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Optimization of DNA extraction of physic nut (*Jatropha curcas*) by selecting the appropriate leaf

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Abstract. *Prayitno E, Nuryandani E. 2011. Optimization of DNA extraction of physic nut (*Jatropha curcas*) by selecting the appropriate leaf. Nusantara Bioscience 3: 1-6. Jatropha curcas* L. has important roles as renewable source of bioenergy. The problem occurs on difficult of DNA extraction for its molecular breeding programs. The objectives of this research were to study which leaf best as source of DNA extraction. Four accession were used, namely J1 and J2 (Jawa Tengah), S1 (South Sumatra), and S2 (Bengkulu). First, third, fifth, seventh, and yellow leaves for each accession were extracted using modification of Doyle and Doyle (1987) method. Visualization and comparation with Lambda DNA, Spectrophotometer UV-Vis and cutting DNA with *Eco*RI enzyme were show quality and quantity of DNA. The result showed that third leaves have sufficient quality and quantity as source of DNA. Third leaves DNA quantity for J1 (19.33 µg/mL), J2 (26.21 µg/mL), S1 (31.20 µg/mL), dan S2 (61.03 µg/mL), and quality for each accession were 1.9063 (J1), 2.0162 (J2), 2.0116 (S1), and 2.0856 (S2).

Key words: *Jatropha curcas,* DNA extraction, appropriate, leaf.

Abstrak. *Prayitno E, Nuryandani E. 2011. Optimalisasi ekstraksi DNA jarak pagar (*Jatropha curcas*) melalui pemilihan daun yang sesuai. Nusantara Bioscience. Nusantara Bioscience 3: 1-6.* Jarak pagar (*Jatropha curcas* L.) mempunyai peran penting sebagai sumber bahan bakar nabati. Usaha pemuliaan tanaman ini secara molekuler sering terkendala sulitnya ekstraksi DNA. Penelitian ini bertujuan untuk mengetahui daun yang sesuai untuk digunakan sebagai sumber DNA. Penelitian ini dilakukan pada empat aksesi jarak pagar yaitu J1 dan J2 (Jawa Tengah), S1 (Sumatera Selatan), dan S2 (Bengkulu). Ekstraksi dilakukan pada daun pertama, ketiga, kelima, ketujuh, dan daun kuning dari setiap aksesi dengan metode Doyle and Doyle (1987) yang dimodifikasi. Kualitas dan kuantitas DNA hasil ekstraksi diketahui melalui visualisasi dengan pembanding DNA lambda, spektrofotometer UV-Vis pada panjang gelombang 260/280, dan pemotongan menggunakan enzim *Eco*RI. Hasil penelitian menunjukkan bahwa daun ketiga memadai untuk digunakan sebagai sumber DNA. Kuantitas DNA daun ketiga J1 (19,33 µg/mL), J2 (26,21 µg/mL), S1 (31,20 µg/mL), dan S2 (61,03 µg/mL). Sedangkan kemurniannya masing-masing yaitu 1,9063 (J1), 2,0162 (J2), 2,0116 (S1), dan 2,0856 (S2).

Kata kunci: *Jatropha curcas,* ekstraksi DNA, daun, sesuai.

INTRODUCTION

Increased economic growth and spur the growth of population of high energy consumption. Energy source the world today is still dominated by fossil fuel that cannot be renewed (unrenewable). Various efforts have been made to solve energy problems (Raharjo 2007). Fuel from the plant has several advantages such as ease of storage and environmentally friendly, therefore biofuels were given priority for development. On January 25, 2006, the President of Indonesia issued Presidential Regulation No. 5/2006 regarding the national energy policy and Presidential Instruction No. 1/2006 concerning the provision and use of biofuels as alternative fules. Then on July 1, 2006, presidential and state officials conducting a retreat in the village of Losari, Grabag subdistric, Magelang district, and decided to develop a bioenergy or biofuel as an alternative energy.

Biofuel can be divided into two major categories, namely bioethanol and biodiesel. Bioethanol is ethanol derived from fermentation of raw materials that contain starch or sugar such as molasses and cassava. This fuel can be used to replace regular gasoline (gasoline). Ethanol can be used is alcohol-free pure water (anhydrous alcohol) and levels of more than 99.5%, or called with a fuel grade ethanol (FGE). Blend of premium and FGE is called gasohol. In Indonesia, Pertamina give biopremium trademark for the product. Biodiesel is a popular name for FAME (fatty acid methyl ester), is a biofuel that is used to power diesel engines as an alternative to diesel. This fuel derived from vegetable oils are converted through chemical and physical reactions, so that the nature of the chemical has changed from its original nature. Currently, Pertamina has issued such a product with trade name which is a blending FAME biodiesel with regular diesel (petrosolar) (Prihandana et al. 2007).

Jatropha curcas is a native plant of Central America (Fairless 2007) and has been naturalized in tropical and subtropical regions, including Indonesia. This species is drought resistant and is commonly planted as a garden fence, but is also useful as an ornamental plant shrubs and herbs. Oil from the seeds is useful for medicine, insecticides, making soap and candles, as well as raw material for biodiesel (Gubitz et al. 1999). The use of castor oil as biodiesel ingredient is an ideal alternative, because it is a renewable oil resources (renewable fuels) and non-edible oil so it does not compete with human consumption requirements, such as palm oil, corn, soybeans and others (Dwimahyani 2005). In addition, Jatropha also contains secondary metabolites which are useful as protectant for plants and as an ingredient for human medicine (Debnath and Bisen 2008) Some of the obstacles encountered in developing castor oil, among others, lack of information about varieties that have beneficial properties such as high production, fast multiplication, high oil yield in seeds, as well as resistance to pests and diseases. This happens because so far the Jatropha plant is only regarded as hedgerows that have low economic value so that research and development of this plant is rarely done. To overcome this, plant breeding has a significant role.

Characterization of jatropha plant in Indonesia is carried out simply and not be universal. Often, the mention of Jatropha plant species is based solely on phenotypic appearance or region of origin. Characterization using morphological or phenotypic description has limitations because it is very influenced by the environment. Different morphological features can be caused by environmental stress, whereas the same genotype, whereas the same morphological features do not necessarily indicate that both types of plants are closely related, because the outer shape of a plant is the result of cooperation between the genotype by environment (Joshi et al. 1999; Karsinah 1999 .) Therefore, it is necessary to develop universal genetic information. Molecular markers can provide information universally because it is not influenced by the environment (Azrai 2005), so that they can answer the problem in the characterization of physic nut plants.

Jatropha curcas is one of the many plants that contain latex, which is a true plant secondary metabolites. The presence of secondary metabolites such as polyphenols, tannins, and polysaccharides can inhibit the action of the enzyme (Porebski 1997; Pirtilla et al. 2001). Isolation of plant DNA at a distance often experienced problems due to high levels of secondary metabolites in the form of polysaccharides and polyphenols. According to Sharma et al. (2002) the presence of metabolites in several crops affect DNA isolation procedure, he was using a modified CTAB to isolate DNA from plant tissue containing high polysaccharide. In line with this Kiefer et al. (2000), Pirtilla et al. (2001) and Sanchez-Hernandes, C. and J.C. Gaytan-Oyarzun (2006), states that the extraction of DNA and RNA from plants containing polysaccharides, polyphenols as well as sap and difficult.

Proper techniques of DNA extraction is needed in the plant breeding process to obtain DNA with a high quality and quantity. To obtain pure DNA from plant sap, generally carried out repeated purification and modification of procedures (Kiefer et al. 2000), thus requiring additional cost and effort. For that, you can use parts of plants that contain little secondary metabolites. The content of secondary metabolites in plant tissues fluctuate in line with its development. Secondary metabolites may vary because of differences in age and plant part (Cirak et al. 2007a, b, 2008; Achakzai et al. 2009). Therefore, to simplify the DNA extraction process jatropha, have done research to learn the parts of plants containing secondary metabolites in small amounts and produce DNA with high quality and quantity.

This research aims to study the jatropha plant leaves at different levels of development that have the potential to produce the best quality and quantity of DNA in the DNA extraction process.

MATERIALS AND METHODS

Time and place of study

Research was conducted at the Open University UPBJJ-Semarang, Central Research Laboratory Tropical Fruit IPB, Bogor, West Java, and Laboratory of Structure and Function of Plant Diponegoro University in March to November 2009.

Plant material

Jatropha plant materials used in this study are the three accessions of jatropha plants originated from areas of Klaten (Central Java) with the code J1 and J2, Palembang (South Sumatra) with codes S1, and Bengkulu, with the code S2.

Procedures

Isolation of DNA. About 0.5 g of leaves from the first, third, fifth, seventh and yellow leaves from each sample was crushed in porcelain bowls by adding 0.1 grams of silica sand to be easily crushed. To prevent network browning by oxidation, polivinilpolipirilidon (PVPP) as much as 40 mg and added extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8, 1.4 M NaCl, 20 mM EDTA) as much as 1 mL is added into a cup containing the sample which has added 1% merkaptoetanol. Samples that have been incorporated into the fine volume of 1.5 mL Eppendorf tube. Subsequently the mixture incubated at 65oC for 30 minutes while inverted, and then added 1 mL solution of chloroform: isoamilalchohol (24:1 = v/v) and divortek for 5 seconds. This solution was then separated using a centrifuge with a speed of 11,000 rpm for 10 minutes at a room temperature. Supernatant was separated from the pellet by putting it into a new Ependorf tube.

DNA in the supernatant was purified by adding 1 mL solution of chloroform: isoamilalkohol (24:1 = v/v) and disentrifuse at a speed of 11,000 rpm for 10 minutes at room temperature. Supernatant was transferred into a tube and added with 1 mL of cold isopropanol, shaken gently until white threads arise, which is DNA. Subsequently DNA was precipitated by incubation for 30 minutes at a

temperature of -20ºC. Solution containing the DNA that has been purified disentrifuse with speed 11 000 rpm for 10 minutes at room temperature and then the supernatant was discarded. DNA precipitate was washed with 70% alcohol and dried at room temperature. Further the DNA samples that was obtained was dissolved in 100 mL TE buffer (10 mM Tris-HCl pH 7.5, 10 mM EDTA) and incubated at 37° C for one hour and then mixed until uniform to further test its quality.

Test the quality and quantity of DNA. The quantity (concentration) and quality of DNA determined by UV-Vis spectrophotometer at wavelength 260 and 280 nm. Determination of the total DNA quantity was calculated based on the value of absorbance at a wavelength of 260 nm. A at 260 = 1.0 equivalent amount of DNA is 50 ug/mL. λ DNA quality is considered good if the value of A260/280 approaching 1.8 to 2. To determine the concentration and quality of DNA, electrophoresis results were soaked in a solution of 1% EtBr and then observed under UV transluminator. The quantity of DNA is based on the thickness of the electrophoresis results of DNA samples are compared with the amount of lambda DNA of known concentration, ie 250 ug/mL. This study also tested the quality of DNA by cutting genomic DNA using *Eco*RI enzyme are visualized by electrophoresis on agarose gel.

RESULTS AND DISCUSSION

Visualization of the extracted DNA

The success of the isolation and extraction process of genomic DNA can be marked with resultant large DNA (high molecular weight DNA), that is not degraded during extraction and purification process, and can be cut by restriction enzymes that has been used (Herison 2003). Results of isolation and extraction of jatropha's DNS employed Doyle and Doyle method (1987) which has been modified to produce the desired genomic DNA bands, although relatively small quantity when compared to lambda DNA. Genomic DNA was seen as a ribbon that lights up at the top sinks electrophoresis results.

In general, smear on DNA extracted from young leaves (code J11, J21, S11, S21) and concentrated look taller than the smear on DNA extracted from the older leaves, then gradually decreasing concentration smear on leaves more old (leaves the third, fifth, and seventh), and smear the least present in yellow leaves, except on J1 where J1k (yellow) has a thicker ribbon smears compared J15 and J17 (Figure 1).

In genomic DNA extracted from young leaves, which are visible smear on the bottom of genomic DNA. Ribbon smear is a molecule with varying weights that can be derived from degraded DNA or other follow-up material that is not known (Herison 2003). Smears indicated that the isolated genomic DNA was not intact anymore, probably dismembered during the extraction takes place (Sisharmini et al. 2001). Genomic DNA damage can be caused by degradation of secondary compounds that are released when the cells were destroyed or damaged due to physical handling. The decline is likely influenced by the smear of secondary metabolites of plants and physical handling. In this case the physical handling for each sample the same can be said for using the same standard procedure, therefore, the greatest influences that cause differences in high and low smear is a secondary metabolite from the leaves of plants (Milligan 1992).

In certain plants, plant metabolites will be seen visually in the form of sap. *Jatropha curcas* is a plant sap, with pink latex (de Padua et al. 1999) or nodes in the young gradually turns cloudy/older if left in free air or dark brown when taken from the older plants (Heyne 1987). Young leaves contain more secondary metabolites than older leaves (Badawi 2006; Mulyani 2006). Young leaves generally contain secondary metabolites and enzymes that high because it requires in the process of growth, development, and division of cells' leaf. In the development of plant secondary metabolite concentrations will gradually decline as the decline in leaf growth activity, and the leaves have yellowed, the concentration of enzymes and secondary metabolites in the leaves decreased significantly due to the ongoing process of senesensi (Salisbury and Ross 1995). At this stage the plant will attract substances and enzymes that are still useful to the plant from old leaves for use in the process of development of the younger plants, so the possibility of plant secondary metabolites present in a very low level so that the DNA is not much degraded by the follow-up compound (Salisbury and Ross 1995; Herison 2003). Although the smear on the older leaves less and less, but the quantity of genomic DNA was also decreased, which lights up genomic DNA bands at the top of the wells that are running low on older leaves.

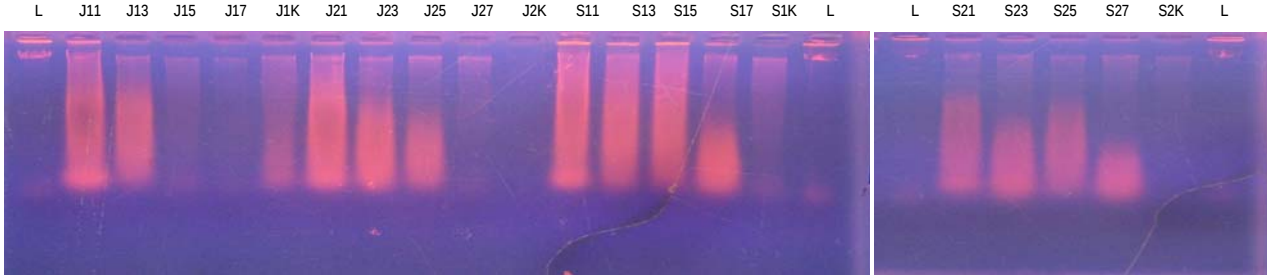

Figure 1. Visualization of the extracted DNA from four accessions of *Jatropha curcas* Klaten (J1, J2), Palembang (S1) and Bengkulu (S2). L = LAMDA (ladder)

The young leaves have a high cleavage activity. In the division process, DNA replication will experience, so the amount of DNA will double itself, thus DNA concentration is relatively high in young leaves. On older leaves, the division process could decrease, until finally stopped altogether. On the leaves that have yellowed, in addition to the absence of the division process, it also exacerbated the death of cells that were old, so the amount of DNA was also decreased dramatically (Salisbury and Ross 1995).

Test the quality and quantity of DNA with UV-Vis spectrophotometer

The quantity (concentration) and quality of DNA determined by UV-Vis spectrophotometer at wavelength 260 and 280 nm. Determination of the total DNA quantity was calculated based on the value of absorbance at a wavelength of 260 nm. The highest DNA purity can be seen in the A260/280 ratio that produces the value of 1.8 to 2. According to Sambrook et al. (1989) DNA with a ratio in the range of figures have met the requirements of purity required in molecular analysis. Spectrophotometer results show relatively good purity DNA that has yet to reach 100% purity in some accessions. The concentration and purity of genomic DNA was analyzed using UV-Vis spectrophotometer can be seen in Table 1.

Genomic DNA which has a purity of 100% contained in the accession J1 was extracted from the third leaf with value ratio of 1.9063. Genomic DNA from the first leaf accession J1 has a value ratios approaching 100% purity with ratio of 2.0131. While the three other leaves, that leaves the fifth, seventh, and yellow leaves have a value ratio of less than 1.8 respectively, 1.7417, 1.2578, and 1.2356. Results DNA extraction leaves first, third, and fifth of the accession J2 has a value closer to purity ratio, respectively 2.0697, 2.0162, 2.0914, while the seventh leaves and yellow leaves have a ratio value that is still far from purity, namely 1.5873 and 1, 1940. On the accession of S1, almost all of the extracted DNA purity approaching leaves, each leaf of the first, third, fifth, and seventh ratio is 2.0768, 2.0116, 2.0792, 2.0225, while the yellow leaves have value ratio far from the purity of 1.4434. DNA extracted first and third leaf from the accession of S2 close to the purity of the value ratio of 2.0611 and 2.0856. While leaf fifth, seventh, and yellow leaves have a ratio that is far from the purity of the respective ratios 2.2187, 2.1782, and 1.5177.

Besides the purity of genomic DNA samples, another consideration that must be considered is the quantity of genomic DNA was generated from the DNA extraction process. Readings A260 = 1 means the concentration of DNA obtained at 50 ug/mL (Herison 2003). The concentration of genomic DNA was extracted was calculated by the formula: DNA concentration (ug/mL) = A260 x dilution factor x 50 ug/mL.

DNA concentration resulting from the extraction process represents the amount of DNA contained in the leaf tissue used for the sample and treatment methods used in each sample is the same. Table 1 below is the concentration of DNA from samples of twenty leaves from four accessions of jatropha plant that is used. From Table 1, note the concentration ratio of genomic DNA from leaf tissue of each first, third, fifth, seventh, and yellow leaves, and comparison of genomic DNA concentration between sections. In general, genomic DNA concentration decreased with increasing age of leaves used as a sample.

Samples from the first leaf shows the quantity of genomic DNA is much larger than the sample leaves the third, fifth, seventh, and yellow leaves. Measurement of the quantity of genomic DNA samples from accessions J1 genomic DNA in Klaten produces relatively little compared to the accession of J2, S1, and S2, which is 27.69 ug/mL for the first leaf, 19.33 ug/mL for the third leaf, 3.68 tg/mL for the fifth leaf, 2.03 g/mL for the seventh leaf, and 4.51 ug/mL for yellow leaves. This is due to a smaller sample size compared to other accessions due to spill some of the samples by laboratory staff who worked on, so that DNA samples that were tested got reduced. While the accession J2, where accession was also derived from the same home with the accession of J1, which was from Klaten, Central Java, and comes from the same parent, the quantity of genomic DNA generated greater than J1, which is 62.06 ug/mL for the extraction of the first leaf, 26.21 ug/mL for the third leaf, 27.69 ug/mL for the fifth leaf, 5.37 g/mL for the seventh leaf, and 4.37 ug/mL for yellow leaves.

The concentration of genomic DNA for S1 accession on the first leaves produced 67.61 g/mL DNA, whereas the third leaf, the concentration of genomic DNA was 31.20 ug/mL, on the fifth leaves of 46.71 ug/mL, on the seventh leaf, 22, 90 ug/mL, and the yellow leaves of 7.59 g/mL. Accession S2 on the first leaves produced 101.35 g/mL genomic DNA, while the third leaf, the concentration of genomic DNA was 61.03 ug/mL, on the fifth leaves of 44.18 ug/mL, leaves the seventh, 26.27 ug/mL , and the yellow leaves of 5.37 g/mL. The Figure 1 shows the concentration of the extracted genomic DNA of

Table 1. Test the quality (purity) and quantity (concentration) of DNA using UV-Vis spectrophotometer in four accessions of jatropha from Klaten (J1, J2), Palembang (S1) dan Bengkulu (S2).

Leaves	DNA purity				DNA concentration (µg/mL)			
	J1	J2	S1	S2	J1	J2	S1	S2
First	2.0131	2.0697	2.0768	2.0611	27.69	62.06	67.61	101.35
Third	1.9063	2.0162	2.0116	2.0856	19.33	26.21	31.20	61.03
Fifth	1.7417	2.0914	2.0792	2.2187	3.68	27.69	46.71	44.18
Seventh	1.2578	1.5873	2.0225	2.1782	2.03	5.37	22.90	26.27
Yellow	1.2356	1.1940	1.4434	1.5177	4.51	4.37	7.59	5.37

diminishing. This is related to the phase of leaf development that has been outlined above.

Results spectrophotometer for quantity of genomic DNA of the above shows that the largest quantity of genomic DNA from four accessions were found in the extraction of the first leaf. But considering the quality of the resulting DNA, the highest purity approaching 100% are found in the sample using the third leaf as a source of genomic DNA, although in terms of quantity, the number is lower than the samples originated from the first leaf.

Comparison of DNA extracted from five types of leaf samples from accessions used in J1 and J2 from Klaten, from the same parent tree can be seen in Table 1. From Table 1 it can be seen that the DNA genome of the first and second leaf (accession J1) and leaves the first, third, and fifth (accession J2) approached the purity, but purity is closest to the third leaf (accession J1 on the ratio of 1, 9063 (purity 100%) and the accession to the ratio of 2.0162 A2). But in terms of quantity, J1 and J2 are not comparable although originating from the same parent because of the sample is not the same J1 J2 terms of number of samples tested for spill samples by the laboratory.

Some researches indicate that generally young leaves are used in DNA extraction because of the ease in getting the DNA with a high quantity. Mansyah et al. (2003) who conducted research on mangosteen states that extraction of DNA from old leaves is more difficult when compared with young leaves, so as to obtain DNA from old leaves with a sufficient quantity is required special treatment, namely with the addition of the extracted leaves up to 2 g and DNA purification with the addition of RNase. While Prana (2003) who perform DNA extraction on taro plants also use the young leaves (in this case the leaf shoots) as the source of DNA.

Test the quality of DNA by using the enzyme *Eco*RI cuts

The purity of DNA can be seen from the absence of a DNA sample can be cut by restriction enzyme such as *Eco*R1 (Figure 2). If a DNA sample has high purity, this DNA would be easy to cut by restriction enzymes. But if this is still contain DNA samples follow-up materials such as secondary metabolites, carbohydrates, proteins, and others, will hinder the work restriction enzymes.

Whether DNA can be cut with restriction enzymes is visible from at least smear results of electrophoresis bands after DNA cut with *Eco*RI enzyme (Herison 2003). *Eco*RI produce DNA bands when smears were electrophoresed because this restriction enzyme included in the frequent cutter (Vos et al. 1995). The result of cutting with *Eco*RI enzyme produces DNA fragments that appear as a smear on some samples, but most other samples can not be cut by this enzyme because of the high follow-up compounds that inhibit enzymes work. Smear only be observed in J13 and J15, while the other samples have not seen a clear smear as a result of enzyme *Eco*RI. Visible is the presence of minor compounds in the lower section sinks. possible follow-up material that inhibits this enzyme *Eco*RI work so as not to cut the genomic DNA tested jatropha.

The description above discussion shows that differences in leaf tissue age used influence the extraction of genomic DNA where the younger leaves will produce a quantity of genomic DNA was higher but also accompanied by the high follow-up material in the form of plant secondary metabolites that inhibit the work in the field of molecular further. Older leaves to produce the amount of genomic DNA are relatively fewer compared to young leaves, but the following secondary metabolites was also reduced in number. This study shows that the third leaf is better used as a source of genomic DNA since the DNA purity is better than the other leaves, and the quantity produced enough DNA to be used for further molecular analysis.

CONCLUSION

The third leaf physic nut plants suitable for use as a source of DNA for molecular analysis of genomes, as in quantity and quality sufficient to produce genomic DNA for further molecular analysis such as PCR. Genomic DNA extracted from the third leaf is generally close to 100% purity and quantity of DNA produced is also large enough to be used for further molecular analysis.

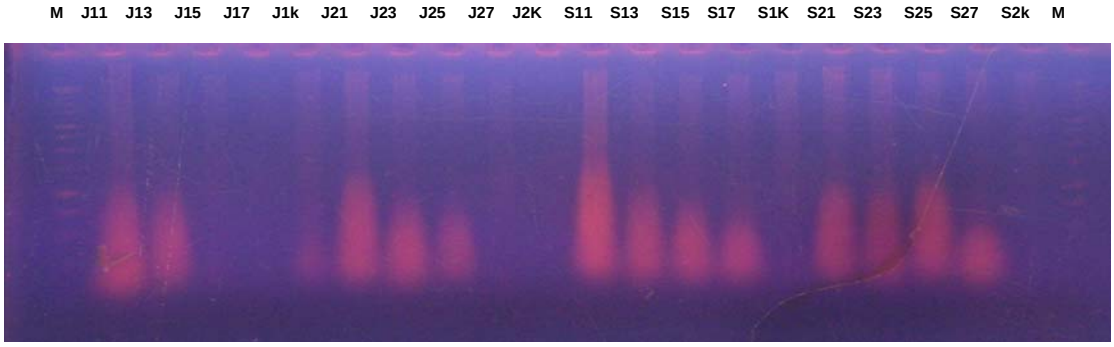

Figure 2. Visualization of results by the enzyme *Eco*RI cuts at the four accessions of jatropha from Klaten (J1, J2), Palembang (S1) and Bengkulu (S2).

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