In silico analysis of effector proteins to elucidate infection strategies of fungal plant pathogens

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Plant pathogenic fungi infect plants to fulfill their nutrient requirements, and this process (pathogenesis) usually leads to disease development. The susceptibility or resistance of host plants against pathogens is partly determined by both pathogens derived elicitors/signal compounds and the corresponding host plant receptors. "Effector proteins" are low molecular weight proteins secreted by microbes during the pathogenesis process and play a significant role as specific elicitors. Recognition of effector proteins (encoded by *Avr* genes) by corresponding receptors (R proteins, encoded by *R* genes) in host plants leads to activation of "Effector triggered immunity (ETI)". The main focus of this study was to conduct an *in silico* analysis to identify and characterize effector proteins of pathogenic fungi and to elucidate their interactions with host receptors. Accordingly, the proteomes of nine fungal pathogens representing three life strategies, viz., biotrophichemibiotrophic and necrotrophic, were downloaded from UniProt knowledgebase and analysed through a predesigned series of various bioinformatics tools (SignalP, PrediSi, Phobius, TMHMM, PredGPI , Wolf psort, and EffectorP) to predict the secretome (collection of all secreted proteins) followed by the effectome (collection of all effector proteins) for each proteome. The predictions resulted in varying numbers of effector proteins for each proteome (155, 180, 57, 69,189, 259, 381, 338, 143 effectors were found in *Cochliobolusheterostrophus*, *Fusariumoxysporum*, *Sclerotiniasclerotiorum*, *Ustilagomaydis*, *Blumeriagraminis*, *Pucciniagraminis*, *Magnaportheoryzae*, *Colletotrichumorbiculare*, *Zymoseptoriatritici* respectively) and showed very low conservation among them. Protein functional domain analysis of effector proteins (using bioinformatics webservers Expasy, InterPro, BLASTp, and HMMER) failed to reveal any life strategy linked functional domains while protein kinase and cell attachment associated domains were distinctly abundant in all nine effectomes No conserved motifs were found among the nine effectomes. The protein docking method used to elucidate interactions between the pi54 resistance protein in *Oryza sativa* subsp. *Japonica* and the putative effector proteins of hemibiotrophic fungal pathogen *Magnaportheoryzae* (based on the gene for gene hypothesis) revealed a number of candidate effector proteins (67 effectors) that could interact with pi54 R protein. This preliminary study underpinned the low conservation among the effector proteins. Effector-receptor interaction studies will continue with the long term focus on developing natural resistance in crop plants.

Keywords: Effector proteins, *Avr* genes, Effector triggered immunity, R proteins.