Simple, rapid and cost-effective DNA extraction method for high quality DNA suitable for PCR based downstream application in mungbean [Vigna radiata (L.) Wilczek]

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Abstract. Mungbean is a widely cultivated pulse crop. It is a source of high-quality protein for human consumption. Several new mungbean varieties have been developed employing molecular breeding tools. The quality and quantity of extracted DNA are very important for PCR amplification, particularly for the inter-simple sequence repeat-anchorered resistance gene analog (ISSR-RGA) marker. In addition, reducing the time required for DNA extraction is essential in cases when large number of genotypes are analysed. Thus, the aim of this study was to compare eight combinations of two homogenization methods (manual grinding and Bullet blender® homogenizer) and four modified DNA extraction protocols. The effectiveness of DNA extraction for PCR amplification was evaluated using 3 PCR based markers, simple sequence repeat (SSR), ISSR and ISSR-RGA, on 6% polyacrylamide gel. Using homogenizer with modified protocol 2 (T6) resulted in a high DNA concentration (1032.60 ng/µl) and an A260/A280 ratio of 1.80, indicating high DNA purity. The PCR amplification of the resulting DNA with three types of molecular markers showed clear DNA bands, suggesting that DNA quality was appropriate for various molecular studies. In addition, homogenizers allowed processing of large number of samples in one go with minimal cost. These results suggest that this simple, rapid and cost-effective DNA extraction method is useful for marker-assisted breeding.

1. Introduction
Mungbean or green gram (Vigna radiata (L.) Wilczek) is an important economic crop in South and Southeast Asia. Currently, it is cultivated in more than 6 million hectares worldwide [1]. Almost 90% of world’s mungbean production comes from Asia—India, China, Pakistan and Thailand are among the most important producers [2]. It is grown mainly for its edible seeds, which are fermented, cooked, roasted, sprouted, or milled [3]. However, mungbean production has reduced due to poor harvest index, lack of genetic variability, abiotic and biotic stresses [4]. Therefore, plant breeders need to develop new varieties to increase yields. Several molecular techniques are currently used in mungbean breeding to facilitate genotyping and selection. Molecular markers are useful in evolutionary, phylogenetic, taxonomical, ecological and genetic studies of plants [5]. Mungbean genetic diversity has been evaluated using several DNA markers to identify polymorphisms among varieties such as SSR, ISSR and amplified fragment length polymorphism (AFLP) [6,7]. Moreover, marker-assisted selection
MAS) has been used to improve disease resistance using several molecular markers including ISSR and ISSR-RGA markers, which improve breeding program speed and accuracy [8-11]. Quality of DNA generally influences target DNA amplification therefore identifying a suitable extraction method for plant genomic DNA is a pre-requisite for molecular plant breeding, especially in mungbean plants with prominent oxidation problems [12].

Although many plant genomic DNA extraction protocols have been developed previously [13-20], they are often specific to tissues and plant species. Some protocols also have high costs, complex procedures, consume excessive time, or result in poor quality and the DNA insufficient for various polymerase chain reaction (PCR) based downstream applications. Recently, genomic DNA extraction protocols of legume crops including mungbean have been developed to generate high quality DNA and were suitable for amplification with diverse DNA markers such as RAPD, RFLP, SSR and ISSR [12,21-24]. However, these procedures still require long manual grinding using a mortar and pestle. This method is not suitable for screening large numbers of progenies in breeding populations. Moreover, some protocols resulted in unclear DNA bands especially with amplification of ISSR-RGA markers. In addition, a high throughput DNA extraction method has never been reported in mungbean. Thus, the present study aimed to find the optimal method of mungbean DNA extraction by comparing eight combinations of two homogenization methods (manual grinding and Bullet blender® homogenizer) and four modified DNA extraction protocols.

2. Materials and methods

2.1. Plant materials
Genomic DNA was extracted from young seedlings [15-20 days after sowing (DAS)] of the Chainat 72 (CN72) variety grown in the Suranaree University of Technology Farm greenhouse, Nakhon Ratchasima, Thailand. Harvested fresh emerging leaves were immediately taken to the laboratory and kept in cool conditions (4°C).

2.2. DNA extraction buffers
The reagents and chemicals used in DNA extraction buffers were partly modified from four published protocols (table 1).

| Protocols | Extraction buffer |
|-----------|-------------------|
| 1 Modified from Lodhi et al [25] | 3% (w/v) cetyltrimethylammonium bromide (CTAB), 1.4 M NaCl, 0.1 M Tris-HCL (pH 8), 0.02 M ethylenediaminetetraacetic acid (EDTA; pH 8), 2% (w/v) polyvinylpyrrolidone (PVP), 0.2% (v/v) β-mercaptoethanol |
| 2 Modified from Dharajiya et al [24] | 2.5% (w/v) CTAB, 1.4 M NaCl, 0.1 M Tris-HCL (pH 8), 0.02 M EDTA (pH 8), 2% (w/v) PVP, 0.2% (v/v) β-mercaptoethanol |
| 3 Modified from Agbagwa et al [23] | 3% (w/v) CTAB, 4 M NaCl, 0.1 M Tris-HCL (pH 8), 0.02 M EDTA (pH 8), 2% (v/v) β-mercaptoethanol |
| 4 Modified from Inglis et al [26] | Sorbitol wash buffer: 0.35 M sorbitol, 0.1 M Tris-HCL (pH 8), 0.05 M EDTA (pH 8), 1% (w/v) PVP, 1% (v/v) β-mercaptoethanol CTAB buffer: 3% (w/v) CTAB, 4 M NaCl, 0.1 M Tris-HCL (pH 8), 0.02 M EDTA (pH 8), 1% (w/v) PVP, 1% (v/v) β-mercaptoethanol |

2.3. Genomic DNA extraction methods
Two homogenization methods consisting of manual grinding (with a pestle and mortar) and homogenizer (Bullet blender®, Next Advance, Inc., NY, USA) were combined with four modified genomic DNA extraction protocols. Extraction buffers used were shown in table 1. In Bullet blender® homogenizer method, 3.2 mm stainless steel beads were used with the extraction buffer without liquid nitrogen in all the protocols. While manual grinding was done in the presence of liquid nitrogen (protocol 1 and 4) or without liquid nitrogen (protocol 2 and 3). The experiment was conducted
following a 2 x 4 factorial completely randomized design (CRD) with 8 treatments and 5 replications. The procedure for each genomic DNA extraction protocol was as follows:

Protocol 1: Leaf samples (50 mg) were ground to fine powder with liquid nitrogen using mortar and pestle. A CTAB extraction buffer (600 µl) was added into the ground tissue and vortexed for 30 s to obtain a homogeneous solution and the mixture was then incubated at 65°C for 30 min. Chloroform:isoamyl alcohol (24:1; 600 µl) was added to the mixture and centrifuged at 13,000 rpm for 20 min at 25°C. The supernatant was then transferred to a new micro-centrifuge tube. This step was repeated once more. The DNA pellets were precipitated with 200 µl of 5 M NaCl and 600 µl of chilled absolute isopropanol and kept at -20°C for 60 min. The samples were centrifuged at 13,000 rpm for 15 min at 25°C. The DNA pellets were washed with 500 µl of chilled 70% and absolute ethanol, and centrifuged at 13,000 rpm at 25°C. The supernatant was then discarded and the pellets were air dried. After air drying, the pellets were re-suspended in 100 µl sterile water. Finally, the RNA was removed by adding 40 µl of 1 mg/ml RNase and incubating at 37°C for 30 min, and the DNA was finally stored at -20°C.

Protocol 2: Leaf samples (50 mg) were ground with 1 ml CTAB extraction buffer without liquid nitrogen using a mortar and pestle. The mixture was then incubated at 65°C for 45 min and allowed to return to room temperature for 5 to 10 min. Chloroform:isoamyl alcohol (24:1; 600 µl) was added to the mixture and centrifuged at 11,000 rpm for 10 min at 25°C. The supernatant was transferred to a new micro-centrifuge tube, and 5 µl of 30 mg/ml RNase were added and incubated at 37°C for 20 min. The extraction step with 24:1 chloroform:isoamyl alcohol was repeated once more. The DNA pellets were precipitated with 150 µl of 3 M sodium acetate and 600 µl of chilled absolute isopropanol and kept at -20°C for 60 min. The samples were centrifuged at 10,000 rpm for 15 min at 4°C. The DNA pellets were washed with 500 µl of chilled 70% and absolute ethanol, and centrifuged at 7,000 rpm for 5 min at 4°C. The supernatant was then discarded and the pellets were air dried. After air drying, the pellets were resuspended in 100 µl TE buffer. The resulting DNA was stored at -20°C.

Protocol 3: Leaf samples (50 mg) were ground with 1 ml CTAB extraction buffer without liquid nitrogen using a mortar and pestle. The mixture was incubated at 65°C for 30 min and allowed to return to room temperature for 5 to 10 min. Chloroform:isoamyl alcohol (24:1; 600 µl) was added to the mixture and centrifuged at 10,000 rpm for 15 min at 22°C. The supernatant was transferred to a new micro-centrifuge tube. This step was repeated once more. The DNA pellets were precipitated with 200 µl of 5M NaCl and 600 µl of chilled absolute isopropanol and kept at -20°C for 60 min. The samples were centrifuged at 6,000 rpm for 8 min at 4°C. The DNA pellets were washed with 500 µl of chilled 70% and absolute ethanol, and centrifuged at 7,000 rpm for 5 min at 4°C. The supernatant was then discarded and the pellets were air dried. After air drying, the pellets were resuspended in 100 µl TE buffer. Finally, the RNA was removed by adding 40 µl of 1 mg/ml RNase and incubating at 37°C for 30 min, and the DNA was stored at -20°C.

Protocol 4: Leaf samples (50 mg) were ground to a fine powder with liquid nitrogen using a mortar and pestle. Sorbitol wash buffer (1 ml) was added into ground tissue and vortexed for 30 s to obtain a homogeneous solution. The mixture was centrifuged at 5,000 rpm for 5 min at 25°C. The supernatant was then transferred to a new micro-centrifuge tube, and CTAB extraction buffer (600 µl) was added. The mixture was incubated at 65°C for 60 min and allowed to return to room temperature for 5 to 10 min. Chloroform:isoamyl alcohol (24:1; 600 µl) was added to the mixture and centrifuged at 5,100 rpm for 10 min at 25°C. The supernatant was transferred to a new micro-centrifuge tube. This step was repeated once more. The DNA pellets were precipitated with 200 µl of 5 M NaCl and 600 µl of chilled absolute isopropanol and kept at -20°C for 60 min. The samples were centrifuged at 13,270 rpm for 10 min at 25°C. The DNA pellets were washed with 500 µl of chilled 70% and absolute ethanol, and centrifuged at 13,270 rpm for 5 min at 25°C. The supernatant was then discarded and the pellets were air dried. After air drying, the pellets were resuspended in 100 µl sterile water. Finally, RNA was removed by adding 40 µl of 10 mg/ml RNase and incubating at 37°C for 30 min, and the DNA was stored at -20°C.

The eight treatment combinations for genomic DNA extraction methods were as follows:
2.4. DNA quantification and purity estimation

The concentration and purity (Optical Density (OD) at 260 and 280 nm) of genomic DNA from mungbean were measured with a ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) and adjusted to a final concentration of 50 ng/μl for PCR amplification.

2.5. DNA quality evaluation by PCR amplification

The quality of DNA was evaluated by PCR amplification using SSR, ISSR and ISSR-RGA markers with 2 primers/ marker pairs for each marker type. The SSR markers were amplified with primer VR393 (Forward: 5’-TGGCAGTTTCCATAACGAA3’, Reverse: 5’-ATCGCAAAAGCTCAGAAAC-3’) and CEDG084 (Forward: 5’-ATCAACTGAGGACATGCA3’, Reverse: 5’-CAA CATTCTAACCTTGGGACAG-3’). The ISSR makers were amplified with primer ISSR 885 (5’-BHB (GA)3’; B = C,G,T; H = A,C,T) and ISSR 816 (5’-(CA)T3’). The ISSR-RGA markers were amplified with primers ISSR 827 (5’-(AC)G-3’) and RLKF (5’-GAYGTNAARCCI GARAA-3’) and primers ISSR 842 (5’-(GA)YG-3’) and P-Loop (5’-(GGI)GTIGGIAAIACIAC-3’). The composition of PCR mixtures are described in table 2. SSR markers were amplified in a T100TM Thermal Cycler (Bio-Rad Laboratories, Inc., California, USA) as follows: initial denaturation at 94°C for 2 min; 35 cycles of denaturing at 94°C for 30 s, annealing at 50-65°C for 30 s, extension at 72°C for 1 min; and final extension at 72°C for 10 min. The amplification of ISSR and ISSR-RGA markers was performed in a T100TM Thermal Cycler, programmed as follows: initial denaturation at 95°C for 5 min; 35 cycles of denaturing at 95°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min; and final extension at 72°C for 10 min. The PCR products were separated on 6% denaturing polyacrylamide gel at 200 V for 50 and 70 min for SSR, ISSR and ISSR-RGA, respectively. DNA bands were detected with silver nitrate staining [27]. These markers are linked to powdery mildew or Cercospora leaf spot resistance genes in mungbean and are useful for marker-assisted selection for disease resistance [28,29].

Table 2. PCR mixture composition of SSR, ISSR and ISSR-RGA markers.

| Markers | PCR mixture | DNA (ng) | Primers (μM) | Taq polymerase (unit) | DNA Buffer | MgCl2 (mM) | dNTP (μM) | Total volume (μl) |
|---------|-------------|----------|--------------|-----------------------|------------|------------|-----------|-----------------|
| SSR     | 150         | 0.5      | 1X Reverse   | 1                     | 1X Buffera| 2         | 200       | 20              |
| ISSR    | 150         | 0.4      | 1X          | 1                     | 1X Buffer  | 3.5       | 250       | 20              |
| ISSR-RGA| 150         | 0.4      | 1X Buffer   | 1                     | 1X Buffer  | 3.5       | 250       | 20              |

*a1X Buffer consists of 50 mM KCl, 10 mM Tris-HCl (pH 9.1) and 0.01% (v/v) Triton® X-100.

3. Results and discussion

When the effect of homogenization methods on DNA concentrations was evaluated, DNA concentrations were significantly variable (F =398.91, df =1, P < 0.01) between the manual grinding and Bullet blender® homogenizer methods. Overall, the homogenizer gave approximately 2.1-fold
higher DNA concentrations (932.1 ng/µl) than manual grinding (447 ng/µl). Similarly, genomic DNA extraction protocols also significantly affected DNA concentrations (F = 83.36, df = 3, P < 0.01). On average, protocol 1 gave the highest DNA concentration (850.91 ng/µl), followed by protocol 2 (707.89 ng/µl). Significantly lower DNA concentrations were obtained from protocol 3 (619.07 ng/µl) and protocol 4 (580.32 ng/µl). The interaction between homogenization methods and genomic DNA extraction protocols was significant (F = 83.36, df = 3, P < 0.01) on DNA concentration. Thus, the combination of both factors should be considered simultaneously to obtain high total DNA yield in mungbean.

The quantity and estimated quality of genomic DNA extracted by different combinations of homogenization methods and genomic DNA extraction protocols are shown in Table 3. From the eight treatments, T7, T6 and T1 gave significantly higher DNA concentrations than other treatments (1069.26, 1032.58 and 920.12 ng/µl, respectively). The concentrations of CTAB and β-mercaptoethanol commonly used in DNA extraction protocols of T1 and T6 were 2.5-3% and 0.2%, respectively, which have been shown to maximize total DNA yield in cereal crops, while, higher concentrations of both caused a reduction on DNA yield in the experiment, however, we found that a high DNA yield can be achieved when using high concentrations of β-mercaptoethanol (2%) in the T7 using the homogenizer. Note that T3 which used similar DNA extraction protocol as T7 but with manual grinding gave a significantly lower DNA yield, substantiating the effects of homogenization methods and the interaction between homogenization methods and genomic DNA extraction protocols.

Table 3. Concentrations and OD (A$_{260}$/A$_{280}$) of mungbean genomic DNA isolated from different genomic DNA extraction methods and the time required for extraction.

| Genomic DNA extraction methods (Treatments) | DNA concentrations (ng/µl) | OD (A$_{260}$/A$_{280}$) | Sample grinding time (min/sample no.) | Total time for 24 samples (min) |
|-------------------------------------------|----------------------------|--------------------------|--------------------------------------|-------------------------------|
| (1) Manual, Protocol 1                    | 920.12 ± 37.12abc$^a$     | 1.90                     | 3/1                                  | 260                           |
| (2) Manual, Protocol 2                    | 383.20 ± 57.40d           | 1.73                     | 3/1                                  | 265                           |
| (3) Manual, Protocol 3                    | 168.88 ± 11.29e           | 1.83                     | 3/1                                  | 198                           |
| (4) Manual, Protocol 4                    | 315.76 ± 5.03d           | 1.43                     | 3/1                                  | 250                           |
| (5) Homogenizer, Protocol 1               | 781.70 ± 12.25c          | 1.90                     | 3/24                                 | 191                           |
| (6) Homogenizer, Protocol 2               | 1032.58 ± 17.86ab         | 1.80                     | 3/24                                 | 196                           |
| (7) Homogenizer, Protocol 3               | 1069.26 ± 44.32a         | 1.95                     | 3/24                                 | 129                           |
| (8) Homogenizer, Protocol 4               | 844.88 ± 46.70bc         | 1.67                     | 3/24                                 | 181                           |

$^a$Results are presented as mean ± SE, n =5. Means identified with the same letters are not significantly different (P > 0.05).

Both T6 and T7 gave high DNA quantity; Nevertheless, the highest purity of DNA estimated from the A$_{260}$/A$_{280}$ ratio was observed in T6. The A$_{260}$/A$_{280}$ ratio of T6 was 1.8, suggesting the best quality of extracted genomic DNA for molecular studies—it likely had a very low contamination level from other compounds such as phenol, proteins and polysaccharides [24]. On the other hand, the ratio of nearly 2 in T7 may indicate RNA contamination because RNA can greatly absorb UV light at 260 nm [31]. By contrast, the aromatic amino acids in proteins can absorb UV light at 280 nm, resulting in a lower A$_{260}$/A$_{280}$ ratio as shown in T4 and T8. Both types of contaminants may be found in the DNA solution of some treatments, affecting DNA quality. In addition, the presence of guanidine may lead to high absorbance at 260 nm. If the A$_{280}$ is used to calculate DNA concentration, it may be overestimated [32]. Although PVP has been suggested to play a major role on extracted DNA purity by removing polyphenolic contamination, preventing polyphenol oxidation and/or browning of DNA [33], the omission of PVP in T3 and T7 did not result in browning. And T7 also gave the highest DNA
concentration in our experiment, possibly due to high concentration of β-mercaptoethanol that prevent the oxidation problem.

Moreover, our study also focused on reducing the extraction time with a Bullet blender® homogenizer. When comparison was made between manual grinding using mortar and pestle (T1-T4) and Bullet blender® homogenizer (T5-T8), the homogenizer did not require liquid nitrogen and can homogenize much faster (3 min per 24 samples), while manual grinding required 3 min per sample and needed liquid nitrogen in T1 and T4. The combination of using the Bullet blender® homogenizer with protocol 2 (T6) gave both high yields and purity of DNA, and required only 196 min/24 samples, indicating that it was the most efficient DNA extraction method from this experiment.

![Figure 1](image_url)  
**Figure 1.** PCR products of genomic DNA extracted by different extraction methods, amplified using SSR, ISSR and ISSR-RGA primer/primer pairs, and separated on 6% polyacrylamide gel. (A, B and C), using SSR (CEDG084), ISSR (885) and ISSR-RGA (827 and RLKfor) primer/primer pairs, respectively. T1-T4 is the combinations of manual grinding with 4 modified DNA extraction protocols. T5- T8 is the combination of Bullet blender® homogenizer with 4 modified DNA extraction protocols.

The quality of genomic DNA isolated from various protocols was further confirmed by amplifying them with various molecular markers. Downstream PCR-based amplification results using 3 types of markers are presented in figure 1. The genomic DNA was amplified using SSR, ISSR and ISSR-RGA primers. The T1, T5 and T6 showed clear DNA bands with all molecular makers. These results indicated that the genomic DNA isolated using these modified methods is of decent quality and purity and can be applied with diverse molecular markers. However, the remaining treatments showed no or unclear DNA bands when amplified with same SSR, ISSR and/or ISSR-RGA primers/primer pairs; This phenomenon might be due to the contamination of plant DNA with polyphenolics, polysaccharides, RNA, and/or proteins, which inhibited DNA amplification in PCR reactions [12,34-36]. This is most obvious with T4 which appeared to have amplification problems in all marker types. Note that the DNA extracted by this method also had the lowest A260/A280 ratio of 1.43, implying contamination by proteins. When the three methods were compared based on all criterions, T5 was found to give lower DNA yield than T1 and T6. In addition, T1 required much longer extraction time (260 min/24 samples) than T6 (196 min/24 samples) and needed liquid nitrogen for grinding the
samples, which is costly. Therefore, T6 was found to be the optimal genomic DNA extraction method for mungbean, it was quick, and yielded both high DNA concentrations and high purity (A260/A280 ratio) suitable for diverse PCR applications. It was also found cost-effective because liquid nitrogen was unnecessary for this method.

4. Conclusion
The genomic DNA extraction method that combines the Bullet blender® homogenizer with modified DNA extraction protocol 2 (T6) is optimal for genomic DNA extraction of mungbean as it yielded abundant and pure genomic DNA. At the same time, this procedure was also simple, rapid and cost-effective as well as suitable for application of diverse molecular markers. Therefore, it can be used efficiently to carry out large scale marker analysis and genotyping, reducing time required and cost in molecular breeding.

Acknowledgment
We thank Dr. Colin Thomas Strine for editing the manuscript. This work was supported by the Office of the Higher Education Commission under NRU project of Thailand and grants from Suranaree University of Technology and Agricultural Research Development Agency (Public Organization), Thailand.

References
[1] World Vegetable Center 2016 Establishing the international mungbean improvement network [on-line] Available: https://avrdc.org/intl-mungbean-network/
[2] Lambrides C J and Godwin I D 2007 Mungbean Genome Mapping and Molecular Breeding in Plants-Pulses, Sugar and Tuber Crops ed C Kole (Heidelberg: Springer) pp 69-90
[3] Malik B A 1994 Grain Legumes Crop Production ed S Nazir et al (Islamabad: National Book Foundation) pp 277-8
[4] Tantasawat P, Trongchuen J, Prajongjai T, Thongpae T, Petkhum C, Seehalak W and Machikowa T 2010 Variety identification and genetic relationships of mungbean and blackgram in Thailand based on morphological characters and ISSR analysis Afr. J. Biotecnol. 9 4452-64
[5] Agarwal M, Shrivastava N and Padh H 2008 Advances in molecular marker techniques and their applications in plant sciences Plant Cell Rep. 27 6173
[6] Singh R, Adriaan W, Heusden V and Ram C Y 2013 A comparative genetic diversity analysis in mungbean (Vigna radiata L.) using inter-simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) Afr. J. Biotechnol. 12 6574-82
[7] Kaur G, Joshi1 A and Jain1 D 2018 SSR-marker assisted evaluation of genetic diversity in mungbean (Vigna radiata (L.) Wilcezk) genotypes Braz. Arch. Biol. Technol. 61 e180613
[8] Maiti S, Basak J, Kundagrami S, Kundu A and Pal A 2010 Molecular marker-assisted genotyping of mungbean yellow mosaic india virus resistant germplasms of mungbean and Urdbean Mol. Biotechnol. 47 95-104
[9] Kabi M, Das T R, Baisak B and Swain D 2017 Resistant gene analogous marker assisted selection of yellow mosaic virus resistant genotypes in Greengram (Vigna radiata) Int. J. Curr. Microbiol. App. Sci. 6 3247-52
[10] Poolsawat O, Kativat C, Arsakit K and Tantasawat P A 2017 Identification of quantitative trait loci associated with powdery mildew resistance in mungbean using ISSR and ISSR-RGA markers Mol. Breed. 37 150-61
[11] Chathiranrat N, Nitisit S, Chaiyapan C, Wansuriwong N, Papan P and Tantasawat P A 2018 Selection of mungbean resistant to powdery mildew in BC1F1 progenies based on ISSR and ISSR-RGA markers Proc. of Advances in Science Engineering and Technology (Macau, Chaina) pp 73-7
[12] Ambawat S, Kumar R, Singh S and Yadav R 2017 An easy, quick and cost effective method of
high quality DNA extraction from mungbean [Vigna radiata (L.) Wilczek] without liquid nitrogen Int. J. Curr. Microbiol. Appl. Sci. 6 2695-703

[13] Saghai-Marooof M A, Soliman K M, Jorgensen R A and Allard R W 1984 Ribosomal DNA-spacer-length polymorphism in barley: mendelian inheritance, chromosomal location and population dynamics Proc. Natl. Acad. Sci. 81 8014-9

[14] Doyle J J and Doyle J L 1990 Isolation of plant DNA from fresh tissue Focus 12 13-5

[15] Scott K D and Playford J 1996 DNA extraction technique for PCR in rain forest plant species Bio. Techniques 20 974-9

[16] Haymes K M 1996 Mini-prep method suitable for a plant breeding program Plant Mol. Biol. Rep. 14 280-4

[17] Piriritilä M A, Hirsikorpi M, Kämäräinen T, Jaakola L and Hohtola A 2001 DNA isolation methods for medicinal and aromatic plants Plant Mol. Biol. Rep. 19 273

[18] Drábková L, Kirschner J and Vlcek C 2002 Comparison of seven DNA extraction and amplification protocols in historical herbarium specimens of Juncaceae Plant Mol. Biol. Rep. 20 161-75

[19] Shepherd M, Cross M, Stokoe R L and Scott L J 2002 High throughput DNA extraction from forest trees Plant Mol. Biol. Rep. 20 425

[20] Mogg J and Bond J M 2003 A cheap, reliable and rapid method of extracting high quality DNA from plants Mol. Ecol. Notes. 3 666-8

[21] Chakraborti D, Sarkar A, Gupta S and Das S 2006 Small and large scale genomic DNA isolation protocol for chickpea (Cicer arietinum L.), suitable for molecular marker and transgenic analyses Afr. J. Biotecnol. 5 585-9

[22] Krasova-Wade T and Neyra M 2007 Optimization of DNA isolation from legume nodules Lett. Appl. Microbiol. 45 95-9

[23] Agbagwa I O, Datta S, Patil P G, Singh P and Nadarajan N 2012 A protocol for high-quality genomic DNA extraction from legumes Genet. Mol. Res. 4 4632-9

[24] Dharajiya D T, Khadia S M, Pagi N K, Khatrani T J, Jasani S V, Khunt A D and Ravindrababu Y 2017 Modified method of high quality genomic DNA extraction from mungbean [Vigna radiata (L.) Wilczek] suitable for PCR based amplification. Indian J. Sci. Technol. 10 1-7

[25] Lodhi M A, Ye G N, Weeden N F and Reisch B I 1994 A simple and efficient method for DNA extraction from grapevine cultivars and Vitis species Plant Mol. Biol. Rep. 12 6-13

[26] Inglis P W, Pappas Md C R, Resende L V and Grattapaglia D 2018 Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications PLoS One 13 e0206085

[27] Sambrook J and Russell D W 2001 Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press)

[28] Arskit K, Papan P, Tharapreukasapong A and Tantasawat P A 2017 Simple sequence repeat markers associated with cercospora leaf spot and powdery mildew resistance in mungbean (Vigna radiata L. Wilczek) Proc. Forum-Agriculture, Biology and Life Science (Kyoto, Japan) pp 27-9

[29] Poolsawat O, Kativat C, Arskit K and Tantasawat P A 2017 Identification of quantitative trait loci associated with powdery mildew resistance in mungbean using ISSR and ISSR-RGA markers Mol. Breed. 37 150-61

[30] Mace E S, Buharivalla H K and Crouch J H 2003 A high-throughput DNA extraction protocol for tropical molecular breeding programs Plant Mol. Biol. Rep. 21 459a-h

[31] Gallagher S 2001 Quantitation of nucleic acids with absorption spectroscopy Curr. Protoc. Protein Sci. 13 A.4K.1-3

[32] Promega Corporation 2019 How do I determine the concentration, yield and purity of a DNA sample [on-line] Available: https://worldwide.promega.com/resources/pubhub/enotes/how-do-i-determine-the-concentration-yield-and-purity-of-a-dna-sample/
[33] Jadhav K P, Ranjani R V and Senthil N 2015 Chemistry of plant genomic DNA extraction protocol BioInfo. 12(3A) 543-8

[34] Murray M G and Thompson W F 1980 Rapid isolation of high molecular weight plant DNA Nucleic Acids Res. 8 4321-5

[35] Shioda M and Murakami-Murofushi K 1987 Selective inhibition of DNA polymerase alpha by a polysaccharide purified from slime of Physarum polycephalum Biochem. Bioph. Res. Co. 146 61-6

[36] Richards E 1988 Preparation of genomic DNA from plant tissue Current Protocols in Molecular Biology ed F M Ausubel et al (New York: Greene Publishing Associates and Wiley-Interscience)