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Poster Abstract - F.10

BOWMAN-BIRK INHIBITOR GENES IN THE *PHASEOLUS VULGARIS* GENE-POOLS: ISOLATION AND COMPARISON

A.R. PIERGIOVANNI*, I. GALASSO**, L. LIOI*

*) Istituto di Genetica Vegetale-CNR, Bari, Italy
**) Istituto di Biologia e Biotecnologia Agraria-CNR, Milano, Italy

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Bowman-Birk inhibitors (BBI) are the most important class of proteinase inhibitors present in legume seeds. The high interest on BBI is due to the several roles attributed to them: reduction of protein digestibility, storage of sulphur amino acids in plant tissues, active role in the towards pest and diseases, effectiveness in preventing or suppressing carcinogenic processes in both *in vitro* and *in vivo* models. BBI isoforms have been isolated and characterised in many legume species. The aim of this study was to isolate and compare BBI genes from Mesoamerican and Andean gene pool of wild and cultivated common beans.

Genomic DNA was extracted from young leaves of cultivated and wild common bean accessions (16 and 6, respectively). BBI sequences were amplified by PCR using two specific primers designed on the gene sequence encoding for the BBI. The amplified product of about 300 bp was gel purified and cloned in pGEM®T plasmid (Promega, USA). Several clones for each sample were sequenced.

By comparing the inserts of about 200 clones, 38 BBI types sharing 91 to 99% of similarity were found. The observed substitutions involved residues located outside as well as within the trypsin and the chymotrypsin binding loops. The analysis of the deduced amino acid sequences of BBI types evidenced: 1) Ile or Arg residues can alternatively occupy the P'_2 position of trypsin binding loop; 2) Leu or Phe were found at the P_1 position of chymotrypsin binding loop; 3) two further regions of variations were observed outside both binding loops. The presence of Arg residue at P'_2 position of trypsin binding loop is uncommon in natural BBI. Studies based on synthetic peptides have shown that distinct active-site variants can influence the protein inhibitory activity. Our data showed that the polymorphism at P'_2 position is a characteristic of both wild and cultivated Mesoamerican accessions. Conversely, among the tested samples only 40% of the cultivated Andean samples and no one of the corresponding wild accessions showed that polymorphism. This suggests that a successive introgression of this character might have occurred in the Andean cultivated gene pool after domestication.

The control of BBI activity is very important from a nutritional point of view because the selection for low BBI content is considered a desirable trait in breeding programmes aimed to increase the nutritional value of animal feed. Moreover, the assay of antitryptic activity showed that generally lower inhibitory activities were associated to Mesoamerican accessions.