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Genome Mining of Secreted Effectors of Fusarium Oxysporum, Fusarium Solani and Rhizectonia Solani

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Introduction

Of the various devastating diseases triggered by necrotrophic fungi, the diseases caused by following fungi Fusarium oxysporum, Fusarium solani and Rhizectonia solani are of particular economic significance in date palm (Phoenix dactylifera L.) growing countries. Undoubtedly date palm is the most important fruit tree cultivated in Arabian Peninsula. Unfortunately date palm cultivation encounter many challenges, such as fungal diseases. It has been reported that Fusarium oxysporum, Fusarium solani and Rhizectonia solani are the major causative of various diseases in Date palm.

For successful colonization of host, necrotrophic pathogens secrete an arsenal of effector proteins that facilitates infection by manipulating host cell structure and function. Thus secretory proteins (SPs) are critical for both short and long range intercellular signaling during infection and colonization.

The present study is conducted on three date palm pathogens, which causing diseases like bayoud disease and other necrotrophic diseases including root rot. This study focused on molecular genetics of necrotrophic pathogenicity in date palm by following an in silico approach to mine putative secreted elicitors from publically available Fusarium oxysporum, Fusarium solani and Rhizectonia solani ESTs.

Methodology

Methodology involved mining of 25213 Fusarium oxysporum ESTs, 12433, Fusarium solani ESTs and 22324 Rhizectonia solani ESTs for protein sequence based features characteristic of SPs (signal peptides). All the ESTs

were collected from NCBI. With using ORF predictor (http://proteomics.ysu.edu/tools/OrfPredictor.html) algorithm, found out all the six frame ORF. Predisi (http://www.predisi.de/) and SignalP (http://www.cbs.dtu.dk/services/SignalP/) algorithms was exploited to infer signal peptide within the ORF. Followed by scanning of transmembrane domain performed with TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) to discard the transmembrane sequences. 0 TM/1TM proteins were selected for further CD (conserved domain) search.

Before going to the mining experiment, the pathogenicity of soil isolated (from northern part of Qatar) fungal pathogen on Date palm was confirmed with detached leaf inoculation method. All the three fungi, Fusarium oxysporum, Fusarium solani and Rhizectonia solani showed necrosis in date palm leaf after three days of infection.

Result and conclusion

Out of 25213 Fusarium oxysporum ESTs 24890 have ORF. 12176 ORF found in Fusarium solani ESTs. Above 60% of all the three fungal ESTs had signal peptide. Number of sequences narrowed down by transmembrane topology search and the sequence doesn't containing transmembrane domain were selected for conserved domain identification.

Subsequent to our searches with sequences lacking transmembrane domains, we could catalog candidate effector sequences based on conserved features common to characterized fungal effectors. In Fusarium oxysporum following protein found to be extracellular secretory protein, this include cellulose binding protein, which possibly involved in in host cell adhesion, qaq like polyprotein: a virulent associated protein, lysin motif: an effector protein, extracellular peptidase, cell wall hydrolase, 150 amino acid cerato platanin sequence (elicitor), alpha amylase, beta 1,3 glucanase and 165 amino acid necrosis inducing protein. These effector proteins have the ability to degrade plant derived compound, this suggest the role of the effectors in pathogenicity.

In addition to these high amount of lipid targeting enzymes and glycoside hydrolase found in all the three phyto pathogenic ESTs. Presents of secretory protease detected in Fusarium solani. Peptidase like superfamily domain found in Rhizectonia solani.

To date, none of these effectors have been cloned and thus we propose that the catalogued effectors will in future be useful for isolation and characterization of these identified proteins. Expression studies are already underway and we expect that the results will provide insights into mechanisms underlying host-Pythium interactions and thereby help in generating new strategies for effective date palm disease control.