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Phylogenetic relationships of selected commercial *Dendrobium* hybrids in Sri Lanka

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Nuclear rDNA-ITS regions and chloroplast *matK* genes are useful in delineating plant species. In this study, genetic relatedness of eight commercial *Dendrobium* hybrids (A-H) with a range of attractive flower colours was studied using nuclear rDNA-ITS and chloroplast *matK* sequences. Genomic DNA was extracted from fresh, young leaves using a modified cetyltrimethylammonium bromide based protocol. rDNA-ITS and *matK* were amplified using PCR in 25 µl reactions containing 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.25 U Taq DNA polymerase, 0.4 µM forward and reverse primers and 1.00 µl of genomic DNA. The optimized thermal cycling conditions were initial denaturation at 95 ºC for 5 minutes, 35 (rDNA-ITS) and 40 (*matK*) cycles of denaturation at 95 ºC for 40 seconds, annealing at 55 ºC (rDNA-ITS) and 48 ºC (*matK*) for 40 seconds, extension at 72 ºC for 40 seconds and final extension at 72 ºC for 10 minutes. rDNA-ITS and *matK* PCR products were subjected to Sanger sequencing. Sequences were manually edited using BioEdit 7.0.5.3. and ContigExpress software. Sequences were aligned to the nucleotide database in the National Center for Biotechnology Information using mega BLAST program. Forty-three related sequences were obtained from GenBank and the sequences were aligned using ClustalW implemented in MEGA 7.0.26 software. Phylogenetic analysis was performed by generating trees of ITS, *matK* and concatenated sequences of ITS and *matK*. The phylogenetic relationships were analyzed using Maximum Likelihood analysis with 1000 bootstrap replications. *Phalaenopsis aphrodite*, *Liparis kumokiri* and *Malaxis spicata* were used as outgroups. Combined gene-tree was estimated using RAxML-HPC BlackBox tool in CIPRES Science Gateway platform. Resulting trees were viewed using Figtree v1.4.3. In the combined gene tree, selected hybrids were clustered into two distinct groups. *Dendrobium* hybrids A, B, C, E and F were clustered with *Dendrobium bigibbum* var *bigibbum* and *Dendrobium phalaenopsis* (72% bootstrap). Hybrids G, H and D were clustered with *Dendrobium nindii* and *Dendrobium taurinum* (79% bootstrap). In *matK* gene tree, all the selected hybrids were clustered together with *Dendrobium kingianum* (90% bootstrap). In rDNA-ITS gene tree, hybrids A, B, C, E and F were clustered with *Dendrobium bigibbum* var *bigibbum* and *Dendrobium phalaenopsis* while hybrids D, G and H were clustered with *Dendrobium taurinum* and *Dendrobium nindii* (81% bootstrap). Therefore, though high variation in floral morphology is observed among the selected imported commercial hybrids, they were represented from a narrow genetic background. This is an indicative of genetic bottleneck most likely due to selective breeding and it is important to incorporate more diverse varieties in future breeding programs to maintain a diverse genetic background.

Keywords: *Dendrobium*, rDNA-ITS, *matK*

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