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Comparison of different RNA extraction protocols: An optimized RNA extraction protocol for tea leaves [*Camellia sinensis* (L.) O. Kuntze]

V. Keerthika[1]*, M. A. B. Ranatunga[2], D. Perera[3] and H. Herath[1]

[1]University of Kelaniya
[2]Plant Breeding Division,Tea Research Institute of Sri Lanka, Talawakelle
[3]Coconut Research Institute, Lunuwila
*keertigavive@yahoo.com

Extracting high-quality RNA is critical for downstream applications such as quantitative real time PCR (qRT PCR), RNA sequence based transcriptomics and a prerequisite for ensuring representation of all expressed genes in a cDNA library. Tea is a popular non- alcoholic beverage crop and tissues contain abundant polysaccharides, phenolic compounds and other metabolites, which hinder isolation of high-quality RNA. Tender two leaves and a bud harvested is the economically important tissues of tea. A large number of extraction protocols have been exploited or modified for tea, *viz* commercial kits, CTAB-based methods and SDS-based methods. However; difficulties were encountered in terms of purity and quantity of isolated RNA, while some of the methods were time-consuming. Hence, the present study was aimed to optimize protocol/s for extracting good quality RNA suitable for downstream applications from two leaves and a bud tissue of tea. Two different RNA extractions protocols based on CTAB and hot borate with modifications along with three commercial RNA extraction kits were used to extract RNA from two tea cultivars. Out of which, a CTAB-based protocol optimized for pomegranate plant tissues with minor modifications resulted in the highest RNA yield, varying from 524 ± 4 to 776 ± 16 µg/ml for cultivars TRI 2023 and TRI 2025 and high integrity as confirmed by Gel electrophoresis (distinct and visible 28S rRNA and 18S rRNA bands). The extracted RNA was further used for cDNA synthesis, expression of 18s house keeping gene through Realtime-polymerase chain reaction (qRT-PCR). Results showed that the yield and quality of total RNA extracted were suitable for qRT-PCR, depicting the quality of RNA for the downstream applications. Commercially available extraction kits despite of giving sufficient amount of RNA, had lesser yield and quality when compared to the CTAB based method. The protocol optimized for pomegranate was modified with omitting washing the RNA pellet with LiCl and replacing the extraction buffer (CTAB, PVP, NaCl, EDTA, Tris HCl, Spermidine, β-mercaptoethanol) with extraction buffer used for grapevines. This protocol enables successful isolation of RNA from two leaves and a bud tissues of tea within two days without the use of toxic and expensive chemicals such as phenol, guanidium isothiocyanate and guanidium hydrochloride. The protocol is efficient, simple, and reproducible and is therefore recommended for RNA extraction from plants with high polyphenol contents.

Keywords: *Camellia sinensis* (L.) O. Kuntze, qRT PCR, Downstream applications, RNA integrity

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