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IN SILICO IDENTIFICATION AND CHARACTERIZATION OF MOLECULES WITH ANTIMICROBIAL ACTIVITY IN LEGUMES FROM THE NORTHEASTERN SEMIARID REGION SUBJECTED TO STRESS

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Abstract: Introduction: Antimicrobial peptides (AMPs) make up the innate defense of plants and can increase expression when subjected to biotic and abiotic stresses. In the semi-arid environment of the Caatinga, there are few molecular studies with native plants, such as the Cenostigma pyramidale (``*catingueira*``), which presents considerable resistance to adverse environmental conditions. Considering the antimicrobial potential of AMPs and their relevance in plant defense, structural analyzes aim to identify and characterize in silico AMPs with great biotechnological potential, through the analysis of omics data using bioinformatics. **Methodology:** sequences were aligned using Clustalômega, with manual analysis using MEGA7 and Jaw view; phylogeny by MEGA7; and gene expression from salt stress over 30 minutes, 2 hours and 11 days. **Results and discussion:** As for gene expression, the action of *cross-talking* on defensin genes was visualized, which were activated after salt stress at 30 minutes, to ensure the plant's defense against other types of stress, such as biotic stress. At 2 hours, the genes were repressed, as the plant prioritizes the activation of other sequences, and, at 11 days, the genes were induced again, as the ``*catingueira*`` accepts high salinity conditions as a new degree of homeostasis. By alignment, it was ensured that these peptides share the conserved 6-cysteine domain as well as the conserved three-dimensional structure, which consists of an α-helix and three antiparallel β-strands. Conclusion: ``*catingueira*`` is an excellent source of antimicrobial peptides and has high biotechnological potential for the development of drugs that do not present resistance to antibiotics.

Keywords: bioinformatics; antimicrobial peptide; northeastern semi-arid; mesquite; ``catingueira``.

INTRODUCTION

During the life cycle of plants, multiple biotic and abiotic stresses interfere with the development, metabolism, fertility and productivity of plants, such as bacteria, fungi, salinity, temperature and exposure to insects. These adverse environmental conditions are limiting to plant growth and development, especially when related to agricultural production (Calanca, 2017). This way, to survive and reproduce, plants have developed the ability to adapt to adverse contexts, through biochemical, physiological and molecular pathways, which modulate the regulation and expression of functional genes (Acevedo et al., 2015; Santos et al., 2017), which trigger specific responses to the stressful conditions imposed, minimizing the damage suffered (Basu et al., 2017).

In evidence in recent decades, there are antimicrobial peptides (Antimicrobial Peptides, AMPs), which make up the innate defense of plants and can increase expression when subjected to biotic and abiotic stresses (Benko-Iseppon et al. 2010; Li et al., 2021). Thus, in a reality where antimicrobial resistance represents a public health problem that has been growing in recent decades, AMPs represent a new class of therapeutic agents, emerging as a promising option against a wide range of pathogenic microorganisms, as they have several properties that favor this applicability, such as its small size, rapid activity and a low chance of developing resistance (Santos-Silva et al., 2021).

In the semi-arid environment of the Caatinga, molecular studies with native plants are scarce. The Fabaceae family, for example, has several endemic species, but with few studies related to omics technologies, such as the target species of this study, Cenostigma pyramidale (catingueira), which present considerable resistance to adverse environmental conditions. Despite

the environmental relevance of these plant species, there are few studies that portray their physiological, molecular and biochemical aspects, which have a high capacity for use in identifying genes with biotechnological potential, capable of presenting new functions or potential, such as AMPs. (Jha et al., 2019; Pandey et al., 2021).

Finally, when considering the antimicrobial potential of AMPs, their relevance in plant defense, as well as their potential use in the development of new drugs, the structural analyses, associated with the modeling suggested in this project, aim to identify and characterize in silico AMPs with great potential biotechnological, based on species adapted to the northeastern semi-arid region, through the analysis of omics data using bioinformatics.

METHODOLOGY

UNDERSTANDING GENE EXPRESSION

The expression of genes encoding plant defensins differs between tissues and after pathogen invasion (De Coninck et al., 2015; Pothana et al., 2019). Plants defend themselves using constitutive and induced mechanisms. Induced defense mechanisms play an important role in plant self-protection (Scheres and van der Putten, 2017). To understand gene expression and the induction/repression pattern of the defensins under study, salt stress was performed on *Cenostigma pyramidale* with evaluation of gene expression after 30 minutes, 2 hours and 11 days, using Fold-Change, P-Value and FDR of each sequence studied.

DIVERSITY ANALYSIS THROUGH PHENETICS

In order to understand the diversity and structural variation, through the characterized candidates of AMPs, a multiple alignment of the amino acid sequences for the different AMPs of the species of *Cenostigma pyramidale* (catingueira) will be conducted, through the contribution of the Clustal Omega program (Sievers et al., 2011). Furthermore, a phenetic tree will be constructed using the MEGAX program (Kumar; Stecher; Tamura, 2016) with the Neighbor-Joining method, with a bootstrap of 1000 replications. Alignment analysis was also performed using the Jaw view program (Waterhouse, A. M. et al., 2009)

RESULTS AND DISCUSSION

GENE EXPRESSION

Plants can identify damage-associated molecular patterns (DAMPs) that are derived from host biomolecules (Meents, A. K., Mithöfer, A., 2020) and can act as danger signals, promoting a series of defense mechanisms; including the induction of resistance responses and the biosynthesis of immune-related proteins. The transcription of most genes related to plant defensin varies according to the pathogen to which the plant has been subjected. For example, Liu Y et al. (Liu, Y. et al., 2020), demonstrated that under low phosphate, seven BnaPDFs defensin genes exhibited differential expression in shoots or roots, and that a greater proportion of DEGs were upregulated.

Regarding the gene expression analysis of the present study, a *Heat Map* was made (Table 1), in order to observe the pattern of induction or repression of the selected sequences, as well as the Fold-Change of each one, from salt stress to over 30 minutes, 2 hours and 11 days. In Table 1, we can see that salinity considerably modifies the gene expression pattern, even of the defensins under study, related to biotic

stresses. This scenario can be explained based on the concept of *cross-talking*, in which the activation of a pathway (in this case, defensin genes against abiotic stresses - salinity) leads to the activation of other cascades (such as defensin genes against biotic stresses - those of the present study).

Based on this *cross-talking* mechanism, the plant provides a guarantee of defense against various types of stress, if they occur concomitantly with abiotic stresses, to ensure protection. The fact that the induction/repression pattern of each sequence is different can be explained by the concept of specific action of defensins, which favors defense against certain pathogens. Following this thought, it can be seen that, after 30 minutes, the defensins under study are activated by cross-talking, in order to guarantee defense against various stresses, in addition to saline; at 2 hours, most of the defensin genes studied are repressed, which can be explained by the idea that the plant prioritizes the activation of genes other than defensins; Finally, after 11 days, the plant activates many of the genes again, as it reaches a new state of homeostasis, considering the high level of salinity as normal, resuming the expression of the defensins under study.

Sequence name	30 min	2h	11 days
TR253412|c0_g1_i1*	9.8	8.4	2.3
TR253412|c0_g1_i3	-1,6		-1,5
TR253412|c0_g1_i2	0,2		-1,6
TR258862|c0_g1_i1	-5,1	-3,1	
TR253807|c0_g1_i1		-2.1	1.3
TR253807|c0_g1_i2	1.7		4.1
TR212973|c0_g1_i1		-3,5	
TR178948|c0_g1_i1	1.2		2.6
TR22755|c0_g1_i1	2.0	2.8	1.2
TR178948|c0_g2_i1			2.1
TR246517|c0_g1_i1			
TR102328|c0_g1_i1		-4.0	
TR21396|c0_g1_i3		-1,6	

Table 1. * significant FDR and P-VALUE; Red: induced genes; Blue: repressed genes

PHENETICS ANALYSIS

Sequence alignment is a fundamental task in bioinformatics, as it is used in several important biological analyses, such as predicting the function and structure of unknown proteins, to identify regions of similarity that may be a consequence of functional, structural or evolutionary relationships between sequences (Amorim, A. R. et al., 2021). It can be seen from Table 2 that these peptides share the conserved 6-cysteine domain as well as the conserved three-dimensional structure, which consists of an α-helix and three antiparallel β-strands. Although plant defensins share a common tertiary structure, there is extensive variation in amino acid sequences and peptide lengths. Such sequence diversity correlates with variation in antimicrobial activity.

Using the MEME Protein tool (Bailey, T. L., Elkan, C., 1995), the Sequence Logo was found, in Figure 1, generated from the multiple alignment performed between the motifs in the data set.

From the alignment carried out, it was possible to find the phylogenetic tree of the defensins under study, using MegaX (Kumar; Stecher; Tamura, 2016), with the Neighbor-Joining method, with bootstrap of 1000 replications, represented by Figure 2. The results showed that the five sequences lower in Figure 2 present more divergent clades than those at the top, representing a greater evolutionary distance than the others.

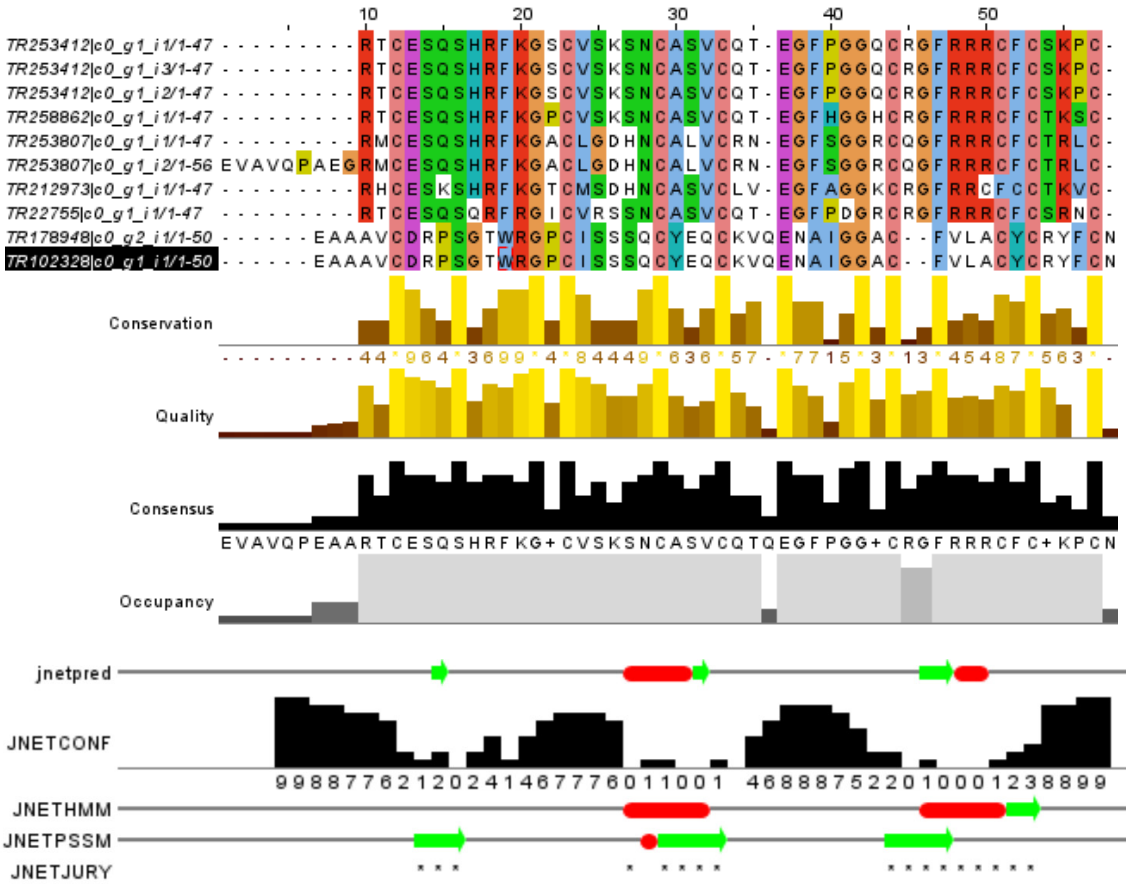

Table 2: alignment of mature sequences, without signal peptide, and converged three-dimensional structure.

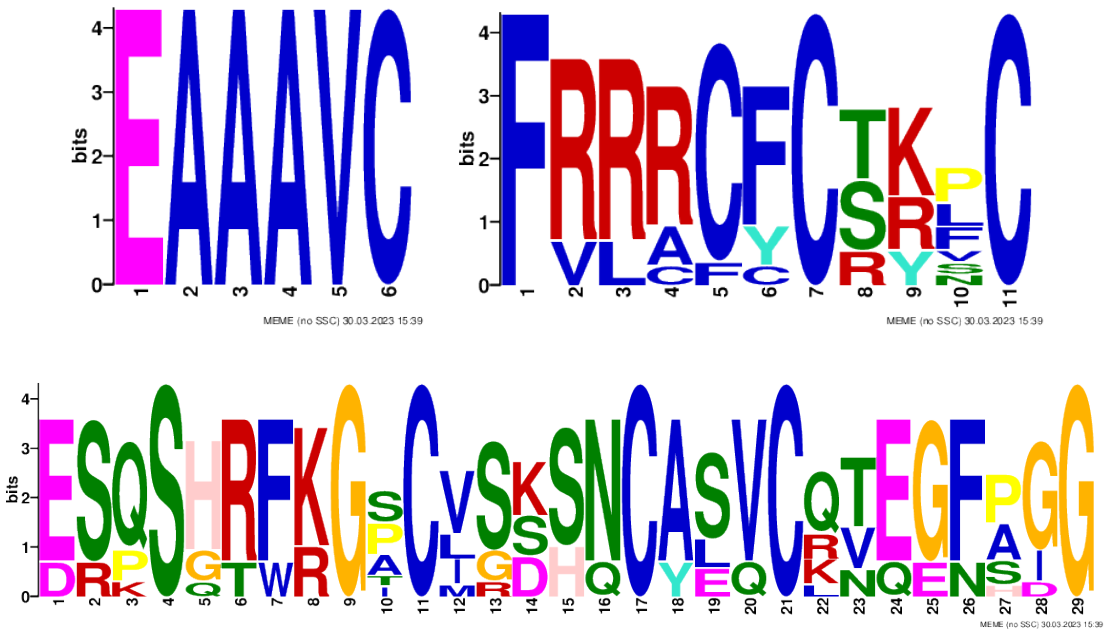

Figure 1: Sequences Logo by MEME Protein.

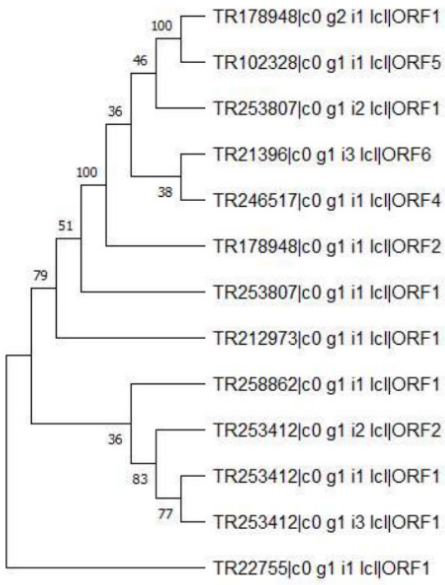

Figure 2: phylogenetic tree of the sequences under analysis, using the MegaX Program.

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