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DNA CONTENT IN DRUPE FRUITS WERE SIGNIFICANTLY MORE THAN THAT IN POME, HESPIRIDIUM, PEPO AND COMPLEX FRUITS BECAUSE OF MORE FLESH IN DRUPE

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ABSTRACT

Genomic DNA extraction is an important aspect of plant molecular biological research. The objective of the study was to recommend the cheap and efficient genomic DNA extraction method for some economically important fruit species of Bangladesh. The modified plant genomic DNA extraction methods explained by drupe, pome, hespiridium, pepo and complex fruits extraction kit (Qiagen) method were applied with nine different fruit species such as *Carica papaya* (Papaya), *Musa aouminata* (Banana), *Scolytus unipinosus* (Grape), *Psidium guajava* (Guava), *Malus angustifolia* (Apple), *Citrus aurantiam* (Orange), *Manilk arazapota* (Whitelead), *Pyrus commonis* (Pear), *Anaras conassus* (Pineapple).Based on the quantity of the extracted genomic DNA tested by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer, quality determined by the ratio of A260 / A280 and the amplifiable quality of DNA determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V, the method explained by Cheng et al and the Genomic DNA extraction kit yielded good quality DNA with satisfactory concentration for all the fruit species tested. Therefore the modified method of Cheng et al, 1987 could be recommended for the efficient and cost effective DNA extraction from fruit species instead of the commercially available expensive and chemically hazardous DNA easy plant kit method. The DNA content in drupe fruits were significantly more that that in pome, hespiridium, pepo and complex fruits (two tailed t-test value 0.26).

INTRODUCTION

The demand for tropical fruits has increased more than 40% during the last decade [Food and Agriculture Organization of the United Nations (FAO), 2010] as consumers seek healthy and more diverse food products.[3]

DNA is an almost universal genetic material, and that genes present in simple viruses, bacteria, plants, and animals are all made of DNA. It was a very long polymer made up of millions of nucleotides.[1] The living cell is an extraordinarily complicated entity producing thousands of different macromolecules and harboring a genome. The methods of molecular biology depend upon an understanding of the properties of biological macromolecules. The systematic comparison of different animal genomics gives a chance of identifying genetic basis for diversity. We are fast entering a golden era of comparative genome analysis.[2] Methods used to isolate the DNA depend on the source, age and size of the sample. Principle behind the separation of DNA which is present in the cells is to make the DNA free from the other cellular components.[3] Isolation of DNA is needed for the genetic analysis, which is used for scientific, medical or forensic purpose. Scientists use DNA in a number of applications, such as introduction of DNA into the cells and animals or plants, or for diagnostic purposes.[4] Many protocol have been used for isolation of plant DNA, but because of chemical heterogeneity of the species many of them could be applied to a limited number of species or even closely related species in some case fail to respond to the same protocol. Plants contain an array of secondary metabolites.

Classifying Fruits

All fruits may be classified into three major groups on the basis of the number of ovaries and the number of flowers involved in their formation.

A. Simple Fruits

Simple fruits develop from a single matured ovary in a single flower. Accessory fruits have some other flower part united with the ovary.

1. Fleshy Fruits, pericarp fleshy at maturity

a. Berry, consisting of one or more carpels with one or more seeds, the ovary wall fleshy

(1) Pepo (an accessory fruit), a berry with a hard rind, the receptacle partially or completely enclosing the ovary. The papaya (*Carica papaya*) is a pepo because of its thick outer rind.

(2) Hesperidium, a specialized berry with a leathery rind. Orange (*Citrus aurantiam*) and Grape (*Scolytusunipinosus*) belongs to this group.

b. Drupe, a stone fruit, derived from a single carpel and containing (usually) one seed. Exocarp a thin skin. Banana (*Musa aouminata*) and Sapodilla (*Manilkarazapota*) belongs to this group.

c. Pome (an accessory fruit), derived from several carpels, receptacle and outer portion. of pericarp fleshy, inner portion of pericarp papery or cartilaginous, forming a core. Apple (*Malus angustifolia*) and Pear (*Pyruscommonis*) belong to this group.

d. Hip (an accessory fruit), several separate carpels enclosed within the fleshy or semi-fleshy receptacle

2. Dry Fruits, pericarp dry at maturity

a. Dehiscent fruits, those which dehisce or split open when fully mature

(1) Follicle, composed of one carpel and splitting along a single suture
(2) Legume, composed of a single carpel and splitting along two sutures
(3) Capsule, composed of several carpels and opening at maturity in one of four ways:
(a) Along the line of carpel union (septicidal dehiscence)
(b) Along the middle of each carpel (loculicidal dehiscence)
(c) By pores at the top of each carpel (poricidal dehiscence)
(d) Along a circular, horizontal line (circumscissle dehiscence)
(4) Silique, composed of two carpels which separate at maturity, leaving a persistent partition between them

b. Indehiscent fruits, those which do not split open at maturity

(1) Achene or akene, a one-seeded fruit with the seed attached to the fruit at one point only
(2) Caryopsis or grain, a one-seeded fruit in which the seed is firmly attached to the fruit at all possible points
(3) Samara, a one- or two-seeded fruit with the pericarp bearing a wing like outgrowth. A modified achene
(4) Schizocarp, consisting of two carpels which at maturity separate along the midline into two one-seeded halves, each of which is indehiscent
(5) Loment, having several seeds, breaking into one-seeded segments at maturity
(6) Nut, a hard, one-seeded fruit, generally formed from a compound ovary, with the pericarp hard throughout

B. Aggregate Fruits

Aggregate fruits consist of a number of matured ovaries formed in a single flower and arranged over the surface of a single receptacle. Individual ovaries are called fruitlets

C. Multiple Fruits

Multiple fruits consist of the matured ovaries of several to many flowers more or less united into a mass. Multiple fruits are almost invariably accessory fruits. Pineapple (*Anarasconassus*), Guava (*PsidiumGuajava*) and Grape (*Scolytusunipinosus*) belong to this group.

The objective of the current study was to establish a DNA extraction procedure. Ten separate plant species were collected---their fruits were chopped in mortar and pestle and transferred for DNA extraction. After extraction of DNA, DNA presence was tested by Agarose gel electrophoresis. With NanoDrop Machine, the DNA content was measured.

MATERIALS

Collection and Preservation of Fruits

The nine type fruit was collected in the kawran bazaar by the professional shop keeper. They made fruits in formalin and transported for marketing all over the country. We purchased about 5kg (mixed fruit) from Kawran Bazaar, Tejgaon, Dhaka in the very early morning from some fresh fruits.

Harvesting of Fruits

The parchead fruits was puted the different fruit in different way. Cut one type fruit into small pieces and use the blender for blending these fruits under liquid nitrogen. After blending collected the juice around 20ml and continous these process another 8types of fruit. These nine type fruit juice was preserved in refrigerator (-4^0C) until use.

DNA Quantification using Nano Drop

The Thermo Scientific NanoDrop™ 1000 Spectrophotometer measures 1 ul samples with high accuracy and reproducibility. The full spectrum (220 nm-750 nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the NanoDrop 1000 Spectrophotometer has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Reagent

Tris-HCl, EDTA, Triton X-100 and 5µl RNase A (10mg/ml) was added into the sample tube and mixed by vortexing. Tris-HCl, EDTA, Triton X-100 are used for the purpose of breaking open cells. Tris-HCl, EDTA, Triton X-100 are added to break up membrane structures.

Procedure

Tissue Dissociation

50 mg of plant tissue was grinded under liquid nitrogen to a fine powder. It was transferred into a microcentrifuge tube.

Lysis

The mixture was incubated at 65° C for 10 minutes to weaken the cell walls and to lyse.

100 µl lysis buffer was added and mixed by vortexing. The lysis buffer contains sodium hydroxide (NaOH) and the detergent Sodium Dodecyl (lauryl) Sulfate (SDS). SDS is to solubilize the cell membrane. NaOH helps to break down the cell wall, but more importantly it disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA (dsDNA) in the cell, including the genomic DNA SDS also denatures most of the proteins in the cells, which helps with the separation of the proteins.

The closed tube was placed in the ice bath using forceps to hold the tube. The tube was kept in the bath for three minutes to freeze. All cells, the basic structural and functional unit of life, consist of living material bounded by layers of membranes made of lipids, proteins, and some other compounds. Cell lysis is the first step in the process of DNA purification. The DNA genome contains all the genetic information of an organism, and is protected from the external environment by the cell membrane. In order to release the genetic material for study and analysis, cells must be broken open, or lysed. There are several methods available for cell disruption including physical and chemical techniques. For this DNA extraction, freeze-thaw was used because it is a very common method used to lyse plant tissue cells.

Cell lysis was followed by precipitation of proteins, which traps chromosomal DNA in insoluble fraction and after centrifugation, a filter column was placed in a 2 ml Collection Tube. The mixture from previous step was applied into the Filter Column. The filter column was centrifuged at full speed (13,000 rpm) for 3 minutes. The filter column was discarded and clarified supernatant was carefully transferred in Collection Tube to a new microcentrifuge tube.

2.2.4.3 DNA Binding

A GD Column was used as 2 ml Collection Tube.
700µl the mixture (including any precipitate) was applied from previous step into the GD Column.

The solution was centrifuged at full speed (13,00 rpm) for 2 minutes. The flow through was discarded in Collection Tube.
Spin column-based nucleic acid purification is a solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions.

2.2.4.4 Washing

400µl of wash buffer was added into the GdColumn. Again it was centrifuged at 10,000 xg (13,00 rpm) for 30 seconds. The flow through was discarded and placed back in the Collection Tube.600µl of Wash Buffer was added into the GdColumn. It was centrifuged at 10,000 xg (\13,00 rpm) for 30 econds. The flow through was discarded and returned into the 2ml Collection tube. It was centrifuged again for 3 minutes at full speed to dry the Column matrix.

DNA Elution

Standard elution volumn is 100 µl. If less sample volumn is used. The elution volume was reduced (30-50 µl) to increase DNA concentration. If higher DNA yield is required, the elution step was repeated to increase DNA recovery and the total elution volume to about 200 µl.

The dried GD Column was transferred into a clean 1.5 ml microcentrifuge tube. 100 µl of preheated Elution Buffer was added onto the center of the column matrix. For 3.5 minutes it was kept standing until Elution Buffer absorbed by the matrix.

It was centrifuged at 13,000 rpm for 30 seconds to elute purified DNA. The composition of Elution Buffer is:10mM Tris-Cl, pH 8.5

RESULTS AND DISCUSSION

Quantity of Extracted Fruits The quality of the Genomic DNA Mini Kit (Plant) is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Genomic DNA is extracted from 20ml fresh fruit juice. The purified genomic DNA (>10 µg) is quantified with a spectrophotometer and checked by electrophoresis.

Table 1: Quantity of Extracted fruits using Nano Drop Machine.

Plant species	Yield of DNA
Carica papaya (Papaya)	5.3ng/µl
Musa aouminata (Banana)	11.8ng/µl
Vitis vinifera (Grape)	6.3ng/µl
Psidium guajava (Guava)	3.3ng/µl
Malus angustifolia (Apple)	3.5ng/µl
Citrus aurantiam (Orange)	5.7ng/µl
Manilkara zapota (White lead)	6.3ng/µl
Pyrus commonis (Pear)	1.4ng/µl
Anaras conassus (Pineapple)	2.8ng/µl

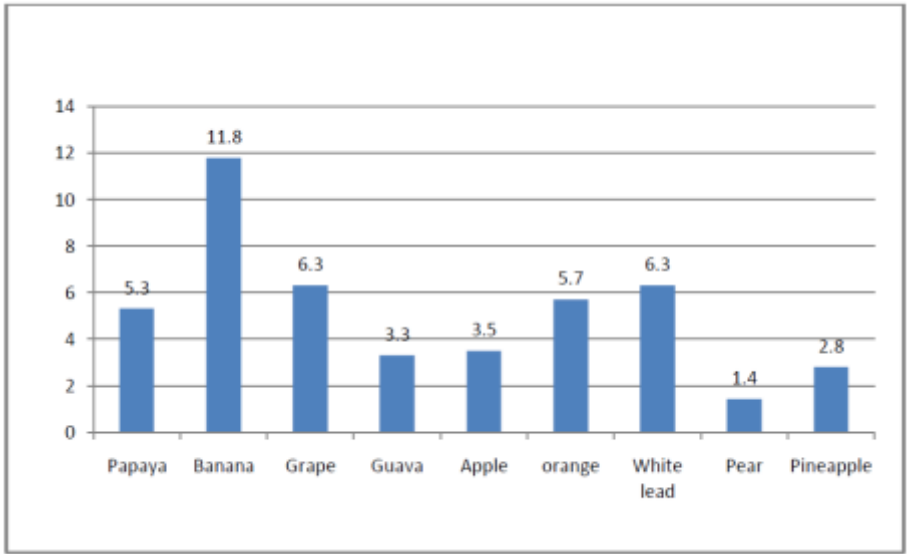

Figure: Quality mean DNA the Different extracted fruit.

Among the fruit species tested, fresh fruits of *Carica papaya* (Papaya), yielded maximum amount of DNA with overall mean of 5.3ngµL-1, *Musa aouminata* (Banana), *Scolytusunipinosus* (Grape), *Psidium Guajava* (Guava), *Malus angustifolia* (Apple) *Citrus aurantiam* (Orange) *Manilkarazapota* (White lead) *Pyruscommonis* (Pear) *Anarasconassus* (Pineapple) maximum amount of DNA with over all mean of 11.8NG/µL, 6.3ng/µl, 3.3ng/µl, 3.5ng/µl, 5.7ng/µl, 6.3ng/µl, 1.4ng/µl,2.8ng/µl for nine type of fruits.

The DNA content in drupe fruits were significantly more that that in pome, hespiridium, pepo and complex fruits (two tailed t-test value 0.26).

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