CTAB'S MODIFICATION: HIGH-QUALITY PLANT DNA EXTRACTION OF TOMATO FOR PCR WITH HEAT SHOCK TREATMENT

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SUMMARY

DNA extraction is a crucial first step in PCR activities, especially for plants that contain secondary metabolites. Secondary metabolites may reduces the quality of the extraction and damages the DNA. The aim of this research was to get a modified of CTAB which can improve the quality of the extraction and storability of the DNA in tomatoes. This research used 6 local genotypes of tomato and primer RAPD (OPH 5). Each genotype was divided into two samples and treated with heat shock and without heat shock treatment. The heat shock treatment uses incubation at 95^0C for 5 minutes and saved at room temperature. PCR-RAPD activities has done every week for 8 weeks. Observation on electrophoresis genome shows that heat shock treatment results of DNA extraction is cleaner than without heat shock treatment. Heat shock treatment also increases the storability of DNA that can be saved at room temperature. RAPD results showed that heat shock treatment give a better consistency results than the amplification without heat shock treatment.

Keywords: CTAB, tomato, heat shock, DNA extraction, room temperature, RAPD, consistency

INTRODUCTION

Tomato is an important vegetable (Minoia *et al.,* 2010). These plants classified as vegetables and into family *Solanaceae* (Wasnowati, 2011). Tomato has polyphenols and tannisa that participate in the degradation of DNA during extraction and there is no universally applicable protocol to extract high quality DNA for plants (Varma *et al.,* 2007). The isolation of good quality DNA is the prerequisite for molecular research. Successful application of PCR based downstream applications requires efficient recovery of good quality and quantity of DNA (Devi *et al.*, 2013). The isolation of pure, intact, and high-quality DNA is very crucial for any molecular studies (Tan and Yap, 2009).

The isolated high quality genomic DNA is amenable to RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats), and amplification of plant barcode

genes with reduced cost and health concerns (Devi *et al.,* 2013). This researsch aimed was to get a modified of CTAB which can improve the quality of the extraction and storability of the DNA in tomatoes.

MATERIAL AND METHODS

Plant material

Fresh and young leaves of six local genotypes of tomato (Kudamati 1, STB GL, Lombok 4, Meranti 2, F1 Kudamati 1 x Lombok 4, dan F1 STB GL x Meranti 2).

DNA extraction protocol

The tomato leaves were grind 0.5-1 gr in 700 µL CTAB and 0.5 gr PVP with mortar. The ground material were then tranfer into 2 mL tube and incubate in water bath at 65^0C for 30 minutes. Incubate tube at room temperature for 10 minutes. Add 700 µL CIA into the tube, vortex, and centrifuge with 12 000 rpm for 5 minutes. Collect the supernatant in new 2 mL tube. Add 700 µL CIA again, vortex, and centrifuge with 12 000 rpm for 5 minutes. Collect the supernatant in new 2 mL tube. Add 1.5 mL ethanol and centrifuge with 8 000 rpm for 5 minutes. Throw the ethanol and collect the pellet. Vaccum the pellet about 30 minutes. Add 200 µL aqua bidestilata steril into the tube.

Heat shock treatment

Each DNA's stock was divided into 2 so we have12 samples were subsequently treated differently, namely : 6samples with heat shock treatment and 6 samples without heat shock treatment. The samples without heat shock treatment was stored in a refrigerator at 4^0C.

The samples with heat shock treatment were incubated at 95^0C for 5 minutes. Then the samples were incubated at 65^0C and allowed to cool slowly. Furthermore, the stock DNA stored at room temperature.

Agarose gel electrophoresis

Electrophoresis of the extracted DNAs was performed in 1.5% agarose with TAE 0.5 x. Electrophoresis was performed at a voltage of 90 V for 30 minute using Mupid 2Plus. Gel was submerged into etidium bromida (0.5 mg/l) for 20 seconds and aquades for 15 minutes. The photographs were taken in a UV transilluminator.

RAPD and PCR

Amplification process was done by using RAPD with OPH5 primer with 5'AGTCGTCCC'3 sequences. PCR activities did every week for 8 weeks. We used working solution from DNA's stok (0.1x with aqua bidestilata steril). Working solution was made everytime before PCR.

PCR was performed in a 10 uL reaction mixture containing 2 µL Buffer A, 0.2 µL $MgCl_2$, 0.2 µL dNTP, 0.04 µL KAPA 2G, 2.56 µL aqua bidestilata steril, 2.5 µL of the working solution of DNA and 2.5 µLprimer. Thermal cycling condition were as follows: pre- denaturation at

94^0C for 5 minutes , followed 45 cycles each of denaturation at 94^0C for 5 seconds , annealing at 32.2^0C for 30 seconds , elongation at 72^0C for 1 minute , and final elongation at 72^0C for 10 minutes.

RESULT AND DISCUSSION

Quality and quantity of genomic DNAs are the one of the most critical factors for many molecular biology studies and molecular marker assisted (MAS) studies (Ince *et al.,* 2011). Successful amplification with reproducible result in PCR, depends upon the quality and quantity of template DNA (Ahmed *et al.,* 2009).

DNA degradation is mediated by secondary plant products such as phenolic terpenoids. The isolation of high quality DNA from plants containing a high content of polyphenolics has been a difficult problem (Kim *et al.*, 1997). A relatively quick, inexpensive and consistent protocol for extraction of DNA from expanded leaf material containing large quantities of polyphenols, tannins, and polysaccharides is very important (Porebski *et al.,* 1997) because it can come into contact with nucleic acids within nucleus and other organelles during the DNA extraction (Peterson *et al.,* 1997). It may covalently bind to DNA, giving it a brown color and making it useless for most molecular research application (Peterson *et al.,* 1997). Reagents such as chloroform isoamyl alcohol (CIA) is used to eliminate contaminants from protein. To clean phenol using PVP (polivynilpyrrolidone) (Kim *et al.,* 1997; Rocha, 2002) and to reduce carbohydrates using CTAB (cetyl trimetyl ammonium bromide) (Rocha, 2002). But using reagent does not eliminate all of contaminants.

Figure 1 shows that heat shock treatment gives a cleaner genome compared without heat shock treatment. Weishing *et al*. (1995) said that one problem encountered in the isolation and purification from plant is enzymatic reaction (directly or indirectly).

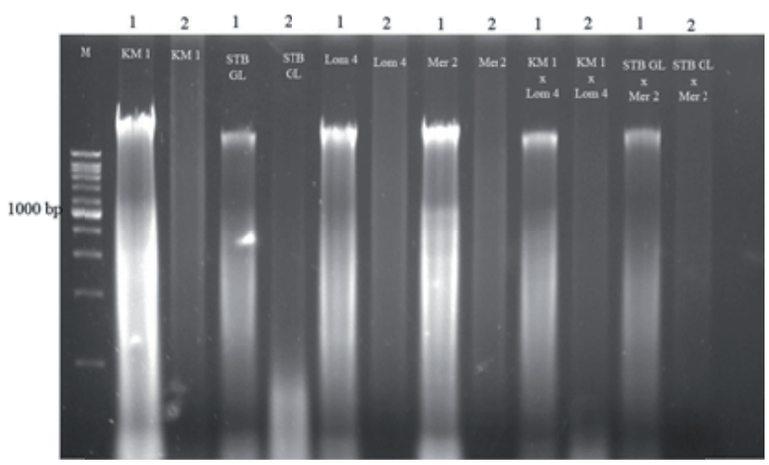

Figure 1. DNA quality, 1 for without heat shock and 2 for heat shock.

Many factors can influence the activity of enzim, such as: concentration of enzim, concentration of substrat, pH, and temperature. Chemistry reaction be slowly in the low temperature and be faster in higher temperature. Enzim is a protein and the highest temperature can makes denaturation that making activity of enzim be damage. Heat shock treatment uses 95^0C that damages the enzim. Using temperature under 95^0C has a probability to increase the activity of enzim. Surely, heat shock treatment does not damage DNA.

One of the major problems commonly associated with DNA is that it does not give reproducible PCR results after long-term storage (Sharma *et al.,* 2002) because of freezing and thawing, which it expected to damage the DNA (Ahmed *et al.,* 2009). Heat shock treatment makes DNA can be saved at room temperature. Those figures (fig.2-9) compares results of weekly PCR from without and with heat shock treatment.

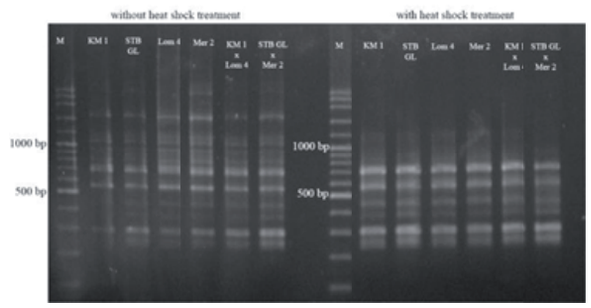

Figure 2. The first week

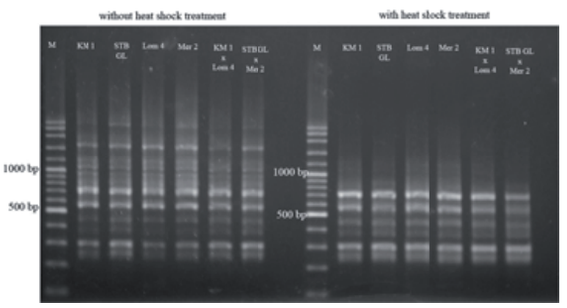

Figure 3. The second week

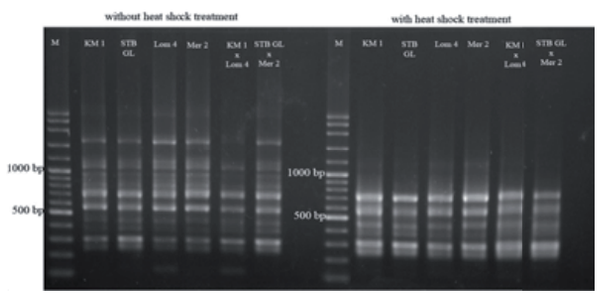

Figure 4. The third week

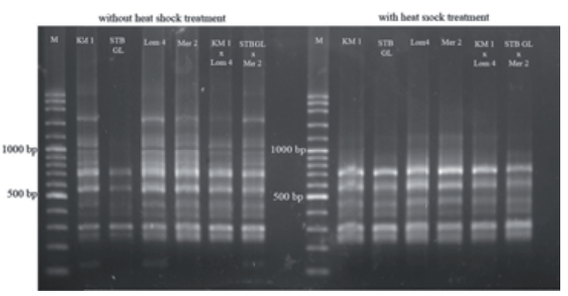

Figure 5. The fourth week

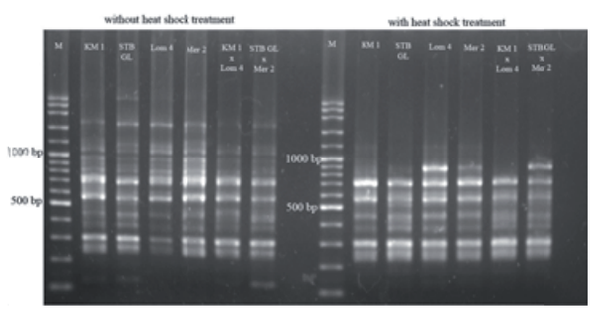

Figure 6. The fifth week

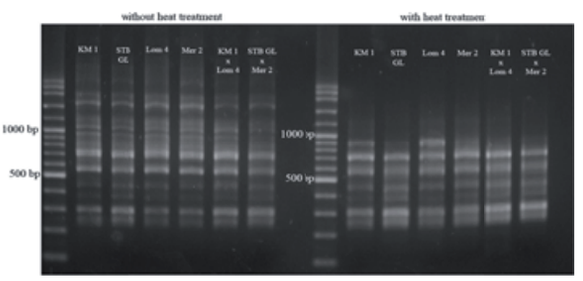

Figure 7. The sixth week

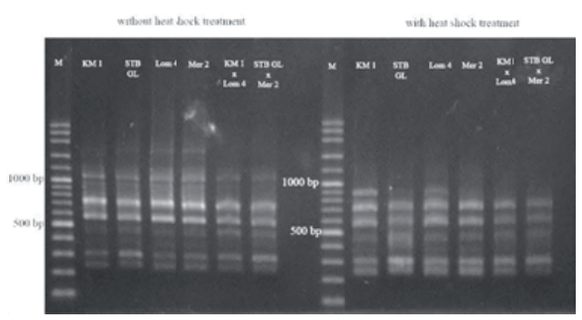

Figure 8. The seventh week

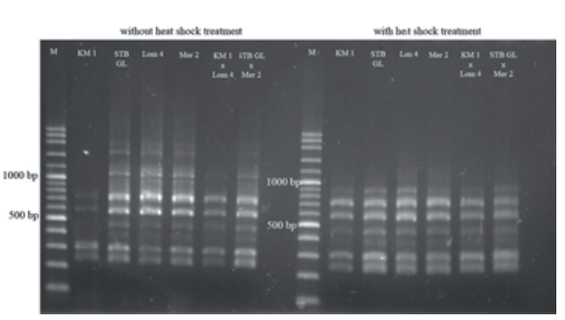

Figure 9. The eighth week

Those figures shows the heat shock treatment give a better consistency compared without heat shock treatment. From the first until fourth week, RAPD's result showed a high consistency. The heat shock treatment also gives the sure band and this is important for scoring.

CONCLUSION

The heat shock treatment does not damaged DNA and also makes its quality be better. It can be a solution for RAPD's unreproducibility. Result showed that heat shock treatment gives a good consistency. Forth, to get a better consistency we can do optimation with condition of PCR.

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