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Full Length Research Paper

A new protocol for extraction of *C0t-1* DNA from rice

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***C_0t-1* DNA, enriched for repetitive DNA sequences, has been proved to be valuable in the studies of plant species differentiation and genome evolution. A new protocol to steadily obtain the aimed range of DNA fragments has been developed by shearing the genomic DNA with the digest system containing DNase I and DNA polymerase I. Comparison through fluorescence *in situ* hybridization (FISH) - *Oryza sativa* L with labeled probe of *C_0t-1* DNA isolated by the autoclave equipment and the new procedure was carried out. FISH result shows that the new procedure can be a feasible method to steadily isolate high-quality *C_0t-1* DNA.**

Key words: *C_0t-1* DNA, Deoxyribonuclease I, DNA polymerase I, rice, repetitive DNA sequence.

INTRODUCTION

Repetitive DNA sequences, dispersed in the whole genomes, are significant components and important characteristic of most eukaryotic genomes (Rokka et al., 1998; Ohmido et al., 2000), usually amounting to more than 50% of higher plant (Flavell et al., 1974; Bennetzen et al., 2005). The DNA sequence of a repeat and its copy number at each chromosome site can all evolve rapidly, leading to the specificity of a certain genome/species, and even a chromosome (Galasso et al., 1995; Wang et al., 1995; Matyasek et al., 1997; Hall et al., 2004). Such changes prove to be valuable in studies of species divergence and genome evolution, in order to determine the putative progenitor and to establish the evolutionary relationships among different species and genomes (Matyasek et al., 1997). In this respect, repetitive DNA probes are far superior to genomic DNA probes. Lan et al. (2006) first presented the new thought to successfully discriminate the closely related A, B, C and D genomes in the genus *Oryza* by using fluorescence *in situ* hybridization (FISH) with *C_0t-1* DNA (DNA enriched for highly and moderately repetitive DNA sequences) of C genome.

C_0t-1 DNA mainly contains highly and moderately repetitive DNA sequences, including satellites, micro-satellites, and those in centromeres and telomeres, which determine the structure and characterization of chromo-some (Zwick et al., 1997). The procedure for isolation of *C_0t-1* DNA developed by Zwick et al. (1997) is applicable for all endogen and dicotyledon and can generate the high-quality *C_0t-1* DNA. However, this pro-cedure is difficult to control and generate the DNA with aimed range due to several factors, such as quality of DNA (Wei and Wan, 1999), disposed time of the DNA-shearing and different DNA-shearing equipments. In order to solve this problem, we have developed a new procedure to steadily obtain the aimed ranges of DNA fragments by shearing the genomic DNA with the digest system containing the DNase I and DNA polymerase I.

MATERIALS AND METHODS

According to nick translation theory, the genomic DNA was digested by DNase I and DNA polymerase I into the fragments of 100 - 1000 bp. DNA was reannealed at 65℃ for the required time calculated according to the formula Cot-1 = 1 = mol/L × Ts. Since highly and moderately repetitive DNA is reannealed faster than low or single copy sequences, some allotted time shall be passed after the reannealing. S1 nuclease is used to degrade low or single copy sequences that have not been reannealed.

Plant materials, chromosome and genomic DNA preparations

O.sativa ssp. indica cv. Guangluai 4 (*Oryza sativa* L.) were provided by Zuogui Zeng Hubei Academy of Agricultural Sciences. Chromo-

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some preparations were performed following the method by Ren et al. (1997). Total genomic DNA was extracted from *O. sativa* L. fresh leaves with cetyl trimethylammonium bromide (CTAB) method.

Isolation of *C_0t-1* DNA

C_0t-1 DNA preparation was based on the work of Zwick et al. (1997).

Procedures

1. DNA shearing with the digest system: The 50 µl mixture consisted of 100 - 1000 µg ml^{-1} total genomic DNA, 10 µl dNTP (dCTP, dGTP, dATP and dTTP mixture, 0.5 M), 1.5 µl DNA polymerase I.5 µl 10×buffer, 1 - 5 µl DNaseI (1 µg ml^{-1}), and the mixture was incubated at 15℃ for 1 - 2 h, then stopped at 65℃ for 10 min. 1 µL mixture was remove and its size determined by electrophoresis in a 1% agarose gel. Ideally, the DNA fragment should range between 100 - 1000 bp in size.
2. Genomic DNA was diluted to a concentration between 100 - 500 ng $µl^{-1}$ using 5 M NaCl and double-distilled H_2O to a final concentration of 0.3 M NaCl. The salt concentration influences the rate of reassociation of single-stranded DNA.
3. The mixture was then removed and put on ice.
4. The time needed for the reannealing reaction was calculated using the formula

$$Cot\text{-}1 = 1 = \text{mol } L^{-1} \times Ts$$

For example, if the sheared DNA is at a concentration of 500 ng $µl^{-1}$, using 1 mole of dNTP equal to an average of 339 g mol^{-1}, then mole of sheared DNA is equal to (0.5 g)/(339 g L^{-1}) = 1.47×10^{-3} mol L^{-1}.
5. After being denatured in boiled water for 10 min, the mixture was immediately chilled in ice for 10 min, and the sheared DNA was re-annealed in 65℃ according to the time calculated in step 4.
6. S1 enzyme was added to the solution and again, mixed thoroughly, but gently. Then the tube was incubated at 37℃ for 1 - 2 h. S1 nuclease can degrade linear single-stranded DNA.
7. The supernatant with an equal volume of phenol-chloroform was extracted twice in order to stop the reaction.
8. The supernatant with an equal volume of chloroform-isoamyl alcohol (24:1) was also extracted twice.
9. The DNA was precipitated overnight using 1/3 volume of 3 M CH3COONa（pH 7.0）and 2 volumes of 100% ethanol.
10. The solution was resuspended in 20 - 30 µl TE (Ph 7.0), and the *Cot*-1 DNA quantified by UV-Spectrometer or Ethidium bromide method. DNA was stored at -20℃ until needed.

FISH

The comparison through FISH - *O. sativa* L. with labeled probe of its own *C_0t-1* DNA isolated by the autoclave equipment and the new procedure - was carried out. The chromosomes were observed with an Olympus BX61 fluorescence microscope, and photographed with Cool-1300QS CCD controlled by Manager Expo 2.1.1 imaging system, karyotype analysis were carried out using the chromosome analyzing system FISH View EXPO 2.0 software.

RESULTS AND DISCUSSION

The early procedures for the isolation of *C_0t-1* DNA required the use of hydroxyapatitc columns, which are not economical and the DNA yields were variable owing to their sensitivity to temperature fluctuations and varying salt concentrations (Britten and Kohne, 1968). Zwick et al. (1997) have developed a method - inexpensive, fast, simple, and reliable - based on the uses of autoclave equipments and S1 nuclease to isolate *C_0t-1* DNA from plants; while the autoclaving method is more generally used than others in experimentation. This procedure is difficult to control and to generate the DNA fragments in aimed size ranges when the genomic DNA was being sheared in special equipments, such as autoclave, French press, or sonicator. The disposed time needed in autoclaving the genomic DNA is dependent on the nature of the DNA (that is, species, A/T-G/C content), concentration, purity and the initial molecular weight (Zwick et al., 1997). There was variation in the disposed time required for the shearing of DNA among different preparations of the same or different species. Otherwise, different equipment and quantity of the samples influenced the autoclaving efficiency. Thus, the physical methods of DNA shearing have poorer experimental repetition. To overcome this problem, we have developed a new procedure to steadily obtain the aimed range of DNA fragments by shearing the genomic DNA with the digest system containing DNase I and DNA polymerase I. In the digest system of the new procedure, working concentration of DNaseI is the key influencing factor for obtaining the appropriate range of DNA fragments. In this study, different concentration of DNase I and different time for the reaction were tried (Figure 1a). The results showed that the concentration of DNaseI is inversely proportional to the minimum value of DNA fragments obtained by the new procedure. This showed that the lower concentration of DNase I working, the bigger the minimum of DNA fragment obtained. It indicated that, the needed DNA fragments could be obtained by choosing the appropriate working concentration of DNase I.

In the digest system with the fixed concentration of DNase I (Figure 1b), the minimum value of DNA fragments would not change with time after 1 h, suggesting that the disposed time will not influence the minimum value of DNA fragments after a period of time.

Generally, the *C_0t*-1 DNA of 100 - 500 bp is the best for blocking DNA; meanwhile, the *C_0t*-1 DNA of 500 - 1000 bp is best for use as probes. With regard to the different purposes needed, e.g different length of DNA fragments, the enzyme-method is easier to control than the way of breaking in autoclave. In order to obtain the proper length of DNA fragments, we just need to adjust the working concentration of enzyme; however using the autoclave-method we cannot get the steady and prospective range length of DNA fragments easily.

The *C_0t-1* DNA isolated by the autoclave equipment and the new procedure were all labeled with Cy3 and used for FISH analyses to characterize the distribution of repetitive sequences on the chromosomes (Figure 2a and b). Figure 2a shows that the cell preparation of

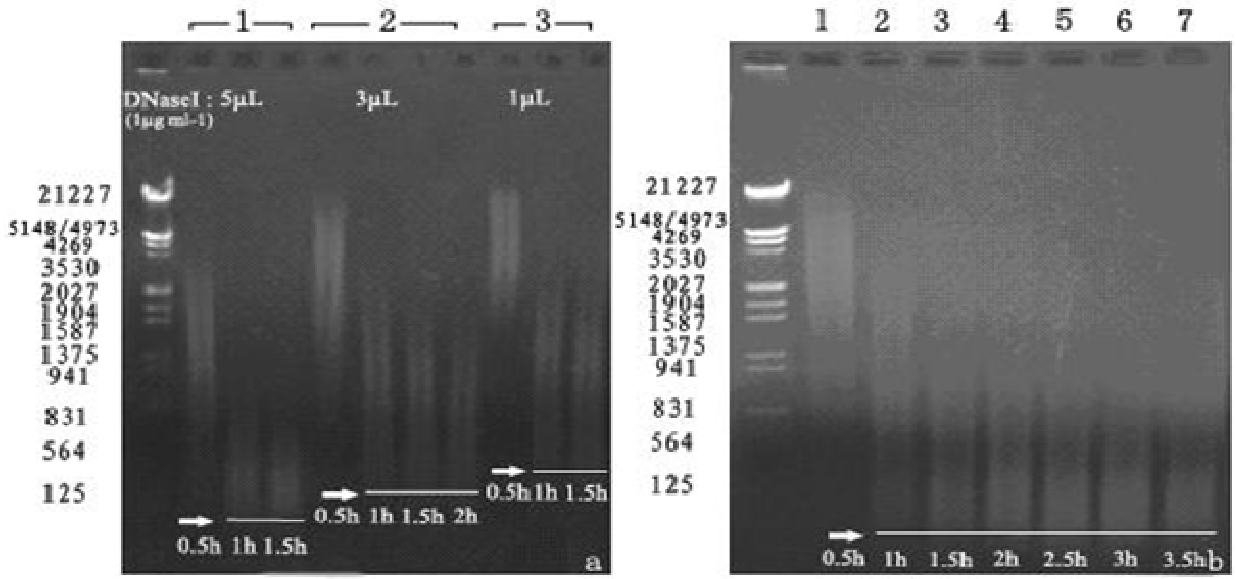

Figure 1. (a) Total genomic DNA of *O. sativa* L was sheared by digest system. From sample 1 - 3, the system contained decreased working concentration of DNase I. The lanes from left to right for each sample were loaded with DNA that has been progressively sheared in 30-min increments; (**b**) total genomic DNA of *O. sativa* L was sheared by digest system containing the fixed working concentration of DNase I. The lanes from left to right were loaded with DNA that has been progressively sheared in 30-min increments. A Lambda DNA/Hind +EcoR marker is shown of both Figure a and b.

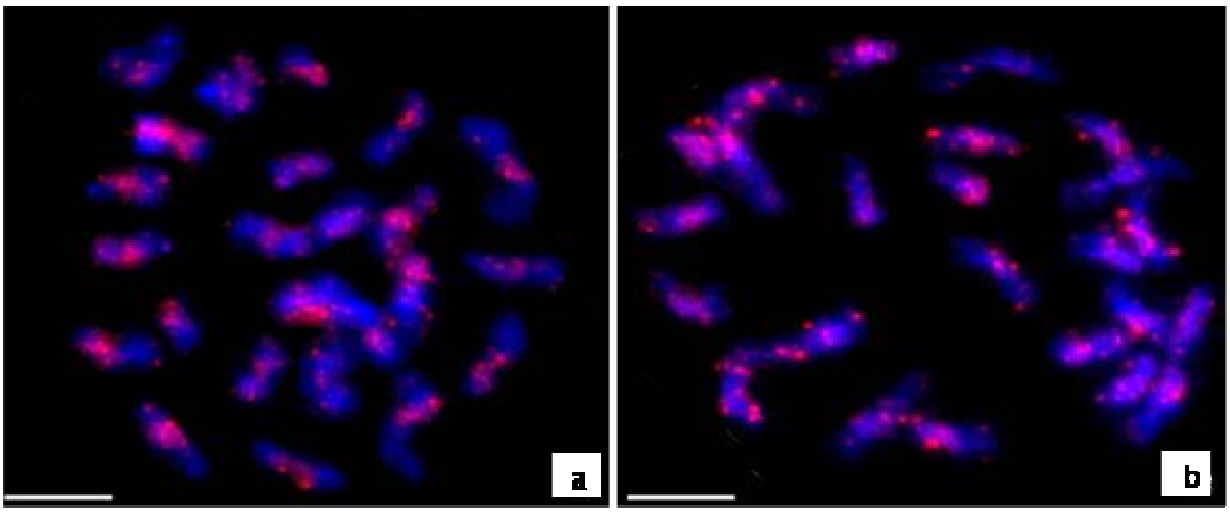

Figure 2. (a) FISH images of *O. sativa* L with labeled probe of its own C_0t-*1* DNA isolated by the autoclave equipment; (**b**) FISH images of *O. sativa L* with labeled probe of its own C_0t-*1* DNA isolated by the new method. Bar = 10 μM.

O. sativa L were probed with labeled probe of its own C_0t-*1* DNA isolated by the autoclave equipment. Figure 2a shows that the cell preparation of *O. sativa* L were probed with labeled probe of its own C_0t-*1* DNA isolated by the new method. The comparison of Figure 2a and b showed that *O. sativa* L chromosomes have similar C_0t-*1* DNA signals by probing two kinds of C_0t-*1* DNA. The result indicated that the method reported in this paper could isolate proper size of C_0t-*1* DNA.

Conclusion

We have developed a method based on the digest system containing DNase I and DNA polymerase I with S1 nuclease to isolate C_0t-*1* DNA from plants. The new procedure has been proved as a feasible method to steadily isolate the high-quality C_0t-*1* DNA. C_0t-*1* DNA could be valuable in studies of species divergence and genome evolution, in order to determine the putative

progenitor and to establish the evolutionary relationships among different species and genomes.

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