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Isolation of retro-transcribed RNA from *in vitro* *Mycosphaerella fijiensis*-infected banana leaves

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ABSTRACT. High polyphenol and polysaccharide levels in plant tissues such as banana fruit and leaves constitute a significant challenge to the extraction of sufficient amounts of high-quality RNA required for cDNA library synthesis and molecular analysis. To determine their comparative effectiveness at eliminating polyphenols, polysaccharides and proteins, three protocols for RNA extraction from *in vitro* banana plantlet leaves were tested: Concert™ Plant RNA isolation kit, a small-scale protocol based on Valderrama-Cháirez, and a modified version of the Valderrama-Cháirez protocol. RNA quantity and purity were evaluated by UV-spectrophotometry using DEPC-treated water and Tris-HCl, pH 7.5. Purity was greater using Tris-HCl. The Concert™ Plant protocol produced the poorest quality RNA. Reverse transcription into cDNAs from RNA isolated from *in vitro* banana plantlet leaves infected with *Mycosphaerella fijiensis* using the modified Valderrama-Cháirez protocol, followed by PCR using primers designed against γ-actin from banana and *M. fijiensis*, yielded products of the anticipated size. In addition, this protocol reduced the processing time, lowered costs, used

less expensive equipment, and could be used for other plants that have the same problems with high polyphenol and polysaccharide levels.

Key words: Banana; *Mycosphaerella fijiensis*; Polyphenols; Polysaccharides; RNA isolation; Black leaf streak disease

