

NUCLEAR AND PLASTID SEQUENCES FOR IDENTIFICATION OF SPECIES OF FLOWERING PLANTS (DNA BARCODING)

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Methods for identifying species by using short orthologous DNA sequences, known as "DNA barcodes," have been proposed and initiated to facilitate biodiversity studies. A Consortium for the Barcode of Life (www.barcoding.si.edu) has been established to stimulate the creation of a database of reference sequences to serve as a universal library. Barcoding will provide vital new tools for appreciating and managing Earth's immense and changing biodiversity. The cytochrome *c* oxidase 1 sequence has been found to be widely applicable in animal barcoding but unfortunately is not appropriate for most species of plants because of a much slower rate of cytochrome *c* oxidase 1 gene evolution in higher plants.

The internal transcribed spacer (ITS) region of the nuclear ribosomal cistron (18S-5.8S-26S) and the *trnH-psbA* plastid intergenic spacer have been recently proposed to be appropriate for barcoding of flowering plants since they a) have significant species-level genetic variability and divergence, b) are appropriately short so as to facilitate DNA extraction and amplification, and c) are flanked by conserved sites for developing universal primers. To evaluate the suitability of these sequences as DNA barcodes, we analyzed some individuals belonging to different plant genera and families. The nuclear and plastid regions were PCR amplified with specific primers and sequenced with an ABI Prism 3730 Automated DNA sequencer. Intraspecific and interspecific sequence variation was evaluated to assess the resolution of the technique. After sequence analysis usually a very low (or absent) intra specific variability was detected, whereas interspecific variability was usually sufficient to identify the species. Moreover some interspecific crosses were performed in the genus *Helianthus*, i.e. *H. argophyllus* x *H. debilis* (and reverse reciprocal cross), *H. annuus* x *H. argophyllus*; *H. debilis* x *H. annuus*. The hybrid progenies and parental species were characterized at the morphological level and at the molecular level using the two DNA sequences. Interspecific variability due to SNPs, Indels and SSR length, was sufficient for an unambiguous identification of each species. In particular *trnH-psbA*, uniparentally inherited, was very useful to identify the maternal origin of each hybrids even at juvenile stage of development or without morphological evidences.