Abstract

Paphiopedilum concolor (Lindl.) Pfitzer is economically important. Moreover, wild populations of this orchid are threatened with extinction. However, there have been very few studies on genetic diversity of this orchid species using molecular tools, partly due to difficulties in obtaining good quality of DNA suitable for polymerase chain reactions (PCRs). This study tested three DNA extraction methods for purification of genomic DNA from *P. concolor* leaves, i.e., GF-1 Plant DNA Extraction Kit (Vivantis Technologies), sodium dodecyl sulfate (SDS)-potassium acetate method, and cetyltrimethyl ammonium bromide (CTAB) method. It was found that the SDS-potassium acetate method produced the highest yield of DNA with the best quality. The CTAB method produced considerably less yield with greater co-purified contaminants that absorbed strongly at 230 nm. The GF-1 Plant DNA Extraction Kit produced the lowest yield of DNA that contained high proportion of contaminants with absorption at 230 nm. Quality of the DNA was also tested by PCRs. The DNA samples suitable for the PCRs were those prepared by the SDS-potassium acetate and the CTAB methods, but not the samples prepared by the GF-1 Plant DNA Extraction Kit. According to this study, the SDS-potassium acetate method was the best method for purification of DNA from fresh leaves of *P. concolor*. The forward primer designed for amplification of the Internal Transcribe Spacer (ITS) region of *P. concolor* was named ITSP-F. Test of this primer and the reverse primer, ITS4 at annealing temperature 55°C, resulted in high quantity of single product suitable for DNA sequencing. Efficient DNA extraction method and ITS-specific primer would be vital to the development of molecular markers in the study of genetic diversity which would be beneficial to the conservation and varietal improvement programs of *P. concolor* and closely-related orchids.