Cloning and Molecular Characterization of Cyclotides Genes from Brazilian Cerrado Plants


Centro de Análises Proteômicas e Bioquímicas, CAPB-UCB, DF, Brazil.

INTRODUCTION: Cyclotides are a specific class of plant cyclic peptides with 28-37 residues and a clear pattern of six cysteines linked by three disulfide bridges, forming a motif known as cyclic cysteine knot. This highly stable structure presents several biological activities including insecticidal, anti-HIV, antitumor and antimicrobial. Rubiaceae and Violaceae plant families are known to harbor a wide variety of cyclotides and thus became a target for genic studies according a remarkable biotechnological potential of such compounds. This work aims to clone and characterize cyclotides genes from *Palicourea rigida* and *Hybanthus lanatus*, prototypes of Rubiaceae and Violaceae families, respectively, that occur in Brazilian Cerrado.

METHODOLOGY: *P. rigida* and *H. lanatus* leaves were macerated in liquid nitrogen, subjected to total RNA extraction, using trizol reagent, and used for cDNA synthesis with Superscript II reverse transcriptase. The cDNA was used as template in PCR with degenerate primers, which are designed by using specific cyclotides sequences obtained in public data banks (NCBI-Genbank). The amplicons were purified and cloned into pGEM-T Easy vector. Several colonies were subjected to PCR and those with fragments over 400bp were submitted to plasmid DNA extraction and further sequencing using Applied Biosystems 3130 Genetic Analyzer.

RESULTS AND DISCUSSION: cDNA amplifications resulted in partial genes isolation from both studied species. In *P. rigida* a gene fragment that encodes the cyclotide named parigidin-br1, with approximately 227bp, was discovered. In *H. lanatus*, an incomplete gene sequence was detected, which encodes a novel cyclotide precursor protein, with approximately 309bp. Both sequences are under investigation for further complete determination through 5'-RACE experiments. Subsequently, genomic DNA amplifications and Southern blot experiments complete the molecular genes characterization. CONCLUSION: In summary the isolation of two novel genes encoding cyclotide precursors from Cerrado plants were here described. These molecules can be used in a near future as biotechnological tools for develop genetically modified (GM) organisms with ability to produce cyclotides in large-scale or GM organisms harboring microbial and/or insect resistance.

Keywords: cyclotides, Rubiaceae, Violaceae, Cerrado, biotechnology.

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