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OPTIMIZATION AND RAPD-PCR PROCEDURE FOR EXTRACTING DNA FROM SEEDS OF SESAME (*Sesamum indicum* L.)

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ABSTRACT

Molecular work on sesame seeds has been complicated due to presences of high level of polysaccharide and secondary metabolites that make it difficult to obtain good quality DNA for such study. To overcome this challenge, an optimized method for the extraction of genomic DNA from the seeds of sesame (*Sesamum indicum* L.) was designed. A standard protocol of CTAB was reviewed and modified for DNA extracted from twenty three sesame samples. The combined use of CTAB/PVP and re-precipitation of supernatants with 70% ethanol (instead of the common isopropanol used) recovered the DNA from the impurities. Aliquots of 4 µL of RNase were added to the samples in the extraction buffer after grinding into a paste and homogenizing well before incubation. A complete removal of impurities and RNA from the total genomic DNA was recorded after checking on 0.8% agarose gel. For confirmation DNA from two sesame samples was subjected to PCR amplification using OPA 10, OPA 18 and ODP 20 primers. Thereafter, the twenty three sesame samples were amplified successfully with OPA 10. The extracted DNA samples were observed under UV illumination using agarose gel electrophoresis after staining with ethidium bromide. The modified protocol employed in this study yielded a good quality DNA and was found to be suitable for PCR and RAPD analyses especially well only sesame seeds are available for molecular study.

Keywords: polysaccharides, genomic, electrophoresis, RAPD, PCR

INTRODUCTION

According to [1] sesame is very popular and widely used in the Central, North Western and North Eastern parts of Nigeria where it is grown. The regions of major production of sesame in Nigeria in the order of importance are Nasarawa, Jigawa and Benue State. Other important areas of production are Yobe, Kano, Kastina, Kogi, Gombe and Plateau States [2]. Sesame is majorly grown in countries such as Sudan, Nigeria, Ethiopia, Uganda, Mexico, Venezuela, India, China, Pakistan, Turkey and Myanmar [3]. Sesame is an important oil seed crop world-wide, and it yields high quality edible and odourless oil that serve as a good source of protein and fat for humans and livestock [4]. Sesame seeds are used in baking and making candy, while its oil in addition to cooking is used in the manufacture of soaps, paints, perfumes, pharmaceuticals and insecticides for ethnobotanical uses [5].

[6] and [7] opined that accurate characterization of the available genetic pool is important in breeding programs. The traditional methods for characterization and assessment of genetic variability based on morphological, physiological and agronomic traits are often not adequate, since these traits are developmentally regulated or influenced by the genotype-environment interaction and agronomic practices [8 and 9]. [10] reported that molecular markers provide an opportunity for genetic characterization and allow direct comparison of different genetic material independent of environmental influences.

Occasionally leaves of sesame may not be readily available for molecular study; this will now leave the researcher with no option than to consider the seeds for such work. The isolation of good quality genomic DNA in the opinions of [11] and [12] is a prerequisite for successful molecular study. However, high amounts of polysaccharides, polyphenols and various secondary metabolites such as alkaloids, flavonoids and tannins in some plant parts usually interfere with DNA isolation. Researchers often modify a protocol or blend two or more different procedures to obtain DNA of the desired quality [13]. Plant contaminants like polysaccharides and phenolic compounds according to [14] and [15] are difficult to separate from DNA and are readily identified as they impart a sticky gelatinous brown color to the DNA isolated which therefore interfere with polymerases, ligases and restriction enzymes.

Prior to this study, no report of DNA isolation from seeds of sesame (*Sesamum indicum* L.) has been reported to the best of my

knowledge because sesame seeds often contain high quantity of polysaccharides, polyphenols, and other secondary metabolites which hamper DNA isolation, amplification, restriction digestion and subsequent molecular cloning. Hitherto various molecular studies on sesame have been carried out on the leaf samples [16, 17, 18, 19, 20, 21 and 22]. [14] reported that molecular techniques require isolation of genomic DNA of suitable purity.

I tested previously established standard DNA isolation protocols on the selected sesame seeds but these methods resulted into DNA with lot of impurities which make them unsuitable for RAPD studies. Modifications were made to minimize polysaccharide and secondary metabolites co-isolation and to simplify the procedure for processing large number of samples. Therefore, the aim of this study is to report an efficient protocol for isolating pure DNA from seeds of sesame for PCR amplification by optimizing the existing protocols for plant DNA extraction.

MATERIALS AND METHODS

Genomic DNA extraction

Genomic DNA was extracted from seeds of twenty three sesame genotypes as shown in table 1 using the CTAB protocol of [23] with some modifications. The modifications were made to improve the quality of the DNA. Standard isolation methods yield DNA with high amounts of contamination (impurities) confirmed to be secondary metabolites and polysaccharides that precipitated along with the DNA. These non-polar impurities remained stocked along with RNA in the well of the gel during electrophoresis. In this method 0.20g of ground sesame seeds were immediately transferred into extraction buffer solution consisting of 2% CTAB (w/v), Tris HCL pH 8.0 (2M): Sodium EDTA pH 8.0 (0.5M); NaCl (0.5M), 2% Poly Vinyl Pyrrolidone (PVP). This was later transferred into 13ml Falcon tubes containing 6ml of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water bath at 65^0C with gentle shaking for 30min and left to cool at room temperature for 5mins. Isopropanol-chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5mins at room temperature to make a homogenous mixture. The cell debris was removed with 70% ethanol instead of the common isopropanol extraction and centrifugation at 5,000r.p.m for 15mins. Immediately after centrifugation, the supernatant was carefully removed without dislodging the DNA pellet. The last drop of ethanol was removed by placing the tube face down on paper towel. The procedure was repeated twice and the resulting highly

viscous solution (containing DNA) was transferred to new sterile tubes.

The nucleic acids in the highly viscous solution were precipitated by adding equal volume of cooled isopropanol. The contents were mixed gently and centrifuged at 5,000 r.p.m for 10mins. The formed DNA pellet was washed twice with 70% ethanol, and the ethanol was discarded after spinning with flash centrifugation. The concentrate was re-centrifuged at 4,000r.p.m for 10mins to re-suspend the DNA into the supernatant. The supernatant was pipetted and re-precipitated with 70% ethanol (instead of the common isopropanol used) to recover the DNA while the residues were discarded. The remaining ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10mM Tris, 1mM EDTA, pH 8) and stored at -20^{0}C for further use. The extracted DNA samples were observed under UV illumination using agarose gel electrophoresis after staining with ethidium bromide.

RNase treatment

Aliquots of 4 µL of RNase were added to the samples in the extraction buffer after grinding into a paste and homogenizing well before incubation. The removal of RNA and quality of the DNA was examined by running the extracted DNA samples on 0.8% agarose gel stained with ethidium bromide. The gel was then visualized and photographed under UV light.

PCR amplification using RAPD primers

Extracted DNA from two sesame samples was subjected to PCR amplification using three primers (viz OPA 10, OPA 18 and ODP 20). Thereafter for confirmation, extracted DNA from the 23 sesame samples was electrophoresis using OPA10 with sequence GGGTAACGCC. PCR reaction was carried out with a profile that started with initial denaturation at 94^{o}C for 3min followed by 35 cycles of 20sec at 37^{o}C, 1min at 94^{o}C and 40sec at 72^{o}C, with a final extension of 7min at 72^{o}C. 1.4% agarose was prepared using 2.8g in 200ml IXTAE, micro-waved to dissolve the agarose and cooled down (at 56^{o}C). The gel was poured into the gel tray that was prefixed with comb. The gel tray was immersed into the electrophoresis tank containing 0.5 XTBE buffer. The comb in the gel was removed to expose the well formed. The amplified DNA (10µl) was loaded into the wells of the gel with the aid of a pipette. The gel was run for 2hrs at 150V and 0.5mAmp and the DNA bands in the gel were observed under UV light. The bands were photographed using a digital camera.

RESULTS

DNA extracted from the twenty three sesame seeds using a modified CTAB method yielded good quality DNA. Results from the agarose gel electrophoresis showed clear DNA bands. Plate 1 revealed the presence of non-polar (not charged) impurities still stocked in wells of the gel. Plate 2 showed photograph of gel electrophoresis of the extracted genomic DNA following the modified DNA extraction procedure with removed impurities but RNA still interfering with the DNA. Plate 3 shows the PCR optimizations for three primers (OPA 10, OPA 18 and ODP 20) using two sesame genotypes amplifying well with the DNAs from the samples, also free from impurities and RNA. Plate 4 showed agarose gel showing amplified DNA patterns for the 23 sesame samples using primer OPA 10 (GGGTAACGCC).

Table 1: Brief Description of the 23 Sesame accessions used for the Study

Accession Numbers	Accession Names	Sample Sources (States)	Geopolitical Zones	Brief Morphological Description of Samples at their Collection centre
1	*03M	Badeggi (Niger)	North Central	Stem erect, green branched, whitish pink flower with light brown seeds.
2	*E8	Badeggi (Niger)	North Central	Stem erect, green branched, whitish pink flower with light brown seeds.
3	*01M	Badeggi (Niger)	North Central	Stem erect, green branched, whitish pink flower with light brown seeds.
4	*02M	Badeggi (Niger)	North Central	Stem erect, green, branched, whitish pink flower with light brown seeds.
5	*EXSUDAN	Badeggi (Niger)	North Central	Stem erect, green, branched, whitish pink flower with light brown seeds.
6	IBA I	Ibadan (Oyo)	South West	Stem erect, green, branched, whitish pink flower with dark brown seeds.
7	IBA II	Ibadan (Oyo)	South West	Stem erect, green, branched, whitish pink flower with light brown seeds.
8	OKE I	Okene (Kogi)	North Central	Stem erect, green, branched, whitish pink flower with light brown seeds.
9	YOL I	Yola (Adamawa)	North East	Stem erect, green, branched, whitish pink flower with light brown seeds.
10	MAI I	Maiduguri (Borno)	North East	Stem erect, green, branched, whitish pink flower with dark brown seeds.
11	KAN III	Kano (Kano)	North West	Stem erect, green, branched, whitish pink flower with white seeds.
12	KAN II	Kano (Kano)	North West	Stem erect, green, branched, whitish pink flower with light brown seeds.
13	KAN I	Kano (Kano)	North West	stem erect, green, branched, whitish pink flower with light brown seeds.
14	MAK I	Makurdi (Benue)	North Central	Stem erect, green, branched, whitish pink flower with light brown seeds.
15	OUT	Otukpo (Benue)	North Central	Stem erect, green, branched, whitish pink flower with light brown seeds
16	ZAR I	Zaria (Kaduna)	North Central	Stem erect, green, branched, whitish pink with dark brown seeds
17	ANY I	Anyigba (Kogi)	North Central	Stem erect, green, branched whitish pink flower with light brown seeds
18	ANY II	Anyigba (Kogi)	North Central	Stem erect, green, branched, whitish pink flower with dark brown seeds
19	OKE II	Okene (Kogi)	North Central	Stem erect, green, branched, whitish pink flower with dark brown seeds
20	ILO I	Ilorin (Kwara)	North Central	Stem erect, purple, branched, purple flower with black seeds.
21	ILO II	Ilorin (Kwara)	North Central	Stem erect, purple, profusely branched, pink flower, black seeds
22	OFF I	Offa (Kwara)	North Central	Stem, erect, green, branched, pink flower, black seeds
23	JAL I	Jalingo (Taraba)	North East	Stem, erect, green, branched, whitish pink flower with light brown seeds

*Improved sesame genotypes.

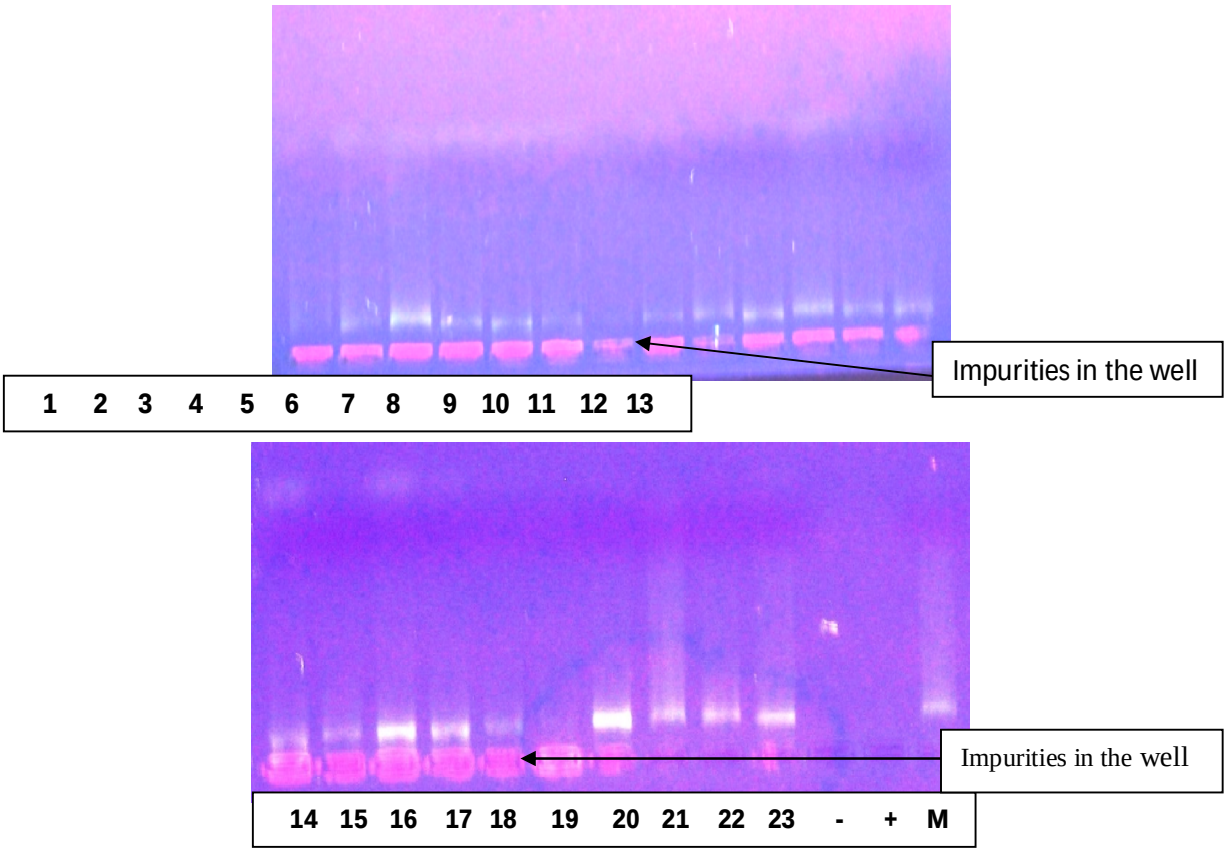

Plate 1: Agarose Gel Electrophoresis of the Genomic DNA isolated showing impurities in the well

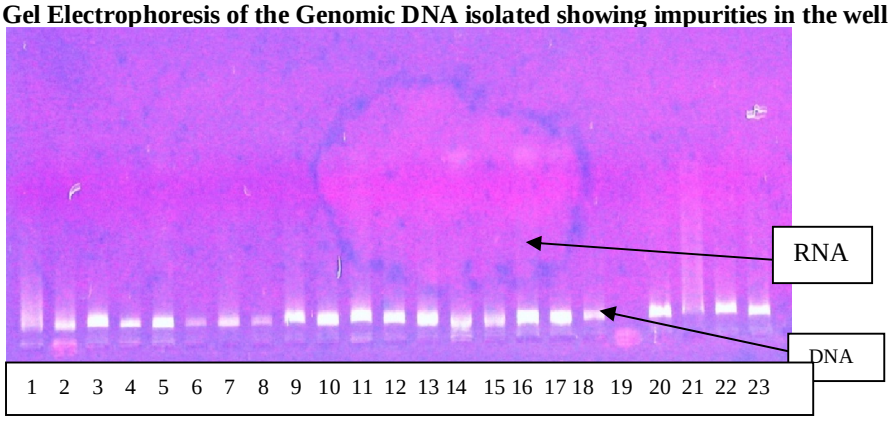

Plate 2: Agarose Gel Electrophoresis of the isolated genomic DNA showing absence of impurities in the well but presence of RNA

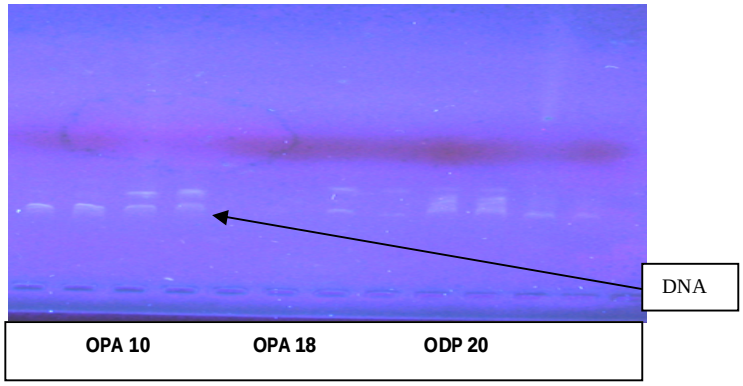

Plate 3: PCR Optimization for three primers using two sesame genotypes free from impurities and RNA.

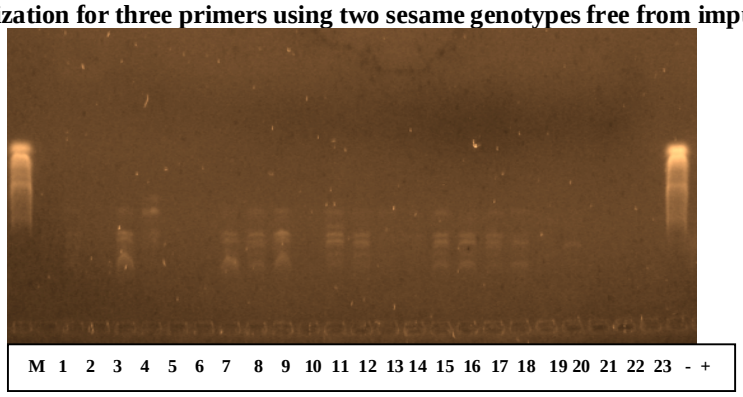

Plate 4: Agarose gel showing the amplified DNA from the 23 sesame samples using OPA 10 (GGGTAACGCC).

DISCUSSION

Seeds are storage organs and hence rich in proteins, lipids, polysaccharides, alkaloids and other secondary metabolites. These compounds can interfere with DNA isolation and successive amplification [15]. In the opinion of [23] the growing number of DNA extraction protocols for specific plant species are not always simple and cannot be reproduced for member of all species. Therefore I initially considered twenty three sesame samples to confirm the universality and consistency of the modified extraction protocol for removing impurities from the plant species studied. In the present study, I have successfully isolated DNA from dry seeds of sesame. The presence of gelling polysaccharides prevents complete dissolution of nucleic acids and imparts a viscous constituency to the DNA making it stick to the wells during gel electrophoresis. The fact that all the twenty three sesame accessions considered in this study revealed impurities precipitating along with the DNA is an indication that seeds of sesame generally is associated with polysaccharides and secondary metabolites. Also, the successful extraction of pure genomic DNA with the use of the modified extraction protocol for all the 23 sesame samples studied further affirmed

the fact that the modified extraction procedure employed in this study is appropriate for standardizing and improving the quality of genomic DNA extracted from sesame seeds. The combined use of CTAB/PVP and re-precipitation of supernatants with 70% ethanol (instead of the common isopropanol used) helped to recover DNA from the impurities. The addition of PVP to CTAB according to [15] helped to removal impurities by forming a complex with them using hydrogen bonds. The quality of DNA was therefore checked by agarose gel electrophoresis. I observed conspicuous intact bands of high molecular weight DNA. Several reports have indicated that the presence of RNA can suppress PCR amplification and lead to non-reproducible and unreliable DNA amplification patterns in RAPD analysis [14]. The treatment of RNase degrades RNA into small ribonucleosides that are not detectable by gel electrophoresis. Without high quality DNA [9] reported that downstream molecular manipulations are not feasible. The removal of RNA with RNAse was earlier reported by [6] to be a reliable method.

The reports of [13] that DNA fingerprint remains the same irrespective of the plant part used make this study a reliable alternative to the dependence of leaf for molecular study in sesame. These modified steps were found to be very useful for standardizing and improving the quality of genomic DNA. The present optimized protocol for DNA isolation and RAPD technique may serve as an efficient tool for further molecular studies especially when only seeds of sesame are available. The absence of RNA, polysaccharides and the amplification of molecular weight bands are evident of a good quality genomic DNA. It is evident that treatment of the samples with the RNase enzyme completely removed RNA from the samples. Therefore, it can be concluded that the present protocol describes a reliable, rapid, simple and consistent DNA isolation method for sesame seed.

CONCLUSION

Although the purity of the extracted DNA was tested exclusively with twenty three sesame samples, it is expected that this protocol will be applicable to all sesame seeds. Therefore, this procedure is a rapid, simple, reliable and consistent DNA isolation method for sesame seed.

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