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Research Article

Development of Rapid and Less Hazardous Plant DNA Extraction Protocol using Potassium Phosphate Buffer

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Abstract

Background and Objective: Protocols commonly used in plant DNA extraction were known to be highly time-consuming and harmful due to the application of some hazardous reagents. Therefore, it was not applicable for such laboratories with limited resources as well as for high-throughput analysis. This study was aimed to develop a rapid yet less hazardous DNA extraction protocol for a plant using potassium phosphate buffer. **Materials and Methods:** Genomic DNA of chili pepper (*Capsicum annuum*) was extracted using potassium phosphate buffer and its efficacy was compared to three widely known protocols (CTAB-based, mini preparation and commercial kit). The extracted DNA from those four methods was evaluated based on its quality, quantity, practicality and cost per reaction. **Results:** Genomic DNA resulted from potassium phosphate buffer-based protocol exhibited comparable quality with adequate concentration for further downstream analysis. Results of PCR and sequencing were also emphasized the amplifiable DNA quality from this developed protocol. Compared to those commonly used protocols, potassium phosphate buffer consisted of 5 main working steps only, thus providing a simple yet rapid plant DNA extraction protocol. Since this protocol used ethanol only, it also offered a less hazardous and low-cost protocol that applicable for those resource-limited laboratories. **Conclusion:** This developed protocol provided a promising alternative of plant DNA extraction that might be applicable for both large scale analysis and any laboratory type. Further investigation was needed to evaluate its efficacy in extracting genomic DNA from various plants with different morphological characteristic.

Key words: Simple protocol, low-cost method, DNA mini preparation, quick preparation, *Capsicum annuum*, potassium phosphate, cetyltrimethylammonium bromide (CTAB)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Protocol of genomic DNA extraction used for *Capsicum* genus was mostly based on conventional cetyltrimethylammonium bromide (CTAB) method, even various modifications applied in several studies[1-3]. Protocol of mini preparation developed by Dellaporta *et al.*[4] was also commonly used in some studies involving molecular detection of *Capsicum*-infecting viruses[5-7]. However, these classic protocols are known to be highly time-consuming and harmful due to the numerous handling steps and hazardous reagents used to achieve a high yield yet pure gDNA[8-10]. Therefore, some novel methods used a different approach to minimize the multi-steps and use of organic solvents in order to provide a highly practical and safe protocol with an acceptable gDNA yield[11,12].

Besides the CTAB buffer, the use of phosphate buffer had been reported in several studies, although its application for plant DNA was less popular compared to the CTAB-based method. Interestingly, this method had been proven as a highly practical DNA extraction protocol due to its time-saving and low laborious properties, including for handling such complex samples (e.g. environmental and human samples)[13,14]. Moreover, Garcia *et al.*[15] highlighted the applicability of this method for on-site testing used to detect GMO maize through the replacement of centrifuge with polypropylene membrane filtration. However, the efficacy of this phosphate buffer-based method in chili pepper had not been documented yet. In this present work, the efficacy of Potassium Phosphate Buffer (PPB)-based method for gDNA preparation of chili pepper was carefully evaluated.

MATERIALS AND METHODS

Study area and plant materials: This present study was conducted in the Laboratory of Plant Biotechnology, Faculty of Agriculture, Universitas Andalas, Indonesia from September-October, 2019. The plant material used in this study was West Sumatra local genotype of chili pepper named *Berangkai*. Plants were maintained in a greenhouse for 4 weeks. Leaves were collected in bulk and sterilized using sterile aquadest before subjected to further analysis.

Preparation of chili pepper genomic DNA: Genomic DNA (gDNA) of chili pepper was prepared using three different manual methods (PPB, mini preparation[16] and CTAB-based[17]) and a commercial kit (GeneJet Plant Genomic DNA Purification Mini Kit, Thermo Scientific™, USA). PPB-based was performed three times, while the remaining methods and commercial kit were performed once.

Extraction of gDNA was performed using 0.5 g finely ground chili leaves for each method. For PPB-based protocol, about 1 mL of 1 M potassium phosphate (KH_2PO_4) buffer was added to the sample and homogenized for 1 min. The suspension was centrifuged at 10,000 rpm for 1 min. A 500 µL supernatant was collected and resuspended with 500 µL potassium phosphate buffer. The sample was then centrifuged at 13,000 rpm for 5 min and the supernatant was transferred into a new 1.5 mL tube. DNA pellet was further precipitated with an equal volume of ethanol and the pellet was air-dried at room temperature for 5 min. DNA was resuspended with 50 µL TE buffer then stored at -20°C. The resulted gDNA from all methods tested was further analyzed for its quality and quantity using a microvolume spectrophotometer (BioDrop, UK).

DNA amplification and sequencing: Chili pepper gDNA extracted from each method was subjected to PCR to detect two different domains of the *NPR1* gene, namely the F3-TA domain and F1-distal promoter. The touchdown PCR technique was carried out using the scheme shown in Table 1. All amplified products were further visualized through electrophoresis using 1% agarose gel. Amplified products of the F3-TA domain and F1-distal promotor representing each method were verified using bi-directional sequencing. Sequence data were verified using Geneious. Furthermore, all sequences were aligned using Clustal Omega (https://www.ebi.ac.uk) to check its similarity coverage.

Table 1: A touchdown PCR condition used to amplify the *NPR1* domains

Step	Temperature (°C)	Duration (sec)	Repetition within step	Cycle repetition
Initial denaturation	94	180		
Denaturation	94	30		14
Annealing	70	15		
Extension	72	90	2	
Denaturation	94	30		24
Annealing	55	15		
Extension	72	96	5	
Final extension	72	600		

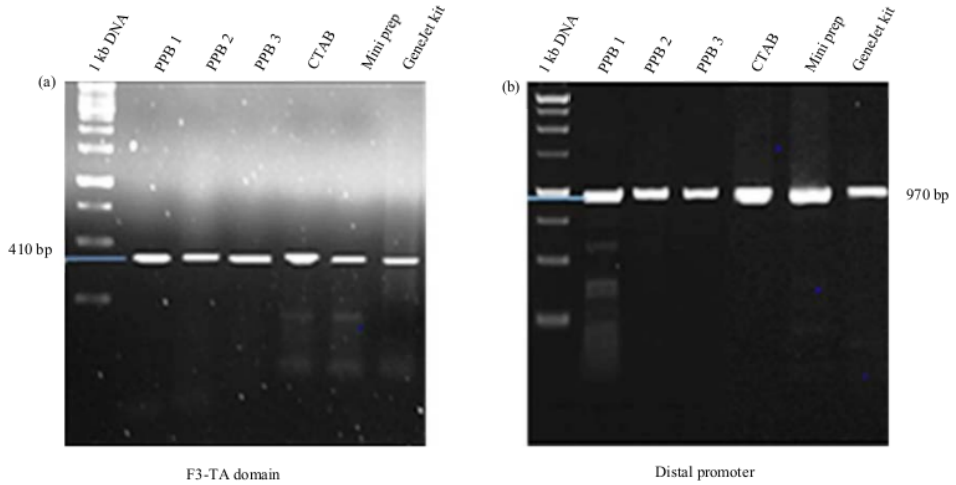

Fig. 1(a-b): Visualization of PCR products of (a) F3-TA domain and (b) Distal promoter generated from four different gDNA extraction methods

Table 2: Comparison of the extracted gDNA concentration and purity between various DNA extraction methods

Methods	Concentration* (ng µL^{-1})	Purity* (A260/280)
Potassium phosphate buffer-based	232.6	1.89
CTAB	868.0	2.05
Mini preparation	3,199.6	1.84
Commercial kit	149.1	1.81

*Values of concentration and purity showed were mean value from three replicates

RESULTS

Quantity and quality of isolated DNA: Of all the methods tested, chili pepper gDNA extracted using the PPB-based method showed comparable quantity and quality compared to other methods (Table 2). Although the resulted concentration was not as high as mini preparation and the CTAB method. However, this method displayed higher purity DNA compared to mini preparation and a commercial kit (Table 2).

DNA quality for PCR and sequencing data: Chili pepper gDNA extracted using all methods exhibited a single yet clear amplicon with the expected size on both primers of the F3-TA domain (~410 bp) (Fig. 1a) and distal promoter (~970 bp) of *NPR1* (Fig. 1b). Supporting this result, the sequencing analysis generated from the PPB method also displayed the expected sequence length of both the F3-TA domain (368 bp) and distal promoter (884 bp) in comparison to other commonly used methods (Table 3). In addition, Fig. 2 also revealed that the sequence visualization generated from the PPB-extracted gDNA was comparable to other protocols indicating the reliability of this developed method in terms of its quality for any downstream analysis, such as PCR and sequencing. Despite the simplicity of PPB-based method execution, this new protocol had proven its promising efficacy as an alternative method for gDNA extraction of chili pepper.

Of the four methods tested, the PPB-based method offered the quickest yet budget-friendly protocol (Table 4). Since this method used PPB and ethanol only, the total duration required to complete this entire protocol was extremely short (18 min) with 5 steps only (Table 4), thus resulting in a cost-effective protocol. It also emphasized that the absence of the most commonly used organic solvents in this new protocol did not affect the quality and quantity of achieved gDNA. Considering these practicality aspects and gDNA quality resulted, this method was highly recommended to be used for high-throughput DNA analysis and safe to be performed in such laboratories with minimum resources. However, the detailed mechanism on how this potassium phosphate buffer could result in proper quality and quantity of gDNA remained unknown.

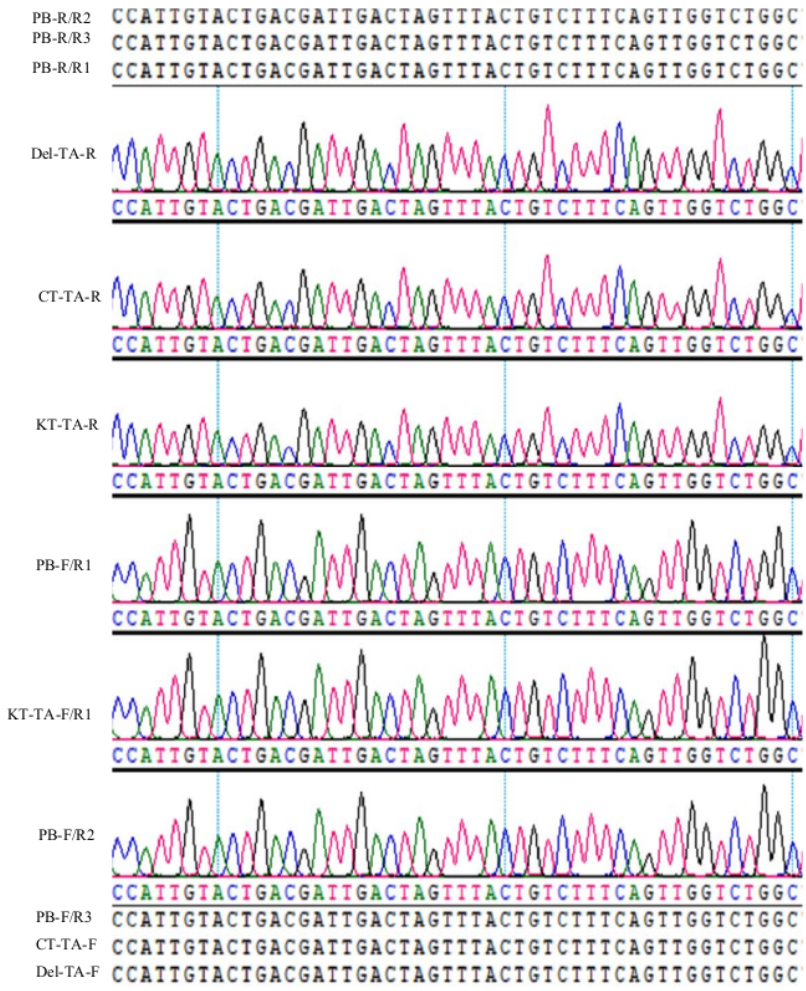

Fig. 2: Comparison of F3-TA domain segment chromatogram generated from the gDNA extracted using various DNA extraction protocols

Table 3: Comparison of the detected F3-TA domain and distal promoter sequences length generated from various DNA extraction methods

	Detected sequences length (bp)					
	TA domain			Distal promoter		
Methods	Forward	Reverse	Mean	Forward	Reverse	Mean
Potassium phosphate buffer-based						
Replicate 1	365	363	368	818	800	884
Replicate 2	368	368		941	923	
Replicate 3	378	368		918	902	
CTAB	365	364	364	886	860	873
Mini preparation	364	366	365	929	909	919
Commercial kit	366	366	366	887	913	900

Table 4: Practicality and cost among four plant DNA extraction methods

Methods	Number of steps	Duration (min)	Cost per reaction (USD*)
Potassium phosphate buffer-based	5	18	0.03
CTAB-based	15	104	0.13
Mini preparation	15	132	0.17
Commercial kit	17	57	4.57

*Price in USD was converted based on the currency rate IDR 14,000, -per USD 1

DISCUSSION

Considering its speed and organic solvents-free, the PBB method has proven its high efficacy to produce an acceptable amount and purity of isolated gDNA. The resulted amount was considered sufficient for any downstream analysis which normally required low DNA concentration (10-50 ng) per reaction[18].

Based on the resulted purity (Fig. 1), it was assumed that PPB played a role in purifying the gDNA. This buffer has been reported as an alternative buffer for the purification of plasmid DNA (pDNA), instead of using ammonium sulfate[19]. Supporting this study, Bonturi *et al.*[20] found that the addition of this buffer resulted in higher purity of pDNA since it triggered lower ionic strength in the elution buffer. Another study reported the use of sodium phosphate buffer for the washing step of DNA isolated from high clay and iron oxide subsoil[21]. However, the use of phosphate buffer for plant gDNA extraction was rarely used, hence the mechanism on how it worked in plant gDNA remained unknown.

Considering the achieved purity of gDNA in this present study, we predicted that the PPB applied was powerful to block the activity of some troublesome compounds, such as polysaccharides and polyphenols. This blockage enabled better DNA dissolution, thus leading to a higher quality of the resulted gDNA for the downstream application. Previous studies had reported that the impurity found in the isolated gDNA was mostly associated with the failure in eliminating the polysaccharides and polyphenols during the DNA extraction[22-24]. Most widely used methods usually utilize hazardous organic solvents, such as phenol, chloroform and β-mercaptoethanol to minimize these contaminations[12]. In contrast, the newly developed protocol had successfully proven the possibility of producing high-quality gDNA without applying any organic solvent. It also improved the practicality feature of this protocol that might be suitable for both limited resources laboratories and high-throughput analysis.

Another practical feature shown by this newly developed protocol was its duration of execution. Table 4 emphasized such a remarkable difference in terms of this duration aspect between the PPB-based and other commonly used protocols. This extremely time-saving feature might be associated with the absence of organic solvents mainly used in common protocols. It also resulted in a significant cost reduction per sample since the high cost applied in common protocols of plant DNA extraction was originated from the utilization of those organic solvents. In line with the results of this study, Tan *et al.*[25] reported a similar duration required for executing these commonly known methods using *Vigna unguiculata*. Several studies also mentioned that the use of commercial kit cost ranging from USD 2-9 per sample[26,27]. Therefore, the comparison of these practicality and cost aspects had emphasized the reliability of the PPB-based method as a promisingly rapid and low-cost protocol for plant DNA extraction.

CONCLUSION

This study has successfully proven the promising capability of the PPB-based method in comparison with those widely used protocols of chili pepper DNA extraction. This developed method should be further optimized by exploring its compatibility to be applied in various types of plant species. This information would be useful to measure the spectrum of its application and determine its chance to substitute such laborious methods. Moreover, some modifications were needed to obtain a higher concentration and amount of gDNA required for any downstream analysis. Therefore, considering its practicality and cost, this newly developed method could be considered as an economically promising method for the DNA extraction of chili pepper.

SIGNIFICANCE STATEMENTS

This present study discovers a promising alternative protocol of plant DNA extraction that provides a rapid, time-saving and cost-effective method with acceptable quality and quantity of the resulted yield. The comparison of this PPB-based method with other widely known protocols had confirmed the reliability of this newly developed protocol. This finding would contribute to a highly applicable and less hazardous protocol for those laboratories with minimum resources due to its organic-solvent free feature. Moreover,

the features of this PPB-based DNA extraction method would be also useful for high-throughput analysis, thus enabling the plant genomic studies with more affordable cost compared to those commonly used protocols.

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