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Full Length Research Paper

Comparative analysis of genomic DNA isolation procedures; hexa decyltrimethylammonium bromide (CTAB) and liquid detergent (morning fresh®) methods from Samsorg 41 (icsv 400)

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Genomic DNA was prepared from germinating seeds of a variety of sorghum in Nigeria (Samsorg 41) using two different isolation protocols (CTAB and morning fresh® method). Highest quantity of DNA was obtained from Samsorg 41 using the morning fresh® method. Genomic DNA obtained using this method had a much lower purity as compared with that obtained using the CTAB method. Both methods produced genomic DNA of high integrity with minimum shearing.

Key words: Genomic DNA, CTAB, morning fresh®, isolation, Samsorg 41, Nigeria.

INTRODUCTION

Sorghum is the fifth most important grain crop in the world after wheat, maize, rice and barley and the second most important cereal crop in sub-Saharan Africa. Like rice and barley, sorghum belongs to the grass family – Gramineae. Sorghum is the main food grown in parts of India and Africa, where it is mainly used in making bread, porridges and opaque alcoholic drinks (Mohammed et al., 2008; Agu et al., 1997; Murty et al., 1996; Rootney et al., 1990). It is classified into four groups; Grain sorghum, Broom sorghum, Grass sorghum, Sweet sorghum, based on their local uses.

Samsorg 41 a variety of sorghum developed at ICRISAT Asia, which is a photosensitive high fertility sorghum plant and it is known to be early maturing at 65 to 75 days (Aba et al., 2005).

Starch is the major storage form of carbohydrate in sorghum, it consist of amylopectin, a branched chain polymer of glucose and amylose, a straight chain polymer. The plant cell wall contains many non-carbohydrate components in addition to lignin, such as protein, lipids and inorganic material and they modify the property of the polysaccharide (Ausubel et al., 1993). Germinating sorghum is known to contain hydrolytic enzymes such as α and β amylase in its embryo (Schulze, 2007; Mathew et al., 2002; Taylor and Robins 1993).

In recent times, sorghum is used as a substitute for barley in Nigeria for brewing. As regards fermentable carbohydrate extracts, sorghum is similar to corn, barley and rice used internationally as brewing adjuncts. The high starch content of sorghum (Haln, 1966), offers not only a rich source of fermentable extract, but it also reduces brewing cost.

The study of gene expression requires the preparation of DNA from the plant. Even though there are several protocols for the preparation of DNA from plants (Byeong-Ha, 2006, http://bioprotocol.bio.com/protocolstools/protocol) these methods are saddled with several disadvantages including contamination of the DNA, yield of DNA and cost of preparation of DNA. Also chemotypic homo-geneity among species may not allow optimal yield with a single protocol; thus even closely related species may

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require different isolation protocols (Weishing et al., 1995). This study was carried out in order to compare

genomic DNA yield and purity from Samsorg 41 (icsv 400) using two different protocols for genomic DNA isolation.

MATERIALS AND METHODS

Samsorg 41 (a variety of brewing sorghum) was obtained from Institute of Agricultural Research Center, Ahmadu Bello University, Zaria. Eco RI restriction endonuclease, 2-mercatoethanol, hexadecyltrimethyl ammonium bromide (CTAB) were supplied by Sigma. Morning fresh® was supplied by PZ Cussons Nigeria. All other reagents were molecular grade. 2 g of Samsorg 41 was germinated for 2 weeks under laboratory conditions.

Genomic DNA preparation using CTAB as detergent

The frozen sprouting leaves of Samsorg 41 were used for the preparation of genomic DNA. Tris-HCl extraction buffer containing 1.4 M NaCl, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 2% hexadecyltrimethyl ammonium bromide (CTAB), 0.2% (v/v) 2-mercaptoethanol was added to 2 g of ground leaves, and the mixture heated to 60°C for 30 min. phenol: chloroform (1:1) mixture was then added to the resultant mixture and then centrifuged at 4000×g for 20 min. DNA was then precipitated from the upper aqueous phase using an equal volume of ice cold 100% ethanol, DNA was then pelleted by centrifuging at 4000× g for 12 min. the pellets were washed with wash buffer containing sodium acetate and 76% (v/v) ethanol, the pellet was reconstituted in TE buffer pH 8.0 containing 10 mM Tris-HCl and 1 mM EDTA. 1 M NaOH pH 8.4 was used to remove RNA. Phenol: chloroform (1:1) mixture was then added to the mixture, and the upper aqueous phase was subjected to precipitation using ice cold absolute ethanol and 7 M sodium acetate. The precipitate was recovered by centrifuging at 4000×g for 10 min. the DNA precipitate was washed using 70% ethanol.

Genomic DNA preparation using liquid household detergent morning fresh®

2 g of Samsorg 41 was homogenized in distilled water containing 0.5 g NaCl. To the homogenate was added 10 ml of morning fresh®. The mixture was subsequently incubated at 60°C for 12 min to inactivate DNAses. The mixture was then cooled in a freezer at 0°C for 15 min. After 15 min the mixture was then filtered through four layers of cheesecloth. 100% ice cold ethanol was used to precipitate genomic DNA in the filtrate, and the genomic DNA was recovered by centrifugation at 4,000 × g for 10 min. genomic DNA was then air dried and re-dissolved in distilled water for further analysis.

Genomic DNA quantification and purity assessment

DNA samples were dissolved in distilled water and its yield was assessed by taking absorbance at 260 nm. DNA purity was also calculated by taking absorbance of samples at 280 nm.

Restriction digest of genomic DNA

Restriction digest was carried out in 50 µl containing 10 u Eco RI restriction enzyme, 100 mM Tris, pH8.0; 100 mM $MgCl_2$; 100 mM NaCl; 100 mM β- mercaptoethanol and 5 µg plant genomic DNA Digestion was done at 37°C overnight. Digested samples were resolved on a 1% agarose gel at a voltage of 49 V, 50 mA current and 2 W of power, compared with a 10 to 50 kb DNA standard marker. The electrophoresis was carried out for 40 min. A UV transilluminator was used to visualize the gel and photograph was taken using a digital camera, and the photograph was analyzed with a gel documentation system.

RESULTS

Comparative physical appearance and properties of isolated genomic DNA from Samsorg41 using morning fresh® and CTAB

Genomic DNA obtained from apical tissues of germinating samsorg 41 sorghum using morning fresh was visible but difficult to spool, and had to be recovered by centrifugation at 4,000× g for 5 min.

Genomic DNA obtained using CTAB as a detergent was also recovered by centrifugation at 4, 000× g for 5 min to get pellets.

Genomic DNA quantity and purity using both morning fresh® and CTAB as detergents

All extractions were done in triplicates of complete independent extractions. And the results are shown in Tables 1 and 2. a higher yield of genomic DNA (36.70 µg) from 2 g of sample was obtained from Samsorg 41 using the morning fresh method® this method also produced DNA of lower percentage purity (68%) as compared to the CTAB method which gave a genomic DNA yield of 25.78 µg DNA from 2 g of Samsorg 41 and 84% purity.

Restriction digest

We also tested the integrity of the genomic DNA obtained and its usability for further molecular studies by subjecting 5 µg of the genomic DNA obtained to 10 units of Eco RI restriction digest. Several bands were resolved on the 0.7% agarose gel. Eco RI generated 5 characteristic bands (105, 100, 89, 43 and 25 kb) as seen in Figure 1. The gel also revealed no signs of shearing of Samsorg 41 genomic DNA.

DISCUSSION

Genomic DNA was successfully isolated from Samsorg 41 using both methods. Both methods produced genomic DNA from Samsorg 41 in sufficient quantities for further studies as was seen from the high yield of genomic DNA from 2 g of Samsorg 41. The method used significantly influenced the yield and purity of genomic DNA obtained from Samsorg 41. Genomic DNA obtained using the morning fresh® method was much lower in purity than that obtained using CTAB, this could be attributable to

Table 1. Quantity and purity of genomic DNA from germinating seeds of Samsorg 41 using morning fresh® detergent technique.

Sample	Absorbance at 260 nm	Absorbance at 280 nm	Purity 260/280	Percentage Purity	Total yield (µg/ml)	Genomic DNA yield (µg)
Samsorg41	1.63 ± 0.38	1.09 ± 0.31	1.49 ± 0.03	68±2	734 ± 171.2	36.70 ± 0.02

Values are mean ± SD of three independent isolation events.

Table 2. Quantity and purity of genomic DNA from germinating seeds of Samsorg 41 using CTAB technique.

Sample	Absorbance at 260 nm	Absorbance at 280 nm	Purity 260/280	Percentage Purity	Total yield (µg/ml)	Genomic DNA yield (µg)
Samsorg41	1.15 ± 0.67	0.71 ± 0.38	1.63 ± 0.10	84 ± 6.11	515.67 ± 304.11	25.78 ± 0.03

Values are mean± SD of three independent isolation events.

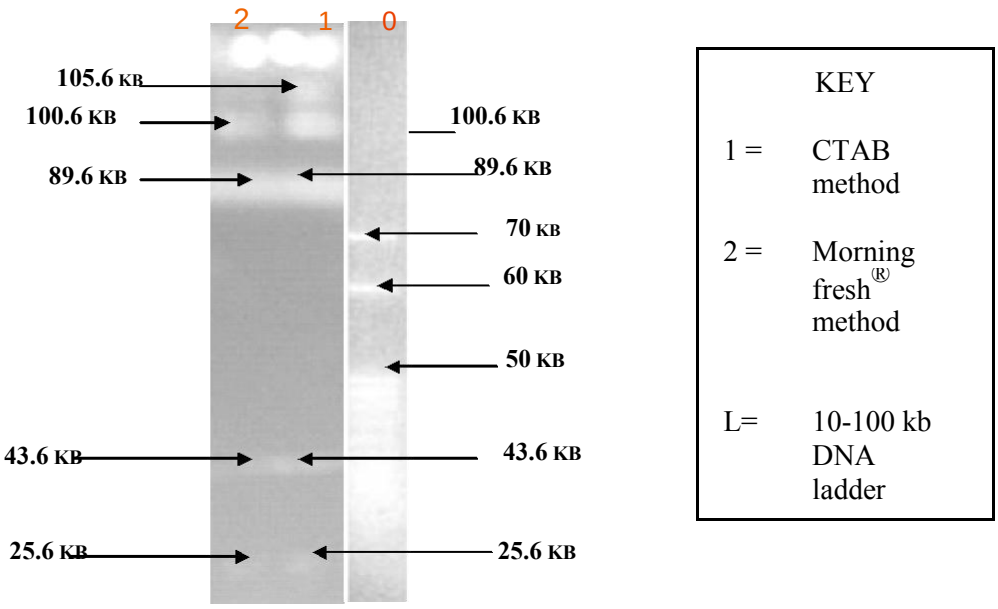

Figure 1. Samsorg 41 (2 µg) genome was digested with 10 units of Eco RI for 1hr at 37°C fragments were separated on 0.7% agarose gel and photographed using a Sony digital camera under UV-light and analyzed using a gel documentation system.

the heterogenic nature of the morning fresh® being composed of several other chemical substances in addition to its major constituents of Sodium dodecyl Sulphate (SDS). Naranayan et al. (2006) has previously demonstrated that using the CTAB method of genomic DNA isolation from plants yielded genomic DNA of higher purity than SDS. The CTAB method produced genomic DNA of acceptable purity which is very desirable in molecular studies (Khan et al., 2007; Narayanan, 2006).

Both methods provide a very rapid and convenient way of preparing DNA from plants considering the ease of DNAse inactivation and protein removal using phenol-chloroform mixture (Sarwat et al., 2006; Sangwan et al., 1998).

Samsorg41 has a very high content of carbohydrate and other polysaccharide (Aba et al., 2005; Do and Adams 1991) which frequently interferes with the preparation of genomic DNA. The use of CTAB or morning fresh as the detergent was able to isolate genomic DNA from the seeds of Samsorg 41 even though there was a clear difference in the quality and quantity of genomic DNA, with the morning fresh method giving a significantly higher yield of genomic DNA as compared to the CTAB method. The morning fresh method also revealed genomic DNA with high protein contamination this is not surprising as the main detergent component of morning

fresh® is SDS and similar results have been demonstrated (Narayanan et al., 2006). High temperature was used to inactivate DNAse enzyme in order to protect genomic DNA from digestion. Also 0.5 M NaOH at a pH of 8.4 was used to degrade RNA which is another common contaminant of genomic DNA from plant sources. The use of temperature to inactivate DNAse and other nucleases capable of degrading genomic DNA as opposed to proteinase K helps to reduce overall cost of genomic DNA preparation. Similarly the use of sodium hydroxide further enhances the cost effectiveness of both methods used.

Eco RI digest of the isolated genomic DNA was done to ascertain the integrity of isolated genomic DNA. The results demonstrate that genomic DNA obtained using both isolation methods are suitable for use in further molecular studies such as restriction digest and PCR studies. Electrophoresis of the restriction fragments revealed similar banding patterns for Eco RI restricted genomic DNA obtained using both protocols with little sharing of the genomic DNA obtained (Sarwat et al., 2006).

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