



SHORT RESEARCH ARTICLE

A modified DNA isolation protocol for high-quality DNA and long-term storability in grasspea (*Lathyrus sativus* L.)

K. R. Ramya, R. Gupta¹, A. Pandey¹, K. Tripathi¹, K. C. Bhatt¹ and R. Singh^{1*}**Abstract**

Grasspeas pose challenges in obtaining high-quality DNA due to their seed and leaves' high protein, phenols, secondary metabolites, and neurotoxins. This study investigated the challenges faced by the CTAB method in DNA isolation, subsequent enhancements in quality and storage with a modified CTAB approach using triton, PVP, high salt TE and sodium acetate to extract the high-quality genomic DNA from grasspea.

Keywords: Grasspea, CTAB, protein, PVP, Triton

Grasspea (*Lathyrus sativus* L.) is an annual pulse crop known for its resilience against various stresses, suitable for arid and semi-arid regions globally (Tripathi et al. 2022). It is a multifaceted legume, contains high protein (26.6–34.6%), only known dietary source of L-homoarginine, neurotoxin β -ODAP (β -N-Oxalyl-L- α , β -diaminopropionic Acid) and a significantly higher amount of micronutrients and macronutrients (Ramya et al. 2022). The conventional CTAB extraction approach proved to be ineffective and unsatisfactory. Given the need for quality genomic DNA for the diverse use of molecular markers, an improved extraction method was sought. The study employed kit method and diverse chemical combinations with CTAB for DNA isolation (Sahu et al. 2012). The experiment was carried out with twelve grasspea accessions and four different methods of DNA isolation are given as follows.

CTAB 1

Leaves were grinded with liquid nitrogen with 2% CTAB, 1% β -mercaptoethanol, followed with C: I wash and RNAase purification; precipitation with C_3H_8O overnight with DNA extraction the next day.

CTAB 2

The above-mentioned procedure with 2% CTAB, 1% β -mercaptoethanol, 10% PVP with twice the C: I wash and P: C: I wash. DNA was extracted using the kit method (FAVORGEN).

CTAB 3

The modified genomic DNA isolation protocol was as follows.

An extraction buffer (CTAB 3%) with pH 8 was formulated using 120 mM Tris-HCl, 1M NaCl, 50 mM EDTA, 30/100 mL CTAB, and 1% PVP40, 1% β -mercaptoethanol added.

2% Triton-X-100 was prepared using 50 mM EDTA, 120 mM Tris-HCl, 1M NaCl, 0.5M sucrose, and 20/100 mL Triton-X-100 and supplemented with 1% PVP40 and 1% β -mercaptoethanol. TE buffer was used for DNA suspension, containing 10 mM Tris-HCl (pH8) and 1 mM EDTA. The high salt TE buffer included 0.5M NaCl, 10 mM Tris-HCl, and 1 mM EDTA. In addition, 3M sodium acetate and a 70% (v/v) ethanol washing solution were prepared and incorporated into the extraction procedure. For detailed instructions on each buffer and chemical usage, refer to the flowchart in Fig.

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1. (Note: *- Himedia; #- Gbiosciences; \$- Sigma).

DNA concentration was assessed using 0.8% agarose gel electrophoresis alongside a 100 kb ladder and quantified via Nanodrop. Comparative gel images of DNA concentration are given in Fig. 3. PCR amplification utilized SSR primers in a 15 µL reaction with 36 cycles. The resulting fragments were visualized in a 3% metaphor gel. Concentration and 260/280 ratio details are provided in Fig. 2. Four SSR markers were used for PCR amplification (Table 1 and Fig. 4). A ratio below 1.8 and above 2.0 signifies DNA contamination.

Interestingly, using the CTAB ½ /kit method, we observed ratios below 1.3, above 2.0, or even negative. In contrast, DNA isolated using the CTAB3 showed an OD ratio ranging from 1.78 to 2.02, which falls within the ideal range.

The extraction process involves precise chemical and detergent buffers. Tris-HCl is a buffering agent to maintain pH stability, while EDTA is a metal chelating agent with high

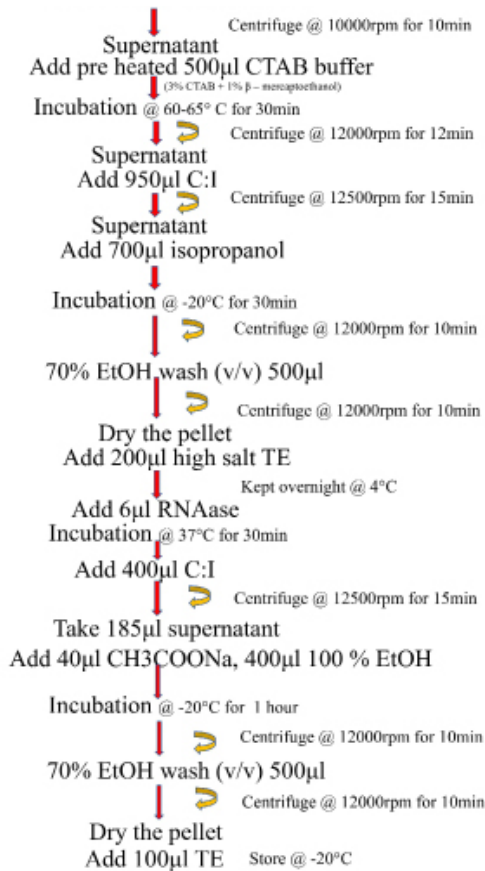


Fig. 1. Modified (CTAB3) DNA isolation protocol

Table 1. SSR primers used in the PCR standardization

S.No.	Repeat motif	Forward Primer (FP)	Reverse Primer (RP)	Ta/C*
1	(AAC)6	CAACCAGAGCAACCACAAGA	GGTTGCAAGAGGTTGCAGAT	56
2	(AAC)6	ACAGCAAGAAGCAGCAACAG	AGTTGGTTGTTGTGTCGTTGT	54.3
3	(AC)18	ATCTTACCGGGATCCATTC	CTTCCCCATTCTCTGGTGTT	56.4
4	(TTG)6	TTTGTGCGTTGATGTTGTT	CTACGTCAGCCCGTCATACC	52

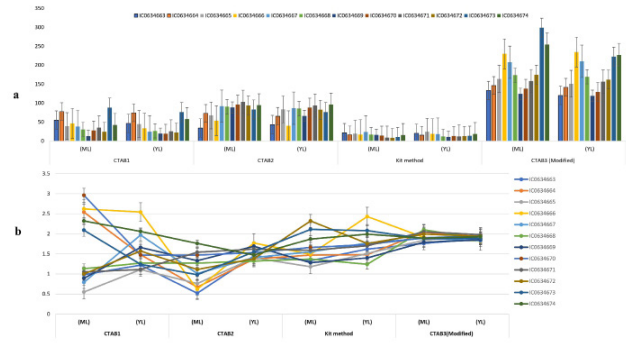


Fig. 2. Comparison of different methods with a) concentration (ng) and b) 260/280 ratio (2)

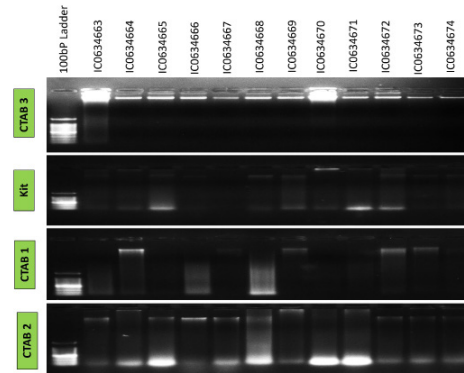


Fig. 3. Comparative gel image of DNA intactness of different methods (5)

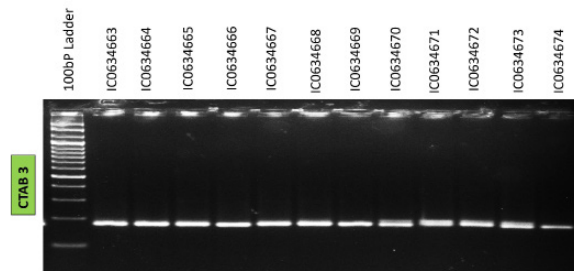


Fig. 4. Gel image of PCR amplification CTAB3 (Modified) using primer 2 (2)

metal ion affinity.

Mg²⁺ plays a crucial role in DNases as a cofactor, impacting DNA synthesis fidelity beyond its charge-related effects. NaCl is instrumental in displacing DNA-bound proteins and neutralizing negative sugar-phosphate backbone charges. PVP efficiently removes phenolic compounds.

Triton X-100, a lab detergent, is a non-ionic surfactant with both hydrophobic and hydrophilic aspects. Its hydrophobic property ensures stability against electrolytic salts, while the hydrophilic end enhances membrane permeability, aiding nucleic acid release. β -mercaptoethanol denatures proteins and eliminates tannins/polyphenols. Sodium acetate precipitates proteins, facilitating DNA renaturation. Samples are suspended in TE buffer for storage. High-salt TE solution facilitates DNA denaturation and neutralization. The incorporation of diverse buffers improved the effectiveness of the adapted CTAB3 method, yielding DNA of exceptional quality. This enhancement considerably extends the DNA's storage potential, outperforming previous methods by addressing technical challenges. In comparison to methods like CTAB 1/2/Kit, the DNA obtained through the CTAB3 method displays remarkable stability, remaining viable for over a year when stored at 4°C which starkly contrasts with earlier methods with reduced maintenance costs and make it as the preferred option for DNA extraction in grasspea.

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