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Tryptic Digested Protein Transglutaminase Elicitor Can Improve Plant Physiological Parameters: A Computational Approach to Insight Molecular Interactions

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ABSTRACT: *Phytophthora melonis* causes horticultural crops' dieback, foot rot, root rot, crown rot, and blight. It possesses multiple pathogenicity factors, and transglutaminase is among the most effective pathogenicity tools of *Phytophthora*. We have isolated the pathogenic enzyme and applied its tryptically digested fragments to tomato plants to exploit effector proteins for the augmentation of plant defenses, physiological parameters, antioxidative machinery, and evapotranspiration. The results revealed that the treatment significantly increased agronomic traits, e.g., root length, shoot length, root biomass, shoot biomass, root dry mass, shoot dry mass, and leaf area, around 30% in each case. Furthermore, an increase of more than 10% was observed in the case of photosynthetic parameters. At the same time, the antioxidative machinery (enzymatic and biochemical) was more than 53% more efficient than the control plants. Similarly, the evapotranspiration and electrolyte leakage improved by more than 18%. Furthermore, the pathogenic enzyme underwent structural and functional characterization. The biochemical tolerance tests revealed the temperature of 37 °C and apH of 6.4 as the optimum circumstances of the enzyme. The Tween-80 application increased activity by 136.72%, although higher temperatures reduced the stability of the enzyme. The structural protein annotations exhibited 2 monomers, including A (260-620) and B(141-219). Elicitation of transglutaminase (TGase) and antifreeze attributes were revealed by monomer A and monomer B, respectively. Additionally, an association of 6 ligand molecules of 3-cyclohexyl-1-propylsulfonic acidic were observed to maintain the enzymatic activity. Overall, the protein was categorized as the freeze-resistant protein that was revealed in three genera, i.e., *Plasmopara*, *Phytophthora*, and *Pythium*, according to the biome distribution analysis. Whereas the protein was also present in some other taxonomic classes (i.e., archaea, and bacteria). The current study is a new approach to exploit the plant metabolic machinery for improved cellular defenses and metabolism. It will also help to interpret the supramolecular interactions between the pathogen and its host, especially in a pathogen from oomycetes.

KEYWORDS: Transglutaminase activity; Structural analysis; Pathogenicity protein; Functional annotation; Tgase.

INTRODUCTION

The transfer of the functional molecules from one molecule to another is an imperative function needed in all signaling and metabolic pathways (Zhang et al., 2018; Ahmad et al., 2021a). All the cell processes and responses to the external stimuli involve the intrinsic function of transferases for being happened (Li et al., 2016). The large group of transferases contains a subgroup of transglutaminases, which takes the acyl group and transfers it from the donor molecule to the acceptor. The specific subgroup is the part of all types of transcription events, especially to the plant cell responses against external stressors (Greene et al., 2015). The process they catalyze is the acylation which involves the transfer of an acyl moiety and happen in all type of organisms, including small pore-forming microbes. The biosynthesis of the toxins and the activation of lethal defensive chemicals need to be acylated before getting functional. Triacylglycerol is an essential part of the membrane synthesis process that requires the enzyme transglutaminase for its production (Sharma et al., 2016).

Phytophthora mlonis, with a wide host spectrum, occupies the position among the top plant pathogens. *P. mlonis* may cause dieback, foot rot, root rot, crown rot and blight in horticultural crops. Production of the zoospores is the characteristic feature of the genus *Phytophthora* (Hashemi et al., 2019). The infections caused by the pathogen range from mild rottening of herbs to death of the trees (Ahmad et al., 2021b), including oaks (Tyler et al., 2006). Nevertheless, the amplified losses on cucumber plants during recent years have become an asterism for the researchers' community.

Besides increased infection intensity, the pathogen has also broadened its host spectrum. The changes in the environmental conditions are also playing a pivotal role in making the pathogen more aggressive (Tingquan, 2019). The increasing temperature accompanied by the humidity provides a favourable environment for pathogenic growth and infection development. In China, the pathogen is famous for causing cucumber blight in coastal areas with high humidity and increased temperature, e.g., Guangzhou. The pathogen has been reported for 80% yield losses in severely infected fields and has been declared as a food security threat in the local agriculture system (Cline et al., 2008; Tingquan, 2019). The pathogen possesses multiple pathogenicity tools to infect crop plants, and transglutaminase is the most powerful pathogenicity factor among them. On the other side, plant cells have a pathogen detection system consisting of various specialized receptors. Plant receptors have the tendency to detect effector proteins and other biochemicals of pathogen origin. Early detection of the pathogen infestation alerts the plant defense system and helps plants to avoid infection. We hypothesized that the digested fragments of Phytophthora (transglutaminase) specialised pathogenicity tool might efficiently activate the plant defenses. Previously, our lab team explored the pathogenicity proteins of *P. melonis* to better understand its pathogenicity tools and functions. The research work explored interesting facts about transglutaminase protein (H3GZF4), which was the main reason for pathogenicity. Although some transglutaminases have been reported for causing infections (Lee et al., 2014; Ahmad et al., 2020b), but there is no previous use of digested enzyme fragments for the improved physiological profiles of the plants. The current study gives a unique approach to exploit a pathogenicity protein for induced resistance, augmented physiological profiles, and antioxidative defenses. Furthermore, the study precisely describes the functional and structural characters of H3GZF4 in detail. In the present study, the functional characterization and structural analyses of H3GZF4 were executed to better understand its kinetics at the supramolecular level. The study will be a milestone in twigging the molecular interactions of a host and an oomycete pathogen.

METHODS

P. melonis strain and culture conditions

Representative pathogenic isolates of *P. melonis* were obtained by the Key Laboratory for the Research of Vegetables, Guangzhou, China. Three isolates were highly pathogenic, while three isolates exhibited mild pathogenicity. All the fungal isolates were maintained on V8 medium incubated at 28 °C. However, Henninger synthetic medium was used to grow the isolates for protein extraction. The composition of the medium is available in the Supplementary Data Set 1. The growth conditions provided to the isolates were 20 °C temperature at continuous agitation of 120 rpm while adjusting the volume of the media one litre for each isolate (Ahmad et al., 2020a).

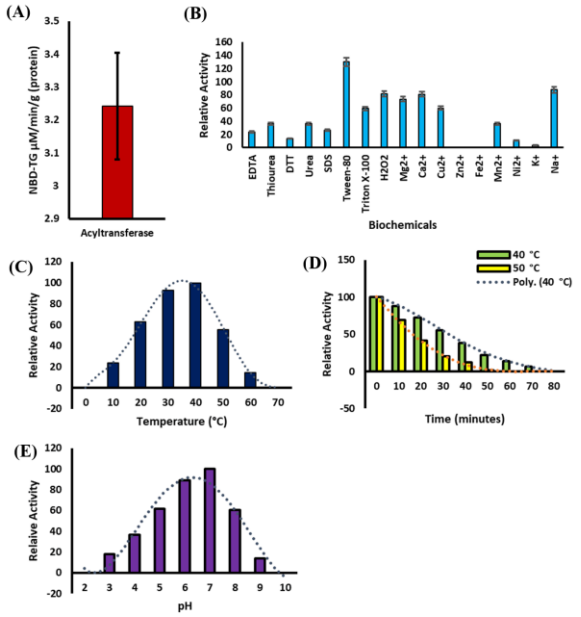

Figure 1: Determination of transglutaminase activity (A). Effect of the biochemical environments on the activity of the enzyme. The effect has been shown in terms of percentage increase or decrease compared to 100 units activity of control treatment (B). Measurement of the enzyme activity under a temperature gradient (C). Enzyme activity loss under high-temperature gradient incubation (D). Enzyme activity under gradient (E). All the enzyme activities were determined under the units NBD-TGµM.min^{-1}.g^{-1}ofprotein. Data presented here is in the form of mean±SE calculated from three independent biological replicates.

Protein extraction and Bioactivity Guided Pathogenicity Assay

After eight days of growth on Henninger medium, the fungal isolated were separated, and the liquid medium was filtered through Whatman filter paper (0.2 µm pore size). To keep the proteins and their activities intact, the filtrates were lyophilized. Pathogenicity assays were conducted for the filtrate of each isolate separately, and the protein profiles were analyzed to mark the proteins responsible for the pathogenicity of *P. melonis*. Pathogenicity-based bioactivity-guided protein extraction was carried out following the method of (Khan et al., 2019). It was accomplished through evaluation of the unidentical expressions of proteins in all profiles and correlating these with the virulence of the pathogenic strains. For protein profile analysis, the protein species were extracted in the phosphate-buffered saline containing NaCl (140 mM), NaH_2PO_4 (1.8 mM), Na_2HPO_4 (10 mM), and KCl (2.5 mM). Protein profiles were acquired through running a protein sample on 2D native polyacrylamide gel electrophoresis by employing the similar technique as it was used during (Ahmad et al., 2013). The identification of the proteins was performed using liquid-chromatography-mass-spectrometry (LCMS). The conditions of LCMS were used identical to

Bashir et al. (Bashir et al., 2016). All the protein profiles of highly pathogenic isolated were averaged and compared with the average protein profile of the low-pathogenic isolates. Matrix plots were constructed showing the profusion behavior of each protein concerning their pathogenicity. The formula of calculating the profusion index was

$$\text{Profusion Index} = \frac{\Sigma\text{Ind}}{\text{Frequency of occurrence}}$$

While Σind was the number of times a particular protein was professed

The most pathogenic protein species were selected from the matrix plot and were searched on the online database Uniprot for functional annotations. The structural analysis of the protein was carried out on the scripting database Pymol. However, the structural and physical characteristics were determined using solution-state nuclear magnetic resonance spectroscopy. The experimental data of NMR was processed on the PSVS software package to verify the functional annotations and structural characteristics.

Protein sample preparation

The protein samples were prepared for lab assays to determine the transglutaminase activity of the protein at different conditions. The resin affinity chromatographic technique (Ni-NTA) was adopted with five beds of resin. A step-wise elution of the sample from different resin beds containing an increasing concentration of imidazole elution buffer (40, 100, and 250 mM). However, the elution rate of the sample was kept one milliliter per minute. The fraction containing purified protein was undergone dialyzation using PBST with a decremental urea concentration (6-0 M).

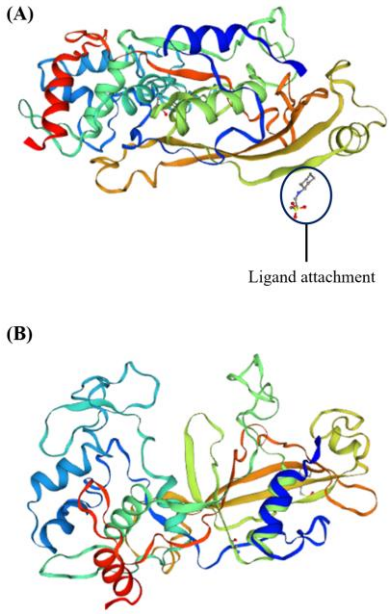

Figure 2: The structural analysis of H3GZF4 transglutaminase (A), and the two monomers merged in the rotating structure (B).

Determination of transglutaminase activity and biochemical properties

Transglutaminase activity was determined using (McFie and Stone, 2011) at different pH (3.0-9.0) and temperature (0-60 °C) treatments. The details of the method have been provided in Supplementary Data Set 1. The biochemical properties were determined under the environment of various biochemicals. The details have been provided in Supplementary Data Set 1. After exposing the enzyme to different chemical treatments, the transglutaminase activity was measured to understand the enzymatic behavior.

Tryptic digestion

TEAB- triethylammonium hydrogen carbonate method was used to undergo protein samples for tryptic digestion. Purified protein fractions were dissolved in 0.1 M TEAB buffer (100 µL). LACβ - bovine β-Lactoglobulin was used as a reference protein, and SDS – sodium dodecyl sulfate was used as a reducing agent. Two microliters of TCEP tris (2-carboxyethyl) phosphine with a concentration of 50 mM was added to the reaction system prior to one-hour incubation at 60 °C temperature. At the next stage, a 30 minutes dark incubation was provided to the reaction system after adding 400 mM iodoacetamide (1 µL), which resulted in the alkylation of the free groups related to the cysteine residues at room temperature. Then, at the last step, trypsin (protein/trypsin ratio 50:1 w/w) was used to digest the protein in the TEAB buffer (0.1 M) at a pH pf 8.0, and temperature 37 °C.

iTRAQ Labeling of the peptides and LCMS/MS analysis

The reagents of the iTRAQ Multiplex Kit (AB Sciex, Foster, CA, USA) were used to tag the peptides with isobaric labels, for which the detailed method has been adapted from Wu et al. (Wu et al., 2020). The peptides collected by OFFGEL fractionation were lyophilized for storage prior to their processing for downstream analyses. Samples were dissolved in eight microliters of CH_3CN (5%) and injected into LCMS/MS consisting of Waters NanoAcquity UPLC system (Milford, USA) and mass spectral system from Thermo Electron LTQ - Orbitrap Velos Pro (San Jose, USA). The rest of the conditions used in the method were identical to (Ahmad et al., 2020b). A freeware MZMine version 2.5 (Pluskal, Okinawa, Japan) was used to process mass spectral data through which the peaks were detected and identified using standard mass spectral databases. All the experiments were conducted in three biological replicates, and the values exhibited in the graphs are the mean values.

Plant Growth and Treatment

Tomato plants were grown by strictly following the method of Akram et al. (Akram et al., 2014). Briefly, tomato seeds were sown in 11 inches plastic pots filled with sandy loam soil and occasionally watered by observing the soil dryness up to 1 cm of its depth. A treatment of enzymes fragments (after tryptic digestion) was prepared by maintaining the protein concentration of 1 ppm in the double-distilled sterilized water. Twenty days old plants were sprayed with the treatment at the rate of 2 mL solution per plant and incubated in a greenhouse at the temperature of 25± 2 ℃. After two months, the data were recorded about the agronomic traits, physiological parameters, and antioxidative machinery by following the standard methods of Li et al. (Li et al., 2021).

Data Analysis

Statistical analyses were performed with the help of Add-On MS-Excel DSAASTAT (Onofri, Italy). Whereas, the analysis of variance was observed at P>0.5, and significance was evaluated by Dunkan's Multiple Range Test (DMRT).

RESULTS

Transglutaminase activity

Transglutaminase activity under standard conditions was recorded 3.81 NBD-TG $\mu M.min^{-1}.g^{-1}$ of the protein mass. The transglutaminase activity was used as a standard of 100 units to compare the performance of the enzyme under various biochemical environments. The enzyme activity was increased 130.1% under the application of Tween-80, and it was the only chemical that could enhance the transglutaminase activity. Under the environment of sodium ions, the activity was reduced up to 92.3%, whereas the hydrogen peroxide also showed a 26.74% reduction in the enzyme activity. The behavior of the enzyme under calcium ions was similar to the hydrogen peroxide and recorded a 26.93% reduction in the enzyme activity. A complete inhibition in the transglutaminase activity was observed under the application of zinc and iron. The activity trend was demonstrated the maximum value at the temperature of 37 °C. However, the experimental value recorded the maximum activity at 40 °C. At the temperature of 64 °C, complete inhibition of the activity was demonstrated. The enzyme showed activity loss at increasing temperatures, and the decline in the activity was more abrupt when it was incubated at gradually elevated temperatures. The thirty minutes of incubation at sixty degrees Celcius temperature completely blocked the enzyme activity. Enzyme pH analysis concluded that the transglutaminase worked better at a slightly acidic pH of 6.4. However, the best experimental value was recorded at pH 7.0 (Figure 1).

The structural analysis of the protein H3GZF4 revealed two distinct globular monomers in it. Both monomers were labeled as monomer A and monomer B. The globular structures of the two monomers were joined by the residues 432, 538, 559, and 593, which were valine, isoleucine threonine, and phenylalanine, respectively. Both monomers originated from the standard templates of 4dt5.1.A and 3tw5.1.A. and had a total of four ligand attachment sites. However, only one ligand, 3-Cyclohexyl-1-Propylsulfonic Acidic, could occupy all the attachment sites. Jointly described with the sequence analysis, monomer A had the range of 361 residues, starting from 260 and ending at 620. However, monomer B was slightly shorter in length ranging from 141 residues to 219 residues (Figure 2).

Table 1: Sequence analysis of H3GZG4 and recorded transglutaminase elicitor.

Domain	Start	End	Bits threshold		Bits Score		E-value	
			Sequence	Domain	Sequence	Domain	Sequence	Domain
TGaseelicitor	184	542	35.00	35.00	627.00	626.70	2.3e-185	3e-185

molecules were found already reported on the Uniprot database under 3TW5 as protein database ID and Q01928_PHYSO as entry number for Uniprot (Table 1).

The residual sequence analysis grouped the enzyme sequence into functional areas and uncovered different regions of similar physical characteristics. There were four aliphatic regions in monomer A and 28 aliphatic regions in monomer B. Monomer A showed three aromatic regions, whereas the similar regions were fourteen in monomer B. monomer B was responsible for the acidic affinity of the enzyme due to slightly positive charge on it. However, the monomer one was completely balanced with reference to charge distribution. Overall, the hydrophobic areas were in

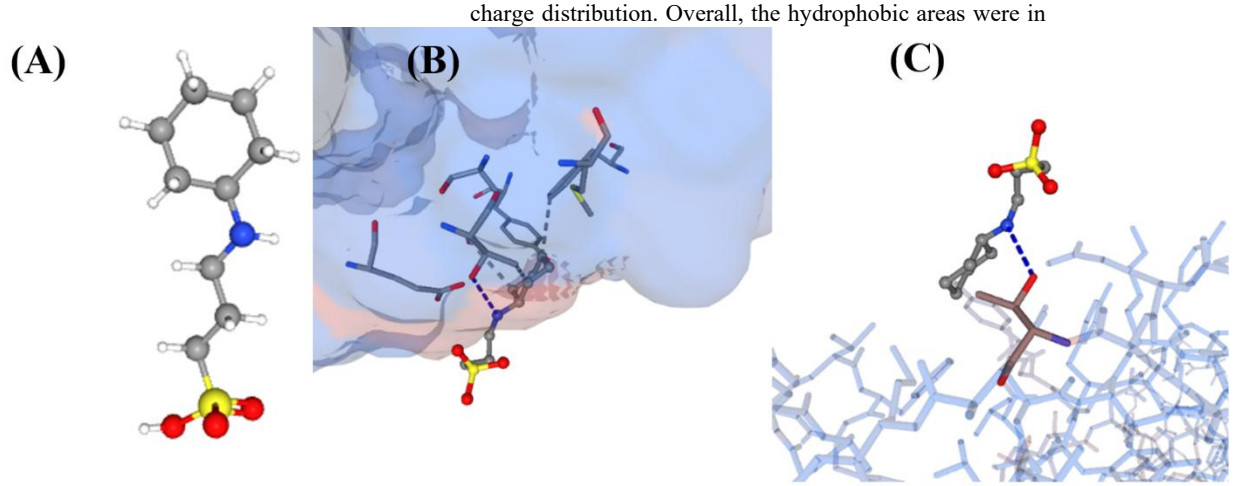

Figure 4: Three-dimensional molecular structure of the ligand molecule (A), ligand molecules interacting with enzyme residues in licorice mode on the surface structure of H3GZF4 (B), hydrogen bonds present in monomer B in licorice mode (C).

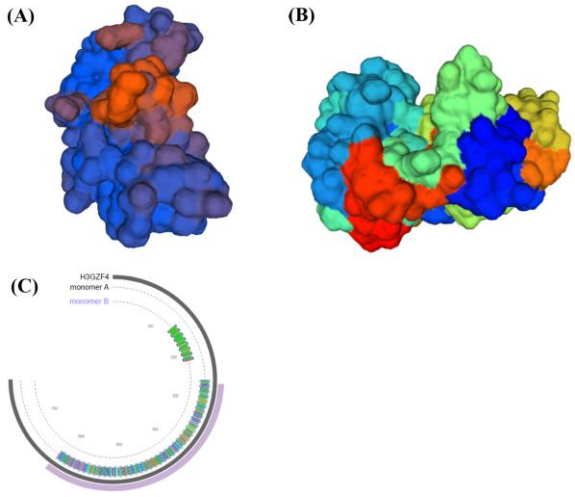

Figure 3: Three-dimensional surface structure of the two monomers of protein H3GZF4 (A, B), and the annotated residues of H3GZF4 protein.

The sequence analysis of the enzyme detected two elicitor units of TGase. The identity of both elicitors was analyzed PF16683and, IPR032048 from their recorded sequences of 359 bases ranging from 266 to 624 residues. Both elicitor the ratio of monomer A. Whereas, monomer B exhibited an increased ratio of hydrophobic areas in comparison to monomer A. Due to the extended sequence, monomer B had a greater polar region in the sequence. However, the residual sequence to polar region ratio was greater in monomer A. Similarly, proline domains were seven in number on monomer B. But, considering the sequence length, proline domain to sequence ratio was higher in monomer A (Figure 3).

Ligand interactions

A total of six ligand molecules of one ligand species CXS were found attached with H3GZF4. Both of the six ligand molecules were attached with monomer B of the enzyme. Two tyrosine residues were engaged in the ligand interactions placed at 272 and 323 in the sequence. One CXS molecule was observed in the interaction with glutamic acid present at 242 in the sequence. One threonine amino acid engaged one ligand molecule of CXS at 244. The remaining two ligand molecules were found attached with methionine at residue 246 and phenylalanine at 255. There were three hydrogen bonds found in the transglutaminase, and all of the three bonds had a distance > 4 Å. The location of the hydrogen bonds was also recorded in monomer B (Figure 4).

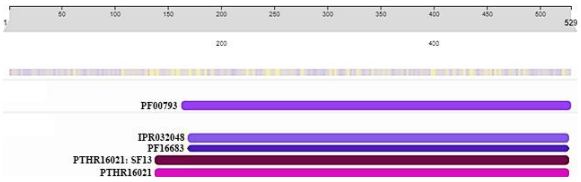

Figure 5: Intra-protein domain mapping of H3GZF4. Mapping was carried out at 100% homology and 100% confidence level compared to the data available at Ebi database UK. Functional analysis of protein sequences was performed to classify them into families and to determine the presence of domains.

Functional group classification of the enzyme revealed a total of five groups in the protein sequence. Two of the groups were categorized in the Panthor family at ID number 16021. However, one of them had an active tag of SF13. There was one transglutaminase elicitor found in the protein, which had a database ID PF16683. One antifreeze protein domain was also located in the enzyme with the database ID IP03248. However, the transferase activity regulating promoter binding region PF00793 was also labeled in the protein H3GZF4 (Figure 5)

The results showed a 35.7% increase in shoot length and 42.3% increase in the root length of the plants treated with transglutaminase treatment. Similarly, a 35.89% increase in the shoot fresh mass was recorded along with the 28.9% increase in leaf area. An identical trend was evident in the case of root fresh mass, shoot dry mass, and root dry mass, where an increase of 26.4%, 45.1%, and 43.6% was recorded, respectively. The analysis showed a significant decrease (68.4%) in the production of H_2O_2. Besides, leaf water potential, transpiration rate and maximum quantum yield of photosystem II were enhanced with a rate of 53.6%, 8.75%, and 46.2%, respectively. SPAD chlorophyll showed an increase of 6.43% with reduced electrolyte leakage of 17.3%. Furthermore, the enzyme treatment almost halved the concentration of malondialdehyde, and increased net photosynthesis rate (27.9%). An increase of 53.6% and a decrease of 46.7% was observed in the case of stomatal conductance and internal carbon dioxide concentration, respectively (Figure 6).

The enzyme treatment showed a positive impact on the other enzyme activities involved in antioxidantive machinery. It elevated the activity of nitrate reductas up to 35.1% along with the increased activities of catalase (63.9%) and superoxide dismutase (48.3%). Similarly, carbonic anhydrase showed a 45.5% increase in its activity, just comparable to the increased activity of peroxide (50.6%). Other biochemical antioxidants also recorded significant increases in their concentrations. An increased concentration of 54.7% was recorded in the case of proline. However, lycopene, β -carotene, and lutein concentrations were increased at the rate of 48.3%, 57.4% and 13.8%, repectively (Figure 7).

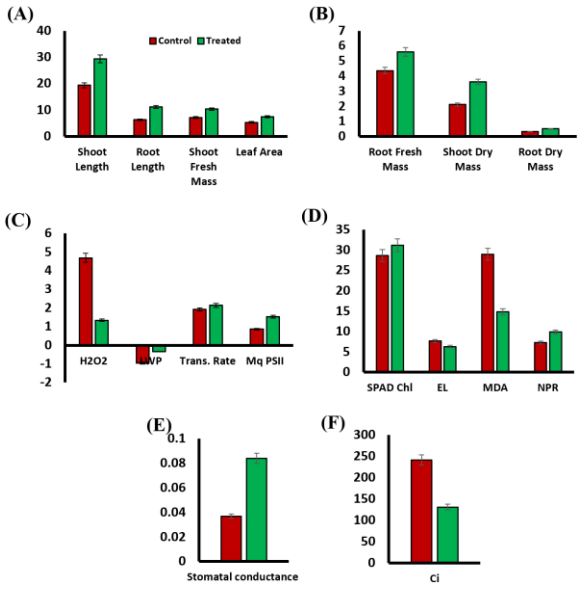

Figure 6: Impact of transglutaminase treatment on agronomic traits of the crop (A, B). The impact of the enzyme treatment on the photosynthetic and metabolic profile of the plant (C-F). Hydrogen peroxide (H_2O_2), leaf water potential (LWP), transpiration rate (Trans. Rate), maximum quantum yield of photosystem II (Mq PSII), SPAD chlorophyll (SPAD Chl), electrolyte leakage (EL), malondialdehyde (MDA), net photosynthesis rate (NPR), and internal carbon dioxide concentration (Ci).

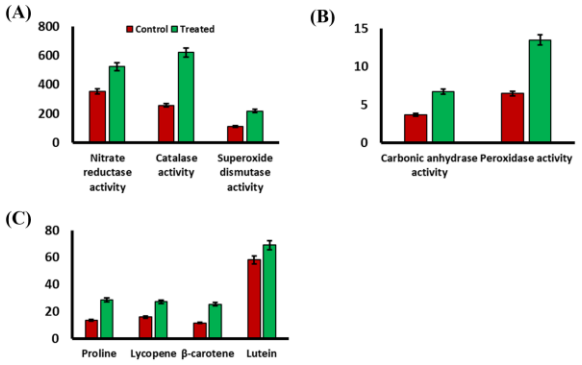

Figure 7: The impact of an transglutaminase treatment on antioxidative enzymatic profile (A, B); and on the biochemical antioxidants (C).

Transglutaminase enzyme H3GZF4 was involved in twenty cellular responses, among which most of the processes are related to pathogenicity or modulating host proteins.Overall, the main function of the enzyme was to regulate transferase activity on different biomolecules, including alanine, agaritine, glutamine, etc. The global distribution analysis of the enzyme showed around 23% of its presence in the

bacterium and 26% among oomycetes. However, other taxa of the organisms also had their representation in the biome distribution of the enzyme, which showed the importance of the enzyme in a living cell. From the true fungi, Ascomycota exhibited around 10 percent share on the enzyme distribution chart. Another important finding was that the bacterial species more commonly had the enzyme transglutaminase than any other kingdom (Figure 8).

DISCUSSION

Researchers have always been conceptualized induced systemic resistance (ISR) as an effective tool against plant diseases (biotic and abiotic). Multiple research efforts have been carried out to find the most promising agent for ISR in agricultural crop plants, and several agents have also been reported. However, most of the reported agents push the plant physiology into a stressed condition, which ultimately impacts the plant yield, and retard the quality of the plant produce (Yousaf et al., 2015; Tariq et al., 2021; Shahzadi et al., 2022). There is a dearth of defense inducers that positively impact crop agronomic and physiological traits, and digested transglutaminase is one of those precious inducers. Plant cells have a specialized mechanism to detect pathogenic invasion by identifying the pathogenicity factors and effectors proteins (Javaid et al., 2014; Ibrahim et al., 2016). Microbes produce these pathogenicity factors to cause diseases to the crops. In the current investigation, we did not apply an intact form of transglutaminase to the plants. Instead, we used tryptically digested fragments of the enzymes to avoid the chance of disease occurrence. Our main target was to provide plants with inert but recognizable parts of the pathogen to modify their physiological processes accordingly. The current technique is new in the field of ISR and can also be opted for other enzymes to induce resistance in agricultural crops.

The role of ligands can never be over-emphasized while study the mechanism of enzymes. Several plant enzymes can not perform their functions properly in the absence of ligands. Furthermore, several enzymes lose their activity or their efficiency if constructed with the deleted/removed ligand molecules (Dai et al., 1992; Payehghadr et al., 2012). Therefore, the presence of ligand plays a pivotal role in the supramolecular kinetics of the enzyme functions (Ahmad et al., 2020c; Hafeez et al., 2020; Li et al., 2021). Furthermore, the transglutaminase is a newly reported pathogenicity protein for which the role of ligands has not been elaborated. On the other side, protein has a high impact on human food security as being the pathogenicity tool of economically important pathogens (Han et al., 2019; Ali et al., 2020; Usman et al., 2021). The current investigation is the first time reporting the type of ligands required to function an transglutaminase, number, and attachment site in the protein. The study will be of assistance to understand the molecular interactions of *Phytophthora* with host defenses.

Previously, it was considered that the Tgase elicitors are only the enzyme of oomycetes, in which they perform the transferase activity of acyl groups. However, the current study has brought a detailed structural and functional analysis of the enzyme. According to the findings of the current investigation, the functional homolog of the protein can be found in bacteria. A few studies have also been previously conducted supporting the notion based upon the whole genome sequences (Reiss et al., 2011). The current study reveals that Tgase present in bacteria is more than one-fourth of the total TGase in the biome.

Both of the monomers are jointed at four residual points in order to remove the chance of constructing a chimera protein. However, based upon the functional annotations, both of them were different in properties and cell responses.

The transglutaminase activity of H3GZF4 is the fastest and the most stable activity ever reported in the literature (Shi et al., 2014). Previously, most of the studies did not measure enzyme activity due to the complex and tricky protein purification procedure. Therefore, the current study is dually novel concerning enzyme isolation and its activity (Akram et al., 2013; Ibrahim et al., 2017; Yasin et al., 2018a).

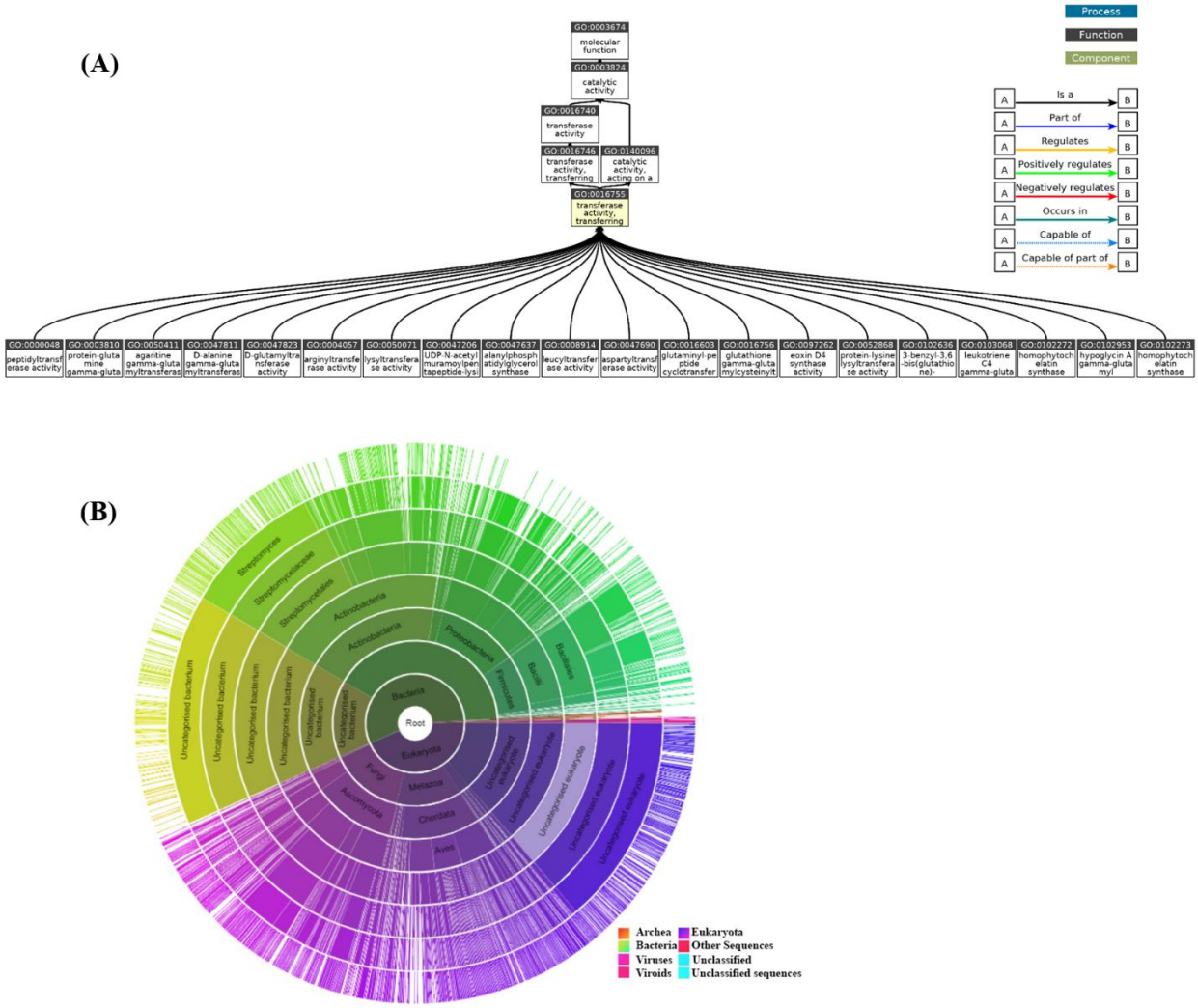

Figure 8: The cell function analysis for the protein H3GZF4 (A), and biome distribution chart of the enzyme (B).

Many studies have categorized transglutaminases in multiple classes. Most of the studies have described a total of eight classes of transglutaminases, including mu, theta, pi, sigma, alpha, zeta, omega, and kappa (Konishi et al., 2005; Zaheer et al., 2017). There is also a lot of diversity in terms of their hydrophobicity, polarity, and electronegativity of the members of transglutaminases; however, this study is the first time distinguishing the two monomers in the protein.

Isolating the transglutaminase enzyme in its intact functional form was the main achievement that can be replicated to study the interaction of the enzyme with different substrates. Furthermore, it can also be helpful to study enzyme behavior in different environments. For example, the study classified monomer A as an antifreeze domain, meaning that the domain will play its role under the chilling temperatures. It also provides a scientific base of the fact that the pathogen

gets established under freezing temperatures (Thompson et al., 2014; Wang et al., 2018).

An enzyme performs its best at optimum conditions (Lwalaba et al., 2021; Mapodzeke et al., 2021). If the same phenomenon is considered *P. melonis*, the oomycete will be the most virulent at the optimum conditions of the transglutaminase enzyme. Therefore, the determination of the optimum conditions of the enzyme is one of the important findings of the study. The research would recommend avoiding high-temperature conditions ≥30 °C, and a low pH environment in this aspect. The optimized activity of the enzyme at acidic pH argues for the enhanced efficiency of the enzyme under adverse conditions. It is a common notion that microbes produce organic acids during their active growth and that an acidic environment becomes the cause of their death (Ahmad et al., 2018; Khan et al., 2018; Siddiqui et al., 2021). However, transglutaminase studied in this research is more active under acidic conditions, which would greatly help the pathogen for combating against adverse conditions and find new hosts. Moreover, Tween solutions also cause an accelerated activity of the enzyme, which is also supported by the previous literature (Liu et al., 2011; Yasin et al., 2018b; Tariq et al., 2021). This study recommends avoiding the use of Tween solutions during the preparation of pathogen control formulations.

Transglutaminases are the primary construction unit of cell membranes. Due to their transverse incorporation in the cell membranes, it is very hard to isolate them without using membrane rupturing elements that also inactivates the enzyme by protein denaturation. Most of the previous studies have been conducted about the transglutaminase activity by using an unpurified mess of proteins containing trace amounts of the enzyme (Ogawa et al., 2018; Shafique et al., 2022). Therefore, a deep functional and structural analysis of the enzyme couldn't be achieved (Garner and Janda, 2010). The current study also describes the method to purify transglutaminase from *P. melonis* in its intact form. Additionally, it also characterized the enzyme in detail with respect to structural kinetics and functional annotations. Moreover, the enzyme is also involved in the biosynthesis of glycophospholipids where it serves as a precursor molecule. Considering this aspect, transglutaminase is also important for the growth and development of the organism.

CONCLUSION

The present investigation reports a unique measure to induce resistance in tomato plants against stressors. Furthermore, it provides a deep characterization of an transglutaminase H3GZF4 from an oomycete *P. melonis*. The optimum conditions for the enzyme activity are high temperature (≥30 °C), and slightly acidic pH. The enzyme also contains an antifreeze monomer and a TGase monomer. The TGase activity needs six ligand molecules of CXS. However, the antifreeze activity needs no ligand at all. More than half of the enzyme's global prevalence is shared by bacteria and oomycetes.

DECLARATIONS

AI Usage Declaration

In line with COPE guidelines, AI-assisted tools were used only for language editing and formatting and did not contribute to scientific content, data, analysis, or conclusions. All responsibility for the manuscript rests with the authors.

Authors' Contribution

All authors equally and actively contributed in the research work and write-up of this article.

Conflict of Interest

There is no conflict of interest among authors concerning this manuscript submission.

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