PAPER ID:I037789

GENETICS RESEARCH PROJECT

STUDIES ON THE RENATURATION KINETICS OF DNA ON ADDITION OF CINNAMON EXTRACT.

ZEHRA FATIMA & BATUL SHABBIR

St. Ann's College for Women

Hyderabad, A.P

IJSER

CERTIFICATE

Certified that ***Ms. Zehra Fatima*** *&* ***Ms. Batul Shabbir*** *from Department of Genetics are bonafide student has carried out this dissertation entitled* ***"STUDIES ON KINETICS OF RENATURATION OF DNA ON ADDITION OF CINNAMON EXTRACT"*** *under my guidance. Further certified that this work either in part or full has not been submitted to any other University / Institute for the award of any Degree / Diploma*

PROF Dr. V. Venugopal Rao

HOD (Department of Genetics)

SIGNATURE:

Dr. K. ANTHONY MARY

Principal

SIGNATURE:

PRINCIPAL
ST. ANN'S COLLEGE FOR WOMEN
Mehdipatnam, Hyderabad-28

IJSER

DECLARATION

We, Ms. Zehra Fatima & Batul Shabbir hereby declare that the dissertation work entitled "KINETICS OF RENATURATION OF DNA ON ADDITION OF CINNAMON EXTRACT" is our original work and has been carried out under the guidance of PROF. Dr. V. VENUGOPAL RAO

Department of Genetics, St. Anns College for Women, Hyderabad.

I also hereby declare that this work in part or full has not been submitted to any other

University / Institution for any Degree / Diploma

Signature:

Zehra Fatima

(ZEHRA FATIMA)

Batul Shabbir

(BATUL SHABBIR)

IJSER

AUTHORSHIP

1. *Ms. Zehra Fatima[1].*
2. *Ms. Batul Shabbir[1].*
3. *Dr. V. Venugopal Rao[2].*

[1] Students of B.sc (Genetics Chemistry Microbiology) II yr.

[2] HOD of Genetics Department.

IJSER

ACKNOWLEDGEMENT

We are grateful for having had the opportunity to work under the guidance of Dr. V. Venugopal Rao, his deep understanding of genetics has led to enlightening discussions not only concerning the work presented in this Abstract but also concerning a wide variety of genetic diseases related to the study of large biological molecules.

Being undergraduate students we have tried our best to come out with good results, keeping in mind that we have done this experiment in our college lab which doesn't have many of the sophisticated tools like photographic techniques etc. we will be obliged to the reviewer if he /she will consider our papers knowing the above mentioned fact.

IJSER

ABSTRACT

Introduction:-

Continuity of species from one generation to other is mainly due to stability of genetic material that is DNA, DNA is a complex self replicating molecule were mistakes during replication may occur or it may be damaged by some intrinsic or extrinsic factors

Example : simple heating, ionizing radiations ,U.V rays or exposure to certain chemicals.

DNA damage can be harmful to cell because their DNA may not be able to replicate over the damaged area hence, cells do not multiply. Even if damage doesn't block replication it can cause mutation which may be lethal or harmful. Therefore cells need mechanisms for the repair of damaged DNA in order to multiply and undergo normal mitosis.

In eukaryotic cell, DNA damage is caused rather frequently. Several genetic diseases in human beings are characterized by cellular hypersensitivity to DNA damage , these diseases also cause increased risk of cancer.

The double stranded DNA is first denatured and converted into single stranded DNA by heating the DNA solution. This is accompanied with increase in optical density, a phenomenon described as ***hyperchromicity.*** *The solution of single stranded DNA thus obtained is then allowed to cool slowly, so that the double stranded DNA will be formed again, which accompanies decrease in optical density* ***(hypocromicity,*** *Fig. 28.2). This process is called reassociation of DNA for which one should maintain fairly high concentration of DNA and a temperature 25°C below the dissociation temperature or melting temperature* $\boldsymbol{T_m}$ *(T_m is a temperature, at which 50% reassociation is achieved). The mixture should be allowed to undergo reassociation for a fairly long time so that sequences may come in contact with their complementary sequences (Fig. 28.3). The amount of repetitive DNA will be determined on the basis of extent of the formation of double stranded DNA in a definite period of time keeping concentration constant. The formation of double stranded DNA is actually measured over different values of a parameter which is described as* ***Cot*** *(conc. x time).*

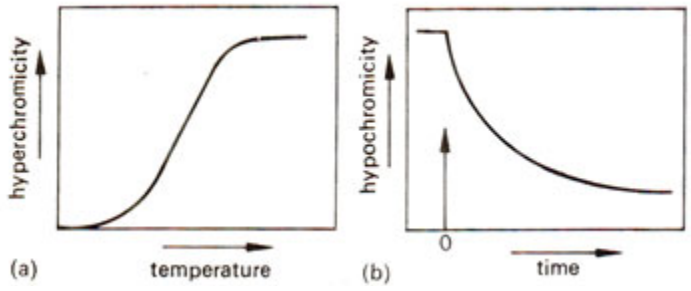
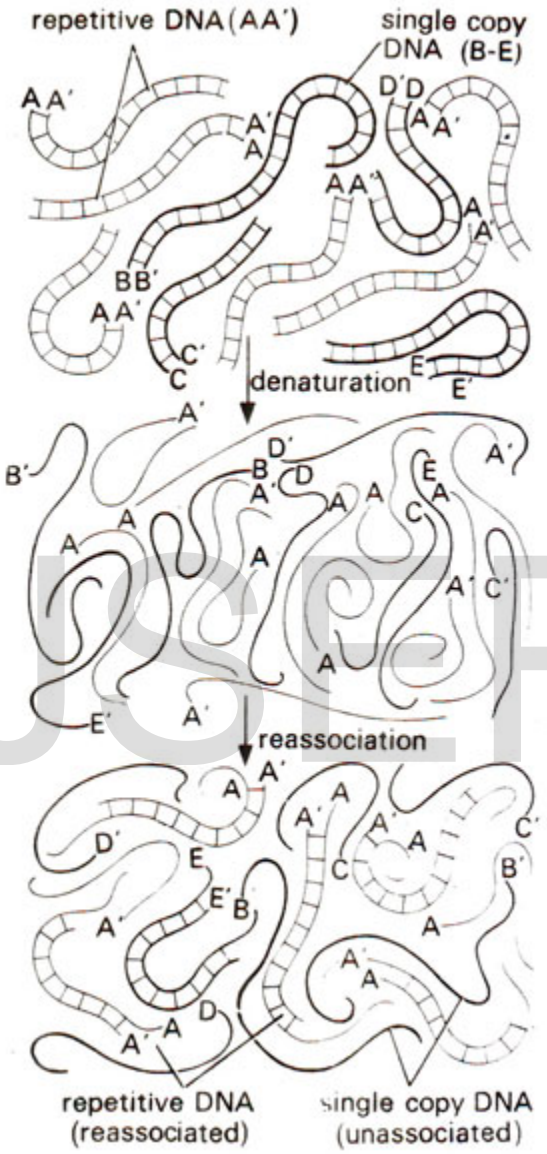


Fig. 1. Hyperchromicity and hypochromicity'of DNA due to denaturation and reassociation respectively.



OBJECTIVES:-

1. Effect of cinnamon extract used at different concentrations on denatured DNA

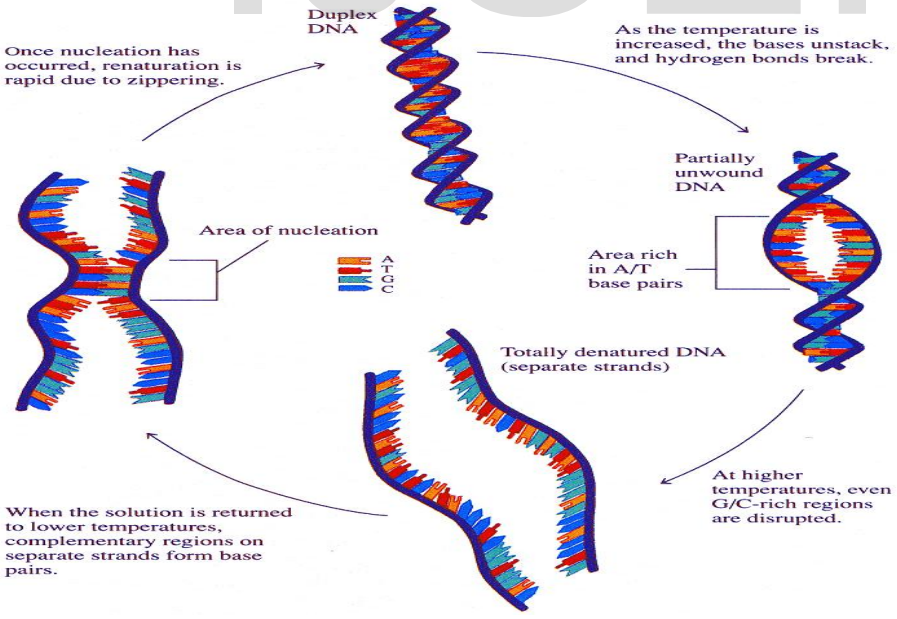
2. Effect of crude extract on cell division (Mitosis) in onion root tips

DEFINITIONS:-

***DENATURATION OF DNA*: DNA denaturation, also called DNA melting, is the process by which double-stranded deoxyribonucleic acid unwinds and separates into single-stranded strands through the breaking of hydrophobic stacking attractions between the bases. Both terms are used to refer to the process as it occurs when a mixture is heated, although "denaturation" can also refer to the separation of DNA strands induced by chemicals**

RENATURATION OF DNA: The reformation of double stranded DNA from thermally denatured DNA. The rate of reassociation depends upon the degree of repetition and is slowest for unique sequences (this is the basis of the Cot value)

IJSER



The Diagrammatical representation

PROCEDURE/PROTOCOL:-

MATERIALS AND METHODS BEING USED:-

Plant Material

Collection of plant materials: CINNAMON STICKS were purchased from the local market of Hyderabad.

Preparation of *Cinnamon* Extract:-

Preparation of alcoholic extract of CINNAMON was carried out by taking 800 g of cinnamon and grounding them with a blender into fine powder with a commercially available food blender. 100mL of 60% ethanol was added to 20g of powder in a sterile well capped flask, left for 3 days at room temperature and then filtered using number 1 filter paper. The extract then evaporated in a rotary evaporator at 40 $^{\circ}$ C until ethanol removing. The extract stored in sterile screw-capped vials in the refrigerator until needed.

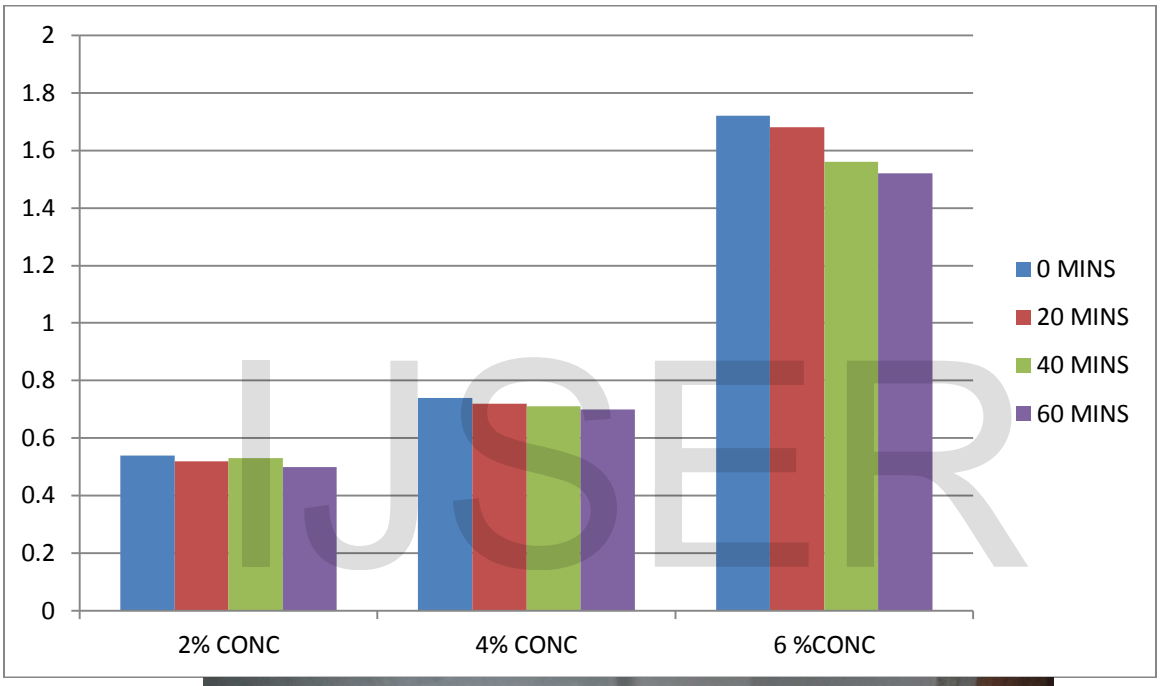
IJSER

After obtaining the extract the extract was used to test renaturation kinetics of DNA by using Onion DNA Solution ,the cinnamon extract was added to DNA solution at different concentrations and the DNA solution was checked for solubility at different interval of time using a colorimeter.

RESULT:-

The following are the observations made at different time intervals at different concentrations of alcoholic extract of cinnamon :-

Time interval	*2% conc.*	*4% conc.*	*6%conc.*
0 mins	*0.54*	*0.74*	*1.72*
20 mins	*0.52*	*0.72*	*1.68*
40 mins	*0.53*	*0.71*	*1.56*
60 mins	*0.50*	*0.70*	*1.52*



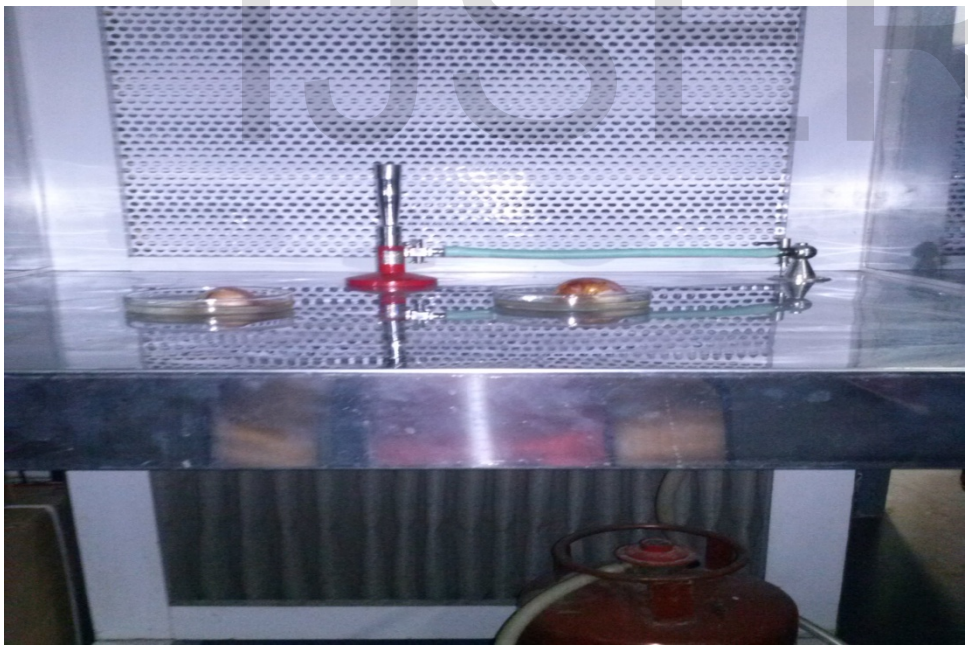
IJSER

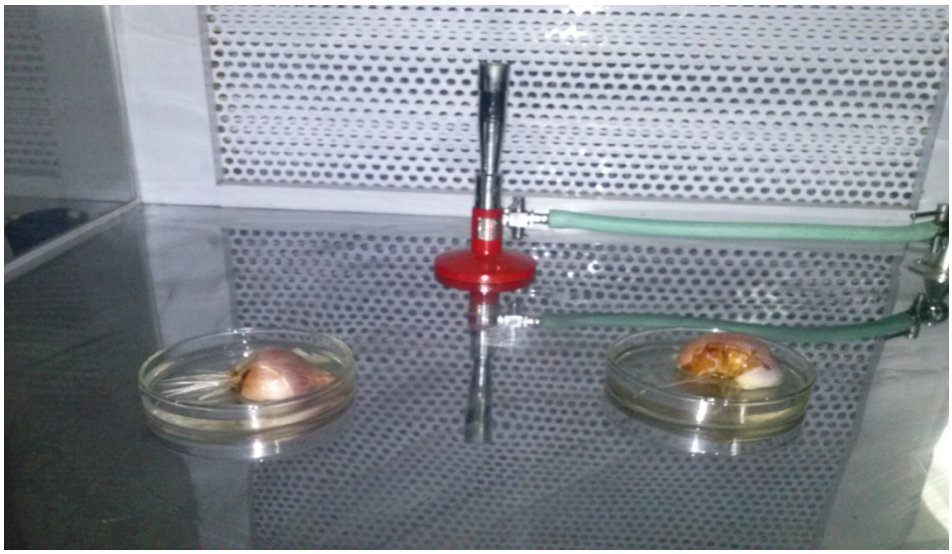
Picture of different concentrations of extract

EFFECT OF CINNAMON EXTRACT ON IRRADIATED ONION ROOT TIPS:-

After the testing cinnamon extract on DNA solution and knowing that the results indicate reassociation or renaturation of DNA with decreased optical density at different time intervals and concentrations of extract another experiment was done to prove that cinnamon helps in DNA repair mechanism with the help of onion bulbs.

IJSER





PROCEDURE:-

1. The onion bulbs were placed in water beaker for 3-4 days for roots to arise.

2. Then the bulbs with fully grown roots were subjected to U.V radiation at a time interval of 15 minutes,

3. From two of the irradiated bulbs one was kept in fixative 1:3 solution and the other was placed in crude cinnamon extract for 30 minutes.

4. After the given time interval the roots from both the onions were cut and dipped in 9 drops of acetocaramine stain + 1 drop of HCL and incubated for 20 minutes now the roots were smashed on glass slides and were examined under the microscope .

RESULTS:-

Case 1.

The slide which contained irradiated roots without cinnamon extract showed no cell division(Mitosis)

IJSER

MITOTIC INDEX = 0÷30×100=0%

Case 2.

The slide which contained irradiated roots treated with cinnamon extract showed cell division (Mitotic stages- Prophase,metaphase,anaphase,and telophase)as well.

MITOTIC INDEX= 7÷30×100=23.33%

Therefore MI of slide 1< MI of slide 2

In this way mitotic index of many slides were calculated and all the observed slides take showed that the mitotic index of slides exposed to extract was greater than slides without extract hence we conclude that cinnamon active part in DNA repair mechanism there by promoting cell division.

BACKGROUND INFORMATION :-

IJSER

Renaturation kinetics and thermal stability of DNA in aqueous solutions of formamide and urea.

Hutton JR.

Abstract

This paper reports the results of a systematic study of the effects of formamide and urea on the thermal stability and renaturation kinetics of DNA. Increasing concentrations of urea in the range 0 to 8 molar lower the Tm by 2.25 degrees C per molar, and decreases the renaturation rate by approximately 8 percent per molar. Increasing concentrations of formamide in the range from 0 to 50 percent lowers the Tm by 0.60 degrees C per percent formamide for sodium chloride concentrations ranging from 0.035M to 0.88M. At higher salt concentrations the dependence of Tm on percent formamide was found to be slightly greater. Increasing formamide concentration decreases the renaturation rate linearly by 1.1% per percent formamide such that the optimal rate in 50% formamide is 0.45 the optimal rate in an identical solution with no formamide. The effects of urea and formamide on the renaturation rates of DNA are explained by consideration of the viscosities of the solutions at the renaturation temperatures.

Abstract

A band of 300 nucleotide long duplex DNA is released by treating renatured repeated human DNA with the single strand-specific endonuclease S_1. Since many of the interspersed repeated sequences in human DNA are 300 nucleotides long, this band should be enriched in such repeats. We have determined the nucleotide sequences of 15 clones constructed from these 300 nucleotide S_1-resistant

repeats. Ten of these cloned sequences are members of the Alu family of interspersed repeats. These ten sequences share a recognizable consensus sequence from which individual clones have an average divergence of 12.8%. The 300 nucleotide Alu family consensus sequence has a dimeric structure and was evidently formed from a head to tail duplication of an ancestral monomeric sequence. Three of the remaining clones are variations on a simple pentanucleotide sequence previously reported for human satellite III DNA. Two of the 15 clones have distinct and complex sequences and may represent other families of interspersed repeated sequences.

SIGNIFICANCE OF THE STUDY:- ***As we all know "Power is the capacity to translate "intention" into reality and then sustain it." Our" intention" before going through this research was to make world a better place for living by contributing our little efforts towards the field of science ….and try to explore the fact that "each and every creation of GOD is beneficial in its own way".***

IJSER

CONCLUSION:-

From the above mentioned Observation & statistical data we there by conclude that cinnamon used at proper concentration in relation to time can act as a drug which helps in DNA repair mechanism of damaged cells on exposure to intrinsic and extrinsic factors hence promoting cell division.

REFERENCES:-

1. GENETICS FROM CLASSICAL TO MORDERN BY P.K GUPTA.

2. INTERNET EXPLORER

IJSER