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Original Scientific Article

A MODIFIED SDS – BASED METHOD APPLIED FOR EXTRACTION OF HIGH-QUALITY DNA FROM RAW CORN AND ROASTED SOYBEAN

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ABSTRACT

The probability of contamination of non-transgenic varieties with genetically modified (GM) products increase as a result of global expansion of areas sown with transgenic crops. DNA-based methods as accurate, efficient and reliable methods are preferable for detection of GM material in raw or highly processed foods. Isolation of high quality DNA with a suitable and efficient DNA extraction protocol is crucial for getting precise results in DNA amplification. In this study, we performed modifications of previously known Sodium dodecyl sulfate (SDS)-based DNA extraction method regarding the incubation period, DNA pellet washing and addition of organic solvent extraction, to improve DNA quality and to reduce costs. Raw corn kernels and roasted soybean seed were used as samples. DNA was extracted following three protocols, modifications of Edwards protocol. The type of detergent used in raw corn sample did not cause significant effects on extracted DNA yield and purity, while in roasted soybean samples the 2% (w/v) SDS lysis buffer gave the highest DNA yield. The additional incubation step raised the DNA yield from raw corn for 121%, while the purest DNA from soybean sample was obtained using organic solvent extraction. Electrophoretic determination of DNA integrity showed varying degree of DNA smearing from roasted soybean. Contrary, all extraction protocols used on raw corn kernels produced a high molecular weight DNA. Thus, our in-house DNA extraction protocol is as efficient but more cost effective compared to commercial kits and can be used for raw corn, while the protocol for roasted soybean needs further improvement.

Key words: sodium dodecyl sulfate, DNA extraction, GMO, corn, soybean

INTRODUCTION

Limited crop resources and climate disorders led to implementing a recombinant DNA technology to genetically modify agricultural crops for potential improvement of harvest quality and productivity (1). Global expansion of the areas sown with transgenic crops, increased the probability of contamination of non-transgenic varieties with genetically modified (GM) products (2). Consumer concerns regarding consumption of GM food has increased widely, mainly regarding the long-term health effects like horizontal gene transfer to gut microflora, antibiotic resistance, toxicity and allergenicity (3, 4, 5). As a result, the European Union (EU) has established biosafety regulations for the proper use of GM crops, requiring compulsory labelling of food products containing more than 0.9% authorized GMO for consumers' information (6, 7). Therefore, an accurate, efficient and reliable analysis method, such as DNA-based methods, are needed for detection of GM material in raw or highly processed foods (8).

The isolation of nucleic acids from intact kernels and roasted beans requires disruption of the cell wall, followed by extraction and subsequent purification. The disruption step requires mechanical, thermal or chemical processing

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and can affect DNA integrity (9). Plants possess varying levels of polysaccharides, polyphenols and other secondary metabolites, that strongly combine with nucleic acids during DNA isolation, affecting the quantity, quality and purity of the extracted DNA (10). In order to obtain consistent, reliable and accurate results in DNA amplification, it is crucial to isolate high quality DNA with a suitable and efficient DNA extraction protocol (8). Hence, choosing the right protocol for extraction of DNA from raw or processed food, considering the variety of food matrices with diverse types of processing, can be challenging (11).

An ideal extraction protocol should optimize DNA yield, minimize DNA degradation, and be efficient in terms of cost, time, labour and supplies. Most commonly used methods from diverse organisms are sodium dodecyl sulphate (SDS) and cetyltrimethyl ammonium bromide (CTAB). Absolute ethanol or isopropanol are routinely used for DNA precipitation in the presence of sodium ions. However, there are several modifications regarding the volume of ethanol or isopropanol, incubation temperature and time used for DNA precipitation (12).

Edwards et al. (13) have developed a method for rapid extraction of plant genomic DNA, applicable to a variety of plant species, that does not require any handling with hazardous organic solvents. In the present work, we tested few modifications of this protocol. We examined the effect of SDS and sodium N-lauryl sarcosine concentrations (0.5% (w/v) and 2% (w/v)) in the lysis buffer, on the quality of the DNA. These are both anionic detergents with a structure similar to phospholipid molecules of the cell membrane, so they can mimic the biological membrane environment and help capturing the lipids that constitute the cell and nuclear membrane. Besides removing the membrane barriers, these detergents denature histones and help release DNA from the nucleoprotein complex. Additionally, we performed modifications regarding the period of incubation, DNA pellet washing step and adding an organic solvent extraction step, to improve DNA yield and quality.

MATERIAL AND METHODS

Sample preparation

For the purpose of this study, we used raw corn kernel and roasted soybean seed samples in order to eliminate the matrix influence on the results. The genomic DNA was extracted following four different protocols, three modifications of the Edwards protocol and one commercially available (GeneSpin, Germany).

All samples used for DNA extraction were finely grinned and tested in duplicate. The sample was mixed with lysis buffer (200 mM Tris HCl, 250 mM NaCl, 25 mM EDTA,0.5% SDS, pH 8.0), where lysis buffer 1 (original Edwards buffer) containing 0.5% SDS, lysis buffer 2 containing 2% SDS (modification 1) and lysis buffer 3 containing 2% N-lauryl sarcosine detergents (modification 2). Then the sample was vortexed and centrifuged at 13.000 rpm/5 min. An aliquot of the supernatant was transferred to a fresh tube, ispopropanol was added, vortexed and left at room temperature for 2 minutes, followed by centrifugation at 13.000 rpm/10 min. The supernatant was discarded and the remaining pellet was washed three times with 70% (v/v) ethanol. After centrifugation, the supernatant was carefully discarded and the pellet was dried at 37°C/30 min. DNA was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). This simplified protocol was modified with additional incubation step, usage of organic solvent extraction and washing steps.

The optimized protocol included an incubation period of 30 minutes at 65°C with constant shaking at 350 rpm after addition of lysis buffer containing 2% SDS. We used organic solvents (n-hexane, chloroform and chloroform: isooctane mixture (3:2)) for protein and lipid removal, followed by a step of agitation at 400 rpm/15 min and centrifugation at 13.000 rpm/5 min. An aliquot of the supernatant was transferred into a fresh tube, followed by the addition of isopropanol and after centrifugation the pellet was washed once with 96% ethanol and three times with 70% ethanol. The pellet was air-dried, suspended in TE buffer (preheated at 65°C) and incubated at 65°C/3 min. Extraction with the GeneSpin, was performed following manufacture's manual. The dissolved pellet was used for Nano Drop measurement and kept at +4°C until electrophoresis performance.

Determination of DNA concentration, purity and structural integrity

DNA quantification was performed using NanoDropTM 2000 Spectrophotometer (Thermo ScientificTM, Germany). DNA purity was estimated by (i) absorbance ratio A260/A280 and (ii) absorbance ratio A260/A230. DNA yield was calculated with the formula:

DNA yield (µg) = DNA concentration (ng/µL) * total sample volume (mL)

The structural integrity was evaluated by electrophoresis in 1.5% agarose with 1x TBE buffer for 2 hours at 70 V, stained with ethidium bromide and visualized under UV light using Gel doc XR+ imager (Bio Rad,USA).

RESULTS

Our results showed that the type of detergent used in raw corn sample did not have significant effects on extracted DNA yield and A260/A280 ratio. Contrarily, the highest value of DNA yield from roasted soybean sample was obtained using 2% SDS in a range between 271.7 – 279.9 µg and a A260/A280 ratio between 1.95-1.97 (Fig. 1).

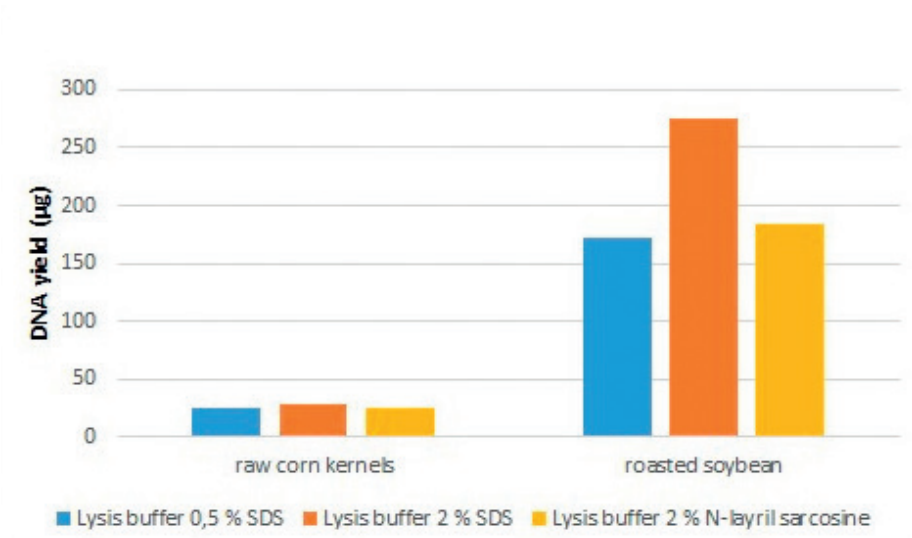

Figure 1. Comparison of DNA yields from raw corn and roasted soybean seed obtained with different lysis buffers

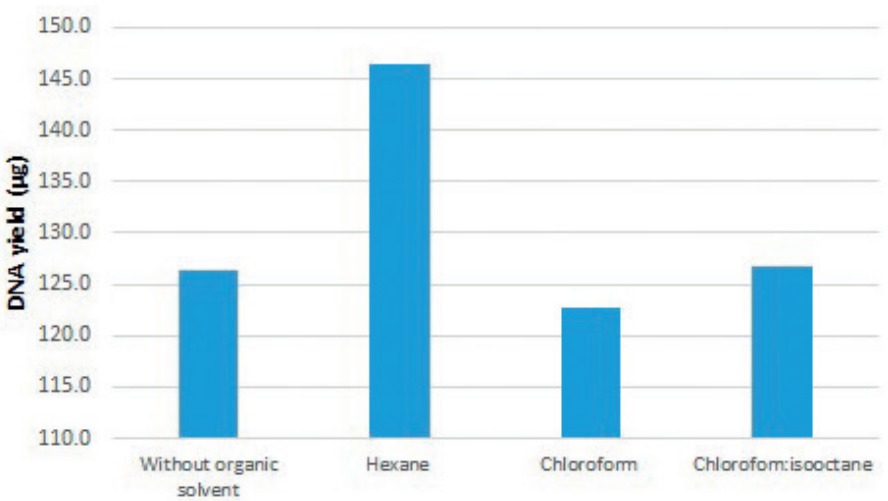

Figure 2. Comparison of DNA yields from roasted soybean with organic solvent extraction step

An additional incubation step was added to the simplified Edward's protocol at 65°C for 30 minutes, using preheated lysis buffer to avoid temperature differences in the test tube. The incubation period had a great impact on DNA yield from raw corn samples increasing the value for 121% (15.1±9.5µg to 33.5±7.0µg), opposite to roasted soybean samples where it did not generate a significant effect (+6%).

Addition of DNA pellet washing step increased the DNA yield from roasted soybean samples when it was applied a single step with 96% ethanol and triple steps with 70% ethanol. We tested addition of 5M NaCl to 96% ethanol but it halved the DNA yield (127.85±32.0 µg to 63.68 ± 5.45 µg). Regarding the raw corn samples, we did not detect a significant outcome for DNA yield.

Moreover, we used few organic solvents like n-hexane, chloroform and chloroform:isooctane (3:2) mixture to eliminate contaminants from extracted nucleic acids. The highest DNA yield from roasted soybean sample was obtained using n-hexane as organic solvent for lipid extraction (127.8-165.0 µg) (Fig. 2). Using only chloroform or mixture of chloroform:isooctane (3:2) did not have significant outcome in terms of DNA yield.

Based on A260/A280 ratio the purest soybean samples were obtained using n-hexane treatment (1.90±0.03), while the best value for A260/A230 ratio was obtained after chloroform treatment (1.77±0.02). In raw corn samples best results regarding DNA purity were obtained using 2% SDS lysis buffer followed by incubation step giving value of A260/A280 ratio 1.94±0.05, while the value of A260/230 gave best results using GeneSpin. (Table 1.)

Table 1. Values of A260/280 and A260/230 ratios as indicators of purity of DNA extract from raw corn kernel and roasted soybean samples

		Raw corn kernels		**Roasted soybean**	
		A260/280	A260/230	A260/280	A260/230
Lysis buffer	0.5% SDS	2.06±0.05	1.55±0.24	1.96±0.02	1.61±0.13
	2% SDS	2.07±0.02	1.64±0.12	1.95±0.01	1.38±0.14
	2% N-lauryl sarcosine	2.03±0.11	1.37±0.31	1.97±0.02	1.47±0.01
Incubation step	Without incubation	2.04±0.04	1.71±0.11	1.96±0.01	1.50±0.14
	With incubation	1.94±0.05	1.45±0.16	1.97±0.01	1.48±0.04
Organic solvent extraction	n-hexane	N/A*	N/A*	1.90±0.03	1.31±0.12
	chloroform	N/A*	N/A*	2.00±0.01	1.77±0.02
	chloroform:isooctane	N/A*	N/A*	1.98±0.01	1.61±0.04
GeneSpin	/	2.08±0.01	2.29±0.01	2.01±0.01	1.60±0.02

N/A* not available data

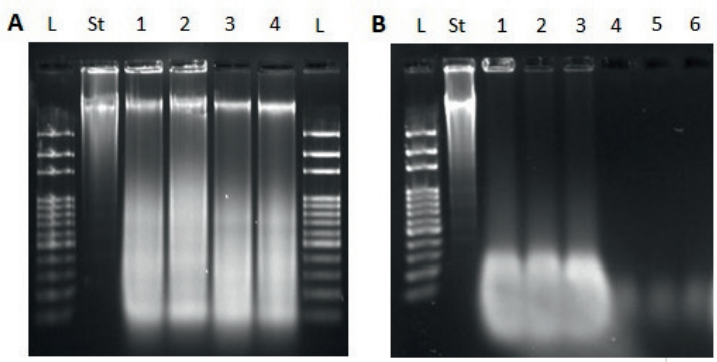

Figure 3. DNA integrity assessed with agarose gel electrophoresis. **A.** Raw corn kernel. Line L-ladder, St-DNA standard, 1-2 corn DNA samples without incubation step, 3-4 corn DNA samples after incubation; **B.** Roasted soybean L-ladder, St-DNA standard, 1- extraction with n-hexane solvent, 2-extraction with chloroform solvent, 3-extraction with chloroform:isooctane solvent mixture, 4,5,6 – diluted samples 1,2,3

Varying degree of smearing was observed in DNA extracts from roasted soybean samples, while in the case of raw corn samples, all extraction protocols produced a high molecular weight DNA (Fig. 3).

DISCUSSION

The original DNA extraction protocol developed by Edwards et al. (13), that uses SDS (0.5% w/v) as detergent for cell lysis, remains simple and rapid method for extraction of small amounts of various plant genomic DNA. In our research, we made some modifications of the original protocol to raise the DNA yield and quality. Successful DNA purification depends on effective disruption of cells and denaturation of nucleoprotein complexes. Hence, during the cell lysis step, we used higher concentration and different type of detergent to improve the cell lysis. For that purpose, we used 2% (w/v) SDS (i) and 2% (w/v) N-lauryl sarcosine (ii) as components of the cell lysis buffer. Fig. 1. shows that the 2% (w/v) SDS lysis buffer gave the highest DNA yield from roasted soybean samples, which is in an agreement with the results of Xia et al. (8). Even when compared to most frequently used CTAB method and commercial kits, Wang et al. (14) gained highest DNA yield from soybean seeds using SDS-based extraction method. The concentration of DNA (25.89±3.2 μg) that we obtained from raw corn kernels did not reveal any significant difference regarding the detergent type and its concentration. Vivekananda et al. (15) used 1% SDS for DNA extraction from corn leaves and they obtained DNA yield approximately 60-105 ng/μL, while Sharma et al. (16) using the same concentration of detergent in lysis buffer obtained much higher DNA yield (870.3±32.4 ng/μL) due to the supplements of lysis buffer.

Furthermore, an additional incubation step at 65°C/30 min/350 rpm was added to increase cell lysis efficiency and to obtain high quality DNA. An additional experimental protocol was performed, with a longer incubation period (1h) at the same temperature, but it did not show any significant recovery of DNA yield (data not shown here). These results coincide with the findings of Akaneme et al. (17) showing that incubation temperature is essential criteria for production of high quality of DNA. They extracted DNA of high purity and quantity by incubating the samples at 60°C that helps the 0.5% SDS lysis buffer to dissolve the cellular proteins.

Many protocols use organic solvent extraction to eliminate contaminants from extracted nucleic acids. Contaminants can be present from the sample itself like proteins, polysaccharides, lipids and polyphenols that co-precipitate with DNA. Moreover, the chemicals used for DNA isolation like detergents, chaotropic salts and organic solvents can influence the DNA purity and could lead to reduced PCR efficiency. Most frequently used organic solvents are phenol:chloroform or phenol:chloroform:isoamyl alcohol mixtures for protein denaturation and lipid purification. Xia et al. (8) optimized the extraction protocol from raw soybean samples using chloroform:isoamyl alcohol (24:1, v/v) mixture for gaining high quality DNA. We obtained the highest DNA yield from roasted soybean using n-hexane (Fig. 2), which is in accordance with many researches that were using the same solvent for DNA extraction from vegetable oils or oil rich samples (18, 19).

Additionally, we verified the quality of each DNA extract spectrophotometrically and on agarose gel electrophoresis. The A260/A280 nm ratio of 1.8 indicates a high purity of extracted DNA, while the lower ratio indicates presence of proteins, phenol or other contaminants that absorb at or near 280 nm (Table 1). We used the A260/A230 ratio as a second indicator of DNA purity, with expected values for “pure” DNA between 2.0-2.2. It reflects contamination of the sample by different molecules that absorb at 230 nm wavelength. The variations in the data for DNA yield and purity are accredited to the effect of extraction method, since the matrix effect was eliminated using the same corn and soybean samples.

Detecting considerable level of DNA degradation from roasted soybean sample regardless of the extraction protocols might mean that food processing has an influence on the DNA integrity (Fig. 3). Many studies have shown that different food processing parameters like temperature, pH, pressure and exposure time demonstrate an immense impact on DNA integrity and can cause DNA fragmentation, which is seen as an expanded smear on a gel and not as a sharp band (20, 21, 22). To distinguish if the smear is a result of RNA contamination of the extracts, additional treatment with RNase is recommended. However, obtaining a single high molecular weight band from raw corn kernels with all extraction protocols is an indicator that the DNA integrity was preserved during the extraction process (Fig. 3). These results are in accordance with the gel electrophoresis of genomic corn DNA, which shows a single, high molecular weight DNA band (23).

CONCLUSION

This study proposes a practical explanation of an in-house method for DNA extraction, based on the Edward's protocol, in which we used different commonly available laboratory reagents and added or modified some of the performance steps of the primary method. Based on our findings regarding the DNA yield and its quality we can conclude that this protocol can be used for DNA extraction from raw corn kernel, since it is more cost effective compared to commercial kits. Still the results that were obtained for DNA extraction from roasted soybean indicated that some further improvement of the performance steps must be made to get more quality DNA from thermally processed food.

CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

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