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Full Length Research Paper

The MAGi DNA extraction method for fresh tissues and dry seeds

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The isolation of un-degraded, high-molecular-mass genomic DNA is essential for many DNA based molecular biology applications including long polymerase chain reactions (PCR), multiplex PCR, endonuclease digestion, Southern blot analysis and genomic DNA library construction studies. Although many protocols are available for the extraction of DNA from plant species, most of them require longer times and the size of extracted DNA is usually below 20 kb. Most of commercially available kits are expensive, unavailable in many part of the world and also require at least 1.5 h including the RNase digestion. In the present study, we report a DNA extraction method using the MAGi reagent. The MAGi DNA extraction method extracts genomic DNAs with excellent spectral readings, efficiently digestible with endonucleases and suitable for enzymatic and multiplex PCR amplification studies. The MAGi DNA extraction method is suitable for multiplex PCR, endonuclease digestion studies and greatly reduces the time required to isolate high intact genomic DNAs from fresh leaf, fruit and fresh and dry seed at different development stages. Using the MAGi extraction protocol, highly pure DNA higher than 50 kb DNA in size can be obtained in approximately 1 h.

Key words: High quality DNA, MAGi solution, marker assisted selection (MAS), multiplex polymerase chain reaction (PCR).

INTRODUCTION

Quality and quantity of genomic DNAs are the one of the most critical factors for many molecular biology studies and molecular marker assisted selection (MAS) studies. Many plant species contain high levels of tannins and other polyphenolic compounds which can come into contact with nucleic acids within nucleus and other organelles during the DNA extraction. Oxidized forms of these compounds, may covalently bind to DNA, giving it a brown color and making it useless for most molecular research applications (Peterson et al., 1997). Although many DNA extraction methods work well for many plant species, the size of extracted DNA, time to complete the extraction of DNA per sample, the number of samples extracted per day per person and the cost of extraction vary among the extraction methods depending on the plant species, tissues and development stages (Peterson et al., 1997; Karaca et al., 2005; Tan and Yiap, 2009).

Fine-resolution genetic mapping of the molecular markers for map-based cloning of genes and marker-assisted selection of economically important traits require DNA isolation from a large number of plants in a short time. For this purpose many commercial all-in-one kits are available today for extraction of many DNA samples in a short time period. However, the cost of analysis per

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Abbreviations: BME, β-Mercapto ethanol; **CAPS,** cleavage amplified polymorphic sequence; **CTAB,** cetyltrimethylammonium bromide; **DAMD-PCR,** directed amplification of minisatellite DNA region polymerase chain reaction; **EtBr-CsCl,** ethidium bromide-cesium chloride; **GTPC,** guanidine thiocyanate-phenol-chloroform; **MAS,** marker assisted selection; **mRNA,** messenger RNA; **PCR,** polymerase chain reactions; **RAPD-PCR,** random amplified polymorphic DNA polymerase chain reaction; **SCAR,** sequence characterized amplified region; **TBE,** TRIS–Borate EDTA; **Td-PCRs,** touch-down polymerase chain reactions.

sample for kits is usually high, the amount of extracted DNA is low and the size of extracted DNAs is usually below to 20 kb (Tan and Yiap, 2009; Turci et al., 2011; Yang et al., 2011).

The most commonly used DNA extraction methods after the very first isolation by Friedrich Miescher in 1869 followed the initial DNA extraction developed from density gradient centrifugation strategies by Meselson and Stahl (1958) are based on ethidium bromide-cesium chloride (EtBr-CsCl) gradient centrifugation, cetyltrimethylammonium bromide (CTAB), guanidine thiocyanate-phenol-chloroform (GTPC) and solid-phase methods, which are based on the use of silica matrices, glass particle and diatomaceous earth (Tan and Yiap, 2009). These methods generally consist of the following main steps; homogenization for the disruption of cells or tissue, denaturation of cell membranes and nucleoprotein complexes to break down the cells and the specified precipitation of DNA (Karaca et al., 2005). However, some of these DNA extraction protocols have several potential drawbacks including risks of cross contaminations due to many steps, lower molecular mass of extracted DNA due to degradation and unsuitable for high-throughput applications due to longer extraction time required (Karaca et al., 2005; Tan and Yiap, 2009; Turci et al., 2011; Yang et al., 2011).

This study reports a new DNA extraction method using the MAGi solution (Ince and Karaca, 2009) for extraction of intact genomic DNA for fresh and dry samples of plant species. This method was called the MAGi DNA extraction method, which is a non CTAB, GTPC, or solid-phase based method, does not require cell degradation enzymes such as proteinase K and use less amount of solvent such as phenol and chloroform. The MAGi extraction completes in approximately 1 h, purifies DNA with excellent spectral readings and higher molecular mass, efficiently digestible with endo-nucleases and suitable for amplification reactions including the multiplex polymerase chain reactions and MAS studies (Ince et al., 2009; Ince et al., 2010a, b, c).

MATERIALS AND METHODS

Plant materials

Plant species tested for DNA extraction studies consisted of *Solanum lycopersicum* L. samples collected from different lines, varieties and two species, *Capsicum annuum* L. samples obtained from different tissues, development stages and organs, *Pisum sativum* L. leaf samples collected from two varieties grown in a greenhouse. Mature seeds of the above species and seeds from *Phaseolus vulgaris* L., *Glycine max* (L.) Merr., *Gossypium hirsutum* L. were also used. Samples of plant materials were collected from each individual plant, brought to a laboratory and stored at -20°C. One g leaf tissues were transferred to a mortar and grinded to a powder in the presence of liquid nitrogen using a pestle. Powdered materials were transferred into 15 ml sterile tubes, and stored at -20°C till the DNA extraction studies. Mature seeds (250 mg) of plants were also grinded to a powder in the presence of liquid nitrogen.

The MAGi reagent

The MAGi solution described in Ince and Karaca (2009) was prepared. Our initial studies revealed that the MAGi solution was stable at room temperature for at least 3 or longer years. This solution has white color and is easily transferable using pipettes or liquid dispensers to sample tubes.

Extraction protocol

This protocol presented in this study describes a midi scale prep of the MAGi DNA extraction method. The solutions and buffers for mini scale preparation can be adjusted at 1/10 volumes of solutions and buffers given in Table 1 for mini scale preparations. Step by step the MAGi DNA extraction protocol is given in Table 1.

Quantification of the extracted DNA using a spectrophotometer

Extracted DNA samples were quantified using a spectrophotometer (Spectro UV–VIS Double Beam, Model UVD-2960; Labomed, Culver City, CA, USA). Spectrophotometric analyses included full scans from A_{1100} to A_{190} nm and individual absorbance readings at A_{230}, A_{260} and A_{280} nm (Ince and Karaca, 2009).

Agarose gel electrophoresis

TRIS–Borate EDTA (TBE) electrophoresis of the extracted DNAs was performed in 1% agarose gels containing 0.5 µg/ml ethidium bromide and 1× TBE buffer (89 mM TRIS–Borate, 2 mM EDTA, pH 8.0), which was also used as a running buffer during the electrophoresis. All the chemicals used are from Amresco Inc., Solon, OH, USA. Electrophoresis was performed at a voltage of 5 V per 1 cm for 90 min.

Restriction enzyme digestions

Extracted DNAs from each sample (2 µg) were incubated in 25 µl reaction volume with 20 units of each *Bam* HI*, Eco* RV, *Hinf* I, *Mse* I, *Alu* I and combination of *Eco* RV and *Bam* HI (Boehringer Mannheim, Mannheim, Germany) in the recommended buffers at 37°C for 3 h. After incubation, DNA digestion was assayed by visual inspection after agarose gel electrophoresis according the procedures described in Ince et al. (2010a).

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), directed amplification of minisatellite DNA region polymerase chain reaction (DAMD-PCR), Multiplex-sequence characterized amplified region (SCAR), sequence characterized amplified region (SCAR) and cleavage amplified polymorphic sequence (CAPS)

Randomly selected plant DNAs were used to investigate their application in random amplified polymorphic DNA polymerase chain

Table 1. A step-by-step the MAGi DNA extraction procedure reported in this study.

Step	Procedure
i	Transfer 1 g fresh or 250 mg dry tissues into a 15 ml tube, add 3.9 ml the MAGi solution and 0.1 ml 14.4 M β-mercaptoethanol (BME, Amresco), vortex 45 s, add 0.5 ml 2 M sodium acetate (pH 7.0) and 1 ml phenol: chloroform: isoamyl alcohol (Amresco, 25:24:1 v/v) vortex as before, add 5 ml chloroform, vortex as before.
ii	Centrifuge at 12000 ×*g* at 5°C for 12 min. Transfer the supernatant to a new tube and add 5 M NaCl in 1/10 volume of supernatant, mix well, precipitate DNA by adding an equal volume of isopropanol and centrifuge at 12000×*g* at 5°C for 12 min.
iii	Discard the supernatant and dissolve the pellet in 0.49 ml TRIS-EDTA buffer (TE, 10 mM TRIS and 01 mM EDTA, pH 7.5), add 10 µl RNase (10 mg/ml) incubate the samples at 37°C for 30 min.
iv	Add 0.05 ml of 2 M potassium acetate (pH 5.5). Precipitate DNA by adding three volumes of 100% ethanol and mix by shaking, and centrifuge the tube at 10000×g at 5°C for 5 min.
v	Discard the supernatant and dissolve the DNA pellet in 0.4 ml TE (pH 8.0), precipitate DNA by adding three volumes of 100% ethanol, centrifuge the tube at 10000×g at 5°C for 5 min. Invert the tube on a sterile Kim wipe. Evaporate the remains of the ethanol using a sterile hairdryer and dissolve the purified DNA in appropriate amount of TE (pH 7.5 or 8.0) buffer.

[a] These steps must be conducted in a fume hood.

Table 2. Primer pairs used in multiplex PCR, SCAR and CAPS analyses.

Primer ID	Sequence (5'=> 3')	Marker Type
OIF	TCCGTGCTGAATGAAGATTCAAAC	Multiplex PCR SCAR
OIR	TCCGTGCTGATAAAACTGTTAGAC	
FuF	GTCCCCACCTACTTACGA	
FuR	CCGCAATCAGGTGTCCA	
C8BF	TACCCACGCCCCATCAATG	
C8BR	TGCAAGAGGGTGAATATTGAGTGC	
TMVF2	GGTGACTGTGTAATTATGTTGTGC	
TMVR2	GGTGACTGTGGTGGAAAATGCAAA	
I2F	CAAGGAACTGCGTCTGTCTG	SCAR
I2R	ATGAGCAATTTGTGGCCAGT	
RexF	TCGGAGCCTTGGTCTGAATT	CAPS
RexR	GCCAGAGATGATTCGTGAGA	

reaction (RAPD-PCR) and directed amplification of minisatellite DNA region polymerase chain reaction (DAMD-PCR) techniques according procedures described in Ince et al. (2010b) and Ince et al. (2010c), respectively. A total of 5 RAPD and 5 DAMD-PCR primers were used in these studies. For sequence characterized amplified region (SCAR) and multiplex SCAR, touch-down polymerase chain reactions (Td-PCRs) were carried out in 25 µl reaction volume containing 80 nanograms genomic DNA as template, 0.5 µM of a primer pair for SCAR or four primer pairs for multiplex SCAR listed in Table 2, 80 mM TRIS–HCl (pH 8.8), 19 mM $(NH_4)_2SO_4$, 0.009% Tween-20 (w/v), 0.28 mM of each dNTP, 2 or 3 mM $MgCl_2$, and 2 units of *Taq* DNA polymerase (MBI Fermentas, Amherst, NY, USA).

Td-PCR amplification profile used in the present study was as follows: initial denaturation at 94°C for 3 min, 10 cycles with denaturation at 94°C for 30 s, annealing at 60°C for 30 s in the first cycle, diminishing by 0.5°C each cycle, and extension at 72°C for 1 min in a 96-well GeneAmp PCR System 9700 (Carlsbad, California, USA). An additional 30 PCR cycles were run using the same cycling parameters with constant annealing at 55°C. Denaturation and extension conditions were the same as indicated previously. The amplifications finished with final extension at 72°C for 7 min. Amplicons were separated using 2 to 3% high resolution agarose gel (Serva, Heidelberg, Germany) electrophoresis according the procedures described in Ince et al. (2010c).

For cleavage amplified polymorphic sequence (CAPS) study 10 µl amplified product obtained using the foregoing PCR conditions was digested with 10 units Taq I restriction enzyme (MBI

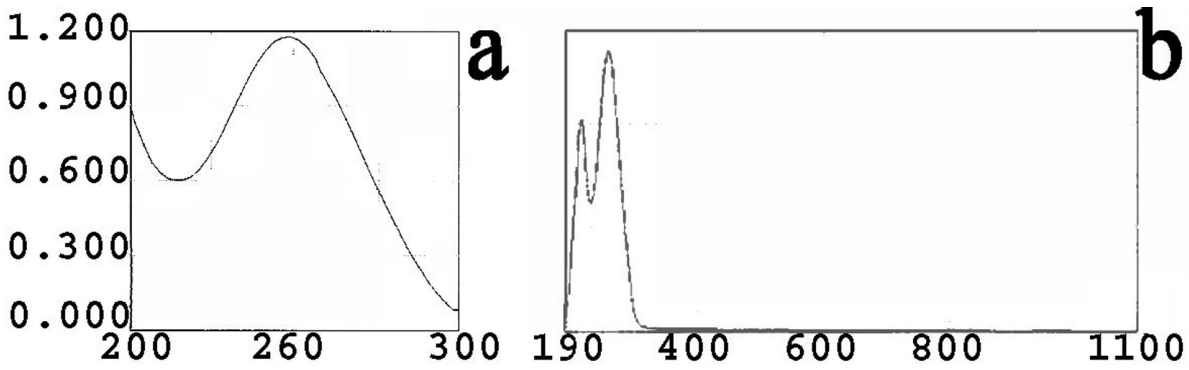

Figure 1. Spectrophotometric measurements of the MAGi DNA extraction method for tomato leaf sample. Panel a shows spectrophotometric measurement between A_{200} and A_{300} while panel b shows full scan measurements. The x-axis represents spectral measurements at various wavelengths between A_{190} and A_{1100} nm, and the y-axis represents intensity measurements, in arbitrary units.

Fermentas, Amherst, NY, USA) according procedure described in Ince et al. (2010a).

RESULTS

A good extraction procedure for the isolation of DNA should yield adequate, intact and pure DNAs in a short time period with low cost and should also be suitable for a large number of plant species (Doyle and Doyle, 1990; Ince et al., 2009; Turci et al., 2011). The MAGi DNA extraction method completes in approximately 1 h and could be used in small scale (mini) using 100 mg fresh issue or in midi scale using 1 g tissue. This method is also suitable for genomic DNA extraction from various fresh tissues, mature and immature seeds. In comparison to other methods (Doyle and Doyle, 1990; Karaca et al., 2005), two sample tubes are required per sample thus reducing the use of disposable plastic materials. In the first step of the extraction (Table 1), the MAGi DNA extraction method uses the MAGi solution originally developed for RNA extraction described in Ince and Karaca (2009). The MAGi solution supplemented with β-mercapto ethanol (BME), NaCl, phenol and chloroform effectively purify DNA from plant species as tested on pepper, tomato and pea plants fresh tissues at different development stages and dry seeds of cotton, pepper, tomato, common bean, and soybean. The MAGi solution consists of sodium lauroyl sarcosine and Triton-X 100 as detergents, sorbitol and Tween-20 as cell disrupters, EDTA as chelator and glycerol as stabilizer. Addition of BME, NaCl, chloroform and phenol remove proteins by denaturation and polysaccharides removed by precipitation from the extract. The MAGi solution itself is non-toxic, easy to prepare, and can be stored at room temperature for several years (Ince and Karaca, 2009).

In the second step of this DNA extraction protocol, the mixture of supernatant, NaCl and isopropanol is centrifuged to precipitate DNA at the bottom of the tube removing the solved proteins and polysaccharides within the supernatant. Third step of the protocol involves in digestive removal of ribonucleic acids using RNase, followed with sodium acetate and ethanol precipitation. The MAGi DNA extraction method extracts a considerable amount of RNAs, therefore, the use of RNase is required. During the final step of the DNA extraction protocol, DNA including the cytoplasmic DNA is washed using ethanol and dissolved in TE buffer.

Quality and quantity of the DNA extracted from 300 samples were confirmed using spectrophotometer measurements, agarose gel electrophoresis, restriction enzyme analyses and several polymerase chain reactions based techniques. DNAs were subjected to spectrophotometric analyses scanned between the wavelengths of A_{190} and A_{1100} nm (Figure 1a, b). Samples clearly exhibited no detectable absorbance of substances from A_{300} to A_{1100} nm. All samples showed the highest absorbance at A_{260} nm (Figure 1a). The mean ratios of A_{260}/A_{280} and A_{260}/A_{230} were 1.87 and 1.97, respectively. The mean readings of A_{230}, A_{260} and A_{280} were 0.19, 0.39 and 0.21, respectively. The mean fresh leaf DNA yield was 741.6 µg/g ranging from 600 to 1600 µg. The mean dry seed DNA yield was 854.4 µg/g ranging from 400 to 1200 µg. The MAGi DNA extraction method also worked well for samples of tomato and pepper consisting of fruits, stems, flower buds, pericarps and immature seeds. Overall results showed that the amount of DNA extracted using the MAGi DNA extraction method is considerable higher than the most commonly used commercial kits, SDS and CTAB methods (Turci et al., 2011).

Agarose gel electrophoresis of undigested and restriction enzyme digested DNAs (example is shown in Figure 2a) clearly indicated that the undigested DNA exhibited little shearing but were larger than 50 kb in size and intact while digested samples showed complete digestion producing DNA fragments between 50 kb to 50 bp. DNA samples stored at -20ºC for more than 1 year also showed the same intact DNA patterns and were completely digestible with the several restriction enzymes (data not shown).

In order to investigate one of the applications of extracted DNA, 4 sets of samples each consisting of 18 samples randomly selected from the tomato samples used for DNA extraction, were used in RAPD-PCR, DAMD-PCR, multiplex-PCR-SCAR, SCAR and CAPS analyses. RAPD and DAMD-PCR amplification were successful across the 18 samples. Consistent results obtained within and between different PCR sets indicating the quality of DNA.

To investigate whether extracted DNAs were useful in marker assisted selection studies, a combination of four primer pairs amplifying the loci showing resistance to *Oidium lycopersicum* (Huang et al., 2000), heat stable *Meloidogyne incognita* (De Castro et al., 2007) and tomato mosaic virus (Ohmori et al., 1995) were amplified using the primer pairs listed in Table 2 in a single PCR (Figure 2b). Also, *I*2 locus resistant to *Fusarium oxysporum* f. sp. *lycopersici* (Staniaszek et al., 2007) and *Mi* gene resistant to *Meloidogyne incognita* were analyzed using SCAR and CAPS analyses, respectively (Figure 2c, d). Analyses clearly indicated that DNA extraction method reported in this study could be used in marker assisted selection studies in both multiplex and single assays.

DISCUSSION

This DNA extraction method uses the MAGi solution which was originally developed for RNA extraction studies of the plant samples (Ince and Karaca, 2009). Results clearly indicated that the MAGi solution could also be used in DNA extraction using the protocol described in this paper. Since the MAGi solution could be used for the extraction of high-quality DNA and RNA from the same biological sample, it is useful for molecular studies involving genotyping and messenger RNA (mRNA) expression analyses. Once prepared, the MAGi solution is stable for several years and can be transferred into the sample tubes with solution dispenser without needing pipettes (Ince and Karaca, 2009).

This method uses two tubes per DNA extraction and the chemical required for the preparation of the MAGi solution is easily available and cheaper in comparison to other methods including the kits (Turci et al., 2011). It uses very little amount of phenol which is toxic and expensive in the mini scale preparations (Aljabani and Martinez, 1997).

In marker assisted breeding programs, large numbers of individuals must be screened for economical traits to allow efficient selection of superior varieties. Recent advances in biotechnology have made many molecular marker techniques available to researchers. Although, PCR based molecular markers are less sensitive to the quality and quantity of the DNAs used as template, reproducibility of the markers depends on the quality of template DNAs (Yamada et al., 2002). For instance, our unpublished studies clearly indicated that the reproducibility of RAPD markers was greatly affected with the quality and quantity of the template DNAs. Polysaccharides and proteins as wells as some other biological molecules such as phenolic compounds, tannins and pigments interfere with several biological enzymes such as DNA polymerases, ligases and restriction endo-nucleases (Karaca et al., 2005). Therefore, the quality of the DNA is one of the most important factors in DNA related studies. The MAGi DNA extraction protocol effectively removes polysaccharides, proteins and pigments.

In general, comparison to intact DNA, fragmented or partially degraded DNAs contain less amount of proteins and polysaccharides and they are efficiently amplified in PCRs. However, degraded DNAs are not suitable for long PCR, endo-nuclease digestion, Southern blot analysis, genomic DNA library construction studies (Aljabani and Martinez, 1997; Michiels et al., 2003). In the present study, it was observed that there were complete digestions with restriction endo-nucleases. The use of several PCR based techniques indicated that polysaccharides and proteins as well as the other compounds preventing PCR were successfully removed during the extraction. The spectral reading at A_{230} nm was low and the ratio of A_{260}/A_{230} was close to 2. However, some DNA of the wells of agarose contained substances that ethidium bromide bound them. However, these substances did not have absorbance at the A_{230} nm and were not present in the agarose wells of the restriction enzyme digested samples. It is known that polysaccharides are difficult to separate from DNA but they are easily identifiable in the DNA preparations as they impart a sticky, viscous consistency to the DNA preparations dissolved in TE buffer (Aljabani and Martinez, 1997). However, there were no indications of polysaccharide contamination or co precipitation in the present study.

In conclusion, in this study a new DNA extraction method reported for the isolation of DNAs from leaves, fruits, pericarps and dry and immature seeds of plant

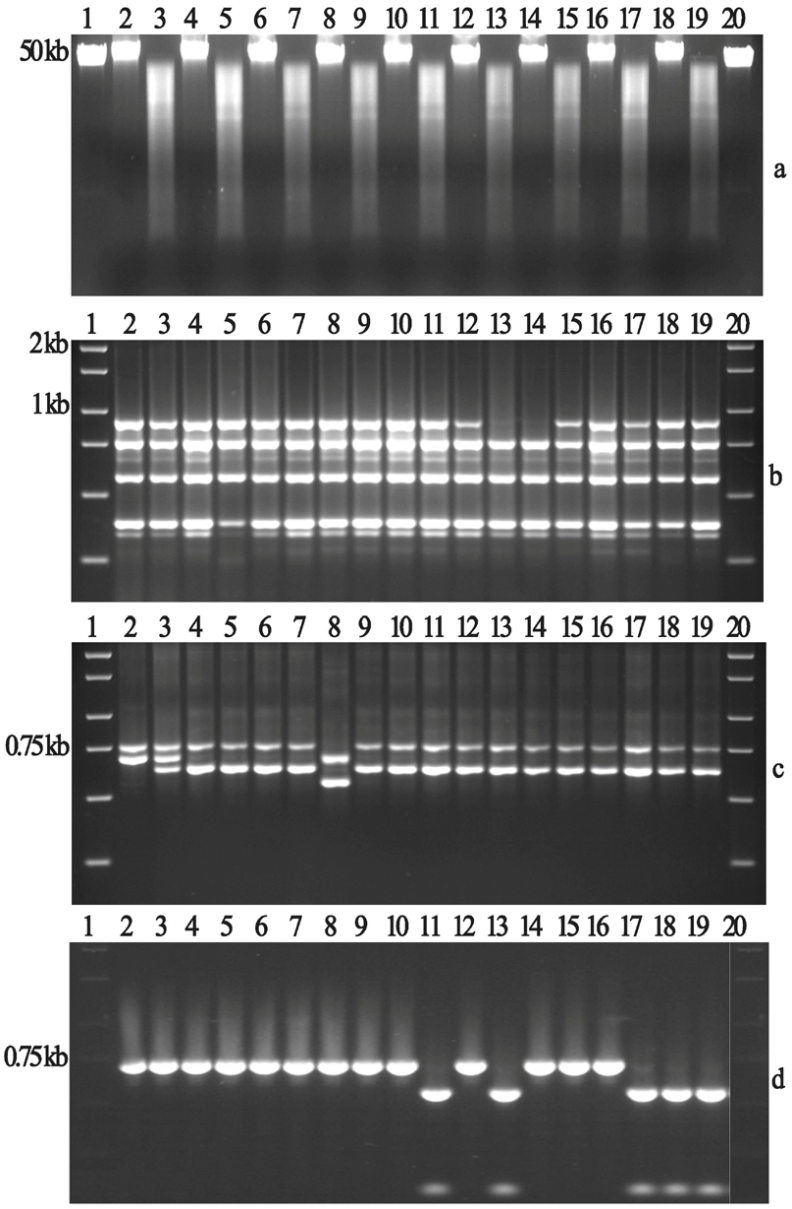

Figure 2. Endonuclease restriction digest of genomic DNAs and application in PCR. Panel a: Genomic and restriction enzyme digested DNA samples from tomato lines and species. Lane 1 and 20 are 50 kb DNA size markers. Lanes 2-19 are genomic and digested DNA samples. Panel b: Multiplex-SCAR-PCR of 18 samples. Lane 1 and 20 are DNA size markers. Panel c: SCAR markers and panel d: CAPS marker. Lanes 1 and 20 in panel c and d are DNA size markers.

species. Extracted DNAs were suitable for enzymatic digestions and various PCR based amplification including the multiplex reactions. The MAGi DNA extraction procedure greatly reduces the time required to isolate DNA from leaves and other tissues. The typical yields and the size of DNAs using this method were high compared with the other methods. The MAGi DNA extraction protocol is simple and completes in approximately 1 h. The MAGi DNA extraction method has several advantages in comparison to other methods including the commercial kits such as it produces un-degraded, high-molecular-mass genomic DNAs higher

than 50 kb in size with excellent spectral readings.

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