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A Simple DNA Extraction Method for PCR Amplification from Dry Seeds of *Brassica napus*

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(The first two authors have same contribution to this paper)

Abstract: A simple and reliable DNA extraction method for dry seeds of *Brassica napus* has been developed in our laboratory. The NaCl and PVP were used to remove polysaccharides and polyphenols during DNA purification. The oil and proteins of dry seeds were removed only through centrifugation in this method. The RAPD amplification patterns have no obviously difference between the DNA extracted from dry seeds and fresh leaves extracted with control method. The good results of SSR molecular markers on the DNA of dry seeds of another 12 *B. napus* indicating that the DNA extracted from dry seeds was freedom from common contaminating compounds. In conclusion, this method could be widely used in DNA extraction from dry seeds of *B. napus*.

Key words: DNA, extraction *B. napus*, seeds, RAPD, SSR

INTRODUCTION

Brassica napus was one of the important oilseed crops in the China and worldwide, which has been the subject of extensive genetic studies due to their cultivation and importance. Recently, this species has been used for molecular analysis, such as genetic diversity analysis (Seyis *et al*., 2003; Pallett *et al*., 2006) and gene mapping (Lombard and Delourme, 2001; Kole *et al*., 2002; Javidfar *et al*., 2006). Most of the plant DNA extraction methods are essentially either a CTAB method (Murray and Thompson., 1980) or an SDS-potassium-acetate method (Dellaporta *et al*., 1983). However, these two methods are not appropriate for a large number of DNA extractions in a short period of time such as for marker-assisted selection in rapeseed breeding for their time consuming. Various simple methods by using the leaves as materials have been developed such as a direct amplification of leaf tissues (Berthomieu and Meyer, 1991), boiling method (Thomson and Henry, 1995; Ikeda *et al*., 2001) and alkali treatment method (Xin *et al*., 2003). In *Brassica* species, we have successfully tested several procedures for DNA extraction by using fresh leaves as materials (Horn, 1992; Li *et al*., 1994), but few methods were developed for dry seeds. In this paper, we reported a simple DNA extraction protocol for dry seeds of *B. napus*, it could be finished very quickly and could produce relatively high quality DNA for RAPD and SSR molecular markers.

MATERIALS AND METHODS

Materials: Dry seeds of *B. napus* were used for DNA extraction. The fresh leaves of one *B. napus* cultivar were as a control.

Methods:
DNA extraction method for dry seeds:

- Grind 0.5 g of dry seeds into fine powder by using mortar and pestle in liquid nitrogen and transfer into the 1.5 mL^{-1} eppendorf tube with 1 mL DNA extraction buffer (1 mol L^{-1} NaCl, 50 mmol L^{-1} This-HCl (pH 8.0), 50 mmol L^{-1} EDTA (pH 8.0), 1% PVP).
- Incubate the sample at 65°C water bath at least 10 min while vortexing 3-4 times to mix powder evenly.
- Centrifuge at 13200 rpm for 5 min at 4°C and three layers were formed, pipette off the middle aqueous phase (about 700 μL) into a new 1.5 mL tube.
- Add 2/3 volume of isopropanol and gently mix, then centrifuge at 13200 rpm for 5 min, remove away the supernatant of aqueous phase after centrifuge, soak the DNA in the tube for about 10 min at 1 mL 75% ethanol.
- Dry the DNA in room temperature or air condition and then add 100-200 μL TE buffer (10 mmol L^{-1} This-HCl (pH 8.0), 1 mmol L^{-1} EDTA (pH 8.0) to dissolve DNA. Add 5 μL RNase A (stock solution: 10 mg mL^{-1}, sigma), incubate at 37°C for 1 h.

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Finally, centrifuge at 13200 rpm for 5 min another time and then pipette off the aqueous phase into another 1.5 mL tube for molecular analysis.

The control DNA was extraction from fresh leaves of *B. napus* by using the method of Horn *et al.* (1992).

RAPD analysis: 8 RAPD primers; S1069, S1070, S1074, S1077, S1084, S1085, S1087 and S1089 (SBC-Shanghai) were used were used for RAPD analysis. The PCR procedure was followed the method of Li *et al.* (2005a). The PCR reaction profiles were as follows: 1 cycle of 3 min at 95°C, 1 min at 50°C, 1.5 min at 70°C; 2 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 70°C, 38 cycles of 1 min at 94° C, 1 min at 40°C and 1.5 min at 72°C followed with 1 cycle of 1 min at 94°C, 1 min at 40°C, 10 min at 72°C. PCR was carried out in a total volume of 20 μL per reaction, containing about 50 ng of genomic DNA, 8 μM of random primers, 0.2 mM of all the four dNTPs, 1×PCR buffer, 1.5 mM of $MgCl_2$ and 1.5 U of *Taq* DNA polymerase.

SSR analysis: The PCR procedure was followed the method of Saal *et al.* (2001) and Li *et al.* (2005b). The PCR reaction profiles were as follows: 94°C at 60 sec followed by 35 cycles of 60 sec at 94°C, 60 s at 61°C and 1.5 min at 72°C, extension at 72°C for 10 min and then held at 4°C. The 20 μL of formamide loading Buffer was added into selective amplification reaction products, the mixed samples were denatured in 95°C for 5 min cooled on ice and then analysis in 6% PAGE gel. The gel was stained with silver staining kits (Promega, Madison, Wis., USA) according to the manufacturer's instructions. Five primer pairs, Ra3-H09, Ra2-H12, Ra3-C04, Ra2-H07 and Na10-E09 were used and it was downloaded from the *Brassica* database (http://www.ukcrop.net).

RESULTS AND DISCUSSION

The materials that used in DNA extraction were fresh leaves in most cases (Murray and Thompson, 1980; Dellaporta *et al*., 1983; Saghai-Maroof *et al*., 1984; Doyle and Doyle, 1987; Lange *et al*., 1998). The DNA extraction method by using the seeds as material was also reported in some crops, such as in rice (Chunwonges *et al*., 1993; Peng *et al*., 2002), corn (McDonald *et al*., 1994) and sorybean (Yang *et al*., 2003). We have obtained higher quality of DNA from dry seeds of *B. napus* by using the protocol outlined above. The fatty acid and proteins was the main compontents of the seeds of *B. napus*. For fatty acid has lower density and non-polar characteristic, it could easily be distinguished from the aqueous phase when it involved into centrifugation. The phenol and chloroform were frequently used for protein removing in custom DNA extraction method; it needed only need a short centrifugation to separate DNA from all the other contaminants in our present protocol, for the most of proteins were removed in the insoluble precipitate. The 1M NaCl was added into extraction buffer to remove the polysaccharides by increasing their solubility in ethanol (Fang *et al*., 1992). In order to remove polyphenols from the dry seeds, the PVP was added to the extraction buffer according to the results of Maliyakal (1992).

By using the DNA extraction protocol outlined above, we have obtained higher quality of DNA from dry seeds of *B. napus* with A260/A280 between 1.7 and 2.0, which indicated that the protein could be removed through centrifugation. The DNA was further analyzed in agarose gel, there was no DNA degradation and the average size of the bands was about 20 kb or so (Fig. 1). The DNA extracted from dry seeds was successfully used in RAPD analysis, the results revealed that the band patterns amplified from the DNA of dry seeds has no obviously difference with the bands amplified from the control DNA of leaves (Fig. 2). In order to testify the applicability of this protocol, the dry seeds of another 12 *B. napus* cultivars were performed the DNA extraction protocol outlined above, the A260/A280 ratio was 1.6-2.0 among those 12 samples. We further analyzed the DNA in agarose gel and found them intact with no DNA degradation. Good amplification results of SSR molecular markers also vertified the good quality of these 12 DNA samples (Fig. 3). RNA could be removed by digesting the sample with Rnase (Horn, 1992). In our experiment, we

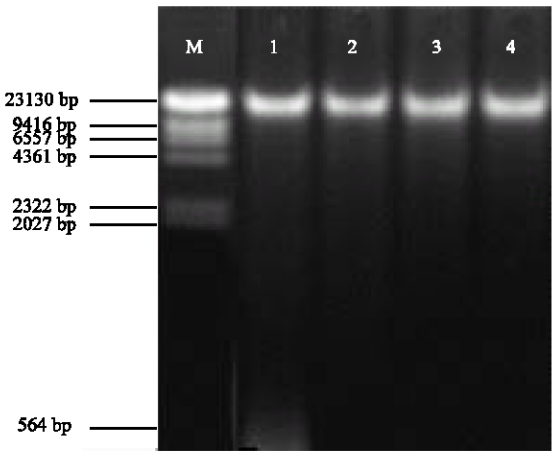

Fig. 1: The DNA extracted from dry seeds and fresh leaves. Lanes 1 and 2 represent the DNA extracted from dry seeds by using the method outlined above; Lanes 3 and 4 represent the DNA extracted from fresh leaves by using the control method of Horn *et al.* (1992)

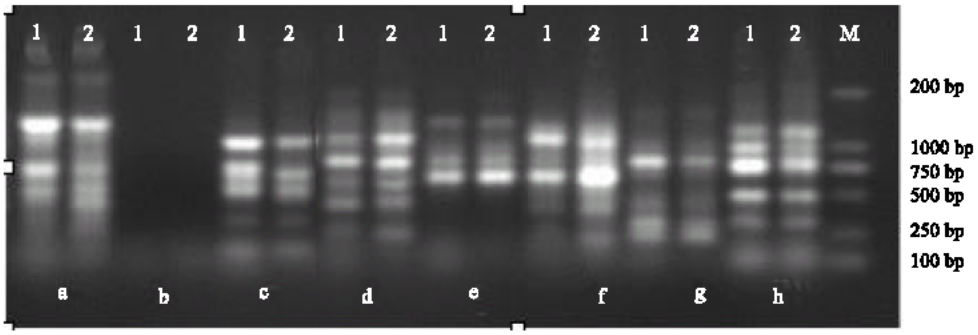

Fig. 2: Amplified band patterns of RAPD molecular markers with total DNA from dry seeds and fresh leaves. Lanes 1 and 2 represent the template DNA extracted from dry seeds and fresh leaves, respectively; a, b, c, d, e, f, g and h represent the RAPD primers of S1069, S1070, S1074, S1077, S1084, S1085, S1087 and S1089, respectively

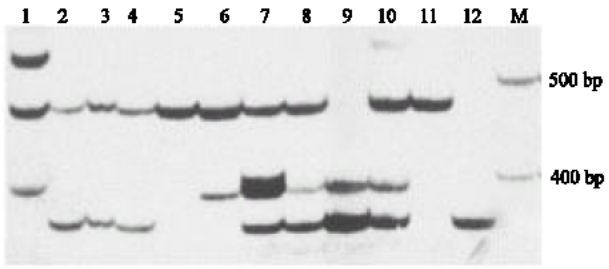

Fig. 3: SSR band pattern of DNA extracted from dry seeds of 12 *B. napus* cultivars by using the primer Ra3-C04. Lanes 1-12 represent different *B. napus* cultivars

found that the RNA did not interfere with marker development (data not shown), so the step for add the RNase A may be removed in our developed protocol. In conclusion, our newly developed DNA extraction protocol can produce clean and high-quality DNA that is suitable for RAPD and SSR molecular analysis in *B. napus*.

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