TÍTULO

UTILIZATION OF THE MEGA X PROGRAM FOR GRAPHICAL REPRESENTATION OF THE EVOLUTIONARY RELATIONSHIPS OF THE PATHOGENESIS-RELATED (PR) PROTEIN FAMILY IN TOMATO PLANTS

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RESUMO

Physalis peruviana has been affected by the Physalis rugose mosaic virus (PhyRMV). To understand the plant-virus interaction, the project aims to collect PR protein (Pathogenesis-related) sequences and construct a phylogenetic tree to analyze the evolutionary relationship of the proteins. The NCBI site was used to gather the proteins. To build the phylogenetic tree the MEGA X 11 program was used. As a result, it is possible to observe how the functions of the proteins are evolutionarily related.

PALAVRAS-CHAVE

Solanaceae, Proteomics, Bioinformatics, Pathogen.

GRANDE ÁREA

CIÊNCIAS AGRÁRIAS (50000004)

ÁREA

AGRONOMIA (50100009)

INTRODUÇÃO E OBJETIVOS

The cultivation of Physalis (Physalis peruviana L., Solanaceae family) has gained importance in the Americas, (FISCHER & MIRANDA, 2012) due to its high commercial value. However, this crop, like others, is susceptible to a variety of pathogens, including viruses. In Brazil, a new Sobemovirus associated with Physalis was recently characterized and tentatively named Physalis rugose mosaic virus (Savi, et al., 2021). In response to pathogen infection, plants alter their metabolism to activate local defense pathways (hypersensitive response) and systemic pathways involving the activation of the expression of defense-related genes (DIXON & HARRISON, 1992), such as several genes belonging to the pathogenesis-related protein (PR) family (DURNER et al., 1997). The aim of this work is therefore to use bioinformatics to characterize PR genes in tomato plants in order to identify orthologous genes in Physalis plants in the future. Tomato plants are used as a model for this study as their genome is already fully sequenced in the database (GenBank: MT811796.1) they share the same family, Solanaceae (CHAVES, 2006). Bioinformatics is a promising field that includes mathematical algorithms, computer knowledge and statistics (FILHO et al., 2002) and started after DNA sequencing in the 1970s. In this project, it becomes an important tool for the search for target genes, such as PR genes, which may be involved in the plant defense response in Physalis plants.

METODOLOGIA

Search for genomic sequences

The NCBI website (https://www.ncbi.nlm.nih.gov/) was used to search for genomic sequences. The PR gene sequences were generated using the database of the tomato plant Solanum lycopersicum. The sequences of the genes (nucleotides) of the proteins (amino acids) were downloaded in FASTA mode and inserted into a Notepad file with the corresponding information. A total of twenty-three amino acid sequences related to Pathogenesis-related (PR) proteins were downloaded in FASTA format and stored in an electronic file.

Alignment (comparative genomics)

The PR protein sequences were aligned using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to check the homology between them. Phylogeny, once the genomic sequences of the PR genes in tomato plants were identified, these sequences were grouped using the program MEGA X11 to obtain a phylogenetic tree using the neighbor-joining method. This program allows evolutionary analysis based on alignments using different methods such as ClustalW and Muscle and shows the results with phylogenetic trees using bootstrap.

After, the swiss-model (swissmodel.expansy.org) was used to build a 3D structure of one of the proteins (PR1a CAA09671.1).

RESULTADOS

After analyzing the database, the sequences of 21 proteins from the PR family were grouped into a phylogenetic tree, which shows the evolutionary relationships between them (Figure 1). The analysis of the phylogenetic tree reveals the presence of well-defined clades among the proteins, indicating the diversification of PR proteins. Each clade of the tree suggests a close evolutionary relationship. The proteins in the initial clade, P4 (AAA03615.1), PR1a (CAA09671.1), and P6 (AAA03616.1), are known for their direct role in pathogen defense (VAN LOON & VAN STRIEN, 1999), with P4 (AAA03615.1) and PR1a (CAA09671.1) being important PR proteins in responses against fungi (VAN LOON & VAN STRIEN, 1999).

In the second clade, there are proteins related to the leaf, with PR Leaf 4 (XP 004246723.1) and PR Leaf 6 (XP 004230669.1) being involved in plant responses in the tomato leaf. The third clade includes PR-1 (XP 004242675.1) and PRBI-3 (XP 004245428.1), indicating their evolutionary relationship as proteins involved in the plant's immune response. Lastly, the clade of PR Transcriptional Activator PTI5 (NP 001233987.1) and PTI6 (NP 001233991.1) highlights these two proteins which, besides their roles related to pathogenesis, can also function as transcriptional activators, a broader regulatory function beyond plant defense (VAN LOON & VAN STRIEN, 1999; WANG et al., 2020).

Also, as a result, a structure form in 3D was made to see one of the proteins (PR1a), using the swiss-model site. This protein has a domain related to cysteine-rich secretory proteins (CAP) located in the extracellular portion which accumulates after infections with pathogens (KITAJIMA & SATO, 1999). In summary, the PR proteins of the tomato plant form a wide range of diverse proteins, reflecting the plant's evolution and adaptation to a variety of different pathogens, including fungi, bacteria, and viruses. Different clades of the tree indicate a complex and extensive defense network for the plant.

CONSIDERAÇÕES FINAIS

Considering the advances in the fields of genomics and bioinformatics, this study demonstrated the importance of identifying and characterizing Pathogenesis-related (PR) proteins in physalis plants through a comparative approach with the genome of the model plant Solanum lycopersicum. The results provided information on the conservation and diversity of these proteins, contributing to a better understanding of plant defense mechanisms against pathogens. Additionally, the methodology used, including phylogenetic analysis, proved to be an effective tool in investigating the evolution and phylogenetic relationships of PR proteins within the same organism. Therefore, this study not only expands knowledge about the plant immune response but also provides a foundation for future research aiming the genetic improvement and the development of disease management strategies in physalis crops and other plants in the Solanaceae family

LINK DO VÍDEO

https://drive.google.com/file/d/1EI5NtsU4Tm9nhIAbGON4GfGkQX3t46oV/view?usp=drive_link

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AGRADECIMENTOS

A equipe do projeto agradece ao Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq e ao Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina – IFSC, pelo apoio recebido, viabilizando a execução das atividades do projeto de pesquisa.