Comparative investigation of DNA extraction methods in black gram *Vigna mungo* (L.)

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**ABSTRACT**

Isolation of intact, double stranded, pure and non-contaminated genomic DNA is a prerequisite for large scale genotyping analysis including DNA-banks. Three methods of DNA isolation (Dellaporta, CTAB and Hi-PurAg DNA isolation kits) from 25 black gram genotypes were compared in terms of the yield, purity, integrity, and stability of extracted DNA. Purity and quantification of isolated DNA samples was confirmed by using the UV nano-spectrophotometer at OD260/280 and the same is confirmed based by agarose gel electrophoresis. The CTAB method showed the best results followed by Hi-PurAg and Dellaporta method. The CTAB DNA extraction method was found to be the most efficient DNA extraction method, capable of providing high quality, pure and stable DNA and could be used for various molecular related works. All the 25 black gram genotypes for this research gave good yield of DNA from the established modified CTAB protocol.

**Key words:** CTAB, Gel electrophoresis Isolation, UV gel documentation, UV nano-spectrophotometer.

**INTRODUCTION**

Black gram [*Vigna mungo* (L.) Hepper] belongs to the family *Fabaceae* and the genus *Vigna*. Only seven species of the genus *Vigna* are cultivated as pulse crops viz. five Asian species of subgenus *Ceratotropis, Vigna mungo* (black gram), *V. radiata* (mung bean), *V. aconitifolia* (moth bean), *V. angularis* (azuki bean) and *V. umbellata* (rice bean) and two African species of subgenus *Vigna, V.unguiculata* (cowpea) and *V. subterranean* (bambara groundnut) (Verdcourt, 1966). It is a staple crop in the central and South East Asia; however, it is extensively used only in India and now grown in the Southern United States, West Indies, Japan and other tropics and subtropics (Delic *et al.*, 2009).

Black gram is one of the most highly prized pulse crop, cultivated in almost all parts of India. It has inevitable market itself as the most popular pulse and can be most appropriately referred to as the “king of the pulses” due to its mouth watering taste and numerous other nutritional qualities. In Japan, the health conscious people eat these seeds by soaking them in water overnight and then serving them as fresh bean sprout salad which is highly nutritious. Black gram is perfect combination of all nutrients, which includes proteins (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins. It stands next to soybean in its dietary protein content. It is rich in vitamin A, B1, B3 and has small amount of thiamine, riboflavin, niacin and vitamin C in it. It contains 78% to 80% nitrogen in the form of albumin and globulin (Das *et al.*, 2016). The dry seeds are good source of phosphorus. It also has very high calorie content. 100 gm of black gram has 347 calories. Therefore, black gram is the cheapest available source of protein for the poor and vegetarians (Tharanathan and Mahadevamma, 2003).

The combination of dal-chawal (pulse-rice) or dalroti (pulse-wheat bread) is an important ingredient in the average Indian diet. The biological value improves greatly, when wheat or rice is combined with black gram because of the complementary relationship of the essential amino acids such as lysine and sulphur containing amino acids methionine and cysteine. Black gram also has medicinal properties, like curing diabetes, sexual dysfunction, nervous disorder, hair disorders, digestive system disorders and rheumatic afflictions. It is valued for its high digestibility and freedom from flatulence effect.

The extraction of good quality DNA with a high yield is a limiting factor in plants’ genetic analysis. DNA quality from each line should be consistent to allow a proper genetic analysis from several plant individuals. High quality of DNA is characterized by predominantly high molecular weight fragments with an A260/280 ratio between 1.8 and 2.0 and the lack of contaminating substances, such as polysaccharides and phenols (Kasem *et al.*, 2008).

The extraction and purification of high-quality DNA is generally difficult due to the presence of polysaccharides, proteins, and DNA polymerase inhibitors such as tannins, alkaloids, and polyphenols (Sarwat *et al.*, 2006). The presence of these compounds affects the quality and quantity of isolated DNA, and therefore, renders the sample non-amplifiable. Polysaccharides, the most commonly found

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contaminants in plant DNA extraction, make DNA pellets slimy and difficult to handle. The anionic contaminants inhibit restriction enzymes and effect enzymatic analysis of the DNA (Braid et al., 2003).

MATERIALS AND METHODS

Plant Samples: A total of 25 genotypes of black gram was collected from the Germplasm maintained at the Department of Genetics and Plant breeding, Annamalai University. Seeds were germinated in the pots in the greenhouse. Genomic DNA isolation methods viz., Dellaporta, CTAB, Hi-PurA DNA isolation kits have been used to extract DNA from black gram genotypes.

Reagents and Chemicals: Cetyl trim ethyl ammonium bromide (CTAB) extraction buffer [2% CTAB, 1% polyvinyl pyrrolidone,100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA], Dellaporta DNA Extraction Buffer [100 mM Tris pH 8; 50 mM EDTA pH 8; 500mM NaCl; 10 mM; 2-mercaptoethanol], TE buffer [10 mMTris–HCl, pH 8.0; 1.0 mM EDTA, pH 8.0], chloroform: isoamyl alcohol (24:1, v/v), iso-propanol, 70% ethanol, 2-mercaptoethanol, liquid nitrogen, sodium acetate and Rnase were used in the study.

DNA Extraction Procedure

CTAB-based method: The genomic DNA was extracted from 2 g of each sample by CTAB-based method according to Doyle and Doyle (1990) with slight modification. Grinded 2 g of plant tissue to a fine paste in approximately 500 µl of CTAB buffer. Transferred CTAB: plant extracts mixture to a micro centrifuge tube. Incubated the CTAB: plant extract mixture for about 15 min at 55°C in a water bath. After incubation, spun the CTAB: plant extract mixture at 12000 rpm for 5 min to spin down cell debris. Transferred the supernatant to clean micro centrifuge tube. Incubated the CTAB: plant extracts mixture at -20°C for 30 min. Centrifuged the supernatant in a 65°C water bath for 10 min. Added 500 µl ice cold 70% ethanol. Removed residual ethanol by air drying. Dissolved the DNA in 20 µl TE buffer.

Dellaporta-based method: The protocol described here is a modification of the method developed by Dellaporta et al. (1983). Weighed 1 g of tissue and grinded using liquid N2 to fine powder in a mortar and pestle. Transferred the fine powder into a 30 mL falcon tube and added 15 mL Dellaporta DNA extraction buffer. Added 1mL of 20 % SDS to each tube. Mixed thoroughly by vigorous shaking, and then incubated tubes in a 65°C water bath for 10 min. Added 5 mL of 5M Potassium acetate and mix thoroughly by vigorous shaking, then incubated the tubes at 0°C (on ice) for 20 min. Centrifuged the tubes at 13000 rpm for 20 min. Poured supernatant into a 30 ml falcon tube containing 10 mM isopropanol and inverted 20 times to mix well and incubated at -20°C for 30 min. Centrifuge tubes at 12000 rpm for 15 min. Gently pour off supernatant and slightly dry DNA pellets by inverting the tubes on paper towels for 10 min. Redissolve each DNA pellet with 0.7 mL of 0.5 M EDTA pH 8.0 and 1 M Tris pH 8. Spin the eppendorf tubes in a micro centrifuge for 10 min at max speed to remove insoluble debris. Transferred the supernatant to a new Eppendorf tube and added 75 µL 3M sodium acetate and 500 µL isopropanol. Mixed well by inverting 20 times and pellet the DNA for 30 sec in a microfuge Washed the pellet with 500 µL 80% ethanol, dried thoroughly and redissolve in 100 µL TE, EB or H2O.

Hi-PurA method: Black gram DNA was extracted using a commercially available kit (HiPurA™ Plant DNA Isolation Kit, Himedia) following the manufacturer’s instructions. The time consumed in this method was about 1.5–2 h for 10 samples.

Quantification of the genomic DNA: The quantification of genomic DNA was achieved using a spectrophotometer (UV-Nano spectrophotometer, Shimadzu, Japan). The yield was determined by measuring the absorbance at A260, A280 and A320 nm. The level of DNA purity was determined by the A260/A280 absorbance ratio (Table 1). Purity determination of DNA interference by contaminants can be recognized by the calculation of ratio of A260/A280, which is used to estimate the purity of nucleic acid.

Table 1: Comparative performance of absorption ratio of OD260/280.

| Genotypes | CTAB | Dellaporta | Hi-PurA |
|-----------|------|------------|---------|
| AUB1      | 1.8  | 1.6        | 2.7     |
| AUB2      | 1.9  | 1.7        | 2.3     |
| AUB3      | 1.9  | 1.6        | 1.8     |
| AUB4      | 2.0  | 2.3        | 1.9     |
| AUB5      | 2.0  | 2.4        | 2.1     |
| AUB6      | 2.0  | 2.1        | 2.0     |
| AUB7      | 1.8  | 1.7        | 2.0     |
| AUB8      | 1.8  | 1.8        | 2.5     |
| AUB9      | 2.0  | 1.6        | 1.7     |
| AUB10     | 2.0  | 1.5        | 1.9     |
| AUB11     | 1.9  | 1.2        | 1.6     |
| AUB12     | 1.9  | 2.5        | 2.3     |
| AUB13     | 1.9  | 2.7        | 2.1     |
| AUB14     | 1.9  | 2.3        | 2.4     |
| AUB15     | 1.9  | 1.8        | 2.6     |
| AUB16     | 2.0  | 1.9        | 2.5     |
| AUB17     | 2.0  | 2.1        | 1.7     |
| AUB18     | 2.0  | 2.0        | 1.3     |
| AUB19     | 2.0  | 2.0        | 1.6     |
| AUB20     | 1.8  | 2.5        | 1.7     |
| AUB21     | 1.9  | 1.7        | 1.6     |
| AUB22     | 1.8  | 1.9        | 2.3     |
| AUB23     | 1.8  | 1.6        | 2.4     |
| AUB24     | 1.9  | 2.3        | 2.1     |
| AUB25     | 1.8  | 2.1        | 1.7     |
DNA quality using agarose gel electrophoresis: DNA purity was further tested by running the extracted genomic DNA samples on 0.8% agarose gel (Fig 1.) stained with 6ul/100 ml ethidium bromide in 1×TBE (Tris base, Boric acid, 0.5M EDTA) gel buffer. The gels were visualized and photographed in gel documentation unit.

RESULTS AND DISCUSSION

A simple, fast and reliable protocol for extraction of genomic DNA from fresh leaves of black gram was established in this study. Most of available DNA extraction protocols were very lengthy, very expensive or not suitable for extracting DNA probably due to the presence of secondary metabolites (Wang et al., 2011; Kotchoni et al., 2011; Margam et al., 2010).

Our results indicated that the CTAB method was more consistent in producing quality DNA than the Dellaporta and HipurA methods. First, the overall amount of DNA extracted using the CTAB method was similar across the 25 black gram genotypes. The quality of each extracted DNA sample was verified using a UV-Nano spectrophotometer. The absorbance profile is useful for detection of contaminants such as protein, salts, and polysaccharides. The 260/280 nm ratio of 1.8 indicated that the extracted DNA had high purity with absence of proteins and phenols (Abdel-Latif and Osman, 2017). Table 1 summarizes the DNA purity range obtained for all sample extracts using the three extraction methods. A 260/280 ratio in this study was found to be in a range of 1.8–2.7. A 260/280 ratio between 1.93 and 2.27 indicates insignificant levels of contamination (Pervaiz et al., 2011). In this study, high quality DNA was extracted with the use of liquid nitrogen. The purity of DNA varied with the method of extraction (Table 1). DNA purity can be severely affected by various components of sample matrices such as polysaccharides, lipids, and polyphenols or extraction chemicals.

The HipurA method produced DNA samples with purity ratios in a range of 1.3–2.7, whereas the purity ratio of samples extracted by Dellaporta was between 1.2 and 2.3. A purity ratio of >1.9 indicates the presence of RNA in the sample. The ratio of >1.8 in few samples of DNA extracted by CATB method suggests the presence of proteins in those samples. High quality DNA is characterized by 260/280 absorbance ratio of approximately 1.8 with a single absorbance peak at 260 nm. The ratio obtained varied from 1.8 to 2.0 indicating that the isolated DNA was free from contamination.

Further, to check the quality of the genomic DNA extracted from different cultivars of black gram using these methods, agarose gel electrophoresis was performed. DNA fragments were clearly obtained from fresh leaves of different black gram genotypes (Fig 1). It was observed that DNA band was clearly visible in gel documentation of agarose gel of modified CTAB method. Whereas in case of Dellaporta and Hipur A methods, DNA bands appeared as smear and degraded to some extent in some of the black gram genotypes. Few genotypes show the RNA contamination in lower portion of agarose gel from Dellaporta method. This might be due to the presence of plant secondary metabolites which affects the quality of DNA. Presence of clear, intact DNA from CTAB was an indication that the DNA extracted using this method was free from plant secondary metabolites e.g. flavonoids, terpenes, and phenolic compounds, which interfere with the yield and quality of the DNA (Porebski et al., 1997).

CONCLUSION

The proposed protocol can be adopted for any PCR-based genetic analysis, especially for large number of plant samples in laboratory settings with limited resources.
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