A Rapid DNA Minipreparation Method Suitable for AFLP and Other PCR Applications

D.-H. CHEN** and P.C. RONALD*
Department of Plant Pathology, 1 Shields Ave., University of California, Davis CA 95616, USA

Abstract. A rapid DNA minipreparation method was developed for rice and other plant species. This method uses an Eppendorf tube and 1-ml pipette tip to grind plant tissues, and requires only one transfer for DNA isolation. In a single day, one person can complete DNA isolation from more than 120 leaf samples. The yields of the DNA samples ranged from 2.3 to 5.2 µg from 25–50 mg fresh leaf tissue. DNA samples extracted using this method from rice were completely digested with five restriction enzymes (EcoRI, EcoRV, HindIII, MseI and PstI) and were successfully used for AFLP and other PCR applications.

Key words: CTAB, DNA extraction

Introduction

PCR-based methods are widely used in plants for marker-assisted breeding and high-resolution mapping. Because these studies require analysis of large populations, a DNA extraction method, which is fast, inexpensive and yields high quality DNA, is desired.

Rapid and efficient rice DNA extraction methods suitable for PCR have previously been developed (Williams and Ronald, 1994; Zheng et al., 1995). These methods, however, do not work well for AFLP analysis as yield is low, quality is poor and there are risks of cross contamination (Chen and Ronald, unpublished).

A rapid CTAB DNA isolation technique for extracting DNA from five plant species and one fungus has been proven effective for PCR analysis.
D.-H. Chen and P.C. Ronald

(Stewart and Via, 1993). Barley DNA prepared using this method was successfully used for AFLP analysis and high-resolution mapping of the barley MLO locus (Büschges et al., 1997). However, since rice leaves are composed of high quantities of cellulose, lignin, silica cells, wax, etc., leaf samples cannot be easily homogenized using this method (Chen and Ronald, unpublished). We have now modified the CTAB method for rapid isolation of DNA from rice and other species. The DNA is suitable for AFLP analysis and other PCR-based applications. Using this method, high quality DNA samples from a segregating population consisting of 2200 rice plants were extracted by a single person in less than twenty days.

Materials and Methods

Leaves of rice, grape, maize, squash, tomato, peppermint, and walnut were collected and set in ice. An Eppendorf 1 ml plastic pipette tip (Out Patient Service, Inc) was bent by pressing the tip against the bench. The bent pipette tip was then mounted on a disposable pellet pestle (VWR Scientific Cat. KT749520-0000) attached to a drill (Black & Decker (US) Inc) to serve as the homogenizing pestle. A fragment of leaf sample (25–50 mg) was rounded into a ball on the end of a forceps and put into a 1.5-ml Eppendorf tube on ice. The leaf segment must be folded or rounded rather than cut into pieces before being placed in an Eppendorf tube to avoid losing the leaf tissue when adding the liquid nitrogen. Using forceps, the uncapped tube with leaf or root tissue was dipped into liquid nitrogen to allow the liquid nitrogen enter the tube. It was then homogenized for 20 s at full speed. Immediately after homogenization, the tissue powder was added to 700 μl pre-warmed (65 °C) extraction buffer [2% w/v CTAB, 1.42 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 2% w/v polyvinylpyrrolidone (PVP-40), 5.0 mM ascorbic acid, 4.0 mM diethyldithiocarbamic acid (Doyle and Doyle 1990)]. 7 μl Rnase A (20 mg/ml) was added and the mixture stirred and incubated at 65 °C for approximately 5 min. 570 μl of a chloroform: isoamyl alcohol mixture (24:1) was then added, and the mixture was shaken by hand and centrifuged at a full speed (13,000 rpm) for 10 min at room temperature. The upper DNA containing phase was transferred to a new Eppendorf tube. DNA was precipitated by adding 0.7 vol of isopropanol, mixing and immediately spinning at full speed for 5 min. The DNA pellet was washed with 70% ethanol, air-dried and suspended in 15 μl of TE. The extracted DNA was subjected to five restriction enzyme digestions (EcoRI, EcoRV, Hind III, MseI and PstI) and to AFLP (Vos et al., 1995) and PCR analysis.