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Optimization of high throughput DNA extraction from fresh leaf tissues of wheat for PCR assay.

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We have checked various protocols and their modifications that were developed and used to isolate quality DNA from wheat in the past (Murray and Thompson 1980; Dellaporta et al. 1983; Saghai-Maroof et al. 1984; Rogers and Bendich 1985; Doyle and Doyle 1990; Suman et al. 1999; Warude et al. 2003; Sarwat et al. 2006; Deshmukh et al. 2007). Three reported protocols (Na-bisulphite, CTAB, and SDS) were used (with some modifications) to isolate and analyze DNA from *T. aestivum* using fresh and dried leaf samples. The basic aim was to optimize a protocol that may be rapid and inexpensive with high quality and throughput.

The DNA isolated using various extraction protocols was compared from preparation in terms of quantity and quality. The DNA obtained was not of sufficient quantity, and the quality was very poor, especially in case of dried leaf tissues in all the tested protocols. The preparations (including DNA) in the test tubes were highly viscous and dirty brown in color, which showed no or very faint bands (or smears of the bands) upon gel electrophoresis and there were no amplification products after PCR analysis. However, the results from the modified CTAB buffer method were encouraging and were far better than the rest of the tested protocols especially in case of fresh leaf tissue without liquid nitrogen. We have stopped further testing for the protocols except CTAB procedure. The modified CTAB buffer method of genomic DNA extraction from fresh leaf tissues of *T. aestivum* as further tested and refined to compare its efficiency in terms of quantity and quality of the DNA for various tissue types. The DNA concentration was measured in a spectrophotometer (UV/VIS), and an absorbance, i.e., A260/A280 ratio of 1.3, was obtained indicating high levels of contaminated proteins and polysaccharides. Total DNA isolated from fresh leaves and dried-seed powder of T. aestivum was checked by means of agarose gel electrophoresis. High-molecular-weight DNA of larger quantities and of good quality was obtained from fresh leaves without using liquid nitrogen and dried seed samples (Figs. 1 and 2). The purity of the DNA samples was confirmed by absorbance (A260/A280) ratio, which was 1.8.

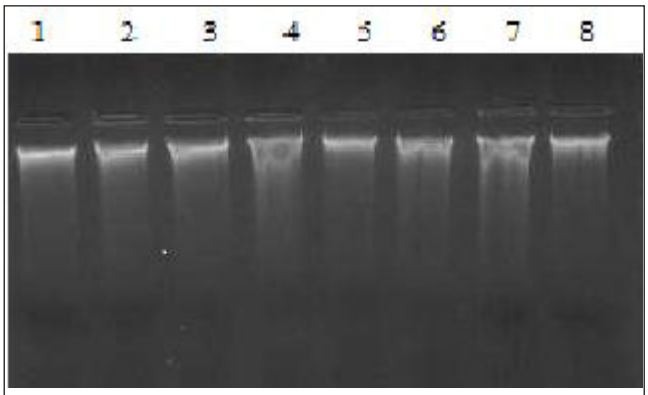

Fig. 1. Ethidium bromide-stained, agarose gel showing total DNA isolated from eight samples of fresh leaf tissue of *Triticum aestivum*.

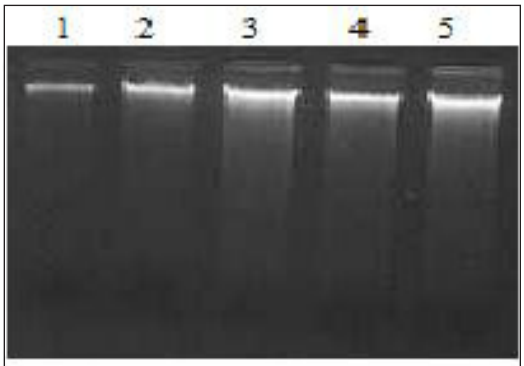

Fig. 2. Ethidium bromide-stained, agarose gel showing total DNA isolated from five samples of fresh leaves of *Triticum aestivum*.

DNA isolation is a primary and critical step for molecular analysis of any plant species. This process becomes even more difficult when the plant species contain high amounts of secondary metabolites and essential oils. These compounds are considered to be as contaminants that cause DNA degradation during preparation and therefore the extraction of genomic DNA from this plant is difficult. Polyvinylpyrrolidone (PVP), a compound known to suppress polyphenolic oxidation, has been used frequently in CTAB extraction protocols (Doyle and Doyle 1990). The modified CTAB buffer containing PVP was also employed to extract DNA from *T. aestivum* using liquid nitrogen (Hills and Van Staden 2002). However, Schneerman et al. (2002) reported that this compound did not significantly increase the yield or prevent contamination of the DNA. SDS-based extraction buffer is being used to break open the cells and isolate DNA, but the quality of DNA obtained is questioned due to precipitation of polysaccharides and proteins. In addition, the SDS might not bind with the proteins in the purification step, thus degrading the extracted DNA (Aljanabi et al. 1999; Deshmukh et al. 2007). Because SDS and

isoamylalcohol methods did not give significant results in either type of leaf tissues that we tested in the case of *T. aestivum*, it is hard to make any conclusive comments on their efficacy or effectiveness. However, the use of PVP in CTAB buffer did not improve the yield or quality rather we obtained significantly better results without its use in our experiment of DNA extraction.

Most of the protocols that we tested recommend extraction of DNA from fresh tissue, but for some areas of the world, the chemicals and resources that are routinely used in many protocols are too expensive to be used for routine DNA extraction. Therefore, it was necessary to establish an inexpensive and less time-consuming protocol for optimizing DNA extraction from fresh leaves of *T. aestivum*. We anticipate that this protocol will be adequate for extracting high-molecular-weight DNA from other species containing large amounts of secondary metabolites and essential oils.

The genetic characterization for improvement of cereals, including wheat, can be achieved by the use of molecular markers only if there is an efficient, rapid ,and less cost effective method of DNA extraction is available. To isolate high-quality DNA from leaf tissue of *T. aestivum*, various standard protocols were tested and modified. For DNA analysis, fresh and dried samples of wheat leaves were used. The DNA obtained from fresh-leaf tissue with a modified cetyltrimethylammonium bromide (CTAB) buffer protocol was of good quality, with no colored pigments or contaminants. We were able to obtain good quality DNA from fresh leaf tissue without using liquid nitrogen. A relatively large amount of DNA also was extracted from the dried tissue, but its quality was not as good as that from fresh leaves. The DNA extracted from fresh leaves was successfully amplified by PCR using STS markers. The same protocol will probably be useful for extracting high-molecular-weight DNA from other plant materials containing large amounts of secondary metabolites and essential oils.

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