Vietnam Journal of Science and Technology ***55*** *(6) (2017) 725-733*

DOI: 10.15625/2525-2518/55/6/9724

OPTIMIZING DNA EXTRACTION CONDITION FROM WOOD USING RESPONSE SURFACE METHODS

Du Phuc Thinh[1], Tang Thi Kim Hong[2], Huynh Van Biet[3, *]

[1]*Department of Biotechnology, Nong Lam University Ho Chi Minh City, 6 Quarter, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Viet Nam*

[2]*Center for Forestry Research and Technology Transfer, Nong Lam University Ho Chi Minh City, 6 Quarter, Linh Trung Ward, Thu Duc District, Ho Chi Minh city, Viet Nam*

[3]*Research Institute for Biotechnology and Environment, Nong Lam University Ho Chi Minh City, 6 Quarter, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Viet Nam*

[*]Email: *hvbiet@hcmuaf.edu.vn*

Received: 25 April 2017; Accepted for publication: 31 October 2017

Abstract. The DNA extraction protocol from fresh wood, dried wood and leaves of *Hopeaodorata* by by CTAB method was investigated. The protocol optimization was performed through Central Composite Design (CCD) using Response Surface Methodology (RSM). The results showed the centrifugation time of 0.15 hour and the volume ratio of isopropanol to solutions containing DNA of 1:1.25 were found to be the optimum conditions for the maximum DNA concentration of 179.89ngµl^{-1}. The DNA extraction protocol with the optimum conditions was succeeded for dried wood samples.

Keywords: DNA extraction, response surface methods, *Hopeaodorata*, psbA – trnH.

1. INTRODUCTION

The DNA extraction is one of the first steps and plays an important role in study on genome of any species on earth. Depending on the purpose and object of study, DNA can be extracted from various tissues. For plants, DNA is usually extracted from the leaves, seeds and young buds, in which these tissues are the best DNA source and can be extracted easily. However, the collection of sample from mature trees, which are generally tall, is difficult and need more facilities. Consequently, it leads to be limited for the study scope. The proposed solution is to use a wood tissue instead of the leaves, seeds and young due to they are easily collected. The problem is very difficult to extract high quality DNA from the wood tissue [1]. However, if the extraction of DNA from wood tissue would be successful, it would open up many research directions and could turn techniques that seemed unfeasible before into the effective solution [2]. Extracting and analyzing DNA from dried wood and processed wood could be developed to explore the possibility of identifying the species and their origin. This could be greatly useful for determining the legality of wood log and wood products, and for deterring trade in illegal wood products [3].

Currently, there are some commercialized kits, enabling DNA extraction from wood more easily, e.g. DN easy Plant Mini Kit (Qi agen), Nucleospin Plant II (Macherey-Nagel), Genomic DNA Purification Kit (Fermentas) and Miniprep Plant DNA Kit (Analytik Jena). However, the commercial Kits are expensive. Meanwhile, there are some cheaper methods, applying for DNA extraction from wood successfully, such as SDS method [4, 5], protein precipitation protocol [6, 7], especially CTAB method, obtaining with a high DNA concentration [8].The DNA extracted by CTAB protocol is less pure; however, they are still suitable to use in molecular biology [1, 9].

The objective of this work was to optimize the conditions of CTAB protocol for DNA extraction from wood. The two parameters, centrifugation time and volume ratio of isopropanol to solutions containing DNA that affect the yield and quality DNA, were explored using the response surface methodology (RSM). The central composite design (CCD) was used to obtain the experimental design matrix. This approach has limited number of actual experiments performed whereas allowing probing into possible interaction between these parameters studied and their effect on the quantity and quality of DNA.

2. MATERIALS AND METHOD

Plant materials

Three types of sample of fresh wood, dried wood and leaves of the species *Hopeaodorata* were used for DNA extraction. The samples were collected in Ho Chi Minh City. For the short trees, the fresh wood samples were taken directly from branches and were taken from the trunk for the tall trees. In the field, soon after the collection, the samples were preserved in ice box, and then stored in refrigerator at -20 ºC until use. The dried wood samples were done by air drying the fresh wood samples in 3 months. They were reached a moisture content of about 20 %.

All of wood samples were removed of bark. For making wood powder, these wood samples were drilled with a depth of about 5 mm, using MultiProTM DREMEL® driller. Then, the wood powder was pulverized with liquid nitrogen and stored at -70 ºC until use.

Chemicals

Chemicals used for DNA extraction contain of CTAB extraction buffer (20 gl^{-1} CTAB; 1.4 mol l^{-1}NaCl; 0.1 mol l^{-1}Tris-HCl; 20 mmol l^{-1} Na_2EDTA), CTAB precipitation buffer (5 gl^{-1} CTAB, 0.04 mol l^{-1} NaCl), solution of1.2 mol l^{-1} NaCl, Ethanol 70 %, Chloroform, Isopropanol and TE buffer (0.01 mol l^{-1}Tris-HCl; 0.001 mol l^{-1} Na_2EDTA).

DNA extraction

The CTAB protocol used for DNA extraction was done based on the studies of Verbylaite et al. [1], Asif and Cannon [2] and Stefanova et al. [10].

Design of experiment

The experiment was designed by the Central Composite Design (CCD). The range and levels used in the experiments are given in Table 1 in which X_1centrifugation time, X_2volume ratio between isopropanol and solutions containing DNA, respectively.

Modeling is represented by a quadratic equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where Y is DNA concentrations (ng μl^{-1}); b_0 is constant; b_1 and b_2 are coefficients level 1; b_{12} is interaction coefficient between the two factors.

In this study, the design of experiment and the response surface methodology were employed using the JMP Software Version 10.

Table 1. Level of variable for DNA concentration by the Central Composite Design (CCD).

Name	Variable	Variable level				
		-α	-1	0	1	α
The centrifugation time (hour)	X_1	0.075	0.1	0.15	0.2	0.225
The volume ratio between isopropanol and solutions containing DNA	X_2	0.125	0.5	1.25	2	2.375

DNA quality evaluation

Separating and analyzing DNA was done by 1 % Agarose gel electrophoresis. The quantitation of DNA was performed by spectrophotometer BioDropμLITE (BioDrop, England).The spectrophotometer was zeroed with a sample of solvent 0.2 µl 1XTE, then 0.2 µl DNA loaded into the sample port. The OD measurement was done to obtain DNA concentration and purity of DNA based on the ratio of A260 / A280.

DNA amplification by PCR

Primers psbA – trnHwas used for amplify DNA as following steps: psbA 5’ – GTTATGCATGAACGTAATGCTC – 3’ (for ward), trnH 5’ – CGCGCATGGTGGATTCA-CAAATC – 3’ (reverse). The volume of each PCR reaction is 25 µl, reactive components include: 12.5 µl 1X Master mix (Bioline), 10 $pmol\mu l^{-1}$ each primer, 50 µl DNA target, 8.5 µl water.

Heat treatment for PCR reaction was as follows: 95°C for 5 minutes; 35 cycles of 95 °C for 1 minute; 48 °C for 1 minute; 72 °C for 2 minutes; 1 cycle of 72 °C for 7 minutes; ends at 4 °C.

RESULTS AND DISCUSSION

3.1 Optimizing conditions for the DNA extraction from wood using RSM – CCD

The experimental design matrix derived from CCD. Two variables (X_1centrifugation time andX_2volume ratio between isopropanol and solutions containing DNA), each with five levels (1 for the factorial points, 0 for the center points and ± for the axial points),was used. Results of 13 experiments including 5 experiments at the center, with α = 1.414 are given in Table 2. However, the centrifuge (Eppendorf AG 22331 Hamburg) can not set up correctly the centrifugation times with α = 1.414, so chose α = 1.5).

The regression equation between the variables (X_1, X_2) and the concentration of DNA (Y) is as following:

$$Y = -101.94 + 168.6 \times X_1 + 2089.02 \times X_2 - 57.8 \times X_1^2 - 6876.7 \times X_2^2$$

Table 2. Independent variables and result for DNA concentration by RSM – CCD.

Experiment	Coded Variables	Actual variable		Response (DNA concentration (ng μl^{-1}))	
		X_1	X_2	Experimental	Predicted
1	00	0.150	1.250	176.3	177,2
2	α0	0.225	1.250	131.8	140.4
3	-α0	0.075	1.250	129.6	136.5
4	0α	0.150	2.375	136.9	131.2
5	-1-1	0.100	0.500	135.4	117.0
6	00	0.150	1.250	172.9	177.2
7	0-α	0.150	0.125	55.60	76.90
8	-1+1	0.100	2.000	133.3	135.3
9	00	0.150	1.250	178.4	177.2
10	00	0.150	1.250	173.8	177.2
11	+1+1	0.200	2.000	155.0	155.8
12	00	0.150	1.250	180.5	177.2
13	+1-1	0.200	0.500	121.3	101.8

Results comparing the DNA concentration from model predictions and DNA concentration from experimental are presented in the Figure 1.

P-values related to Fisher testing in Table 3 less than 0.05 (P = 0.0019) shows the compatibility of experimental models and statistical significance.

Table 3. ANOVA analysis.

Source	DF	Sum of squares	Mean square	F ratio
Model	5	12949.014	2589.80	13.1770
Error	7	1375.779	196.54	Prob > F
C. total	12	14324.793		0.0019*

Regression coefficient (R^2) was calculated as 0.904, which means that there are 90.4 % compatible experimental data with data in the model predictions. Besides, larger R^2 value of 0.85 and predictive value was 0.835 R^2 (R^2 calculated from the model) proved compatible model with experimental data (Table 4).

Table 4. Parameters evaluated regression models.

RSquare	0.904
RSquareAdj	0.835
Root Mean Square Error	14.019
Mean of Response	144.675
Observations (of sum Wgts)	13

Response surface (Figure 2) shows the interaction of two factors is the centrifugation time and volume ratio between isopropanol and solutions containing DNA. From this chart one can determine the optimum value of each factor leading to achieve maximum response functions. In the survey area, the regression equation showed that the concentration of DNA is affected at the level 1 and level 2 of both factors X_1, X_2. However, the concentration of DNA is not affected by pairs factors $X_1 \times X_2$ (Table 5).

Table 5. Evaluation of the parameters in the regression equation.

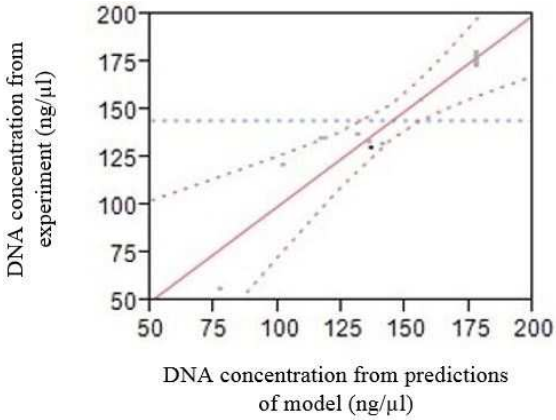
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-57.143	58.553	-0.98	0.3616
X_1	132.749	35.999	3.69	0.0078*
X_2	1790.947	636.776	2.81	0.0261*
$X_1 \times X_2$	238.433	186.924	1.28	0.2428
$X_1 \times X_1$	-57.763	8.659	-6.76	0.0003*
$X_2 \times X_2$	-6876.708	1948.332	-3.53	0.096*

Volume ratio between isopropanol and solution containing DNA is the largest positive impact on the concentration of DNA at the level 1, but the main factor is also the largest negative impact at the level 2.The cause of this effect is due to isopropanol have an important role to precipitate DNA. In the solution, the final concentration of isopropanol needs to be around 35 % and 0.5 M salt is DNA falls out of solution. This means that for the typical precipitation protocol, isopropanol is added from between 0.7–1 volumes of sample (solution containing DNA) may be precipitated DNA [9]. In this study we use of sodium chloride. In the solution, the positively charged sodium ions neutralize the negative charge on the PO^{3-} groups on the nucleic acids, making the molecule far less hydrophilic, therefore much less soluble in water and leading to DNA precipitation. The increase in volume of isopropanol in the solution to reduce the salt concentration, leading to Na^+ cation difficult combined with PO^{3-}anions. The result is that a certain ratio of DNA remains dissolved in water leading to reduced DNA precipitated.

The centrifugation time has the positive impact on level 1 and level 2 in the negative for DNA concentration. The reason is that the need to have time long enough to centrifugal DNA in the form of precipitation can fall down and clinging to the bottom of the Eppendorf. However, if the recommended centrifugation time or speed is exceeded, the DNA in the form of precipitates

can be more difficult to re-suspend back to DNA preservation solution [11] so the amount of DNA collect is impaired.

The model predicts the maximum DNA concentration is 179.89 DNA (ng μl^{-1}) when isopropanol is added 1.25 volumes of solution containing DNA and centrifugal time is 0.15 hours (Figure 3).



P = 0.0019; RSq = 0.904; RMSE = 14.019

Figure 1. Comparison between the DNA concentration from model predictions and experimental.

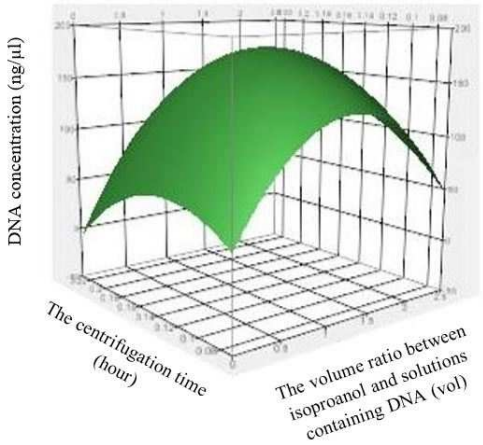


Figure 2. Response surface chart showing the dependence of DNA concentrations (ng µl-1) by the centrifugation time and volume ratio between isopropanol and solution containing DNA.

Solution

Variable	Critical Value
X1(0.5,2)	1.4704472
X2(0.1,0.2)	0.1547105
Solution is a Maximum	
Predicted Value at Solution	179.8916

Figure 3. The conditions to achieve maximum response functions

3.2. Results of DNA quality assessment

After selecting the optimal conditions, the DNA extraction was carried out on three types of samples according to the process proposed as follows:

- Heat the CTAB extraction buffer to 65 °C.
- Each sample (200 mg) was transferred to a 1.5 ml sterile reaction tube, followed by addition of 1.4 µl of CTAB extraction buffer. Vortex the sample for 15 sec.
- Incubate at 65 °C for 60 min with periodic gentle swirling. After that Centrifugation at 12000×g for 9 min. The supernatant (750 µl) was transferred to a new 1.5 µl sterile reaction tube.
- Add 1 vol. of chloroform. Mix well and centrifuged at 12000×*g* for 9 min. Pipette the aqueous (top) phase into a new 1.5 µl sterile reaction tube.
- Add2 vol. of CTAB precipitation and incubated for 60 min at room temperature.
- Centrifugation at 12000×g for 9 min, the supernatant was discarded.
- The precipitate was dissolved in 350 µl of 1.2 mol l^{-1}NaCl and extracted with an equal volume of chloroform. The mixture was then centrifuged at 12000×g for 9 min. Pipette the aqueous (top) phase into a new 1.5 µl sterile reaction tube.
- Add 1.47vol of isopropanol and incubated for 20 min at room temperature. After that centrifugation at 12000×g for 9 min, the supernatant was discarded.
- Add 500 µl of 70% ethanol to wash the pellet. Mix gently but not thoroughly. After that centrifugation at 12000×g for 9 min, the supernatant was discarded (be careful that the pellet dose not slide out).
- The pellet was dried overnight at room temperature. The dry pellet was dissolved in 50 µL of TE buffer and stored at -20 ℃.

After obtaining the total DNA, conduct the electrophoresis DNA with 1% agarose gel.

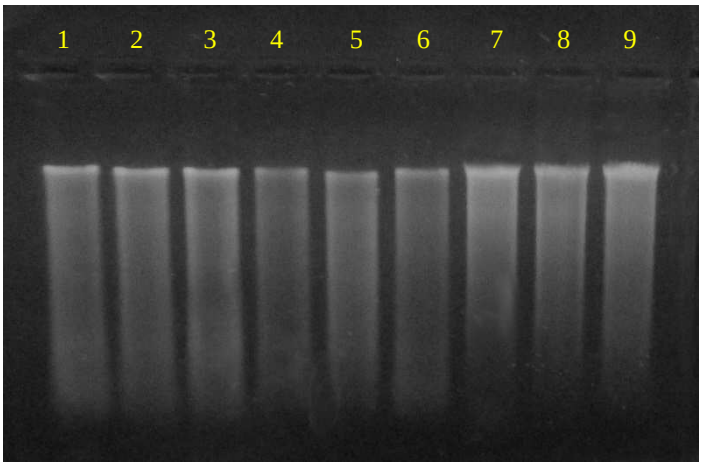


Figure 4. Result of electrophoresis total DNA with 1 % agarose gel; 1, 2, 3: fresh wood samples; 4, 5, 6: dry wood samples; 7, 8, 9: Leaves samples.

From the result of electrophoresis of total DNA with 1 % gel agarose (Figure 4) it is shown that the brightest bands are 7, 8, 9 corresponding to leaves samples, followed by the bands 1, 2, 3 corresponding to fresh wood samples; finally the bands 4, 5, 6 corresponding to dry wood. Results of the visual observations showed that the DNA extraction process performed successfully on all three types of samples.

Results of the DNA Quality are presented in Table 6. The DNA quality of the fresh wood samples is very good with high purity. The DNA quality of dry wood samples is the lowest due to the death of cells and storage times as well as the microbial decomposition lead to the degradation of DNA in these samples [12]. The DNA from the leaves samples obtained the highest quality and purity (A260/A280 around 1.7 – 2).

Table 6. Results of DNA quality when measured by the spectrophotometer BioDrop.

Symbol	Samples	DNA concentrations (ng µl-1)	A260/A230	A260/A280
1	Fresh wood	153.330	2.925	1.996
2		145.045	3.463	2.002
3		149.890	3.260	2.108
4	Dry wood	37.535	1.873	2.040
5		44.755	2.037	2.043
6		42.665	1.985	2.031
7	Leaves	158.090	1.679	1.764
8		166.500	2.266	1.820
9		164.280	2.239	1.787

Results of electrophoresis of PCR products using primers psbA - trnH on 1.5 % agarose gel (Figure 5) showed that the quality of the DNA extracted from the three sample types are met the requirements to perform PCR reactions. The bands are bright and have an equal size about 380 kb.

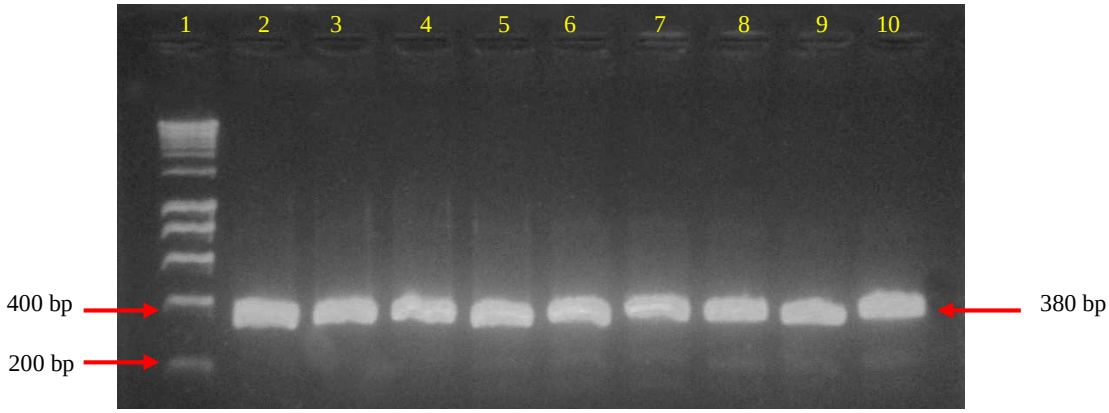


*Figure 5.*Results of electrophoresis of PCR products with primers psbA - trnH on 1.5 % agarose gel:

1: ladder 1kb; 2, 3, 4: fresh wood samples; 5, 6, 7: dry wood samples; 8, 9, 10: leaves samples.

4. CONCLUSION

The DNA extraction using CTAB protocol was applied successfully in the fresh wood, dried wood and leaves samples of *Hopeaodorata*. The optimal conditions of the DNA extraction established by RSM-CCD are as follows: the centrifugation time of 0.15 hour and the volume ratio of isopropanol to solutions containing DNA of 1:1.25 reveal the maximum DNA concentration of 179.89 $ng\mu l^{-1}$.

REFERENCES

1. Asif M. J. and Cannon C. H. – DNA extraction from processed wood: a case study for the identification of an endangered timber species (*Gonostylus bancanus*), Plant Molecular Biology Reporter, 23, International Society for Plant Molecular Biology, Canada, 2005, pp. 185-192.
2. David D. M. and Dennis D. – Preparation and Analysis of DNA, Current Protocols in Molecular Biology (2002) 201-203.
3. Dellaporta S. L., Wood J. and Hicks J. B. – A plant DNA minipreparation version II, Plant Molecular Biology Reporter **1** (1983) 19-21.
4. Doyle J. J. and Doyle J. H. – Isolation of plant DNA from fresh tissue, Focus **12** (1987) 13-15.
5. Edwards K., Johnstone C. and Thompson C. – A simple and rapid method for the preparation of plant genomic DNA for PCR analysis, Nucleic Acids Research **19** (1991) 1349.
6. Fang G., Hammar S. and Grumet R. – A quick and in expensive method for removing polysaccharides from plant genomic DNA, Biotechniques **13** (1992) 52–56.
7. Goodwin D. And Lee S. – Microwave miniprep of total genomic DNA from fungi, plants, protists and animals for PCR, Biotechniques **15** (1993) 441–444.
8. Promega Corporation – Protocol and applications guide, Madison, USA, 1996 pp. 170-207.
9. Rachmayanti Y., Leinemann L., Gailing O. and Finkeldey R. – DNA from processed and unprocessed wood: Factors influencing the isolation success, Forensic Science International: Genetics **3** (2009) 185–192.
10. Stefanova P., Taseva M., Georgieva T., Gotcheva V. and Angelov A. – A modifified CTAB method for DNA extraction from soybean and meat products, Biotechnol. & Biotechnol **27** (3) (2013) 3803 – 3810.
11. Tsumura Y., Kado T., Yoshida K., Abe H., Ohtani M., Taguchi Y., Fukue Y., Tani N., Ueno S., Yoshimura K., Kamiya K., Harada K., Takeuchi Y., Diway B., Finkeldey R., Na'iem M., Indrioko S., Kevin K. S. Ng., Muhammad N. and Lee S. L. – Molecular database for classifying Shorea species (*Dipterocarpaceae*) and techniques for checking the legitimacy of timber and wood products, Journal of Plant Research **124** (2011) 35–48.
12. Verbylaite R., Beišys P., Rimas V. and Kuusiene S. - Comparison often DNA Extraction Protocols from Wood of European Aspen (*Populus tremula* L.), Baltic Forestry **16** (1) (2010) 35-42.