GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Comparison of five DNA extraction methods for molecular analysis of Jerusalem artichoke (*Helianthus tuberosus*)

T. Mornkham[1], P.P. Wangsomnuk[1], P. Wangsomnuk[1], S. Jogloy[2], A. Pattanothai[2] and Y.B. Fu[3]

[1]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
[2]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
[3]Plant Gene Resources of Canada, Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada

Corresponding author: P.P. Wangsomnuk
E-mail: Prepua@kku.ac.th

Genet. Mol. Res. 11 (1): 572-581 (2012)
Received August 25, 2011
Accepted November 25, 2011
Published March 8, 2012
DOI http://dx.doi.org/10.4238/2012.March.8.5

ABSTRACT. DNA extraction is an essential step for molecular analysis of an organism, but it is difficult to acquire a sufficient amount of pure DNA from plant tissue with high levels of phenolic compounds, carbohydrates, proteins, and secondary metabolites. Jerusalem artichoke (*Helianthus tuberosus*) has high levels of such substances. We compared five commonly used methods of extracting genomic DNA in tests made with leaves and seed of four Jerusalem artichoke genotypes: 1) modified method of Tai and Tanksley, 2) method of Doyle and Doyle, 3) method of Porebski, 4) modified method of Štorchová, and 5) Plant DNA Kit of Omega Bio-tek. The quality and quantity of extracted DNAs were assessed by photometric assay, electrophoresis on 1% agarose gel and a PCR-based technique. The modified method of Tai and Tanksley was found to be superior for both young leaves and seed. The quality of the extracted DNA was confirmed by sequence-related am-

plified polymorphism. This information will be useful for molecular analyses of Jerusalem artichoke and other related *Helianthus* species.

Key words: DNA extraction; Phenolic compound; Jerusalem artichoke

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.), a member of family Asteraceae, is originated from North America. The plant can be grown for human consumption, alcohol production, fructose syrup production, and livestock feed. Jerusalem artichoke tubers accumulate inulin as a carbon source that can be used as raw material to produce a variety of value-added products (Cosgrove et al., 2000). However, molecular analyses of Jerusalem artichoke such as DNA fingerprinting may be hindered by poor DNA extracted from its tissue, as the Jerusalem artichoke plants are known to contain a high amount of polyphenolic compounds and polysaccharides as well as high lipids in seed (Seiler and Brothers, 1999) and these phytochemicals can interfere with DNA amplification reactions (Khan et al., 2004). The phenolic compounds and polysaccharides can firmly bind to nucleic acids during DNA extraction. The fatty acids, the main component of the seed, could be easily contaminated in the aqueous phase when they are subjected to centrifugation due to their lower density and non-polar characteristics (Nelson et al., 2008). Therefore, an effective method of DNA extraction is warranted for Jerusalem artichoke, which is generally considered to be a recalcitrant species for DNA fingerprinting (Li et al., 2007).

There are several commonly used methods for extracting genomic DNA from plant tissue. The method developed by Doyle and Doyle (1990) has been widely used in horticulture and fruit trees. Porebski et al. (1997) proposed a method for use in strawberry (*Fragaria* spp) to overcome the effects of polysaccharides. Štorchová et al. (2000) introduced an effective method to extract genomic DNA from the leaf samples of the genus *Hieracium*. This protocol has also been successfully applied for the herbarium specimens of the family Juncaceae (Drábkova et al., 2002). Tai and Tanksley (1990) presented a DNA extraction method for dehydrated plant tissues. The method is rapid and inexpensive and has been routinely used in our laboratory with some modifications on various applications in plants species such as tapioca (*Manihot esculenta* Crantz), orchids (*Eulophia herbacea* Lindl., *E. andamanensis* Rchb.f., *Spathoglottis affinis* de Vriese, *Geodorum recurvum* (Roxb.) Alston, and *Paphiopedilum exul* (Ridl.) Rolfe), sugarcane (*Saccharum* spp) and several herb species in Thailand. The modifications include the use of an extraction buffer without β-mercaptoethanol and the procedure re-precipitating DNA with ammonium acetate and sodium acetate. However, no specific assessment has been previously made on the effectiveness of various extraction methods for these plant species assayed in our laboratory, including Jerusalem artichoke.

To facilitate the molecular analyses of Jerusalem artichoke germplasm and the development of effective molecular tools for Jerusalem artichoke breeding programs, we performed a comparative assessment of five commonly used methods of extracting genomic DNA from the leaf and seed of four Jerusalem artichoke genotypes: 1) modified method of Tai and Tanksley (1990), 2) method of Doyle and Doyle (1990), 3) method of Porebski et al. (1997), 4) modified method of Štorchová et al. (2000), and 5) Plant DNA Kit of Omega Bio-tek. The quality and quantity of extracted DNA were assessed by means of photometric assay, electrophoresis on 1% agarose gel and a PCR-based technique.

MATERIAL AND METHODS

DNA extraction

The Jerusalem artichoke genotypes JA102, JA7, JA37, CN52867, HEL65, and HEL335 were kindly donated by the plant genetic resources of Canada at Saskatoon Research Center (PGRC) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) of Germany.

Young leaves of Jerusalem artichoke genotypes JA102, HEL335 x JA37 and F_1 seeds of CN52867 x HEL65 and JA7 were used in this study and extracted for DNA with five independent replications following the five extraction methods below.

Modified method of Tai and Tanksley (1990)

Solutions

- Extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5 M NaCl, 1.25% SDS, 8.3 mM NaOH and 0.38% Na bisulfate).
- T5E (50 mM Tris-HCl, pH 8.0, 10 mM EDTA).

Protocol

Young leaf tissue (100 mg) or seed (30 mg) was ground with mortar and pestle in the presence of liquid nitrogen and 0.7 mL extraction buffer was added and mixed by vortexing. Each sample was incubated at 65°C for 20 min and 0.22 mL 5 M potassium acetate was added and mixed well. The tube was placed at -20°C for 30 min, and then centrifuged at 10,000 rpm for 3 min in a microcentrifuge. The supernatant was transferred to a new tube. The DNA was precipitated by adding a 0.7 volume of isopropanol, mixed well, and centrifuged at 10,000 rpm for 3 min. The supernatant was poured off and the pellet rinsed with 70% ethanol. The pellet was resuspended in 300 µL T5E by briefly vortexing, incubating at 65°C for 5 min, and re-vortexing. Then, 150 µL 7.4 M ammonium acetate was added and mixed well before centrifugation for 3 min and removal of the supernatant to a new tube. The DNA was precipitated by mixing with 330 µL isopropanol and centrifuged for 3 min. The pellet was rinsed with 70% ethanol and resuspended in 100 µL T5E by vortexing, incubating at 65°C for 5 min, and re-vortexing. The DNA was re-precipitated by adding 10 µL 3 M sodium acetate and 75 µL isopropanol and mixing well, followed by centrifugation for 3 min. The remaining pellet was washed with 70% ethanol and then dried before adding 25 µL TE. The DNA solution was stored at -20°C until used.

Method of Doyle and Doyle (1990)

Young leaf tissue and seed of Jerusalem artichoke were extracted in CTAB isolation buffer according to the protocol of Doyle and Doyle (1990).

Solution

- CTAB isolation buffer [2% (w/v) CTAB (hexadecyltrimethylammonium bromide),

100 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 0.5 M NaCl, 1% (w/v) PVP 40].
- Chloroform:isoamyl alcohol (24:1)
- Isopropanol
- Ethanol
- TE buffer

Protocol

Young leaf tissue (100 mg) or seed (30 mg) was ground with mortar and pestle in approximately 500 µL CTAB buffer. The CTAB/plant extract mixture was transferred to a microfuge tube. Each sample was incubated at 60°C for 45 min. After incubation, 600 µL chloroform:isoamyl alcohol (24:1) was added into the mixture. The sample was centrifuged at 10,000 rpm for 15 min. The supernatant was transferred to a clean microfuge tube, followed by adding 500 µL isopropanol and leaving at -20°C for 30 min. The pellet was rinsed with 70% ethanol and resuspended in 25 µL TE buffer. The DNA solution was stored at -20°C until used.

Method of Porebski et al. (1997)

Solution

- Extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 2% (w/v) CTAB, 0.05% (w/v) PVP and 0.3% β-mercaptoethanol).
- Chloroform:isoamyl alcohol (24:1).

Protocol

Young leaf tissue (100 mg) or seed (30 mg) was ground with mortar and pestle in the presence of liquid nitrogen. Then, 500 µL of the extraction buffer was added, mixed by inversion, and incubated at 65°C for 30 min. The protein contaminant was removed by adding 400 µL chloroform:isoamyl alcohol (24:1), mixed well, and centrifuged at 10,000 rpm for 3 min. The supernatant was transferred to a new tube. Then, 250 µL 5 M NaCl was added and mixed well, and 2 volumes of ice-cold ethanol were added before centrifugation for 3 min. The pellet was rinsed with 70% ethanol and resuspended in 25 µL TE. The DNA solution was stored at -20°C until used.

Modified method of Štorchová et al. (2000)

This protocol was modified from Štorchová et al. (2000), with the addition of mannitol instead of sorbitol.

Solution

- Extraction buffer (100 mM Tris-HCl, pH 7.5, 50 mM EDTA, pH 8.0, 0.35 M mannitol and 0.3% β-mercaptoethanol).

- Lysis buffer (200 mM Tris-HCl, pH 7.5, 50 mM EDTA, pH 8.0, 2 M NaCl, 2% (w/v) CTAB).
- Chloroform:isoamyl alcohol (24:1).

Protocol

Young leaf tissue (100 mg) or seed (30 mg) was ground with mortar and pestle in the presence of liquid nitrogen. Then, 700 µL extraction buffer was added, mixed by inverting the tube, and incubated at room temperature for 20 min. The DNA precipitation was done by centrifugation at 10,000 rpm for 3 min. The supernatant was poured off and the pellet was resuspended in 300 µL lysis buffer and incubated at 65°C for 15 min. After incubation, 600 µL chloroform:isoamyl alcohol (24:1) was added into the mixture. The sample was centrifuged at 10,000 rpm for 3 min and the supernatant was transferred to a clean microfuge tube. Then, 500 µL isopropanol was added, mixed and left at -20°C for 60 min. The DNA precipitation was performed with centrifugation at 10,000 rpm for 3 min and the pellet was rinsed with 70% ethanol and then resuspended in 25 µL TE. The DNA solution was stored at -20°C until used.

E.N.Z.A. Plant DNA Kit (Omega Bio-tek, USA)

Solution

- Buffer P1, P2, P3 wash buffer and E available from E.N.Z.A. Plant DNA Kit.
- Ethanol.

Protocol

Young leaf tissue (100 mg) or seed (30 mg) was ground with liquid nitrogen to optimize for sufficient homogenization. Extraction was done following the instructions of the Plant DNA Extraction Kit (Omega Bio-tek).

Comparison of the extraction protocols

The DNA extractions from all five protocols were measured from the 260/280 nm absorbance ratios obtained from a spectrophotometer and agarose gel electrophoresis. An aliquot of 2 µL total genomic DNA was used in the spectrophotometer NanoDrop™ (Thermo Scientific) according to manufacturer instructions. The variation in the efficiency of DNA extractions with respect to tissue used (leaf and seed) was analyzed using Statistix 8 (Analytical Software, 2003). The quality of the extracted DNA was assessed with the SRAP-PCR technique (Li and Quiros, 2001). Several combinations of SRAP primers (synthesized by 1st base, Singapore) were used (Table 1). A 10-µL final volume in a PCR contained 1 µL 2.0 mM dNTPs (Fermentas), 1 µL 2 mM $MgCl_2$ (Fermentas), 0.4 U Taq DNA polymerase (Vivantis), 1 µL 10X PCR buffer [750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20; Fermentas], 0.5 µM of each forward and reverse primers and sterile water. The PCR condition for SRAP primers was as follows: at 94°C for 1 min; 5 cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 2 min; subsequently by 35 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min,

with a final extension at 72°C for 5 min. PCR was performed in a "CG1-96" thermocycler (Corbett Research, Germany). The PCR products were stored at 4°C, analyzed on 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. The photograph was taken using Vilber Lourmat (France).

Table 1. List of SRAP primers used to amplify DNA of Jerusalem artichoke.

Primer	Sequence (5'-3')	Annealing temperature (°C)
ODD4	AGGGTAGCGTCTGAGGA	50
BG68	AAAGGGAGACAGATATTTACA	50
ME2	TGAGTCCAAACCGGACG	50
EM6	GACTGCGTACGAATTCCA	50

RESULTS AND DISCUSSION

The basic idea behind the DNA extraction is relatively simple, but it is difficult to deliver reliable quantity and purity of DNA for molecular analysis of an under-exploited plant species. This is the case for Jerusalem artichoke, which contains a high level of polysaccharides, phenolic compounds, tannins, and secondary metabolites (Seiler and Brothers, 1999). Figure 1 shows the variation in efficiency of five DNA extraction methods. The absorbance values of double-stranded DNAs in the wavelength of 260 nm showed the differences in DNA yield ranging from 20.84 to 74.59 µg/g tissue. The modified method of Štorchová et al. (2000) provided the highest DNA yield, and the lowest DNA yield was obtained when using the CTAB method containing a high concentration of NaCl (Porebski et al., 1997). A higher DNA yield was obtained from the modified method of Štorchová et al. (2000), but the resulting DNA samples were still contaminated and thus were not pure.

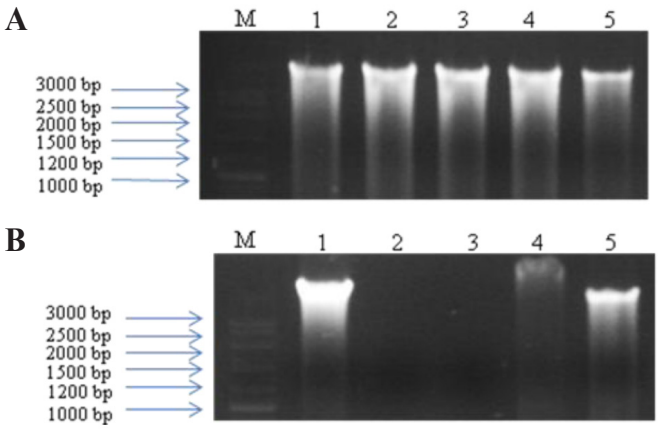

Figure 1. Electrophoresis on agarose gel for the DNA extracted from young leaves and seeds of Jerusalem artichoke using five extraction protocols. **A.** and **B.** are for *Helianthus tuberosus* DNA extracted from young leaves of JA102 and from seeds of CN52867 x HEL65, respectively. *Lane M* = 100-bp DNA ladder plus. The numbers from left to right indicate the extracted protocols: *lane 1* = modified method of Tai and Tanksley (1990); *lane 2* = CTAB DNA extraction method; *lane 3* = modified CTAB with high NaCl concentration; *lane 4* = modified method of Štorchová et al. (2000); *lane 5* = E.N.Z.A. Plant DNA Kit.

The purity of extracted DNAs also varied among extraction protocols as determined by the absorbance ratio of A260/A280 nm (Table 2). The modified method of Tai and Tanksley (1990) showed satisfactory results for both quantity and quality, with the lower amount of potential contaminants compared to those from other techniques (Figure 1 and Table 2). An average of 63.44 and 148.60 µg genomic DNA was extracted from 1 g leaf and seed, respectively. According to Li et al. (2007), the CTAB-based method is an efficient method for DNA extraction from mature leaf of sunflower compared to the SDS-based method. However, nucleic acids could be selectively precipitated with CTAB and the polysaccharides are insoluble over this salt. Our assessment revealed that DNA extraction from leaf samples using the modified method of Doyle and Doyle (1990) also gave the satisfactory absorption from both JA102 and HEL335 x JA37 genotypes with an average yield of 61.27 and 62.19 µg DNA/g, respectively. However, this method failed to extract DNA from the seeds (Table 3). The DNA extraction from the method of Doyle and Doyle (1990) with the addition of PVP to the CTAB solution can enhance the removal of polysaccharides from nucleic acids (Fang et al., 1992). The other protocol with CTAB includes β-mercaptoethanol and high NaCl concentration. These chemicals are used to denature the sulfate linkage in protein structures and help remove the proteins. However, our assessment showed that the DNA purity was reduced from the CTAB method with NaCl when compared to those with only the addition of PVP.

Table 2. Comparison in quality and quantity of DNA extracted from young leaves of Jerusalem artichoke among five DNA extraction methods.

Extraction methods	JA102		HEL335 x JA37	
	DNA concentration (µg/1 g tissue)	Absorption ratio (260/280 nm)	DNA concentration (µg/1 g tissue)	Absorption ratio (260/280 nm)
Modified method of Tai and Tanksley (1990)	63.44^{ab}	1.98^{a}	58.28^{ab}	1.98^{a}
CTAB	61.27^{ab}	1.96^{ab}	62.19^{ab}	1.96^{a}
CTAB with NaCl	20.84^{c}	1.90^{b}	24.46^{c}	1.87^{b}
Modified method of Štorchová (2000)	73.37^{a}	1.83^{c}	74.59^{a}	1.84^{b}
E.N.Z.A. Plant DNA kit	53.70^{b}	1.79^{c}	53.21^{b}	1.77^{c}
LSD	*	*	*	*

Values with different superscript letters within column are significantly different at $P \leq 0.05$ by the least significant difference (LSD) test. *Significant at the $P \leq 0.05$ probability level.

Table 3. Comparison in quality and quantity of DNA extracted from seeds of Jerusalem artichoke of two DNA extraction methods.

Extraction methods	CN52867 x HEL65		JA7	
	DNA concentration (µg/1 g tissue)	Absorption ratio (260/280 nm)	DNA concentration (µg/1 g tissue)	Absorption ratio (260/280 nm)
Modified method of Tai and Tanksley (1990)	148.60^{a}	1.94^{a}	163.28^{ab}	1.93^{a}
E.N.Z.A. Plant DNA Kit	43.45^{b}	1.92^{b}	44.92^{b}	1.92^{a}
LSD	*	*	*	ns

Values with different superscript letters within column are significantly different at $P \leq 0.05$ by the least significant difference (LSD) test. *Significant at the $P \leq 0.05$ probability level. ns = nonsignificant.

Proteinase K and RNase are used to remove protein and RNA contamination in most DNA extraction protocols available. Extraction by the modified method of Tai and Tanksley (1990) does not use these reagents. Re-precipitation with variable stringency of salt was used

to eliminate contaminants from genomic DNA. This strategy displayed some advantage over the addition of PVP or reduction of agents such as β-mercaptoethanol that limit cross linking of phenolics to nucleic acids as reported by several authors (e.g., Katterman and Shattuck, 1983; Hugo et al., 1998).

Seiler and Brothers (1999) reported that *H. tuberosus* seeds contained a large amount of lipids (up to 49%). The presence of unusual compounds could hamper DNA extraction and downstream molecular analysis via inhibiting enzyme activity. We have showed that only the modified method of Tai and Tanksley and E.N.Z.A. plant DNA extraction kit (Omega Bio-tek) were able to extract DNA from seeds (Figure 1). Also, the modified method of Tai and Tanksley (1990) generated 3.4 and 3.6 times more DNA with improved purity when compared to those from the latter method on genotype CN52867 x HEL65 and JA7, respectively (Table 3).

The SRAP analysis following Li and Quiros (2001) also showed the effectiveness of the extracted DNA from the modified method of Tai and Tanksley (1990) in the detection of genetic variation (Figure 2). The amplicons displayed clear and high-intensity bands after agarose gel electrophoresis. These SRAP-PCR fragments could be cut, cloned and sequenced (Li and Quiros, 2001; Mutlu et al., 2008; Lui et al., 2011). Also, the DNA samples extracted from this method were successfully amplified in the analysis of amplified fragment length polymorphism in our laboratory (results not shown).

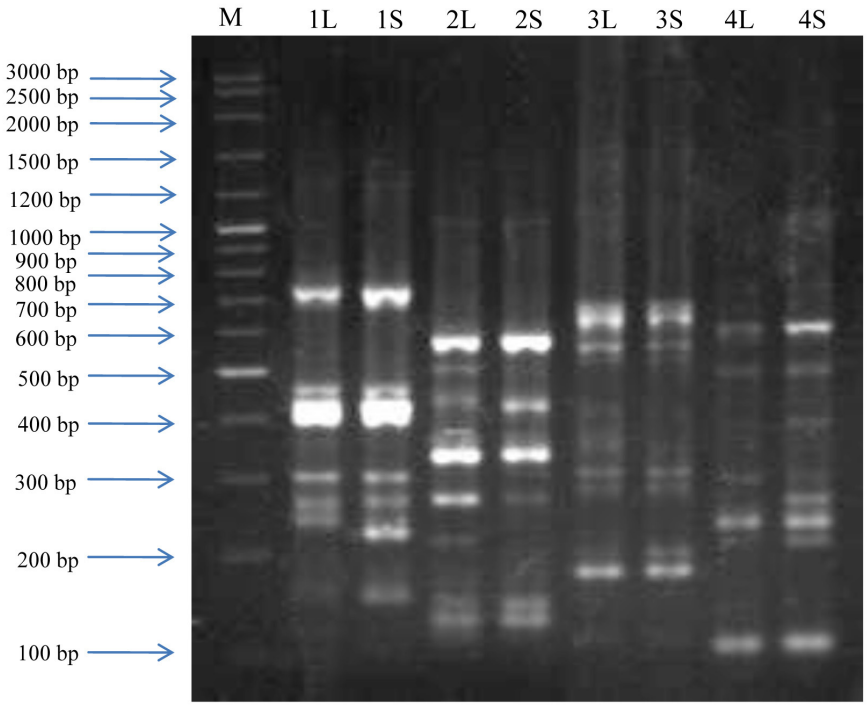

Figure 2. PCR amplification on 1% agarose gel with the SRAP primers of young leaf and seed DNA extracted using the modified method of Tai and Tanksley (1990). *Lane M* = DNA ladder, 100-bp DNA ladder plus. The numbers indicate primer combinations: *1* = ODD4/BG68; *2* = ME2/ODD4; *3* = ME2/BG68; and *4* = EM6/BG68. *L* = extracted DNA from young leaves of JA102; *S* = extracted DNA from seeds of CN52867 x HEL65.

The results presented here may be applicable to other *Helianthus* species in the family Asteraceae, as these species may have similar levels of phenolic compounds, polysaccharides, oil, tannin, and secondary metabolites as in the Jerusalem artichoke genotypes assayed here. The superiority of the modified method of Tai and Tanksley (1990) also provided an empirical confirmation on the effectiveness of its extensive use in our laboratory to extract DNA from many other plant species as mentioned earlier.

CONCLUSIONS

Our preliminary assessment revealed that the modified method of Tai and Tanksley (1990) appeared to be superior among the five extraction methods assayed in DNA extraction of Jerusalem artichoke tissue. This finding is useful for future molecular analyses of Jerusalem artichoke and related *Helianthus* species.

ACKNOWLEDGMENTS

Research supported by the Higher Education Research Promotion and the National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster of Khon Kaen University.

REFERENCES

Analytical Software (2003). Statistix 8, Analytical Software. Tallahasee, USA.

Cosgrove DR, Oelke JD, Doll DW and Davis DJ (2000). Jerusalem Artichoke (Online). Available at [http://www.hort.purdue.edu/newcrop/afcm/jerusart.html]. Accessed August 22, 2011.

Doyle JJ and Doyle JL (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 11-15.

Drábkova 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: 161-175.

Fang G, Hammar S and Grumet R (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biotechniques* 13: 52-4, 56.

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

Katterman FR and Shattuck VI (1983). An effective method of DNA isolation from the mature leaves of *Gossypium* species that contain large amounts of phenolic terpenoids and tannins. *Prep. Biochem.* 13: 347-359.

Khan IA, Awan FS, Ahmad A and Khan AA (2004). A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. *Plant Mol. Biol. Rep.* 22: 89a-89e.

Li G and Quiros CF (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor. Appl. Genet.* 103: 455-461.

Li JT, Yang J, Chen DC and Zhang XL (2007). An optimized mini-preparation method to obtain high-quality genomic DNA from mature leaves of sunflower. *Genet. Mol. Res.* 6: 1064-1071.

Lui HJ, Xu H, Yu X and Jiang TB (2011). Application of SRAP and SSR molecular markers in genetic diversity of DaXing' anling area wild *Auricularia auricular*. *Adv. Mat. Res.* 183-184: 1118-1122.

Mutlu N, Boyaci FH, Gocmen M and Abak K (2008). Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a *Fusarium* wilt resistance gene in eggplant. *Theor. Appl. Genet.* 117: 1303-1312.

Nelson DL, Lehninger AL and Cox MM (2008). Lehninger Principles of Biochemistry. W.H. Freeman, New York.

Porebski S, Grant B and Boum BR (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.* 15: 8-15.

Seiler GJ and Brothers ME (1999). Oil concentration and fatty acid composition of Achenes of *Helianthus* species (Asteraceae) from Canada. *Econ. Bot.* 53: 273-280.

Štorchová H, Hrdličková R, Chrtek J and Tetera M (2000). An improved method of DNA isolation from plants collected

in the field and conserved in saturated NaCl/CTAB solution. *Taxon* 49: 79-84.
Tai TH and Tanksley SD (1990). A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Biol. Rep.* 8: 297-303.

