Molecular identification of selected Dendrobium cultivars

W. E. R. Silva, R. Attanayake and S. P. Senanayake*

Department of Botany, Faculty of Science, University of Kelaniya, Sri Lanka
Email: priyangi@kln.ac.lk

The family Orchidaceae includes more than 25,000 species, and the genus *Dendrobium* consists of over 1,450 species around the world. Today many unidentified Orchid cultivars are available in the market and growers use different vernacular names. Authentication of parental materials is important for conservation and selecting cultivars as parental materials in breeding experiments. However, Dendrobiums are well known for their difficulty in identification due to vegetative similarity among different species and morphological dissimilarity among same species. Since DNA barcoding has been proposed to be one of the most promising tools for accurate identification of taxa, this project was initiated with the objectives of identifying selected commercial Dendrobium cultivars and to determine their phylogenetic relatedness. Twelve commercial Dendrobium cultivars were selected based on the flower morphology. Genomic DNA was extracted from young leaves using a modified Cetyltrimethylammonium bromide (CTAB) method. PCR amplification of DNA was performed using universal ITS 1 and ITS 4 primers. PCR products were sequenced at Genetech Pvt. Ltd., Sri Lanka. Sequences were manually edited using BioEdit software version 7.1.9. Out of 12 samples, 9 samples produced non-specific amplification and only 3 samples produced good quality sequences of nearly 700 bp length. BLAST analysis was performed and sequences were deposited in the GenBank (MF535341, MF535342, and MF535343). Sequences of the current study with other 26 sequences from the GenBank were used in maximum likelihood analysis implemented in Mega 6.0 software with 1000 bootstrap replications. *Liparis kumokiri* (AY907087) was used as the out group for the analysis. Dendrobium cultivar Triple Fantacy (MF535341) resulted 99% similarity to *Dendrobium bigibbum* var. *bigibbum* and *Dendrobium bigibbum* var. *superbum* (KP142215 and KP142213) in the BLAST analysis. Unidentified Dendrobium cultivar (MF535343) was 94% similar to *Dendrobium bigibbum* var. *bigibbum* (KP142215) and *Dendrobium bigibbum* var. *superbum* (KP142214). In addition, both Triple Fantacy and unidentified Dendrobium cultivar, were clustered together with *Dendrobium bigibbum* var. *bigibbum* and *Dendrobium bigibbum* var. *superbum*. Therefore, Dendrobium cultivars, both Triple Fantacy and unidentified Dendrobium cultivar were identified up to species level as *Dendrobium bigibbum*. Dendrobium cv. Thailand Tommy (MF535342) resulted 99% similarity to *Dendrobium nindii* (AY239985) and clustered with *Dendrobium nindii* with 99% bootstrap support. Thus, the identity of Dendrobium cv. Thailand Tommy was confirmed to be *Dendrobium nindii*. In summary, DNA barcoding with ITS sequence was successfully used in resolving species identity of selected commercial Dendrobium cultivars in Sri Lanka.

Keywords: Dendrobium, DNA barcoding, rDNA-ITS