AGARICUS: Advances Agriculture Science & Farming
Vol. 3 No. 1 June 2023, Page. 6 - 10
E-ISSN: 2797-0884

Optimization of Fruit DNA Extraction by Kitchen Kit Method with Isopropanol and Absolute Ethanol

Umi Kulsum Nur Qomariah
Agroekoteknologi, Universitas KH. A. Wahab Hasbullah
*Email: umi.kulsum@unwaha.ac.id

ABSTRACT

Fruits such as papaya, guava, banana, strawberry are high-value food commodities that are in great demand by the wider community, so they have the potential to be developed. It is necessary to study various scientific disciplines, one of which is the molecular biology approach. DNA is a basic element in molecular biology research. The DNA extraction technique greatly determines the quality and quantity of the DNA produced. The chemical solvent used in the precipitation is an important factor in producing the quality and quantity of DNA, so optimization is required. The purpose of the research to investigate the effect of isopropanol and ethanol on fruit DNA extraction. The DNA extraction method used is the kitchen kit method which is used on 4 types of soft fruit: papaya, guava, banana and strawberry. The principles of DNA extraction are lysis, precipitation and purification. The lysis process was carried out chemically with a solution of detergent and NaCl, and physically by means of a blender until it was homogeneous, then separation was carried out using filter paper. The aquos phase is collected to be chemically precipitated. Precipitation was carried out with cold absolute ethanol and cold isopropanol at -4^{0}C. The results of extraction with isopropanol showed the consistency and quantity of fruit DNA: papaya had rather dense fiber, slightly soft guava, slightly faded thin strawberry and rather dense fibrous banana. The results of absolute ethanol extraction showed the consistency of fruit DNA: papaya fiber was rather dense, guava fiber was medium, strawberry was rather dense and banana fiber was moderate. DNA extraction with ethanol precipitation produces more optimal DNA clumps compared to isopropanol precipitation.

Keywords: *DNA extraction; precipitation; Optimization; fruit DNA; simple method*

INTRODUCTION

The development of science and technology to meet human food needs is mostly done through plant breeding. The agricultural sector has benefited the most so that at this time various fruit plants have been produced which can be harvested in a relatively short time with superior fruit quality when compared to natural types. It is necessary to study various scientific disciplines, one of which is the molecular biology approach. The DNA molecule is the basic key in molecular biology and biotechnology research, which can be obtained from plant materials through an extraction process. DNA extraction is the process of separating DNA molecules from cell components such as proteins, protoplasts, organelles, polysaccharides, lipids, cell walls and other substrates. The principle of genomic DNA isolation can be carried out by physical and chemical cell lysis methods (Murtiyaningsih, 2017). Physical lysis was carried out using mechanical forces, namely freeze thaw, bead mill homogenization and resonance. Chemical lysis is carried out with lysis buffer in the form of chemical compounds that can damage the integrity of the cell wall barrier, for example with SDS (*Sodium Dedocyl Sulfate*) and CTAB (*Cetyl Trimethyl Ammonium Bromide*) (Cheng et al., 2003).

So far, DNA extraction is considered an expensive and difficult procedure with modest resources. But actually the DNA extraction process can be done with a simple kit that is easy and inexpensive (Purwoko, 2018). DNA visualization needs to be shown at the learning stage so that students believe that the DNA molecule is real. The DNA extraction process is simple, carried out using ingredients and kitchen equipment that are easy to obtain and prepare and do not require a high cost (Marek et al., 2009). Through the kitchen kit method, the learning experience of DNA extraction can be carried out with

simple materials and without having to follow preparation protocols that require sterile and complicated conditions (Mardiyyaningsih, 2013).

The materials used for DNA isolation basically come from parts of living things. In eukaryotic living things, DNA is located in the cell nucleus and several other organs in the cell such as mitochondria and chloroplasts (Hairuddin, 2013). Soft fruit in this case can be an ingredient in DNA extraction. Materials used in simple DNA extraction include liquid soap, table gram and protease from meat tenderizers and ethanol or isopropanol (Marek et al., 2009). In the presence of sodium ions derived from salt, absolute ethanol or isopropanol is generally used for the precipitation of DNA from the aqueous phase (Chen et al., 2010). Absolute ethanol solution which is quite expensive can be replaced with isopropanol which is more affordable for use in the precipitation process. However, differences in the chemical properties of ethanol and isopropanol can result in different quality and quantity of DNA for each type of fruit sample. The purpose of this study to investigate the different effects of alcohol and isopropanol on DNA extraction.

METHOD

DNA extraction in this study was carried out using the kitchen kit preparation method. Materials used as DNA sources include papaya, banana, strawberry and red guava. The **lysis** buffers include liquid soap and table salt. The **precipitation** buffer uses absolute ethanol and cold isopropanol at -4^0C. The tools used include blender, volume pipette, filter paper, measuring cup, funnel, erlenmeyer glass, and slide glass.

Preparation: Soft fruit DNA was carried out by selecting fresh and healthy milk bananas, papaya, strawberries and red guava, then peeled and washed thoroughly under running water. The fruit is then weighed as much as 50 grams for each type of fruit sample. The knife and cutting board used for each type of fruit is different to prevent contamination.

The DNA **extraction procedure** begins with preparing a lysis buffer solution consisting of a mixture of 20 ml of liquid soap, 10 g of table salt and 50 ml of distilled water, then stirred gently until homogeneous. The extraction stage begins with **lysis,** which is carried out physically and chemically, namely 50 grams of papaya fruit sample is cut into small pieces then added lysis buffer that has been prepared beforehand, then blended until homogeneous. Then it was filtered and the aquos phase was collected in an Erlenmeyer glass. The filtering process should not be suppressed, wait for the water phase to drop slowly. Subsequent **precipitation** was carried out by taking 10 ml of the aqueous phase into two test tubes, then adding cold ethanol into one tube and adding cold isopropanol into the other tube at a ratio of 1:1. Wait and observe the process of DNA thread formation for 5 minutes. The collected DNA genome was then taken carefully using a pipette and observed on a glass slide and then **analyzed** for quantity, quality and consistency visually. The same protocol was also carried out for DNA extraction of milk banana, red guava and strawberry.

RESULT AND DISCUSSION

The results of genomic DNA extraction can be observed with the naked eye without using tools. Genomic DNA was visualized with a set of DNA strands formed in a test tube and then observed for quantity, concentration and consistency. The soft fruit genomic DNA that was formed was observed and then summarized in Table 1. The visual genomic DNA produced by the kitchen kit preparation method is documented and presented in Figure 1.

Result

The results showed that the DNA extraction kitchen kit preparation method can be applied to isolate genomic DNA. The test tube shows a clear separation between DNA and supernatant in observing the precipitation process. Each fruit DNA supernatant that was filtered from the lysis process was divided into two tubes and then precipitated using cold absolute ethanol and cold isopropanol respectively.

Papaya fruit genomic DNA precipitated with absolute ethanol has the characteristics of moderate concentration, rather dense fiber consistency and quite a lot of quantity. Papaya fruit genomic DNA precipitated with isopropanol has the characteristics of low concentration, dense fibrous consistency and small amount. Banana milk genomic DNA precipitated with absolute ethanol has the characteristics of high concentration, dense fibrous consistency and large amount. Banana genomic DNA precipitated with isopropanol has the characteristics of low concentration, small amount and rather dense fibrous consistency. Red guava genomic DNA precipitated with absolute ethanol has the characteristics of high

DNA concentration, soft fiber consistency and a very large amount. Guava genomic DNA precipitated with isopropanol produces DNA that has a low concentration, soft fibrous consistency and small amount. Strawberry genomic DNA precipitated with absolute ethanol has the characteristics of moderate DNA concentration, rather dense fiber consistency and small amount. Strawberry genomic DNA precipitated with isopropanol exhibits very low concentration characteristics, brittle consistency due to fading thin films and very small amounts (Figure 1).

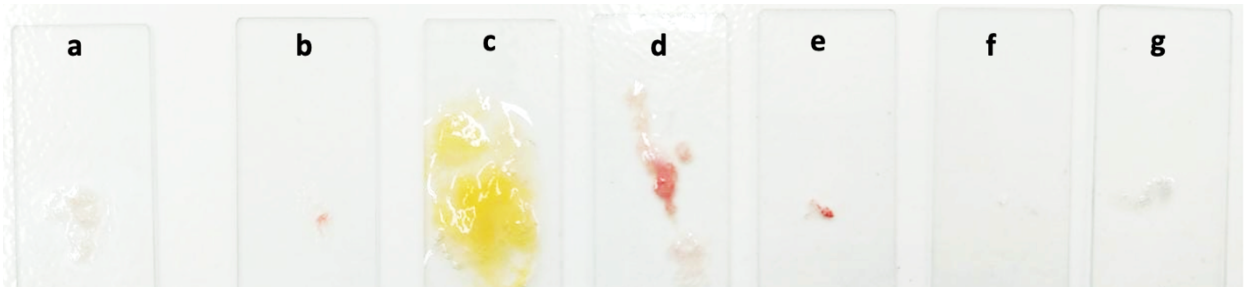

Figure 1. Visualization of soft fruit genomic DNA extracted using the kitchen kit method

a. strawberry DNA + absolute ethanol, b. Papaya DNA + isopropanol, c. Papaya DNA + absolute ethanol, d. Guava DNA + absolute ethanol, e. Guava DNA + isopropanol, f. Banana DNA + isopropanol, g. Milk Banana DNA + absolute ethanol.

Observation of the consistency of soft fruit genomic DNA was carried out by drying the DNA threads that had been dripped on a glass slide for approximately 15 minutes until some of the solvent evaporated so that only DNA remained on the slide. The collection of genomic DNA on glass slides was then observed for its density and consistency with the help of a toothpick to feel the level of density. The quantity of DNA produced was observed by comparing all extracted fruit DNA arranged in such a way. Data from visual observations of genomic DNA are then summarized in Table 1.

Table 1 Summary of soft fruit DNA visualization observations

Sample	DNA concentration	DNA consistency	Quantity
Papaya + ethanol	+++	Fibrous rather dense	Numerous
Papaya + isopropanol	++	Fibrous dense	Slight
Banana + ethanol	++++	Fibrous dense	moderate
Banana + isopropanol	++	Fibrous rather dense	Slight
Guava + ethanol	++++	Fibrous soft	Much
Guava + isopropanol	++	Fibrous soft	Slight
Strawberry + ethanol	+++	Fibrous rather dense	Moderate
Strawberry + isopropanol	+	Fibrous fragile	Slight

Description of DNA concentration:
+ not concentrated, ++ slightly concentrated, +++ concentrated, ++++ very concentrated

In general, the aqueous phase of soft fruit precipitated with absolute ethanol resulted in a higher concentration of DNA strands than that precipitated with isopropanol. DNA threads precipitated with ethanol solution, yielded a greater amount than that precipitated with isopropanol as a whole. The resulting DNA consistency varied, some were precipitated with ethanol resulting in a denser consistency compared to isopropanol deposition such as banana and strawberry DNA. However, it is different from papaya DNA which produces a denser consistency by precipitating isopropanol compared to precipitating with ethanol. The consistency of guava DNA showed the same consistency whether precipitated with ethanol or isopropanol, namely soft fibers.

Discussion

In principle, extraction of soft fruit genomic DNA includes lysis, precipitation, and purification. In this study, lysis and precipitation were carried out but no purification was carried out. Cell lysis was carried out by mixing each soft fruit together with lysis buffer. The blending process aims to damage and break down the cell walls mechanically and physically. The addition of lysis buffer made from liquid

soap and salt serves to lyse the cell membrane which is composed of lipid and protein components. The mechanism of cell lysis is carried out by breaking the cell membrane that separates the cell from the outside environment and lysing the nuclear membrane (Mardiyyaningsih, 2013). This study is in line with the recommendations for a simple DNA isolation protocol conducted by (Marek et al., 2009) that liquid detergents can be used to lyse membranes. In this study, the fruit samples used were ripe fruit because it can produce enzymes that help lyse cell walls (Rante et al., 2021).

The use of table salt together with liquid soap aims to lyse the nuclear membrane and remove the contents of the cell nucleus, where it is known that the nuclear DNA is in the cell nucleus. The fruit DNA of eukaryotic cells, such as the soft fruit in this study, is present in the nucleus, which is involved in various DNA-binding proteins (Hofmann & Clokie, 2018). The use of detergent or liquid soap in the process of extracting DNA from this fruit also serves to open the cell membrane and the cell nucleus membrane, so that the DNA will exit the nucleus and then enter the solution or water phase. The filtering process with filter paper serves to separate cellulose, cell walls, proteins, lipids and carbohydrates. Even though it is filtered, DNA molecules can still pass through the porous filter paper along with the aqueous phase which is accommodated in a test tube.

The precipitation process is carried out by adding cold ethanol and cold isopropanol. The addition of the precipitate solution was carried out slowly through the test tube wall so as not to damage the fragile DNA structure. After adding the precipitate solution, 2 layers were formed. The bottom layer is a fruit and soap solution, while the clear top layer is isopropanol and ethanol in the other tube. Isopropanol and ethanol solutions are on the top layer because they have a lighter density compared to the aquos phase.

The addition of cold absolute ethanol solution decreases the solubility of DNA, so that the DNA will precipitate or concentrate towards the ethanol or isopropanol layer in another tube, while fat and protein molecules precipitate in the solution at the bottom layer (Waldron et al., 2016). The precipitation process using the kitchen kit method in this study was waited for less than 5 minutes to see DNA molecules in the form of fibrous threads floating between the aquos phase and the ethanol phase. The ethanol solution used is cold at -4^0C, which aims to increase precipitation. In line with this research (Rante et al., 2021) reveal that if the ethanol used was not cold enough it would result in the formation of imperfect precipitates.

The DNA extraction results produced in Figure 1 show different amounts of each sample. This can be affected by adding the same volume of buffer during DNA deposition, even though each sample may contain different DNA. Goes along with it, (Rizko et al., 2020) reveal that the washing process with ethanol could also affect the concentration and purity of DNA. The more samples that were washed with ethanol, the higher the DNA purity, but the DNA concentration decreased.

The addition of ethanol solution in precipitation can reduce the solubility of large molecules such as carbohydrates, proteins and nucleic acids (Gokarn et al., 2016).

But of the three polymers, nucleic acid is the most soluble in ethanol. In this study, the quantity of fruit DNA extracted by precipitation with cold absolute ethanol was greater than that of isopropanol in banana, guava, papaya and strawberry. In the DNA isolation method, DNA precipitation occurs at an ethanol concentration of 67-70% (Rizko et al., 2020). However, in this study DNA precipitation was formed properly using enough cold absolute ethanol solution as in papaya genomic DNA.

Differences in concentration and amount of extracted DNA can be caused by differences in the water content of each sample. Indeed, the volume of buffer mixed in each sample is the same, but the natural water content in each sample may be different. For example, the water content of guava is less than that of strawberries, bananas and papayas. The water content of bananas is less than that of papayas and strawberries Figure 1. This of course can result in the formation of an aquos phase which has a different concentration level, so that the amount of DNA produced is also different. This fact is in accordance with research (Rizko et al., 2020) that a less concentrated solution causes less DNA to precipitate. Cold isopropanol is better to use than 70% alcohol. Thus, the amount of extracted DNA is different due to differences in water content.

Precipitation using 100% isopropanol in this study resulted in fewer clumps of DNA strands than precipitation using ethanol. When isopropanol is added in the aqueous phase, the solution begins to spin, and clumps of DNA threads appear to coil floating between the aqueous phase and the isopropanol phase. Isopropanol is used as an alternative to ethanol in the DNA precipitation process because the efficiency of this chemical is higher than ethanol (Sambrook & Russel, 2001). However, the results of DNA extraction in this study showed that the amount of DNA precipitated with isopropanol was less than that precipitated with ethanol. This is possible because the ethanol used is of the PA type (proanalyst), while the

isopropanol used is of the technical isopropanol type. The pro-analyst chemicals have been analyzed and their concentration levels checked in the laboratory, while the level of purity of the engineered chemicals is not as high as the pro-analyst chemicals. Technical chemicals are generally used for production processes. Even so, isopropanol can still be used as a DNA extraction agent even though it only produces less DNA.

DNA extraction in this study was only carried out in two stages, namely lysis and precipitation, while the purification process was not carried out. Fruit DNA extract using the kitchen kit preparation method has a low level of purity. Contaminant substrates such as proteins, RNA, polysaccharides and lipids may still be contained in the DNA produced by this simple method. Precipitation can be carried out with ethanol or isopropanol, whichever is available. Precipitation with pro-analytical chemicals can produce DNA in larger quantities than technical chemicals. Ethanol can precipitate papaya, banana, strawberry and guava fruit DNA with higher quality than isopropanol. However, DNA extraction with isopropanol can also be carried out in the DNA recognition process for early-level students so they can visually identify the shape of DNA.

CONCLUSION

Plant DNA extraction can be done in a simple way with materials that are easily available. In the lysis process, CTAB buffer can be replaced with liquid soap and NaCl can be replaced with table salt. The precipitation process with pa absolute ethanol produces more DNA and is more optimal than technical isopropanol. Pro-analyst chemicals are best used for DNA extraction rather than technical chemicals.

REFERENCES

Chen, H., Rangasamy, M., Tan, S. Y., Wang, H., & Siegfried, B. D. (2010). Evaluation of Five Methods for Total DNA Extraction from Western Corn Rootworm Beetles. *PLoS ONE*, *5*(8), e11963. https://doi.org/10.1371/journal.pone.0011963

Cheng, Y.-J., Guo, W.-W., Yi, H.-L., Pang, X.-M., & Deng, X. (2003). An efficient protocol for genomic DNA extraction fromCitrus species. *Plant Molecular Biology Reporter*, *21*(2), 177–178. https://doi.org/10.1007/BF02774246

Gokarn, K., Sarangdhar, V., & Pal, R. B. (2016). Research Article Ethanol Extraction Methode for DNA Isolation from Mycobacterium Smegmatis. *International Journal of Current Research*, *8*(9), 39013–39015.

Hairuddin, R. (2013). Analisis DNA Pada Tanaman Gandum (*Triticum aestivum* L.). *Jurnal Dinamika*, Vol 04 (02), 41-46.

Hofmann, A., & Clokie, S. (2018). *Principles and Techniques of Biochemistry and Molecular Biology* (8th edition). Cambridge University Press.

Mardiyyaningsih, A. N. (2013). Teknik Isolasi DNA Sel Hati Ayam Secara Tradisional. *Jurnal Pendidikan Matematika dan IPA*, *2*(1). 23-28. https://doi.org/10.26418/jpmipa.v2i1.2175

Marek, E., Mulvihill, C., & Bell, D. (2009). *Extraction The Max from A DNA Extraction.* Science Scope. Oklahoma: 36-39

Murtiyaningsih, H. (2017). *Isolasi DNA Genom dan Identifikasi Kekerabatan Genetik Nanas Menggunakan RAPD (Random Amplified Polimorfic DNA), Agritrop, 15 (01), 83-93*

Purwoko, M. (2018). Purifikasi DNA Manusia dengan Teknik Kitchen Preparation Menggunakan Ekstrak Jahe (Zingiber officinale Rosc). *Syifa'MEDIKA:Jurnal Kedokteran dan Kesehatan*, *9*(1), 39. https://doi.org/10.32502/sm.v9i1.110

Rante, H., Djide, M. N., Yulianty, R., Pakki, E., Fatmawaty, A., Tayeb, R., & Wahyudin, E. (2021). *Pengenalan Bioteknologi Melalui Pelatihan Isolasi dan Ekstraksi DNA Pada Guru dan Siswa SMA Negeri 13 di Kabupaten Bone Sulawesi Selatan*. *5*(4).

Rizko, N., Kusumaningrum, H. P., Siti, R., Pujiyanto, S., Erfianti, T., Mawarni, S. N., Tri, H., & Khairunnisa, D. (2020). *Isolasi DNA Daun Jeruk Bali Merah (Citrus maxima Merr.) dengan Modifikasi Metode Doyle and Doyle*. Vol 3(2), 1-7.

Sambrook, J., & Russel, D. (2001). *Molecular Cloning: A Laboratory Manual, 3rd Edition*. Cold Spring Harbor Laboratory Press.

Waldron, I., Spindel, L., & Doherty, J. (2016). *DNA*. University Pennsylvania.