

Genomic DNA Analysis from cereals: Isolation and Quantification

Muhammad Junaid^{1*}, Muhammad Abbas², Syed Asim Shah¹, Syed Masood Shah¹, Muhammad Alamgir², Yasir Ali², Abdul Jalal²

¹Department of Agriculture, University of Swabi, Anbar Swabi, Swabi, Khyber Pakhtunkhwa, Pakistan

²Department of Horticulture, University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan

*Corresponding author: Muhammad Junaid

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Email: mjunaidkhan@aup.edu.pk

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Abstract

The review briefly clarifies and summarize some of the major research works on cereals at molecular level. Some basic knowledge about cereals and the isolation and quantification of DNA along with various researches that are previously reported are gathered and presented briefly.

Keywords: cereals, isolation, quantification.

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INTRODUCTION

Cereals are grown all over the world the grain of wheat is used to make flour for breads, cakes, cookies, nodules, pasta and fractionated into grain components (starch, gluten, and oil) for human consumption. It is also used for fermentation to make alcohols and beer [1]. Today's changing environment due to Biotic and Abiotic stresses caused 25% losses in yield. In order to improve the yield, quality and quantity of the crops it has been argued that a new level of understanding the structure and function of genomes. Genome sequencing is a widely accepted mechanism for these objectives and enables more rapid genetic improvement [2]. Marker analysis have been used to characterize molecular diversity [3].

Genomic DNA has great importance in cloning, PCR template, genomic libraries, gene sequencing, analysis of genomic organization, detection of abnormalities or mutation, DNA fingerprinting, microarrays, genomic diversity, gene and structure analysis of genome composition [4]. Genomic DNA is used as a candidate for common reference especially to identify genes differentially expressed in various growth stages [5]. DNA extraction has unique challenges that requires kits to deals with carbohydrates, phenolic, tannins and other compounds abundant in plant tissues that can affect DNA quality and inhibit downstream reactions.

Previously reported research works on wheat genomic DNA quantification and isolation

Heinz Hahn and Doris Servos, (1979) conducted a study to describe the method which

facilitated the rapid mass isolation of nuclei from wheat embryos (wheat germ). Routinely 5×10^7 nuclei/g wheat embryos were obtained. The nuclei showed a DNA: RNA: protein ratio of 1: 0.3: 3.5. DNA-dependent RNA polymerases were solubilised from isolated nuclei by sonication in TGED buffer including 0.5 M (NH₄)₂SO₄ the isolation of all classes of RNA polymerases was achieved by affinity chromatography on heparin-Sepharose 4B containing 400 {tg covalently bound heparin/ml wet gel. Using heparin-Sepharose and DEAE-cellulose chromatography it could be shown that nuclei of quiescent wheat embryos contained all three classes of RNA polymerases (<<the tripartite transcriptive system>>). RNA polymerase II which was further purified showed the typical subunit pattern as revealed by 10-15 % acryl amide SDS-gel electrophoresis. The combination of heparin-Sepharose affinity chromatography and conventional chromatography on DEAE-cellulose and DEAE-Sephadex as a general procedure for the simultaneous isolation of all three classes of eukaryotic RNA polymerases is discussed.

Dai *et al.*, [6] evaluated a study on DNA methylation of cytosine residues, catalyzed by DNA methyltransferases, is suggested to play important roles in regulating gene expression and plant development. In this study, we isolated four wheat cDNA fragments and one cDNA with open reading frame encoding putative DNA methyltransferases and designated TaMET1, TaMET2a, TaMET2b, TaCMT, TaMET3, respectively. BLASTX searches and phylogenetic analysis suggested that five cDNA belonged to four classes (Dnmt1, Dnmt2, CMT and Dnmt3) of DNA methyltransferases

genes. TaMET2a encoded a protein of 376aa and contained eight of ten conserved motifs characteristic of DNA methyltransferase. Genomic sequence of TaMET2a was obtained and found to contain ten introns and eleven exons. The expression analysis of the five genes revealed that they were expressed in developing seed, during germination and various vegetative tissues, but in quite different abundance. It was interesting to note that TaMET1 and TaMET3 mRNAs were clearly detected in dry seeds. Moreover, the differential expression patterns of five genes were observed between wheat hybrid and its parents in leaf, stem and root of jointing stage, some were up-regulated while some others were down-regulated in the hybrid. We concluded that multiple wheat DNA methyltransferase genes were present and might play important roles in wheat growth and development.

Fredlund *et al.*, [7] conducted a trial for Identification of Fusarium species by traditional methods requires specific skill and experience and there is an increased interest for new molecular methods for identification and quantification of Fusarium from food and feed samples. Real-time PCR with probe technology (Taqman) can be used for the identification and quantification of several species of Fusarium from cereal grain samples. There are several critical steps that need to be considered when establishing a real-time PCR-based method for DNA quantification, including extraction of DNA from the samples. In this study, several DNA extraction methods were evaluated, including the DNeasy Plant Mini Spin Columns (Qiagen), the Bio robot EZ1 (Qiagen) with the DNeasy Blood and Tissue Kit (Qiagen), and the Fast-DNA® Spin Kit for Soil (Qbiogene). Parameters such as DNA quality and stability, PCR inhibitors, and PCR efficiency were investigated. Our results showed that all methods gave good PCR efficiency (above 90%) and DNA stability whereas the DNeasy Plant Mini Spin Columns in combination with sonication gave the best results with respect to Fusarium DNA yield. The modified DNeasy Plant Mini Spin protocol was used to analyse 31 wheat samples for the presence of *F. graminearum* and *F. culmorum*. The DNA level of *F. graminearum* could be correlated to the level of DON ($r^2 = 0.9$) and ZEN ($r^2 = 0.6$) whereas no correlation was found between *F. culmorum* and DON/ZEA. This shows that *F. graminearum* and not *F. culmorum*, was the main producer of DON in Swedish wheat during 2006.

Zhang *et al.*, [8] stated that DNA is one of the most basic and essential genetic materials in the field of molecular biology. To date, isolation of sufficient and good quality DNA is still a challenge for many plant species, though various DNA extraction methods have been published. In the present paper, a recycling DNA extraction method was proposed. The key step of this method was that a single plant tissue sample was recycled for DNA extraction for up to four times, and

correspondingly four DNA precipitations (termed as the 1st, 2nd, 3rd and 4th DNA sample, respectively) were conducted. This recycling step was integrated into the conventional CTAB DNA extraction method to establish a recycling CTAB method. This modified CTAB method was tested in eight plant species, wheat, sorghum, barley, corn, rice, *Brachypodium distachyon*, *Miscanthus sinensis* and Tung tree. The results showed that high-yield and good-quality DNA samples could be obtained by using this new method in all the eight plant species. The DNA samples were good templates for PCR amplification of both ISSR and SSR markers. The recycling method can be used in multiple plant species and can be integrated with multiple conventional DNA isolation methods, and thus is an effective and universal DNA isolation method.

Ahmed *et al.*, [9] studied that Polymerase chain reaction has found wide applications in modern research involving transformations and other genomic studies. For reproducible PCR results, however, the quantity and quality of template DNA is of considerable importance. A simple and efficient plant DNA extraction procedure for isolation of high-quality DNA from plant tissues is presented here. It requires maceration of plant tissue of about 1.0 cm² (e.g. of a leaf blade) in DNA extraction buffer (100 mM Tris-HCl, 100 mM EDTA, 250 mM NaCl) using 1.5-mL microfuge tubes, followed by cell lysis with 20% SDS, and DNA extraction with phenol: chloroform: iso-amyl alcohol (25:24:1). Hydrated ether is then used to remove polysaccharides and other contaminants from the DNA preparation. Average DNA yield is 20–30 µg cm⁻² for fresh tissues, and ratio of absorbance at 260 nm to absorbance at 280 nm is 1.5–1.8. The DNA is quite suitable for PCR using microsatellites, RAPD and specific markers for recombinant selection. Amplifications have been obtained for these markers by using template DNA extracted from fresh as well as frozen leaf tissues of various plants, including barley, oat, potato and tomato. DNA stored for more than 2 years has been successfully amplified with microsatellite markers, which shows suitability of this method after long-term storage of DNA. Besides, the ease of use and cost-effectiveness make the procedure attractive.

Moslemi *et al.*, [10] stated that Pomegranate (*Punica granatum* L.) is a plant rich in polysaccharides, polyphenols and secondary metabolites, which make it difficult to obtain high quality DNA. The present study reports a quick, simple and inexpensive method to isolate genomic DNA suitable for amplified fragment length polymorphism (AFLP) analysis and other PCR-based applications. This method is a modification of a protocol described by Doyle and Doyle [11]. It is a cetyltrimethyl ammonium bromide (CTAB)-based protocol modified by the use of potassium acetate (KoAc) and polyvinylpyrrolidone (PVP) to remove polyphenols and polysaccharides and a high

concentration of β -mercaptoethanol to reduce oxidation. Moreover, the final optimized protocol was then compared with three different methods, which are routinely used for many plant species. The results show that our modified CTAB protocol produced a high yield (>500 ng/ μ l) of good-quality DNA ($A_{260}/A_{280} >1.8$) compared to the other three methods. The DNA purity was further confirmed by complete digestion with *EcoRI* and *MseI* enzymes. The modified CTAB protocol used in this study could be a useful protocol for extraction of high quality DNA not only for pomegranate but also for other plants rich in polysaccharides, polyphenolics and secondary metabolites. Using this method, DNA was extracted from 67 accessions of pomegranate. The DNA was then used for AFLP analysis. To optimize the AFLP protocol, the effects of $MgCl_2$ concentration during selective amplification, the dilution level of pre-amplified DNA and the cycle number used in the pre-amplification were studied. After optimization of the reaction conditions, AFLP was used to study genetic diversity among Iranian pomegranate accessions.

Zhanguo Xin and Junping Chen [12] Investigated that Background Preparation of large quantity and high quality genomic DNA from a large number of plant samples is a major bottleneck for most genetic and genomic analyses, such as, genetic mapping, TILLING (Targeting Induced Local Lesion IN Genome), and next-generation sequencing directly from sheared genomic DNA. A variety of DNA preparation methods and commercial kits are available. However, they are either low throughput, low yield, or costly. Here, we describe a method for high throughput genomic DNA isolation from sorghum [*Sorghum bicolor* (L.) Moench] leaves and dry seeds with high yield, high quality, and affordable cost. Results: We developed a high throughput DNA isolation method by combining a high yield CTAB extraction method with an improved cleanup procedure based on MagAttract kit. The method yielded large quantity and high quality DNA from both lyophilized sorghum leaves and dry seeds. The DNA yield was improved by nearly 30 fold with 4 times less consumption of MagAttract beads. The method can also be used in other plant species, including cotton leaves and pine needles. Conclusion: A high throughput system for DNA extraction from sorghum leaves and seeds was developed and validated. The main advantages of the method are low cost, high yield, high quality, and high throughput. One person can process two 96-well plates in a working day at a cost of \$0.10 per sample of magnetic beads plus other consumables that other methods will also need.

Ambroz et al., [13] Studied that New technology is allowing marker-assisted selection to fulfil the promise of increasing efficiency of cultivar development. However, these techniques depend upon the ability to extract DNA from large populations of plants. The objective of this project was to develop a

high-throughput DNA extraction procedure without the need for greenhouse space or growing wheat (*Triticum aestivum* L.) plants. A sodium hydroxide rapid DNA extraction was modified for a 96- well format to reduce costs. Seeds were germinated in 8-well tissue culture plates, and 4-d-old seedling tissue was used to extract DNA by means of sodium hydroxide methodology. Approximately 1 g of genomic DNA per 10 mg of tissue was isolated at a cost of about \$0.10. The DNA quality was verified by amplification of microsatellite markers. Results were consistent with either fresh or stored tissue extracts. This technique allows one person to extract nearly 1000 Storage-stable DNA samples daily, while keeping costs at a minimum.

Elsalam et al., [14] evaluated an experiment that a simple, rapid and efficient method for isolating genomic DNA from germinated seeds of wheat that is free from polysaccharides and polyphenols is reported. DNA was extracted, treated with RNase, measured and tested for completeness using Agarose gel electrophoresis. DNA purification from wheat grains yielded abundant, amplifiable DNA with yields typically between 100 and 200 ng DNA/mg. The effectiveness and reliability of the method was tested by assessing quantity and quality of the isolated DNA using three PCR-based markers. Inter-simple sequence repeats (ISSRs) were used to assess the genetic diversity between different wheat varieties. Specific PCR primer pair Tox5-1/Tox5-2 and a loop-mediated isothermal amplification (LAMP) procedure were used to detect genomic DNA of *Fusariumgraminearum* contaminated wheat seeds. In this method there is no need to use liquid nitrogen for crushing germinated seedlings. The protocol takes approximately one hour to prepare high quality DNA. In combination with the LAMP assay it is a fast and cost-effective alternative to traditional diagnostic methods for the early detection of toxigenic *Fusarium* in cereals.

Liu et al., [15] Isolation, physical mapping and polymorphism of chromosome specific DNA sequences in wheat are reported. Following the micro dissection of the long arm of chromosome 5B (5BL) of common wheat, its DNA was amplified by degenerate oligonucleotide-primed PCR and directly cloned into plasmid vectors. Characterization of the chromosome arm library showed that ~55% of the inserts are of low-copy nature. Southern analysis using aneuploidy lines of common wheat revealed that five of 11 low-copies inserts analyzed map to chromosome arm 5BL; four of these are 5BL-specific. By deletion mapping, the 5BLspecific sequences were located to sub-chromosome arm regions. Based on the hybridization patterns of three 5BLspecific sequences to DNA from a diverse collection of goat-grass (*Aegilops*) and wheat (*Triticum*) species, it was concluded that these sequences emerged at different times in the course of evolution of this group of plant species.

Lee *et al.*, [16] presented that DNA electrophoresis gel is an important biologically experimental technique and DNA sequencing can be defined by it. Traditionally, it is time consuming for biologists to exam the gel images by their eyes and often has human errors during the process. Therefore, automatic analysis of the gel image could provide more information that is usually ignored by human expert. However, basic tasks such as the identification of lanes in a gel image, easily done by human experts, emerge as problems that may be difficult to be executed automatically. In this paper, we design an automatic procedure to analyze DNA gel images using various image processing algorithms. Firstly, we employ an enhanced fuzzy c-means algorithm to extract the useful information from DNA gel images and exclude the undesired background. Then, Gaussian function is utilized to estimate the location of each lane of A, T, C, and G on the gels images automatically. Finally, the location of each band on the gel image can be detected accurately by tracing lanes, renewing lost bands, and eliminating repetitive bands.

Dhakshanamoorthy, D and Selvaraj, R., [17] Isolate Genomic DNA from leaves of three species of *Jatropha*, namely *J. glandulifera*, *J. gossypifolia* and *J. curcas*. The objective of our study was to use alcohol as a fixing solution, making liquid nitrogen unnecessary for isolation of genomic DNA from *Jatropha* species. The spectral quality of genomic DNA isolated using this method as measured by the A260/A280 absorbance ratio ranged from 1.83 to 1.94 for all three species of *Jatropha*. DNA quality and quantity were comparable to those isolated with liquid nitrogen. The purity of the isolated DNA was further confirmed by PCR (RAPD) using 4 decamer primers. DNA samples prepared by this method were consistently amplifiable in the RAPD reaction and gave reproducible profiles. This method does not require for fixation or grinding in liquid nitrogen, making it advantageous over common protocol.

Stein *et al.*, [18] Conducted an experiment that Gene mapping and marker-assisted selection in complex, polyploidy genomes still relies strongly on restriction fragment length polymorphism (RFLP) analysis, as conversion of RFLP to polymerase chain reaction (PCR) markers can be very difficult. DNA extraction in amounts suitable for RFLP analysis represents the most time consuming and labour-intensive step in molecular marker analysis of plant populations. In this paper, a new efficient method for plant DNA extraction is presented. It allows a high-throughput of samples in a short time without the need for freezing or lyophilizing the plant material. The method allows the isolation of genomic DNA with a yield of 100 µg for a minimal amount of 200 mg of leaf material. This is sufficient for work with large-genome plant species such as hexaploid wheat, where 20 µg of genomic DNA are required for a single RFLP analysis.

CONCLUSION

It is concluded at the end that the review provides basic knowledge about the quantification and isolation of DNA along with the previous research works on genomic DNA specifically focuses on cereals.

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