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A modified CTAB method for extraction of genomic DNA from *Piper nigrum* L. (black pepper)

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Black pepper (*Piper nigrum* L) is known as the "King of spices" and belongs to the family, *Piperaceae*. Isolation of high quality DNA from pepper plant tissues is difficult due to high levels of polysaccharide and polyphenolic compound formation during homogenization of tissues. Contamination of DNA by polysaccharide and polyphenols inhibit most of the commonly used molecular biological enzymes, such as polymerases, ligases and restriction endonucleases. Most of the commonly used plant DNA extraction procedures fail to extract high quality DNA from pepper. The objective of this study was to isolate high quality DNA from black pepper by using a modified CTAB method.

The modified CTAB method was practiced with different plant types which were obtained from the local black pepper cultivar of GK49. Tender leaf, mature leaf, stem and root samples (2 g from each) were separately ground to a fine powder in liquid nitrogen. The ground sample was added to 20 ml of pre warmed (65 ^{0}C) extraction buffer constituted with 2 % CTAB (w/v), 1 % PVP – 40,000 (w/v), 1.4 M NaCl, 20 mM EDTA ; pH = 8.0, 0.5% β mercaptoethanol and 125 µg/ml of RNase. Samples were incubated at 65 ^{0}C for 30 min. The incubated samples were allowed to cool at room temperature and extracted twice with chloroform / isoamyl alcohol 24:1 mixture (CHISM) and centrifuged at 4500 rpm for 15 min. The aqueous layer was separated and DNA was then precipitated by adding 0.7 volume of isopropanol to the aqueous layer followed by centrifugation at 13,000 rpm for 15 min. The pellet was washed with 70% ethanol, dried and dissolved in TE buffer. The CTAB method described by Doyle and Doyle (1990) was also practiced as a control. The number of replicates in each method was 20.

Brown colour large gelatinous DNA pellets were given by the CTAB method and white colour tiny pellets were given by the modified CTAB method. Brown colour DNA occurred due to contamination of polyphenol. The amount of β mercaptoethanol was increased up to 0.5% and 1% PVP was added to the extraction buffer of modified CTAB protocol to improve the quality of DNA by reducing polyphenol contaminations. A large gelatinous DNA pellet was obtained from the CTAB method due to embedding of polysaccharides on DNA. CHISM extraction was practiced twice in the modified CTAB method to reduce polysaccharide contamination. In this study the highest yield of DNA (5 µg) was obtained by the modified CTAB method from tender leaves and this DNA also gave significant results for AFLP analysis.

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