

Comparison of small scale methods for the rapid and efficient extraction of mitochondrial DNA from wheat crop suitable for down-stream processes

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ABSTRACT. We evaluated and compared 2 mitochondrial DNA (mtDNA) extraction methods in terms of DNA quality and success of subsequent polymerase chain reaction (PCR) amplifications from etiolated leaves of wheat crop (*Triticum aestivum*). mtDNA extraction is difficult because the presence of metabolites interfere with DNA isolation procedures and downstream applications such as DNA restriction, amplification, and cloning. The method (with modification) involved inactivation of genomic DNA by DNase I enzyme, RNA by RNase enzyme, contaminant proteins by using proteinase K, and precipitation of polysaccharides in the presence of a high salt concentration. The DNase I and RNA enzyme ratio was adjusted to 10:8 μ L. The purity of mtDNA was confirmed by PCR amplification of genomic, mitochondrial, and chloroplast (*rbcL*) gene. The mitochondrial COXIII gene of 400 bp was amplified; the β -actin and chloroplast genes were not amplified. A_{260}/A_{280} (1.89) and A_{260}/A_{230} (2.07) ratios were calculated using a spectrophotometer. The isolated mtDNA was amenable to amplification and

restriction digestion. The technique is fast, reproducible, and suitable for PCR-based markers.

Key words: Extraction method; Mitochondrial DNA; Molecular markers; *Triticum aestivum*