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High-resolution melting traceability of black pepper adulteration with papaya seeds, chili and/or other potential plants

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Black pepper (*Piper nigrum* L.) is a valuable medicinal spice and one of the premier exports in Sri Lanka. The black pepper industry in Sri Lanka faces a major threat due to the adulteration of Ceylon pepper with inferior quality substitutes like papaya seeds and chili powder. Therefore, frequent testing of black pepper products is essential to retain the reputation of Ceylon pepper. Application of morphological and chemical methods have limitations in adulteration detection, especially for admixtures in powdery and processed forms. Available molecular techniques also have limitations of being expensive, time consuming and less reliable. The research was aimed at developing an assay based on DNA barcoding coupled High Resolution Melting Analysis (Bar-HRM) which is sequencing-free, reliable, yet faster and more economical than DNA barcoding to report the spice authentication results. DNA isolation from dried storage tissues is extremely difficult due to the presence of polysaccharides, polyphenols, proteins like compounds and due to the scarcity of DNA. A modified CTAB method was developed along with a phenol extraction to extract and amplify the required DNA regions from the dried processed admixture of black pepper and its major adulterants. Although the DNA quality of the product varies among different samples, the capability of PCR amplification from any material including powdered admixture affirms the validity of the tests being developed in adulterant detection. Two novel gene-specific primer pairs were designed targeting the assay development and both newly developed *rbc*L markers were successful in PCR amplification. Subsequently, a relatively novel, high throughput technique called Bar-HRM was applied to detect the black pepper adulteration. According to the results melting profiles of pure samples of black pepper, papaya and chili were clearly separated so that they can be differentiated by HRM analysis. HRM data were further examined using Principal Component Analysis (PCA) and the results showed that HRM analysis successfully differentiates three species, separating them into three different clusters. Then the optimized HRM conditions were applied to admixtures and HRM curves of the adulterated samples were clearly deviated from the pure samples. It could be concluded that developed technique is a very first HRM based high-throughput system to authenticate black pepper adulteration with papaya seeds and chili. Although as a proof of concept this technique was developed to detect papaya and chili adulteration, novel system has the potential to detect other black pepper adulterants as well.

Keywords: Admixture, Adulteration, Black pepper, HRM analysis, *rbc*L

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