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METHOD

Recycling Isolation of Plant DNA, A Novel Method

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ABSTRACT

DNA is one of the most basic and essential genetic materials in the field of molecular biology. To date, isolation of sufficient and good-quality DNA is still a challenge for many plant species, though various DNA extraction methods have been published. In the present paper, a recycling DNA extraction method was proposed. The key step of this method was that a single plant tissue sample was recycled for DNA extraction for up to four times, and correspondingly four DNA precipitations (termed as the 1st, 2nd, 3rd and 4th DNA sample, respectively) were conducted. This recycling step was integrated into the conventional CTAB DNA extraction method to establish a recycling CTAB method. This modified CTAB method was tested in eight plant species, wheat, sorghum, barley, corn, rice, *Brachypodium distachyon*, *Miscanthus sinensis* and tung tree. The results showed that high-yield and good-quality DNA samples could be obtained by using this new method in all the eight plant species. The DNA samples were good templates for PCR amplification of both ISSR and SSR markers. The recycling method can be used in multiple plant species and can be integrated with multiple conventional DNA isolation methods, and thus is an effective and universal DNA isolation method.

KEYWORDS: Plant; DNA isolation; Tissue recycling; Molecular marker; PCR amplification

INTRODUCTION

Isolation of sufficient and high-quality genomic DNA is vital to DNA-based molecular biology experiments. Various intracellular materials in plants interfere with isolation of clean DNA. To date, many DNA isolation methods have been published. These methods aimed to improve the quality and yield of DNA isolation in different plant species, and mainly included sodium dodecyl sulfate (SDS) method (Marmur, 1961), cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990), high salt low pH method (Guillemaut and Marechal-Drouard, 1992), salt extraction method (Aljanabi and Martinez, 1997), and NaOH method (Hill-Ambroz et al., 2002). Recently high-throughput DNA isolation methods and even the automatic facility are available (Stein et al., 2001; Hill-Ambroz et al., 2002; Mogg and Bond, 2003; Post et al., 2003; Xin et al., 2003; Allen et al., 2006). Specific DNA isolation methods were developed for the major crop species, sorghum (Woo et al., 1995), sugarcane (Honeycutt et al., 1992), rice (Ahmadikhah, 2009), corn (Oard and Dronavalli, 1992), tomato (Fulton et al., 1995), barley (Saini et al., 1999), cotton (Li et al., 2001), peanut (Sharma et al., 2000) and pepper (Ogunkanmi et al., 2008), and a few minor plant species such as moss (Schlink and Reski, 2002), chocolate and date palm tree (Haymes et al., 2004).

However, chemotypic heterogeneity of different tissues often does not allow the direct application of an extraction method for a specific species to DNA isolation of other species. Thus some modifications should be made with the purpose of reaching an optimum DNA isolation. Furthermore, many plant species are inherently difficult for DNA extraction. Most of the medicinal, aromatic and woody plants contain high level of secondary metabolites such as alkaloids, flavonoids, polyphenols, gummy polysaccharides (Loomis, 1974; Scott

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and Playford, 1996; Shepherd et al., 2002; Vural, 2009; Bharmauria et al., 2010). These components are quite complicate and always disturb DNA isolation. They can be co-isolated with DNA, add color to the DNA sample, cause malfunction of the pipettor and interfere with the downstream DNA-based experiments (Merlo and Kemp, 1976; Murray and Thompson, 1980; Demeke and Adams, 1992; Fang et al., 1992; Pich and Schubert, 1993; Pandey et al., 1996; Khanuja et al., 1999; Pirttilä et al., 2001; Ogunkanmi et al., 2008).

In general, efficient elimination of the non-DNA chemical components plays a key role in isolation of high-quality DNA from plants tissues. There are three main components, including polysaccharides, polyphenols and proteins, which should be responsible for the increased difficulties in DNA isolation. To solve this problem, several reagents were widely used to decrease the negative influence of these chemical components on quality and yield of DNA isolation. For instance, to reduce polysaccharides, sorbitol (Li et al., 2001), mannitol, glucose (Paterson et al., 1993), sucrose (Permingeat et al., 1998) and higher concentration NaCl (Khanuja et al., 1999) were applied into the DNA extraction buffer. High concentration of polyvinylpyrrolidone (PVP) and β-mercaptoethanol were used to remove the polyphenols (Ribeiro and Lovato, 2007). Proteinase K was chosen to degrade the protein existing in the DNA solution (Goldenberger et al., 1995). These reagents and chemicals were efficient to some extent for some species, but could not meet the demands of removing large amount of complex intracellular chemicals.

Therefore, a novel and universal DNA isolation method is eagerly needed to eliminate interference of the complex chemicals in plant cell. Here in this study, we reported a novel method of DNA extraction, i.e., recycling DNA isolation. The major principles were that a sample was repeatedly treated by CTAB extraction buffer for four times, and four corresponding DNA precipitations were obtained. This method was tested in genomic DNA isolation of eight plant species.

RESULTS

Effect of extraction buffer volume on DNA yield

The main steps of the recycling DNA isolation method were graphically illustrated in Fig. 1. The yield of wheat genomic DNA was basically the same between the two volumes, 500 μL and 1000 μL, for all the four cycles of extraction (Fig. 2). The similar results were obtained in barley. Therefore, no obvious effect of buffer volume was observed on DNA yield when the two levels of extraction buffer volume were tested.

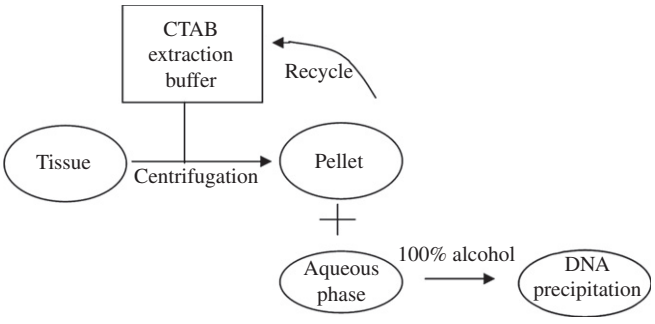

Fig. 1. The simplified flow chart of the recycling DNA isolation method.

DNA yield and quality of eight plants species

Using agarose gel electrophoresis and λDNA of known concentration as standards, DNA concentration of all the samples were determined. As shown in Fig. 2, DNA of the 1st DNA samples for the eight plant species was not detectable except for wheat and rice. But the 2nd, 3rd and 4th DNA samples for each species were obviously detectable except for the 4th DNA samples of sorghum and *B. distachyon*. Furthermore, the brightness of DNA bands was different among the eight species, indicating efficiency variation of DNA isolation among plant species. Table 1 showed that DNA concentration varied in the range of 100−500 ng/μL except those undetected in agarose gel (Fig. 2).

The DNA concentration was also measured by ultraviolet spectrophotometer. As shown in Table 1, concentration value of the 1st DNA for all the eight species was the highest, especially for wheat (2455.8 ng/μL), sorghum (1160.0 ng/μL), barley (1901.7 ng/μL) and *B. distachyon* (1784.2 ng/μL). Concentration value of the 2nd, 3rd and 4th DNA for each species was decreased gradually with the increase of isolation cycle. Furthermore, there is an obvious difference among the eight plant species for DNA concentration variation range from the 1st DNA to the 4th DNA samples (Table 1). For instance, the concentration variation range was 2455.8−344.2 ng/μL for wheat, 1160.0−88.3 ng/μL for sorghum, and 309.2−160.0 ng/μL for *M. sinensis*. Using ultraviolet spectrophotometer, two parameters, i.e., the absorbance ratios of $OD_{260/280}$ and $OD_{260/230}$ showing purity of DNA solution, for each DNA sample were also recorded (Table 1). $OD_{260/280}$ absorbance ratios of the extracted DNA samples (except for corn) varied mainly in the range of 1.8−2.0, indicating low protein and RNA content in the DNA samples. Meanwhile, except for all the four corn DNA samples, the 1st DNA of sorghum, and the 3rd and 4th DNA of rice, $OD_{260/230}$ absorbance ratios were all greater than 2.0, indicating low contents of polyphenols and carbohydrates in the DNA samples (Table 1). Therefore, using the recycling DNA isolation method, high-quality and high-yield DNA could be obtained from multiple plant species.

To graphically illustrate difference for DNA concentrations detected by the two determination methods above, line and scatter plots (Sigma Plot 10.0) were made (Fig. 3). As shown in Table 1 and Fig. 3, the 1st DNA concentration discrepancy between the two methods was huge, 76 times for barley, 12 times for *M. sinensis*. Discrepancy for the 2nd DNA concentration determined by the two methods were also observed, but largely decreased in comparison with the 1st DNA, the biggest discrepancy (8.3 times) for *B. distachyon* and the smallest discrepancy (1.93 times) for corn. When DNA isolation reached the 3rd and 4th cycle, difference between the two concentration determination methods became small and the ratio of DNA concentration by the two methods was close to 1.