Optimization of DNA Extraction Protocol for *Mormodica charantia*

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Bitter gourd is a monoecious crop that belongs to the Curcubitaceae family. It has significant agricultural and medicinal value. High levels of bioactive substances such as phenolic compounds and polysaccharides, along with other contaminants, restrict obtaining high-quality and higher-quantity DNA from these plants. To minimize contaminants and to ensure the integrity of extracted DNA, extraction protocols must be modified. The aim of this study is to optimize the DNA extraction protocol for *Mormodica charantia* by using variations of the CTAB method. This protocol mainly depends on the buffer that is used to lyse cells and separate DNA from other cellular compounds with phenol, chloroform, and isoamyl alcohol. Three variations of the protocol were followed. Tender leaves were used for DNA extraction. UV spectrophotometry was used in the following wavelengths, A230, A260, A280, and A320, to evaluate the quantity and quality of the isolated DNA obtained through each protocol, and the results were compared. The results from UV spectrophotometry were further validated by agarose gel electrophoresis. The first protocol was the standard CTAB extraction method, which yielded 0.884±0.245 (µg/150mg) DNA with a concentration of 17.68±4.89 (ng/µl). The second protocol was the modified CTAB with cold treatment. Leaves were ground in a cold motor and pestle, which was surrounded by ice cubes, that yielded 0.3±0.079 (µg/150mg) DNA with a concentration of 5.994±1.582 (ng/µl). The third protocol was modified CTAB with cold treatment and RNAse treatment; this method yielded 0.461±0.265 (µg/150mg) DNA with a concentration of 13.23±6.92 (ng/µl). The optimized protocol 3 was found to be more suitable and comparatively gave high yields of pure DNA with minimal RNA contamination in the presence of the RNAse enzyme, as ensured by Agarose gel electrophoresis. Furthermore, the DNA templates generated using the modified method could facilitate genetic studies, marker-assisted breeding, and molecular breeding efforts in bitter gourd to enhance desirable traits in the future. This protocol can be further refined and applied to other plants that have similar biochemical properties.

Keywords: DNA extraction, *Momordica charantia*, Spectrophotometer, Gel Electrophoresis, CTAB