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Isolation of High-Quality DNA from a Desert Plant *Reaumuria soongorica*

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1. Introduction

The desert plants, widely distributed in arid and semi-arid regions, have the significant ecological functions of combating desertification and maintenance of the ecosystem stability in the desert ecological system, and also have an irreplaceable role in restoration and reconstruction of vegetation in the sand. With the development of modern molecular biology, molecular markers analysis such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (ISSR), and especially full genome sequencing technique are widely used in the study of biological diversity, genetic transformation and the conservation of genetic resources of desert plants. The isolation of high molecular weight and high purity genomic DNA is a pre-requisite for these molecular techniques. Now there are many extraction methods of genomic DNA from biomaterials. The commonly employed DNA isolation methods involve the use of enzymes such as lysozyme and proteinase K (Lockhart et al., 1989), cetytrime-thylammonium bromice (CTAB) (Ghosh et al., 2009; Moyo et al., 2008; Khanuja et al., 1999; Novaes et al., 2009; Singh et al., 2000) or sodium dodecyl sulfate (SDS) (Kaufman et al., 1999; Dellaporta et al., 1983) treatment and extraction with organic solvents, detergent-induced lysis in conjunction with proteinase K and lysozyme (Perera et al., 1994) or lysis using guanidinium isothiocyanate (GITC)-containing solutions (Boom et al., 1990; Noordhoek et al., 1995; Chakravorty & Tyagi, 2001), among which, guanidinium thiocyanate has been shown to be a powerful agent in the purification of DNA because of its potential to lyse cells and its potential to inactivate nuclease (Boom et al., 1990; Chomczynski et al., 1987; Zeillinger et al., 1993). However, high amounts of gummy polysaccharides, polyphenols and other various secondary metabolites such as alkaloids, flavonoids, terpenes and tannins in the desert plants usually hamper the DNA isolation procedures and reactions such as DNA restriction, amplification and cloning (Moyo et al., 2008; Khanuja et al., 1999; Pang et al., 2011; Zhang K., 2011; Ji & Li, 2011). The main problems encountered in the isolation and purification of high

molecular weight DNA from plant species include degradation of DNA due to endonucleases and high levels of contaminants (polyphenols or polysaccharides) that co-precipitate with DNA. Endonucleases released from the vacuoles during the cell lysis process, which are co-isolated with highly viscous polysaccharide, lead to the degradation of DNA and remarkably reduce the yield of extracted DNA (Khanuja et al., 1999. Polyphenols released from the vacuoles during the cell lysis process are oxidized by cellular oxidases and undergo irreversible interactions with nucleic acids causing browning of the DNA (Varma et al., 2007; Moyo et al., 2008; Khanuja et al., 1999; Porebski et al., 1997). The presence of gelling polysaccharides prevents complete dissolution of nucleic acids and imparts a viscous constituency to the DNA making it stick to the wells during gel electrophoresis (Barnell et al., 1998; Diadema et al., 2003; Varma et al., 2007). Furthermore, inhibitor compounds like residual polyphenols, polysaccharides and other secondary metabolites inhibit enzymatic reactions such as restriction endonuclease cleavage (Raina and Chandlee, 1996) or Taq DNA polymerase amplifications (Shioda and Murakami-Murofushi, 1987; Tigst and Adams, 1992; Pandey et al., 1996) or ligase links (Moyo et al., 2008; Khanuja et al., 1999, Weishing et al., 1995). Thus, though several successful genomic DNA isolation protocols for high polyphenol and polysaccharide containing plant species have been developed, none of these are universally applicable to all plants (Varma et al., 2007), because qualitative and quantitative differences in the levels of polysaccharides, phenols and secondary metabolites in various plant tissues significantly alter the efficiency of nucleic acid extraction and purification procedures. Therefore researchers often modify a protocol or blend two or more different procedures to obtain DNA of the desired quality (Varma et al., 2007).

Reaumuria soongorica (Pall.) Maxim, an extreme xeric semi-shrub of Tamaricaceae, is a constructive and dominant species of desert shrub vegetation (Liu et al. 1982; Wang et al., 2011; Bai et al., 2008). It is distributed widely on a large area of sand wasteland (Fig. 1a) and saline land (Fig. 1b) in arid and semiarid regions of central Asia from the western Erdos, Alaskans, Hexi Corridor, Qaidam Basin to Tarim Basin and Jungar Basin (from the east to the west) and forms the vast and distinctive landscape of the salt desert (Liu and Liu, 1996). The distribution of *R. soongorica* in desert in northwestern China is shown in (Fig. 2) (Ma et al., 2007). It can inhabit on the alluvial plains of piedmont, hilly lands, eroded monadnocks, piedmont gravel mass, gravel alluvial fan and the Gobi. It is distributed on large span, wide range, and complex habitat where there are different climatic conditions among regions, especially with significantly different water conditions, such as the average annual rainfall in Lanzhou with 327.7 mm, Shapotou with 188.2 mm, and Ejina with 35.1 mm as it possesses the characteristics of drought resistance, salt tolerance, barrenness tolerance, and dune fixation. It is such a good candidate of desert plants that it is very significant for us to study its biological diversity and the mechanism of adverse environments resistance. However, the leaves of *R. soongorica* are evolved into the form of pellets suitable for arid environment, which are very hard in texture and contain high level of polysaccharides, polyphenols and secondary metabolites that co-precipitate with DNA, making DNA isolation difficult.

A good isolation protocol should be simple, rapid and efficient, yielding appreciable levels of high quality DNA suitable for molecular analysis. Krizman et al. (2006) were of the opinion that, among other factors, the amount of plant sample extracted could be critical in keeping an extraction procedure robust. In the present study, our objective was to create an improved DNA extraction procedure amenable for the isolation of high quality DNA in the

desert plant *R. soongorica*. Four methods for extracting DNA were tested in this study and they included the TianGen Plant Genomic DNA Kit, the modified TianGen Plant Genomic DNA Kit, the modified CTAB-A method and the modified CTAB-B method herein promoted by us. The results showed that the modified CTAB-B method was a relatively quick and inexpensive method and it was the best method for extraction DNA from leaf materials containing large quantities of secondary metabolites. Furthermore, it was further tested that the modified CTAB-B method for isolating DNA from leaves of *R. soongorica* yields DNA in a quantity and quality suitable for PCR amplification, DNA marker analysis and restriction digestion.

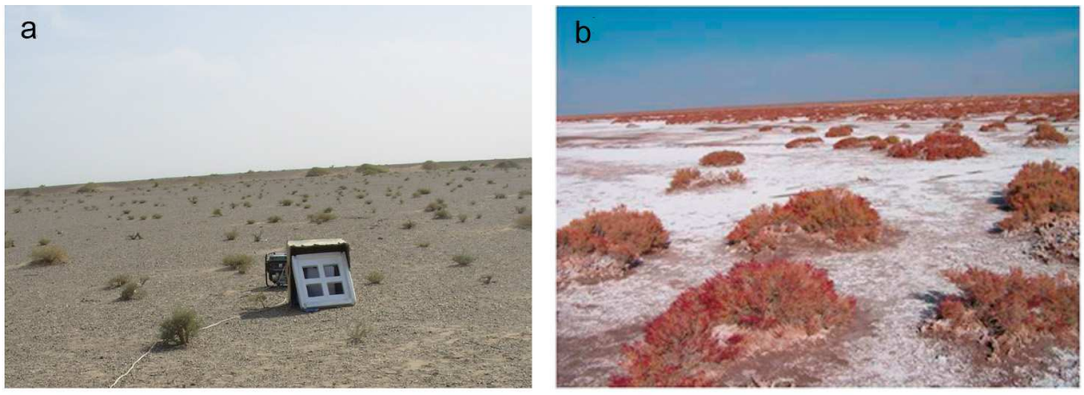

Fig. 1. The natural habitats of *Reaumuria soongorica*. a Populations of *R. soongorica* in sand wasteland or Go; b Populations of *R. soongorica* in saline land with a white visible salt on the ground

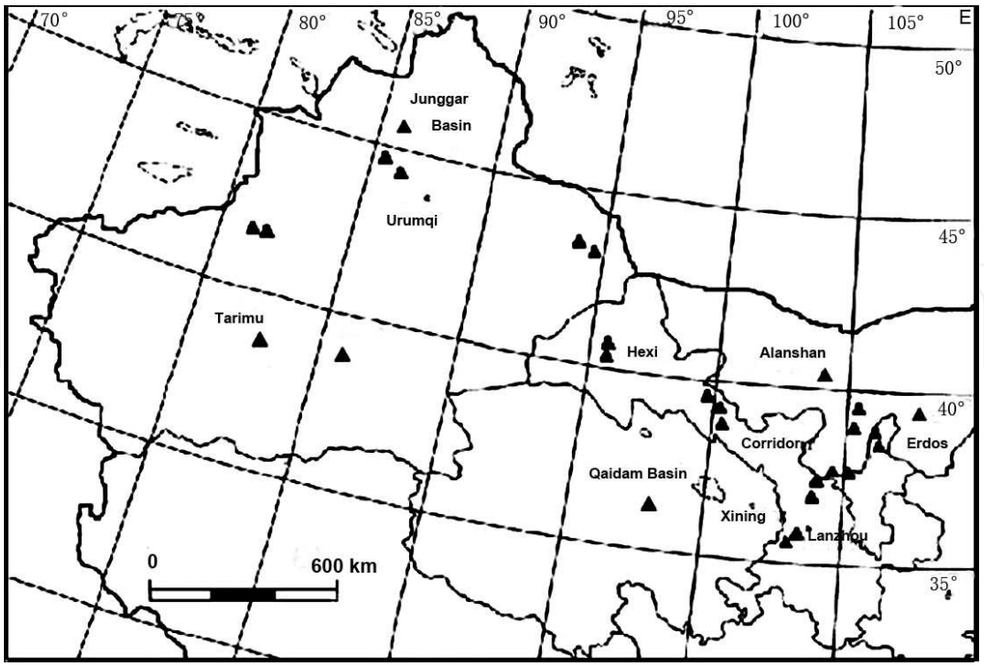

Fig. 2. Distribution map of the study plant *Reaumuria soongorica* in northwest China (the triangle symbol indicates the major distribution area)

2. Materials and methods

2.1 Plant materials

Tender *R. soongorica* leaves were collected from Ejina in Mogo, China and snap-frozen in liquid nitrogen. The frozen leaves were transported in liquid nitrogen and stored at -80ºC upon reaching the laboratory.

2.2 Equipments and solution preparation

Mortars, pestles, glassware and plasticware was autoclaved prior to use. The CTAB extraction buffer was composed of 2.0% CTAB (High Purity grade, Amresco), 100 mM Tris-HCl (pH 8.0)) (Ultra pure grade, Amresco), 2 M NaCl (Biotechnology grade, Amresco), 25 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0) (High purity Grade Amresco) in 0.1% ultra pure water. The components in the extraction buffer were mixed and autoclaved. The 5% PVPP (Sigma P-6755) was added when the material was grounded and the 5% beta-mercaptoethanol (Biotechnology grade, Amresco) was added before DNA extraction. The final solution was warmed in a water bath to 65°C for use in DNA extraction. TE buffer was prepared with 10 mM Tris-HCl (pH 8,0) and 1.0 mM EDTA (pH 8.0). A phenol (pH 5.0)/chloroform /isoamyl alcohol mixture (25/24/1) (Biotechnology grade, Amresco) and a chloroform /isoamyl alcohol mixture (24/1) were prepared before use, and all other solutions including 3 M sodium acetate (NaAc) (pH 5.2) (Biotechnology grade, Amresco), 1 M NaCl, pre-cooled 75% ethanol were prepared with ultra pure water and autoclaved. DNase-Free RNase (Ultra pure grade) was purchased from Amresco Corporation.

2.3 Grinding

The frozen fresh samples were transferred into a mortar with liquid nitrogen and the ceramic pestle was pre-chilled for grinding. By providing liquid nitrogen as a cooling jacket, the samples were sprinkled with PVPP and ground vigorously to fine powder using the ceramic pestle. This powder was used for the following extraction protocols of the CTAB methods. And the commercial DNA isolation kit was ground in liquid nitrogen free of adding to PVPP.

2.4 DNA extraction

For our modified CTAB method, the steps of this protocol was carried out as follows:

1. The ground powder sample (100 mg) was transferred to 2 ml micro-centrifuge tubes filled with 700 µl of pre-warmed CTAB extraction buffer containing 35 µl β-mercaptoethanol, following by incubation at 65°C for 30 min in a warm water bath. The mixture was regularly mixed three to four times by gently inversion during the incubation..
2. 200 µl 3 mol/L sodium acetate (NaAc) (pH 5.2) was added to the incubated mixture and mixed gently by inversion and incubated on ice for 30 min.
3. An equal volume of chloroform/isoamyl alcohol (24/1) was added to the homogenate and mixed thoroughly for 2 min, following by centrifugation at 12,000×g for 10 min at

room temperature. The upper aqueous phase was carefully collected from each sample without disturbing the interface. This step was repeated twice.

4. 2.5 volumes of absolute ethanol was added to the recovered supernatant and precipitated 30 min at -20ºC. A precipitate formed at this stage and the mixture was centrifuged at 12,000×g for 10 min at 4°C. DNA pellet was recovered by decanting the supernatant.
5. The crude nucleic pellet was dissolved in 1 ml of 1 M NaCl instead of dissolving it in Tris-EDTA (TE) buffer. The entire solution was transferred to a 2 ml microcentrifuge tube and treated with RNase at 37°C for 1 h. RNase contamination was removed by adding an equal volume of phenol (pH 5.0)/chloroform /isoamyl alcohol (25/24/1) and the aqueous phase was collected in a fresh microcentrifuge tube after centrifugation at 12,000×g for 5 min at room temperature.
6. An equal volume of chloroform/isoamyl alcohol (24/1) was added and mixed thoroughly. The samples were centrifuged at 14,000×*g* for 5 min at room temperature and the top aqueous phase was transferred to a fresh tube.
7. A double volume of absolute ethanol and 0.1 volumes of 3 M (pH 5.2) sodium acetate were added into the collected aqueous phase and were mixed gently by inversion. The samples were incubated at -80ºC for 30 min, followed by centrifugation at 12,000×g.
8. The DNA pellet was washed with 75% ethanol, absolute ethanol, air-dried and finally the purified DNA pellet was dissolved in 100 µl of TE buffer. and stored at -20°C.

Initial tests for DNA isolation from the leaves of *R. soongorica* were carried out with the modified CTAB-A method and the Plant Genomic DNA Kit (TIANGEN Biotech Co., Ltd., Beijing). The modified CTAB-A method was modified based on the classical Doyle and Doyle (1987) method. The steps of the modified CTAB-A are similar to those of the CTAB-B method before step 4 (the precipitate of crude nucleic pellet). The main difference is that the crude nucleic pellet in CTAB-A method was solved in TE and extracted by chloroform/isoamyl alcohol (24/1) again instead of being treated with DNase-free RNase. Briefly, the crude nuclei pellet was dissolved in 500 µl of TE buffer, followed by the steps 3 and 4 of the CTAB-B method repeatedly. The protocols for the commercial DNA isolation kit was performed according to the manufacturer' procedures on their website:http://www.tiangen.com/newEbiz1/EbizPortalFG/portal/html/ProductInfoExhibit.html?ProductInfoExhibit_ProductID=c373e923ec4bc4d68f7efc2e13bcb309&ProductInfoExhibit_isRefreshParent=false. The protocol of the modified TianGen Plant Genomic DNA Kit was based on those of the TianGen Plant Genomic DNA Kit with some slight modifications. The modifications were listed as follows: (1) The plant materials were ground free of liquid nitrogen, but were added to the cooled sterile mortar and ground with Gp1 buffer poured into the mortar. (2) The ground tissue was transferred to 2 ml micro-centrifuge tubes prepared a warm (65°C) Gp1extraction buffer, and then the 5% beta-mercaptoethanol and 10 µl DNase-free RNase were added to the mixture immediately and mixed gently by inversion. The other steps are carried out by the instructions of the kit. For each method, three independent experiments were done, and three samples were prepared in each independent experiment.

2.5 Testing the quality of the genomic DNA

Three microliters of each genomic DNA sample is examined by electrophoresis and remnant DNA sample is stored at –70°C. Mixing 1 µl of 5× DNA loading buffer (TIANGEN Biotech

(Beijing) Co. Ltd.) with 3 µl of genomic DNA at room temperature for 1 min. Then the sample was loaded on 0.8% agarose formaldehyde denaturing gels stained with ethidium bromide (EtBr) (Biotechnology grade, Amresco), and run on gels in the 1× formaldehyde electrophoresis buffer at 5-7 V/cm.

2.6 Assessment of the purity and the yield of the genomic DNA

Two microliters of each genomic DNA sample was diluted into 200 µl of sterilized ultra pure water (pH 7.0). The absorbance of each diluted genomic DNA sample was evaluated at 260 and 280 nm using a ND-2000C (Thermo, America). The yield of genomic DNA was calculated according to the formula: DNA yield = 50 × OD_{260} × dilution factor × volume of sample in milliliters/material weight (g). Measured the values at the wavelengths of 260, 280 nm and 230 nm and calculated the ratios of A260/A280 and the A260/A230.

2.7 ISSR amplification

The DNAs isolation from different *R. soongorica* populations by our promoted CTAB-B protocol were used as template for inter simple repeat sequence primers (ISSR) amplification (Gajera et al., 2010). ISSR amplification reactions were performed in 20 µl reaction volume containing 1 µl gDNA template, 0.25 mmol/L of each dNTPs, 2.5 mmol/L $MgCl_2$, 1×PCR buffer (10mmol/L Tris-HCl pH8.3, 5mmol/L KCl), 1 U Taq DNA polymerase and 0.5 mmol/L of UBC-807 primer (AGA GAG AGA GAG AGA GT). The amplification reaction were carried out on a thermocycler (Biometra) and programmed for an initial pre-denaturing at 94°C for 5 min, followed by 35 cycles of 1 min at 94°C (denaturation), 1 min at 48°C (annealing temperature), and 1.5 min at 72°C (extension) followed by a final extension step at 72°C for 10 min. Amplification products (5 µl) were electrophoresed in 1.5% agarose in 1× TBE buffer and stained with ethidium bromide.

3. Results and discussion

3.1 DNA isolation methodology

Commercial DNA isolation kits are widely used for their single-step methods and the relatively short amount of time required (usually about 1-2 h). These kits have also proven effective for isolating DNA from common plants such as rice, barley and Arabidopsis. We first attempted to isolate DNA from the leaves of *R. soongorica* using three commercial DNA isolation kit: the Plant Genomic DNA Kit (TIANGEN Biotech Co., Ltd., Beijing) which is designed specifically to extract DNA from plant tissues rich in secondary metabolites. In our hands, this kit was not able to isolate any DNA from the leaves of *R. soongorica* (data not shown). Then, we carried out the improved kit method according to the suggestion of this company' technical assistance employee, which yielded a small amount of DNA, but it was seriously contaminated (Fig. 3, lanes 1,2). The failure of the kit may be explained by the DNA likely formed a sticky, a glue-like gel in complex with these secondary metabolites and this could not be properly separated into two phases by centrifugation.

During the course of the RNA isolation, none of the kits were able to isolate any RNA from the leaves of *R. soongorica* (Wang et al. 2011), so we did not try more other commercial DNA kits to isolate DNA, but attempted to use a improved CTAB method to extract DNA from

the leaves of *R. soongorica*. When we carried out the modified CTAB-A method based on the classical Doyle and Doyle (1987) method, which consistently resulted in significant RNA contamination of the DNA samples (Fig. 3, lanes 3,4). To remove RNA contaminants, additional purification steps must be performed, which not only reduce DNA yield but also increase the time required for DNA extraction.

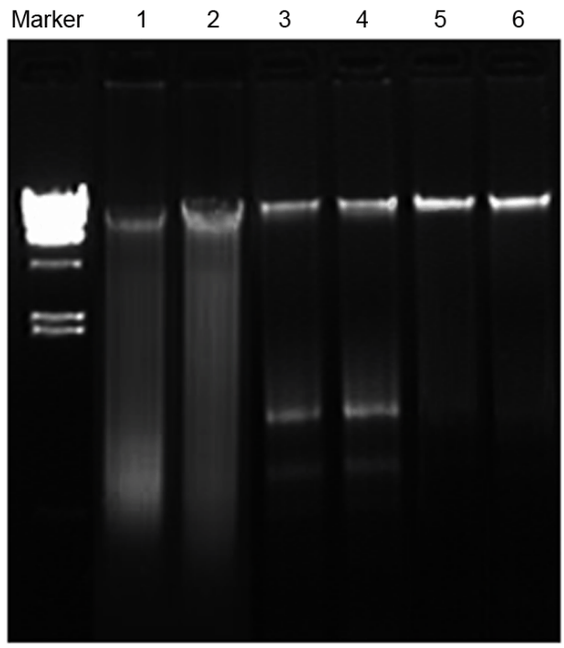

Fig. 3. Electrophoretic analysis of *R. soongorica* genomic DNA isolated using various extraction methods. The genomic DNA (3 µl) of each sample was loaded into the different well, and then was run on a 0.8% agarose gel stained with ethidium bromide in 1×TAE buffer. Marker indicates the Molecular weight marker – 1 kb DNA ladder. Lanes 1 and 2, the modified TianGen Plant Genomic DNA Kit; lanes 3 and 4, the modified CTAB-A method; lanes 5 and 6, our promoted CTAB-B method. As the TianGen Plant Genomic DNA Kit was able to isolate any DNA from the leaves, the photos were not shown in Fig. 3.

The present study was motivated by the need for better methods of extracting sufficient quantities of high-quality DNA from plant tissue rich in secondary metabolites for use in molecular marker assays. The promoted CTAB-B protocol described here efficiently eliminates most of the interfering molecules, including polyphenols, polysaccharides, and proteins, and it yields translucent and water-soluble DNA pellets without RNA contamination. The main protocols made in this method were grounding PVPP together with the plant material, an increase in the volume of high salt extraction buffer, adding 3 mol/L sodium acetate (NaAc) in extraction buffer, dissolving the crude pellet in 1 M NaCl followed by RNase treatment, the purification of acid phenol extraction (phenol: chloroform: isoamylal alcohol (PCI)=25:24:1) and the use of pre-cooled ethanol and sodium acetate in precipitation and all these modifications helped to remove the interference of secondary metabolites in the DNA isolation. PVPP was sprinkled directly onto the frozen fresh leaf tissue in the mortar and vigorously ground with the leaf tissue in the presence of liquid nitrogen, which can avoid the oxidation of released polyphenols into

quinines, which in turn bind to nucleic acids and hinder the isolation of high quality DNA. Increase in the volume of extraction buffer can completely break down the cell walls and make more nucleotide acid released resulted in increasing the yield of DNA isolation. Krizman et al. (2006) postulated that the plant tissue amount per volume of extraction buffer has an effect on DNA quality and yield. Since the extraction buffer is responsible for the lysis of membranes and liberation of DNA from cellular organelles (Weising et al., 2005), the smaller the quantity of plant tissue per unit volume, the more optimal the lysis process. Striking the correct balance between plant tissue amount and extraction buffer volume would reduce the probability of co-precipitation of contaminants with the DNA pellet as the saturation concentration during precipitation is less likely reached or exceeded (Krizman et al., 2006). During the extraction, 3 mol/L sodium acetate (NaAc) added combined with chloroform/isoamyl alcohol extraction can reduce markedly the co-precipitation of polysaccharides with the nucleic acids and remove most proteins, polysaccharides, polyphenols and other impurities for the first time. The crude nucleic pellet was dissolved in 1 ml of 1 M NaCl instead of dissolving it in Tris-EDTA (TE), which ensured further reduction of viscosity of the mucilaginous substances (Chen and Chen, 2004; Ghosh et al., 2009). DNase-free RNase was added to crude DNA samples dissolved in1 ml of 1 M NaCl to completely clear residual RNA. After RNase treatment, the DNA solution requires purification with an acid-phenol: chloroform : isoamyl alcohol (25: 24: 1) extraction because small amounts of protein in DNA pellets and salts in the RNase reaction buffer and stop solution both influence downstream molecular procedures such as restriction endonuclease digestion, ISSR-PCR amplification and full the genomic sequencing. Thus, we used acid-phenol to remove residual protein and the remaining salts after the RNase treatment. Finally, it is necessary to precipitate DNA simultaneously with sodium acetate (pH 5.2) and absolute ethanol which can completely remove the residual polysaccharides from the DNA sample resulted in increasing the yield of DNA isolated.

3.2 Assessment of the quality and quantity of the total DNA

The success of an DNA isolation protocol may be judged by the quality and quantity of DNA recovered. The quality of DNA was assessed by gel electrphoresis, spectrophotometry, restriction endonuclease digestion and PCR amplification. The mean yield of DNA extracted by our promoted method was approximately 60.29 ± 20.16 μg/100mg of fresh leaves, which was higher than that of the modified CTAB-A method (35.72 ± 15.41 μg/100mg) and the modified TianGen Plant Genomic DNA Kit (20.54 ± 8.43 μg/100mg) (Table 1). The DNA isolated by our promoted method also exhibited good purity. DNA absorbs UV light maximally at 260 nm, whereas protein absorbs at 280 nm and other contaminants including carbohydrates, phenol, and aromatic compounds generally absorb around 230 nm. Therefore, the A260/A280 and the A260/A230 ratios are often used as indicators of DNA sample purity. Generally, ratio values of A260/A280 in the range of 1.8 – 2.0 indicate high-purity DNA; the ratio values of A260/A280 less than 1.8 indicate protein contamination in DNA samples; the ratio values of A260/A280 more than 2.0 indicate much RNA or many DNA fragments in DNA samples. With our method, the A260/A280 and A260/A230 ratios were 1.86 ± 0.16 and 1.92 ± 0.13, respectively, indicating that the DNA was free of protein and polysaccharides/polyphenol contamination (Table 1). In addition, there were no other bands visible in the bands (Fig. 1, lanes 5,6), indicating that the DNA was free of genomic RNA contamination. In contrast, the DNA from the CTAB-A method had poor purity as assessed by A260/A280 (2.12 ± 0.18) and the A260/A230 ratios (1.55 ± 0.36 μg/100mg), and

the yield was lower than that obtained with our promoted CTB-B method and the DNA was badly contaminated with RNA because there were two other visible bands on the lane (Fig. 1, lanes 3,4; Table 1). The TianGen Plant Genomic DNA Kit was not able to isolate any DNA from the leaves of *R. soongorica*. Furthermore, even the improved kit method only yielded a small amount of DNA (20.54 ± 8.43 μg/100mg), and it was seriously contaminated (Fig. 1, lanes 1,2). The above results show that the improved CTAB-B protocol described herein efficiently eliminates most of the interfering molecules (including polyphenols, polysaccharides, proteins and salts), and it also provides a higher yield of DNA pellets that are translucent, water-soluble and lack RNA contamination, indicating that it is superior to the CTAB-A method and the commercial kits.

Method	Purity		Yield
	$A_{260/280}$	$A_{260/230}$	(μg/100mg)
Modified TianGen DNA kit	1.31 ± 0.15	0.87 ± 0.32	20.54 ± 8.43
Modified CTAB-A	2.12 ± 0.18	1.55 ± 0.36	35.72 ± 15.41
Modified CTAB-B (promoted by us)	1.86 ± 0.16	1.92 ± 0.13	60.29 ± 20.16

Table 1. The genomic DNA purity and yield in *R. soongorica* leaves by different methods

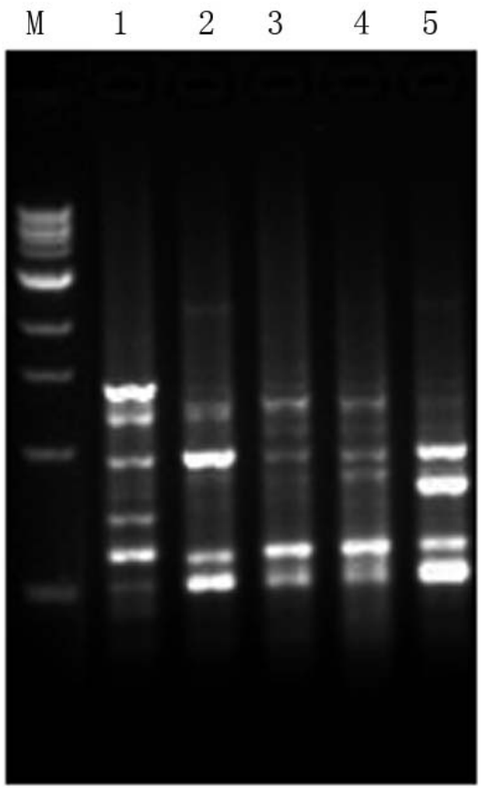

Fig. 4. ISSR-PCR profiles of the genomic DNAs isolation from different *R. soongorica* populations by our promoted CTAB-B protocol using the UBC-807 primer were analyzed on a 1.5% agarose gel stained with ethidium bromide in 1×TAE buffer. "M" represents the Molecular weight marker – DNA marker DL2000.

The suitability of extracted DNA for downstream molecular processes was further verified by molecular markers ISSR-PCR amplification. AS shown in Fig. 4, the genomic DNA of five different *R. soongorica* populations were highly amplifiable by ISSR-PCR as indicated by the amplification products resolved on 1.5% agarose gel. This further confirmed the purity of DNA, free of polysaccharide and polyphenol contamination, which would otherwise inhibit Taq DNA polymerase and restriction endonucleases (Ahmad et al., 2004). Plant molecular applications such as RAPD and AFLP necessitate the successful isolation of high quality DNA (Michiels et al., 2003; Ahmad et al., 2004), devoid of contaminants. Without high quality DNA such downstream molecular manipulations are not feasible (Varma et al., 2007). To confirm the applicability of our method, this DNA extraction method has also been found to be efficient in other desert plants, including *Tamarix ramosissima*, *Nitraria tangutorum* and *Caragana korshinskii* Kom. (data not shown).

4. Conclusion

Our results showed that the modified CTAB-B method promoted here was of high quality, purity and yield and was suitable for downstream molecular assays. Based on a CTAB method, the protocol has been improved as follows: the more volume of extraction buffer was used to completely break down the cell walls; the samples were ground with PVPP to effectively inhibit the oxidation of phenolics; during the extraction, 3 mol/L sodium acetate (NaAc) was added to reduce markedly the co-precipitation of polysaccharides with the nucleic acids; contaminating RNA was removed with RNase I; acid phenol extraction (phenol: chloroform: isoamylal alcohol (PCI)=25:24:1) was used to effectively removes the residual proteins and inhibitors in the RNase reagent. Thus, despite the high levels of secondary metabolites in the leaves of *R soongorica*, the high quality DNA is isolated from the nuclei without interference. Moreover, the new protocol is also suitable for isolating genomic DNAs from other desert plant species and tissues that are rich in secondary metabolites.

5. Acknowledgments

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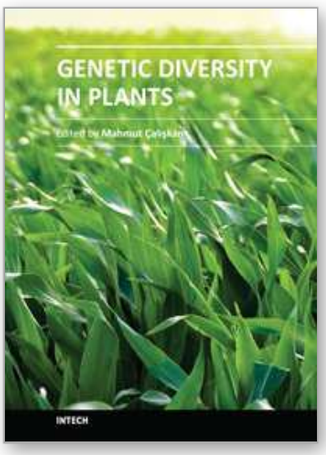

Genetic Diversity in Plants

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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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