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A valid measure to eliminate the influence of polysaccharides and polyphenols in recalcitrant longan (*Dimocarpus longan* L.) during DNA isolation

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Large amounts of polysaccharides, polyphenols, tannins, proteins, and other secondary metabolites in recalcitrant longan leaves make it difficult to obtain high quality genomic DNA during extraction. To obtain good quality of nucleic acids from local longan leaves and for its downstream applications, a new protocol was developed. It consists of rapid isolation of stable nuclei, which hinders covalent interactions with phenolics, followed by DNA extraction. The yield and quality of the resulting DNA were satisfactory and suitable for PCR analysis and digestion with a restriction enzyme. Here, a valid combination measure (β-mercaptoethanol, PVP40 and PVPP were used at different stages) was created to eliminate the influence of polysaccharides and polyphenols in recalcitrant longan during DNA extraction, which will facilitate the development of molecular quantitative genetics of longan.

Key words: Longan, extraction buffer, DNA isolation, PCR products.

INTRODUCTION

Longan (*Dimocarpus longan* L.) is an evergreen tree of the Sapindaceae family, which is widely cultivated in Southern China, India, and Southeast Asia (Jiang et al., 2002). Longan fruit is one of the most favored tropical fruits in China (Zheng et al., 2012). In traditional medicine, the flesh of the fruit is administered as a stomachic, febrifuge (antipyretic) or vermifuge (anthelmintic), and is regarded as an antidote for poison. A decoction of the dried flesh is taken as a tonic or as treatment for insomnia or neutrasthenic neurosis (Sudjaroen et al., 2012).

However, leaves of recalcitrant longan have high polysaccharides, polyphenols, tannins, proteins, and other secondary metabolites, which contribute to the difficulty in DNA extraction and present a major problem during the purification of longan DNA. When cells are lysed, nucleic acids come into contact with these polysaccrides (Sun et al., 2010). In the oxidised form,

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these polyphenols bind covalently and irreversibly to proteins and nucleic acids (Maréchal-Drouard and Guillemaut, 1995), resulting in a brown gelatinous material that reduces the yield and quality of the extracted DNA (Porebskim et al., 1997) and prevents it (DNA pellet) from redissolving completely. Furthermore, DNA that is able to dissolve in the presence of these polysaccharides is shown to inhibit PCR amplification and the activity of several restriction enzymes (Sahu et al., 2012).

The availability of high quality intact genomic DNA is a precondition for almost every molecular genetic analysis. Many plant tissues, especially in recalcitrant longan, are rich in polysaccharide contaminants, making isolation of good quality DNA for PCR, gene mapping, diversity assessments, and other molecular analyses a challenge. The isolation of DNA from tissues with high levels of polysaccharides and polyphenols had been reported using traditional methods (Dabo et al., 1993; Lodhi et al., 1994; Permingeat and Romagnoli, 1998; Leftort and Douglas, 1999; Chaudhry et al., 1999; Khanuja et al., 1999; Zhang and Stewart, 2000; Michiels et al., 2003; Puchooa, 2004; Zidani et al., 2005; Cota-Sanchez et al., 2006; Kotchoni and Gachomo, 2009; Azmat et al., 2012)，however, these methods did not give satisfactory results with longan, because they were not effective in eliminating the effects of polysaccharides and polyphenols in the process of DNA isolation.

PVPP was mainly applied in the beverage industry, where polyphenol adsorption leads to the stabilization of beer, wine, and fruit juices (Sarioglu 2007; Leiper et al., 2005). A research of comparison of polyvinylpoly-pyrrolidone (PVPP), silica xerogel and a polyvinyl-pyrrolidone (PVP)–silica co-product for their ability to remove polyphenols from beer was carried out (Mitchell et al., 2005), demonstrating that the PVPP had the greater binding capability compared to the PVP-silica co-product. Many researches on DNA isolation from environmental samples were conducted, however, high quality DNA could not be achieved using previous methods because these samples often contains enzyme inhibitors disruptive to downstream molecular applications; to eliminate these inhibitors from sediment samples or cells collected from freshwater ecosystems, PVPP was used to eliminate the influence of polysaccharides and polyphenols during DNA extraction and the yield and quality of the resulting DNA were satisfactory and suitable for PCR analysis, along with cloning and gene sequencing (Berthelet et al., 1996; Arbeli and Fuentes, 2007; Yilmaz and Phlips, 2009). These results indicate that PVPP was a valid reagent to eliminate the influence of polysaccharides and polyphenols tannins, proteins, and other secondary metabolites during DNA isolation. However, PVPP was not used to remove polysaccharides and polyphenols in previous methods of recalcitrant fruits DNA extraction.

Due to problems with DNA isolation, only a few studies at the molecular level in longan were carried out. Thus, an appropriate protocol for DNA isolation of longan is necessary to be developed. After following published protocols and failing to obtain high quality DNA free of polyphenolic compounds, we developed an improved genomic DNA extraction protocol for longan.

In the present study, we report a valid measure to eliminate the influence of polysaccharides and polyphenols in recalcitrant longan (*Dimocarpus longan* L.) during DNA isolation and successfully collected good quality DNA from longan validated for PCR applications. The protocol developed in the present study will facilitate the development of molecular quantitative genetics of longan.

MATERIALS AND METHODS

Reagents for extraction of genomic DNA

The protocol for extraction of genomic DNA used in our experiment was modified from Paterson Paterson et al. (1993). Extraction buffer (adjusted pH to 8.0 after sterilization) included 2.0% CTAB (w/v), 2.8% PVP-40 (polyvinylpyrrolidone, molar weight 40 000) (w/v), 1.5 M NaCl, 20 mM thylenediaminetetraacetic acid (EDTA), 100 mM Tris-HCl (pH 8.0), β-mercaptoethanol 5% (added just before use); chloroform-isoamyl alcohol 24:1 (v/v); ethanol (75%, 100%); CTAB/NaCl (including 0.14 M CTAB, 0.5 M NaCl); 3 M sodium acetate; RNase A 10 mg/mL; TE buffer [including 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)]; PVPP (polyvingypyrrolidone) (solid powder).

Plant materials

Young leaves from local commercial cultivars of longan (Table 1) were collected from the National Field Genebank for Tropical Fruit (Zhanjiang), meanwhile, banana leaves contained a moderate amount of polysaccharides and polyphenols were used as control. Leaf samples were stored at −80°C until analysis.

Extraction protocol for genomic DNA

The following protocol was employed to extract good quality genomic DNA from young leaves of longan: (1) The extraction buffer was preheated at 65°C. (2) 2-3 g of leaf sample and 0.25 g of PVPP (polyvingypyrrolidone) (solid powder) were placed together in a pre-cooled pestle and mortar and ground to a fine powder using liquid nitrogen; (3) The powdered leaf sample was transfer to a 15 ml centrifuge tube and 5 ml of hot extraction buffer added to the tube before the frozen powder starts to thaw. (4) The tube was several times inverted to mix the ingredients thoroughly and incubated at 65°C for 60 min. (5) An equal volume of chloroform-isoamyl alcohol (24:1, v/v) was added and mix gently by inverting the tube to form an emulsion. (6) It was spined for 7 min at 10,000 rpm at room temperature. (7) The supernatant (top aqueous phase) was carefully transfered to a new centrifuge tube, and 1/10 volume of CTAB/NaCl added to equal volume of chloroform-isoamyl alcohol (24:1, v/v), and mix gently by inverting the tube to form an emulsion. (8) It was spun for 10 min at 10,000 rpm at room temperature. (9) The supernatant (top aqueous phase) was carefully transferred to a new centrifuge tube, 5 ml of chilled isopropanol added, and mix gently by inverting the tube. Tubes were placed at −20°C for 30 min

Table 1. DNA yield and purity from mature or young leaves of ten longan cultivars

S/N	Cultivar name	Concentration(ng/μl)	A260/A280
1	Youxuanshixia	158	1.80
2	Benzhan	162	1.84
3	Guixiang	173	1.92
4	Chailuo	168	1.83
5	Liqiuben	180	1.97
6	Nanhujiaohe	172	1.96
7	Xipuben	185	1.81
8	Cizaozaobai	175	1.95
9	Xuezhuangben	186	1.84
10	Caohuiben	190	1.86

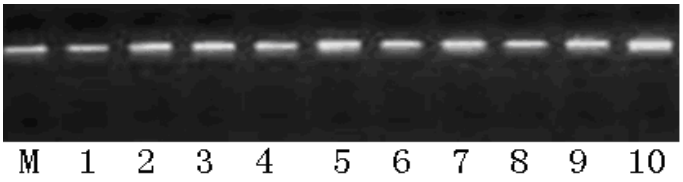

Figure 1. Agarose gel electrophoresis of DNA extracted from 10 cultivars of longan. M, λ DNA; concentration: 150 ng/ul; 1, Youxuanshixia; 2, Benzhan; 3, Guixiang; 4, Chailuo; 5, Liqiuben; 6, Nanhujiaohe; 7, Xipuben; 8, Cizaozaobai; 9, Xuezhuangben; 10, Caohuiben.

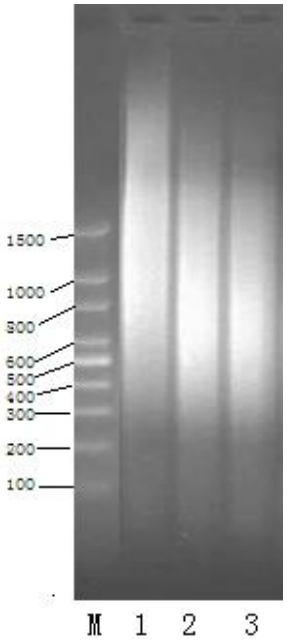

Figure 2. Genomic DNA of longan digested with EcoR I restriction enzyme (M, 100 bp DNA ladder marker).

(the precipitated DNA will be visible at this step). (10) It was spun at 10,000 rpm for 5 min and discarded the supernatant. (11) Wash the pellets 2-3 times with 75% ethanol. (12) The tube was inverted on a paper towel and the pellets air-dried. (13) The DNA in 500 μl TE buffer was resuspended, and treated with 25 μl RNase A, and incubated at 37°C for 30 min. (14) 500 μl of chloroform-isoamyl alcohol was added and mixed gently. (15) It was spun for 10 min at 10,000 rpm and the supernatant transferred into a new 1.5 ml eppendorf tube. (16) 1/10 volume 3 M sodium acetate was added, mix and precipitate the DNA with two volumes of chilled 100% ethanol precipitated. (17) It was spun for 5 min at 8000 rpm, supernatant discarded, and the pellet washed with 75% ethanol. (18) The pellet was air-dried and dissolved in 200 μl TE. (19) The DNA concentration was measured by taking absorbance at 260 nm or by running aliquots on a 1% agarose gel. (20) The DNA concentration and purity of these samples were determined by estimating the ratio of absorbance at 260 nm to that at 280 nm (A260/A280).

The extracted DNA was validated for PCR application as described by Mei et al. (2004). Preparation of polyacrylamide gel and electrophoresis of PCR products were carried out according to Benbouza et al. (2006). Gels were silver stained according to the steps described by Liang et al. (2014).

RESULTS AND DISCUSSION

The quality and concentration of longan DNA prepared with developed procedure was tested for yield and quality using UV spectrophotometer; the A260/A280 of the extracted DNA ranged from 1.8-2.0 (Table 1) and the result indicated that we achieved high quality longan genomic DNA. The use of adjusted β-mercaptoethanol, PVP40 and PVPP specifically helps the release of DNA from polysaccharides and polyphenols. The poly-saccharides settle in the bottom with NaCl while the precipitated DNA remains suspended in the upper isopropanol layer. The quality of the extracted DNA was observed by 1% agarose gel electrophoresis (Figure 1).

The purity, quality and intactness of the extracted DNA were also evaluated by digestion with restriction enzymes. Briefly, each digestion in a total volume of 20 μl (4 h at 37°C) with EcoR I (10 unit/μl) according to Barzegari et al. (2010), the result indicated that the quality of the extracted DNA was suitable for digestion with restriction enzymes (Figure 2).

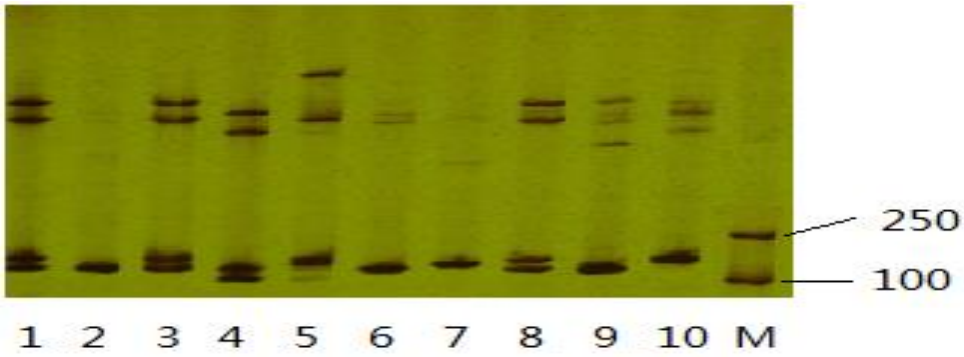

Figure 3. Revelation of the amplification products of SSR primer pair on polyacrylamide gel SSR primer sequences: Forward AAAAGGGGCCAAAATG. Reverse GGCAGAGTTCGGGATTTT. 1, Youxuanshixia; 2, Benzhan; 3, Guixiang; 4, Chailuo; 5, Liqiuben; 6, Nanhujiaohe; 7, Xipuben; 8. Cizaozaobai; 9. Xuezhuangben; 10. Caohuiben; M: DNA marker.

In order to further detect the quality of the extracted longan DNA, SSR primers (Figure 3) were used to amplify genomic DNA of longan; PCR conditions were as described by Benbouza et al. (2006). PCR products were silver stained according to the steps described by Liang et al. (2014). The results (Figures 2 and 3) show that extracted DNA was suitable for PCR analysis.

We presented an efficient and reliable method to extract high-quality genomic DNA from longan. The tissues of longan have large amounts of polysaccharides, polyphenols, tannins, proteins, and other secondary metabolites. Therefore, the most critical issue is to remove interfering substances that often coprecipitate with the extracted DNA, which presents a major contamination problem in the purification of longan genomic DNA. Previous procedures described by Dabo et al. (1993), Lodhi et al. (1994), Permingeat and Romagnoli (1998), Leftort and Douglas (1999), Chaudhry et al. (1999), Khanuja et al. (1999), Zhang and Stewart (2000), Michiels et al. (2003), Puchooa, (2004), Zidani et al. (2005), Cota-Sanchez et al. (2006), Kotchoni and Gachomo (2009) and Azmat et al. (2012) were used to isolate gemomic DNA of longan and banana (which contained a moderate amount of contaminants used as control). Unfortunately, these procedures were inefficient for extracting genomic DNA from mature longan leaves, making it useless for most of molecular manipulations, however, we obtained high quality banana genomic DNA (supplementary Figure 1). These results show that polyphenols and polysaccharide were the main reason for failure of longan DNA extraction, the presence of these metabolites can hamper the DNA isolation procedures and reactions such as DNA restriction, amplification and cloning. In order to eliminate the effects of polysaccharides and polyphenols during genomic DNA extraction, we adjusted the amount of β-mercaptoethanol and PVP40 in the DNA extraction buffer of longan (reagents for extraction of genomic DNA); β-mercapto-ethanol and PVP40 were effective and commonly used reagents for removing polyphenols and polysaccharide. However, the improvement effect of DNA extraction was not obvious. These results show that relying only on the β-mercaptoethanol and PVP40 could not eliminate effectively the influence of the polyphenols and poly-saccharide during longan genomic DNA extraction.

PVPP was first reported and used effectively to remove the brown algal phlorotannins by Toth and Pavia (2001); the results indicated that insoluble polyvinylpolypyrrolidone (PVPP) can be used to specifically remove tannins and phlorotannins from plant and algal phlorotannins. Nevertheless, PVPP was not used to remove polysaccharides and polyphenols in previous methods of DNA extraction. In order to detect the extraction effects of longan DNA using PVPP only, longan and banana leaf sample of 2-3 g respectively and 0.25 g of PVPP together in a pre-cooled pestle and mortar were ground to fine powder using liquid nitrogen; when no β-mercaptoethanol and PVP40 were added into traditional DNA extraction solution, the same result occurred (supplementary Figure 2). Interestingly, combination (β-mercaptoethanol and PVP40 were added into traditional DNA extraction solution, meanwhile, PVPP were used at grinding step stage) was adopted to eliminate the influence of polysaccharides and polyphenols, longan genonmic DNA degradation was avoided and DNA with good spectral qualities was obtained (Figure 1).

Leaves of recalcitrant longan have high polysaccharides, polyphenols, tannins, proteins, and other secondary metabolites, which present a major problem during the purification of longan DNA. Previous methods were tried to isolate genomic DNA of longan leaves but failed to obtain intact DNA due to irreversible binding of phenolic

compounds and coprecipitation of polysaccharides when cells were lysed nucleic acids come into contact with these polysaccrides. Therefore, the main problem of longan genomic DNA extraction was how to eliminate the influence of polysaccharides and polyphenols which degrade genomic DNA at different stages of extraction. In present experiment, a valid combination (β-mercaptoethanol, PVP40 and PVPP were used at different stages) was adopted to eliminate the influence of polysaccharides and polyphenols. From perspective of elimination of polysaccharides and polyphenols, the present method and measure was efficient and reliable.

Present research provided an opportunity to successfully collect good quality DNA for PCR applications in recalcitrant longan. This protocol has the potential to extract DNA from the young and mature leaves of other species high in polysaccharides and polyphenols as well (data not shown). The improved method has been used successfully to extract DNA from mango, litchi and so on in our laboratory, those plants with high in polysaccharides and polyphenols likewise. The protocol developed in the present study will facilitate the development of molecular quantitative genetics of recalcitrant longan.

Author contributions

QL LD, Conceived and designed the experiments. QL DW DG, Performed the experiments, QL DW DG, analyzed the data. DW DG, contributed reagents/materials and QL DW wrote the paper. All the authors discussed the results and commented on the manuscript.

Conflict of interests

The author(s) did not declare any conflict of interest.

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REFERENCES

Arbeli Z, Fuentes CL (2007). Improved purication and PCR amplication of DNA from environmental samples. FEMS Microbiol. Lett. 272: 269-275.

Azmat MA, Khan IA, Cheema H, Rajwana IA, Khan AS (2012). Extraction of DNA suitable for PCR applications from mature leaves of *Mangifera indica* L. Zhejiang Univ-Sci B (Biomed & Biotechnol), 13: 239-243.

Barzegari A, Vahed SZ, Atashpaz S, Khani S, Omidi Y (2010). Rapid and simple methodology for isolation of high quality genomic DNA from coniferous tissues (*Taxus baccata*). Mol. Biol. Rep. 37: 833–837

Benbouza H, Jacquemin JM, Baudoin JP, Mergeai G (2006). Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. Biotechnol. Agron. Soc. 10:77-81.

Berthelet M, Whyte LG, Greer CW (1996). Rapid, direct extraction of DNA from soils for PCR analysis using polyvinylpolypyrrolidone spin columns. FEMS Microbiol. Lett. 138:17-22.

Chaudhry B, Yasmeeen A, Husnain T, Riazuddin S (1999). Mini-scale genomic DNA extraction from cotton. Plant Mol. Biol. Rep. 17:1-7.

Cota-Sanchez J, Remarchuk K, Ubayasena K (2006). Ready-to-Use DNA extracted with a CTAB method adapted for Herbarium specimens and mucilaginous plant tissue. Plant Mol. Biol. Rep. 24: 161-167.

Dabo SM, Mitchell J, Melcher U (1993). A method for the isolation of nuclear DNA from cotton (*Gossypium*) leaves. Anal. biochem. 210:34-38.

Jiang Y, Zhang Z, Joyce DC, Ketsa S (2002). Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). Postharvest Biol. Tec. 26:241-252.

Khanuja S, Shasany AK, Darokar MP, Kumar S (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Mol. Biol. Rep. 17:1-7.

Kotchoni SO, Gachomo EW (2009). A rapid and hazardous reagent free protocol for genomic DNA extraction suitable for genetic studies in plants. Mol. Biol. Rep. 36: 1633-1636.

Leftort F, Douglas GC (1999). An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species acer, fraxinus, prunus and quercus. Ann. For. Sci. 56:259-263.

Leiper KA, Stewart GG, McKeown IP, Nock T (2005). Optimising beer stabilisation by the selective removal of tannoids and sensitive proteins. J. Inst. Brew. 111:118-127.

Liang QZ, Wen DQ, Xie JH, Liu LQ, Wei YZ, Wang YC and Shi SY (2014). A rapid and effective method for silver staining of PCR products separated in polyacrylamide gels. Electrophoresis, 35: 2520-2523.

Lodhi MA, Ye GN, Weeden NF, Reisch B (1994). A simple and efficient method for DNA extraction from grapevine cultivars and vitis species. Plant Mol. Biol. Rep. 12: 6-13.

Maréchal-Drouard L, Guillemaut P (1995). A powerful but simple technique to prepare polysaccharide-free DNA quickly and without phenol extraction. Plant Mol. Biol. Rep. 13: 26-30.

Mei M, Syed N, Gao W, Thaxton PM, Smith CW (2004). Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). Theor. Appl. Genet. 108:280-291.

Michiels A, Ende W, Tucker M, Riet L.V, Laere AV (2003). Extraction of high-quality genomic DNA from latex-containing plants. Anal. biochem. 315:85-89.

Mitchell AE, Hong YJ, May JC, Wright CA, Bamforth CW (2005). A comparison of polyvinylpolypyrrolidone (PVPP), silica xerogel and a polyvinylpyrrolidone (PVP)–silica co-product for their ability to remove polyphenols from beer. J. Inst. Brew. 111(1):20-25.

Paterson HA, Brubaker LC, Wendel FJ (1993). A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. Plant Mol. Biol. Rep. 11:122-127.

Permingeat HR, Romagnoli MV (1998). Vallejos R.H., A simple method for isolating high yield and quality DNA from cotton (*Gossypium hirsutum* L.) leaves. Plant Mol. Biol. Rep. 16: 1-6.

Porebskim S, Bailey LG, Baum BR (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Rep. 15:8-15.

Puchooa D (2004). A simple, rapid and efficient method for the extraction of genomic DNA from lychee (*Litchi chinensis* Sonn.). Afr. J. Biotechnol. 3:253-255.

Sahu SK, Thangaraj M, Kathiresan K (2012). DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. Mol. Biol. 1: 1-6.

Sarioglu K (2007). Synthesis of octadecyl acrylate-co-ethylene glycol dimethacrylate resin for removal of dark-colored compound from apple juice and comparison with polyvinylpolypirrolidone (PVPP). Eur. Food Res. Technol. 225:443-449.

Sudjaroen Y, Hull WE, Erben G, Würtele G, Changbumrung S (2012). Isolation and characterization of ellagitannins as the major polyphenolic components of Longan (*Dimocarpus longan* Lour) seeds. Phytochemistry, 77:226-237.

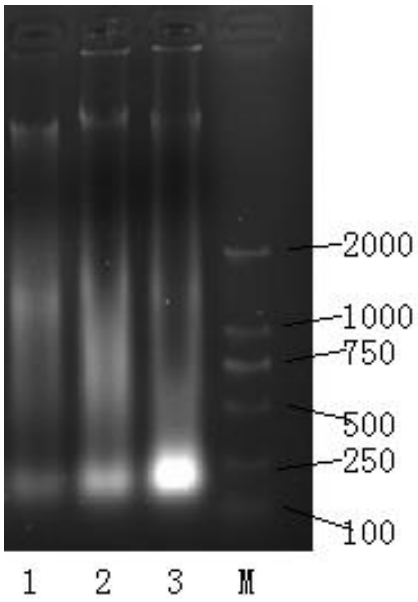
Sun J, Zhang E, Xu L, Li Z, Wang Z (2010). Comparison on characterization of longan (*Dimocarpus longan* Lour.) polyphenoloxidase using endogenous and exogenous substrates. J. Agr. Food. Chem. 58:10195-10201.

Yilmaz M, Phlips EJ (2009). Improved methods for the isolation of cyanobacterial DNA from environmental samples. J. Phycol. 45:517-521.

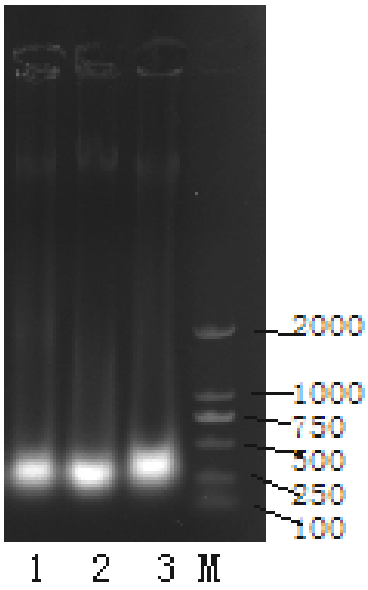
Zhang J, Stewart M (2000). Economical and rapid method for extracting cotton genomic DNA. J. Cotton. Sci. 4: 193-201.

Zheng G, Wei X, Xu L, Li Z, Liu G, Zhang X (2012). A new natural lactone from *Dimocarpus longan* Lour. Seeds. Molecules, 17: 9421-9425.

Zidani S, Ferchichi A, Chaieb M (2005). Genomic DNA extraction method from pearl millet (*Pennisetum glaucum*) leaves. Afr. J. Biotechnol. 4: 862-866.



Supplementary Figure 1. DNA extraction from mature leaves of longan using traditional method.



Supplementary Figure 2. DNA extraction solution including PVPP only; no β-mercaptoethanol and PVP40 were added