Computational proteomics analysis reveals pathogen secreted carbohydrate active enzymes in tomato xylem sap

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Abstract

Fusarium oxysporum f. sp. *lycopersici* (Fol), a causal organism of Fusarium wilt in the tomato plant, secretes cell wall degrading enzymes, also known as carbohydrate-active enzymes (CAZymes). These are crucial during colonization and pathogenesis, as evidenced by several proteomic studies, revealing the importance of these CAZymes in virulence and pathogenicity. However, few of them have been done *in-planta*, exhibiting differences in the expression of these cell wall degrading enzymes compared to *in-vitro* studies. Therefore, to explore the CAZymes involved in pathogenesis while residing in the host plant, an *in-planta* (xylem sap) proteomics of a susceptible tomato variety affected with Fol was done. Most of these CAZymes belonged to the hydrolase and oxidoreductase families having no significant homology with tomato proteins. Nearly 90% of them were predicted to be soluble and extracellular. The core CAZymes families with interactional evidence identified were AA3, GH3, GH18, GH20, GH28, GH43, GH47, GH55 and CE8. Thus, apart from annotating some of the hypothetical proteins to be CAZymes, the study sheds light on CAZymes families that may have a role in the pathogenesis and survival of this fungus in the susceptible tomato plant.

Introduction

Fusarium oxysporum species complex (FOSC) causing wilt and rot disease in various plant species is generally host-specific and known as *formae speciales*, ff. spp. (singular *formae specialis*, f. sp.). These plant pathogenic *formae speciales* follow a similar infection mechanism in the host plant, which includes penetration through root and colonization in the xylary tract (Lagopodi et al. 2002; Jiménez-Fernández et al. 2013; Lu et al. 2013; Upasani et al. 2016; Dong et al. 2019), resulting in wilting in some cases, root rot of susceptible plants leading to necrosis. One such *formae specialis* is f. sp. *lycopersici*, affecting the important vegetable crop tomato grown worldwide (<http://www.fao.org/faostat/en/#data/QC/visualize>).

To penetrate the root for successful colonization, f. sp. *lycopersici* requires crossing the cell wall barrier of the host plant. These cell wall barriers are composed of cellulose, hemicellulose, pectin, lignin, and other proteins, essentially present for structural integrity (Rose et al. 2004; Cosgrove 2005). The process of cell wall deconstruction by the pathogen is achieved by the enzymes known as plant cell wall degrading enzymes (PCWDE), which within the host plants help degrade the plant tissues for liberating mono- and oligosaccharides required for their growth (Kubicek et al. 2014). Some of these enzymes are also needed for cell wall remodeling (Beauvais and Latgé 2018) and at the later stage of colonization (Gibson et al. 2011). These CWDEs are a subset of enzyme families known as Carbohydrate active enzymes (CAZymes), which according to the CAZy database (<http://www.cazy.org/>) (Lombard et al. 2014), are divided into five modules, i.e., Auxiliary activities (AA), carbohydrate Esterase (CE), Glycosyl Hydrolase (GH), Glycosyl Transferase (GT), Polysaccharide Lyase (PL) and one associated module, Carbohydrate-Binding Modules (CBM), consisting of several families based on functional, structural and sequence similarities. The families in these modules are further divided into four broad categories based on their activities, such as cellulose-degrading (mostly GH family), hemicellulose degrading (GH and CE), pectin (GH, CE, and PL) and lignin-degrading (mostly AA). In contrast, members of the GT family are involved in the biosynthetic process. The FOSC and other sordariomycetes harbor a plethora of CAZymes in their genome (<https://mycocosm.jgi.doe.gov/mycocosm/annotations/browser/cazy/summary;85ATbn?p=dothideomycetes>) with a majority of them belonging to GH families followed by GT, AA, CE, PL and being necrotrophic, codes for a significant amount of PCWDE as well as secretory CAZymes in *Fusarium oxysporum formae speciales* (Roy et al. 2020).

Over the last decade, fungal proteomics have taken the lead in the field of omics due to the availability of full-length fungal genomes, high throughput transcriptomic data and advancements in protein mass spectrometry, which gave us a valuable insight into the pathogenicity of the plant fungal pathogens. Although proteomics analysis of host plants in response to *Fusarium oxysporum formae speciales*, such as banana (Li et al. 2013; Lu et al. 2013; Dong et al. 2019), cabbage (Pu et al. 2016), cucumber (Zhang et al. 2016), pea (Castillejo et al. 2015), tomato (Gawehns et al. 2015; Silva et al. 2017; de Lamo et al. 2018) and strawberry (Fang et al. 2013) have been done, but several proteomics studies were also performed in *Fusarium* species, including the *Fusarium oxysporum* ff. spp. For instance, an exoproteome study on *Fusarium graminearum* grown on the cell wall of *Humulus lupulus* L. (Phalip et al. 2005) revealed Cellobiohydrolase (belonging to GH6 and GH48) to be pre-dominant in pathogen-plant interaction. Similarly, in a study (Paper et al. 2007) on the same fungal species, *in-planta* and *in-vitro* analyses evidenced the presence of hydrolase, lyase, and esterase in the former, suggesting their role in pathogenesis. Likewise, another study on *Fusarium proliferatum* also showed inhibition of CWDEs consisting of glucanase, cellulase, and glucanosyltransferase (Li et al. 2017) to be influenced by high pH, therefore affecting its growth.

Among the *F. oxysporum* ff. spp., an *in vitro* comparative proteomics studies on the races of f. sp. *cubense* and f. sp. *conglutinans* enabled the identification of proteins involved in carbohydrate metabolism, in which chitinase and α -galactosidase were revealed to be differentiating the virulence pattern in former (Sun et al. 2014), whereas the glycosidase protein was observed to differ among the races of the latter (Li et al. 2015). Moreover, some CWDE in f. sp. *cubense*, such as glucanase and cellobiose dehydrogenase, were found to be downregulated by deleting a transcription factor resulting in the reduction of the pathogenicity (Hou et al. 2018). Simultaneously, some cell wall modifying enzymes were also expressed during conidial germination (Deng et al. 2015), depicting their role in growth. Likewise, another comparative proteomics analysis of different isolates of f. sp. *lycopersici* revealed the importance of endo- β -N-acetylglucosaminidases (GH85) in imparting the virulence (Manikandan et al. 2018). Although most proteomics work is done so far to identify and decipher the role of cell wall degrading enzymes has been through *in-vitro* studies. Still, very few of them were reported in-planta using *Fusarium* species. Concerning the *formae speciales*, any study reported so far was only on f. sp. *lycopersici* assessing the pathogenicity of chromosomes (Schmidt et al. 2013). Thus, to narrow down this gap with the process of annotating and implicating the role of CAZymes in pathogenesis, a downstream proteomics analysis was done using publically available proteomics data of tomato xylem sap infected with *Fusarium oxysporum* f. sp. *lycopersici*. Further, using computational tools to substantiate the importance of these cell wall degrading enzymes which would otherwise may not be expressed at the protein level under *in-vitro* conditions in this pathogenic fungus.

Material And Methods

Data retrieval

The LTQ Orbitrap label-free mass spectrometric publically available data of tomato xylem sap with accession: PXD011072 (<https://www.ebi.ac.uk/pride/archive/projects/PXD011072>) was downloaded from PRIDE (<https://www.ebi.ac.uk/pride/>) which constituted the experiments performed elsewhere (Gawehns et al. 2015; de Lamo et al. 2018). This study includes a differential proteomics analysis of a resistant and susceptible tomato variety infected with *Foxysporum* f. sp. *lycopersici* (Fol). Samples consisting of a susceptible host infected with Fol and a mock treated of the same tomato cultivar were taken for further analysis, based on the hypothesis proposed in the current study. A total of eight samples, i.e., four replicates each (FR24 to FR27) considered as treated (T) and (FR12 to FR15) considered as mock treated (M) in PXD011072 were taken. These experiments were done in a climate controlled greenhouse at 25 ° C with a relative humidity of 65% and 16 hours photoperiod. The pathogen used was Fol race 1 (fol007) and the susceptible tomato plant cultivar C32. Protein sequences of the organisms Tomato (*Solanum lycopersicum* assembly SL3.0) consisting of 37660 proteins and *Fusarium oxysporum* (*Fusarium oxysporum* f. sp. *lycopersici* 4287 assembly ASM14995v2) consisting of 27347 proteins were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/genome/>) database.

Data processing

All the raw files of a susceptible plant treated with Fol and mock treated were processed in the MaxQuant version 1.6.17.0 (Tyanova et al. 2016a) for protein identification using an inbuilt search engine against the protein database created by taking Fol and tomato proteins mentioned above with an in-built contaminant database (246 proteins). Label-free quantification was done using default parameters (Cox et al. 2011, 2014) with a match between runs enabled and label-free quantification (LFQ) minimum ratio count set to 1. An extra variable modification- deamidation (NQ), performed by original authors (de Lamo et al. 2018) was also included for the protein quantification. The data generated were further filtered and analyzed on Perseus 1.6.14.0 (Tyanova et al. 2016b), where all the peptide counts less than 2 identifying a protein group were filtered out. The LFQ intensities were further $\text{Log}_2(x)$ transformed to visualize the data. The proteins identified in the tomato were removed for subsequent analysis retaining the proteins identified by at least one unique and one unmodified peptide for the Fol proteins.

Gene Ontology (GO) analysis

The Fol proteins identified in the xylem sap were subjected to gene ontologies (GO) annotation using Blast2GO 5.2.5 suite (Götz et al. 2008) with an in-built InterPro tool by keeping default settings to see the categorical representation of these proteins in individual studies. The proteins with GO annotations were further visualized using WEGO 2.0 (<http://wego.genomics.org.cn/>) (Ye et al. 2018) for the Fol proteins identified in the experiment. Additionally, GO term enrichment analysis was also performed to visualize the functional enrichment using web-based tool g: Profiler (<https://biit.cs.ut.ee/gprofiler/gost>) (Raudvere et al. 2019) after selecting the organism *Fusarium oxysporum* f. sp. *lycopersici* and with the default options for the enrichment process.

Carbohydrate-active enzymes (CAZymes) annotation

A web-based meta server as well as a stand-alone tool- dbCAN2 (<http://bcb.unl.edu/dbCAN2/index.php>) (Zhang et al. 2018) was used to annotate the CAZymes among the identified protein groups in Fol. This web-based meta server annotates CAZymes using three integrated search tools, HMMER (<http://hmmer.org/>), DIAMOND (<https://github.com/bbuchfink/diamond>) and HotPep (Busk et al. 2017) for the annotation of CAZymes with their default parameters from the search databases viz. dbCAN, CAZy and PPR databases, respectively. This meta server tool suggests retaining only those CAZymes, which are annotated with a minimum of 2 integrated tools for better prediction confidence.

Secretory protein and subcellular localization analysis

As the xylem sap collected in these datasets were devoid of any physical presence of fungus. Therefore, to ascertain if CAZymes annotated in the identified Fol proteins were secretory or extracellular in origin, two online tools, namely, SignalP 5.0 (<http://www.cbs.dtu.dk/services/SignalP/>) (Almagro Armenteros et al. 2019) and DeepLoc 1.0 (<http://www.cbs.dtu.dk/services/DeepLoc/>) (Almagro Armenteros et al. 2017), were used. These two tools work on deep- and recurrent- neural network approaches, respectively. The former predicted the signal peptides by keeping the organism group- Eukarya, and the latter identified the subcellular localization by keeping PROFILES as a protein-coding option for accurate prediction.

Metabolic pathway enrichment and ortholog interaction network analysis

The CAZymes identified were analyzed for their chromosomal location by taking the respective gene accessions of the identified CAZymes through the genome view option and metabolic pathways enrichment was also done with a p-value <0.05 using FungiDB (<https://fungidb.org/fungidb/app>) (Basenko et al. 2018). Further, an evidence-based protein interaction network was analyzed using STRING 11.0 (<https://string-db.org/>) (Szklarczyk et al. 2019) by taking clusters of orthologous groups (COG), Eukaryotic orthologous group (KOG) and non-supervised orthologous groups (NOG) for the corresponding CAZymes families with the settings: minimum required interaction score of 0.700 (high confidence) and a maximum number of five interactors in the first shell to see the existing interaction among these ortholog groups of the identified CAZymes.

Results

Identification of protein groups and statistical analysis

The proteins identified in the individual sample dataset after filtering (i.e., the removal of contaminants, reverse hits, and proteins identified only by site) revealed the number of proteins identified in tomato and Fol is summarized in Table 1. The $\text{log}_2(x)$ transformed LFQ intensities were plotted for each of the experiments (Fig.1) with an average mean of 22.6 and 20.0, median of 22.4 and 19.8 in all the four replicates of treated (T) and mock (M), respectively

(Supplementary Table 1). The histogram revealed the data to be more or less normally distributed. These replicates showed a Pearson correlation of more than 0.8 in both mock and treated samples (Fig. 2).

Functional assessment of Fol proteins

All the Fol proteins identified in the susceptible tomato plant have proceeded for GO annotation. A total of 36 out of 53 protein accessions were assigned for some GO terms. Among these GO terms, 32 GO terms were assigned for biological functions, whereas 36 GO terms were assigned to some molecular functions with 3 GO terms belonging to cellular functions. The histogram of GO categories revealed the majority of the GO categories were involved in primary and organic substance metabolic processes followed by the oxidation-reduction process with a function of hydrolase and oxidoreductase activity (Fig. 3a).

In gene enrichment analysis, the proteins in each experiment were assigned to their respective gene accessions. The enrichment analysis using g:SCS threshold, which is an algorithm developed by the authors of the tool utilized for multiple testing correction, revealed the enriched biological process to be carbohydrate metabolic process (GO: 0005975) and response to oxidative stress (GO: 0006979) with most significant molecular functions of hydrolase activity (GO: 0016798, GO: 0004553, GO:0016787), polygalacturonase activity (GO:0004650) catalase activity (GO:0004096), peroxidase activity (GO:0004601), oxidoreductase activity (GO:0016684), antioxidant activity (GO:0016209) and catalytic activity(GO:0003824) (Fig. 3b).

Annotated and core CAZymes in Fol proteins

After filtering the CAZymes annotated by ≥ 2 tools from dbCAN2 annotation results (Fig. 4; Supplementary Table 2), altogether, 30 protein accessions were annotated as CAZymes. Most of the CAZymes belonged to the families of GH, followed by AA and only one CE family, i.e., CE8 (Table 2). Among these protein accessions annotated for CAZymes, 16 of them were hypothetical proteins (HP). Further, the GO annotation from the already annotated protein groups in the previous step revealed 24 protein accessions coding for CAZymes families were having GO terms associated with them (Supplementary Table 3). Interestingly, most GO categories were identified to be involved in primary and organic substance metabolic processes having hydrolase and oxidoreductase activities.

Secreted protein and subcellular localization of identified CAZymes

To confirm the CAZymes identified above belonged to secretory proteins and extracellular in function, which apparently may influence the process of pathogenesis and colonization after infecting the host plant, they were subjected to secretory protein analysis. Interestingly, nearly 90% of the CAZymes were found to have standard signal peptides cleaved by signal peptidase I (Table 2, Supplementary Table 4 and 5) and extracellular in nature except for GH47 and GH72, though harboring signal peptide. Most of these extracellular CAZymes combined together were generally hemicellulose degrading (GH18, GH20, GH43, GH93, GH32), followed by cellulose/hemicellulose degradation (GH45, GH3), oxidoreductase (AA3, AA5) and pectin degrading (CE8, GH28).

Chromosomal location, metabolic pathway enrichment and interaction network of CAZymes

The CAZymes identified were found to be located on chromosome 1, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13 and 14 (Table 3), with half of them to be located on the reverse strand. In the metabolic enrichment process, the pathway in which these CAZymes involved were pectin degradation (CE8 and GH28), choline degradation (AA3_2) and chitin degradation (GH18, GH20, GH45, GH47) with a p-value from Fisher's exact test, Bonferroni adjusted p-value n and Benjamini Hochberg false discovery rate (FDR) cut-off <0.05 (Supplementary Table 6). The CAZymes families were further studied for an evidence-based interactional network to elucidate a relationship among these CAZymes present in xylem sap of the host plant infected with pathogen Fol. Hence, an interaction network of ortholog groups, COG, KOG and NOG corresponding to the gene accessions of each protein group was created, which resulted in 7 CAZymes families encompassing 12 protein accessions (Table 4) having direct and indirect interaction within them. The interaction network with a combined interaction score ranged from 0.7-0.9 (Supplementary Table 7) and revealed six of the CAZymes families, i.e., GH3, GH18, GH20, GH28, GH43 and CE8, having direct interaction evidence. In contrast, AA3 linked with GH18 through an enzyme Acyl-CoA reductase (COG1012), GH3 linked with GH18 through pectate lyase (COG3866), GH32 and GH55 were connected through a glucanase (GH17 family, COG5309) whereas, GH74 was interlinked with a mannosidase (KOG2204) and a transferase (KOG1413), revealed to be involved in the polysaccharide degrading process (Fig. 5).

Discussion

The plant pathogen *Fusarium oxysporum* f. sp. *lycopersici*, a causal organism of tomato wilt, like many other members of FOSC, follows the same mechanism of entry and colonization process in the xylem before the wilting of plants takes place. For the successful infection and colonization in the host plants, a complex and coordinated interplay of enzymes is required, including cell wall degrading enzymes, which probably act as key players in the pathogenesis and growth. A difference in the proteins was found among the data taken, i.e., presence of proteins in the treated compared to mock samples, especially the proteins belonging to hydrolase and oxidoreductase families and the activities of these enzymes had shed light on the mechanism of infection while inside the host plant as also reported in case of *Fusarium graminearum* (Paper et al. 2007). Further, the carbohydrate metabolism process was found significant with hydrolase and oxidoreductase activity, reflecting the importance of these CAZymes during the pathogenesis.

Perhaps, most of the CAZymes annotated in the Fol proteins were described as hypothetical proteins (HP); therefore, the functional assignment to these HP was also achieved in the present study. Interestingly, the CAZymes identified, such as AA3 (Schmidt et al. 2013), belonged to gene accessions that were present on conditionally dispensable chromosomes (CDC), also known as supernumerary chromosomes (SC) or lineage-specific (LS) chromosomes, though not on Fol CDC but GH3 and GH43 were reported to be present on legume infecting *formae speciales* CDC (Williams et al. 2016). These chromosomes are known to determine the pathogenicity in *Fusarium oxysporum formae speciales* (Ma et al. 2010; Vlaardingerbroek et al. 2016; Williams et al. 2016; van Dam et al. 2017). At the same time, the majority of CAZymes were predicted to be soluble and extracellular proteins involved in the degradation of polysaccharides, either of plant or fungal origin. However, two CAZymes families: GH47 (alpha-mannosidase) and GH72 (β -1,3-glucanosyl transglycosylase), were membrane proteins harboring signal peptides.

Among these non-secretory CAZymes, GH72 (β -1,3-glucanoglucosyl transglycosylase), a membrane protein, was revealed to help in fungal cell wall formation and attachment of glycoprotein into the cell wall (Ao and Free 2017; Kar et al. 2019). Likewise, another membrane protein, GH47 (α -mannosidase) was also reported to be involved in cell wall morphogenesis with a secretory CAZyme GH20 (β -hexosaminidase) (Wymelenberg et al. 2010). However, not being extracellular but the presence of these CAZymes in the xylem sap suggest them to be important in fungal growth and survival while inside the plant.

The secretory CAZymes reported to be involved in fungal cell wall degradation, GH55 (β -1,3-glucanase) seemed to have a role in cell wall morphogenesis and germination (Millet et al. 2019). Moreover, an important family and much explored CAZyme, GH18 (Chitinase), identified in the present study signified its role in morphogenesis, defence, or parasitism (Oyeleye and Normi 2018) because of its involvement in the breakdown of chitin, an essential component of the fungal cell wall for cell growth and plasticity (Langner et al. 2015). Further, the presence of these two CAZymes viz. GH55 and GH18 were also reported to be found in the secretome of *Aspergillus niger* (Nitsche et al. 2012). Meanwhile, the GH families, especially GH20 (β -hexosaminidase), was reported to be involved in the trans-glycosylation process (Muschiol et al. 2020) and helps in fungal hyphal growth and branching (Rast et al. 1991). Additionally, the role of this family had also been shown to assist the chitinase for the complete degradation of chitin (López-Mondéjar et al. 2009). Moreover, the family GH32 (invertase) involved in the hydrolysis of sucrose reported here, perhaps, indicated its significant role in an increased level of pathogenicity (Ruiz and Ruffner 2002; Voegelé et al. 2006). However, its actual role in plant-pathogen interaction remains unclear (Chang et al. 2017).

Further, the rest of the extracellular CAZymes, including AA3, an aryl-alcohol or glucose oxidases, was identified to play a role in biomass degradation as in the case of a necrotrophic fungus, *Botrytis cinerea* (Petrasch et al. 2019). Likewise, AA5 (a galactose, raffinose or alcohol oxidase) that oxidize oligogalacturonides simultaneously was reported to reduce the activation of plant immune response (Ferrari et al. 2013).

Pectinolytic enzymes are very crucial in the process of pathogenesis because of their role in plant cell wall degradation and the CAZyme, CE8 (pectin methylesterase), the only CE family identified with GH28 (polygalacturonase) was identified to be involved in pectin degradation by a de-esterification process, which in turn, allows the pectin accessible to other pectinases such as polygalacturonase (Pelloux et al. 2007), while GH28 was reported to be engaged in the breaking of α -linked galacturonic acid bonds (Markovič and Janeček 2001) in the pectin. Moreover, the presence of these two CAZymes families was also reported to be co-expressed in a basidiomycetes fungus (Miyachi et al. 2016) and identified in the secretome of an ascomycetes species (Couturier 2016). Other CWDEs, GH3 (β -glucosidase), reported having a role in chitinolytic activity (Yang et al. 2014) was observed to be involved in cellulose as well as hemicellulose degradation. Further, the coordinated effort of hemicellulose degrading CAZymes GH43 (β -xylosidase) and GH93 (α -arabinanase) was also reported in *Aspergillus fumigatus* (de Gouvêa et al. 2018).

To get an insight into the coordination of these CAZymes, an interaction network of the CAZymes based on the evidence from various sources resulted in the identification of six CAZymes, i.e., GH3, GH18, GH20, GH28, GH43 and CE8 to be directly involved in the interactions out of which excluding GH3, the other CAZymes were enriched for a metabolic pathway involved in pectin and chitin degradation. Although enriched for and belonging to different cell wall degrading categories, i.e., cellulose/hemicellulose, hemicellulose and pectin, their coordination might be playing a significant role in fungal growth and pathogenesis. Besides this, GH3 also possessing chitinolytic activity and interacting with GH18, may involve in the chitin degrading mechanism (Yang et al. 2014), suggesting their significant role in the process of morphogenesis of the fungus. Meanwhile, their interconnected network, along with the contribution of CE8, which also assist other polygalacturonases, is found to be interacting with a pectate lyase and a xylosidase, where both of these families in a broad sense considered as plant cell wall degrading enzymes. Therefore, the coordination of these CAZymes in the network (including GH43, hemicellulose degrading CAZymes) and their presence in the xylem sap implicates a role in fungal colonization, most probably by degrading complex sugars into simpler sugar forms for growth and morphogenesis. Another CAZymes, i.e., GH55, although not directly reported to be involved in the main network, but was observed to be linked with a GH17 family, perhaps contributing to the phenomenon of cell morphogenesis and conidiation (Millet et al. 2018, 2019), which is further connected to GH32 (invertase), an enzyme involves in breaking down of sucrose sugar abundantly found in plants.

Hence, within the intricate mechanism of cell wall degradation during the process of colonization by this fungus, some of the crucial CAZymes families, i.e., AA3, GH3, GH18, GH20, GH28, GH43, GH55 and CE8, were identified in the xylem sap of a susceptible host plant, which could be key players in the pathogenesis and also gave a glimpse on the mechanism of colonization among the interacting CAZymes through available evidence. Therefore, extracellular CAZymes identified in the xylem sap might have been involved in the growth, maintenance and colonization process of this pathogen.

Conclusion

Although several secretomes and transcriptomic studies in various pathogenic fungi were done, most of them were restricted to *in-vitro* set up only; hence an attempt was made to fulfil this lacuna. Therefore, to identify and annotate the cell wall degrading CAZymes at the protein level and revealing the role of these CAZymes in the process of successful pathogenesis through cell wall degradation and also in fungal survival, the present study of *in-planta* proteomics analysis of two independent data sets was done to elucidate cell wall degrading CAZymes families of fungal origin. Some of the families belonging to AA, CE and GH were identified and their potential role in fungal colonization and pathogenicity as understood was discussed. Hence, this study provides a future scope for detailed studies on these candidate CAZymes within the *formae speciales* and to depict their elaborate mechanisms during pathogenesis.

Declarations

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Conflicts of interest/Competing interests:

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material

All data generated or analysed during this study are included in this published article (and its supplementary information files)

Code availability NA

Authors' contributions

AR and PTV designed the study. AR BK and AJ performed the analysis. AR, BK, AA and PTV wrote the final manuscript. All the authors analyzed and approved the manuscript.

Ethics approval NA

Consent to participate NA

Consent for publication NA

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Tables

Table 1. Number of proteins identified in the xylem sap proteomics data of susceptible tomato plant

	M	T
Proteins after processing*	473	413
Proteins identified in tomato	473	360
Protein identified in Fol	00	53

(* removal of contaminant, reverse hits, identified by site and peptide count <2. These proteins were belonging to majority protein groups after processing in each experiment having four replicates were identified by the peptides present in more than two replicates)

Table 2. CAZymes annotation in the Fol proteins with their subcellular localization and presence/absence of secretory peptides.

Fol major protein groups accession	Gene id	Given Protein name	CAZymes	First activity in the family	Signal peptide
XP_018247179.1; XP_018247180.1	FOXG_09774	hypothetical protein	AA3_2	glucose oxidase	OTHER
XP_018253950.1	FOXG_14236	hypothetical protein	AA3_2	glucose oxidase	SP(Sec/SPI)
XP_018253982.1	FOXG_14258	hypothetical protein	AA3_2	glucose oxidase	SP(Sec/SPI)
XP_018246910.1	FOXG_09570	sialidase-1	AA5_2	galactose oxidase	SP(Sec/SPI)
XP_018250887.1	FOXG_12330	hypothetical protein	CE8	pectin methylesterase	SP(Sec/SPI)
XP_018247509.1	FOXG_10034	hypothetical protein	GH18	chitinase	SP(Sec/SPI)
XP_018248633.1;XP_018248634.1	FOXG_10748	chitinase	GH18	chitinase	SP(Sec/SPI)
XP_018235878.1;XP_018235879.1;XP_018235880.1	FOXG_02349	beta-glucosidase	GH3	β -glucosidase	SP(Sec/SPI)
XP_018250106.1	FOXG_11736	hypothetical protein	GH43_22	β -xylosidase	SP(Sec/SPI)
XP_018254051.1	FOXG_14489	hypothetical protein	GH43_24	β -xylosidase	SP(Sec/SPI)
XP_018235838.1; XP_018235839.1; XP_018235840.1	FOXG_17741	hypothetical protein	GH55	β -1,3-glucanase	SP(Sec/SPI)
XP_018256212.1	FOXG_15793	hypothetical protein	GH93	exo- α -L-1,5-arabinanase	SP(Sec/SPI)
XP_018231736.1; XP_018231737.1; XP_018231738.1	FOXG_00102	beta-N-acetylhexosaminidase	GH20	β -hexosaminidase	SP(Sec/SPI)
XP_018257975.1	FOXG_17088	hypothetical protein	GH28	polygalacturonase	SP(Sec/SPI)
XP_018251964.1	FOXG_12535	hypothetical protein	GH28	polygalacturonase	SP(Sec/SPI)
XP_018252069.1	FOXG_12585	hypothetical protein	GH28	polygalacturonase	SP(Sec/SPI)
XP_018253513.1	FOXG_14004	polygalacturonase	GH28	polygalacturonase	OTHER
XP_018237179.1	FOXG_03211	beta-fructofuranosidase	GH32	invertase	SP(Sec/SPI)
XP_018248486.1; XP_018248487.1	FOXG_10638	endoglucanase type K	GH45	endoglucanase	OTHER
XP_018250156.1	FOXG_11769	mannosyl-oligosaccharide	GH47	α -mannosidase	SP(Sec/SPI)

		alpha-1,2-mannosidase			
XP_018239045.1	FOXG_18811	hypothetical protein	GH72	β-1,3-glucanosyltransglycosylase	SP(Sec/SPI)

(Fol: *Fusarium oxysporum* f. sp. *lycopersici*, GH: Glycosyl hydrolase, AA: Auxillary activities, CE: Carbohydrate esterase)

(**C:GO:0005576** - extracellular region; **C:GO:0016020** - membrane; **F:GO:0004553** - hydrolase activity, hydrolyzing O-glycosyl compounds; **F:GO:0004563** - beta-N-acetylhexosaminidase activity; **F:GO:0004571** - mannosyl-oligosaccharide 1,2-alpha-mannosidase activity; **F:GO:0004650** - polygalacturonase activity; **F:GO:0005509** - calcium ion binding; **F:GO:0005515** - protein binding; **F:GO:0008061** - chitin binding; **F:GO:0008810** - cellulase activity; **F:GO:0016614** - oxidoreductase activity, acting on CH-OH group of donors; **F:GO:0030248** - cellulose binding; **F:GO:0030599** - pectinesterase activity; **F:GO:0050660** - flavin adenine dinucleotide binding; **P:GO:0005975** - carbohydrate metabolic process; **P:GO:0005975** - carbohydrate metabolic process; **P:GO:0042545** - cell wall modification; **P:GO:0055114** - oxidation-reduction process)

Table 3. Chromosomal location of the accessions annotated for CAZymes

Gene Accessions	CAZymes	Sequence accession	Chromosome	Length of sequence (base pairs)	Core/dispensable chromosome
FOXG_09774 (F)	AA3	CM000599.1	11	2337134	Core
FOXG_09570(R)	AA5				
FOXG_10034 (R)	GH18				
FOXG_12535 (F)	GH28	CM000591.1	3	5634991	Core
FOXG_12585 (F)	GH28				
FOXG_17741(F)	GH55	CM000593.1	5	4914260	Core
FOXG_02349 (F)	GH3				
FOXG_17088 (R)	GH28	CM000594.1	6	4589937	Core
FOXG_14004 (R)	GH28				
FOXG_10638 (R)	GH45	CM000595.1	7	4347182	Core
FOXG_10748(F)	GH18				
FOXG_03211 (R)	GH32	CM000596.1	8	3984410	Core
FOXG_15793 (R)	GH93				
FOXG_11769 (F)	GH47	CM000598.1	10	2896840	Core
FOXG_11736 (R)	GH43				
FOXG_14236 (F)	AA3_2	CM000602.2	14	1838362	Dispensable
FOXG_14258 (F)	AA3_2				
FOXG_00102 (R)	GH20	CM000589.1	1	6854980	Core
FOXG_18811 (F)	GH72	CM000592.1	4	5212762	Core
FOXG_14489 (R)	GH43_24	CM000600.1	12	2233407	Core
FOXG_12330 (R)	CE8	CM000601.1	13	1751499	Core

(F: on forward strand; R: on reverse strand)

Table 4. Gene accessions for the CAZymes corresponding to the cluster of ortholog (COG), Eukaryotic ortholog (KOG) and non-supervised ortholog (NOG)

CAZymes	CAZymes name	Accession (Fol)	Alias accession (<i>Fusarium oxysporum</i>)	COG/KOG/NOG accession	Description	No. of ortholog proteins	No. of organisms
AA3	Glucose oxidase	FOXG_09744; FOXG_14236; FOXG_14258	FOQG_11064; FOQG_19211	COG2303	Choline dehydrogenase or related flavoproteins	13768	2812
CE8	Pectin methyl esterase	FOXG_12330		COG4677	Pectin methyl esterase and related acyl-coA thioesterase	5988	927
GH3	β -glucosidase	FOXG_02349	FOQG_08375	COG1472	Periplasmic β - glucosidase and related glycosodase	14784	3914
GH18	Chitinase	FOXG_10034; FOXG_10748	FOQG_16748; FOQG_17261	COG3325	Chitinase, GH18 family	6649	1906
GH20	β -hexosaminidase	FOXG_00102	FOQG_07894	COG3525	N-acetyl β - hexosaminidase	5282	1906
GH28	Polygalacturonase	FOXG_12535; FOXG_12585; FOXG_14004; FOXG_17088	FOQG_19426	COG5434	Polygalacturonase	9473	2060
GH32	Invertase	FOXG_03211		NOG10950	GH family 32 C terminal	22	18
GH43	β -xylosidase	FOXG_11736	FOQG_07578	COG3940	β -xylosidase, GH43 family	1425	754
GH47	α -mannosidase	FOXG_11769	FOQG_07554	KOG2431	α -1,2- mannosidase	873	426
GH55	β -1,3 -glucanase	FOXG_17741	FOQG_08398	NOG259137	Pectate lyase superfamily	286	122

Figures

Figure 1

Histogram of $\log_2(x)$ transformed LFQ intensity (x-axis) vs. protein counts (y-axis) and corresponding profile box plot of $\log_2(x)$ transformed LFQ intensities, in the replicates of (a) Mock treated susceptible tomato variety C32 plant (control) (b) Fol007 (race 1) treated tomato cultivar C32 (treated). Light blue bars and lines are the LFQ intensities of the Fol proteins. (Fol: Fusarium oxysporum f. sp. lycopersici, LFQ: Label free quantification)

Figure 2

Multiple scatter plot of log₂(x) transformed LFQ intensities with Pearson correlation values above them in (a) Control, top to bottom from left to right each row: M1 vs. rest; M2 vs. rest; M3 vs. rest and M4 vs. rest (B) top to bottom from left to right each row: T1 vs. rest; T2 vs. rest; T3 vs. rest and T4 vs. rest

Figure 3

(a) Category-wise comparative plot of GO annotation of the identified proteins in the using WEGO online tool. (x-axis: GO categories with their functions, y-axis: percentage and number of proteins in each GO categories. (b) GO enrichment plot of gene accessions for identified Fol proteins. The circles which are darkened are significant molecular function (9) and biological process (2) in each experiments at padj < 0.05 and (-log₁₀ (padj) > 1.4. (padj: adjusted p-value)

Figure 4

Venn diagram of annotated CAZymes. identified by Diamond, HMMER and HotPep, generated online through dbCAN2 annotation server with parameters: e-value < 1e-05 and query coverage of > 35% for HMMER, e-value < 1e-102 for DIAMOND and frequency > 2.6 for HotPep.