GENETIC TRACEABILITY OF PLANT VARIETIES BELONGING TO SPECIES OF AGRI-FOOD INTEREST BY MEANS OF DNA BARCODING: A CASE STUDY

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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 recognize different Phaseolus species and to assess genetic distinctiveness of P. vulgaris varieties. It is largely accepted that the mitochondrial gene COI can be considered the core of the global biodentification system for animals, while for the plant kingdom the research of the barcoding markers is slowing down by the difficulty of finding the gene analogous to COI. Currently, the most promising markers for plants are from the chloroplast genome because it owns the same attributes of the mitochondrial one: it is an unparentally inherited, non recombinating and structurally stable genome. This project deals with the study of the potentials of DNA barcoding applied to several pure lines of Phaseolus vulgaris species belonging to wild, domesticated and cultivated common beans by means of multilocus approach that consisted in amplifying and sequencing plastid genic regions (rbcL, trnL and matK) and intergenic spacers (rpoB-trnC, atpB-rbcL, trnT-trnL and psbA-trnH) along with the nuclear internal transcribed spacers (ITS1 and ITS2). In particular several Italian pure lines and Mesoamerican and Andean landraces were arbitrarily selected as representative of gene pools on the basis of morphological seed traits and plant descriptors, along with a few P. coccineus, P. lunatus and Vigna unguiculata accessions adopted as reference standards and out-types. Our main goals were i) to test how different markers perform as DNA barcodes, mainly below the level of species; ii) to investigate the differentiation among varieties and how we can use barcode data to reconstruct where modern "Italian" varieties come from; iii) to evaluate how well different methods (tree based versus character based) help us answer the previous questions. Among all the sequences tested, the best performance as barcoders at varietal level was attributable to trnH-psbA intergenic spacer and trnL intron, while the other regions provided few point mutations. Regarding the method, the phenetic approach confirmed to be a powerful technique to correctly separate different species and to cluster accessions corresponding to members of the same species, while at varietal level DNA barcoding standard tree-building method revealed to be scarcely informative to discriminate gene pools and to identify varieties within P. vulgaris. Thus a second approach, the character-based system, was tested and it revealed to be useful to detect within P. vulgaris species a total of 16 haplotypes over all cpDNA regions corresponding to as many subgroups, each one made up by Mesoamerican or Andean accessions along with Italian accessions that clustered with one or the other gene pool. An important finding is that haplotypes of most domesticated and cultivated
accessions were clustered in tight sub-groups, with the exception of few haplotypes shared only by wild and ancestral accessions, irrespectively of their Mesoamerican or Andean gene pool of origin. In conclusion, the DNA barcoding confirmed to be a very powerful technique to distinguish different plant species, but revealed to be poorly informative for the genetic traceability of single plant varieties.