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ISOLATION, QUALITATIVE AND QUANTITATIVE ESTIMATION OF DNA FROM VARIOUS SOURCES

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ABSTRACT

DNA was isolated from various sources such as banana, cauliflower, muscle and spleen of goat. The entire DNA that has been extracted was found to differ in their molecular weight and was seen as separate bands when viewed under UV light. The concentration of the DNA was estimated by using the UV spectrophotometer. The concentration of DNA was found to be more from onion and the ratio of their absorbance at 260 and 280 nm, was 1.88 which showed slight contamination. The concentration of DNA was found to be the least from spleen and ratio was found to be 1.48 indicating the presence of contamination. The DNA isolated from the cauliflower was found to be 100% pure and free from contamination.

Keywords: Genomic DNA Isolation, Spectrophotometry, Banana, Spleen, Muscle, Cauliflower

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. (Charlotte *et al.,* 2004). 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 (Watson *et al.,* 1953). 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 (Saenger, 1984).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. (Ammayappan *et al.,* 2011). 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. (Soni *et al.,* 2011). The compounds which make them interesting for molecular biology studies and hence, for DNA isolation themselves interfere with the DNA isolation procedure. The present study deal with the modern approaches to develop a simple, reliable and cost – effective method for isolation, purification and quantitative estimation of DNA from various sources of the different species such as muscle, banana. Onion and spleen.

MATERIAL AND METHODS

Reagent and chemical

0.15MNaCl, 0.015M Sodium Citrate, CTAB Buffer, Phenol, Chloroform, Isoamyl Alcohol, sodium chloride, proteinase K, solvent, buffer saline

DNA Extrction from Banana and Cauliflower

Take 0.2gm of onion, crushed it in 2ml of CTAB buffer. Take it on microcentrifuge and put it on water bath at 60^0C for 20 min. Spin at 13,000 rpm for 5 min. at 4^0C.Supernatant transferred to microcentrifuge tube & add equal vol. of phenol:chloroform:isoamyl alcohol(24:24:1).invert the mixture 4-5 times. Aqueous layer transferred to separate tube, add absolute alcohol and keep it at -20^0C for overnight, spin at 13,000 rpm for 1 min. drain off ethanol & blot dry the pellet of DNA.(Harborne, 1991).

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DNA Extracted from Spleen and Muscle

Chop 50 gm of spleen and muscle into small fragments. Homogenize with 200ml of buffer saline, centrifuge 5000rpm for 15 min. Discard supernatant and add buffer saline in equal vol. then vortex. Centrifuge for 5000 rpm for 50 min. discard the supernatant add NaCl , equal vol. or each 1 ml then vortexing. Centrifuge at 5000rpm for 3 min. the collect supernatant and add equal volume of distill water, settle down for 30 min. add proteinase K then collect the supernatant and add isoamyl alcohol and chloroform, centrifuge at 5000rpm for 15 min., collect supernatant add solution 1ml again centrifuge ppt. the DNA by slowly stirring 2 volume of ice cold ethanol with the supernatant and collect the mass of fiber on the glass rod stirring. Centrifuge 5000rpm for 5 min, remove rod and gently press the fibrous DNA against side if beaker to expel in solvent. Wash the ppt by dipping the rod into solvent and then dry and remove trace of ether by standing DNA add the dye weigh dry DNA and dissolve by stirring in buffer saline dilute one in 10 with distill water.

Qualitative Estimation of DNA

Killer-Killani Test: Sample with 1ml of glacial acetic acid containing one drop of 1%ferric chloride solution. Under lay the mixture with 1 ml of concentrated sulphuric acid from side wall of tube, a brown ring at the interfece indicates deoxy-sugar (pentose sugar) characteristics of every nucleic acid. (Heidcamp, 2009).

Diphenylamine (DPA) Test: Sample with DPA reagent[1gm DPA + 50 ml glacial acetic acid+ 2.5 ml concentrated sulphuric acid]Place above mixture in boiling water bath for few min. blue color observed confirm the presence of DNA.(Devenish *et al.,*1982).

Agarose gel electrophoresis

Agarose gel electrophoresis was used for the separation of the DNA fragments by using 0.7% of agarose. The sample DNA which was isolated from various sources are mixed with ethidium bromide, a marker dye and then loaded well in agarose gel which was then kept in position in the electrophoresis chamber filled with buffer and current was applied (typically 100 V for 30 min). The marker dye had a low molecular weight and migrated faster than the DNA. When the marker dye approached the end of the gel, the current was stopped and viewed under ultra violet light (Moyo *et al* 2008).

Quantitative Estimation of the purity of the DNA

Dissolve deoxyribose in 5mM NaOH solution to prepare standard solution. Mix equal volume of stock DNA solution with 1 N perchloric acid and heat it for 15min. at 70^0C. Measure absorbance at 260nm and 280nm using a spectrophotometer of different concentration of standard DNA solution plot a graph or standard curve. Usually, the absorbance is measured at 260 nm at which wavelength of absorbance A 260 of 1.0 corresponds to 50 μg take a small volume of DNA extract in test tube and put into 0.5 N perchloric acid so that it may be diluted and contain 0.02-0.25m mole of DNA-phosphate/ml. take 2 ml of DNA sample in a test tube and add4 ml of DPA reagent. Incubate the mixture at 25-30^0C for 15-17 hrs. Measure absorbance of the solution at 260 and 280nm after calibrating the spectrophotometerr with blank tube and standard containing the similar volume of perchloric acid.(Dubey *et al* 2008) As the DNA concentration increased the OD value also increased. OD is directly proportional to DNA concentration (Psifidi *et al* 2010).

RESULTS AND DISCUSSION

The differences quality and quantity of isolated DNA observed in gel electrophoresis and stained with ethidium bromide. The dye intercalates into DNA double helix, and the intensity of fluorescence induced by UV light is proportional to the amount of DNA in the corresponding lane. (Figure 1). Modified method good quality of DNA. This method was determine to be the best method for banana and cauliflower DNA isolation . Each species of animal has a characteristic content of DNA. Eukaryotes vary greatly in DNA content and contain more DNA then prokaryotes. The isolated DNA was qualitative estimated by Killer- Killani Test and it gives brown ring at interfece that means pentose sugar is present. DPA reagent gives blue colored obsereved. (Table 1. & Figure 2).

Figure 1 : Agarose gel electrophoresis of isolated DNA(Lane 1:Spleen, Lane2: muscle, Lane3:Cauliflower, Lane4:banana)

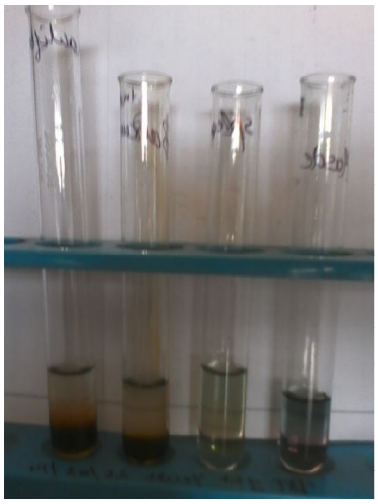

Figure 2: Qualitative test of isolated DNA

Table1: Qualitative estimation of DNA

S No.	Test	observation	Interfere
1	Killer-killani test	A brown color observed	Pentose sugar
2	Diphenylamine test	Blue color observed	Presence of DNA

Table 2. Characterization of extracted DNA from various sources

S. No.	Sample	Absorbance at 260nm	Absorbance at 280 nm	Concentration of DNA(μg/ml)	A260/A280
1	Banana	0.68	0.36	1.8	1.88
2	Cauliflower	0.45	0.29	3.1	1.55
3	Muscle	0.37	0.23	2	1.66
4	Spleen	0.43	0.29	1.4	1.48

The concentration of the DNA was estimated by UV spectrophotometer using the formula 50 * OD *´ 20 μl/4000 and the values were recorded in Table 2. The concentration of DNA was found to be more from banana and the ratio of their absorbance at 260 and 280 nm is 1.88 which showed slight contamination, the concentration of DNA was found to be least from Spleen of goat and ratio was found to be 1.48 showed the presence of contamination. The DNA which was isolated from the cauliflower and muscle was found to be 100% pure which was free from contamination.(Ammayappan *et al.,*2009). In molecular biology, isolation of purified DNA is required for numerous manipulations. For large scale DNA isolation, several procedures relatively rapid mini preparations have been developed. The time required for extraction and purification depends upon the purity of DNA and its suitability for various procedures such as restricted enzyme cleavage, ligation and cloning (Mandelkern *et al.,*1981) plant and animal which is the source of natural products or bioactive amines produced a large number of primary and secondary metabolites. The of plant are known to contain high concentration of polysaccharides and its active metabolites. (Khanuja Suman *et al.,*1999)

CONCLUSION

In conclusion these results show that plants and animal are major source for DNA isolation that contains high quantities of primary and secondary metabolites. Fruits and vegetables from the succulent plant easier to crush and grind under detergent solution as well as lyses in buffer. The isolated DNA was separated by gel electrophoresis and measures the total estimation of nucleic acid. This method

involve measuring absorbance at 260 and 280 nm .A good quality DNA sample should have a A260/A280 ratio of 1.7-2 is desired when purifying nucleic acid. A ratio less than1.5 means there is probably contaminant in the solution, typically either protein or phenol. In all the tissue types, DNA has a greater stability at high temperature and differentiation of closely related species is offered by the genetic code (de Kloet, 1984). This protocol will be used in future to isolate genomic DNA from tested and other related plants for downstream molecular biology studies and can probably be extended also other species.

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