COMPARISON OF PURIFIED DNA BY USING FIVE PURIFICATION METHODS FROM THE LEAVES OF *CAPSICUM ANNUUM* L. (NGA-YOKE)*

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Abstract

DNA extraction is one of the most important parts of molecular genetics. In this study, the genomic DNA were extracted from the tender leaves of *C. annuum* L by using modified Dellaporta method (Sharma *et al.*, 2000). Then, the quality of extracted genomic DNA was amplified by Polymerase Chain Reaction (PCR) technique and checked by gel electrophoresis and NanoDrop spectrophotometer analysis. The result of NanoDrop spectrophotometer revealed that DNA was yielded 336.7 ng/µL concentration with A_{260}/A_{280} ratio of 1.97 and with A_{260}/A_{230} ratio of 2.26. That isolated DNA has no protein and others impurities contaminations. The gel electrophoresis gave the intact DNA band and PCR product had the expected band (~500 bp). Moreover, the isolated DNA was purified by five different methods. It was revealed that protocol 1 (PEG 8000) obtained 166.32 ng/µL with 1.78 ratio of A_{260}/A_{280} that indicate the good quality of DNA from the tender leaves of *C. annuum* L.

Keywords: DNA, PCR, NanoDrop spectrophotometer, PEG 8000, *Capiscum annuum* L.

Introduction

The extraction of deoxyribonucleic acid (DNA) and purified DNA are the basic steps for many biotechnological techniques, such as molecular markers and genetic engineering. Basically, it is difficult to extract and purify high-quality DNA from certain plants, because of secondary metabolites (tannins, alkaloids, and polyphenols), polysaccharides and proteins. These compounds can interfere DNA, thus degrading its quality and reducing yields. The best quality DNA from mature leaf is problematic, particularly, due to the presence of phenolic compounds and polysaccharides. Mature leaves have higher quantities of polyphenols, tannins, and polysaccharides that can contaminate DNA during isolation. Some researchers have been reported the rapid and reliable procedure for extracting good quality and high quantity of genomic DNA for PCR and molecular analysis (Roomi *et al.,* 2013).

The concentration and quality of extracted genomic DNA can be evaluated by using NanoDrop spectrophotometer, gel electrophoresis and PCR. The quality of DNA concentration and purity is important to downstream processes like sequencing, restriction enzyme digestions, PCR and qPCR (Olson and Morrow, 2012).

PCR technology is based on DNA sequence, to be amplified with the synthetic DNA two chain end complementary two oligonucleotide primers, *in vitro* to be detected DNA sequences (template) were amplified in enzymatic action. The primer can be used rbcL, matK and psbA-trnH depending on the using template (Yu *et al*., 2017). For sequencing, DNA can be purified either from PCR product or from gel slice. The PCR product can be purified using polyethylene glycol (PEG), sodium acetate (NaOAc) and isopropanol, ethanol etc. There are many methods for purification of gel slice by using ethanol precipitation (Oswald, 2007; Michael and Sambrook, 2016), spin filtration (Grey and Brendel, 1992) and home spin column or freeze squeeze methods

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instead of commercial kits. The excised gel should as small as possible to avoid diluting the recovered DNA. The presence of gel after purification can interfere downstream processing (Abraham *et al.,* 2017). All these methods have their advantages and disadvantages. A fast and simple methods result in low yield and poor DNA quality; while methods that result in pure DNA are time-consuming, laborious, and expensive (Kurien and Scofield, 2002).

Recovery of DNA fragments from agarose gel with TAE buffer is one of the most common methods in molecular biology laboratories for downstream applications. If the purification of small fragments of DNA (<100 bp), can use TBE (Tris-Boric acid-EDTA) buffer (Santos *et al.,* 2017). To date, many methods have been published to extract the desired DNA band from the agarose gel. The commercial kits include binding resins such as silica, Silica-based methods are fast, but low yield and high cost, which may interfere in sequencing. Cost is a serious limitation, especially in developing countries (Abraham *et al.,* 2017). Therefore, the present investigation was to establish using inexpensive and un-harmful chemicals, to safe and reduce time-consuming protocol for DNA purification, to obtain good quality of DNA in a relatively purified form which can be used for further investigations.

Materials and Methods

Collection of Samples

Capsicum annuum L. is one of the Capsicum species used for genomic DNA extraction and purification. The samples were collected from the Vegetable and Fruit Research and Development Center, Hlegu Township, Yangon Division.

Extraction of genomic DNA (Sharma *et al.*, 2000)

Apparatus: 1.5 ml Eppendorf tube, Pipette, Mortar and pestle, Vortex, Refrigerated Centrifuge, Microcentrifuge, Beaker, Water bath, Freezer, Refrigerator

Required chemicals: Sodium Dodecyl Sulfate (SDS), Sodium chloride (NaCl), Tris hydroxymethyl aminomethane (Tris), Ethylenediaminetetraacetic acid (EDTA), Potassium acetate (KOAc), Polyvinylpyrrolidone (PVP)

Reagents and solutions

DNA extraction medium: (1 M tris, pH 8.0; 500 mM EDTA, pH 8.0; 5 M NaCl; 10 % SDS), 5 M potassium acetate buffer: pH 4.8, 20% SDS, cold isopropanol, cold 70% ethanol, RNase A, TE buffer (10 mM Tris,1 mM EDTA (pH 8.0)

Procedure

The sample 0.13 g of harvested leaf stored in -20°C was ground firstly with PVP powder and the addition of 600 µL of extraction buffer and the mixture was transferred into 1.5 ml sterilized microcentrifuge tube and vortexed. The tube was vortexed briefly for 5 mins to mix the contents well and added 100 µL of 5 M potassium acetate and 100 µL of 20% SDS. After that vortex about 1 min, the tube was incubated the tube at 65°C for 30 mins. The sample was cooled for 10 mins at room temperature and centrifuged at 15,000 rpm for 30 mins at 4°C. Amount of 200 µL of supernatant was transferred into the new tube and added 180 µL of isopropanol and then inverted the tube. The tube was incubated at room temperature for 10 mins and centrifuged at 15,000 rpm for 60 mins at 20°C to pellet out and was observed at the bottom of the tube. The supernatant was discarded and the pellet was washed with 500 µL of cold 70% ethanol and centrifuged at 15,000

rpm for 10 mins at 20°C. The step 6 was repeated. The pellet was air dried and dissolved in 50-100 µL of TE buffer and 0.5 µL of RNase A was added and incubated for 1hr at 37°C to remove the RNA. Finally, the DNA sample was stored at -20°C.

Purification Protocols

Protocol (1) PCR purification using PEG (Mikheyev, 2009**)**

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Micro centrifuge, Beaker, Water bath, Freezer, Refrigerator

Required chemicals: Polyethylene glycol (PEG) 8000, NaCl

Reagents and solutions: 3 g PEG 8000 and 2.19 g NaCl up to 15 ml with DW and chilled absolute ethanol. Lasts for at least 30 days at 4°C, 80% ethanol, nuclease free water

Procedure

The amount 120 µL of PCR product was taken to the tube and added 1:1 volume of PEG to PCR product. The tube was briefly vortexed the tube. The tube was warmed at 37°C for 15 mins. The tube was centrifuged at 15,000 rpm for 15 mins at 25°C. The supernatant was discarded carefully. The pellet was washed with excess 150 µL of 80% cold ethanol. Then, the tube was centrifuged at 15,000 rpm for 10 mins at 25°C. The supernatant was discarded and washed with 300 µL of 95% cold ethanol. Then, the tube was centrifuged at 15,000 rpm for 10 mins at 25°C. The supernatant was pipetted off and air-dried the pellet about 5-10 mins at room temperature depending on the pellet out. The pellet was dissolved in 30 µL of nuclease free water and incubated at 4°C or -20°C.

Protocol (2) PCR purification using 3M sodium acetate and chilled absolute ethanol

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Micro centrifuge, Beaker, Water bath, Freezer, Refrigerator

Required chemicals: Sodium acetate (NaOAc)

Reagents and solutions: 3 M sodium acetate (NaOAc), pH 4.5, chilled absolute ethanol, 70% ethanol, nuclease free water

Procedure

The tube containing 120 µL of PCR product was added 120 µL of 3M sodium acetate (NaOAc), pH 4.5 and 240 µL of chilled absolute ethanol. The tube was vortexed and then stored it at -20°C for 30 mins. The tube was centrifuged at 14,000 rpm for 30 mins at 25°C. The supernatant was removed carefully and don't disturb the pellet. The pellet was washed with 300 µL of 70% ethanol and centrifuged at 14,000 rpm for 5 mins at 25°C. The supernatant was discarded and then air-dried the pellet at room temperature. Re-suspend the pellet in 30 µL in nuclease free water and incubated at 4°C or -20°C.

Protocol (3) PCR purification by using Combination of PEG and sodium acetate (Lis, 1980)

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Micro centrifuge, Beaker, Water bath, Freezer, Refrigerator, Ice pack

Required chemicals: Polyethylene glycol (PEG) 8000, NaCl, Sodium acetate (NaOAc)

Reagents and solution: 5.0 M NaCl, 22% PEG 8000, 0.3 M sodium acetate (NaOAc)95% ethanol, 70%, ethanol, nuclease free water

Procedure

After running PCR, the 120 µL of PCR product was precipitated with 1:3 volume of 95% ethanol. The tube was incubated on ice for 10 mins and centrifuged at 15,000 rpm for 15 mins at 20°C. The supernatant was discarded and air-dried the pellet. The precipitated fragment was dissolved in 32 µL of nuclease free water. The tube was added 8 µL of 5.0 M NaCl and 40 µL of 22% PEG 8000 and mixed the tube (11% PEG will precipitate all DNA fragments larger than 180 bp). The tube was incubated on ice at least 30 mins and then centrifuged at 4°C for 5 to 10 mins. The supernatant was discarded the supernatant. The pellet was dissolved in 20 µL 0.3 M NaOAc, and added 2.5 volumes of 95% ethanol and mixed, on ice for about 15 mins, then centrifuged the tube at 15,000 rpm for 15 mins at 4°C. Carefully the supernatant was aspirated and discarded. The pellet was rinsed with 300 µL of 70% ethanol and centrifuged at 15,000 rpm for 5 mins at 4°C. The supernatant was removed and then, air-dried the pellet for 3 mins at room temperature. The pellet was dissolved in 20 µL of nuclease free water, and stored at 4°C or -20°C.

Protocol (4) Gel purification using home spin column (Abraham *et. al.*, 2017)

Apparatus: Syringe needle, Cotton, 1.5 mL Eppendorf tube, 2 mL Eppendorf tube Microwave oven, Microcentrifuge, Omni Doc Gel Documentation System**,** Centrifuge, Beaker, Tweezer

Reagents: 0.5X TAE buffer

Procedure

To purify the selected DNA bands from agarose gels, a 1.5 mL Eppendorf tube punctured in the center bottom with a syringe needle and packed with a small tassel of cotton was used. The cotton was embedded with 0.5X TAE buffer and squeezed until no liquid came out. The tube was placed into another 2 mL Eppendorf tube. The piece of DNA-containing gel was laid on the cotton filter in the tube and then centrifuged at 5,000 rpm for 7 min at room temperature. The DNA in the collected liquid was stored at 4°C or -20°C.

Protocol (5) Gel purification using GeneJet Gel Extraction Kit (Thermo Fisher Scientific)

Apparatus: 1.5 mL Eppendorf tube, Pipette, Vortex, Centrifuge, Microcentrifuge, Beaker**,** Water bath, Freezer, Refrigerator, Microwave oven, Surgical blade, Omni Doc Gel Documentation System, Tweezer

Required chemicals: Agarose gel

Reagents and solution: 1X TAE buffer, SYBR Safe DNA gel stain, Binding buffer, Wash buffer, Elution buffer, Isopropanol

Procedure

After running gel electrophoresis, the gel containing the DNA fragment was excised using a clean scalpel or razor blade. Cutting was made as close to the DNA as possible to minimize the gel volume. The gel slice was placed into a pre-weighed 1.5 mL tube and weighed again. The weight of the gel slice was recorded. The ratio 1:1 volume of Binding Buffer was added to the gel slice (volume: weight) (e.g., add 100 µL of Binding Buffer for every 100 mg of agarose gel). The gel mixture was incubated at 50-60°C for 10 min or until the gel slice was completely dissolved.

The tube was mixed by inversion every few minutes to facilitate the melting process to ensure the gel was completely dissolved. The gel mixture was vortex briefly before loading on the column. The solubilized gel solution was transferred up to 800 µL to the GeneJET purification column. The column was centrifuged at 12,000 rpm for 1 min at 25°C. Discarded the flow-through and placed the column back into the same collection tube. The column was added 100 µL of Binding Buffer. The column was centrifuged at 12,000 rpm for 1 min at 25°C. Discarded the flow-through and placed the column back into the same collection tube. The column was added 700 µL of Wash Buffer. The column was centrifuged at 12,000 rpm for 1 min at 25°C. Discarded the flow-through and placed the column back into the same collection tube. The empty GeneJET purification column was centrifuged for an additional 1 min to completely remove residual wash buffer. The GeneJET purification column was transferred into a clean 1.5 mL microcentrifuge tube. The column was added 30 µL of Elution Buffer to the center of the purification column membrane. The column was centrifuged at 12,000 rpm for 1 min at 25°C. and stored the purified DNA at -20 °C.

Analysis of DNA using NanoDrop spectrophotometer

The amount of 1µL of DNA sample from extraction was used to measure the nucleic acid concentration (Quantity) and DNA quality by means of measuring the absorbance ratio of A_{260}/A_{280} for protein contamination and that of A_{260}/A_{230} for other impurities involved in the sample. For DNA, the range of A_{260}/A_{280} must be between 1.7- 2.0 and that of A_{260}/A_{230} must be greater than 2.

Preparation for Polymerase Chain Reaction (PCR)

EmeraldAmp® Max PCR Master Mix was used to isolate part of the psbA-trnH gene (~400 bp) for amplification. The sample of DNA from extraction was diluted to 5 ng/ µL. The PCR buffer was prepared by adding 9.5 µL of dH2O, 1 µL of each forward primer (psbA-F(5' to 3'): GTTATGCATGAACGTAATGCTC) and reverse primer (trnH-R (3' to 5'): CGCGCATGGTGGATTCACAATCC) and 12.5 µL of enzyme mixture. The amount of 24 µL of this PCR buffer was needed for 1 µL of DNA Template for PCR amplification. As a PCR thermal cycler, a TechneTM PCRmax Alpha Cycler 1 PCR Machine (PCR max, UK) was used. PCR condition must be at 95°C for 5 minutes for initial temperature, at 95°C for 30s for denaturing, at 55°C for 30s for annealing Temperature, at 72°C for 40s for extension, at 72°C for 10 minutes for final extension and for hold (10°C for infinity). The reaction was repeatedly cycled for 35 times. The psbA (forward primer) and trnH (reversed primer) were intergenic spacer regions and also universal primers (~400bp). These primers possess conserved flanking sites, short sequences and discriminate between species. So, it was suitable for running of PCR. (Kress and Erickson, 2012)

Preparation of TAE Buffer for Gel Electrophoresis

Firstly, a 50X concentration of TAE stock solution was made. For this, 242 g of Tris was dissolved in 500 mL of distilled water. The amount of 18.61 g of EDTA (pH- 8) was weighed and dissolved in 100 mL of distilled water and stirred with magnetic stirrer and then added some NaOH up to pH- 8. These two solutions were combined and 57.1 mL of acetic acid was added to it. Then distilled water was added to bring the final volume to 1 L.

Preparation for Gel electrophoresis

Amount 0.17 g of agarose gel (1% agarose) for a small casting tray was weighed and put in a 200 mL beaker and then 17 mL of TAE buffer was added and then the dissolved in the microwave for about 5 min until the agarose powder completely dissolved. After that, 0.6 µL of

SYBR Safe DNA gel stain (Thermo Fisher Scientific, USA) was added and slowly shook the beaker. Then the gel solution was poured into the gel tray and the comb was put and then waited for about 30 min. The comb was then removed and the prepared gel tray was placed on the gel bed of the migration tank containing the 1X TAE buffer. For genomic DNA, 2.5 µL of DNA sample with 1 µL of loading dye was loaded into the wells for protocols. For PCR products, each 2.5 µL of PCR products was loaded into the well of an agarose gel containing SYBR stain. Then, the 100bp marker (Thermo Fisher Scientific, USA) was placed into the well for comparing with the size of the samples and run for 15 min at 100 voltages. And then, the gel was placed in the Omni Doc Gel Documentation System which is already connected with the computer. The exposure was adjusted to 3 and switched to UV light then took the photo of the gel.

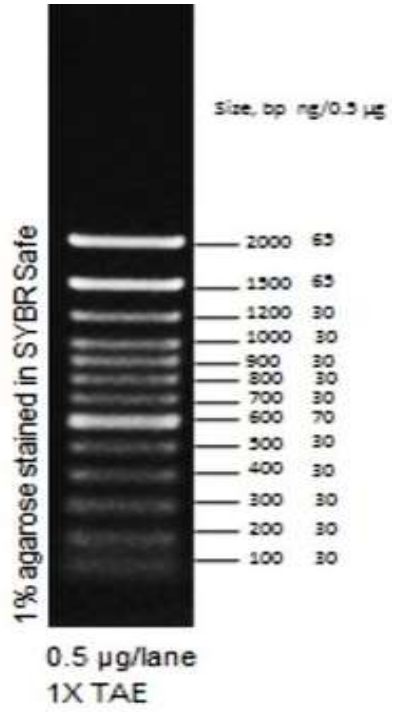

Figure 1.100 bp ladder (Invitrogen, Thermo Fisher Scientific)

Results

The species *Capsicum annuum* (cv) was collected from the Vegetable and Fruit Research and Development Center, Hlegu Township, Yangon Division during the flowering period of June - August, 2019.

Outstanding Characters of *C. annuum* (cv)

Annual or perennial herbs; pubescence of simple hairs. Stems branched. Leaves solitary or paired, petiolate; leaf blade simple, entire or sinuate. Inflorescences solitary; peduncle absent. Flowers are nodding or erect, actinomorphic. Pedicle erect or nodding. Calyx broadly campanulate to cup-shaped, denticulate, sometimes slightly enlarged. Corolla white, campanulate or rotate, divided halfway or more. Stamens are inserted near the distal end of the corolla tube; filaments are slender; anthers are yellow or purplish, ovoid, and dehisce longitudinally. Ovary 2- (-3)- locular; ovules numerous. Style slender; stigma small, capitate. Fruit is a moist berry, sometimes large, erect, nodding or reflexed. Seeds are yellowish, discoid; embryo coiled, subperipheral.

Figure 2. Habit of Capsicum annuum (cv)

Genomic DNA

i. Quality and Quantity Assessment Using NanoDrop Spectrophotometer

The genomic DNA of tender leaves of *C. annuum* L. was extracted by using five different methods in plant analytical laboratory. The quality and quantity assessment of extracted DNA was evaluated by the NanoDrop spectrophotometer, gel electrophoresis and PCR. According to the result of the NanoDrop spectrophotometer, the genomic DNA was yielded a 336.7 ng/µL concentration with A_{260}/A_{280} ratio of 1.97 and with A_{260}/A_{230} ratio of 2.26. The DNA has no protein but a little other impurity contamination.

ii. Quality and Quantity Assessment Using Gel Electrophoresis and PCR

According to the results of gel electrophoresis, the whole genomic DNA of *Capsicum* sp. might be between 3-3.5 Gb. The result showed that the genomic DNA was free from protein contamination and a little degraded. The Gel electrophoresis result were shown in Figure 3.

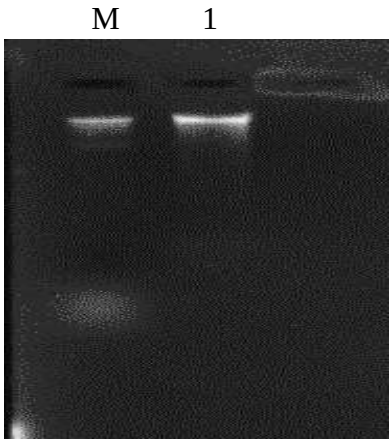

Figure 3. Checking Genomic DNA Quantity by using Gel Electrophoresis. M (λ DNA/Hind III Fragments), Lane 1. DNA sample was loaded 2.5 µL per lane.

In addition, the result of PCR product demonstrated that the DNA concentration diluted to 50 ng/µL had expected band (~500 bp) were shown in Figure 4. The result of PCR product by NanoDrop spectrophotometer was 1154.43 ng/µL concentration with A 260/280 ratio of 1.88 and with A_{260}/A_{230} ratio 1.93.

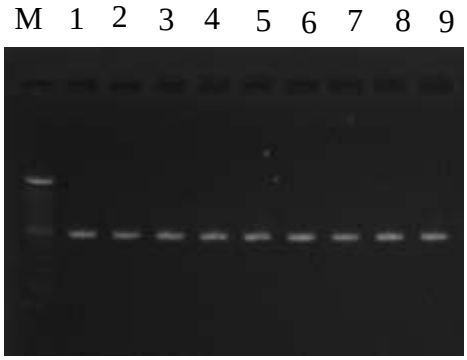

Figure 4. Checking of PCR products of genomic DNA quantity by using PCR. M (100bp ladder), Lane 1 to 9. DNA sample was loaded 5 ng per lane

Purified DNA

i. Quality and Quantity Assessment Using NanoDrop Spectrophotometer

The purified extracted DNA can be used for various types of molecular analysis such as sequencing, electrophoresis, PCR and molecular cloning. At first, the quality, purity and concentration of purified DNA must be taken by using NanoDrop spectrophotometer which measure A_{260}/A_{280} and A_{260}/A_{230}. The A_{260}/A_{280} ratio is used as an indicator of DNA purity. Weising *et al.*, 2005 stated that A_{260}/A_{280} ratio should be between 1.7 and 2.0 that means free from ethanol and other chemicals contamination (DNA purification, Promega corporation). According to the NanoDrop spectrophotometer, the purified DNA obtained by protocol 1 was 166.32 ng/µL with 1.78 the ratio of A_{260}/A_{280} and 2.33 ratio of A_{260}/A_{230}. This protocol was free from ethanol and other chemicals contamination. Protocol 2 gave 198.14 ng/µL with the ratio of A_{260}/A_{280} was 1.65 but the ratio of A_{260}/A_{230} was 1.25 of the purified DNA. The protocol 2 was a little contamination. Purified DNA obtained from protocol 3 was 126.43 ng/µL with 1.72 of the ratio of A_{260}/A_{280} and 1.51 ratio of A_{260}/A_{230} protocol 3 was better than protocol 2 and protocol 4 because of free from chemicals contamination. DNA from protocol 4 gave 37.98 ng/µL with 1.45 ratio of A_{260}/A_{280} that means many contamination and 0.5 ratio of A_{260}/A_{230}. Purified DNA yielded from protocol 5 gave 46.91 ng/µL with 1.93 ratio of A_{260}/A_{280} and 0.37 ratio of A_{260}/A_{230}. This protocol was free from contamination. The NanoDrop result of all protocols were as shown in Table 1.

Table 1. Purified DNA Concentration and Quality Using NanoDrop Spectrophotometer

Protocol	DNA Concentration (ng/µL)	Indication Ratio for Protein Contamination (A_{260}/A_{280})	Indication Ratio for Impurities Contamination (A_{260}/A_{230})
1	166.32	1.78	2.33
2	198.14	1.65	1.25
3	126.43	1.72	1.51
4	37.98	1.45	0.5
5	46.91	1.93	0.37

(All DNA concentration, ratios for protein contamination and impurities contamination were average values.)

ii. Quality and Quantity Assessment Using Gel Electrophoresis and PCR

The results of protocol 1, 2, 3 and 5 demonstrated that the purified DNA by using gel electrophoresis was free from chemical contamination. The DNA of protocol 4 was contaminated with other impurities. And the protocol 1 gave intact DNA band. The result of protocols was shown in Figure 5.

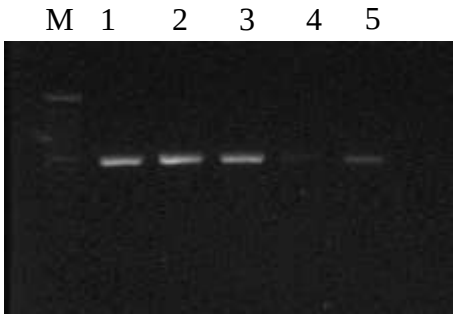

Figure 5. Checking the purified DNA Quantity by using Gel electrophoresis. M (100 bp ladder), Lane 1 (P1), Lane 2 (P2), Lane 3 (P3), Lane 4 (P4) and Lane 5 (P5). DNA sample was loaded 2.5 µL per lane

In addition, the result of PCR product revealed that 5 ng/µL dilution of all protocols had expected band (~500 bp) were shown in Figure 6.

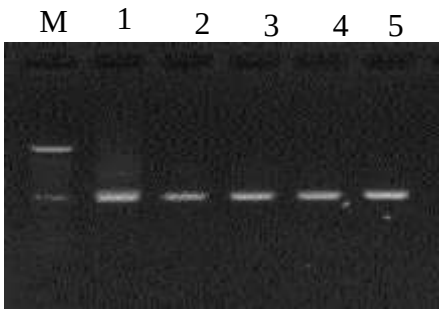

Figure 6. Checking the PCR product of purified DNA quantity by using PCR. M (100 bp ladder), Lane 1 (P1), Lane 2 (P2), Lane 3 (P3), Lane 4 (P4) and Lane 5 (P5). DNA sample was loaded 5 ng per lane.

The estimate cost on purification processes of *C. annuum* (cv) was shown in Table 2.

Table 2. Comparison of yield and quality of DNA recovered with different methods

Protocol	PEG	NaOAc	PEG+ NaOAc	Home spin column	GeneJet Gel extraction kit
Time for purification (minutes)	40	70	105	7	15
Total DNA amount (ng/µL)	1154.43	1154.43	1154.43	1154.43	1154.43
Final DNA concentration obtained (ng/µL)	166.32	198.14	126.43	37.98	46.91
Volume obtained (µL)	30	30	30	99	30
Total DNA after purification (ng)	4989.6	5944.2	3792.9	1253.34	1407.3

Protocol	PEG	NaOAc	PEG+ NaOAc	Home spin column	GeneJet Gel extraction kit
PCR results	Good	Good	Good	Good	Good
Cost per sample (MMK)	25	25	23	21	2400

Discussion and Conclusion

This research was conducted with the objectives to develop a simple and rapid method to purified the extracted DNA from leaves of *C. annuum* (cv). It was also intended to provide information for the best DNA purification protocol from plants with inexpensive, safe and reducing time consuming. The quality of extracted DNA sample was verified spectrophotometrically using a NanoDrop instrument, gel electrophoresis and amplified by PCR. The NanoDrop absorbance profile was useful for detection of contaminants such as protein, salts and polysaccharides, which can inhabit and interfere in DNA purification. The A_{260}/A_{280} nm ratio of 1.97 and the A_{260}/A_{230} nm of 2.26 indicated that extracted DNA had high purity with absence of proteins and phenols. According to the Sharma *et al.*, 2000; Dehestani and Kazemi. 2007; Doyle and Doyle, 1987, the A_{260}/A_{280} nm ratio varied between 1.7 and 1.9, 1.69-1.91, 1.76-1.93 respectively. The PCR product demonstrated that the DNA had the expected band (~500 bp). Lavanya and Arun., 2019 reported that the DNA of *Capsicum* sp. had the expected band was ~530 bp.

The protocol 1, polyethylene glycol (PEG) 8000 method produced the purified DNA with the yield of 141.2 to 186.2 ng/µL A_{260}/A_{280} ratio in this study was found to be in a range of 1.77 to 1.79 whereas the protocol 2 (NaOAc), the protocol 3 (PEG+NaOAc), the protocol 4 (home-spin method) gave the DNA with the yield of 37.98 to 198.14 ng/µL, the ratio obtained varied from 1.45 to 1.72 respectively.

Weising *et al.*, 2005, Lis, 1980 and Mikheyev, 2009 reported that the absorbance ratio obtained varied from 1.6 to 1.8 indicating that the isolated DNA was free from contamination. The DNA yielded from protocol 5 (GeneJET Gel Extraction Kit method) was 46.91 ng/µL with 1.93 ratio of A_{260}/A_{280}. The advantages of this method was less time consuming. The gel electrophoresis results of protocol 1,2 ,3 and 5 gave the intact DNA and the PCR results of all protocols had the expected band (~500 bp). According to the Abraham *et. al.*, 2017 that the expected PCR product was 312 bp in microorganism of banana leaf. The DNA extracted with optimized protocol presented a reduced degradation and an excellent overall quality. The developed procedure was fast and reproducible.

In conclusion, DNA extraction method isolate high quality of DNA. The result showed that DNA extraction using modified boiling, centrifugation time as well as percent of chemical used was more effective. From the result of purification of DNA, it can be concluded that the GeneJET Gel Extraction Kit using procedure, which was easy and rapid, could be applied for the isolation of analytical quality DNA from *C. annuum* (cv). Also the protocol 1 used PEG 8000 for DNA purification gave better yield of large DNA fragment, safe, inexpensive, no harmful chemicals. Thus, PEG method can be used instead of commercial kit as the kits are expensive for developing countries. Therefore, the PEG method can be also recommended for the isolation of analytical quality DNA from *C. annuum* L.

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