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ISOLATION AND AMPLIFICATION OF GENOMIC DNA FROM POWDERED ADMIXTURE OF BLACK PEPPER AND ITS MAJOR ADULTERANTS

H.M.P. Herath[1], M.A.L.M. Peiris[2] and W.R.P. Wijesinghe[1,2*]

[1]*Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka*
[2]*Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka*
[*]*priyangaw@pdn.ac.lk*

Ceylon black pepper (*Piper nigrum* L.) is traded internationally at a premium price as it is quite rich in piperine. Pepper industry in Sri Lanka faces a major threat due to the adulteration with inferior substitutes, such as papaya seeds and chili. Based on morphological characteristics, it is difficult to distinguish these adulterants since they are converted to visually undetected forms during processing. The use of molecular approaches is the most attractive strategy under these circumstances. However, it is challenging to extract sufficient amount of DNA from this admixture since pepper and papaya seeds are recalcitrant storage tissues containing high level of polyphenols, polysaccharides and proteins. Therefore, isolated DNA is contaminated with a brownish, sticky and viscous matrix. Current protocols for isolation of DNA from black pepper require specific chemicals, such as PEG 6000, liquid nitrogen and commercial kits, and labor-intensive steps to eliminate inhibitors, such as polyphenols. In this research, the original CTAB DNA extraction protocol devised by Doyle and Doyle was modified by increasing the concentration of β-mercaptoethanol up to 0.25%, PVP 2% and a phenol extraction step, to yield high quality DNA from black pepper, papaya and chili. The concentration of DNA of all samples obtained from 0.2 g of starting material exceeded 250 ng μL^{-1}. The isolated DNA was PCR amplified using universal primers, *rbcL* and *psbA-trnH*, and the results supported the efficacy of the protocol to extract good quality DNA for further molecular analysis. The capability of PCR amplification from any material including powdered admixture of black pepper, papaya and chili affirms the validity of the tests being developed in adulterant detection. This may also be applicable to other adulterants in traded black pepper including wild varieties, such as *P. galeatum* and *P. attenuatum*.

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