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In Silico Assessment of the Relatedness of Blight Resistance Protein Sequences in Different Crop Species

Nworie Paul O. and Afiukwa Celestine A.

Department of Biotechnology, Faculty of Science, Ebonyi State Unversity, P.M.B. 053 Abakaliki, Ebonyi State, Nigeria.

Corresponding author:paulolisa90@gmail.com

ABSTRACT

Blight disease caused by *Phythopthorainfestans* is one of the most devastating disease of most crop species, affecting the output of farmers and the economic value of the affected crops. The objective of this study is to ascertain the relatedness of blight resistance genes among selected crop species which include tomato, potato, pepper and sesame species. Sequences of the blight resistance proteins were retrieved in FASTA format and aligned using the multiple sequence light alignment tool (CLUSTAL Omega version 2.1) online.The percentage identity and the conserved regions of the blight resistance proteins were determined using the Conserved Domain Architecture Retrieval Tool (CDART) in the NCBI database. Phylogenetic tree was generated by Neighbor joining methodusing CLUSTALOmega tool. The results did not show high sequence similarity but revealed the presence of a disease resistance protein domain (NB-ARC domain)in the retrieved disease defense proteins of all the crop species. This suggests that disease resistance proteins in different crop species are closely related.

Keywords: *In silico* analysis, blight disease, disease resistant protein, NB-ARC protein domain

INTRODUCTION

Successful disease combat in crop species is crucial forbumper return to farmers. Limitation in conventional disease management approach, especially in cases of late blight disease of many staple cropspecies such as rice, tomato, potato, etc. has triggered this study for an alternative pathway for effective disease management [1,2,3,4]. The world population is expected to rise from 6.1 billion up to 9.1 billion in the year 2050. The rapid increase in human population is expected to increase the demand for food production to satisfy the increasing global needs [5]. While the paddy agro-ecosystems are created to fulfill this demand, it is evident that those ecosystems are also exposed to both biotic and abiotic stresses. One of the main biotic stresses that affect majorcrop species in Nigeria is the bacterial leaf blight [6,7]. This research paper, through computer simulation, retrieved and compared the relatedness of the blight resistance protein sequences among different crop species with a view to revealing potential sources of the resistance traits for future breeding purposes[8].

MATERIALS AND METHODS

The protein product sequences of complete coding sequences (cds) of blight disease resistance genes of different crop species including *Solanum habrochaites, Solanum stoloniferum, Solanum verrucosum, Sesamum indicum, Sesamum orientale, Solanum lycopersicum, Capsicum annuum,* were retrieved from the NCBI database (http://ncbi.nlm.nih.gov) and analyzed for similarity using Multiple Sequence Alignment tool (CLUSTAL Omega)version 2.1. The percentage identity among the sequences were recorded and the conserved regions in the protein sequences was determined using Conserved Domain Architecture Retrieval Tool (CDART) in the NCBI database. Phylogenetic tree was constructed by neighbor joining method from the CLUSTAL Omega server following the multiple sequence alignment.

RESULTS

Table 1 shows the percentage similarities among the blight resistant protein sequences from the different crop species. The result showed high percentage similarity (74-77%) among some varieties of tomato (*Solanum stoloniferum* and *Solanum habrochaites*) and pepper (*Capsicumanum*) species, but in lowrelationship (22–30%) with sesame species. It was observed that the blight resistant proteins fromall the *Solanum spp.*were 80-84% identical to each other, except the sequence from *Solanum verrucosum* which did not show significant association (32–34%) with those of other *solanum spp.* Similarly, the blight resistant sequences of *Sesamum indicum* and *Sesamum orientale* did not show high similarity,just 30% identity. However, all the blight defense protein sequences were found to contain a nucleotide binding protein domain (NB-ARC) with the structure shown in Figure 2.

Table 1: Percentage identity matrix of the selected species

Crop species	*Sesamumindicum*	*Sesamumorientale*	*Solanumverrucosum*	*Capsicum annum*	*Solanumstoloniferum*	*Solanumhabrochaites*	*Solanumlycopersicum*
Sesamumindicum	100.00						
Sesamumorientale	30.05	100.00					
Solanumverrucosum	22.30	28.48	100.00				
Capsicum annum	23.12	26.33	32.93	100.00			
Solanumstoloniferum	24.17	24.69	32.34	77.17	100.00		
Solanumhabrochaites	23.78	25.29	34.12	**75.09**	**80.56**	100.00	
Solanumlycopersicum	23.68	24.85	31.75	**74.67**	**81.01**	**83.56**	100.00

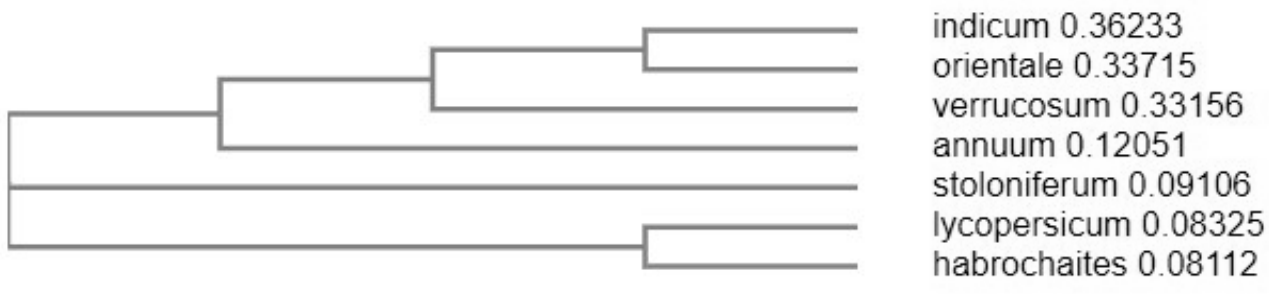

Figure 1: Phylogenetic tree showing evolutionary relationship among the disease defense proteins in different crop species

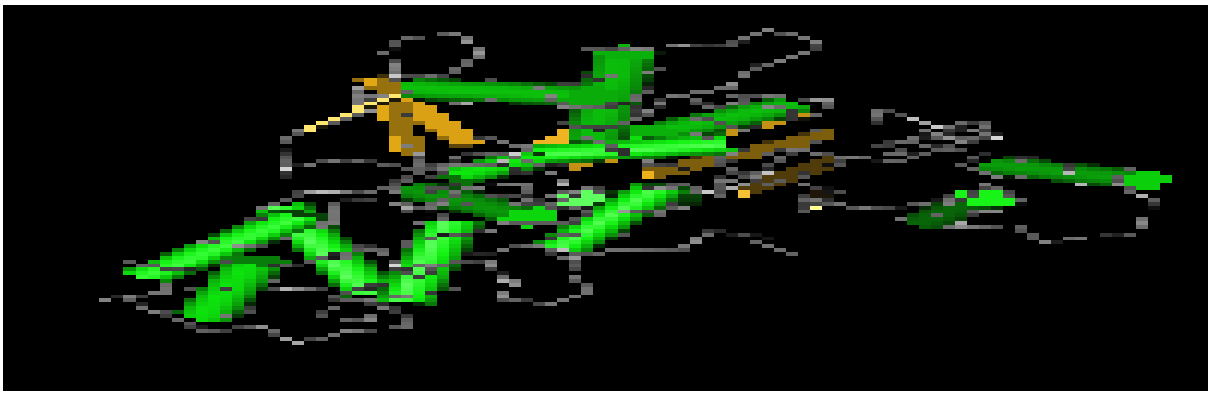

Figure 2: Structure of NB-ARC Protein Domain in all the Disease Defense Proteins from Different Crop Species

DISCUSSION

Percent identity refers to a quantitative measurement of the similarity between two sequences (DNA, amino acid or otherwise). Closely related species are

expected to have a higher percent identity for a given sequence than would more distantly related species, and thus percent identity to a degree reflects relatedness. The percent identity of the tomato disease defense proteins (*Solanum lycopersicum* and *Solanum habrochaites*), asa well as potato defense protein (*Solanum stoloniferum*) are the most closely related, having percentage identities between 80.6 and 83.6%, but the disease defense protein of *Solanum verrucosum* (wild potato) did not show significant sequence identity with the other*Solanum spp.* Similarly, the disease resistant protein sequences of *Sesamumindicum* and *Sesamumorientale* did not show significant identity (only 30%) even when both crops varieties belong to the same species. The study however revealed significant identity among *Solanum stoloniferum* (potato) , *Solanum habrochaites* (tomato) and *Solanum lycopersicum* (tomato) and *Capsicum annum* (pepper) (74 – 77%).

This suggests that the entire sequence is not important in the disease defense action of the proteins, rather it is the protein domain (NB-ARC domain) that is relevant [6] opined that the ARC component of the domain recognizes pathogen effectors, while NB domain initiates hypersensitive reaction against the pathogen.In accordance with the work of [7], when similarity is observed in sequences, it implies that such sequences did not arise independently, rather they arose from a common ancestor. Common ancestry explains high level of sequence similarity. Previous studies that estimated percent identity among blight resistance proteins put the full sequence identity at 95-99% using different computational tools. Although the blight defense proteins from different crop species did not really show high sequence similarities, they all contained a NB-ARC domain. The ARC component of the domain is believed to be responsible for pathogen recognition, while NB component initiates hypersensitive reaction against the pathogen [6]. This suggests that the entire sequence is not relevant to the defensive actions of the proteins, rather it is the NB-ARC domain.

Protein structure is conserved during evolution much better than protein sequence. There are numerous examples of proteins that show little sequence similarity but still adopt similar structures, contain identical or related amino acid residues in their active sites, and have similar catalytic mechanisms. These shared features support the notion that, despite low sequence similarity, such proteins are homologous in structure. Conserved domain analysis of the blight resistant proteins revealed that there exists an NB-ARC domain which is a functional ATPase domain and its nucleotide state is proposed to regulate activity of the defense protein (R protein). R proteins in plants are involved in pathogen recognition and subsequent activation of innate immune responses [7]. This report is however in accord with the works of [6] which states that blight resistance genes are located in the same parts of the genome with similar structure (nucleotide-binding site, NB-ARC) as R genes involved in pathogen recognition and activation of innate immune responses in plants. Also dominant among the blight resistance genes is the leucine rich repeats which is a characteristic feature of defense related proteins. R genes encode five classes of proteins [5]. R genes for resistance to *P. infestans* in potato encode proteins that belong to the major R protein class NB-LRR (also called NBS-LRR where NBS stands for Nucleotide Binding Site) [6]. NB-LRR consists of a Nucleotide Binding (NB) domain and a Leucine Rich Repeat (LRR) domain [5]. LRR domain recognizes the pathogen effectors, and NB domain initiates hypersensitive reaction [3].

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