

An inexpensive and rapid method for extracting papilionoid genomic DNA from herbarium specimens

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ABSTRACT. Three DNA extraction protocols were compared for their ability to yield DNA from the leaves of herbarium specimens of nine species from nine genera of the Papilionoideae. We tested two protocols that use classic procedures for lysis and purification with cetyl trimethylammonium bromide (CTAB); a third protocol used a Nucleospin Plant kit. DNA obtained from all three procedures was quantified and tested by PCR. Test results indicated the superiority of one of the CTAB protocols. We made some modifications, developing a protocol that produced high-quality DNA from all nine species. The modification involved the use of a lower EDTA concentration (20 mM instead of 50 mM) and a higher β -mercaptoethanol concentration (1% instead of 0.4%) in the extraction buffer. The modified protocol avoids the necessity for

a second DNA precipitation step. This new CTAB protocol includes the use of 1.4 M NaCl, 20 mM EDTA and 1% β -mercaptoethanol in the extraction; DNA precipitation time is reduced. A reduction in contaminating metabolites (such as PCR inhibitors) in the sample mixtures and lower costs for reagents are characteristics of this modified protocol; the cost of analysis per sample was lowered, compared to previous options. The quality of DNA was suitable for PCR amplification. This is a practical alternative to more difficult, time-consuming and expensive protocols.

Key words: Papilionoideae; DNA extraction; Herbarium specimen; PCR amplification; CTAB