Comparison Of CTAB Method And Wizard Genomic Dna Purification System Kit From Promega On DNA Isolation Of Local Varieties Of Rice Of South Sumatera

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ABSTRACT

Research in the molecular field requires DNA with a high degree of purity. Local rice varieties of South Sumatra have different leaf texture so that in isolation process to obtain DNA with high purity level required the right method. This study aims to compare the quality and quantity of purity of local rice insulation of local rice varieties of South Sumatra with different methods of CTAB and Wizard Genomic DNA Purification System Kit from promega. The research was conducted from August 2015 to December 2015 at the Laboratory of Microbiology and Genetics and Biotechnology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University. The method used is DNA isolation method Wizard Genomic DNA Purification System Kit from promega and CTAB. Based on research that has been done result of DNA visualization showed that DNA isolate CTAB method still have smears while DNA isolate method of Wizard Genomic DNA Purification System Kit from promega not. The average DNA isolate concentration of Wizard Genomic DNA Purification System Kit method is A260/280 = 1.853 μg/ml. The average DNA isolate concentration of CTAB method is A260/280 = 1.705 μg/ml. Isolation of Rice DNA of local variety of South Sumatera using DNA method of Genomic DNA Purification System Kit from promega has higher quality and quantity compared to CTAB method.

Keywords: CTAB, Local Rice Varieties of South Sumatra, Promega

1. INTRODUCTION

Local rice varieties of South Sumatra have superior properties that are not owned by superior rice varieties. These properties are stored in genes. This property is an important source of germplasm to be maintained. In addition, genetic information can be used as a basic material in the process of plant breeding.

The genetic information of a plant variety can be known by the PCR technique. DNA isolation was the first step in molecular analysis of PCR (Restu et al., 2012). The purity of target DNA is very important in the PCR process, since impurities of DNA suspension can affect the amplification reaction and may inhibit the action of DNA polymerase enzymes.

Selection of the target to be amplified should take into account the genetic stability of the targeted region or nucleotide sequence (Fatchiyah et al., 2011). This study aims to compare the results of isolation of local rice varieties of rice with different methods of CTAB method and Wizard Genomic DNA Purification System Kit from Promega. So as to determine which type of method is appropriate for DNA isolation. High quality and high quantity of DNA and free of contaminants can be used for other studies using PCR.

2. EXPERIMENTAL SECTION

2.1. Materials and Equipments

The tool used in this research is stationery, aluminum foil, analytical scale, ice box, glove, mortar, pestle, mikropipet, yellow tip, microcentrifus, microtube size 0.2 ml and 1.5 ml, hot plate, spectrophotometer, thermo-cycler, waterbath, and Wizard Genomic DNA Purification System Kit from Promega and CTAB.

Materials needed are for DNA isolation is the local rice leaf of local varieties of South Sumatra (Table 1), ice, ethanol 70%, ethanol 96-100%.

2.2. DNA Isolation method Wizard Genomic DNA Purification System Kit from Promega

The leaves are frozen in the freezer as much as 0.1 gr. Then finely crushed while added Nuclei Lisis Solution (NLS) 600 μL, then put in eppendorf tube size 0.5 μL and in vortex for 3 second. Incubated...
in water bath for 15 minutes at 65°C. Added 200 μL of ethanol then for 20 seconds. The supernatant is transferred into 600 μL isopropanol (0.6) volume. Mix the solution until you see the white thread of DNA. Then centrifuged at 13,000 rpm for 1 minute. Supernatant was removed and 600 μL of ethanol was added 70%. Wash the DNA by centrifuging at 13,000 rpm for 1 minute. Discard the supernatant and then dry the DNA for 15 minutes. Finally added 100 μL of sterile water and store DNA at a temperature of 2-8°C.

2.3. CTAB

Conducted by method of Doyle and Doyle (1987) in Restu et al. (2012) modified. A total of 200 mg of leafless rice leaves plus 0.02 g of PVP were crushed until smooth (flour). The crushing results were then transferred into a 1.5 ml eppendorf tube, plus 0.5 ml of CTAB extraction buffer (1.4 M NaCl, 2% CTAB, 50 mM EDTA, 1 M Tris-HCl pH 8.0 and 0.2% β-mercaptoetanol). The process of lysis of the cell wall is done by incubating the tube containing the leaf sample into the temperature waterbath of 65°C for 60 minutes. The tube is lifted from the waterbath and allowed for several minutes until the sample temperature in the tube decreases. Subsequently added chloroform: isoamilalcohol (CIA 24: 1) 500 μl. The tube is shaken using a vortex, then centrifuged at 10,000 rpm for 10 minutes. The supernatant was transferred into a bar tub and then cold isopropanol was added as much as 1 volume, slowly flipped through until the DNA thread was visible. The sample was then allowed to settle overnight in the refrigerator at 4°C. After being deposited overnight, the sample was then centrifuged for 15 minutes at 10,000 rpm. The pellets of DNA formed at the bottom of the tube are then dried. After that 100 μL of water was added and stored in refrigerator (-4°C).

2.4. Electrophoresis

Weighed 0.5 gr of agarosa and then put into a erlenmeyer flask containing 50 ml of TAE while shaking to dissolve. Insert a magnetic stir into the flask and heat on the hotplate until cooked. Poured into a well comb which amounts to 8 wells on the first comb and 16 wells on the second comb slowly so as not to form bubbles. Let stand until the gel or gel for ± 30 minutes and after cool comb then lifted. The 10 μL DNA samples were pipetted and then inserted into the wells contained in the gel using a microphone. Added 0.7 μL loading dye in the well. The gel is then fed into an electrophoresis tank that has been filled with a TAE buffer. Electrode connected with power supply at 100 volt voltage with amper 400 ohm for 30 minutes then running done for 30 minutes. After running is done then pour 200 ml of TAE and add a drop of EtBr into aluminum foil coated container. Inserted agarose gel into the container and then soaked for 15 minutes.

2.5. Electrophoresis Visualization of Electrophoresis Results

Visualization of the results running on the gel is done by using a UV transilluminator and the results are photographed as documentation.

2.6. Quantification and Quality of Isolated DNA Result

Prior to use in PCR reactions, DNA quantification was done to determine the purity and concentration of DNA. Quantification is done using GeneQuant (Life Science, Ltd., UK). A 2 μL isolated DNA sample was fed into a quartz containing 1998 μL sterile aqubides and mixed homogeneously. The sample then calculated its concentration at wavelength (λ) 260 and 280 nm. Comparison of concentrations at each wavelength is used as a benchmark of DNA purity.

DNA purity is determined on the basis of the ratio of A260 / 280 = 1.8-2 [8]. DNA quality testing can also be performed with agarose gel electrophoresis on genomic DNA prior to PCR. DNA quality is good when the DNA bands appear clear, thick, there is only one band and no shadow under the band (smear) when compared to marker (Sambrook et al., 1989).

3. RESULTS AND DISCUSSION

3.1. CTAB

Based on the results of electrophoresis (Figure 1) and reinforced with the result of quantity test and DNA quality test (Table 2) shows the isolated DNA with CTAB method can still be used for the analysis of genetic variation by RAPD method. Although its purity is not in accordance with the specified standards. This is based on the ratio of concentration at each wavelength used as a benchmark of DNA purity.

The impure DNA of the mold still contains RNA which is enzyme serves to polymerize the DNA while the molded DNA that shows the isolated DNA with CTAB method can still be used for the analysis of genetic variation by RAPD method. Although its purity is not in accordance with the specified standards. This is based on the ratio of concentration at each wavelength used as a benchmark of DNA purity.

Table 1. List of Local Varieties of Rice Soyth Sumatra

| No. | Varieties | Code |
|-----|-----------|------|
| 1   | Padi Pegagan  | PP   |
| 2   | Padi Dayang Rindu | DRMK |
| 3   | Padi Dayang Kuning | DKMK |
| 4   | Padi Seluang  | SMK  |
| 5   | Padi Panak/Pendek | PMK  |
| 6   | Padi Pamulan/Empat Bulan | PEBMK |
| 7   | Padi Talang   | TB    |
| 8   | Padi Sanapi   | SB    |
| 9   | Padi Ketan Itam | KIB  |
| 10  | Padi Ketan Putih | KPB  |
| 11  | Padi Ketan Abang/Ketan Merah | KAB |
| 12  | Padi Dayang Telash | DTLLG |
| 13  | Padi Hitam    | HLLG  |
| 14  | Padi Pulut/Meto Tomok | PLLG1 |
| 15  | Padi Pulit    | PLLG2 |
| 16  | Padi Pengagat | PLLG3 |
| 17  | Padi Dayang Rindu | DRLLG |
| 18  | Padi Pulut    | PM2SI |
| 19  | Padi Panjang  | PM2S2 |
| 20  | Padi Jambat Thehas | JTM2S |
| 21  | Padi Beram    | BM2S  |
| 22  | Padi Stik     | SK    |
many have fragmentation can eliminate the primary attachment site. Based on the results of electrophoresis (Figure 2) and reinforced by the result of quantity test and DNA quality test (Table 3) shows the isolated DNA with Wizard Genomic DNA Purification System Kit method from Promega can be used for analysis of genetic variation by RAPD method. This is based on the ratio of concentration at each wavelength used as a benchmark of DNA purity. DNA purity was determined based on the ratio of A260 / A280 = 1.8-2 (Sambrook et al., 1989).

3.2. DNA Purification System Kit from Promega

One of the advantages of the diversity analysis utilizing PCR technology is that the quantity of DNA required is small (Arif et al., 2010). The molded DNA concentration used ranges from 1.5 to 25 ng in each PCR reaction. Determination of the concentration of molded DNA greatly affects the attachment of primers to the molded DNA. High DNA mold concentrations will result in poor DNA amplification because the primers used are difficult to stick to. Conversely, if the concentration of the molded DNA is too low, there will be a competition where the primer attaches to the mold causing one of the fragments to be amplified in large quantities while the other fragment is slightly (Pharmawati, 2009). The optimization of isolation and purification technique of DNA on the betel nut plant (Reutalis trisperma (Blanco) obtained DNA quantity ranged from 68.80-5.031.39 ng/μl with DNA purity in the range of 1.8-1.9 with electrophoresis result showed intact DNA marked with absence of DNA smears) (Syafarudin and Santoso, 2011). The ratio is too low (<1.8) contains too much RNA, while the ratio is too high (> 2.2) indicates the presence of many protein contaminants in the DNA solution.

DNA purity of the template (DNA template) greatly affects the intensity of amplified DNA bands. DNA molds containing compounds such as polysaccharides and phenolic compounds will result in a faint or unclear amplification DNA band (Sambrook et al., 1989). Genetic markers (RAPD) are very sensitive to reaction conditions and DNA purity of the mold (Young et al., 2000).

CONCLUSION

Isolation of Rice DNA of local variety of South Sumatera using DNA method of Genomic DNA Purification System Kit from...
promega has higher quality and quantity compared with CTAB method isolate.

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