Protocol Optimization ofDNA Extraction from Banana Fruits

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ABSTRACT

Background: DNA extraction is the process by which the DNA is extracted and purified from the living or dead cells or tissues of organisms. There are many different kitscommercially available for DNA extraction, with varying protocols. This study aimed at determining the optimal conditions for the extraction of high yield DNA from banana fruits.

Methodology:DNA was extracted from banana fruits by precipitation method. The DNA yield was determined using theNanodrop DNA quantification technique.

Results: The optimal sodium chloride salt concentration for DNA extraction from banana fruits was 4mM and the amount of DNA extracted increased as the temperature decreased. The highest yield was obtained between4 ^{0}C to 0 ^{0}C.

Conclusion: We report for the first time a protocol with an optimal salt concentration (NaCl, 4mM) for quality DNA yield from banana fruits. A critical consideration is required to establish an optimal range of moderate temperatures and DNA concentrations for better quality and desired yields. There may not be a universal protocol for DNA extraction from all plant tissues.

Keywords: DNA extraction; banana fruits; sodium ion salt concentration; temperature.

1. INTRODUCTION

DNA extraction is the process by which the DNA is purified from the living or dead cells or tissues of organisms. Currently, DNA extraction is a routine procedure in molecular biology(1). These biomolecule can be isolated from any biological material for subsequent downstream processes, analytical, or preparative purposes(1, 2).There are many different protocols and, kits commercially available for DNA extraction from different plant tissues(2-10). Varying amounts and integrity of DNA are obtained due to the inherent properties as it were, and, differences in the optimization of the protocols for DNA extraction(11, 12).

Water is a polar molecule that has a partial negative charge near the oxygen atom due to the unshared pairs of electrons, and partial positive charges near the hydrogen atoms(13). Because of these charges, polar molecules like DNA can interact electrostatically with the water molecules, allowing them to easily dissolve in water(13). DNA is hydrophilic due to the negatively charged phosphate (PO3–) groups along the sugar-phosphate backbone hence being soluble in water (13). The role of the salt is to neutralize the charges on the sugar-phosphate backbone(13). Varying concentrations of salts are used when extracting DNA using different protocols. In this study we determined the optimal salt concentration that yields high amount of DNA from banana fruits.

Incubation of the nucleic acid/salt/ethanol mixture at low temperatures (–20° or –80°C) is universally cited as a necessary step in the DNA extraction protocols. Nevertheless, there are still discontents to this this establishment due to the fact that at concentrations as low as 20 ng/Ml, nucleic acids precipitate at 0–4°C(14). In addition, there are different DNA extraction kits in phase with the outstanding discrepancy (8, 9). This study addressed the differences in the incubation temperatures and effect of salt concentration optimal for the extraction of DNA,particularlyfrom banana fruits.

2. METHODOLOGY

2.1. Determination of the effect of NaCl concentration on the DNA yield

A mixture of 25g of peeled banana(*Musa acuminata)* fruitand 50 ml of nuclease free water (Cat.W4502, Sigma Aldrich, Darmstadt, Germany) was blended to dissociate cells and strained to yield a banana fruit solution. Ten (10) ml of 10% Sodium Dodecyl Sulfate SDS (Cat. MB-15, Rockland, Inc., USA) was added into the strained banana fruit solution to lyse the cells and release the DNA. The solution was stirred for 10 minutes at room temperature.The mixture was centrifuged for 5 minutes at 8000 rpmto separate the debris from the solution.The supernatant was aliquoted into 20eppendorf tubes each containing 1ml of the supernatant.Different amounts of sodium chloride salt were calculated, weighed, and dissolved in each of the solutions to make different salt concentrations (0.25mM, 0.50mM, 0.75mM, 1.00mM,1.25mM, 1.50mM, 1.75mM, 2.00mM, 2.25mM, 2.50mM, 2.75mM, 3.00mM, 3.25mM, 3.50mM, 3. 75mM, 4.00mM, 4.25mM, 4.50mM, 4.75mM, and5.00mM). Each solution was stirred for 5 minutes.Then, 2ml of 99.5mMisopropanol (Cat. I9516, Sigma Aldrich, Darmstadt, Germany) kept at a given temperature was addedgently down the side of the respective tubes to precipitate DNA in the solution. The solution was applied into the QIAamp Mini column and centrifuged at 8,000 rpm for 1 minute to bind the DNA to silica column. The DNA was washed using 500μL of Qiagen DNA wash buffers. The DNA was eluted in 100μL of nuclease free water. DNA concentration was determined using NanoDrop™ 2000 Spectrophotometer (Cat. ND-2000, ThermoFisher) and the total amount of DNA extracted was determined.

2.2. Determination of the effect of temperature on the DNA yield

The DNA extraction was performed as described in section 2.1 while varying the temperature of 99.5% absoluteisopropanol from -10 ^{0}C to 100 ^{0}C in 5 ^{0}C increments at a constant concentration of NaCl of 4 mM. These temperatures were achieved using an incubators and extreme low and high temperatures were achieved using fridge/freezer and oven respectively while monitoringusing a thermometer.

2.3. Data analysis

The Pearson's product moment correlation coefficient was used to determine the relationship between the concentration of the sodium chloride salt used and the amount of DNA extracted and the relationship between the temperature and the amount of DNA extracted because the variables are bivariate, and the salt concentrations and isopropanol temperatures were measured on a fixed interval.

3. RESULTS

3.1. Effect of salt concentration on the DNA yield

We established that 4mM was the optimal salt concentration at which the maximum yield of DNA was obtained(**Figure 1A**).This can be rationed as the mass of salt to mass of sample solutionof 1:25**(Figure 1A).** There was a very strong positive and significant correlation between the sodium chloride concentration and the amount of DNA extracted before the optimal concentration of sodium chloride (**Figure 1B**; ***r***= 0.998; ***P*** *=0.001*). There was also a very strong negative and significant correlation between the sodium chloride concentration and the amount of DNA extracted after the optimal concentration of sodium chloride salt **(Figure 1C**; ***r***= -0.995; ***P****=0.001*).

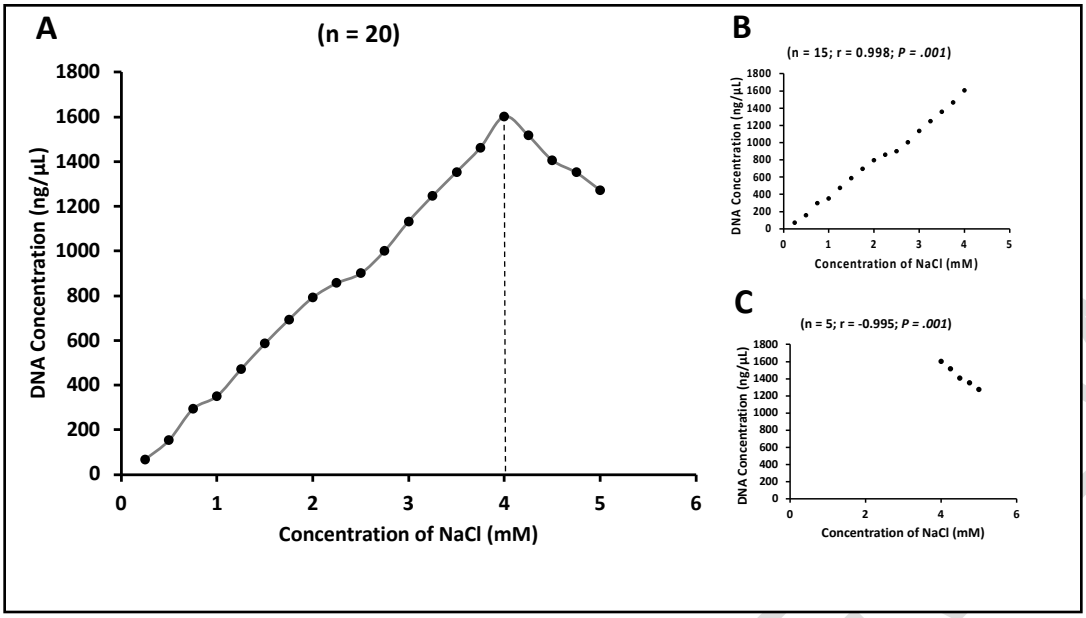

Figure 1. Effects of salt concentration on the DNA yield: **A** *is the DNA yield across various salt concentrations,* **B** *is the correlation between the sodium chloride salt concentration and the amount of DNA extracted before the optimal concentration of sodium chloride salt (r = 0.998; P = .001) and***C** *is correlation between the salt concentration and the amount of DNA extracted after the optimal concentration of salt (r = -0.995; P = .001).*

3.2. Effect of temperature on the DNA yield

There was a very strong negative and significant correlation between the temperature and the amount of DNA extracted (**Figure 2**; ***r*** = -0.997; ***P***= *0.001*).

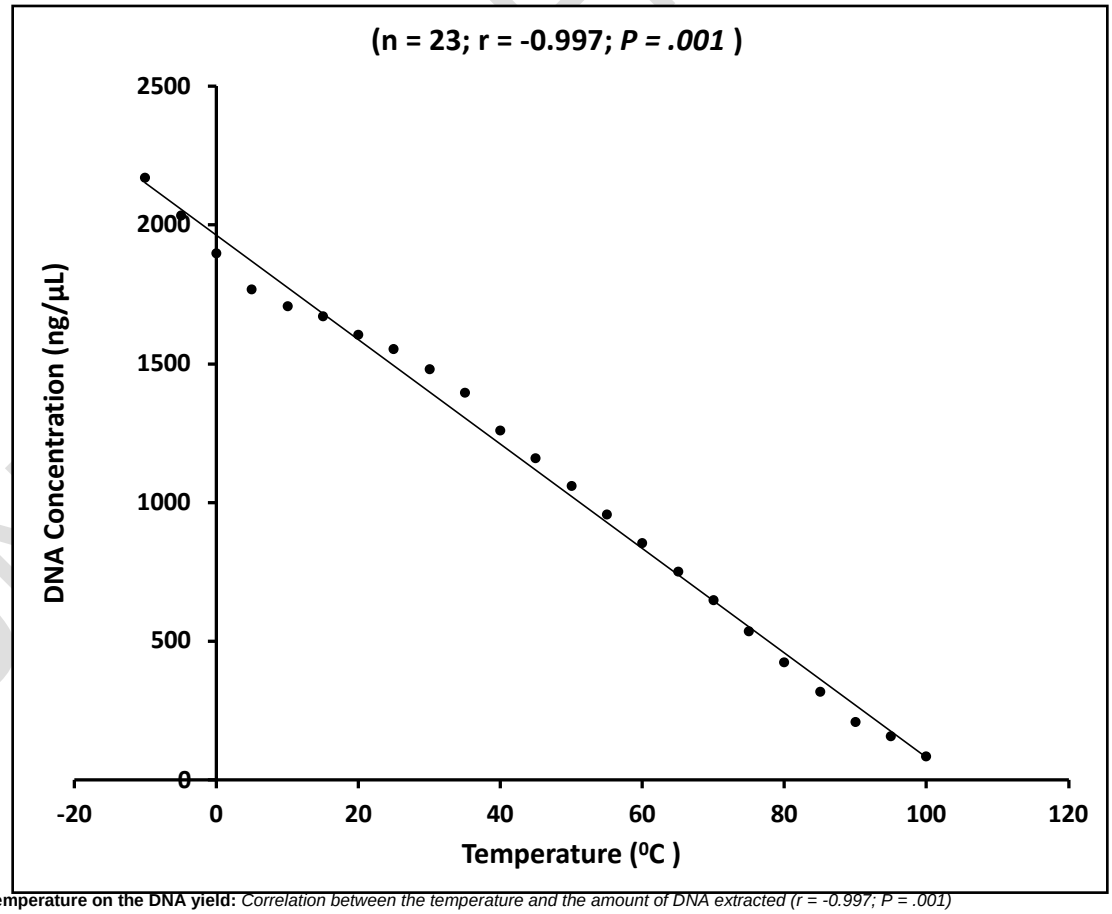

Figure 2. **Effect of temperature on the DNA yield:** *Correlation between the temperature and the amount of DNA extracted (r = -0.997; P = .001)*

4. DISCUSSION

Salt concentration is critical for quality DNA extraction especially from plant tissues (3-7, 10, 15-18). In fact, there are many different protocols and kits considering the importance salt concentration which have been used for DNA extraction from different plant tissues including banana (3-7, 10, 16, 17, 19, 20). We report for the first time a protocol with an optimal salt concentration (NaCl, 4mM) for quality DNA yield from banana fruits. This result is in phase with other reported protocols for DNA extraction from other plant tissues(6, 7, 10). On the other hand, we report a higher optimal salt concentration than earlier on reported(4, 5). There may not be a universal protocol for DNA extraction from all plant tissues as has been claimed (4, 5). Indeed, plant tissues defer in complexities, thus, DNA extraction protocols may have to vary to remain relevant to the desired quality yields(9).

The effects of temperature DNA concentration, stability and yield have been elucidated(15, 21, 22).We report a very strong negative and significant correlation between the temperature and the amount of DNA extracted. Our finding is consisted with the previous reports(12, 19, 21). It is, therefore, critical to establish anoptimal range of moderate temperatures and DNA concentrations for better quality and desired yields.

5. CONCLUSION

We report for the first time a protocol with an optimal salt concentration (NaCl, 4mM) for quality DNA yield from banana fruits. A critical consideration is required to establish an optimal range of moderate temperatures and DNA concentrations for better quality and desired yields. Nevertheless, DNA yield from plant tissues is not solely dependent on the salt concentration and temperature. There are also other factors including the plant complexity and tissue type that could influence the yield and quality of DNA extracted from a given plant sample. There may not be a universal protocol for DNA extraction from all plant tissues.

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