

Transcriptomic analysis of cyclotides in Sri Lankan *Viola betonicifolia*

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Plants belong to Violaceae family are known to contain stable bioactive cyclic peptides known as cyclotides. Eight species of Violaceae plants can be found in Sri Lanka: three from genus *Rinorea*, three from genus *Viola* and two from genus *Hybanthus*. *Rinorea* species were thought to be extinct in Sri Lanka until the recent discovery of *Rinorea decora* and *Rinorea bengalensis*. However, the chemical constituents and bioactivities of Sri Lankan *Viola* plants have not been investigated. Aim of the study was to characterize cyclotides in Sri Lankan *Viola* plants using a complementary tool of transcriptomics. Out of all Violaceae plants, *V. betonicifolia* was selected for transcriptome *de novo* sequencing and analysis to identify cyclotide sequences. RNA from *V. betonicifolia* plant tissues were extracted and sequenced by the platform Illumina HiSeq 2000 sequencing system with the library TruSeq RNA. Analysis of precursor sequences and putative processing sites provides further insight into cyclotide biosynthesis in *V. betonicifolia* and may also help to distinguish cyclotides from other cysteine-rich plant proteins. A total of 28 new (vibe 1-28) and three known (kalata S, viba 17, viba 11) cyclotide sequences were identified, which includes acyclotide and both cyclotide subfamilies Möbius and bracelet. Three precursors differ significantly in these common processing sites; vibe 10, vibe 22 and vibe 25 lack the common asparagine or aspartic acid in their cyclotide domain at C termini, vital for cyclisation. BLAST search of cyclisation enzyme, asparagine endopeptidase in the *V. betonicifolia* transcriptome showed most similar to the predicted enzyme from *V. uliginosa* transcriptome (93%) followed by butelase 1 (71%) and OaAEP1b (68%).

Keywords: Violaceae, Cyclotides, Transcriptome *de novo* sequencing, Cyclisation enzyme

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