Utilization of corn silk for GMO detection through real time PCR

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Research Article

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Abstract

Purpose: Corn silk is a fiber rich byproduct obtained during corn processing. Present investigation deals with the purpose of GMO detection and quantification and comparison of DNA extraction in corn silk that is considered generally as a waste product.

Method: A comprehensive analysis of extracted DNA quality, extraction cost, and time and PCR amplification was performed. Samples were homogenized by three different methods i.e. liquid nitrogen (CSK-LN), tissue lyzer (CSK-TL) and by grinder (CSK-RG). Afterwards, DNA extraction and quantitative analysis of extracted DNA was done using three different Kits QIAGEN DNeasy Mericon Food Kit, eurofins|GeneScan GENESpin DNA isolation Kit and PENICON EZ-10 Spin Column Genomic DNA Miniprep Kit .Furthermore, the agarose gel electrophoresis assay of corn silk was performed. Also scanning electron microscopy revealed observable differences between all 3 samples.

Results: CSK-TL showed finest appearance due to the nature of treatment received. Among all kits, kit 3 (PENICON EZ-10 Spin Column Genomic DNA Miniprep Kit) displayed the most economical one for extraction, however in terms of DNA quantification kit 1 (QIAGEN DNeasy Mericon Food Kit) showed highest % yield of 122.4 ng/ul followed by tissue lyzer technique. In the result of gel electrophoresis assay, 1000bp plus band size observed with different consistency according to different kits.

Conclusion: DNA extracted using waste material (corn silk) can be used for GMO analysis through Real-Time PCR technique. Among different grinding techniques compared, Tissue lyzed sample (CSK-TL) displayed highest quantified DNA extracted. Hence, provided techniques could be helpful in determining DNA in waste products.

Statement Of Novelty

Present research primarily focused on the use of corn silk to detect GMO. By using this method, we can save food product currently use for testing.

1. Introduction

Corn (*Zea mays*), also called Indian corn or maize, is the third most significant crop produced and consumed globally after rice and wheat. It has versatile applications in industries including pharmaceutical, food and non food industries etc [1]. In Pakistan the key season crops; rice and maize, are harvested by mid-December. According to FAO stats, total harvested area, yield and production for maize in Pakistan was 1413246 ha, 51203hg/ha and 7236313 tons, respectively in 2019 [2]. However, the 2020 paddy output was recorded as 12.3 million tons [2] .

Waste utilization has always been an attractive subject for all researchers. Processing of yellow or green maize (*Zea mays L.*), also yields some byproducts i.e. husk, cobs, silk and stalks. Among all, corn silk (*Stigma maydis*) is the most common waste material produced during corn processing that is not utilized,

and is typically discarded. Nutritionally, it consists of protein, fat, CHO, steroids, saponin, tannin etc [3]. However, natural medicinal importance of corn silk is effective to treat nephritis, hypertension, prostatitis, and urinary tract infections [4]. Many countries around the world including China, Turkey, the United States, and France have used it as a common medicine. It's also used to treat cystitis, edema, kidney stones, bladder diseases and obesity [5]. Its potential therapeutic benefits are due to chemical composition and mode of action of bioactive elements including phenolics and terpenoids, as well as polysaccharides and glycoproteins. Flavonoids in corn silk have shown varied pharmacological effects. These are known for their antioxidant and free radical scavenging capability [6].

Maize has the most approved events (single and stacked traits) among Genetically Modified (GM) crops and is the second largest crop in terms of global adoption, after soybean. According to the Novel Food Regulation, the distinction between traditional food and food containing of GMOs should be detected using suitable scientific methods. The Polymerase Chain Reaction (PCR) has been found to be suitable for food analysis and seems to be the preferred method for detecting GMOs in food [7]. Many genetically modified (GM) crops and products have been introduced into the market for food and feed use, due to rapid advancement of recombinant DNA techniques [8]. Globally, 53.6 million hector of GMO maize were planted in 2015, representing about a third of the 185 million ha of maize planted worldwide [9].

The aim of present investigation is to use corn wastage i.e. corn silk, for the detection of GMO using Real Time (qPCR) technique. Commonly, corn kernel is used for GMO detection but keeping in mind the current situation of world hunger and food fraud, the study focused on optimizing GMO detection method by utilizing wastage instead of using food part. To best of our knowledge, little study has been in field of GMO detection from corn silk. Secondly three different Kit based methods were also compared in terms of extraction of DNA. Thirdly effects of grinding methods for DNA isolation were also analyzed with respect to its morphological difference by help of scanning electron microscope. Later the time and cost estimation of each Kit was also calculated.

2. Materials And Methods

2.1 Sample collection and preparation

For the extraction of DNA and morphological study of corn silk, samples (A1, A2 and A3) were collected from three different areas of Karachi, Pakistan. All samples were ground and oven dried at 55^{o}C overnight. All samples were homogenized/ground using three methods a) simply by grinding in laboratory grinder (CSK-RG) (Ciatronic, KSW 3306), b) grinding with TissueLyser II (CSK-TL) (QIAGEN, Germantown, MD, US) at frequency 30 (s^{-1}) for 10 to 15 seconds and c) thirdly with liquid nitrogen (CSK-LN) using mortar and pestle. Samples were stored in corning tubes (Nest, Biotechnology Co., Ltd, China, 15ml) at (-20^{o}C) (SIEMENS, Munich Germany, GS32NA23) until the completion of the analysis.

2.2 Morphological characteristics

Morphological analysis of all corn silk samples (grounded by different methods) was performed through analytical scanning electron microscopy (JSM, 6380A, Jeol, Japan). All samples were precipitated on aluminum specimen holder and then thinly coated with gold by ion splutter (JFC-1500) in vacuum evaporator. After gold coating, the stubs were placed and fixed onto sample holder and then placed in sample chamber of SEM. Corn silk samples were then examined under magnifications of 200X, 1000X, 3500X, 5000X and 10,000X, respectively.

2.3 Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of starches were obtained by a Tensor II Bruker spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector using attenuated total reflectance (ATR). For each spectrum 64 scans at 4 cm^{-1} were recorded.

2.4 DNA Extraction

The genomic DNA (gDNA) of all samples were extracted using three different Kits:

First Kit (Kit 1) used for DNA extraction was QIAGEN DNeasy Mericon Food Kit (QIAGEN, Germantown, MD, US). Approximately 2 g of each sample was weighed on analytical balance (RADWAG, XA 110/X). After grinding in blender and TissueLyser II, the gDNA was extracted by protocol provided by the Kit [10].

Second Kit (Kit 2) used for DNA extraction was eurofins|GeneScan GENESpin DNA isolation Kit (eurofins GeneScan technologies GmbH, Germany). 200mg of sample was taken then 1ml of lysis buffer and 10ul of proteinase K was added. Recovery of purified DNA after washing with wash buffers containing guanidine hydrochloride (24–36%) and ethanol (36–50%), and then elution was done by using EB (elusion buffer) contain Tris EDTA (TE) buffer to obtain pure DNA.

Third Kit (Kit 3) used was PENICON EZ-10 Spin Column Genomic DNA Miniprep Kit (BIO BASIC INC, Canada). All samples were ground approx 100mg to fine powder and transfer to 15ml tube. For lysis 600ul of plant cell lysis buffer with 12ul of β-mercaptoethanol added. Afterwards, 0.6ml of chloroform was added and centrifuge at 12000x g for 2 minutes afterwards, 400ul of supernatant was mixed with binding matrix along with absolute ethanol. All the mixture was then transferd to silica based column to bind DNA with column. Washing was done to remove impurities by wash solution. Lastly, to gain maximum yield of DNA elution was done by tris EDTA (TE) buffer.

2.5 Quantification

To study the relation of homogenization method to Kit, samples were quantified by Qubit Fluorometer 3.0 (invitrogen Life Technologies, US). Qubit™ ds DNA HS standard 1 and Qubit™ ds DNA HS standard S2 were prepared by adding 189ul of buffer with 1ul of fluorescent dye and 10ul of each standard provided with the Kit [11]. For sample preparation, 198ul of buffer was dispensed into tube (Qubit Assay Tubes, Thermo Fisher SCIENTIFIC) containing 1ul of dye. 1ul of extracted sample was added and the tubes were then incubated at room temperature (25^{o}C) for 1 minute and inserted in Qubit fluorometer for

observation. Thus, the quality of the isolated gDNA was evaluated by preparing 1.5% agarose gel electrophoresis.

2.6 Real time PCR

For the detection of genetically modified organisms (GMO), DNA isolation Kit (eurofins|GeneScan GMO*Screen* RT PCR Kit) was used. The real-time PCR assays were performed on real time PCR (QIAGEN Rotor-Gene Q, Germany) under the following conditions. An initial denaturation step (10 min at 95°C), 45 cycles at 95°C for 15 s, and annealing and extension at 60°C for 90 s [12]. The results were interpreted with the help of QIAGEN rotor gene Q series software (version 2.3.1).

2.7 Agarose gel electrophoresis

Whole gDNA samples were analyzed by using agarose gel electrophoresis for the evaluation of band size. 1.5% of Agarose was prepared in 1x TAE (Tris Acetate EDTA) buffer with 3ul ethidium bromide [13]. The conditions set on electrophoresis assembly (Cleaver Scientific Ltd, nanoPAC-300) was set on 120 Volt for 60 minutes. Interpretation of results was done by visualizing agarose gel on Gel Documentation (azure Biosystems c150) using software (Second Generation C series capture).

2.8 Estimation of Cost and Time

The time period (hr.) and expected cost (USD) for extraction of one sample using three Kit methods was estimated. Sample preparation and pretreatment steps were eliminated. Based on the cost of DNA extraction Kits, chemical reagents, enzymes and disposable items, the cost of each method was calculated. Comprehensive time & cost was also calculated by using following formula [14].

$$=\left(\frac{\text{Expected cost of one method}}{\text{maximum expected cost}} \times \frac{\text{Expected time of one method}}{\text{maximum expected time}}\right)$$

3. Results And Discussion

3.1 Fourier transform infrared (FTIR) spectroscopy

The spectral scanning of all corn silk samples is displayed in Fig. 1 (a-c). Three basic and simpler modes in corn silk structure observed in all samples i.e. finger print region (800-1500cm^{-1}), C-H stretching (2800-3000cm^{-1}), O-H stretching (3000–3600) cm^{-1}. All samples displayed nearly similar behavior confirming that the grinding technique does not alter the functional profile of corn silk.

3.2 Scanning Electron Microscopy (SEM)

In order to understand the granular image of corn silk samples, scanning electron microscopy (SEM) was performed that represents the morphological images of corn silk samples obtained from different grinding techniques. Results observed through SEM in Fig. 2 (a-c) revealed that varying the grinding technique leads to different physical appearance with respect to size and shape. Among all, corn silk

ground by tissuelyser (CSK-TL) displayed the finest appearance, more compact structure (Fig. 2c). The surface of tissue lyzed samples was more shiny and smooth. However, (CSK-RG) showed enlarged fiber particles with clusters and thread like appearance in Fig. 2a. However, at higher magnification, globular appearance is more obvious on surface of corn silk in CSK-RG (Fig. 2a). In Fig. 2b (CSK-LN) sample displayed compact like structure at low magnification. However, at higher magnification rough surface is visible that defines erosion of particles with liquid nitrogen treatment. Hence it can be suggested that CSK-TL decreased particle size of sample. Even at 10,000X the particles shape is less damaged as compared to CSK-LN or CSK-RG. It hence indicates that tissue lyser is the most efficient technique for grinding and homogenization as compared to remaining ones.

3.3 Quantification

Quantification of DNA in all samples was done by Qubit Fluorometer 3.0. It is a sensitive technique to measure the concentration of DNA even at nanogram level. The extracted samples were quantified in the range of wavelength from 430nm–495nm (blue) and 510nm–580nm (green) in order to determine the yield and purity of DNA in all samples. Comparison between three homogenization techniques with respect to different extraction Kits are presented in Table 1.

Table 1
Comparison of grinding methods and DNA extraction by three different kits

KITS	Samples	CSK-TL (ng/ul)	CSK-RG (ng/ul)	CSK-LN (ng/ul)
KIT 1	A1	110.3	75.1	80.3
	A2	122.2	61.8	96.5
	A3	105.6	56.7	91.6
KIT 2	A1	73.0	35.2	10.71
	A2	85.2	53.6	12.5
	A3	99.6	67.2	14.8
KIT 3	A1	10.56	3.32	9.60
	A2	40.1	26.2	14.9
	A3	15.6	9.82	11.6
A1, A2, A3 = Sample collected from different local areas.				
CSK-TL = Corn silk sample obtained by grinding through tissue lyzer.				
CSK-RG = Corn silk sample obtained by grinding through simple grinder.				
CSK-LN = Corn silk sample obtained by crushing through liquid nitrogen.				
KIT 1 = QIAGEN DNeasy mericon food kit				
KIT 2 = eurofins|GeneScan GENESpin DNA isolation Kit				
KIT 3 = PENICON EZ-10 Spin Column Genomic DNA Miniprep Kit				

On the basis of results observed, QIAGEN DNeasy Mericon Food Kit gave highest efficacy in all three homogenization methods due to efficient purification with no carryover inhibitors, as compared to other Kit that has lesser potency to extract DNA and minimize inhibitors in samples.

Unlike homogenization by blender and Liquid nitrogen in mortar and pestle, which just disrupts the material, the TissueLyser II performs both disruption and homogenization of corn silk, ensuring that high molecular weight cellular proteins and carbohydrates are not sheared [15]. Moreover, tissue lyzed samples showed more DNA yield as compared to samples which were grinded by normal blender and crushed by Liquid nitrogen because the process of homogenization with tissue lyzer is based on stainless steel jars that converted sample into fine powder that result in high quality DNA.

3.4 Real time PCR

qPCR technique is one of the most commonly used method for the detection of genetic material and gene quantification. This technique gives more precise and accurate results then conventional PCR. 35S promoter, ABII and Nos terminator was selected as GM events in corn silk [16]. Results are shown Fig. 3.

After amplification, the GM events showed no calculated threshold (Ct) values in all three samples of corn silk indicating negative results for GMO events 35S promoter, ABII and Nos terminator. Whereas, internal positive controls having ct values in each target as mentioned in Fig. 3.

Figure 3 shows all channels with no sample peaks as well as Ct values in targets 35S promoter, ABII, Internal Controls and Nos terminator that shows negative results of GMO in corn silk. Peaks at (30.38, 30.82, 30.21 and 30.07) Ct values were determined as positive controls of the Kit.

3.5 Agarose gel electrophoresis

The technique of agarose gel electrophoresis helps to separate DNA on the basis of molecular weight and charges. All three samples were loaded on gel and allowed to run for approximately 1 hour at 120 Volts. Afterwards, bands were observed as shown in Fig. 4.

DNA with short fragments moves faster than the long fragments [17]. In Fig. 4, all three Kits showed same band size but different consistency that resulted in lighter and darker band color. The bands from all Kits appeared above the standard ladder. Among all Kits, DNA extracted by Kit 1 gave maximum DNA yield due to which it showed darker bands. Kit 2 and Kit 3 both are not much sensitive or efficient to lyse the cell which results in impurities and less DNA yield. Whereas, the band size of all samples were more than 1000 bp.

3.6 Estimation of cost and time

The time and cost of all three Kits was estimated on the basis of extraction time and cost of Kits. As shown in gel electrophoresis, Kit 1 gave maximum DNA yield therefore it is more efficient Kit than other. All three Kits take approximately 1 to 2 hour for extraction of DNA. The time evaluation and cost estimation is shown in Table 2.

Table 2
Estimation of time and cost with respect to three different DNA extraction kits

KITS	Expected Time (hr)	Expected Cost (USD)	Comprehensive time and cost*
KIT 1	1.5	348.77	0.75
KIT 2	2	270.59	0.776
KIT 3	1	108.24	0.155
1USD = 166.30 PKR			
*(Expected cost of one method / maximum expected cost) × (Expected time of one method/ maximum expected time)			
KIT 1 = QIAGEN DNeasy mericon food kit			
KIT 2 = eurofins|GeneScan GENESpin DNA isolation Kit			
KIT 3 = PENICON EZ-10 Spin Column Genomic DNA Miniprep Kit			

Cost of Kit 1 was higher as compared than other two Kits, as it has more favorable solvents such as lysis buffer that rupture the cell wall to extract DNA or elusion buffer that gives higher efficacy. But after time estimation, Kit 3 gave the DNA yield in 1 hour due to its less time duration. When both time and cost were analyzed together, Kit 3 showed the most economical outcome followed by Kit 1 and then Kit 2 (Table 2).

4. Conclusion

DNA extraction is the most critical step in the field of molecular research. Results quality should be sufficient enough to conduct further experimentations. In the present study, different Kit based DNA extraction protocols were compared by utilizing byproduct of corn i.e. corn silk for the detection of GMO using Real Time PCR technique. Results confirmed the absence of GMO. Furthermore, by comparing different grinding techniques; tissue lyzed samples (CSK-TL) displayed highest quantity of DNA extracted. However, in terms of DNA amount, QIAGEN DNeasy Mericon Kit gave the highest efficacy. Hence, based on the obtained results, present investigation could provide a new insight in utilizing waste as a medium for GMO analysis.

Declarations

Conflict of Interest

There is no conflict of interest between the authors

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References

1. Gwirtz, J.A., Garcia-Casal, M.N.: Processing maize flour and corn meal food products. Ann. N Y Acad. Sci. 1312(1): 66. (2014) https://dx.doi.org/10.1111%2Fnyas.12299
2. FAO, S.: 2019
3. Wan Rosli, W.I., Nurhanan, A.R.: Dehydrated Maydis stigma enhances nutritional and antioxidative capacities of butter cookies. Adv. Environ. Biol. **9**(27), 192–198 (2015)
4. Al-Oqail, M.M., Al-Sheddi, E.S., Farshori, N.N., Al-Massarani, S.M., Al-Turki, E.A., Ahmad, J., Al-Khedhairy, A.A., Siddiqui, M.A.: Corn silk (Zea mays L.) induced apoptosis in human breast cancer (MCF-7) cells via the ROS-mediated mitochondrial pathway. Oxid. Med. Cell. Longev. (2019). https://doi.org/10.1155/2019/9789241
5. Hasanudin, K., Hashim, P., Mustafa, S.: Corn silk (Stigma maydis) in healthcare: a phytochemical and pharmacological review. mol. **17**(8), 9697–9715 (2012). https://doi.org/10.3390/molecules17089697
6. Žilić, S., Janković, M., Basić, Z., Vančetović, J., Maksimović, V.: Antioxidant activity, phenolic profile, chlorophyll and mineral matter content of corn silk (Zea mays L): Comparison with medicinal herbs. J. Cereal Sci. **69**, 363–370 (2016). https://doi.org/10.1016/j.jcs.2016.05.003
7. Vollenhofer, S., Burg, K., Schmidt, J., Kroath, H.: Genetically modified organisms in food screening and specific detection by polymerase chain reaction. J. Agric. Food Chem. **47**(12), 5038–5043 (1999). https://doi.org/10.1021/jf990353l
8. Li, Y., Xiao, F., Liu, B., Gao, H., Wu, Y., Li, J., Wu, G.: Rapid screening of genetically modified ingredients in soybean and cotton processing by-product and waste using direct qPCR. Oil. Crop. Sci. **5**(3), 142–148 (2020). https://doi.org/10.1016/j.ocsci.2020.07.006
9. Pellegrino, E., Bedini, S., Nuti, M., Ercoli, L.: Impact of genetically engineered maize on agronomic, environmental and toxicological traits: a meta-analysis of 21 years of field data. Sci. Rep. **8**(1), 1–12 (2018). DOI:10.1038/s41598-018-21284-2
10. Abdel-Latif, A., Osman, G.: Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant. Methods. **13**(1), 1–9 (2017). DOI 10.1186/s13007-016-0152-4
11. Demeke, T., Jenkins, G.R.: Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. Anal Bioanal Chem. 396(6): p. 1977–1990. (2010) DOI 10.1007/s00216-009-3150-9
12. Dalla Costa, L., Bozzoli, M., Pompili, V., Piazza, S., Broggini, A.L., Patocchi, G., Malnoy, A.: M.: Development of a Taqman real-time PCR method to quantify nptII in apple lines obtained with ‘established’or ‘new breeding’techniques of genetic modification. Eur. Food Res. Technol. **245**(3), 643–652 (2019). https://doi.org/10.1007/s00217-018-3187-0
13. Rabiei, M., Mehdizadeh, M., Rastegar, H., Vahidi, H., Alebouyeh, M.: Detection of genetically modified maize in processed foods sold commercially in Iran by qualitative PCR. Iran. J Pharm Res : IJPR **12**(1), 25 (2013)

14. Wang, Y.S., Dai, T.M., Tian, H., Wan, F.H., Zhang, G.F.: Comparative analysis of eight DNA extraction methods for molecular research in mealybugs. PloS one. **14**(12), e0226818 (2019). https://doi.org/10.1371/journal.pone.0226818
15. Khanna, R., Singhal, K., Mohanty, S.: Quantification of single drosophila fly genomic dna using uv spectrophotometry, nanodrop and qubit fluorometry. Pranikee J. of Zoological Society of Odisha. XXVIII. (2016)
16. Mano, J., Hatano, S., Nagatomi, Y., Futo, S., Takabatake, R., Kitta, K.: Highly sensitive GMO detection using real-time PCR with a large amount of DNA template: single-laboratory validation. J. AOAC Int. **101**(2), 507–514 (2018). https://doi.org/10.5740/jaoacint.17-0197
17. Lee, P.Y., Costumbrado, J., Hsu, C.Y., Kim, Y.H.: Agarose gel electrophoresis for the separation of DNA fragments. JoVE, (62): e3923. (2012) doi:10.3791/3923

Figures

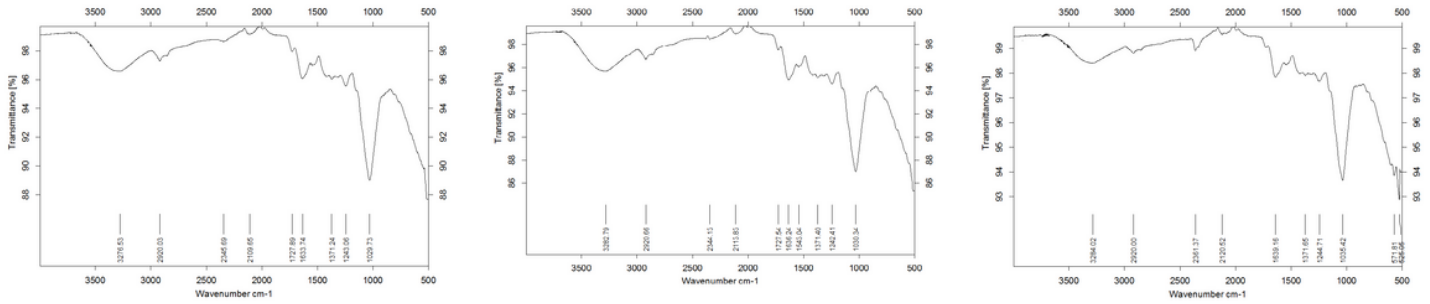

Figure 1

a FTIR spectra of corn silk raw grounded (RG) sample in region of 3500-500 cm^{-1}

b FTIR spectra of corn silk Liquid Nitrogen (LN) sample in region of 3500-500 cm^{-1}

c FTIR spectra of corn silk TissueLyser (TL) sample in region of 3500-500 cm^{-1}

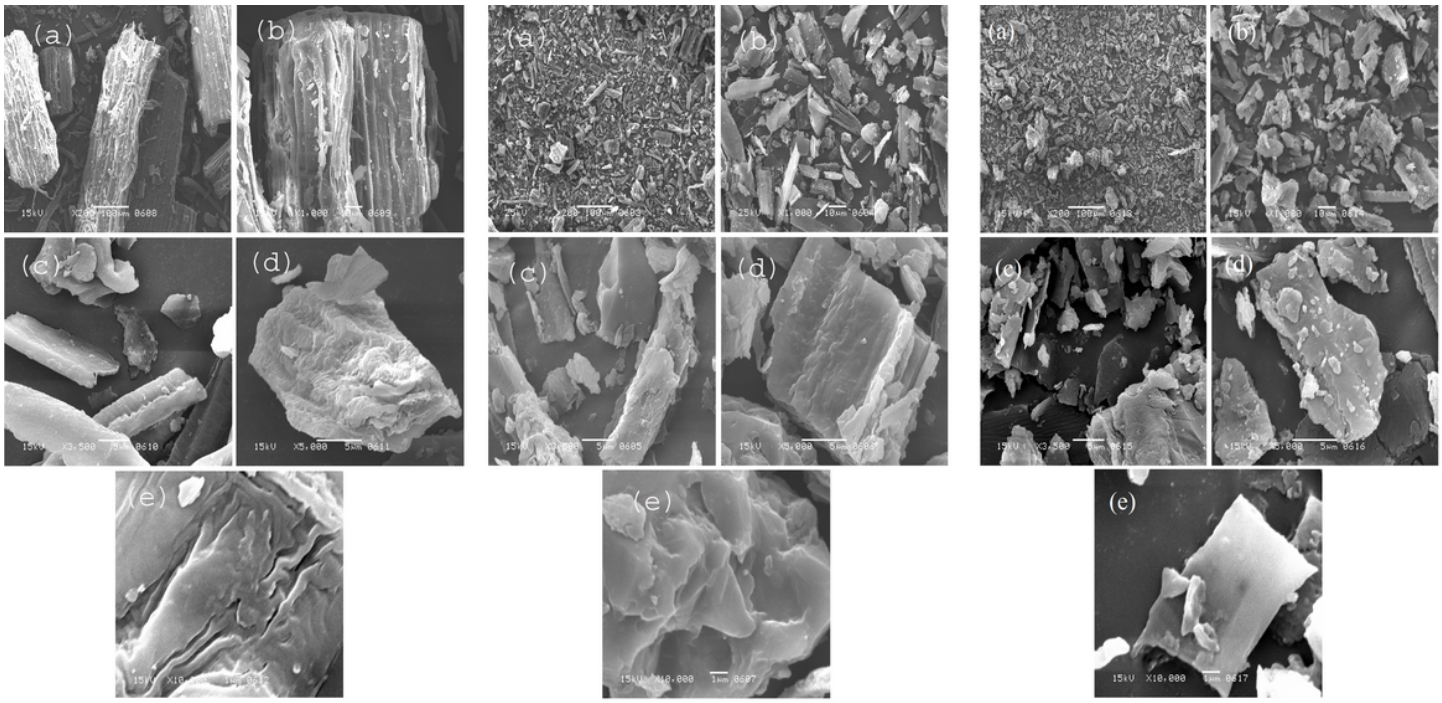

Figure 2

a Scanning electron microscopy SEM of corn silk raw grounded (RG) sample at magnification a) 200X b) 1000X c) 3500X d) 5000 e) 10000X

b Scanning electron microscopy SEM of corn silk grinded by Liquid Nitrogen (LN) at magnification a) 200X b) 1000X c) 3500X d) 5000 e) 10000X

c Scanning electron microscopy SEM of corn silk grinded by TissueLyser (TL) at magnification a) 200X b) 1000X c) 3500X d) 5000 e) 10000X

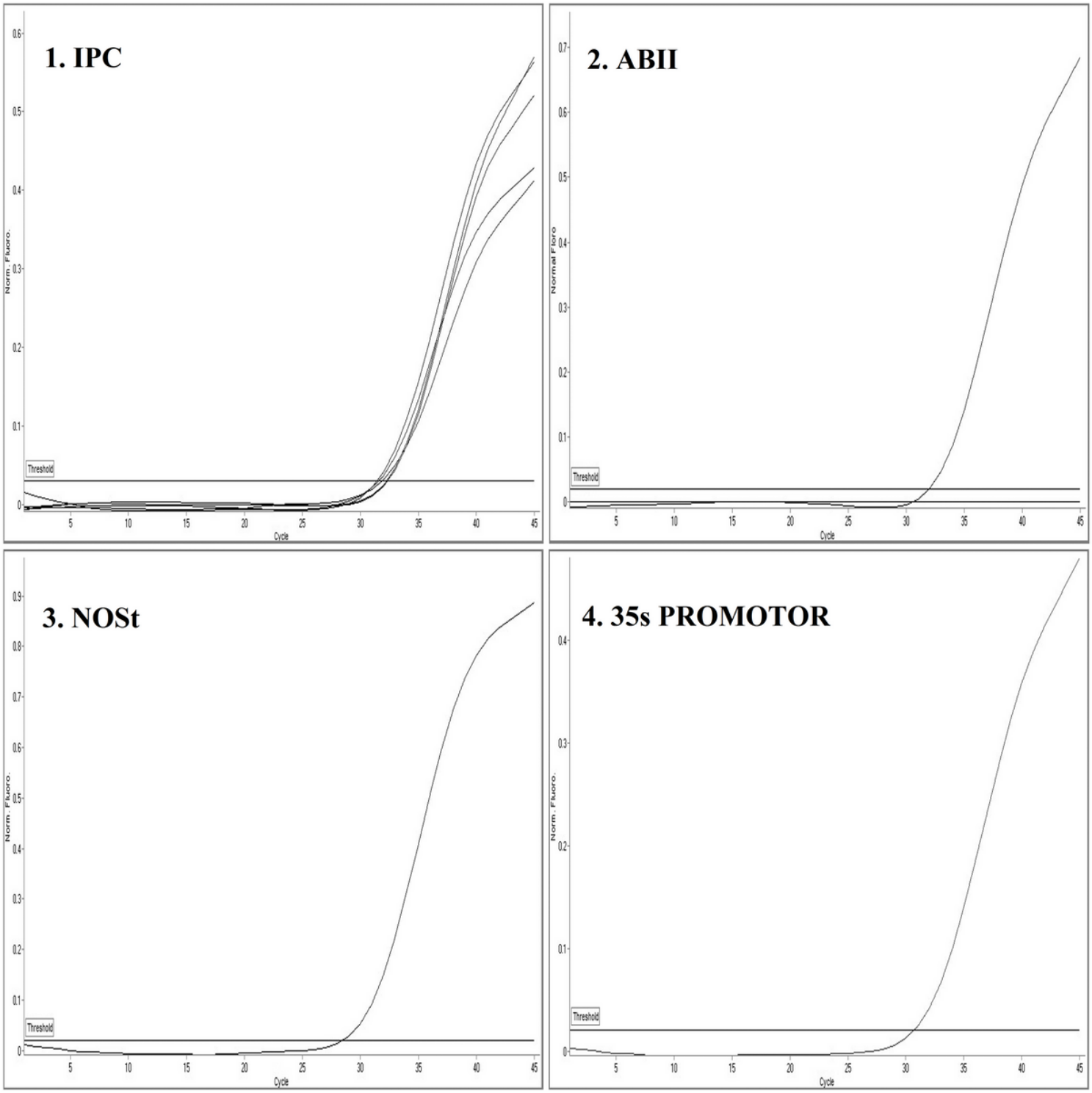

Figure 3

Detection of GMO using corn silk wastage by Real Time PCR technique

1.IPC= internal positive control

2.ABII= (Agroborder II) GMO target

3.NOSt= (Nos terminator) GMO target

4.35s promoter= GMO target

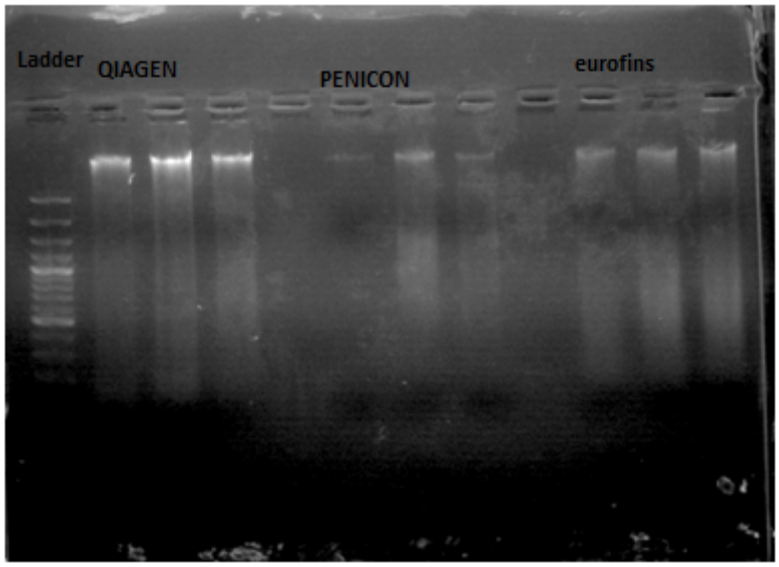

Figure 4

Effect of DNA extraction methods with three different kits specify DNA bands.

Supplementary Files

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- GraphicalAbstract.png