ISBN: 978-93-83083-65-7

A Simple and Efficient Method for High Quality Genomic DNA Isolation and RAPD-PCR Optimization from Cannabis Sativa Containing High Amount of Polyphenols

Deepika Dahiya

Assistant Professor, Department of Biotechnology Shoolini University of Biotechnology

Abstract Isolation of high quality DNA from plants with higher secondary metabolite content has been a challenging effort since long. Present methods of DNA isolation from plants have their own limitations such as highly sophisticated procedures, contamination of DNA with secondary metabolites and a lesser amount of isolated DNA. A novel method of DNA isolation has been optimized that is quick, inexpensive and consistent protocol for extraction of DNA as compared to contemporary counterparts. The method involves modified CTAB extraction using, 200 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% (w/v) CTAB, 2% (v/v), β- Mercaptoethanol (added just before use) and 500 mg of PVP (100 mg/ gm leaf sample). DNA obtained; using this method was intact and of quality (260/280 = 1.8 ± 0.02) routinely yielding 89.5 µg/ml. DNA extracted was used to optimize RAPD-PCR, using 50 ng DNA, 1.5 mM $MgCl_2$, 0.75 µM of RAPD primer (10 µM), 0.75 mM of dNTP's mix (80 mM stock) and 1.5 U *Taq* DNA Polymerase for 20 µL PCR reaction volume per tube. Annealing temperature of 42^0 C resulted in reproducible amplified products free from contamination. The results indicate that the optimized DNA isolation process and RAPD-PCR protocol can be used for further work on genetic diversity analysis, phylogenetic studies and also in developing conservation strategies for this plant and for reducing confusion among different *Cannabis* varieties to be used separately for medicinal and fibre purpose.

Keywords: Cannabis sativa; Secondary metabolites; PCR amplification; RAPD; DNA extraction