Original Research Article

Rapid methodforgenomic DNA isolation of mungbean [*Vigna radiata* (L.) Wilczek]

Abstract

The most significant pulse crop in India is the mungbean [*Vigna radiata* (L.) Wilczek], which constitutes almost 16 % of the national pulse area. For biotechnological interventions, it is prerequisite to develop a rapid and cost-effective method for genomic DNA isolation suitable for PCR. In present investigation,modified CTAB method was used to extract the genomic DNA from the leaf tissues of mungbesn. By crushing the leaves of several mungbean genotypes in a pre-heated CTAB extraction buffer, genomic DNA was extracted. Further, SSR markers were used to assess the efficiency of the extracted DNA.The SSR marker-based PCR amplification findings showed that the DNA extracted using this approach was of good quality and suitable for SSR analysis.

Keywords: Mungbean [*Vigna radiata* (L.) Wilczek],Genomic DNA, Cetyl Trimethyl Ammonium Bromide (CTAB),SSR Marker, PCR Amplification.

Introduction

Mungbean [*Vigna radiata*(L.) Wilczek] is one of the most important pulse crops in the Asiatic region. Mungbean, often known as greengram, is a crucial source of protein for humans and the health of the soil since it fixes atmospheric nitrogen. It is well-liked by people all over the world because of its high protein content, simple digestion, and lack of flatulence**(Anwar*et al*., 2007)**. The grains, whether split or whole, are made into dal or flour. Cattle are given straw and husk as feed. Mungbean's susceptibility to biotic stresses is the main reason for the crop's low yield**(Mishra *et al*., 2020).** The advent of DNA markers resulted in an inevitable revolution in plant genetics and breeding**(Amiteye,2021).**Majority of DNA extraction protocols suggests the use of liquid nitrogen which is expensive and difficult to obtainas and when required.

The recent deployment of molecular markers has tremendously increased the requirement of rapid and PCR quality DNA in very short period of time for crop improvement **(Prasad*et al*.,**

2018). While the availability of DNA markers associated with qualities of interest has expanded, current DNA extraction technologies have limited the number of samples that can be processed. **(Prasad*et al*., 2018; Rehman *et al*., 2007).** The DNA extraction methods from mungbean are not time efficient, since they require several steps, like the tissues be ground in liquid nitrogen, followed by precipitation of the DNA pellet in ethanol, washing and drying the pellet, etc. However, a plethora of DNA extraction methodologies have been reported and although some of these are undoubtedly rapid, many require the use of expensive, often environmentally hazardous chemicals, and specialized laboratory equipment **(Kang and Yang, 2004; Amani*et al*., 2011).**The need for a rapid and simple procedure is urgent, especially when hundreds of samples need to be analyzed. Here, we describe a rapid, cheaper and efficient genomic DNA extraction method for PCR amplification from leaf tissue

Materials and Methods

Materials used

Leaves samples (young immature leaves) of mungbean plant were used for DNA extraction.The seeds were germinated under favourable conditions with adequate water and sunlight. After 10-12 days, leaves (0.5 g) were taken for genomic DNA extraction.

Reagents used

- 2X CTAB Extraction Buffer: The extraction buffer consisted of 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 1% (w/v) PVP, and 2% CTAB. Warm CTAB buffer in a water bath for 5 minutes just before using it.
- 5X CTAB Buffer
- 70% Ethanol
- Chloroform: Isoamyl alcohol (24:1)
- 0.5 M EDTA (pH-8.00)
- Isopropanol
- Ethanol
- 1M Tris-Cl (pH-8.00)
- 0.1X TE Buffer: 1 mM Tris-HCl (pH 8.0), and 0.1 mM EDTA (pH 8.0)

- 10X TBE Buffer

Genomic DNA Extraction

The leaf tissues are ground in a clean mortar and pestle with the addition of 2ml of warm 2X CTAB extraction buffer. The ground sample was transfered into a nuclease's free microcentrifuge tube(2 ml) and incubated in a water bath for 5 minutes at 65°C. After incubation, equal amount of chloroform: isoamylalcohol mixture (24:1) were added, and mixed by gentle inversion followed by centrifugation at 12000 rpm for 5 minutes. The supernatant (uppermost aqueous layer) was carefully transferred to a new microcentrifuge tube using a pipette. One fifth volume of 5X CTABBuffer was added to the supernatant and mix well followed by extraction through chloroform: isoamylalcohol asdescribed above. DNA was precipitatedby addition of either ethanol (2.5 vol) or isopropanol (0.6 vol) and incubated on ice for 10-15 min. DNA pellet was obtained by centrifugation at 12,000 rpm for 5 min. Supernatant was discarded and DNA pellet was washed with 70% ethanol (1 ml) to remove excess salt. The washed DNA pellet was air dried and dissolved in 30 ul of nucleases free water and stored at 4°C. The quality of extracted genomic DNA from mungbean genotypes were analysed using agarose gel electrophoresis (0.8 %) percent,

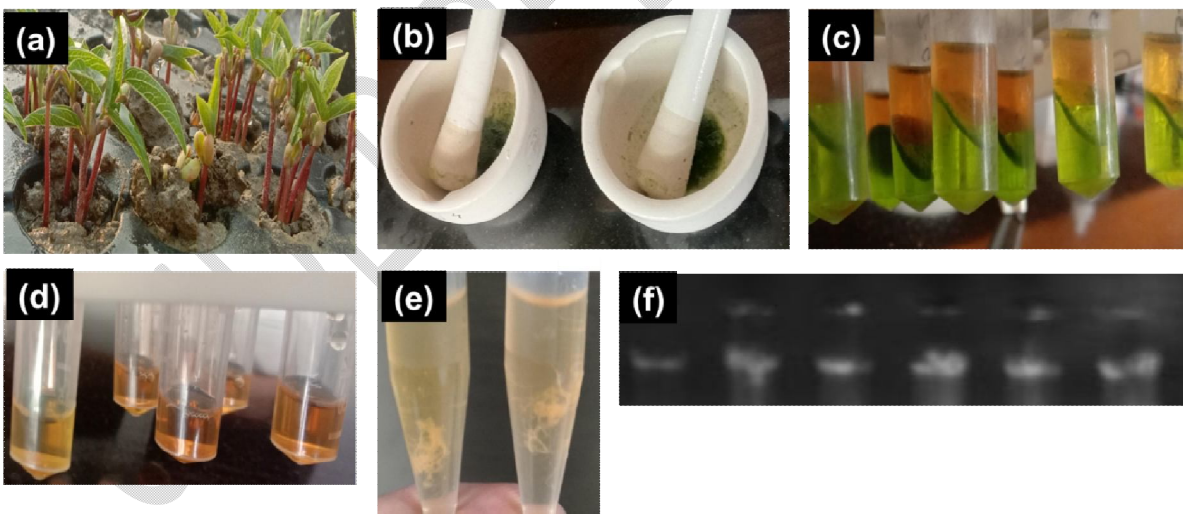

Figure (1): Overview of genomic DNA extraction steps from the leaves of mungbean.

(a) Grown mungbean seedlings; (b) grinding of leaf tissue in warm DNA extraction buffer in mortar with pestal; (c) Three layers separation after Chloroform: isoamyl alcohol treatment, (d) carefully transferred upper or aqueous phase into new micro centrifuge tubes, (e) precipitation of

thread like structure of DNA after addition of either ethanol or isopropanol, (f) visualization of genomic DNA band on agarose gel.

SSR analysis

The extracted genomic DNA was diluted in a 1:10 ratio and 1ul of diluted DNA was used for SSR marker analysis. The SSR amplification was carried out as described previously **(Mishra *et al*., 2020)**. In brief, SSR marker amplification was set up in total 10 µl system volume containing 5 µlAll-in-One PCR SuperMix (Helix), 0.2 µM forward and reverseprimer and 50 ng of genomic DNA in a thermal cycler. The amplification reaction involved an initial 940C for 2 min for denaturation followed by 30cycles of 1 min at 94°C, 30 sec at 51°C, 1 min at 72 °C and final extension at 72°C for 3 min. Amplified products were electrophersised on 2.5 % agarose gel containing 0.5 µl ethidium bromide and the gels were visualized under UV light. The list of SSR markers used in this study is mentioned in Table 1.

Table (1):List of SSR markers used in this study

S. No.	Primer Name	Forward Primer Sequence (5 to 3')	Reverse Primer Sequence (5' to 3')
1.	**MBM00389**	GCAAGTGTTTCGACGAGGTT	GCTACACGCCATTGTTTTGA
2.	**MBM0003**	GGAAGGGAAGGAAAAAGGAA	ATCCAGAAACCGAATCGTTG
3.	**MR7322B**	TCAGTCAGTGTCGATAGCATAGC	GACACAGAGAGAGAGAGAGAG

Results and Discussion

In recent year use of SSR marker in diversity as well as marker assisted breeding (MAB) programme in mungbean have tremendously increased **(Mishra *et al*., 2020; Tabasum*et al*., 2020).**In the present investigation, we aimed to develop a rapid and high throughput DNA isolation protocol for molecular marker analysis in mungbean. The developed protocol was found to be suitable for the rapid isolation of DNA and the suitability of isolated DNA for downstream marker analysis was checked using an SSR marker. The rapid method of DNA isolation has also been developed in other crop plants **(Prasad *et al*., 2018; Ahmadikhah, 2009; Liang*et al*., 2016).**

In present investigation four mungbean genotypes were used for SSR marker analyses. The amplification wasobserved in all the four genotypes in all the three SSR marker used (Figure 2).

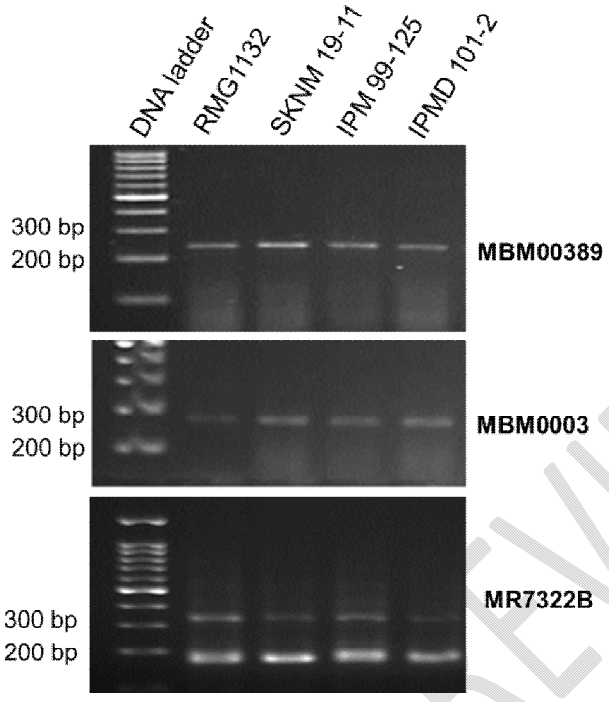

Figure (2): PCR amplification for SSR markers in mungbean genotypes.

The reproducibility this method was checked by repeating the extraction of DNA and PCR several times. Numerous protocols for DNA isolation is available in literature but most of them either uses Liquid nitrogen and other carcinogenic reagents **(Sharma *et al*., 2002).** Our modified protocol doesn't require use of liquid nitrogen and phenol. Additionally, this modified protocol doesn't require any specialized apparatus (e.g., the matrix mill) **(Hill-Ambroz*et al*., 2002).** The commercially available kits are one of the alternatives to traditional methods of DNA isolation, but their higher cost is the limiting factor **(Kang and Yang, 2004; Ahmed *et al*., 2009).**The major advantage of our modified protocol is its rapidity without the use of liquid nitrogen and phenol, a dangerous organic solvent.

Conclusion

Our results clearly demonstrated that the quality of extracted DNA is good enough to perform SSR marker analysis. This method has provided a convenient, rapid, low-cost and safe DNA extraction method for SSR analysis in mungbean.

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