

Understanding Generic Affinities among some of the *Coffea* species and related taxa using ISSR and RAPD markers

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Abstract

Coffee-tree belongs to the genus *Coffea*, comprising of two main cultivated species *C. arabica* (the only tetraploid species with $2n=44$) and *C. canephora* (diploid species having $2n=22$), and more than 100 related wild species that differ greatly in their size, morphology, adaptation and geographical distribution. In addition there are many species belonging to the genus *Psilanthus* that is the sister genera of *Coffea* in the tribe *Coffeae* of *Rubiaceae* family. Importantly, many of these diverse species hybridize readily with each other and thus are a rich and valuable source of desirable genetic variability for improvement of cultivated coffee germplasm. There are a total of 15 *Coffea* species (including the 2 cultivated forms) and 4 indigenous species putatively belonging to *Psilanthus* genus, that are available in the coffee gene bank maintained in India. The present investigation was carried out to elucidate the phylogenetic relationships of these species using DNA markers. The representative samples of each species, individual as well as pooled, were amplified using 20 arbitrary decamer primers and 10 ISSR primers to generate Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) markers, respectively. The resulting markers were scored as presence/absence binary data that were used directly in Wanger parsimony method (MIX) and also for calculating Nei's distance matrices followed by distance based clustering algorithms (UPGMA and NJ), to infer phylogenetic relationships. The most parsimonious trees were used to construct a strict consensus tree. The bootstrap analysis was done in each case to appraise the consistency of the resulting clusters.

The study largely supports the generic affinities of some of the species that were analyzed earlier and provides newer insights about the taxonomic status of six of the species that were analyzed for the first time. Among the diploid species, as expected *C. canephora* was found to be phylogenetically most close to *C. arabica* followed by the cluster comprising of *C. congensis*, *C. liberica* and *C. dewevreii*. Four of the Pachycoffea species that were studied for the first time in the present study appeared as a coherent cluster within which a strong geographical correspondence was observed for the species. The results further validate the placement of the two analyzed indigenous species under the related Paracoffea genus *Psilanthus*.

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