Optimization of PCR Protocols for Amplification of ITS1, ITS2, rbcL and matK Genomic Regions of *Alpinia galanga* and Related Species

H.M.A.M. Herath, L.M.H.R. Alwis and G.N. Ranasinghe

Department of Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka

Polymerase Chain Reaction (PCR) is a method widely used in molecular biology to make many copies of a specific DNA segment of which a single copy of a DNA sequence is exponentially amplified to generate many copies. Conditions required to amplify specific genomic regions are different for plant to plant. The aim of this study was to optimize PCR protocols to amplify ITS1, ITS2, rbcL and matK genomic regions of *Alpinia galanga, Alpinia calcarata, Alpinia malaccensis, Alpinia purpurata, Hedychium coronarium and Hedychium coccineum*. The protocols of PCR were optimized by changing concentrations of template DNA, dNTPs, Mg^{2+}, Taq DNA polymerase, primer and primer annealing temperatures. At optimized conditions, reproducible amplifiable products were obtained and observed using 2% agarose gel electrophoresis. All genomic regions showed amplification in *Alpinia galanga* and other related species. For better performance in PCR high quality genomic DNA was required. In low annealing temperature and high primer concentrations primer dimers were observed. The developed PCR protocol was a total volume of 30 µl of PCR reaction mixture contained a final concentration of 1x buffer, 0.2 mM Mg^{2+}, 2 U of Taq DNA Polymerase. For ITS1 final forward and reverse primer concentrations were 3 µM. For ITS2, rbcL and matK final concentrations of forward and reverse primers were 1 µM. The optimized PCR program was; initial denaturation at 94 ^{0}C for 5 minutes, 42 cycles of denaturation at 94 ^{0}C for 1 minutes, annealed at 56 ^{0}C for 1 minutes (ITS1), 54 ^{0} C for 1 minute (ITS2 and rbcL) and 47 ^{0}C for 1 minute (matK), extension at 72 ^{0}C for 2 minutes followed by final extension step at 72 ^{0}C for 7 minutes. The protocols established could be employed in producing reproducible DNA fragments for determining the diversity of *Alpinia galanga* and related species and studies relating to DNA barcoding and phylogeny.

Keywords: *Alpinia,* ITS1, ITS2, matK, rbcL, PCR