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Appendix

Obtaining Genomic DNA for Construction of Genomic Library

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Keywords: Genomic library, dragon fruit, restriction digest, high molecular weight DNA, Cactaceae

Abstract

The isolation of pure genomic DNA from the white and red dragon fruits, *Hylocereus undatus* and *Hylocereus polyrhizus* (Cactaceae) for the construction of a genomic library is described. These vine cacti originate from northern South America, Central America and Mexico, and are cultivated as new exotic fruit crops in SE Asia. Successful establishment of a genomic library serves as a platform for molecular studies, such as gene characterization and gene analysis, but intact and high molecular weight DNA of good quality can be difficult to obtain. Furthermore, presence of polysaccharides in DNA extracts interferes with downstream processes such as restriction digest and polymerase chain reaction (PCR). Genomic DNA can be extracted using various plant parts from 2–3-week-old seedling leaves, roots and stems. DNA quality is checked using a spectrophotometer and digested using *Bam*H1. Genomic DNA so isolated had the highest quality following restriction digestion analysis.

INTRODUCTION

Dragon fruits of the genera *Hylocereus* and *Selenicereus* which originate from northern South America, Central America, and Mexico, are currently being grown as new exotic fruit crops in SE Asia. The fruit 'flesh' largely includes a mucilaginous placenta in which is found thousands of small seeds (Le Bellec *et al.*, 2006).

At present, only three species are being cultivated on a commercial scale which is *Hylocereus undatus*, *Hylocereus polyrhizus* and *Selenicereus megalanthus* in Colombia, Israel, Vietnam and Nicaragua (N. Tel-Zur *et al.*, 2005). Vietnam is the biggest producer of the *Hylocereus* spp. However, only *H. undatus* and *H. polyrhizus* are being cultivated in small scale in Malaysia.

These three species are distinguished by peel and pulp colour. *H. polyrhizus* fruit has red peel and red pulp while *H. undatus* fruit has red peel and white pulp. *S. megalanthus* has yellow peel and white pulp. The red colouration is due to the presence of betalain pigments. Plantations of such non-native fruit species are affected by various diseases that lead to poor fruit yield, threatening the income of small-scale farmers who resort to application of pesticides and herbicides. This problem can be solved by establishing a genomic library that serve as a platform to study the gene interaction. The overall procedure involved in genomic library construction includes isolation of genomic DNA, generation of DNA fragments for cloning, packaging and transduction.

MATERIALS AND METHODS

Plant material

Red and white dragon fruits were obtained from a local market. Their seeds were separated from the pulp and air-dried. The seeds were allowed to germinate for about two weeks and seedling leaves were used for DNA extraction.

Reagents and solutions

DNA isolation buffer [0.15M Sorbitol, 0.125M Tris-base, 0.05M EDTA, $8.3x10^{-3}$M Sodium Bis-sulfide, 0.83M NaCl, 2% w/v hexadecyltrimethly-ammonium bromide (CTAB), 0.83% v/v Sodium Sarkosyl, 1% w/v Polyvinylpyrollidone (PVP) (MW 40,000), 1% v/v β-mercaptoethanol)] Chloroform: isoamyl alcohol (24:1), 5M NaCl, Isopropanol, 70% ethanol, TE buffer [10mM Tris-HCl (pH 8.0), 1mM EDTA (pH 8.0)], RNase A (0.1µg/µl)

DNA isolation

A total of 400µl of DNA isolation buffer was added into each 1.5-ml microfuge tube. Leaf fragments from 2-week-old seedlings were weighed and pulverized in liquid nitrogen. 0.20g of leaf sample was transferred into each buffer-added microfuge tube. Microfuge tubes were quick-spun. An additional 300µl of DNA isolation buffer was added. All tubes were gently flicked and inverted to thoroughly mix the sample and incubated at 65°C for 40 minutes (tubes were gently flicked and inverted at 10-minute intervals). 700µl of chloroform: isoamyl alcohol (24:1) was added to each microfuge tube and tubes were gently inverted until an emulsion formed. Samples were centrifuged at 10,000 rpm for 15 minutes. The aqueous phase was gently transferred into new 1.5ml microfuge tubes. The chloroform: isoamyl alcohol procedure was repeated. Half volume of 5M NaCl was added and microfuge tubes were gently inverted. Two volumes of cold isopropanol (-20°C) was added into each microfuge tube and tubes were gently inverted before being incubated on ice for 10 minutes. Samples were centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded. DNA pellets were washed twice with 500µl of cold 70% ethanol (-20°C). Samples were centrifuged at 10,000 rpm for 1 minute. The supernatant was discarded and the pellet washing procedure repeated. DNA pellets were air-dried at room temperature, and then dissolved in 30 to 50µl of TE buffer (depending on their solubility). RNase A was added to each sample (1µl/10µl DNA sample), which was then incubated at 37°C for 1 hour. After RNase A treatment, total DNA was run on a 0.8% agarose gel against a marker of known molecular weight, and stained with ethidium bromide for visualization (Figure 1). The samples were stored at -20°C.

Analytical analysis

Extracted DNA samples were quantified using a spectrophotometer at A_{260nm} while the purity of the DNA was checked through A_{260nm}/A_{280nm} (Table 1).

Partial digestion of the Genomic DNA

Partial digestion of the genomic DNA was carried out with the enzyme *Bam*H1 according to the Stockinger Lab 06/01/05 protocol. After digestion, the product was analyzed through 0.8% agarose gel electrophoresis (Figure 2).

RESULTS AND DISCUSSION

There are several published works that have reported on the use of different plant parts for obtaining genomic DNA, such as callus tissue and roots. Roots were chosen as the source tissue due to the lower viscosity of the extracts relative to that from other plant parts. Leaf callus, essentially an unorganized and undifferentiated cell mass, lacks synthesis of secondary metabolites and so may be more easily dealt with (Diadema *et al.,* 2003 and N. Tel-Zur *et al.,* 1999).

White and red dragon fruits contain high amounts of polysaccharides in their tissues generally. The polysaccharides bind to the DNA, rendering it viscous and glutinous after precipitation of the DNA. This makes the DNA unsuitable for downstream applications such as PCR and restriction digest, as the applied enzyme cannot access the DNA (N. Tel-Zur *et al.,* 1999, Barnwell *et al.,* 1998 and Puchooa, 2004). However, DNA extraction with CTAB in high salt condition suppresses the co-precipitation of polysaccharides and DNA (Murray and Thompson, 1980; Lodhi *et al.,* 1994).

In plant cells, there is an abundance of secondary metabolites. To prevent the interaction of these compounds with DNA, β-mercaptoethanol and/or polyvinyl pyrrolidone (PVP) is often added to the buffer solution. PVP forms complexes with polyphenolics through hydrogen bonding and β-mercaptoethanol reduces oxidation of phenolic compounds (Micheils *et al.,* 2003, De la Cruz *et al.,* 1997 and Maliyakal, 1992).

High yield and quality of DNA from leaf extractions were attributed to active cell division, which produces high cell density and little or no synthesis of secondary metabolites. In addition, the extracted genomic DNA can be restricted using enzyme *Bam*H1.

Table 1 show that DNA extraction using leaves provides higher purity compared to other parts of the plants. Figure 1 also shows that DNA extraction using young leaves yielded a higher amount of DNA. However, when the same extraction protocol was carried out on stem tissue, the extract was viscous throughout the extraction process due to the presence of high amount of polysaccharides (N. Tel-Zur *et al.,* 1999). The root extraction carried out in this study follows the protocol by N. Tel-Zur *et al.,* 1999 and resulted in a brown pellet. This may be caused by the binding of secondary metabolites to the DNA which results in low yield of DNA. Furthermore, Figure 2 confirms that the DNA extract from leaves is of high quality, implying that the enzyme efficiently digested total DNA into smaller fragments.

CONCLUSION

From the experiment conducted, young seedling leaves gave the best quality of DNA and yield. This was confirmed through spectrophotometer and gel electrophoresis assessments. The ratio of absorbance at 260nm to 280nm fall between 1.8-2.0 range and the extracted genomic DNA can be restricted.

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Tables

Table 1. DNA purity and DNA yield using different plant materials

Plant material	A_{260nm}	A_{280nm}	Ratio (A_{260nm}/ A_{280nm})	DNA yield (µg/g)
Stem	0.008	0.007	1.14	20.0
Root	0.023	0.018	1.28	57.5
Leaves (White dragon fruit)	0.081	0.042	1.92	202.5
Leaves (Red dragon fruit)	0.093	0.049	1.90	232.5

Figures

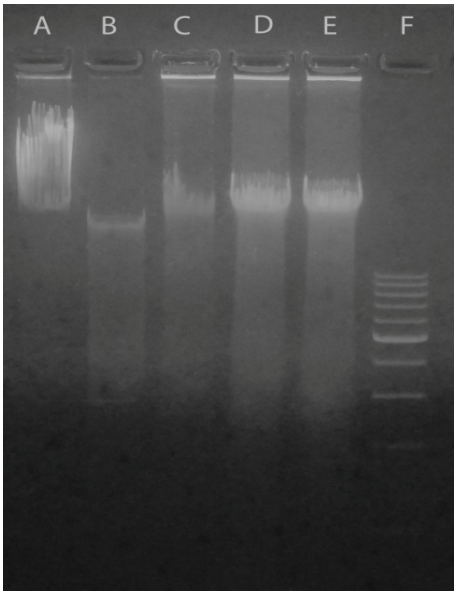

Fig. 1. Electrophoresis of genomic DNA extracted using different plant materials. A- Lamda DNA ; B- stem ; C- Root; D- Leaves *Hylocereus undatus* ; E- Leaves *Hylocereus polyrhizus* ; F = 1kb DNA ladder

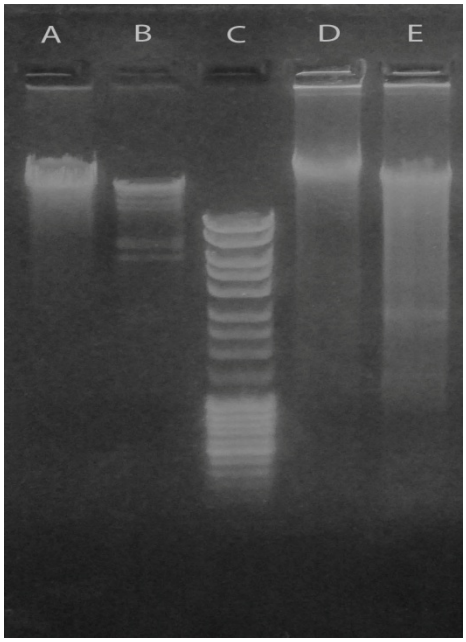

Fig. 2. Electrophoresis of *Bam*H1 restricted genomic DNA extracted from *Hylocereus* leaves. A- Lambda DNA ; B- Lambda DNA digested with *Bam*H1; C- 1kb DNA ladder; D- Total DNA *Hylocereus* ; E- Total DNA *Hylocereus* digested with *Bam*H1