

Abstract

DNA barcoding method was applied to identify and study the phylogenetic relationships existing between 15 species of saffron collected in Iran including 4 crop and 11 wild species. DNA extraction was done by modified Delorapurta method. A total of 60 sequences were obtained and registered in NCBI database. In particular, 25 of these sequences (*rbcL*, n=11; *matK*, n=4; *trnH-psbA*, n=5; ITS, n=5) were not already present in the scientific library. Nucleotide polymorphic sites were counted for each barcode gene (*rbcL*, n=16; *matK*, n=15; *trnH-psbA*, n=46; ITS, n=71). Each sample could be distinguished from the others in the phylogenetic trees developed based on the data obtained by single barcodes (*rbcL*.*matK* and *trnH-psbA*) genes to genus level. *rbcL* sequence has low resolution at the species level, it can contribute to interspecific studies in combination with *matK* sequence. The disadvantages of *trnH-psbA* sequence, are presence of repetitive positions and inappropriate sequence alignment due to its variable length among samples. *matK* phylogenetic tree seemed to enjoy greater discrimination power compared to *rbcL* and *trnH-psbA* data. It can therefore be used as a complementary barcode with other barcodes. In general, ITS sequence, indicating high resolution at the genus and species level, appeared as the best barcode sequence of the present study. In addition, a phylogenetic tree based only on plastid information (*trnH-psbA* + *rbcL* + *matK*) and another created on the data resulting from both nuclear and plastid genomes (*trnH-psbA* + *rbcL* + *matK* + ITS) were also generated. In general, using combination barcode sequences (*trnH-psbA+rbcL+matK+ITS*), indicating high resolution at both genus and species levels and were used to reconstruct phylogenetic events in *Crocus* genus. Phylogenetic analysis demonstrated the genetic relationship between crop saffron and wild *Crocus* species. As a result of this study based on 15 species of *Crocus* family, wild species *C. sp. Eslamabad* and *C. cancellatus* were close to Iranian saffron. Phylogenetic analysis based on 41 species of *Crocus* family through ITS barcode revealed the genetic relationship between *Crocus sativus* and *Crocus* wild species, correctly and with high resolution. As a result, the closest relatives of Iranian saffron are *C. cartwrightianus* and *C. oreoreticus* and *C. cartwrightianus* is the ancestor of *C. sativus*. Our investigation also indicated that the different ecotypes of *C. sativus* may have evolved through independent events probably due to geographic and environmental pressures.

Keywords: phylogeny; DNA barcoding; *Crocus*; *rbcL*; *matK*; *trnH-psbA*; ITS



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