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DEVELOPMENT OF AN EFFICIENT GENOMIC DNA EXTRACTION PROTOCOL AND IDENTIFICATION OF TRUE HYBRIDS OF MANGO USING SSR MARKERS

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Mango is one of the most popular tropical fruits in the world. Due to high demand, development of hybrid mango is one of the research objectives in Department of Agriculture. Compared to other woody plants, extraction of high-quality DNA is more difficult in mango due to the presence of high concentrations of phenolic compounds, proteins, polysaccharides and other secondary metabolites. Present study was conducted to optimize the DNA extraction protocol for mango and to identify true hybrids using SSR markers. Total genomic DNA extraction was done using three different leaf maturity stages (dark brown colored, soft and partially expanded; light brown, soft and expanded; greenish, semi soft and fully expanded) and using four different protocols reported previously with modifications. Out of four DNA extraction protocols, three protocols, followed to remove phenolic compounds and proteins, have shown intense genomic DNA bands in agarose gel electrophoresis at all three leaf stages. Use of 2-3% β-mercaptaethanol, different combinations of 2-2.5 % PVP, 1 % SDS and more than one phenol treatment can be identified as the sources to remove phenolic compounds, proteins, polysaccharides and other contaminants from leaf materials when extracting DNA. PCR amplification was done using extracted genomic DNA as templates. Based on the quality of DNA and the resolution of amplified PCR products, protocol 01 that contained 1.4M NaCl, 0.2 % (v/v) β-mercaptoethanol, 2% PVP, 2 % CTAB, phenol, 10 mM ammonium acetate and protocol 04 that contained 3M NaCl, 3 % (v/v) β-mercaptoethanol, 2.5 % PVP and 4 % CTAB, 1 mg/ml proteinase K, 3M sodium acetate were the most suitable protocols to extract DNA from mango. No differences were observed in DNA quantity and quality of three different leaf maturity stages between these two protocols. Hybridity testing of six mango crosses was performed using three SSR primers. The SSR markers used in this study were not enough to discriminate between two parents of six mango crosses. Further experiments with more polymorphic SSR markers are necessary for the hybridity assessment of the tested mango crosses.