

## Study of Bowman-Birk inhibitors (BBIs) in the genus *Vigna* (Fabaceae) and their binding affinity with trypsin and chymotrypsin

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### Abstract:

Bowman-Birk inhibitors (BBIs) are a family of serine-protease inhibitors mainly found in seeds of Fabaceae plants, which are commonly consumed in human diet. In plants, BBIs serve various functions, in particular protecting against insect attacks by inhibiting digestive enzymes such as trypsin and chymotrypsin. They also contribute to the plant's resistance to different types of biotic and abiotic stresses. Moreover, BBIs have been observed to play a role in prevention of different human pathology: cronic inflammation, neurodegeneration, hypertension and different types of cancer.

From a molecular perspective, mature proteins contain 70 amino acids, including cysteines that form 7 disulfide bridges, providing the molecule with high stability. BBIs have a double-head structure with independent domains. Features of one amino acid in each domain, P1, confer interaction specificity, so BBIs can bind, with competitive way, trypsin in both domain (TT) or trypsin in the first domain and chymotrypsin in the second one (TC).

In order to evaluate genetic variability between TT and TC genes, DNA was extracted from 42 different species, amplified by PCR and sequenced using the Sanger method. TT and TC sequences were then aligned independently. Successively, we performed computational analysis to study the interaction energy of the resulting protein sequences to find isoform with higher substrate binding affinity.

In addition, we did a phylogenetic study with ancestral sequence reconstruction using the maximum likelihood method, and then we investigated their binding affinity to enzymes.

The aim of this project is to evaluate proteins with greater potential for further examination, with the goal of developing new cultivable plant varieties which have improved nutritional quality and more resistance to biotic and abiotic stresses. Furthermore, these proteins could be tested *in vitro* and *in vivo* to observe their effect on human health.