

Evaluation of RNA extraction methods in rice and their application in expression analysis of resistance genes against *Magnaporthe oryzae*

ABSTRACT

Extraction of RNA of high quality and integrity is essential for gene expression studies and all downstream RNA-based techniques. The leaves of 16 merit Malaysian rice varieties were used to isolate total RNA using five different methods. The quantity, quality and integrity of extracted RNA were confirmed using three different means. The ratios of A260/280 ranged from 2.12 to 2.20. Electrophoresis (1.5% agarose gel) was performed, illustrating intact and sharp bands representing the 28S, 18S, 5.8S and 5S ribosomal subunits of RNA, presenting intact RNA. RNA quality was verified using semi-quantitative polymerase chain reaction (sqPCR). The objective of this study was to identify different genes involved in the resistance of rice plants using high-quality RNA extracted 31 h after inoculation of *Magnaporthe oryzae* pathotype P7.2. The expression levels of eight blast resistance genes, Pikh, Pib, Pita, Pi21, Pi9, Os11gRGA8, OsWRKY22 and OsWRKY45, were evaluated by real-time PCR (RT-PCR). Real-time PCR was performed to identify candidate genes using RNA extracted by the TRIzol method, which showed the highest score compared with other methods in terms of RNA quantity, purity and integrity. In addition, the results of real-time PCR confirmed that the up-regulation of seven blast resistance genes may confer stronger resistance for the MR 276 variety against *M. oryzae* pathotype P7.2.

Keyword: *Magnaporthe oryzae*; Real-time PCR; Resistance genes; Semi-quantitative PCR