Comparison of Six Commercial DNA Extraction Kits for DNA Extraction from Wheat

Buğdaydan DNA Ekstraksiyonu için Altı Ticari DNA Ekstraksiyon Kitinin Karşılaştırılması

Research Article

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ABSTRACT

In this study, six commercial DNA extraction kits were compared with each other in terms of DNA yield, DNA purity, amplification efficiency, the time spent and the cost per extraction. Extracted DNA samples were controlled by agarose gel electrophoresis and spectrophotometric measurement. The DNA samples were used as a template for 18SrRNA and chloroplast trnL–trnF intergenic spacer region amplifications to evaluate the PCR efficiency of DNA samples. Results showed that SIGMA GenElute™ Plant Genomic DNA Miniprep Kit and QIAGEN DNeasy Plant Mini Kit are the most efficient commercial kits for DNA isolation from both wheat seed and leaf samples.

Key Words
Wheat, DNA extraction kits, DNA isolation, Plant DNA

ÖZET

Bu çalışmada, 6 adet DNA ekstraksiyon kiti DNA verimi, DNA saflığı, amplifikasyon etkinliği, harcanan zaman ve ekstraksiyon başına düşen fiyat açısından karşılaştırılmıştır. Ekstrakte edilen DNA örnekleri, agaroz jel elektroforezi ve spektrofotometrik ölçümler ile kontrol edilmiştir. DNA örneklerinin PCR etkinliğini değerlendirmek için, ekstrakte edilen DNA örnekleri 18SrRNA kloroplast ve trnL–trnF genler arasındaki bölgenin amplifikasyonları için kalıp olarak kullanılmıştır. Sonuçlar, SIGMA GenElute™ Plant Genomic DNA Miniprep ve QIAGEN DNeasy Plant Mini kitlerinin buğday tohumundan ve yapraklarından DNA izolasyonunda en etkili ticari kitler olduğunu göstermiştir.

Anahtar Kelimeler
Buğday, DNA ekstraksiyon kitleri, DNA izolasyonu, Bitki DNA'sı

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INTRODUCTION

DNA extraction methods constitute the most important step in molecular biology applications. Successful extraction of DNA is vital for further analytical techniques like cloning, sequencing, southern blot and various PCR applications [1-3]. Quality and quantity of DNA affect the efficiency of these molecular techniques. Therefore, choice of the DNA extraction method has a key importance for the success of many molecular genetic analyses [4-6].

DNA extraction mainly comprises of cell lysis, dissolving of membrane lipids by using detergents and precipitation of DNA by alcohol. Although they are similar in principle, DNA extraction methods may have some modifications according to the target tissue which DNA is extracted from. As plant materials have cell wall, polysaccharides and other organic compounds like polyphenols, it is hard to obtain DNA from plant tissues. Liquid nitrogen is usually used for breakdown of the cell wall. Also, organic compounds like chloroform combined with cetyltrimethylammoniumbromide (CTAB) are used to remove polysaccharides. Conventional methods like CTAB are time consuming and require using of toxic substances [7-9]. Presence of handicaps in conventional methods makes molecular geneticists to use commercial DNA extraction kits. The kits generally achieve the isolation by single use chromatographic columns which are silica based ion exchange resins. Molecular geneticists frequently prefer the kits since they are easier to use and give better results in a shorter time when compared to conventional extraction methods. In addition to these, the kits provide standardization so the results obtained are reproducible [10, 11].

Choice of the kit for a researcher is usually a problem since there are many plant DNA extraction kits in the market. In this study, we evaluated the effectiveness of six commercial DNA extraction kits at extraction of DNA from wheat seeds and wheat leaves. The resulting DNA was controlled with spectrometric analysis, agarose gel electrophoresis and PCR amplification. The kits were compared with each other in terms of DNA quality, DNA quantity, the time spent and the cost of money.

MATERIALS AND METHODS

Materials

Wild type wheat was used as a source of leaves and seeds. Leaves were obtained from germinated wheat seeds.

DNA Extraction

Six different commercial DNA extraction kits used in this study were SIGMA GenElute™ Plant Genomic DNA Miniprep Kit, QIAamp® DNA Stool Mini Kit, OMEGA E.Z.N.A.® Plant DNA Kit, VIVANTIS GF-1 Plant DNA Extraction Kit, QIAGEN DNeasy® Plant Mini Kit, BIOLINE Isolate II Plant DNA Kit. DNA extractions were performed according to manufacturers' instructions. The amounts of the samples used for the extraction were 10 mg for dried samples and 100 mg for fresh samples. The exception was QIAamp® DNA Stool Mini Kit. 200 mg starting material was used to extract DNA using QIAamp® DNA Stool Mini Kit. All samples were grounded in liquid nitrogen with mortar and pestle before they were used in the extraction kits. After extraction, DNA samples were stored at -20°C until use.

Quantification of DNA Samples

DNA quality was controlled by spectrophotometric measurements. Optical density of DNA samples at 260 nm and 280 nm were recorded by a Quawell spectrophotometer (San Jose, CA, USA). Then the concentrations of DNA samples (ng/ml) and ratio of A_{260}/A_{280} were determined. The ratio of A_{260}/A_{280} should be between 1.8 and 2 for pure DNA samples [12]. DNA samples were diluted to a concentration of 100 ng/μl before using in PCR.

Agarose gel electrophoresis

Each DNA sample was applied to agarose gel electrophoresis system to investigate if obtained DNA is enough and pure. 1% agarose gel and the TBE buffer system were used in the electrophoresis experiments. Gels were stained with ethidium bromide and were visualized by image analyzer (Syngene Gene Genius, Synoptics, Cambridge, UK).

Amplification of 18SrRNA and trnL-trnF intergenic spacer in Chloroplast DNA

In order to detect if there is any inhibitors to inhibit amplification, genes of 18SrRNA and trnL-trnF intergenic spacer site in chloroplast were amplified from each isolated DNA. The primers used for amplification of DNA samples were given in Table 1. PCR was performed on ABI GeneAmp PCR System (Foster City, CA, USA). Amplification products were controlled by agarose gel electrophoresis.

Table 1. The primers used for amplification of DNA samples.

Primer Sequence 5′ - 3′	Target element
F:5′-TCTGCCCTATCAACTTTCGATGGTA-3′ R:5′-AATTTGCGCGCCTGCTGCCTTCCTT-3′	18S rRNA
F:5′-CGAAATCGCTAGACGCTACG-3′ R:5′-GGGGATAGAGGGACTTGAAC-3′	Chloroplast

Statistics

Statistical analyses were done with Prism 5 for Windows (GraphPad Software Inc., CA, USA). One way ANOVA analyses were used to compare concentration and purity of DNA isolated from six different kits. As a post-hoc analysis, Tukey's test was performed to determine variation between groups. A p value <0.05 was considered significant.

RESULTS

DNA yield and quality

Extracted DNA from leaves and seeds of wheat were evaluated in terms of DNA quality and yield. DNA yield obtained from each commercial extraction kit was shown in Figure 1. Also the purity of DNA samples were given as the ratio of A_{260}/A_{280} in Figure 2. Quantity of DNA isolated from different commercial kits were significantly different both leaf and seed samples but purity of DNA samples were only significant in seed samples results ($p<0.05$). According to the results given in Figure 1, maximum DNA yields both for seed and leaf were obtained from SIGMA GenElute™ Plant Genomic DNA Miniprep Kit. Minimum DNA yield for seed was obtained from OMEGA E.Z.N.A.® Plant DNA Kit and for leaf from QIAGEN QIAamp DNA Stool Mini Kit. DNA yield obtained from OMEGA E.Z.N.A.® Plant DNA Kit and VIVANTIS GF-1 Plant DNA Extraction Kit were equal both for seed and leaf samples. The difference between the DNA yield obtained from seed and leaf was too large for QIAGEN QIAamp® DNA Stool Mini Kit.

As well as DNA yield, purity of DNA is very important for the success of PCR reaction and other molecular analysis. As shown in Figure 2, although the DNA yield was not the highest, the value of ratio of A_{260}/A_{280} was optimum for QIAGEN DNeasy® Plant Mini Kit. Therefore, contamination of RNA and protein is low for QIAGEN DNeasy® Plant Mini Kit. When the results considered, it is obvious that the ratio of A_{260}/A_{280} is very high in seed sample for OMEGA E.Z.N.A. Plant DNA Kit and in leaf sample for QIAGEN QIAamp® DNA Stool Mini Kit. RNA

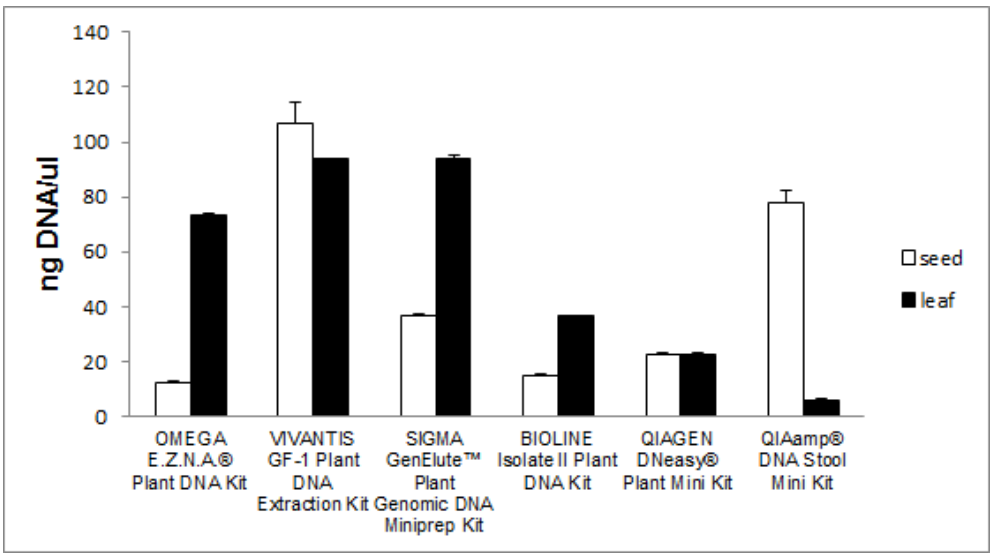

Figure 1. DNA yield obtained from each commercial extraction kit. Open bars represent seed and filled bars represent leaf samples. Mean ±SEM, N=3.

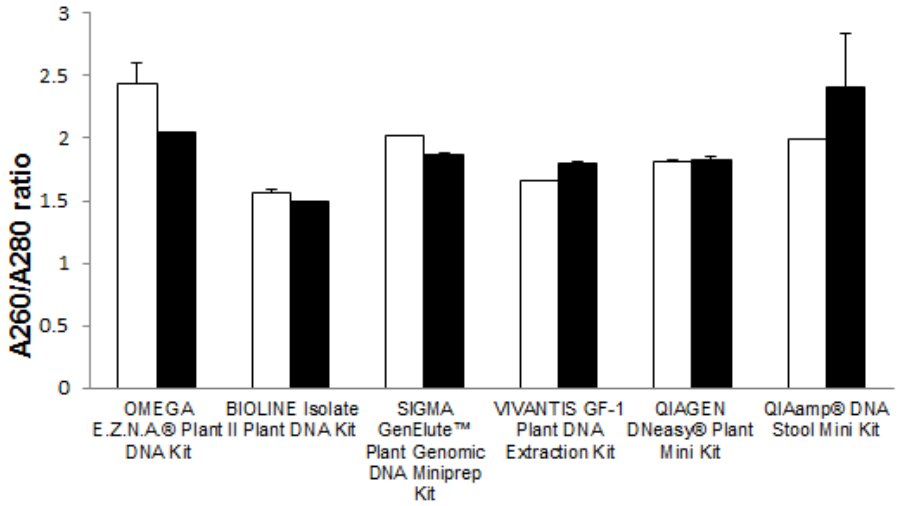

Figure 2. Ratio of A_{260}/A_{280} of DNA samples. Open bars represent seed and filled bars represent leaf samples. Mean ±SEM, N=3

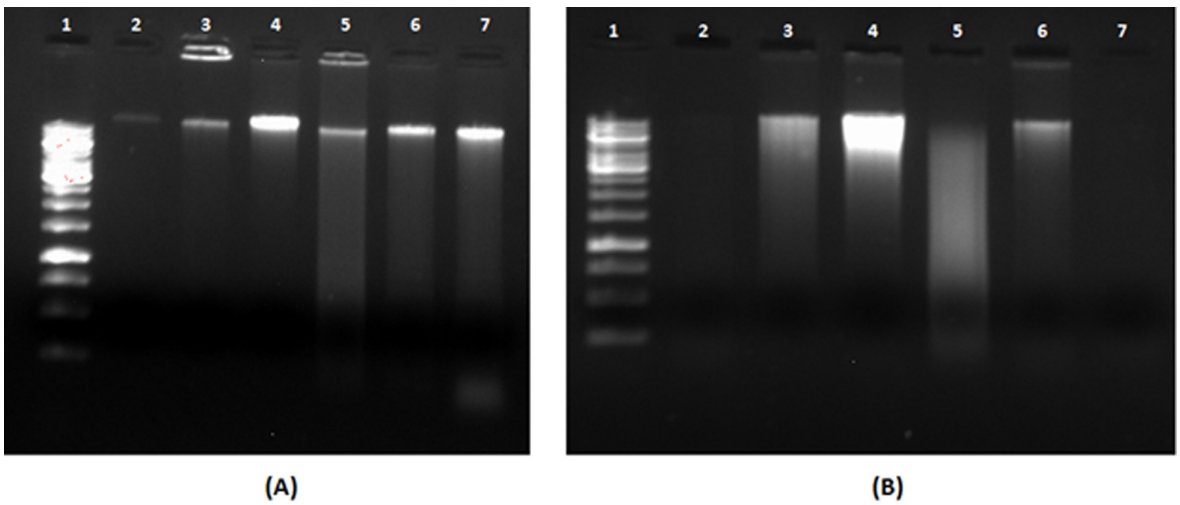

Figure 3. Agarose gel electrophoresis images of DNA samples extracted from seed (A) and leaf (B). Marker (1), OMEGA E.Z.N.A. Plant DNA Kit (2), BIOLINE Isolate II Plant DNA Kit (3), SIGMA GenElute™ Plant Genomic DNA Miniprep Kit (4), VIVANTIS GF-1 Plant DNA Extraction Kit (5), QIAGEN DNeasy Plant Mini Kit (6), QIAGEN QIAamp® DNA Stool Mini Kit (7).

contamination could be the reason of this situation. Quality and purity of extracted DNA samples obtained by using the kits were shown in Figure 3. As clearly seen in Figure 3, SIGMA GenElute™ Plant Genomic DNA Miniprep Kit has the best results for seed and leaf samples. OMEGA E.Z.N.A.® Plant DNA Kit gave the least brightest band for seed sample and also gave a very weak band in leaf sample. Also, QIAGEN QIAamp® DNA Stool Mini Kit gave no band in leaf sample but has the second brightest band in seed sample. Although VIVANTIS GF-1 Plant DNA Extraction Kit gives a smear band in agarose gel for leaf sample, it has adequate efficiency in isolation both from seed and leaf samples according to spectrophotometry results. The smear view can be explained by DNAse contamination during isolation.

Time and cost analysis

The time spent for each kit and costs of the kits per sample were evaluated and the results were shown in Table 2. Time is an important parameter for evaluating the efficiency of a method or of a kit. About one hour was enough to complete the process for almost each kit. BIOLINE Isolate II Plant DNA Kit is the fastest with less than 40 minutes completion. On the contrary VIVANTIS GF-1 Plant DNA Extraction Kit has the longest isolation time with over 2 hours. Price of the each kit per one sample extraction was

Table 2. Time and cost analysis of the kits.

	OMEGA E.Z.N.A® Plant DNA kit	VIVANTIS GF-1 Plant DNA extraction kit	SIGMA GenElute™ Plant Genomic DNA Miniprep Kit	BIOLINE Isolate II Plant DNA Kit	QIAGEN DNeasy® Plant Mini Kit	QIAamp® Stool Mini kit
Cost per extraction ($)	2.49	1.49	2.73	2.16	3.73	4.14
Approximate processing time (h)	<60 min	>2 hours	<40 min	30 min	<60 min	50 minutes

shown in Table 2. According to the results, VIVANTIS GF-1 Plant DNA Extraction Kit has the minimum price and QIAGEN QIAamp DNA Stool Mini Kit has the maximum price.

PCR amplification results

Chloroplast trnL-trnF intergenic spacer region and 18SrRNA PCR amplification results of seed and leaf samples were shown in Table 3 and Table 4 respectively. According to the results, SIGMA GenElute™ Plant Genomic DNA Miniprep KitDNA, VIVANTIS GF-1 Plant DNA Extraction Kit and QIAGEN DNeasy® Plant kits gave the best amplification results. When PCR amplification results of BIOLINE Isolate II Plant DNA Kit was evaluated for chloroplast trnL-trnF intergenic spacer region, this kit showed weak amplification for leaf samples and partial amplification for seed samples. In the contrary, this kit has the 4th brightest band for leaf samples. When the A_{260}/A_{280} ratios were checked for this kit it can be seen that the score is lower than the required 1.80 level for intact and clean DNA.

DISCUSSION

There are many procedures and protocols developed for DNA isolation from plants. These include conventional CTAB isolation or cesium chloride density gradients. But all of these methods are time consuming and includes hazardous chemicals. Today many commercial kits were developed for fast and safe DNA isolation from plants. But the main problem is how to choose the correct one.

In this study, six commercial DNA isolation kits were compared for extraction efficiency, cost and time effectiveness. Although VIVANTIS GF-1 Plant DNA Extraction Kit has the 4 th. highest concentration on spectrophotometric measurements for leaf samples, when the isolated DNAs were controlled on agarose gel it showed a smear band. However, some double-stranded DNAs were likely denatured to single-stranded ones, which lead to spectrophotometric overestimation because of the hyperchromic effect, as was reported with Chelex 100, Alkali, or AlkaliX methods [13]. Also 260/280 ratio is a very important criteria for isolated DNA. This value determines the quality of DNA which is much more important than the quantity. DNA of high quality gives better results in amplification like further applications. In conclusion, there are many alternative kits in the market for plant DNA isolation and it is very hard to point only one kit as the best one. But SIGMA GenElute™ Plant Genomic

Table 3. Chloroplast trnL-trnF intergenic spacer PCR amplification results of seed and leaf samples for six DNA extraction kits.

	OMEGA E.Z.N.A® Plant DNA kit	VIVANTIS GF-1 Plant DNA extraction kit	SIGMA GenElute™ Plant Genomic DNA Miniprep Kit	BIOLINE Isolate II Plant DNA Kit	QIAGEN DNeasy® Plant Mini Kit	QIAamp® Stool Mini kit
Seed	++	++	++	+	++	++
Leaf	++	++	++	+	++	+

Note: ++ represents successful amplification, + represents weak amplification.

Table 4. 18SrRNA PCR amplification results of seed and leaf samples for six DNA extraction kits.

	OMEGA E.Z.N.A® Plant DNA kit	VIVANTIS GF-1 Plant DNA extraction kit	SIGMA GenElute™ Plant Genomic DNA Miniprep Kit	BIOLINE Isolate II Plant DNA Kit	QIAGEN DNeasy® Plant Mini Kit	QIAamp® Stool Mini kit
Seed	++	++	++	++	++	++
Leaf	+	++	++	++	++	++

Note: ++ represents successful amplification, + represents weak amplification, and represents no amplification.

DNA Miniprep Kit and QIAGEN DNeasy® Plant Mini Kit gave the most sensitive results for extracting plant DNA from wheat seeds and leaves in this study. This study could be a guide for researches to choose a suitable kit for extracting DNA from plants.

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