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Research Article

QUANTITATIVE ESTIMATION OF DNA ISOLATED FROM VARIOUS PARTS OF *ANNONA SQUAMOSA*

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ABSTRACT
Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Annona squamosa Linn* is a multipurpose tree with edible fruits & is a source one of the medicinal & industrial products. *Annona squamosa Linn* is used as an antioxidant, antidiabetics, hepatoprotective, cytotoxicactivity, genetoxicity, antitumor activity, antilice agent. It is related to contain alkaloids, flavonoids, carbohydrates, fixed oils, tannins & phenolic. Genetic variation is essential for long term survival of species and it is a critical feature in conservation. For efficient conservation and management, the genetic composition of the species in different geographic locations needs to be assessed. Plants are attracting more attention among contemporary pharmacy scientists because some human diseases resulting from antibiotic resistance have gained worldwide concern. A number of methods are available and are being developed for the isolation of nucleic acids from plants**.** The different parts of *Annona squamosa* were studied for their nucleic acid content by using spectrophotometric analysis. In order to measure DNA content of the Leaves,friuts and stems of *Annona squamosa*, Spectrophotometry serves various advantages *i.e.* non-destructive and allows the sample to be recovered for further analysis or manipulation. Spectrophotometry uses the fact that there is a relationship between the absorption of ultraviolet light by DNA/RNA and its concentration in a sample. This article deals with modern approaches to develop a simple, efficient, reliable and cost-effective method for isolation, separation and estimation of total genomic DNA from various parts of the same species.
Key words: *Annona squamosa*, Genomic DNA extraction, Spectrophotometric

INTRODUCTION

The family (Annonaceae), is a large family which comprising about 130 genera over 2000 species; the most important genera having a largest number of species are *Annona*, with 120species, from genera, the species of *Annona squamosa* commonly known as custard apple is cultivated throughout India, mainly edible fruit. *Annona squamosa syn.* Arabic (gishta); Bengali (ata);German (Rahm Annone,Rahmapfel,Zimtapfel,Süßsack); Hindi (sitaphal,ata,sharifa); Lao (Sino-Tibetan) (khièb); Malay (nona sri kaya,sri kaya,buah nona); Mandarin (fan-li-chi); Portuguese (atta,fructa do conde); Sanskrit (sitaphal); Spanish (candongo,chirimoya,fructo do conde,anón,anona blanca,pinha,saramuya,anona).The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has anti fertility, anti tumor and abortifacient properties[1-4]. *Annona squamosa* is a small, semi-deciduous tree, 3-7 m in height, with a broad, open crown or irregularly spreading branches; bark light brown with visible leaf scars and smoothish to slightly fissured into plates; inner bark light yellow and slightly bitter; twigs become brown with light brown dots (lenticels)[5]. DNA is polymer found in all living cells.DNA contains all genetic information needed for controlling cellular growth and development. Many protocols have been used in plant DNA isolation, but because of the chemical heterogeneity of the species many of them could be applied to a limited number of species or even closely related species in some cases fail to respond to the same protocol[6].Plants, especially medicinal plants contain an array of secondary metabolites. The compounds which make them interesting for molecular biology studies and hence, for DNA isolation, themselves interfere with the DNA isolation procedure. The objective of many bioassay methods is to selectively quantitate a single biomolecule, such as a particular enzyme or antibody, or to determine the presence or absence of a known DNA sequence in an unknown sample[7]. The present study deals with modern approaches to develop a simple, efficient, reliable and cost-effective method for isolation and estimation of total genomic DNA from various parts of the same species.

Fig1: ***Annona squamosa***

MATERIALS AND METHODS

Plant material

To facilitate better homogenization leaves, fruit endosperm and stems were used for the experimental study. For comparing DNA concentrations plant material was collected from the same plant. The plant material was sterilized with distilled water and external moisture from the leaves & stem were allowed to dry.

Reagents and chemicals

The following chemicals and reagents were used: lysate buffer (autoclaved) [1.4 M Sodium Chloride, 20 mM EDTA, 0.02 M Sod. Citrate, 2% CTAB and 100 mM Tris-HCl pH8]. Ethanol, Diphenylamine and Glacial acetic acid. All chemicals were obtained from Shyam brothers, 27- Sindhi market, Bhopal (M.P.).

DNA isolation protocol

The plant materials were cut into small pieces of about 2-3 mm sq. [1.4 M NaCl, 20 mM EDTA, 0.02 M sod. Citrate, 2% CTAB and 100 mM Tris-HCl pH8]. Above tissues separately suspended into prepared lysate buffer & homogenized in blender. The mixture was centrifuged at 5000 rpm for 10 minutes and the aqueous phase was transferred to a new tube containing 0.2 volume CTAB Solution (5%w/v CTAB and 0.7 M NaCl). They were mixed together and added 0.01% of pepsin enzyme solution. Again centrifuged and collected the aqueous phase to a new tube. When the supernatant had become clear, DNA was precipitated using double volumes of 95% cold ethanol. The test tubes were left for 5 min and observed the white webby mucus like interference formation which was

separated by using micropipette into another test tube. This was best stored in PBS (pH=7.4) or 0.9% saline[8].

Qualitative estimation of Nucleic acid

Killer –Killani Test: Sample with 1 ml of glacial acetic acid containing one drop of 1% ferric chloride solution. Under lay the mixture with 1 ml of concentrated sulphuric acid from the side wall of tube, a brown ring at the interface indicates a deoxy-sugar (Pentose sugar) characteristic of every nucleic acid[9].

Diphenylamine (DPA) Test: Sample with DPA reagent [1 gm DPA + 50 ml glacial acetic acid + 2.5ml conc.H_2SO_4].Placed above mixture in boiling water bath for few min. A blue colour observed confirm the presence of DNA[10].

Gel Electrophoresis

1.2% (w/v) agarose was dissolved in 1X TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) by heating in microwave oven for about 2 minutes. It was then cooled to about 50^{o}C before 1 mg/ml Ethidium bromide (EtBr) was added. EtBr was included in the gel matrix to enable fluorescent visualization of the DNA fragments under UV light. The warm gel solution was poured onto casting tray to solidify. The DNA samples were mixed with 2 µl loading dye (50% (v/v) glycerol, 1 mM EDTA, 0.4% (w/v) bromophenol blue, 0.4% (w/v) xylene cyanol) and loaded into the sample wells. Agarose gel was submerged in electrophoresis buffer (TAE buffer) in a horizontal electrophoresis apparatus. The gel was run at 80 volt for about 45 minutes. When electrophoresis was done, the gel was placed on a UV illuminator (Jyoti Scientific Ltd.) to visualize the fluorescent bands of ethidium bromide-stained DNA separation[11].

Quantitative estimation of DNA

100 mg of calf thymus DNA (Oxford Lab. Reagent) was dissolved in 100ml distilled water (1mg/ml Primary stock solution) then pipette out 1ml primary stock solution and made up the volume to 10ml with distilled water. Now prepared different dilution ranging from 20-100µg/ml. The absorbance was measured at 270 nm by using UV-Spectrophotometer (Shimadzu-1700). In this method, the absorbance of the unknown sample in a 1-cm cuvette was measured at 230,260,270 and 280 nm. The A_{260}/A_{230} and A_{260}/A_{280} nm values were determined[12].

RESULTS AND DISCUSSION

The differences in quality and quantity of isolated DNA observed in gel electrophoresis. DNA samples are subjected to agarose gel electrophoresis and subsequently stained with ethidium bromide. The dye intercalates into the DNA double helix, and the intensity of fluorescence induced by UV light is proportional to the amount of DNA in the corresponding lane. Comparison to a dilution series of standards, e.g., λ-DNA, gives an estimate of the amount of DNA in an unknown sample. Modified CTAB method gave good quality of DNA. This method was determined to be the best method for *Annona squamosa* DNA isolation. This is because; it could be clearly seen from the gel electrophoresis (figure 4) that the DNA band obtained from the modified procedure yield the highest quantity of DNA. There was no smear of protein interference for the DNA obtained using CTAB. The size of isolated DNA was about 80-550 bp). In leaves, fruits and stem explants portion of plant A_{260}/A_{280} ratio of ranges 1.6 to 1.9 (Average about 1.8) indicating the level of purity of DNA(Table 2 & Table 3). The DNA obtained was unshared, showing little or no RNA contamination[13]. UV absorbance ratio for protein contamination ($A_{260/280}$) and carbohydrate contamination ($A_{260/230}$). For a good and clean preparation of nucleic acid, the $A_{260/280}$ ratio, which represent protein contamination, should be between 1.8 to 2.0 while the $A_{260/230}$ ratio, which represent carbohydrate contamination, should be more than 2.0[14].The quantization of the obtained DNA from leaves, stem and fruit were found to be 4.377, 3.19 & 3.377 µg/ml respectively (Table 4). Poor stem DNA quantity could be due to certain reasons like mixing of RNA or protein, improper expression of transcription factor or secondary metabolite interferences. *Annona squamosa* plant which is the source of natural products or bioactive substances produced a large amount of secondary metabolites and substances of medicinal importance. The cells of the plant are known to contain high concentrations of polysaccharides in addition to the active metabolites, complicating the problem of DNA isolation. Thus, problems are encountered arising from the presence of polyphenols, polysaccharides and other secondary metabolites[15].This indicated that the isolated DNA was amenable to further processing in cloning experiments as well as DNA fingerprinting. The isolated DNA can be amplifying for producing molecular marker. Molecular markers have been shown to be useful for genetic variation of plant species. Several different PCR- based techniques have been developed during the last decade, each with specific advantages and disadvantages. The randomly amplified polymorphic DNA (RAPD) markers technique is quick, easy and requires no prior sequence information; it detects nucleotide sequence polymorphisms using single primer of arbitrary nucleotide sequence[16]. RAPD marker has been extensively used for DNA fingerprinting.

CONCLUSION

In conclusion, these results show that leaves can be an alternative source for total genomic DNA from medicinal and succulent plants that contain high quantities of secondary metabolites. Leaves from succulent plants were easier to crush and grind under liquid nitrogen as well as lyses in buffer than succulent tissues. The isolated genomic DNA was of high molecular weight and the amount increased proportionally as the amount of petals tissue increases. This technique measures the total amount of nucleic acids in a sample (including DNA, RNA, oligonucleotides, and mononucleotides). It is therefore only useful for pure DNA preparations of a reasonably high concentration. This technique allows, at the same time DNA quantization, estimation of the extent of contamination by RNA, evaluation of DNA quality and integrity (i.e., the extent of degradation). DNA fingerprinting has used to elucidate genetic relationships at various taxonomic levels and also helpful in phylogeographic studies which can be based on information from nuclear DNA, mtDNA, and cpDNA. Phylogenetic variations were also determined in *Annona squamosa* species by DNA typing. This protocol will be used in future to isolate genomic DNA from tested and other related plant species for downstream molecular biology studies and can probably be extended also to other angiosperm species.

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Table 1. Qualitative estimation of DNA

S.No.	Test	Observation	Inference
1.	Killer –Killani Test	A brown ring at the interface	Indicates a deoxy sugar (Pentose sugar)
2.	DPA Test	Blue colour observed	presence of DNA

Table 2. Comparison of DNA quantity obtained following the present plant DNA isolation protocol with other routine methods.

S.No.	Type of the tissue	Absorbance at 260nm	Absorbance at 280nm	$A_{260/280}$	Inference
1.	Leaves	0.376	0.235	1.6	The DNA obtained was unshared, showing little or no RNA contamination
2.	Stem	0.316	0.0176	1.8	The DNA obtained was unshared, showing little or no RNA contamination
3.	Fruit	0.3296	0.206	1.6	The DNA obtained was unshared, showing little or no RNA contamination

Table 3. Comparison of DNA quantity obtained following the present plant DNA isolation protocol with other routine methods(Carbohydrate contamination).

S.No.	Type of the tissue	Absorbance at 260nm	Absorbance at 230nm	$A_{260/230}$	Inference
1.	Leaves	0.376	0.268	1.4	The DNA obtained was unshared, showing little or no carbohydrate contamination
2.	Stem	0.316	0.225	1.4	The DNA obtained was unshared, showing little or no carbohydrate contamination
3.	Fruit	0.3296	0.235	1.4	The DNA obtained was unshared, showing little or no carbohydrate contamination

Table 4. Quantitative estimation of DNA

S.No.	Type of the tissue	Absorbance at 270nm	Statistical Analysis	Concentration (µg/ml)
1.	Leaves	0.267	Correlation coefficient R^2= 0.998 Straight Line equation y= 0.061x	4.377
2.	Stem	0.195		3.19
3.	Fruit	0.206		3.377

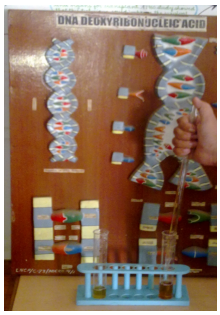

Fig 2: Addition of chilled ethanol

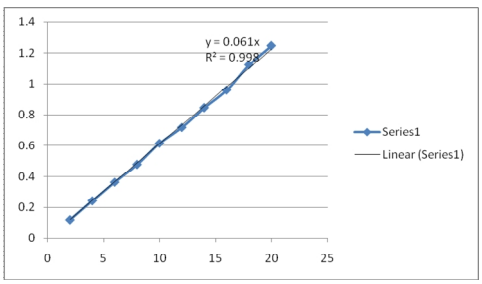

Fig 3: Standard curve of DNA

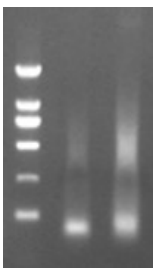

Fig :4 DNA isolated resolved on agarose gel.

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