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Poster Abstract – A.36

BIODIVERSITY STUDIES IN *PHASEOLUS* SPP. BY DNA BARCODING

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DNA barcoding is a technique for identifying species by obtaining a short DNA sequence from a known gene and comparing it with databases of orthologous sequences from species of established identity. Our study deals with the use of DNA barcoding as a new tool to assess genetic relationships among *Phaseolus* species and genetic distinctiveness of *P. vulgaris* varieties. While in a range of animals, the mitochondrial genes, such as the COI, have been proved to be suitable for DNA barcoding, in other organisms they are not useful. Land plants, especially angiosperms, seem to be problematic for DNA barcoding since most mitochondrial DNA regions have exceedingly low levels of variation to distinguish between taxa. The mitochondrial genome in plants undergoes significant rearrangement and horizontal transfer of genes, both at intra and interspecific levels. Standard genic and intergenic regions from the chloroplastic genome can offer for DNA barcoding in plants what the mitochondrial genome does for animals: it is an uniparentally inherited, non recombining and structurally stable genome.

A total of 54 accessions of *Phaseolus vulgaris* were arbitrarily selected as representative of gene pools on the basis of passport information and previous molecular investigation data, along with a few *P. coccineus*, *P. lunatus* and *Vigna unguiculata* accessions adopted as reference standards and out-types. In particular, 24 Italian pure lines, 18 Mesoamerican landraces and 12 Andean landraces of *P. vulgaris* were characterized by amplifying and sequencing four plastid genic regions (rbcL and trnL) and intergenic spacers (atpB-rbcL and psbA-trnH), along with the nuclear internal transcribed spacers (ITS1 and ITS2). The experimental strategy included the following steps: i) retrieving nucleotide sequences of the selected DNA regions from the NCBI databases in the *Fabaceae* family; ii) performing serial local multiple sequence alignments; iii) designing of specific primer pairs in highly conserved short stretches (300-500 bp) flanking the most variable regions; iv) characterization by amplifying and sequencing of the distinct cpDNA regions along with the ITS1-ITS2 for rDNA regions; v) editing and alignment of sequences by Sequencer software; vi) clustering of sequences by UPGMA and NJ methods supported by bootstrapping analysis using PAUP software for phylogenetic analysis.

On the whole, all designed primers proved to be highly specific for the amplification of target DNA sequences. The occurrence of SNPs in either *Phaseolus* spp. or *P. vulgaris* was much lower in plastid DNA sequences than nuclear ITS regions (overall, 36 vs. 72 and 5 vs. 10, respectively). Most importantly, a total of 17 different haplotypes were identified for the 54 common bean accessions. It is worth mentioning that most domesticated Andean varieties were clustered apart from Mesoamerican varieties, whereas wild Andean and Mesoamerican accessions were grouped into two tightly related subclusters. In conclusion, the DNA barcoding confirmed to be a very

powerful technique for phylogenetic purposes in plant species, but revealed to be poorly informative for the genetic traceability of single plant varieties. As a matter of fact DNA barcoding provides an accurate method for the genetic identification of bean species by using a standardized genic or integenic region as molecular tag. The research is in progress with the main goal of discovering additional SNPs and reconstructing haplotypes to be exploited for the identification of varietal groups.