

In-silico structural and functional analysis of cysteine proteinase inhibitors in legumes

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ABSTRACT

Cysteine Proteinase Inhibitors (CPI) play critical role in plant defense and protease regulation, yet their evolutionary and functional diversity across legumes remains incompletely understood. In this study, we performed a comprehensive sequence, structural, and biochemical analysis of CPI protein from eight legume species *viz.* *Arachis hypogaea*, *Arachis duranensis*, *Cajanus cajan*, *Cicer arietinum*, *Vigna radiata*, *Pisum sativum*, *Glycine max*, and *Phaseolus vulgaris*, with *Medicago sativa* as a divergent outlier. Multiple sequence alignment across legume species revealed strict conservation of core inhibitory motifs and cysteine residues forming disulfide bonds, whereas N-terminal signal peptides and reactive loops exhibited species-specific variability, reflecting functional adaptation. Phylogenetic and biochemical property analysis identified two primary evolutionary clusters and highlighted *Medicago* as a structurally and chemically distinct CPI variant. Structural predictions confirmed a conserved β -trefoil-like fold, with flexible loops enabling dynamic protease interactions. Physicochemical profiling demonstrated a balance between hydrophilicity, stability and thermostability, while correlation analysis revealed an inverse relationship between hydrophobicity and conformational flexibility. Collectively, these findings indicate that legume CPIs maintain a conserved structural and functional core while evolving adaptive features to support diverse role in plant defense, stress responses and protein homeostasis. Furthermore, potential CPIs in *A. hypogaea* were analyzed based on available expression databases. These genes can serve as potential candidates for developing biotic stress resistance, particularly against bruchid infestation during legume storage, with specific relevance to peanut as a major food and oil crop.

Keywords: *Biochemical properties, Cysteine Proteinase Inhibitor, legumes, Phylogenetics, Sequence conservation and Structural analysis*