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Full Length Research Paper

A simple, rapid and efficient method of isolating DNA from Chokanan mango (*Mangifera indica* L.)

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Total DNA of Chokanan mango (*Mangifera indica* L.) was extracted from the leaf for the construction of total genomic library. However, the quality of the extracted DNA was often compromised by the presence of secondary metabolites, thus interfering with the analytical applications. Improvement on the quality of the extracted DNA was achieved through the optimization of leaf harvesting stage and modification on the cetyl trimethylammonium bromide (CTAB) DNA extraction procedure. Fully expended, soften and purplish leaf was proved to yield good DNA quality while the addition of polyvinylpyrrolidone (PVP) and β-mercaptoethanol was effective in the removal of secondary metabolites, particularly polyphenolic compounds. The incorporation of high salt washing step was also efficient in removing polysaccharides. This simple, inexpensive and yet reliable method was proved to be successful in yielding sufficient quality and quantity of DNA for the construction of genomic library.

Key words: Mango, DNA isolation, genomic library, secondary metabolites, polyphenolic compounds.

INTRODUCTION

Mango (*Mangifera indica* L.) is popularly known as the 'king of fruits' especially in the tropical regions (Singh, 1996). Mango play a major role in the global trade as it constitutes approximately 50% of all tropical fruits produced worldwide, equivalent to 5.5% of all fruit produced globally (Jedele et al., 2003). Lately, there has been an increase in the demand for Chokanan mango, particularly due to its aromatic and sweet characteristics.

Crop improvement facilitated by modern biotechnology has largely been acknowledged as a key strategy for achieving food security and sustainable agriculture (Hautea and Escaler, 2004). The advent of genomic based approaches to crop improvement will then provide an unprecedented opportunity to make significant genetic advances in mango. Thus, researchers are starting to study all the varieties in the germplasm in order to cope with the mango crop improvement program with respect to high productivity as well as countering disorders and diseases (Vasanthaiah et al., 2007). The isolation of a workable germplasm for analytical study is the most crucial and challenging step in the construction of genomic library. This is due to the presence of high level of contaminants, mainly polyphenolic compounds, tannins and polysaccharides (Maria et al., 2001). The presence of these contaminants tends to suppress and inhibit analytical studies on the isolated DNA. Polyphenolic compounds are known to increase the resistance of DNA against restricttion enzymes and interact irreversibly with proteins and nucleic acids (Manoj et al., 2007), while polysaccharides are particularly problematic as they inhibit the activity of many commonly used molecular biological enzymes, such as polymerases, ligases and restriction endonucleases (Sharma et al., 2002).

The aim of this study is to focus on the modification of the DNA extraction procedure by Doyle and Doyle (1990) after previous attempts of using reported methods which proved to be unsuitable for Chokanan mango (Dellaporta et al., 1983; Rogers and Bendich, 1988; Doyle and Doyle, 1990; Deshmukh et al., 2007). In addition, the determination of best leaf growth stage as starting material was also investigated as previous work on grapevine cultivars by Lodhi et al. (1994) revealed the importance of proper choice of leaf tissue for obtaining good quality of DNA. This DNA isolation study is reliable as it is quick, simple, inexpensive and more importantly, it avoid the use of dangerous chemicals.

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Abbreviations: PVP, Polyvinylpyrrolidone; **CTAB,** cetyl trimethylammonium bromide; **EDTA,** ethylenediaminetetra-acetic acid.

Figure 1. Chokanan mango leaf of different growth stages used in the isolation of DNA. (a) Dark purplish, soft and partially expended leaf; (b) light purplish, soft and fully expended leaf; (c) greenish, hardened and fully expended leaf.

MATERIALS AND METHODS

Plant material

Chokanan mango leaves were collected from the Rimba Ilmu garden in University of Malaya, Malaysia. Leaves were harvested at three different growth stages: (i) Dark purplish, soft and partially expended (Figure 1a); (ii) light purplish, soft and fully expended (Figure 1b); greenish, hardened and fully expended (Figure 1c). All harvested leaves were placed in an ice-box and kept away from sunlight before transporting to the laboratory.

Solutions and solvents

The solutions and solvents used for the assay include Chloroform : Isoamyl alcohol (24:1), 5 M NaCl, Isopropanol, 70% ethanol, TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)], RNase A (0.1 µg/µl).

DNA isolation and analysis

Leaves were washed gently with distilled water in order to remove all surface particles. All midribs and secondary veins were removed using an alcohol-sterilized knife. 400 µl of DNA isolation buffer containing [0.15 M Sorbitol, 0.125 M Tris-base, 0.05 M ethylenediaminetetraacetic acid (EDTA), 8.3 x 10^{-3} M sodium bis-sulfide, 0.83 M NaCl, 2% w/v hexadecetyltrimethly-ammonium bromide (CTAB), 0.83% v/v sodium Sarkosyl, 1% w/v polyvinyl-pyrollidone (PVP) (MW 40,000), 1% v/v beta-mercaptoethanol] was added into each 1.5 ml microfuge tube. 0.20 g of leaf sample was weighed and pulverized in liquid nitrogen. It was then transferred into each buffer-added microfuge tube. After a quick-spin, another 300 µl of DNA isolation buffer was added. All tubes were gently flicked and inverted thoroughly to mix the sample, and were incubated at 65°C for 40 min. 700 µl of chloroform: isoamyl alcohol (24:1) was added to each microfuge tube and gently inverted until emulsion was formed. Samples were centrifuged at 10,000 rpm for 15 min and the aqueous phase was gently transferred into a new 1.5 ml microfuge tube. The chloroform: isoamyl alcohol procedure was repeated. Half volume of 5M NaCl was added followed by two volume of cold isopropanol (-20°C) and contents were gently inverted before incubating on ice for 10 min. Samples were centrifuged at 10,000 rpm for 5 min. Supernatant was discarded. DNA pellets were washed twice with 500 µl of cold 70% ethanol (-20°C). The resulting DNA pellets were air-dried at room temperature and dissolved in 30 µl of TE buffer. RNase A was added to each sample (1/10 µl DNA sample) and was incubated at 37°C for 1 h. Samples were stored in -20°C.

The coloration of the extracted DNA pellets from each growth stage of leaf was checked visually. Extracted DNA samples were quantified by using a spectrophotometer at A_{260}, while the purity of the DNA was checked through A_{260}/A_{280}. DNA samples were digested by using *Eco*R1 enzyme and were analyzed through 0.5% agarose gel electrophoresis (Figure 3).

RESULTS AND DISCUSSION

DNA of *Mangifera* leaf was previously extracted by Yamanaka et al. (2006) using the Doyle and Doyle (1990) method. However, DNA purification kit (Mag-Extractor Genome Kit) was needed to obtain a pure and workable DNA, thus making it as cost ineffective. On the other hand, DNA extraction method on mango by Kashkush et al. (2001) and Ukoskit, (2007) was not suitable for this study either, especially in yielding polyphenolic-free DNA sample.

Proper choice of leaf tissue is crucial in obtaining good quality of extracted DNA. Mauro et al. (1992) has identified that rapidly expanding leaves, one or two nodes from the shoot tip is the best starting material. This was supported by Couch and Fritz (1990) and Lodhi et al. (1994), in which the best results were obtained from partially expanded *Theobroma cacao* and grapevine leaf, respectively. Lodhi et al. (1994) also managed to get equally good results with fully expanded leaves when PVP was added to the extraction

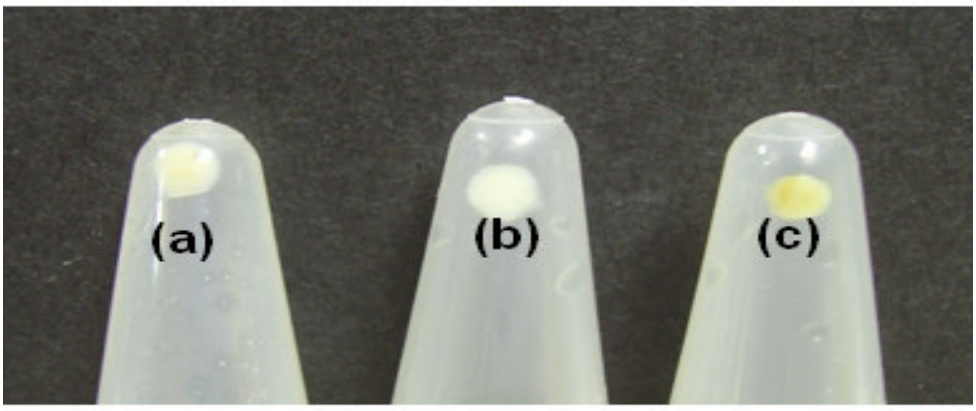

Figure 2. The coloration of the DNA pellet extracted from (a) Dark purplish, soft and partially expended leaf; (b) light purplish, soft and fully expended leaf; (c) greenish, hardened and fully expended leaf.

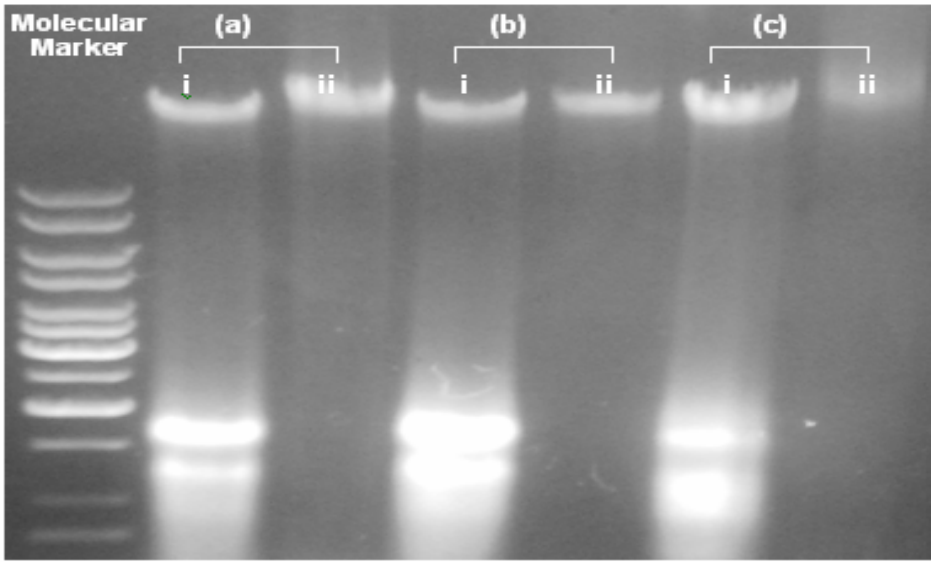

Figure 3. Image of agarose gel electrophoresis of DNA extracted from (a) Dark purplish, soft and partially expended leaf; (b) light purplish, soft and fully expended leaf; (c) greenish, hardened and fully expended leaf by using (i) Doyle and Doyle (1990) method and the (ii) modified method.

buffer, although Puchooa (2004) reported that younger leaf tend to constitute less plant metabolites (contaminants). Mature leaves are usually undesirable as they are thicker and tougher, making the initial grinding step more difficult, apart from the formation of a thick white band in the cesium chloride density gradient which complicates the removal of DNA band (Couch and Fritz, 1990). Howland et al. (1991) also reported that the mature leaves tend to contain higher amount of polyphenolic compounds.

In this study, light purplish, soft and fully expended leaf (Figure 1b) was determined as the most suitable starting material for DNA isolation as whitish DNA pellet was consistently obtained through the modified method (Figure 2b). On the other hand, DNA extracted from the partially expended dark purplish (Figure 1a) and the fully expended greenish leaf (Figure 1c) showed undesirable characteristic, as they exhibited yellowish and brownish colouration, respectively. This was further supported by the fact that whitish DNA pellet tend to dissolved more rapidly in TE buffer as compared to the yellowish and the brownish pellet.

Yellow and brownish coloured DNA pellets obtained were likely to be due to the contamination of polyphenolic compounds and polysaccharides. Couch and Fritz (1990) reported that polyphenols become oxidized and irreversibly react with the protein and nucleic acid fractions when cells are homogenized and disrupted. These compounds have also been reported to cause difficulty in DNA purification in other plant species, such as polysaccharides (Murray and Thompson, 1980; Fang et al., 1992; Lodhi et al., 1994; Manoj et al., 2007; Deshmukh et al., 2007); polyphenolic compounds (Katterman and Shattuck, 1983; Couch and Fritz, 1990;

Howland et al., 1991; Collins and Symons, 1992; Lodhi et al., 1994; Puchooa, 2004; Manoj et al., 2007; Deshmukh et al., 2007) as well as sticky and resinous materials (Webb and Knapp, 1990). The presence of these compounds often increases the viscosity of the extracted DNA samples, thus inhibiting its downstream analysis.

Thus, PVP and beta-mercaptoethanol were added to the DNA isolation buffer as they were previously reported to be effective in removing polyphenols from mature, damage and improperly stored leaf tissues (Doyle and Doyle, 1987; Howland et al., 1991; Dawson and Magee, 1995; Clark, 1997; Zidani et al., 2005). According to Maliyakal (1992), PVP forms complex hydrogen bonds with poly-phenolic compounds which can be separated from DNA by centrifugation. Furthermore, PVP tend to reduce the oxidation of polyphenols (Howland et al., 1991). PVP 40 (MW 40,000) was preferred to PVP 360 (MW 360,000) as the latter tend to precipitate with the nucleic acid, thereby being present as a contaminant (Puchooa, 2004).

On the other hand, polysaccharide-like contaminants are more difficult to remove (Zidani et al., 2005). They are known to inhibit the activity of certain DNA-modifying enzymes as well as interfering with the quantification of DNA by spectrophotometric methods (Wilkie et al., 1993; Deshmukh et al., 2007). In this study, DNA in aqueous solution was washed with 5 M NaCl prior to precipitation to effectively remove polysaccharides as reported by Fang et al. (1992).

Midrib and the secondary veins were removed prior to DNA extraction in order to mediate the leaf pulverization process. The presence of these two components tends to slow down the grinding process as they are not easily grounded.

Clean and white-colored DNA pellets were reproducible through the modified DNA extraction protocol. On the other hand, check on the purity of DNA samples by A_{260}/A_{280} also gave satisfactory results as the ratio was consistently between the ranges of 1.75 to 2.10. DNA samples were also successfully digested by *Eco*R1 restriction enzyme. This suggests that DNA derived from the modified method is more intact, cleaner and purer. This is utmost important as a good extraction procedure for the isolation of DNA should yield adequate and intact DNA of reasonable purity, besides being inexpensive, quick and simple (Zidani et al., 2005).

Conclusion

The selection of proper growth stage of leaf and the modification on the DNA isolation procedure was critical in yielding good quality of Chokanan mango DNA.

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