 bp long with primers *tom1-left/tom1-right* for two isolates (FO-5 and FO-19), while with primer *tom2-left/tom2-right* the amplified DNA fragment was 200 bp for two isolates (FO-14 and FO-19). The DNA fragment amplified was 339 bp long with primers *tom3-F/tom3-R* for only one isolate (FO-19).

Sequencing and identification of tomatinase gene

All amplified PCR products were sequenced at real biogene biotechnology services company, Alexandria, Egypt to AGCT- Advanced Genoming Computing Technologies in Germany (a commercial sequencing facility). The newly identified nucleotide sequences of *tomatinase* gene and reported in the present study, have been deposited in the GenBank nucleotide sequence database. The produced nucleotide sequences for the three isolates (FO-5, FO-14 and FO-19) were aligned, using the program ClustalW, with other *tomatinase* genes published on the GenBank: *FO-tom1* from *F. oxysporum* (AB08006) and *tom1* gene from *F. oxysporum* f. sp. *lycopersici* (AJ012668). The phylogenetic relationship between tomatinase nucleotide sequence showed that the three isolates of FOL divided into two clusters, first one contained each of the FO-14 which was isolated from pepper plants and FO-5 which was isolated from tomato plants grown in Egypt compared with *tom1* gene (*F. oxyporum* f. sp. *lycopersici*: AJ012668) obtained from the Genbank. Cluster II divided into two sub cluster. Sub cluster 1 included isolate FO-19 compared with *FO-tom1* gene (*F. oxysporum*: AB083006 (Figure 1)

Relying on the predectied sequences of amino acid of the tomatinase genes, the family 10 glycosyl hydrolases demonstrated a high degree of identity, indicating a relationship between these proteins. According to the amino acid sequences aligned with those of *F. oxysporum* tomatinases, CAA101121 of *F. oxysporum* f.sp. *lycopersici*, EXL41919 of *F. oxysporum* f. sp. *radices-lycopersici* 26381 and the protein BAB88659 from *F. oxysporum.*

Phylogentic relationship between amino acid sequences revealed that isolate FO-19 was too close with the compared amino acid sequence all CAA101121 (*F. oxysporum* f. sp. *lycopersici*, glycosyl hydrolase family 10), EXL41919 (*F.*

oxysporum f. sp. *radicis-lycopersici* 26381) and BAB88659 (tomatinase from *F. oxysporum*) (Figure 2).

Prediction of Phosphorylation Sites

For the three deduced amino acids of tomatinase, the Phosphorylation site (serine, threonine, and tyrosine) and Kinase site were predicted by the NetPhos 3.1 server. The results of computational prediction showed that there are different phosphorylation sites in the deduced amino acids of tomatinase. 6 positions for isolate FO-5, 7 positions for isolate FO-14 whereas 17 positions in isolate FO-19 (Figure 3).

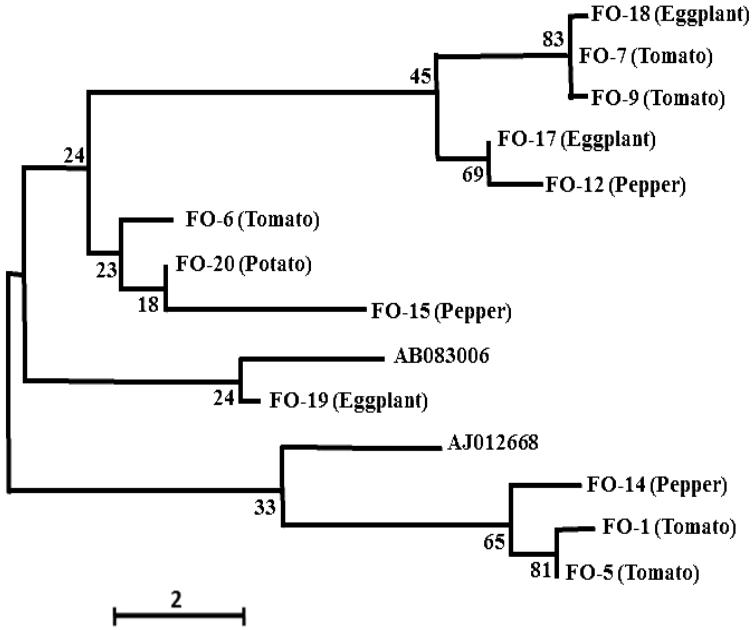

Figure 1. Phylogenetic relationship between tomatinase nucleotide sequences. The tomatinase gene was amplified with *tom3*-F/*tom3*-R primer. Using MEGA6 software, the neighbour-joining approach produced the phylogram. The bootstrap value support is shown by the numbers on the branches.

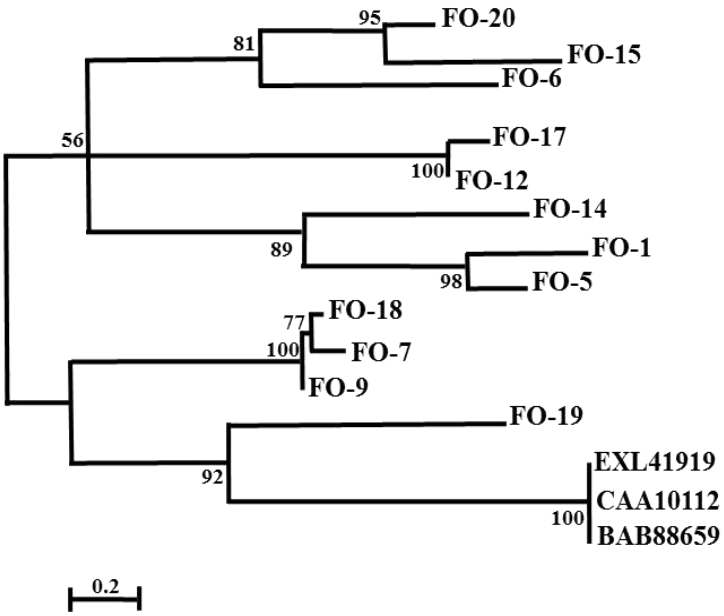

Figure 2. Phylogenetic relationship between tomatinase from *F. oxysporum*. Amino acid sequences were mapped with ClustalW. Tomatinase (*F. oxysporum* f. sp. *lycopersici*) CAA10112, glycosyl hydrolase family 10 (*F. oxysporum* f.sp. *radices-lycopersici* 26381) EXL41919 and tomatinase (*F. oxysporum*) BAB 88659. Numbers at the branch locations represent the bootstrap value. A scale bar illustrating the genetic distance (substitution per site) is represented

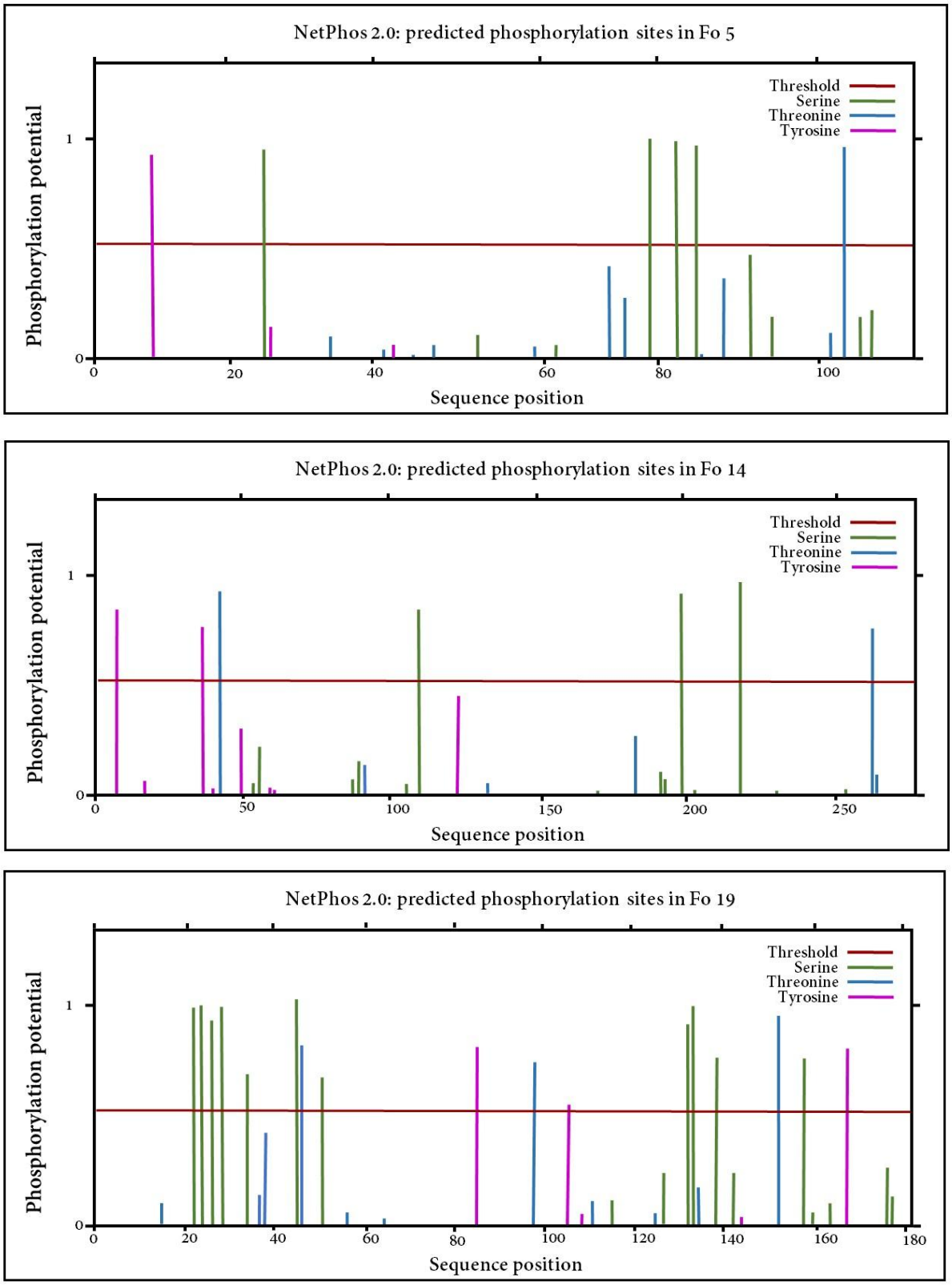

Figure 3. Prediction of phosphorylation sites in the deduced amino acids of tomatinase of three *F. oxysporum* isolates FO-5 (top), FO-14 (middle) and FO-19 (button).

SDS-PAGE profile of proteins of *F. oxysporum* isolates

The SDS-PAGE profile of the extracted proteins from *F. oxysporum* isolates FO-5, FO-14 and FO-19 is demonstrated in Figure (4). The variations in protein bands are obvious in FO-19 isolate as compared with the other isolates. A monomorphic protein band with molecular weight of 27 KDa was detected

in isolates FO-5 and FO-14. On the other hand, six polymorphic bands were detected in the highly aggressive isolate FO-19 with molecular weights of 63, 45, 40, 35,30, and 25 kDa.

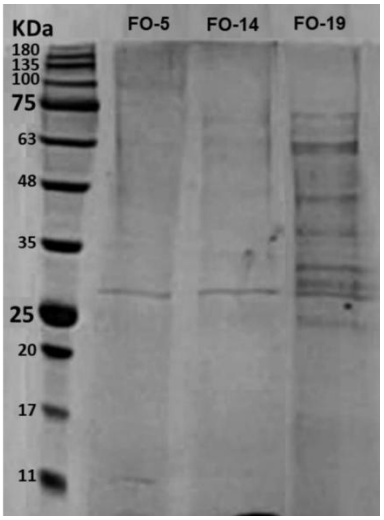

Figure 4. SDS-PAGE profile of proteins extracted from the *F. oxysporum* isolates (FO-5, FO-14, and FO-19). The left lane represents a middle molecular weight protein marker.

Tomatinase activity assay

Tomatinase activity in *F. oxysporum* pathogenic isolates (FO-5, FO-14 and FO-19) was assayed; the isolates were grown for 48 hours in liquid CA in the presence of tomatine extract. Data presented in Table (2) and Figure (5) showed that in the existence of tomatine, the activity of tomatinase was significantly increased in all isolates. The highest activity was detected in FO-19 followed by FO-5 and FO-14.

Table 2: Tomatinase activity in the presence of tomatine aqueous extract.

Treatment	Tomatinase activity OD 525nm		
	FO-5	FO-14	FO-19
Control (tomatine free)	0.0475*b	0.0233b	0.0660b
+ Tomatine	0.627a	0.366a	0.737a

* Figures with the same letter in a column are not significantly ($P \leq 0.05$) different.

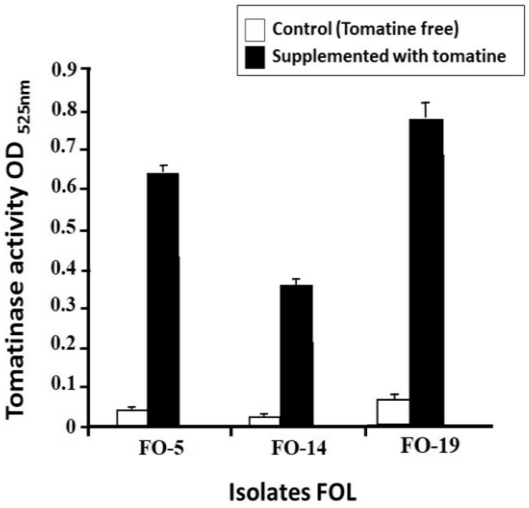

Figure 5. Total activity of tomatinase enzyme of *F. oxysporum* pathogenic isolates. Each column represents the mean of the three replicates with standard error.

DISCUSSION

Plant defense mechanisms including production of secondary metabolites and compounds, such as α-tomatine, are key factors in building plant disease resistance, whereas microbial pathogens have evolved a wide range of tactics to engage with host cells, control their actions, and ultimately endure and spread (El-Bebany *et al.,* 2013; Daayf *et al.,* 2012). The extracellular tomatinase produced by the tomato pathogen *F. oxysporum* is required not only for detoxification of α-tomatine but also for circumventing defense mechanisms of host (Ito *et al*., 2004). Previous studies reported that tomatinase enzymes of tomato pathogens such as *F. solani*, *Septoria lycopersici*, *Botrytis cinerea* and *Verticillium alboatrum* were inducible by tomatine and essential for pathogen virulence. Pathogenesis involves the phosphorylation of proteins on hydroxyl amino acids during various stages, such as cell interactions, microbial entry, and infection-induced changes in host cells. Discovering the link between protein-tyrosine phosphorylation and virulence factors has transformed the study of microbial pathogenesis, leading to new treatments and therapies for infections (Cozzone *et al.,* 2004 and Kuban-

Jankowska *et al.*, 2022). The study revealed differences in the protein patterns of *F. oxysporum* isolates with varying levels of aggressiveness. The polymorphic protein bands in isolate FO-19 appear to be linked to its high pathogenicity. Three tomatinase genes were identified through gene sequencing of three different *F. oxysporum* isolates. Isolate FO-19 was the most virulent, showing stronger hydrolytic activity of α-tomatine compared to the other isolates. The aggressiveness of FO-19 may be due to the presence of phosphorylates sites (serine/threonine and tyrosine) as predicted by bioinformatics. Phosphorylation, as post-translational modification, is a crucial many biological functions, particularly in plant pathology. It regulates the virulence of pathogens that cause plant diseases, influencing protein activity, stability, and interactions. Phosphorylated effector proteins help pathogens suppress plant immune responses, allowing successful infection and disease spread (Zhang *et al.*, 2022). Ge and Shan (2011) highlighted the role of bacterial phosphorylation in potential pathogenicity. The current findings align with El-Bebany *et al.*, (2010) on *Verticillium dahliae*, explaining the essential role of isolate FO-19's aggressiveness and virulence. Pathogens use phosphorylation in their signal transduction pathways, like the MAPK cascades, to adapt to host conditions and express genes necessary for infection (Jiang *et al.*, 2022). This process also affects plant defense mechanisms, where kinases trigger immune responses (Lang and Colcombet, 2020). However, pathogens can counteract these defenses by dephosphorylating plant proteins. These findings lay the groundwork for understanding plant-pathogen interactions. By comprehending phosphorylation's role in pathogen virulence and plant defense disease-resistant crops and innovative plant protection strategies can be developed.

CONCLUSIONS

Tomatinase is a vital enzyme in the virulence of *Fusarium oxysporum*, playing a key role in neutralizing host defenses and facilitating fungal invasion. Its ability to degrade alpha-tomatine underpins the pathogenic success of *Fusarium oxysporum* in a variety of host plants. By elucidating the molecular mechanisms of tomatinase action and its role in host-pathogen interactions, researchers can develop targeted strategies to combat Fusarium-induced diseases, thereby improving crop resilience and reducing agricultural losses. The continued study of tomatinase and its genetic regulation remains critical for advancing our understanding of fungal pathogenesis and enhancing plant protection measures.

REFERENCES:

Abdallah, NA., Shah, D., Abbas, D. and Madkour, M. (2010). Stable integration and expression of a plant defensin in tomato confers resistance to fusarium wilt. *GM Crops*, 1(5): 344-350. doi:10.4161/gmcr.1.5.15091

Alba, JM., Glas, JJ., Schimmel, BCJ. and Kant, MR. (2011). Avoidance and suppression of plant defenses by herbivores and pathogens. *J Plant Interact*, 6: 1–7. doi: 10.1080/17429145.2010.551670

Bradford MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*,72: 248-54. doi: 10.1006/abio.1976.9999.

Cataldi, TR., Lelario, F. and Bufo, SA. (2005). Analysis of tomato glycoalkaloids by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom*, 19 (21): 3103-10. doi: 10.1002/rcm.2176.

Claeys, H. and Inzé, D. (2013). The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiol*, 162 (4): 1768-1779. doi:10.1104/pp.113.220921

Cozzone, AJ., Grangeasse, C., Doublet, P.

and Duclos B. (2004). Protein phosphorylation on tyrosine in bacteria. *Arch Microbiol*, 181:171. doi: 10.1007/s00203-003-0640-6.

Daayf F., El Hadrami, A., El-Bebany, AF., Henriquez, MA., Yao, Z., Derksen, H., El-Hadrami, I. and Adam, LR. (2012). Phenolic compounds in plant defense and pathogen counter-defense mechanisms. *In Recent Advances in Polyphenol Research.* doi: 10.1002/9781118299753.ch8

Di Pietro, A., Madrit, MP., Caracuel, Z., Delgado-Jarana, J. and Roncero, MIG. (2003). *Fusarium oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Mol Plant Pathol*, 4 (5): 315-325. doi: 10.1046/j.1364-3703.2003.00180.x

Dóczi, R. and Bögre, L. (2018). The quest for MAP kinase substrates: gaining momentum. *Trends in Plant Sci*, 23: 918–932. doi: 10.1016/j.tplants.2018.08.002

Duffey, S. and Stout, M. (1996). Antinutritive and toxic components of plant defense against insects. *Arch Insect Biochem*, 32 (1): 3-37. doi:10.1002/(SICI)1520-6327

Edel, V., Steinber, GC., Gautheron, N., Recorbet, G. and Alabouvette, C. (2001). Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. *FEMS Microbiol Ecol*, 36:61–71. doi: 10.1111/j.1574-6941.2001.tb00826.x

El-Bebany, AF., Rampitsch, C., and Daayf, F. (2010). Proteomic analysis of the phytopathogenic soil-borne fungus *Verticillium dahliae* reveals differential protein expression in isolates that differ in aggressiveness. *Proteomics*, 10: 289–303. doi: 10.1002/pmic.200900426

El-Bebany, AF., Adam, LR. and Daayf, F. (2013). Differential accumulation of phenolic compounds in potato in response to weakly and highly aggressive isolates of *Verticillium dahlia*. *Can J Plant Pathol*, 35(2): 232–240. doi: 10.1080/07060661.2013.773943

Ge, R., and Shan, W. (2011). Bacterial phosphoproteomic analysis reveals the correlation between protein phosphorylation and bacterial pathogenicity. *GPB*, 9(4-5):119-27. doi: 10.1016/S1672-0229(11)60015-6.

Ito, S., Eto, T., Tanaka, S., Yamauchi, N., Takahara, H. and Ikeda, T. (2004). Tomatidine and lycotetraose, hydrolysis products of α-tomatine by *Fusarium oxysporum* tomatinase, suppress induced defense responses in tomato cells. *FEBS Lett*, 571:31-34. doi: 10.1016/j.febslet.2004.06.053

Jiang, M., Zhang, Y., Li, P., Jian, J., Zhao, C. and Wen, G. (2022). Mitogen-activated protein kinase and substrate identification in plant growth and development. *Int J Mol Sci*, 23(5):2744. doi: 10.3390/ijms23052744.

Kuban-Jankowska, A., Kostrzewa, T., and Gorska-Ponikowska, M. (2022). Bacterial protein tyrosine phosphatases as possible targets for antimicrobial therapies in response to antibiotic resistance. *Antioxidants*, 11(12):2397. doi: 10.3390/antiox11122397.

Lairini, K. and Ruiz-Rubio, M. (1998). Detoxification of α-tomatine by *Fusarium solani*. *Mycol Res*, 102: 1375–1380. doi: 10.1017/S095375629800656X

Lairini, K., Perez-Espinosa, A., Pineda, M., and Ruiz-Rubio, M. (1996). Purification and characterization of tomatinase from *Fusarium oxysporum* f. sp. *lycopersici*. *Appl Environ Microbiol*, 62(5):1604-1609. doi: 10.1128/aem.62.5.1604-1609.1996

Lang, J. and Colcombet, J. (2020). Sustained incompatibility between MAPK signaling and pathogen effectors. *Int J Mol Sci*. 21(21):7954. doi: 10.3390/ijms21217954.

Manzo, D., Ferriello, F., Puopolo, G., Zoina, A., D'Esposito, D., Tardella, L., Ferrarini, A., and Ercolano, MR. (2016). *Fusarium oxysporum* f. sp. *radicis-lycopersici* induces distinct transcriptome reprogramming in resistant and susceptible isogenic

tomato lines. *BMC Plant Biology*, 16, 53. doi:10.1186/s12870-016-0740-5

Miller, GL. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *J Anal Chem*, 31: 426-428. doi: 10.1021/ac60147a030

Nirmaladevi, D., Venkataramana, M., Srivastava, RK., Uppalapati, SR., Gupta, VK., Yli-Mattila, T., Tsui, KMC., Srinivas, C., Niranjana, SR., and Chandra, NS. (2016). Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. *Sci Rep*, 6, 21367. doi:10.1038/srep21367

Nunez-Rodriguez, JC., Ruiz-Roldán, C., Lemos, P., Membrives, S. and Hera, C. (2020). The phosphatase Ptc6 is involved in virulence and MAPK signaling in *Fusarium oxysporum*. (2020). *Mol. Plant Pathol*., 21(2):206-217. doi:10.1111/mpp.12889

Pareja-Jaime, Y., Roncero, MI., and Ruiz-Roldán, MC. (2008). Tomatinase from *Fusarium oxysporum* f. sp. *lycopersici* is required for full virulence on tomato plants. *MPMI*, 21(6):728-736. doi: 10.1094/mpmi-21-6-0728.

Sandrock, RW. and Vanetten, HD. (1998). Fungal sensitivity to and enzymatic degradation of the Phytoanticipin α-Tomatine. *Phytopathology*, 88(2):137-143. doi:10.1094/PHYTO.1998.88.2.137

Shahda, WT., EL-Bebany, AF., Rasmy, MR. and Gomaa, FH. (2015). Molecular characterization of *Fusarium oxysporum* isolates from Egypt using IGS sequencing. *Asian J Agric Food Sci*, 3(6): 704-711

Singh, VK., Singh, HB., and Upadhyay, RS. (2017). Role of fusaric acid in the development of 'Fusarium wilt' symptoms in tomato: Physiological, biochemical and proteomic perspectives. *Plant Physiol Biochem*, 118:320–332. doi: 10.1016/j.plaphy.2017.06.028

Srinivas, C., Nirmala Devi, D., Narasimha Murthy, K., Mohan, CD., Lakshmeesha, TR., Singh, B., Kalagatur, NK., Niranjana, SR., Hashem A., Alqarawi, AA., Tabassum, B, Abd Allah EF. and Chandra Nayaka, S. (2019). *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity- A review. *Saudi J Biol Sci*, 26(7):1315-1324. doi: 10.1016/j.sjbs.2019.06.002.

Vitrac, H., Mallampalli, VKPS. and Dowhan, W. (2019). Importance of phosphorylation/dephosphorylation cycles on lipid-dependent modulation of membrane protein topology by posttranslational phosphorylation. *J Biol Chem*, 294(49):18853-18862. doi: 10.1074/jbc.RA119.010785.

Wu, J., Wang, J., Hui, W., Zhao, F., Wang, P., Su, C., and Gong, W. (2022). Physiology of plant responses to water stress and related genes: A review. *Forests*, 13(2): 324. doi:10.3390/f13020324

Zhang, S., Li, C., Si, J., Han, Z. and Chen, D. (2022). Action mechanisms of effectors in plant-pathogen interaction. *Int J Mol Sci*, 23(12):6758. doi: 10.3390/ijms23126758.