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A Simple and Rapid Leaf Genomic DNA Extraction Method for Pearl Millet (*Pennisetum glaucum* L. R. Br)

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Abstract

Pearl millet is widely grown as a multi-purpose cereal grain crop for feed, fodder, fuel and mulch predominantly in the semi-arid tropics. It is highly adapted to drought, representing an essential component of the food security and livelihood of many million poor farmers. DNA extraction is unusually difficult in some plants due to the presence of secondary metabolites that interfere with DNA isolation making it very laborious and time consuming. An effective genomic DNA extraction should be simple, cost effective, with good yield and high purity. The protocol used in this study involved the extraction of genomic DNA from fresh leaves using the Sodium dodecyl sulphate (SDS) method with slight modifications including absence of use of liquid nitrogen which is difficult and expensive to obtain in the developing World. Genomic DNA obtained from the ten pearl millet samples using this procedure was good.

Keywords: *Pennisetum glaucum*, Genomic DNA extraction, Sodium Dodecyl Sulphate, Secondary metabolites, Fresh leaves

1. Introduction

Pearl millet (*Pennisetum glaucum* L. R. Br.) which belongs to family poaceae (graminae), is a tall, warm season, annual grass widely grown for feed, fodder, fuel and mulch in more than 26 million hectares mostly in the semi-arid tropics of sub-Saharan Africa and India (FAO & ICRISAT, 1996). It is the sixth most important coarse-grain cereal grown in these regions (Gari, 2002). Pearl millet also plays a critical role in food security, because it is has the highest levels of tolerance to heat and drought among tropical cereals (Khairwal et al., 2007),

Genomic DNA extraction is usually a prerequisite for molecular and forensic analysis. Different methods have been used in isolating genomic DNA, these methods include, the Cetyl Trimethyl Ammonium Bromide (CTAB) method and its modifications (Huang et al., 2000; Doyle et al., 1987), which is the most routinely used method, the Sarkosyl Nitrogen method, the Sodium dodecyl-sulfate (SDS) method, the Phenol/chloroform method and the use of kits e.g. Promega wizard™ genomic DNA purification (Hasan et al., 2009). Though these methods have been widely used and successful in isolating DNA from different plant species, they are time consuming, laborious and not cost effective especially in resource limited laboratories. Some of these protocols include the use of liquid nitrogen and DNA isolation kits which is not easy to obtain due to high cost (Amani et al., 2011; Ahmed et al., 2009)

The presence of metabolites in plants has made genomic DNA extraction a difficult process. These secondary metabolites include alkaloids, flavonoids, phenolic compounds, gummy polysaccharides, terpenes and quinine are co-extracted with the DNA during isolation which makes purification difficult (Shepherd et al., 2002). For downstream applications such as DNA restriction, amplification, and cloning, “clean” DNA isolation is required. While the degree of purity and quantity of DNA is important, it varies between applications. A good extraction procedure for the isolation of DNA should yield adequate and intact DNA of reasonable purity. The procedure should also be quick, simple and cheap (Zidani et al., 2005). The aim of this study, therefore, was to determine a genomic DNA extraction protocol suited for isolation of reasonably pure DNA in sufficient amount from pearl millet fresh leaves which is simple and rapid, without the use of expensive chemicals and specific equipment.

2. Materials and Methods

2.1 Plant Material

Ten samples of Pearl millet (*Pennisetum glaucum* [L.] R. Br.) Seeds were collected from Lake Chad Research Institute (LCRI) Maiduguri, Nigeria, germinated and grown for 7 days. The tissues used were young fresh leaves.

2.2 Solutions

An extraction buffer consisting of 5% SDS, 100mM Tris (pH 8.0), 50 mM EDTA (pH 8.0), and 500m M NaCl, and 5 M potassium acetate, was prepared. In addition, phenol-chloroform, isoamyl-alcohol (25:24:1), 70% and 100% ethanol and a TE buffer consisting of 10 mM Tris (pH 8.0) and 1 mM EDTA (pH 8.0) were also prepared

2.3 DNA Extraction Protocol

0.3 g of young fresh leaves were harvested and chopped into smaller pieces in a mortar. 800

µl of extraction buffer was added and without liquid nitrogen, round quickly, using a pestle to obtain a homogenous mixture. The homogenate was transferred into 1.5 ml eppendorf tubes. 200 µl of the extraction buffer was added again and mixed well by inversion. It was centrifuged at 4 °C for 2 minutes at 12, 000 rpm. The supernatant was transferred into new eppendorf tubes. 200 µl of 5 M potassium acetate was added and mixed well. Equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was then added and mixed well by inversion. The tube was centrifuged at 4 °C for 5 minutes at 12,000 rpm.

The supernatant obtained was carefully transferred into a new eppendorf tubes without disturbing the interface. 800 µl of absolute ethanol was added, mixed well by hand and kept on ice for 20 minutes. The tubes were removed from ice and centrifuged again at 4 °C for 5 minutes at 12, 000rpm. The supernatant was discarded and the DNA pellet obtained was washed three times with 70% ethanol and air dried until the ethanol evaporated completely. The pellet was re-suspended in 100 µl of TE buffer and 2 ul of RNase was then added. The tube was incubated at 37 °C for 30 min (Ferdous et al., 2012)

2.4 Amount and Purity of DNA

The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm using a UV spectrophotometer (Eppendorf Biophotometer). The yield of DNA per gram of leaf tissue was also determined. Electrophoresis was carried out on DNA samples from the leaf tissues on a 0.8% agarose gel, according to Sambrook et al. (1989). Gels were stained with ethidium bromide and viewed and photographed using the gel documentation system (Alpha innotech).

3. Result and Discussion

The DNA extracted from ten varieties of pearl millet, were dissolved in 100 µl of T.E. Each of the DNA samples optical density (OD) ratio 260 nm/280 nm was calculated from the readings obtained from the readings at UV wavelength of 260 nm and 280 nm using a spectrophotometer (Bio photometer, Eppendorf). The values varied between 1.7 and 1. 9 (Figure 1).

DNA quality is considered good with an OD_{260}:OD_{280} ratio between 1.7 and 2.0 (Maniatis et al., 1982, Gulia et al., 2010). Extracted DNA concentrations of the samples ranged between 1500 to 1700 ng per extraction, which is sufficient for various downstream applications.

The quality of the extracted DNA was also evaluated using agarose gel electrophoresis. The sharp and distinct bands obtained after running on a 0.8% agarose gel, stained with ethidium bromide and visualized with UV light is shown in Figure 2.

Extraction of genomic DNA using this method was simple and easy, it was not time also consuming compared to other DNA extraction methods. The steps involved in isolation of DNA took approximately 30minutes.

This protocol also did not include the use of liquid nitrogen; the continuous supply of which is a problem in many resource limited laboratories in developing countries due to the cost and availability. Young fresh leaf tissues were used in this method, it was found that using younger leaves instead of older ones reduced nucleic acid contamination by plant metabolites that interfere with solubilisation of precipitated nucleic acids. A three-time wash combined with a short-run centrifugation was sufficient for DNA purification and removal of

endogenous nucleases or other proteins. The purity of genomic DNA is dependent on the number of washes (Puchaa, 2004).

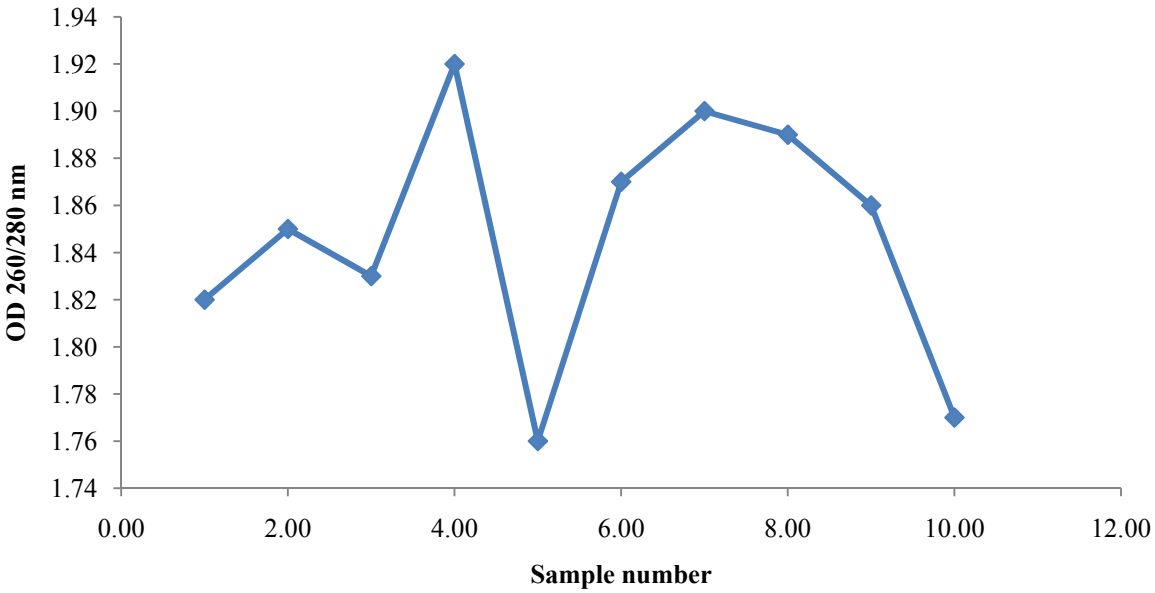

Figure 1. Optical density (OD) ratio of DNA samples extracted from the ten Pearl millet varieties

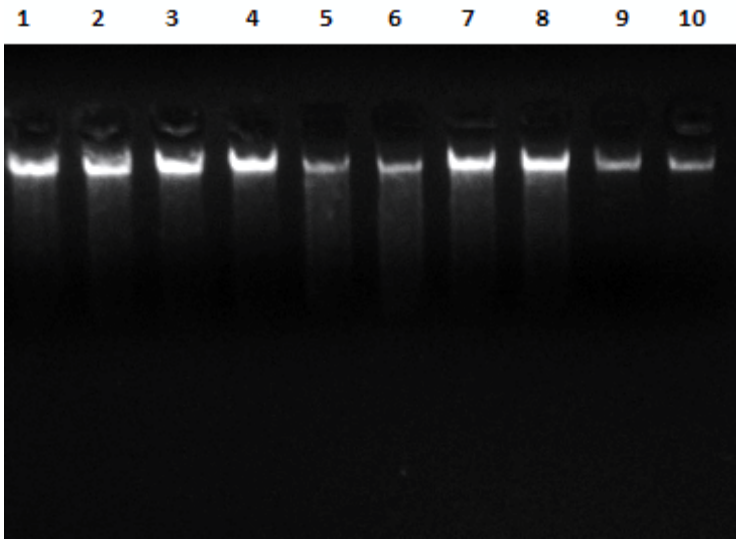

Figure 2. Bands obtained after agarose gel electrophoresis showing total DNA isolated from ten samples of Pearl millet (*Pennisetum glaucum* L. R. Br)

4. Conclusion

The efficiency, speed and the use of inexpensive facilities makes this protocol suitable for the extraction of DNA from leaf tissues of pearl millet and possibly other cereals. These results show that the DNA produced by this simple and rapid protocol can be used in PCR-based

techniques and other applications

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