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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 base 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.

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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, microsentrifus, 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 eppendof tube size 0,5 µL and in vortex for 3 second. Incubated

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 Telasih	DTLLG
13	Padi Hitam	HLLG
14	Padi Pulut/Meto Tomok	PLLG1
15	Padi Putih	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

in water bath for 15 minutes at 650C. Added 200 µL PPS then divortex for 20 seconds. Then centrifuged at 13,000 rpm for 3 minutes. Supernattan 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. Supernattan was removed and 600 µL of ethanol was added 70%. Wash the DNA by centrifuging at 13,000 rpm for 1 minute. Discard the supernattan and then dry the DNA for 15 minutes. Finally added 100 µL of DRS DNA and store DNA at a temperature of 2-80 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% ß-mercaptoetanol). The process of lysis of the cell wall is done by incubating the tube containing the leaf sample into the temperature waterbath of 65oC 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: isoamilalkohol (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 H2O was added and stored in refrigerator (-40 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 stirel 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 ampere 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 quartet containing 1998 µl sterile aquabides 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. DNA purity was determined on the basis of the ratio of A260 / 280 = 1.8-2 (Sambrook *et al.*, 1989).

The impure DNA of the mold still contains RNA which is marked by the presence of Smears on the visualization result of the DNA band. This will disrupt the primary attachment of the site and will inhibit the activity of DNA polymerase enzymes. This enzyme serves to polymerize the DNA while the molded DNA that

Table 2. Quantification of DNA CTAB Method

No	Sample	Quantity
1	Padi Pegagan	1,74
2	Padi Dayang Rindu	1,73
3	Padi Dayang Kuning	1,92
4	Padi Seluang	1,72
5	Padi Panak/Pendek	1,68
6	Padi Pamulan/4 bulan	1,65
7	Padi Talang	1,75
8	Padi Sanapi	1,68
9	Padi Ketan Itam	1,66
10	Padi Ketan Putih	1,71
11	Padi Ketan Abang	1,67
12	Padi Meto Tomok	1,68
13	Padi Dayang Telasih	1,71
14	Padi Pengagat	1,60
15	Padi Hitam	1,69
16	Padi Putih	1,57
17	Padi Pulut	1,72
18	Padi Dayang Rindu	1,67
19	Padi Panjang	1,76
20	Padi Jambat Thehas	1,74
21	Padi Beram	1,72
22	Padi Stik	1,74

Table 3. Quatification of DNA Using Promega

No	Sample	Quantity
1	Padi Pegagan	1.806
2	Padi Dayang Rindu	1.86
3	Padi Dayang Kuning	1.839
4	Padi Seluang	1.889
5	Padi Panak/Pendek	1.863
6	Padi Pamulan/4 bulan	1.802
7	Padi Talang	1,820
8	Padi Sanapi	1.985
9	Padi Ketan Itam	1.828
10	Padi Ketan Putih	1.808
11	Padi Ketan Abang	1.804
12	Padi Meto Tomok	1.848
13	Padi Dayang Telasih	1,857
14	Padi Pengagat	1.897
15	Padi Hitam	1.877
16	Padi Putih	1.893
17	Padi Pulut	1.852
18	Padi Dayang Rindu	1.815
19	Padi Panjang	1,881
20	Padi Jambat Thehas	1,898
21	Padi Beram	1.843
22	Padi Stik	1.804

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 / 280 = 1.8-2 (Sambrook *et al.*, 1989).

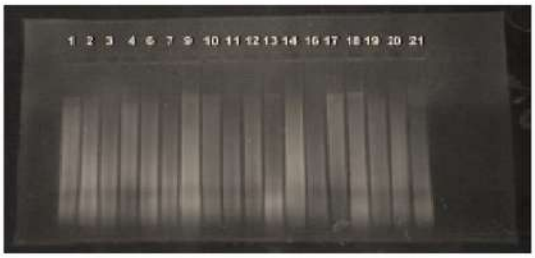

Figure 1. Result of DNA Band Visualization CTAB Method

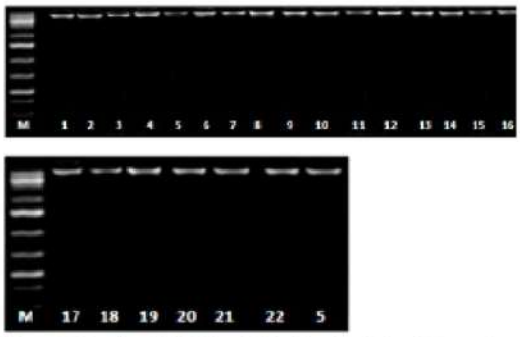

Figure 2. Result of DNA Band Visualization Wizard Genomic DNA Purification System Kit Promega.

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.

REFERENCES

Arif, I.A., Bakir, M.A., Khan, H.A., Al Farhan, A.H., Al Homaidan, A.A., Bahkali, A.H., Al Sadoon, M. and Shobrak, M. 2010. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Genet. Mol. Res*. **9(4)**: 2191-9198.

Fatchiyah, A.E.L., Widyarti, S., dan Rahayu, S. 2011. *Biologi Molekuler: Prinsip Dasar Analisis*. Jakarta: Erlangga xxiv + 191hlm.

Karmana, Wayan, I. 2009 Adopsi Tanaman Transgenik dan Beberapa Aspek Perkembangannya. *GeneC Siwara*. **3(2)**: 12-21.

Pharmawati M. 2009. Optimalisasi Ekstraksi DNA dan PCR-RAPD pada *Grevillea spp.* (Proteaceae). *Jurnal Biologi*, **8(1)** : 12 -16.

Poerba, Yuyu., S., Martanti, D. 2008. Keanekaragaman Genetik berdasarkan Marka Random Amplified Polymorphic DNA pada *Amorphophallus mueller*i Blume di Jawa. *Biodiversitas*. **9(4)**: 245-249.

Restu M, Mukrimin, dan Gusmiaty. 2012. Optimalisasi Teknik Ekstraksi dan Isolasi DNA Tanaman Suren (*Toona Sureni* Merr.) untuk Analisis Keragaman Genetik berdasarkan *Random Amplified Polymorphic* DNA (RAPD). *Jurnal Natur Indonesia*, **14(2)**: 138-142.

Sambrook, J., Fritsh, E.F. and Maniatis T. 1989. *Molecular Cloning*. Cold Spring Harbor Laboratory Press. New York.

Young Et., Sloan JS., Van Riper K. 2000. Trinucleotide Repeats are Clustered in Regulatory Genes in *Sacchavomyches cerevisiae*. *Genetics*. **154(3)**: 1053-68.

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