

Comparative Analysis of Six DNA Extraction Methods in Cowpea (*Vigna unguiculata* L. Walp)

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Received: April 7, 2013 Accepted: May 7, 2013 Online Published: June 15, 2013

doi:10.5539/jas.v5n7p82

URL: <http://dx.doi.org/10.5539/jas.v5n7p82>

Abstract

High quality DNA extractions are a prerequisite for genetic studies of a variety of plants including cowpea (*Vigna unguiculata*). Nowadays, there are a great number of plant DNA extraction methods, and commercially available extraction kits are also becoming more and more popular. It appears that different procedures work best for different plant groups. Thus in the genetic studies of cowpea, which DNA extraction method to choose becomes a concern. To solve this problem, five classic plant DNA isolation methods, including three CTAB methods and two SDS methods, were compared and evaluated while isolation using a commercial kit was also undertaken. The DNA extracted by these six methods from two-week-old cowpea seedlings were analyzed according to their cost and time, yield, purity, integrity, and functionality in restriction endonuclease digestion and PCR (polymerase chain reaction) based downstream analysis. After the evaluation, one most suitable method, described by Dellaporta et al. (1983) was selected and chosen for isolating DNA from young leaves of cowpea seedlings. The cost and time required in this method was relatively low. In addition, the quantity and the quality of the DNA extracted by this method were high enough to perform hundreds of PCR-based reactions.

Keywords: CTAB, SDS, commercial kit, DNA extraction, comparison, *Vigna unguiculata*

1. Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, Leguminosae ($2n = 2x = 22$), is an essential food crop in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, and Central and South America (Singh et al., 1997). The planting area is more than 10.9 million ha, with a production of more than 5.5 million metric tons world-wide in 2010 (FAO, 2010). Cowpea has a wide range of uses and is grown primarily for human consumption as a dry grain legume, fresh shelled 'peas', fresh pods ('snap beans'), and fresh and dried leaves (Diouf et al., 2005). Due to its good protein quality and high nutritional value, cowpea is often referred to as the "poor man's meat" (Jackai et al., 1986). Additionally, cowpea is a drought-tolerant crop that thrives in dry environments, thus makes it the crop of choice in the semi-arid/arid zones of West and Central Africa (Agbicodo et al., 2009). Also, the crop fixes 80% of its nitrogen requirement for growth from the atmosphere (Asiwe et al., 2009), thereby reducing nitrogen fertilizer demand and costs of crop production. In a word, cowpea is a versatile crop that plays an important role in the development of agriculture.

For genetic studies of cowpea, such as gene mapping, genetic fingerprinting, population studies, and phylogenetic analyses, extraction of DNA presents the first essential step for all subsequent genetic analysis, which are frequently PCR based methods. In the past thirty years, plant DNA isolation methods have been described by numerous authors, each contributing a different method to overcome the problems that arise when extracting DNA. The cetyl trimethylammonium bromide (CTAB) method was first presented by Murry and Thompson (1980), who employed cesium chloride density gradients to eliminate enzyme inhibiting polysaccharides. But this method needs expensive equipments like ultracentrifuge and includes some hazardous reagents like ethidium bromide. What's more, the process is complex, time-consuming, and not well suited for assaying large number of samples. Then Saghai-Marroof et al. (1984) reported a modification of the method of Murry and Thompson without the use of cesium chloride density gradients, and simplified the procedure. The protocol was based on lyses and purification with CTAB that selectively precipitates DNA while maintaining the solubility of many polysaccharides (Ribeiro et al., 2007). In 1985, Rogers et al. (1985) developed a technique based on the CTAB nucleic acid extraction procedures of Murray and Thompson (1980) and Taylor and Powell

(1982), which made it possible to extract purified high molecular weight plant DNA without the use of expensive equipment and/or time-consuming procedures. Afterwards, Doyle et al. (1987) made a simple modification of the DNA isolation procedure described by Saghai-Marooof et al. in 1987. They did some further cleaning to the DNA yield by the protocol of Saghai-Marooof et al., and obtained high quality and pure DNA. Since then, the Doyle method has become a popular plant DNA isolation method.

The first SDS method applied in plant DNA isolation was reported by Dellaporta et al. (1983), which was adapted from a procedure commonly used for yeast DNA preparation (Davis et al., 1980). This protocol used sodium dodecyl sulfate (SDS) as detergent and the addition of potassium acetate resulted in the removal of some polysaccharides and proteins as a complication with the potassium-SDS precipitate (Ribeiro et al., 2007). Later in 1995, Jobses et al. (1995) made an improvement of the standard proteinase K-SDS method. The procedure alleviated the time and expense of CTAB and caesium chloride methods by utilizing polyvinylpyrrolidone (PVP) to bind the polyphenolic compounds. And in 1997, Aljanabi et al. reported a universal and rapid salt-extraction of high quality genomic DNA from different organisms. The method was also based on SDS, and did not require expensive and environmentally hazardous reagents and equipment.

DNA isolated by the Doyle method (Ba et al., 2004; Tosti et al., 2005; Sarutayophat et al., 2007), the Dellaporta method (Fatokun et al., 1992; Myers et al., 1996), the Rogers method (Zannou et al., 2008; Malviya et al., 2012), the Saghai-Marooof method (Tantssawat et al., 2010) and the commercial kit (Ghalimi et al., 2010; Fang et al., 2007) have been already used in the genetic analysis of cowpea. However, there is a lack of comparative research of these DNA extraction methods.

It appears that different procedures work best for different plant groups. The reason might be the great diversity of plant secondary compounds that may interfere with a particular method of DNA isolation (Doyle et al., 1987). Another reason to compare is for the following step to use the extracted DNA. The residues of chemicals used in an extraction might affect what you will do next, e.g., PCR reaction. Therefore, it is necessary to compare and optimize some widely used DNA isolation methods in a specific species. In this study, five classic DNA extraction methods reported by Dellaporta et al., Saghai-Marooof et al., Rogers et al., Doyle et al. and Aljanabi et al., respectively, were compared while isolation using a commercial kit was also undertaken. All the DNA extractions were evaluated according to their cost and time, yield, purity, integrity and functionality, in order to find an ideal method which is suitable for isolating high yield and high quality DNA from cowpea.

2. Materials and Methods

2.1 Material Preparation

The cowpea cultivar (Cheng-jiang 7), which belonged to *Vigna unguiculata ssp. sesquipedalis*, was provided by the Chengdu Academy of Agriculture and Forestry Sciences, China. Seeds were grown under greenhouse conditions and leaves were harvested from two-week-old seedlings for DNA isolation.

100 milligram of fresh leaf tissue was weighed in an electronic balance and ground into a fine powder in liquid nitrogen by a pestle and mortar, then transferred into a 1.5 ml microfuge tube and temporarily stored at -20°C.

2.2 DNA Extraction

DNA isolation procedures were completely followed by Dellaporta et al. (1983), Saghai-Marooof et al. (1984), Rogers et al. (1985), Doyle et al. (1987) and Aljanabi et al. (1997). However, a little modification was made on the amount of reagents used in each method.

Method 1: described by Dellaporta et al. Reagents in this protocol included: 750 µl of Extraction buffer (100 mM Tris, pH 8.0, 50 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0, 500 mM NaCl, 10 mM 2-mercaptoethanol), 50 µl 20% SDS, 250 µl 5 M potassium acetate, 700 µl supernatant, 500 µl isopropanol, 700 µl (50 mM Tris, 10 mM EDTA, pH 8.0), 500 µl supernatant, 75 µl 3M sodium acetate and 500 µl isopropanol, 400 µl 80% ethanol.

Method 2: described by Saghai-Marooof et al. Reagents in this protocol included: 400 µl of extraction buffer (50 mM Tris, pH8.0, 0.7 M NaCl, 10 mM EDTA, 1% CTAB, 0.1% 2-mercaptoethanol), 400 µl Chloroform/octanol (24:1, vol/vol), 400 µl supernatant, 265 µl isopropanol, 400 µl washing buffer (76% ethanol, 10 mM NH₄OAc).

Method 3: described by Rogers et al. Reagents in this protocol included: 100 µl 2× CTAB extraction buffer (2% CTAB (w/v), 100 mM Tris, pH 8.0, 20 mM EDTA, pH 8.0, 1.4 M NaCl, 1% PVP, MW 40000), 100 µl chloroform/ isoamyl alcohol (24:1), 90 µl supernatant, 9 µl 10% CTAB (10% CTAB (w/v), 0.7 M NaCl), 70 µl supernatant, 70 µl CTAB precipitation buffer (1% CTAB, 50 mM Tris, pH 8.0, 10 mM EDTA, pH 8.0), 200 µl high salt TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0, 1 M NaCl), 400 µl ethanol, 400 µl 80% ethanol.

Method 4: described by Doyle et al. Reagents in this protocol included: 400 µl extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% 2-mercaptoethanol), 400 µl chloroform/ isoamyl alcohol (24:1), 400 µl supernatant, 265 µl cold isopropanol, 400 µl wash buffer (76% EtOH, 10 mM ammonium acetate), 100 µl TE (10 mM Tris-Cl, pH 7.4, 1 mM EDTA, pH 8.0), RNase A (final concentration, 10 µg/ml), 200 µl TE, 150 µl ammonium acetate (final concentration, 2.5 M), 250 µl cold ethanol.

Method 5: described by Aljanabi et al. Reagents in this protocol included: 400 µl extraction buffer (0.4 M NaCl, 10 mM Tris-HCl, pH 8.0, and 2 mM EDTA, pH 8.0), 40 µl 20% SDS (2% final concentration), 8 µl of 20 mg/ml proteinase K (400 µg/ml final concentration), 300 µl 6 M NaCl (NaCl saturated H₂O), 600 µl supernatant, 600 µl isopropanol, 400 µl 70% ethanol.

In addition, total DNA was also isolated using E.Z.N.A.[®] Plant DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer's protocol. At the end of each method, DNA was air dried for 30 min (except for the commercial kit) and diluted in 100 µl of TE buffer (10 mM Tris-cl, pH 7.4, 1 mM EDTA, pH 8.0). Each method was replicated ten times.

2.3 Cost and Time Estimation

The method to evaluate the cost and time in the DNA extraction was followed by Chen et al. (2010). The cost for each method was estimated based on the price of chemicals, enzymes, and disposable items (including microfuge tubes and pipette tips) consumed in one extraction from 100 mg leaf tissue. The minimum time required to finish one extraction from 100 mg leaf tissue using each method was estimated based on the procedures used in this study, including the time for incubation, centrifugation and 30 min for DNA drying if necessary. The time spent grounding samples in liquid nitrogen and for solution preparation in all the methods was excluded.

2.4 DNA Analysis

DNA concentration was measured photometrically at 260 nm wavelength (OD₂₆₀) with an Eppendorf Bio Photometer (BioPhotometer Plus, Eppendorf Co. Ltd., Germany). Total DNA yield was calculated as the DNA amount in µg in the complete extraction volume.

Purity was determined by measuring additionally at 280nm wavelength (OD₂₈₀) and computing the ratio between the two values (OD_{260/280}).

To evaluate the DNA integrity and functionality, the extracted DNA were digested with *Hind III*, which were performed using approximately 1 µg of DNA and 15 units of enzyme in a total volume of 20 µl and incubated at 37°C for 2 hours. Afterwards 5 µl undigested DNA and 5 µl digested DNA were subjected to electrophoresis on a 0.8% agarose gel at 90 V for 100 min, then stained in ethidium bromide for 20 min, and photographed in GeneGenius Imaging System (Syngene, A Division of Synoptics Ltd., UK).

The DNA functionality was further tested by PCR amplification of a 773 bp fragment from the chloroplast gene for the photosystem II protein D1 (*PSBA*) of *Vigna unguiculata*. Primers were designed from the *PSBA* photosystem II protein D1 sequence of *Vigna unguiculata* taken from Genbank database (GeneID: 13080510, <http://www.ncbi.nlm.nih.gov/gene/13080510>) using Primer-BLAST software (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>): forward primer PD1_F with sequence 5'-GGCCAAGCAGCTAGGAAGAA-3', reverse primer PD1_R with sequence 5'-ACCAGCACCGAAAATCGTCT-3'. The primers were synthesized by Life Technologies Corporation.

PCR (50 µl volumes) contained: Approximately 100 ng of genomic DNA, 0.25 µl Taq (5 U/µl, Fermentas), 5 µl 10× PCR reaction buffer, 3 µl MgCl₂ (25 mM), 4 µl dNTP Mixture(2.5 mM), 1 µl each primer (20 µM), and ddH₂O up to 50 µl.

The amplification was performed in a Peltier Thermal Cycler (BIO-RAD DNAEngine). Cycling conditions consisted of a 5 min initial denaturation at 95°C followed by 1 min denaturing at 95°C, 1 min annealing at 55°C and 1 min extension at 72°C repeated for 40 cycles and with 5 min extension at 72°C. Subsequently, 6 µl PCR products and 1 µl 6×loading buffer were subjected to electrophoresis on a 1.5% agarose gel at 75 V for 75 min, stained in ethidium bromide for 20 min, and photographed in GeneGenius Imaging System (Syngene, A Division of Synoptics Ltd., UK).

2.5 Data Analyses

The general linear model (GLM) was applied to test the effect of extraction method on the DNA yield and on the OD_{260/280} ratio. Tukey's pairwise comparisons with the confidence interval of 95% were used to compare the yield or the ratios between the methods. The statistical analyses were accomplished by using the MINITAB[®] software Release 16 (<http://www.minitab.com>).

3. Results and Discussion

3.1 Choice of the Material

Proper choice of the leaf tissue is very important for DNA extraction (Lodhi et al., 1994). In this research, leaf tissue harvested from two-week-old cowpea seedlings was used for DNA extraction because fresh, young leaf tissue was preferable since it may contain less polyphenolic and terpenoid compounds than older tissue (Rosenthal et al., 1979). Generally, mature plant tissues are not preferred for DNA extraction due mainly to the presence of high concentrations of polysaccharides, polyphenols, and other secondary metabolites (Dabo et al., 1993; Zhang et al., 2000).

3.2 Cost and Time Consumed

In order to perform the PCR technique routinely, low cost and time per assay is very important. Studies based on DNA markers require large amounts of quality genomic DNA, emphasizing the need for inexpensive, rapid and simple DNA extraction methods (Weishing et al., 1995).

The estimated cost in US dollar (USD) and time in hours for each method to extract DNA from 100mg leaf tissue of cowpea are presented in Table 1. The expenses of the laboratory-prepared SDS and CTAB buffers in the five classic methods were much lower (approximately, 0.0003–0.003 USD per sample). As a result, these methods were less costly than the commercial kit. The E.Z.N.A.[®] Plant DNA Kit was the most expensive among the six methods but the least extraction time was required.

Table 1. DNA yield, OD 260/280 ratios, and estimated cost and time used for one cowpea extraction from 100 mg leaf tissue by six extraction methods (The results from each method were from 10 replications of each extraction method)

Method	DNA yield (mean ± SE) (µg/100mg)	OD _{260/280} (mean ± SE)	OD _{260/280} range	Time (hr)	Cost (USD)
Commercial Kit	19.48±2.51	1.82±0.03	1.72-1.90	0.7	2.08
Saghai-Marooft et al.	12.53±1.40	2.05±0.02	1.98-2.10	1.5	0.008
Doyle et al.	4.49±0.32	1.76±0.05	1.65-1.92	2.2	0.01
Dellaporta et al.	61.5±7.50	2.03±0.04	1.90-2.18	2.4	0.012
Rogers et al.	16.47±2.43	2.12±0.03	1.96-2.19	0.7	0.005
Aljanabi et al.	72.00±9.70	1.97±0.03	1.85-2.05	3.3	0.162

3.3 DNA Yield

Yield of the DNA extracted by the six methods were listed in Table 1. The extraction method had a significant effect ($F = 29.20$, $df = 5$, $P < 0.01$) on the DNA yield. The DNA yield by the SDS methods (Aljanabi et al., 1997; Dellaporta et al., 1983) were significantly higher than those obtained by the CTAB methods (Saghai-Marooft et al., 1984; Rogers et al., 1985; Doyle et al. 1987) and E.Z.N.A.[®] plant DNA Kit (Tukey's, $P < 0.05$). But according to Doosty et al. (1994), DNA extracted with the SDS method described by Dellaporta et al. had not good quantity and quality. This extraction method for *Satureja khuzistanica* did not show acceptable results because the SDS buffer attached to the secondary metabolite and prevented extraction DNA with high quality. Furthermore these results were in agreement with the findings of Ziegenhagen and Scholz (1993). In our research, a high yield of DNA was obtained from cowpea using the Dellaporta method, probably because the young cowpea seedlings contain less secondary metabolite.

The lowest DNA yield was obtained by the method reported by Doyle et al. This result accorded with Ostrowska et al. (1998), Abu-Romman (2011) and Doosty et al. (1994). By using the Doyle method, Ostrowska et al. (1998) yield 48-67 µg per g (equal to 4.8-6.7 µg per 100mg) DNA from *Pinus radiata*, Abu-Romman got the lowest DNA yield and poor quality from sage (*Salvia officinalis*), and Doosty et al. obtained negligible DNA from *Satureja khuzistanica*. According to Doosty et al., DNA extracted with this method was surrounded by Ammonium acetate which causes formed gelatinous deposit.

3.4 Purity

The assessment of the purity of a nucleic acid sample is often performed by a procedure commonly referred to as

the OD_{260/280} ratio. Although this procedure was first described by Warburg and Christian (Warburg et al., 1942) as a means to measure protein purity in the presence of nucleic acid contamination, it is most commonly used today to assess purity of nucleic acid samples (Held, 2006). A pure sample of DNA has the ratio at 1.8 (Chen et al., 2010).

The mean OD_{260/280} ratios for the four methods described by Saghai-Marooof et al., Aljanabi et al., Rogers et al. and Dellatporta et al. were higher than 1.9. In these four methods, RNA disposal was not involved, hence there existed some RNA residues, as determined by the electrophoresis on agarose gel (Figure 1, there were clear main bands above but with smear at the bottom of lane 3, 7, 9 and 11)

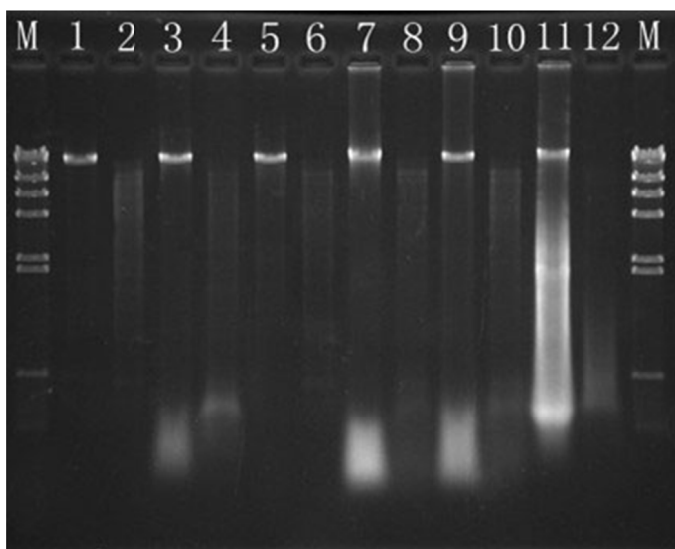


Figure 1. Agarose gel of undigested and digested DNA extracted from cowpea seedlings. The isolated DNA was digested by the restriction enzyme *Hind III*. Lanes designated (M) are lambda/*Hind III* molecular weight markers (Fermentas). Lanes 1 and 2 are the DNA isolated by E.Z.N.A.® Plant DNA Kit, Lanes 3 and 4 are the DNA isolated by Saghai-Marooof et al., Lanes 5 and 6 are the DNA isolated by Doyle et al., Lanes 7 and 8 are the DNA isolated by Dellaporta et al., Lanes 9 and 10 are the DNA isolated by Rogers et al., Lanes 11 and 12 are the DNA isolated by Aljanabi et al., alternating undigested and digested DNA

Proteins from the cell soup are generally removed during extraction by denaturation and precipitation using chloroform and/or phenol (Vinod, 2004). But according to Aljanabi et al., Proteinase K was used to purge the protein instead of chloroform-isoamyl alcohol (24:1), and RNA removal step was not included. Lane 11 (Figure 1) indicated that the protein was not completely removed and RNA was still present in the DNA solution.

The mean OD_{260/280} ratios of Doyle method and E.Z.N.A.® Plant DNA Kit were the closest to 1.8 among the six methods, indicating the isolated DNA was relatively free from RNA and protein contamination. RNase was used to remove RNA from DNA in these two protocols. RNA could also be removed by selective precipitation with lithium chloride (Jobes et al., 1995).

The OD_{260/280} ratio was significantly affected by the six extraction methods ($F = 15.69$, $df = 5$, $P < 0.01$). Statistically, the ratio means of the four methods described by Saghai-Marooof et al., Aljanabi et al., Rogers et al. and Dellatporta et al. were higher than those of the Doyle method and E.Z.N.A.® Plant DNA Kit (Tukey's, $P < 0.05$).

3.5 Integrity

The integrity, i.e. presence of high molecular genomic DNA, was determined by electrophoresis on a 0.8% agarose gel. High molecular DNA bands with no smear were obtained from Doyle method and the E.Z.N.A.® Plant DNA Kit (Figure 1, lane 1, 5), indicating that the DNA were intact and pure. While DNA isolated from the methods described by Saghai-Marooof et al., Aljanabi et al., Rogers et al. and Dellatporta et al. showed high molecular DNA bands with smear at the bottom of lane 3, 7, 9 and 11 (Figure 1), demonstrating that the DNA were intact but there existed some RNA or protein residues.

3.6 Functionality

The functionality of the DNA is the most important factor that determining whether an isolation method is valid or not. Without high quality DNA, the downstream molecular manipulations like RAPD and AFLP, are not feasible (Varma et al., 2007).

There are at least three main contaminants associated with plant DNA: polyphenolic compounds, polysaccharides, and RNA (Jobes et al., 1995). Polysaccharides, which are difficult to separate from DNA (Murray et al., 1980), interfere with several biological enzymes such as polymerases, ligases and restriction endonucleases (Shioda et al., 1987; Richards, 1988). Moreover, Lodhi et al. (1994) found that when polysaccharides were not removed, the DNA would not amplify in PCR reaction.

As shown in Figure 1, the DNA was completely digested with Hind III restriction enzyme, as evidenced by the characteristic "smearing" and the absence of the high molecular weight bands seen in the adjacent lane of undigested DNA. Besides, the target fragment from the *PSBA* photosystem II protein D1 sequence of *Vigna unguiculata* was successfully amplified from all the DNA extractions (Figure 2). This further confirmed the purity of the DNA, free of polysaccharide and polyphenol contaminations. Complete digestion with restriction endonuclease and successful amplification in PCR indicated that all the DNA extractions were of high quality and functionality.

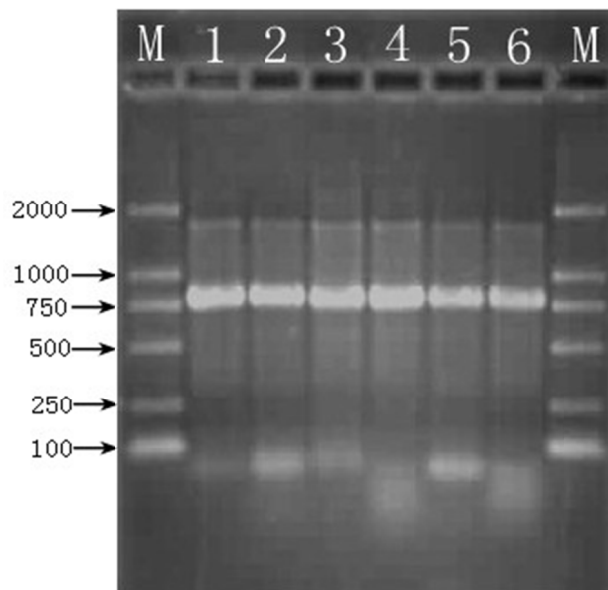


Figure 2. PCR amplification of the partial *psbA* gene from the DNA extracted from cowpea seedlings. Lanes marked (M) are 2000 bp molecular weight markers (Fermentas). Lanes 1-6 are the *psbA* gene fragment amplified from DNA isolated using the methods described by E.Z.N.A.® Plant DNA Kit, Saghai-Marouf et al., Doyle et al., Dellaporta et al., Rogers et al. and Aljanabi et al., respectively

There are two different viewpoints on the effect of RNA residue. Some researchers hold the opinion that contaminants like RNA often inhibit restriction endonuclease digestion and/or PCR amplification (Couch et al., 1990; Guillemaut et al., 1992; Richards et al., 1994). There is also new data indicating that RNA contamination can reduce the effectiveness of many enzymatic processes (Storts, 1993; Yoon et al., 1993; Mejjad et al., 1994). Furthermore, the RNA degrades at high temperature in the presence of magnesium ions and the release nucleotides inhibit deoxynucleotide incorporation in the PCR reaction.

While others argue that the presence of the RNA in DNA extracted is not a major problem as this usually does not interfere with PCR or restriction digestion (Murray et al., 1980; Vinod, 2004). Because RNA is, by nature, transient and unstable unlike DNA. RNA is ubiquitously degraded with striking efficiency in all cells (Houseley et al., 2009). Much of the RNA is cut by ribonucleases or RNases that are released when the cells are broken open and the rest will not last in an environment outside the cell and will degrade anyways even without RNase.

In this study, the RNA residue in the DNA isolated by the four methods described by Saghai-Marooof et al., Aljanabi et al., Rogers et al. and Dellaporta et al., did not inhibit the digestion with restriction enzyme *Hind III* and the amplification of the target gene fragment, suggesting that in most cases, the RNase treatment is not certainly needed.

4. Conclusion

In this study, five classic methods and a commercial kit for isolating DNA from cowpea (*Vigna unguiculata*) were compared and analyzed from the following perspectives: cost and time demands, yield of DNA, the purity of DNA acquired, intactness, and functionality. All the six methods compared in this study turned out to be suitable to extract DNA from cowpea. In summary, the conclusions in this research are as follows:

- 1). The cowpea DNA yield by the SDS methods (Aljanabi et al., 1997; Dellaporta et al., 1983) were significantly higher than those obtained by the CTAB methods (Saghai-Marooof et al., 1984; Rogers et al., 1985; Doyle et al., 1987) and E.Z.N.A.[®] plant DNA Kit (Tukey's, $P < 0.05$).
- 2). The extraction method had a significant effect on the DNA yield ($F = 29.20$, $df = 5$, $P < 0.01$) and $OD_{260/280}$ ratio ($F = 15.69$, $df = 5$, $P < 0.01$).
- 3). When extracting DNA for restriction enzymes digestion or PCR based downstream molecular manipulations, the RNase treatment is not needed.
- 4). After evaluating the cost and time, yield, purity, integrity and functionality among the six methods, the SDS method described by Dellaporta et al., was considered an ideal protocol to isolate DNA from *Vigna unguiculata*. The cost and time required in this method was relatively low. Besides, the quantity and the quality of the DNA extracted by this method were high enough to perform hundreds of PCR-based reactions and also to be used in other DNA manipulation techniques such as restriction digestion, Southern blot and cloning. In addition, it had the added advantage of not requiring any phenol or chloroform extraction.

Acknowledgments

This work was supported by 'Shuang-Zhi Plan' of Sichuan Agricultural University.

References

- Abu-Romman, S. (2011). Comparison of methods for isolating high quality DNA from sage (*Salvia officinalis*). *J. Med. Plants Res.*, 5(6), 938-941.
- Agbicodo, E. M., Fatokun, C. A., Muranaka, S., Visser, R. G. F., & Linden-van-der, C. G. (2009). Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. *Euphytica*, 167, 353-370. <http://dx.doi.org/10.1007/s10681-009-9893-8>
- Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res.*, 25(22), 4692-4693. <http://dx.doi.org/10.1093/nar/25.22.4692>
- Asiwe, J. A. N., Belane, A., & Dakora, F. D. (2009). *Evaluation of cowpea breeding lines for nitrogen fixation at ARC-Grain Crops Institute*. Potchefstroom, South Africa.
- Ba, F. S., Pasquet, R. S., & Gepts, P. (2004). Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. *Genet. Resour. Crop Ev.*, 51, 539-550. <http://dx.doi.org/10.1023/B:GRES.0000024158.83190.4e>
- Chen, H., Rangasamy, M., Tan, S. Y., Wang, H., & Siegfried, B. D. (2010). Evaluation of Five Methods for Total DNA Extraction from Western Corn Rootworm Beetles. *Plos One*, 5, 1-6. <http://dx.doi.org/10.1371/journal.pone.0011963>
- Couch, J. A., & Fritz, P. J. (1990). Isolation of DNA from plants high in polyphenolics. *Plant Mol. Biol. Rep.*, 8, 8-12. <http://dx.doi.org/10.1007/BF02668875>
- Dabo, S. M., Mitchell, E. D., & Melcher, U. (1993). A method for the isolation of nuclear DNA from cotton (*Gossypium*) leaves. *Anal. Biochem.*, 210, 34-38. <http://dx.doi.org/10.1006/abio>
- Davis, R. W., Thomas, M., Cameron, J., John T. P., Scherer, S., & Padgett, R. A. (1980). Rapid DNA isolation for enzymatic and hybridization analysis. *Method Enzymol.*, 65, 404-411.
- Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A Plant DNA Miniprep: Version II. *Plant Mol. Biol. Rep.*, 1, 19-21. <http://dx.doi.org/10.1007/BF02712670>

- Diouf, D., & Hilu, K. W. (2005). Microsatellite and RAPD markers to study genetic relationships among cowpea breeding lines and local varieties in Senegal. *Genet. Resour. Crop Ev.*, 52, 1957-1967. <http://dx.doi.org/10.1007/s10722-004-6107-z>
- Doosty, B., Drikvand, R., Salahvarzi, E., Amiri, H., & Hadian, J. (2012). Comparative Analysis and Optimization of Different DNA extraction protocols in *Satureja khuzistanica*. *International Journal of Biology*, 4, 111-116. <http://dx.doi.org/10.5539/ijb.v4n4p111>
- Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11-15.
- Fang, J. G., Chao, C. T., Roberts, P. A., & Ehlers, J. D. (2007). Genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis. *Genet. Resour. Crop Ev.*, 54, 1197-1209. <http://dx.doi.org/10.1007/s10722-006-9101-9>
- FAO. (2010). *FAO statistical databases*. Retrieved from <http://faostat.fao.org>
- Fatokun, C. A., Menancio-Hautea, D. I., Danesh, D., & Young, N. D. (1992). Evidence for Orthologous Seed Weight Genes in Cowpea and Mung Bean Based on RFLP Mapping. *Genetics*, 132, 841-846.
- Ghalmi, N., Malice, M., Jacquemin, J. M., Ounane, S. M., Mekiche, L., & Baudoin, J. P. (2010). Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.) Walp.) landraces. *Genet. Resour. Crop Ev.*, 57, 371-386. <http://dx.doi.org/10.1007/s10722-009-9476-5>
- Guillemaut, P., & Marechal-Drouard, L. (1992). Isolation of plant DNA: A fast, inexpensive, and reliable method. *Plant Mol. Biol. Rep.*, 10, 60-65. <http://dx.doi.org/10.1007/BF02669265>
- Held, P. (2006). *Nucleic Acid Purity Assessment using A260/A280 Ratios*. Retrieved from <http://www.biotek.com/resources/articles/nucleic-acid-purity-a260a280.html>
- Houseley, J., & Tollervey, D. (2009). The Many Pathways of RNA Degradation. *Cell*, 136, 763-776. <http://dx.doi.org/10.1016/j.cell.2009.01.019>
- Jackai, L. E. N., & Daoust, R. A. (1986). Insect pests of cowpeas. *Annu. Rev. Entomol.*, 31, 95-119. <http://dx.doi.org/10.1146/annurev.en.31.010186.000523>
- Jobs, D. V., Hurley, D. L., & Thien, L. B. (1995) Plant DNA isolation: A Method to Efficiently Remove Polyphenolics, Polysaccharides, and RNA. *Taxon*, 44, 379-386.
- Lodhi, M. A., Ye, G. N., Weeden, N. F., & Reisch, B. I. (1994) A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. *Plant Mol. Biol. Rep.*, 12, 6-13. <http://dx.doi.org/10.1007/BF02668658>
- Malviya, N., Sarangi, B. K., Yadav, M. K., & Yadav, D. (2012). Analysis of genetic diversity in cowpea (*Vigna unguiculata* L. Walp.) cultivars with random amplified polymorphic DNA markers. *Plant Syst. Evol.*, 298, 523-526. <http://dx.doi.org/10.1007/s00606-011-0545-9>
- Mejjad, M., Vedel, F., & Ducreux, G. (1994). Improvement of DNA preparation and of PCR cycling in RAPD analysis of marine macroalgae. *Plant Mol. Biol. Rep.*, 12, 101-105. <http://dx.doi.org/10.1007/BF02668370>
- Murray, M. G., & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, 8, 4321-4325. <http://dx.doi.org/10.1093/nar/8.19.4321>
- Myers, G. O., Fatokun, C. A., & Young, N. D. (1996). RFLP mapping of an aphid resistance gene in cowpea (*Vigna unguiculata* L. Walp). *Euphytica*, 91, 181-187. <http://dx.doi.org/10.1007/BF00021068>
- Ostrowska, E., Muralitharan, M., Changdler, S., Volker, P., Hetherington, S., & Dunshea, F. (1998). Optimizing conditions for DNA isolation from *Pinus Radiata*. *In Vitro Cell Dev-Pl*, 34, 108-111. <http://dx.doi.org/10.1007/BF02822773>
- Ribeiro, R. A., & Lovato, M. B. (2007). Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia*. *Genet. Mol. Res.*, 6, 173-187.
- Richards, E. (1988). *Preparation of genomic DNA from plant tissue*. In: F. M. Ausubel, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, & K. Struhl (Eds.), *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience, New York.
- Richards, E., Reichardt, M., & Rogers, S. (1994). Preparation of genomic DNA from plant tissue. In: F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, K. Struhl (Eds.), *Current protocols in molecular biology*. New York.

- Rogers, S. O., & Bendich, A. J. (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.*, 5, 69-76. <http://dx.doi.org/10.1007/BF00020088>
- Rosenthal, G. A., & Janzen, D. H. (1979). *Herbivores, their interaction with secondary plant metabolites*. Academic Press, New York.
- Saghai-Marooif, M. A., Soliman, K. M., Jorgensen, R. A., & Allard, R. W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *PNAS*, 81, 8014-8018.
- Sarutayophat, T., Nualsri, C., Santipracha, Q., & Saereprasert, V. (2007). Characterization and genetic relatedness among 37 yardlong bean and cowpea accessions based on morphological characters and RAPD analysis. *Songklanakarin J. Sci. Technol.*, 29, 591-600.
- Shioda, M.; & Murakami-Murofushi, K. (1987). Selective inhibition of DNA polymerase alpha by a polysaccharide purified from slime of Physarum polycephalum. *Biochem. Bioph. Res. Co.*, 146, 61-66.
- Singh, B. B., Chambliss, O. L., & Sharma, B. (1997). *Recent advances in cowpea breeding*. In: B. B. Singh, D. R. Mohan-Raj, K. E. Da-shiell, & L. E. N. Jackai (Eds.), *Advances in cowpea research*. Co-publication of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and Japan International Research Centre for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, Japan.
- Storts, D. (1993). *Promega technical manual 24: fmo1[®] DNA cycle sequencing system*. Retrieved from <http://www.ebiotrade.com/buyf/productsf/Promega/tm024.pdf>
- Tantssawat, P., Trongchuen, J., Prajongjai, T., Seehalak, W., & Jittayasothorn, Y. (2010). Variety identification and comparative analysis of genetic diversity in yardlong bean (*Vigna unguiculata* spp. *sesquipedalis*) using morphological characters, SSR and ISSR analysis. *Sci. Hortic-Amsterdam*, 124, 204-216. <http://dx.doi.org/10.1016/j.scienta.2009.12.033>
- Taylor, B., & Powell, A. (1982). Isolation of plant DNA and RNA. *Focus*, 4, 4-6.
- Tosti, N., & Negri, V. (2005). On-going on-farm microevolutionary processes in neighbouring cowpea landraces revealed by molecular markers. *Theor. Appl. Genet.*, 110, 1275-1283. <http://dx.doi.org/10.1007/s00122-005-1964-1>
- Varma, A., Padh, H., & Shrivastava, N. (2007). Plant genomic DNA isolation: an art or a science. *Biotech. J.*, 2, 386-392. <http://dx.doi.org/10.1002/biot.200600195>
- Vinod, K. K. (2004). *Total genomic DNA extraction, quality check and quantitation*. In: Proceedings of the training programme on "Classical and modern plant breeding techniques-A hands on training". Tamil Nadu Agricultural University, Coimbatore, India.
- Warburg, O., & Christian, W. (1942). Isolation and crystallization of enolase. *Biochem. Z.*, 310, 384-421.
- Weishing, K., Nybom, H., Wolff, K., & Meyer, W. (1995). *DNA isolation and purification*. In: DNA fingerprinting in plants and fungi. Boca Raton, Florida: CRC press.
- Yoon, C. S., & Glawe, D. A. (1993). Pretreatment with RNase to improve PCR amplification of DNA using 10-mer primers. *Biotechniques*, 14, 908-910.
- Zannou, A., Kossou, D. K., Ahanchede, A., Zoundjihekpon, J., Agbicodo, E., & Struik, P. C. (2008) Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA. *Afr. J. Biotechnol.*, 7, 4407-4414. <http://dx.doi.org/10.5897/AJB08.856>
- Zhang, J., & Stewart, J. M. (2000). Economic and rapid method for extracting cotton genomic DNA. *J. Cotton Sci.*, 4, 193-201.
- Ziegenhagen, B., & Schulz, F. (1993). A procedure for mini-preparation of genomic DNA from needles of silver fir (*Abies alba* Mill). *Plant Mol. Biol. Rep.*, 11, 117-121.

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