OPEN ACCESS
Scholars Bulletin
Abbreviated Key Title: Sch Bull
ISSN 2412-9771 (Print) |ISSN 2412-897X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsbulletin.com/

Subject Category: Plant Genetics

Isolation and Quantification of Genomic DNA from Wheat Leaves

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| **Received:** 23.01.2019 | **Accepted:** 04.02.2019 | **Published:** 13.02.2019
DOI:10.21276/sb.2019.5.2.3

Abstract

Background: The manuscript is originally the internship report of Muhammad Junaid, submitted for the partial fulfilment of the degree of BSc (h) to University of Swabi. The internship was completed at NIFA (Nuclear Institute of Food and Agriculture), Peshawar, KP-Pakistan under the supervision of Syed Tariq Shah, Senior Scientist, NIFA Peshawar in 2014. The results obtained are from the original project of Syed Tariq Shah, Senior Scientist, NIFA Peshawar. ***Methodology:*** Ten advance lines of wheat were surface sterilized by 70% ethanol for two minutes and then rinse with double distilled water three times. Again washed with mercuric chloride and washed with double distilled water for three times. The sterilized seeds were cultured in Petri dish and kept it in growth chamber at 25^{o}C. When the plants achieved optimum size after germination, the flag leaf were collected on the basis of weight and stored at -80 for molecular analysis. Genomic DNA was extracted from these leaves using DNeasy plant mini kit. Agarose gel was run after the DNA extraction and then the Spectrophotometric Determination was done. ***Results:*** The results literally focused on to study an efficient method for extraction of genomic DNA and to determine the quality and quantity of genomic DNA. After the extraction of genomic DNA from the plant tissues the quantification, quality and concentrations of isolated genomic DNA were verified on 1% of Agarose gel. RNA and other polysaccharides proteins contamination in the Data is not recoded providing a very rapid and sensitive means of estimating the nucleic acid concentration. DNA concentration was also measured and quantified by ultraviolet spectrophotometer using ultraviolet spectrophotometer. Results showed the differences in DNA yield and DNA quality among the advanced lines of ten wheat samples. Purified genomic DNA has *A*260/*A*280 ratios of 1.5–1.77, and absorbance confirming high purity. L-10 shows the highest yield of DNA that is 462.47 and the lowest DNA yield is of L-7 that is 355.54, the OD260/280 of L-3 is high then the remaining nine lines. ***Conclusion:*** DNeasy plant mini kit is an efficient for genomic DNA extraction and purification.

Keywords: Genomic DNA, DNA extraction, DNeasy plant mini kit, Spectrophotometric Determination, Agarose gel.

INTRODUCTION

Wheat is an annual, long day, self-pollinated crop and technically known as (*Triticum aestivum* L) belongs to family Poaceae [1]. Wheat is a stable and becoming important food in the developing world [2]. Wheat is originated from the Fertile Crescent of Middle East. The modern wheat is originated by a spontaneous hybridization between tetraploid cultivated wheat of (*Triticum turgidum* L) with the diploid wild species (*Aegilops tauschii* L) in the main centre of diversity [3-5]. Complete genome of the wheat consists of 16 billion base pairs of DNA. Wheat genome consists of more than 80% of repetitive DNA sequences [6].

Wheat is grown all over the world the grain of wheat is used to make flour for breeds, 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 [7]. 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 [8]. Marker analysis have been used to characterize molecular diversity [9].

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 [10]. Genomic DNA is

used as a candidate for common reference especially to identify genes differentially expressed in various growth stages [11]. 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.

The present study was conducted to study an efficient technique for extraction of high quality of Genomic DNA from crops plant to study an efficient method for extraction of genomic DNA and to determine the quality and quantity of genomic DNA.

MATERIALS AND METHODS

The experiment was conducted in Nuclear Institute of Food and Agriculture (NIFA) Peshawar, KP-Pakistan in the year of 2014. The wheat lines were provided by NIFA Peshawar, KP-Pakistan.

Seed Sterilization

Ten advance lines of wheat were surface sterilized by 70% ethanol for two minutes and then rinse with double distilled water three times. Again washed with mercuric chloride and washed with double distilled water for three times.

Growth Conditions

The sterilized seeds were cultured in Petri dish and kept it in growth chamber at 25^{o}C. When the plants achieved optimum size after germination, the flag leaf were collected on the basis of weight and stored at -80 for molecular analysis.

DNA Extraction

DNeasy plant mini kit were used for genomic DNA extraction. Wheat leaves were grind to fine powder in mortar and pestle with the help of liquid nitrogen. The sample powder were transferred into 2ml micro tube and allow the liquid nitrogen to evaporate for protecting the sample to thaw. Wheat plant leaves tissue is grinded to fine powder with liquid nitrogen using a pestle and a mortar. Then add AP1 buffer upto 400 µl and 4 µl RNase and vertexes the tube vigorously at 65^{o}C for 10. After mixing the sample 130 µl of AP2 buffer is added in the tube mixed it and incubated on ice for 5 minutes. The sample were then centrifuge at 14500 rpm for 5 minutes at room temperature. After this the supernatant is poured into the QIA sherdder mini spin column and centrifuge the sample at 14500 rpm at room temperature for 2 minutes. The fraction filtrate is poured passed through the new 2 ml micro tube column. Greater the capacity of AP3/E buffer upto 1.5 times and mixed with micro pipette. Now 650 µl of mixed solution is poured into the new mini spin column and centrifuge at 8000 rpm for 1 minutes at room temperature. After centrifugation the filtrate is discarded and 500 µl AW buffer were added in the new mini spin column and centrifuge at 8000 rpm at room temperature for 1 minutes. Again the filtrate is discarded and 500 µl AW buffer is added and centrifuge at 14500 rpm for 2 minute and then dried the filtrate. DNeasy Mini spin column was put in the new 1.5 ml micro tube. Buffer AE of 100 µl was added to the column and incubated for 5 minutes on room temperature. It is then centrifuged at 8,000 rpm (6,000xg) for 1 minute at room temperature. Then the DNeasy Mini spin column was removed from tube and the genomic DNA solution present in the tube was eluted and preserved at -70°C.

Agarose Gel Protocol

For making 1% of Agarose gel 1g Agarose is added to 100ml distilled water. Boiled it in oven and cooled. Now Ethidium bromide is added and placed it in the gel tray before loading the gel. 6µl DNA markers and 10µl DNA sample is added to the gel. Electrophoresis is done with it from negative pole to positive from 35 to 45 minutes. DNA can be visualized using a UV transilluminator and quantified in comparison with the fluorescent yield of the standards. For SSR and RAPD analysis, it is more important to have good quality DNA samples (unsheared/ undegraded DNA), than high quantities of DNA.

Spectrophotometric Determination

1 ml TE buffer is taken in a cuvette and calibrate the spectrophotometer at 260nm as well as 280nm. 10 µl of each DNA sample is added to 900µl TE (Tris-EDTA buffer) and mixed well. TE buffer is used as a blank in the other cuvette of the spectrophotometer. The OD_{260} and OD_{280} values are noted on spectrophotometer and the OD_{260}/OD_{280} ratio is calculated. The amount of DNA is quantified using the formula:

$$\text{DNA concentration (µg/ml)} = \frac{OD_{260} \times 100 \text{ (dilution factor)} \times 50 \text{ µg/ml}}{1000}$$

RESULTS AND DISCUSSIONS

DNA yield or quantity is one of the most important criteria in efficiency evaluation of DNA extraction methods. After isolation of DNA, quantification and analysis of quality are necessary to ascertain the approximate quantity of DNA obtained and the suitability of DNA sample for further analysis. This is important for many applications including digestion of DNA by restriction enzymes or PCR amplification of target DNA. The most commonly used methodologies for quantifying the amount of nucleic acid in a preparation are: (i) gel electrophoresis; and (ii) spectrophotometric analysis. If the sample amount is less, the former method is usually preferred [12].

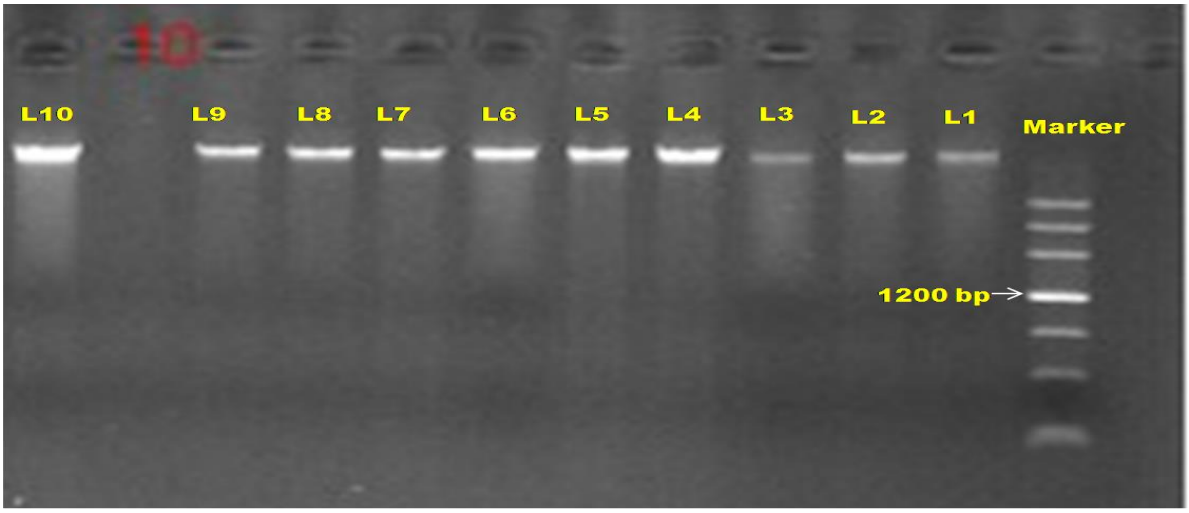

Fig-1: Genomic DNA quantification on Agarose gel

After genomic DNA was extracted from the plant tissues the quantification, quality and concentrations of isolated genomic DNA were verified on 1% of Agarose gel (Fig-1). 6µl DNA markers of high concentration 3000bp and 8 µl DNA sample and 2 µl buffer solutions is loaded to 1%Agaros gel. Run Agarose gel on Electrophoresis for 30 to 35 minutes from negative to positive poles. Agarose gel show different bands of high and low concentration (Fig-1). Quantification is done on basis of ethidium bromide fluorescent staining of DNA. Ethidium bromide is a fluorescent dye, which intercalates between the stacked bases. RNA and other polysaccharides proteins contamination in the Data is not recoded as shown in (Fig-1). This provides a very rapid and sensitive means of estimating the nucleic acid concentration. A large number of samples with as little as 1-5ng of DNA can be quantified. Besides quantification, it also allows provides the advantage of analyzing the quality of the DNA preparation. Native DNA, which migrates as a tight band of high molecular weight (≥40 kb), presence of RNA, and degraded/sheared DNA, if any, can be visually identified on the gel.

DNA concentration was also measured and quantified by ultraviolet spectrophotometer (Table-1). Using ultraviolet spectrophotometer, a parameter i.e., the absorbance ratios of OD260/280 showing purity of DNA solution, for each DNA sample were also recorded. Results in (Table 1) showed the differences in DNA yield and DNA quality among the advanced lines of ten wheat samples. Purified genomic DNA has *A*260/*A*280 ratios of 1.5–1.77, and absorbance confirming high purity. The OD260/A280 ratio and DNA yield (ng/µl) of 1-10 wheat samples (Advance lines) is given in table 1. L-10 shows the highest yield of DNA that is 462.47 and the lowest DNA yield is of L-7 that is 355.54, the OD260/280 of L-3 is high then the remaining nine lines (Table-1).

Table-1: Genomic DNA quality and quantification from wheat leaves on spectrophotometer

Wheat Advance Lines	A260/A280 ratio	DNA yield (ng/µl)
L-1	1.61	384.84
L-2	1.59	362.52
L-3	1.77	298.22
L-4	1.45	435.11
L-5	1.62	415.66
L-6	1.56	295.38
L-7	1.64	355.54
L-8	1.5	403.57
L-9	1.56	289.33
L-10	1.61	462.47

CONCLUSION AND RECOMMENDATIONS

Genomic DNA is used for Preparation of genomic libraries, PCR template, Cloning, Gene/DNA sequencing, Analysis of genomic organization, Study gene structure, DNA fingerprinting, Analysis of genome composition and detection of abnormalities or mutations. DNeasy plant mini kit is an efficient for genomic DNA extraction and purification.

REFERENCES

1. Huang, S., Sirikhachornkit, A., Su, X., Faris, J., Gill, B., Haselkorn, R., & Gornicki, P. (2002). Genes encoding plastid acetyl-CoA carboxylase

and 3-phosphoglycerate kinase of the Triticum/Aegilops complex and the evolutionary history of polyploid wheat. *Proceedings of the National Academy of Sciences*, *99*(12), 8133-8138.

2. Morris, M., Dreher, K., Ribaut, J. M., & Khairallah, M. (2003). Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. *Molecular Breeding*, *11*(3), 235-247.
3. Mergoum, M., Singh, P. K., Anderson, J. A., Pena, R. J., Singh, R. P., Xu, S. S., & Ransom J. K. (2009). Spring Wheat Breeding. Springer Science and Business Media, LLC.
4. Kihara, H. (1944). Discovery of the DD-analyser, one of the ancestors of Triticum vulgare. *Agric. Hort.*, *19*, 13-14.
5. McFadden, E. S., & Sears, E. R. (1946). The origin of Triticum spelta and its free-threshing hexaploid relatives. *Journal of heredity*, *37*(3), 81-89.
6. Bennett, M. D., & Smith, J. B. (1976). Nuclear DNA amounts in angiosperms. *Phil. Trans. R. Soc. Lond. B*, *274*(933), 227-274.
7. Bergthaller, W. (1997). New uses of wheat gluten and non-starch wheat components. In *International Wheat Quality Conference 1997, AM Manhattan/Kansas (USA), 1997*. [np].
8. Gill, B. S., Appels, R., Botha-Oberholster, A. M., Buell, C. R., Bennetzen, J. L., Chalhoub, B., ... & Li, W. (2004). A workshop report on wheat genome sequencing: International Genome Research on Wheat Consortium. *Genetics*, *168*(2), 1087-1096.
9. Szabo, L. J., & Kolmer, J. A. (2007). Development of simple sequence repeat markers for the plant pathogenic rust fungus Puccinia triticina. *Molecular Ecology Notes*, *7*(4), 708-710.
10. Salumbre, R. L. (2009). Genomic DNA Isolation and Applications. Molecular Biology Laboratory Discussion. http//www. scribd. Com/dox 113890135/Genomic-DNA-Isolation and quantification Application.
11. Gadgil, M., Lian, W., Gadgil, C., Kapur, V., & Hu, W. S. (2005). An analysis of the use of genomic DNA as a universal reference in two channel DNA microarrays. *BMC genomics*, *6*(1), 66.
12. Hoisington, D., Khairallah, M., & Gonzalez-de-Leon, D. (1994). Laboratory protocols: CIMMYT applied biotechnology center. *Mexico, CIMMYT*, 94.