International Journal of Basic Science and Technology October 2015, Volume 1, Number 1, Pages 27 - 30

Preservation of Oil Palm (*Elaeis guineensis* Jacq.) leaf tissues for DNA Extraction

[1]Odenore V. D., [1]Aisagbonhi E. P., [1]Eke C. R., [2]*Shittu H. O.

[1]Physiology Division, Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State, Nigeria
[2]Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria

Article Information

Article history:
Received: 16 March, 2015
Accepted: 22 June, 2015
Available online:

Keywords:
Preservation,
Elaeis guineensis,
DNA extraction,
Spectrophotometric analysis,
Gel electrophoresis

Abstract

Plant sample preservation is critical in maintaining the integrity of tissues prior to DNA extraction, especially in cases where the tissue collection site is distanced from the laboratory. This study attempts to investigate methods of preserving oil palm (*Elaeis guineensis* Jacq.) leaf tissue prior to DNA extraction. Oil palm was preserved using physical method by storing leaf samples in the freezer (-20°C), fridge (4°C), room temperature (25°C) and sun drying (37°C). Furthermore, some samples were preserved using chemical method by soaking them in 95% Ethanol, Salted ethanol, SDS buffer and 1X TAE buffer. Both methods were for duration of 2, 4, 6 and 8 days, prior to nucleic acid extraction. Both DNA yield and DNA purity were determined with Spectrophotometric analysis. Also, the purity of the DNA extract from tissues preserved using physical method was confirmed with Agarose gel electrophoresis. When compared with nucleic acid extracts from unpreserved fresh plant tissue (control), the results obtained indicated that preservation at -20°C and storage in Salted ethanol were most effective for long term storage of oil palm leaf samples. Preservation in TAE buffer seemed to be the least effective preservation method as there was low DNA yield obtained from the tissues stored in it. The purity of DNA extracts was not affected by both the physical and chemical preservation methods under investigation.

***Corresponding author:** Shittu, H.O., olalekan.shittu@uniben.edu

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a diploid, monocotyledonous plant, belonging to the family Arecaceae. It is economically an important tree, as it is the source of palm oil. Palm oil is the most traded vegetable oil in the international market (Corley and Tinker, 2003). Oil palm is widely cultivated in the tropical zones which include Malaysia, Indonesia, Nigeria, Ivory Coast, Columbia and Thailand (Wahid *et al.*, 2005). It is the highest producing oil seed crop in the world. Some cultivars produce as much as 5-9 tonnes of oil per hectare per annum; three times the yield of coconut and ten times that of soya bean per hectare. In addition, another 0.5 tonnes of kernel oil per hectare per annum can also be obtained (Jalani *et al.,* 1997). The large amount of oil produced in the oil palm fruit is a unique biological characteristic of this palm species (Hartley, 2000).

Continuity in harnessing the biological potential of oil palm and maintaining its competitive edge is dependent on adequate research and understanding of the crop. Research objectives are broadly similar between the oil palm research workers globally. These goals are higher yields, agronomic best practices including pest and disease control, as well as product diversity within the context of sustainability and environmental protection (Wahid *et al.,* 2005). Molecular studies are being carried out in oil palm research laboratories to investigate the genetic makeup of this crop in relation to its phenotypic characters. Yield character, including oil yield and productivity, is the target of oil palm genetic improvement through breeding programs and biotechnology (Cochard *et al.*, 2005).

Molecular studies begin with extraction of high quality genomic DNA required in downstream reactions. Plant tissue preservation is critical in maintaining the integrity of the tissues prior to DNA extraction, especially in cases where the tissue collection site is distanced from the laboratory. Also, when a researcher is working with a large amount of samples, a method of preserving collected tissues prior to nucleic acid extraction becomes necessary. The most commonly used preservation methods are freezing plant tissues in liquid nitrogen (-196°C) and/or storage at -80°C (Michaud and Foran, 2011), although, the use of such procedures are limiting in developing countries, including Nigeria. The aim of the present study is to investigate the effect of physical and chemical preservation methods on yield and purity of DNA, extracted from oil palm leaf tissue.

Materials and methods

Plant material

Oil palm (*Elaeis guineensis* Jacq.) leaves used in this study were obtained from a single plant at the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria.

Reagents

For chemical preservation method, the reagents used were: 95% Ethanol; Salted ethanol, which was prepared by dissolving 2 g of potassium acetate in 100 ml absolute ethanol; 1X TAE buffer, which was made up of 1M Tris HCl, 0.89 M Acetic acid and 0.5M Sodium Ethylenediaminetetraacetic acid; and Sodium Dodecyl Sulphate (SDS) Buffer which was composed of 0.3% SDS in 0.14 M NaCl, 0.05 M Na acetate and the pH value was adjusted to 5.1.

Preservation of oil palm leaf tissues

Collected leaf tissues were subjected to either physical or chemical preservation method for duration of 2, 4, 6 or 8 days. The physical method of preservation involved storing leaf samples at different temperatures, which include storage in the freezer (-20°C), fridge (4°C), room temperature (25°C) and sun dried (37°C). The chemical method involved soaking plant samples in 95% Ethanol, Salted ethanol, 1x TAE and SDS Buffer.

DNA extraction and agarose gel electrophoresis of extracts

The study was carried out using the facilities of the Biotechnology Unit of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State. DNA extraction from the various preserved plant tissues was carried out using the modified SDS method (Shittu *et al.*, 2015) adapted from (Robb and Nazar, 1996). Also, DNA extract was prepared from freshly harvested leaf tissues, which served as unpreserved control for the experiment (0 day preservation time). The DNA extracts were analyzed using agarose gel electrophoresis. The 2% gel matrix was made by dissolving 1.4 g of agarose powder in 70 ml of TAE buffer. The solution was heated in a microwave for 1 minute and allowed to cool. Afterwards, Ethidium bromide (10 mg/l) was added to the solution. The solution was then poured on a gel tray with a comb inserted in it. After the gel has solidified, the comb was removed creating wells on the gel. The Electrophoresis tank

International Journal of Basic Science and Technology October 2015, Volume 1, Number 1, Pages 27 - 30

was filled with TAE buffer and the gel tray was put in it. Using a micro pipette, 1 µl of loading dye was added to 9 µl of each DNA extract and the mixture was loaded into the wells. The tank was connected to a power source at 90 V for 60 minutes. The gel photograph was taken using a digital camera.

Spectrophotometric analysis of extracts

DNA purity and yield were assessed using spectrophotometric analysis. An aliquot of 1.5 ml of water was put in a cuvette and used to set the equipment to blank. To determine the purity and concentration of extracts from the various tissues, 20 µl aliquot was dissolved in 1.48 ml of water, transferred to the cuvette and placed in a UV spectrophotometer. Absorbance readings were taken at 260 nm and 280 nm wavelengths. DNA purity (quality) and DNA yield (quantity obtained from a tissue) were determined according to Shittu *et al.* (2015).

Statistical analysis

Each treatment was replicated 3 times and results represent mean ± standard error. The statistical tools used in this study include one and two way analysis of variance (ANOVA), Duncan's New Multiple Range (DMR) and Student T- Test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 14.

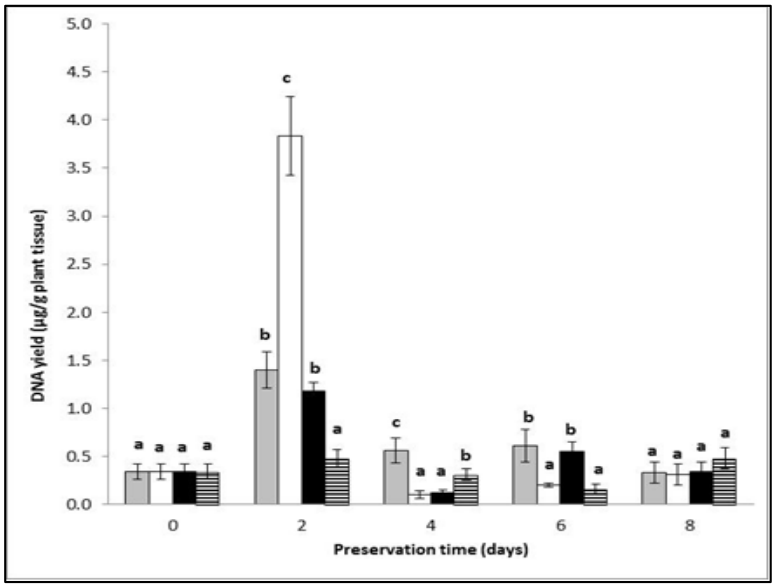

Figure 1: Effect of preservation temperature on yield of DNA extracted from oil palm leaf tissues.

Tissue storage at: -20°C (**Grey bar**); 4°C (**White bar**); 25°C (**Black bar**); 37°C (**Hatched bar**).

Each value is a mean ± standard error of three replicates. Means followed by the same letter in each vertical column are not significantly different according to Duncan's multiple range text (p = 0.5)

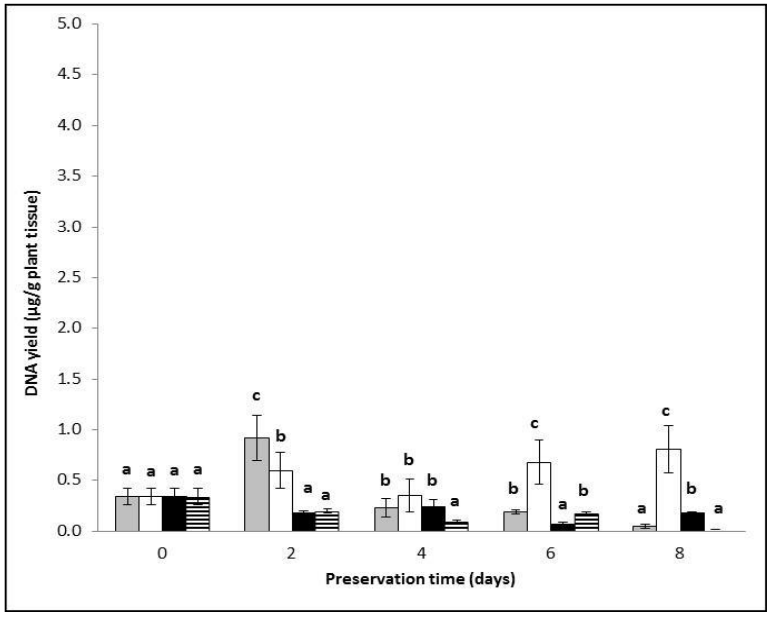

Figure 2: Effect of chemical preservation temperature on yield of DNA extracted from oil palm leaf tissues.

Tissue storage in: 95% Ethanol (**Grey bar**); Salted Ethanol (**White bar**); SDS buffer (**Black bar**); TAE buffer (**Hatched bar**).

Each value is a mean ± standard error of three replicates. Means followed by the same letter in each vertical column are not significantly different according to Duncan's multiple range text (p = 0.5).

Results

The effect of preservation temperature on yield of DNA extract from oil palm is presented in Figure 1. In relation to the control (DNA extract from unpreserved fresh tissue), higher DNA yields were obtained at all temperatures examined when leaf samples were preserved for two days. Throughout the preservation period, there was higher DNA extract from samples preserved at -20°C in relation to the control, except at 8 days (0.33 ± 0.11 µg/g plant tissue), which was not significantly different from the control (0.34 ± 0.08 µg/g plant tissue). The highest DNA yield was obtained at 4°C preservation temperature for 2 days, while the lowest extract also was from tissue preserved at 4°C for 4 days, with values of 3.83 ± 0.41 and 0.10 ± 0.04 µg/g plant tissues, respectively. Chemical preservation (Figure 2) indicated that Salted Ethanol gave the best result. The amount of DNA yield was

International Journal of Basic Science and Technology October 2015, Volume 1, Number 1, Pages 27 - 30

greater than the control for all the preservation days. TAE seemed to be the least effective preservation chemical as there were low amount of DNA extracts from tissues preserved in it throughout the duration of the study. The least amount of DNA recovered from leaf tissue was obtained in sample preserved in 1X TAE for 8 days. Surprisingly, the highest amount of DNA yield (0.81 ± 0.23 µg/g plant tissues) was obtained from leaf tissue preserved in Salted Ethanol after 8 days.

The quality of the DNA extracts was not affected by the preservation methods under study, as revealed by the Spectrophotometric analysis. The values obtained from the absorbance at 260 nm and 280 nm ratios were relatively the same for all extracts obtained from tissues preserved by either temperature or chemical methods, and were within purity range of 1.7 - 1.9. The agarose gel electrophoresis of extracts preserved at different temperatures (Figure 3) also supported the Spectrophotometric analysis for DNA purity. There were distinct bands, which where devoid of smear in all the lanes that corresponded with each preservation conditions.

Discussion

Plant leaf tissues are made up of cells and the constituents of the cell are being protected from external forces by the cell bio-membranes (Nester *et al.,* 2001). Once a leaf is harvested from the plant, degradation of its cells begins starting from the cell wall and membrane. This study demonstrated the effectiveness of temperature and chemical in the preservation of oil palm leaf tissues. It can be deduced that the optimal temperature at which the tissues can be stored for maximum DNA yield (Figure 1) and high quality (Plate 1) is at 4°C for 2 days. A possible explanation for this could be that after 2 days of harvesting the leaf samples, the cell walls of the tissues might have been degraded but the nucleic acid was still intact. During tissue grinding with lysis buffer, there was less obstruction in getting to the DNA, as such there was high yield. Preservation days greater than 2, at 4°C, degradation process and nucleolytic activities of some enzymes may account for yield reduction. The same observation may also account for the tissues preserved at room temperature for 4 days. For longer storage of leaf tissue for up to 6 days, -20°C was preferable. This observation is similar to the findings of Rahimah *et al.* (2006) in their study to determine the effect of freeze drying oil palm leaves on the quality of DNA extract. Their result demonstrated that freeze dried oil palm leaves can be stored at -20°C and 4°C for up to 18 months. It can be deduced that the temperature at -20°C is low enough to inhibit enzymatic activities and maintain cell integrity. A similar observation was made by Nuri *et al.* (2014) who found that cassava leaf tissue can be preserved at 4°C for one week without significant loss in DNA yield or quality. Plant tissues can also be preserved in Ethanol or buffer solution prior to DNA extraction (Michaud and Foran, 2011).

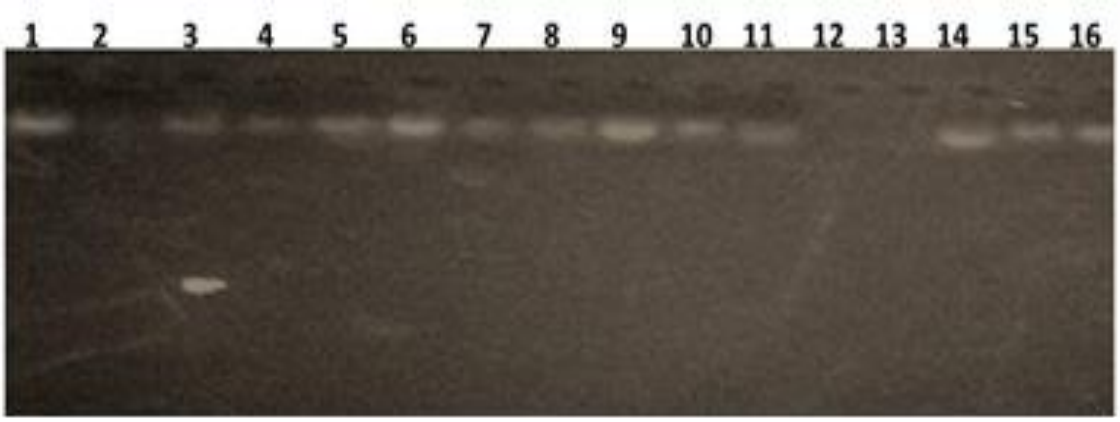

Figure 2: Gel electrophoresis photograph of DNA extracted from oil palm leaf tissues preserved at -20°C, 4°C, 25°C and 37°C.

Preservation of leaf tissues for 2 days at 1: -20°C; 2: 4°C; 3: 25°C; 4: 37°C
Preservation of leaf tissues for 4 days at 5: -20°C; 6: 4°C; 7: 25°C; 8: 37°C
Preservation of leaf tissues for 6 days at 9: -20°C; 10: 4°C; 11: 25°C; 12: 37°C
Preservation of leaf tissues for 8 days at 13: -20°C; 14: 4°C; 15: 25°C; 16: 37°C

The tissues preserved in Salted ethanol gave a high yield of DNA throughout the duration of the study, followed by Ethanol preservation when compared to the unpreserved control. From the study of Akindele and coworkers (2011) on the effect of ethanol pretreatment on DNA yield, the yield obtained from tissues soaked in ethanol was more than the yield obtained from fresh tissues. Bressan *et al.* (2014) in their study on the extraction of high-quality DNA from ethanol-preserved tropical plant tissues also observed that leaf samples can be successfully preserved in ethanol for long periods (30 days) as a viable method for fixation and conservation of DNA from leaves. It was postulated from their study that the success of this technique was likely due to reduction or inactivation of secondary metabolites that could contaminate or degrade genomic DNA. The preservation in buffer solutions on the other hand did not aid the extraction of a high quantity of DNA. The yield obtained was low for all the preservation duration examined. This may be as a result of the high water content in SDS and TAE buffer, as such creating an enabling environment for nucleolytic enzymes to act on the nucleic acid in leaf tissues. This finding is in line with Bressan *et al.* (2014) who implicated a dehydrated solution to guard against the activity of nucleolytic enzymes when DNA was extracted from tropical plant species. The results obtained in this study suggest that long term storage of plant samples in buffer solution is not effective for DNA extraction.

Conclusion

The current study suggests that quality DNA extract with high yield can be obtained from oil palm leaf tissues stored at much lower temperatures such as 4°C and -20°C, as well as storage in Salted ethanol and 95 % Ethanol. Storage in 95 % Ethanol will give a high DNA yield between a 2 - 4 days period and preservation at 4°C will give a high DNA yield when stored for 2 days, but for longer periods, preservation in Salted ethanol or at -20°C is preferred for maximum DNA yield.

References

Akindele, A., Xin, B. J., Minsheng, Y. and Dietrich, E. (2011). Ethanol pretreatment increases DNA yields from dried tree foliage. *Conservation Genetic Resources,* **3:**409–411.

International Journal of Basic Science and Technology October 2015, Volume 1, Number 1, Pages 27 - 30

Bressan, A.E., Rossi, M.L., Gerald, L.T.S. and Figueira, A. (2014). Extraction of high-quality DNA from ethanol-preserved tropical plant tissues. *Research Notes*, **7:**268

Cochard, B., Amblard, P. D. and Gasselin, T. (2005).Oil palm genetic improvement and sustainable development. *Oil Crop Leaders*, **12:** 141-147.

Corley, R.H.V., Tinker, P.B. (2003). *The Oil palm.* Blackwell publishing, United Kingdom, 765p Hartley, C.W.S. (2000). *The Oil palm* (*Elaeis guineensis* jacq.) Longman Publishing, London, 808p.

Jalani, B.S., Cheah, S.C., Rajanaidu, N., and Darus, A. (1997). Improvement of palm oil through breeding and biotechnology. *Journal of African Oil Crops Society*, **74:** 1451-1455.

Michaud, C.L. and Foran, D.R. (2011). Simplified field preservation of tissues for subsequent DNA analysis. *Journal of Forensic Sciences*, **56**:846-852.

Nester, E.W., Anderson, D.G., Roberts, C.E., Pearsall, N.N and Nester, M.T. (2001). *Microbiology: A human Perspective*. McGraw hill publishers, America, 820p.

Nuri, K., Nawar, L.S., Pradita, M., Etik, S., Mirza, R.P., Yudiansyah, Nurul, K., Sintho, W. A. (2014). Sample preservation procedures and simple DNA isolation protocols for the tuberous crop, cassava (*Manihot esculenta* Crantz**.**) *Journal of Molecular Biology Biotechnology, **22 (1):** 164-170.*

Rahimah, A. R., Cheah, S. C. and Rajinder, S. (2006). Freeze-drying of oil palm (*Elaeis Guineensis*) leaf and its effect on the quality of extractable DNA**.** *Journal of Oil Palm Research*, **18:** 296-304.

Robb, E. J. and Nazar, R. N. (1996). Factors contributing to successful PCR-based diagnostics for potato and other crops. In: Diagnostics in Crop Production. G, M. (Eds.), British Crop Protection Council, Surrey, 216p.

Shittu, H. O., Durojaye, A. P., Uzama, O. M., Odenore, V. D. and Eke, C. R. (2015). Optimizing nucleic acid extraction from African yam bean (Sphenostylisstenocarpa) tissues without lyophilization or liquid nitrogen grinding. *Nigerian Journal of Life Science,* 4(2) (in press).

Wahid, M.B., Abdullah, S.N.A. and Henson, I.E. (2005). Oil palm Achievements and Potentials. *Plant Product Science,* **8:** 288-297.