Chloroplast DNA barcoding of *Spathoglottis* species for genetic conservation

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**Abstract:**
*Spathoglottis* is one of the most popular terrestrial orchids because it is attractive, easy to cultivate and continuously blooms throughout the year. It is a native orchid of Malaysia and listed as an endangered species. Therefore, this species needs to be conserved for commercial and environmental purposes. DNA barcoding is a technique for species identification, which uses a short DNA sequence from a standard and agreed-upon position in the genome. This technique plays an important role in biodiversity conservation for animals but is not yet well established in plants. Chloroplast DNA was chosen for this study aimed at building a DNA barcoding of *Spathoglottis* species. Seven species of *Spathoglottis* were used to carry out this study. They were *Spathoglottis plicata*, *Spathoglottis gracilis*, *Spathoglottis aurea*, *Spathoglottis plicata alba*, *Spathoglottis unguiculata*, *Spathoglottis kimbaliana* and a *Spathoglottis* hybrid. Eight regions (i.e., *accD*, *matK*, *ndhJ*, *rpoB*, *rpoC1*, *ycf5*, *rbcL-a* and *trnH-psbA*) in the chloroplast genome and two regions (i.e., *ITS1* and *ITS2*) in the nuclear genome were selected and screened in order to define a universal barcoding region across all the seven selected species. The study found that the four chloroplast regions (i.e., *matK*, *rbcL-a*, *rpoB* and *rpoC1*) were successfully amplified from all the tested species. The DNA sequencing from each chloroplast region was compared among the species and analyzed to differentiate the intra- and inter-genetic variations. The analysis of chloroplast regions was done for single regions and as a combination of the four regions. The haplotypes for the multiple analyses showed sufficiently high resolution to enable differentiation between the selected *Spathoglottis* species. In conclusion, this study showed that the chloroplast DNA regions had high potential to be developed for DNA barcoding of *Spathoglottis* species. This is the first step towards the development of universal DNA barcoding technique for all native orchids in Malaysia.