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Optimization of DNA Extraction and PCR Protocol to Explore Molecular Polymorphism of Artocarpus heterophyllus

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Abstract

The *Artocarpus heterophyllus*, or jackfruit, is a tropical fruit tree that belongs to the Moraceae family and grows in Bangladesh, the East Indies, Malaysia, Southern China, India, Burma, and Sri Lanka. This crop is extremely valuable due to its ability to produce wood, medicine, and food. While morphological and molecular marker studies on the jack tree have been performed in other countries, there is a noticeable absence of similar studies in Sri Lanka. Recent discoveries of molecular markers have greatly expanded the possibilities for in-depth genetic research and increased the effectiveness of plant breeding initiatives. The current study was performed to optimize the DNA extraction procedure and PCR protocol of 10 jack tree varieties in Colombo, Gampaha, and Kalutara districts of the Western province of Sri Lanka to fill this gap. DNA was extracted using the standard CTAB method, which was improved with 1% 2-mercaptoethanol. After DNA purification, a NanoDropTM spectrophotometer was used to quantify the DNA. The ISSR analysis used primer (TC)10G 5'TCTCTCTCTCTCTCTCTCTCG3' and the amplified DNA fragments were confirmed and visualized using gel electrophoresis. In molecular study, the best extraction efficiency was shown by 2nd node and 3rd node leaf samples weighing between 150 300 mg, because of the relatively low polyphenol contents in immature leaves. The effective prevention of polyphenol oxidation of DNA results in clear bands in the gel with the use of 1% 2 βmercaptoethanol in the extraction procedure. DNAs with RNA contaminations were purified by using 1% RNaseA. 3µl of RNaseA was added to each 50µl DNA sample. DNA yield ranges from 197.8 to 898.2 ng/µl, and purity ranges from 1.50 to 1.69, after genomic DNA samples tested between 260 and 280 nm in wavelength. In PCR amplification, the best results were obtained in a 25µl reaction mixture using 10X PCR buffer with 17.5mM $MgCl_2$, 10mM dNTP mixture, 10pM ISSR primer, 1U Taq polymerase, and 40ng of genomic DNA. The thermocycler was programmed for an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute 30 seconds, extension at 72°C for 1 minute 50 seconds, and a final extension at 72°C for 10 minutes. PCR products were resolved in 2% (w/v) agarose gel, visualized and documented using a Biobase gel documentation system. Four bands with sizes ranging from 250 to 500 bp obtained from the experiment show the target PCR amplification. Furthermore, the distinct band visible in the spermidine-treated sample indicates that spermidine reduces the PCR inhibitory effects of phenolics. Further studies are required to gain a better understanding of this species, providing useful insights for agricultural practices, conservation efforts, and future genetic research.

Key words: DNA extraction; ISSR; Jackfruit; Molecular markers; PCR

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