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Studies on the DNA Damage in *Azadirachta indica* Induced by Various Environmental Agents

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Abstract- In all the living organisms, except a few, DNA acts as the genetic material. Though DNA is quite stable inside a cell; many physical, chemical, biological or environmental factors can cause some abnormal changes in the DNA structure and function, which is termed as DNA damage. Amongst the environmental factors, UV radiation and high temperature can damage DNA to a large extent. The present study focuses on the effect of UV rays and temperature on DNA damage of *Azadirachta indica* plant.Gel electrophoresis studies revealed that an exposure of the plant DNA to UV light of 260 nm for 30 minutes and to a temperature of 100^{o}C was sufficient for complete damage of DNA. The findings of the study will help to understand the extent and nature of DNA damage which can be done to plants by major environmental parameters.

Keywords- *Azadirachta indica*, DNA damage, Gel electrophoresis, genetic material, Temperature, UV radiation

1. INTRODUCTION

The main genetic material in almost all the living organisms is DNA. Though the DNA molecule is stable inside an organism, but its stability is sometimes challenged. These stability challenging factors may be a component of the living system or of the environment which cause DNA damage. The most promising environmental factors in this respect are UV radiations and various temperature shifts.

Plants are thus continuously exposed to UV radiations as well as atmospheric temperature shifts (Tuteja *et al.,* 2009). UV radiation damages DNA, proteins, lipids and various other biomolecules on all living organisms (Rastogi*et al.,* 2010, Esnault *et al*., 2010). Cellular DNA has been considered as an important target for UV induced genetic damage (Tuteja *et al*., 2009, Gill *et al.,* 2010, Biedermann *et al.,* 2011). Sunlight contains energy rich UV-A, UV-B and UV-C (Biedermann *et al.,* 2011). But UV-B is the most damaging to plant DNA(Xiong *et al.,* 2001, Frohnmeyer *et al.,* 2003). UV-B can penetrate and damage plant genome by inducing oxidative damage, DNA-protein and DNA-DNA crosslinks (Tuteja *et al.,* 2001).

Another important environmental factor leading to DNA damage in plants is temperature. Thermal stress affect several biochemical parameters, leads to oxidative stress and DNA damage (Cheng *et al.,* 2018, Antunes *et al.,* 2000).

In the present investigation the effects of UV radiation and temperature on overall integrity of DNA in *Azadirachta indica* (Neem) were studied. *Azadirachta indica* (Neem) has been used in Ayurvedic medicine for 4000 years due to its medicinal properties. It is known to have antiallergic, antidermatic, antifeedent, antifungal, anti-inflammatory, cardiac, diuretic, insecticidal, spermicidal and many other biological activities (Lokeswar *et al.,* 2011, Bandyopadhyay *et al*., 2004).The main objectives were to extract the genomic DNA from the Neem leaves and to see the effect of UV radiation and temperature changes on *Azadirachta indica* DNA *in vitro.*

2. MATERIALS AND METHODS

2.1 Collection of the plant sample

50 g of fresh Neem leaves were collected from Guwahati, Assam. The leaves were then surface sterilized using sterile distilled water and subjected to DNA isolation.

2.2 Isolation of the genetic material

Extraction of the genetic material was carried out followingDoyle and Doyle method (1990). 2 g of finely grounded leaf was taken in a tube, to which 10 ml of CTAB extraction buffer was added along with 4 µl of β mercaptoethanol. The CTAB extraction buffer was prepared by dissolving 20 g of CTAB, 100 ml of 100mMTrisHCl, 40 ml of 20mM EDTA, 81.8 g of 1.4 M NaCl, 5 g of 1% PVP to 1000 ml of distilled water

and autoclaved at 121^{o}C for 15 minutes. The tube wasthen incubated at 55^{o}Cin water bath for 30 minutes with shaking between intervals of 10 minutes. Further the tube was cooled down to room temperature and 10 ml of Chloroform:Isoamyl alcohol (24:1) was added to it, followed by vigorous shaking. It was then centrifuged at 10,000 rpm for 10 minutes. The supernatant wastransferred into a fresh tube and added to it equal volume of chilled isopropanol. This setting was left undisturbed in cold condition for 30 minutes. The precipitate was then spooled out, transferred to another tube, added 1 ml of 70% ethanol and subjected to centrifugation at 10,000 rpm for 5 minutes. The supernatant was discarded and the pellet obtained was air dried for 20 minutes in order to remove the ethanol. 2 drops of TE buffer was added to the tube to dissolve the pellet.

1% Agarose gel was prepared and the isolated DNA was run along with Hind III λ DNA as marker. The DNA was allowed to run for 1 hour in 100 Volt. The gel was visualized in UV transilluminator.

2.3 DNA damage by ultra violet radiation

The extracted DNA was taken in four different tubes. Three tubes were then subjected to exposure of UV radiation of 260 nm, for a period of 10 minutes, 20 minutes and 30 minutes respectively. The fourth tube served as control. Immediately after exposure the DNA was subjected to agarose gel electrophoresis.

2.4 DNA damage by high temperature

For this purpose four different tubes containing the extracted DNA were prepared. Three of those were subjected to treatment of a high temperature of 50^{o} C, 75^{o} Cand 100˚C for 10 minutes respectively. The tubes were incubated in a hot water bath maintained at the aforesaid temperatures respecively. The treated DNA was then subjected to agarose gel electrophoresis. The fourth tube served as control.

3. RESULTS

3.1 Isolation of genetic material

When the isolated DNA was electrophoresed, visible and clear bands with negligible shearing were seen (Fig. 1). Hind III digested λ DNA was run as standard marker.

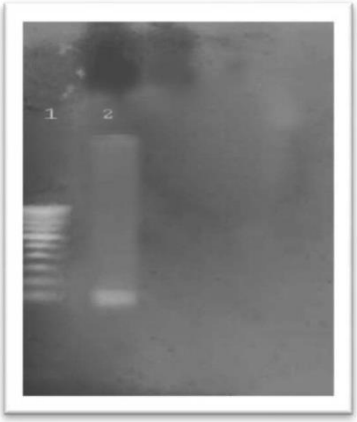

Fig. 1. Gel Electrophoresis of Neem DNA (lane1: Marker DNA, lane 2: Neem DNA)

3.2 Damage by Ultra Violet radiation

DNA extracted from Neem leaves were exposed to UV radiations of 260nm for different time intervals. It was found that an exposure to UV radiation for 10 minutes lead to shearing of the DNA at many points. The level of shearing increased with an increase in the exposure time. Maximum shearing was observed after 30 minutes of exposure (Fig 2)

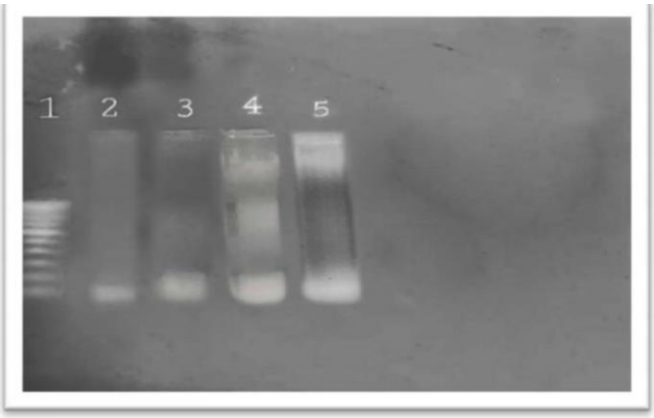

Fig. 2. DNA damage by UV radiation (lane 1: Marker, lane 2: Control DNA, lane 3: 10 min exposure, lane 4: 20 min exposure, lane 5: 30 min exposure)

3.3 Damage by high temperature

Temperature stress can damage DNA to a large extent. It was found that at temperature 75^{o}C, very little damage to the DNA occurred. But at temperature 100^{o}C the *Azadirachta*DNA got completely damaged which was evidenced by presence of continuous stretch of

sheared DNA in the agarose gel (Fig 3). At 50^{o}C, no damage was seen.

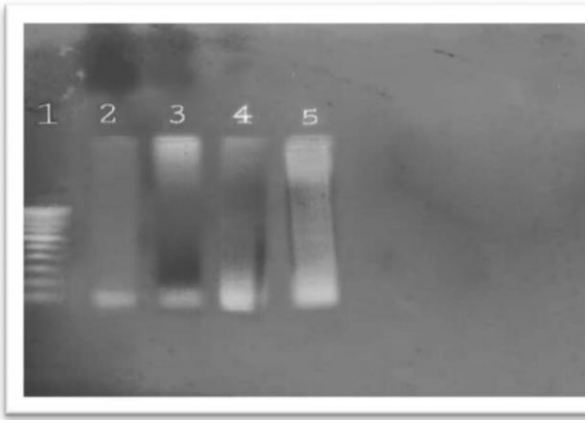

Fig. 3. DNA damage by high temperature (lane 1: Marker, lane 2: Control DNA, lane 3: 50^{o} C exposure, lane 4: 75 ^{o} C exposure, lane 5: 100 ^{o} C exposure

4. DISCUSSION

UV radiation induces two of the most abundant mutagenic and cytotoxic DNA lesions such as cyclobutane–pyrimidine dimers (CPDs) and 6–4 photoproducts. In addition, the ultraviolet rays can also cause the cross-linking of DNA complementary strand and DNA with protein. Double strand breaks are also lethal as they affect both strands of DNA and lead to the loss of genetic information (Ashraf *et al.*, 1994). In the present study it was observed that around 10 minutes exposure to UV radiation (260 nm) is sufficient to produce DNA lesions. When the exposure time was increased to 30 minutes, the extent of damage was found to be very high. DNA appeared as clear stretching band rather than an intact solid band in all treatments as visualized in the electrophoretic gel. This indicates the total disintegration of the *Azadirachta* DNA.

Temperature stress can damage DNA to a large extent. It was found that at temperature 75^{o}C, very little damage to the DNA occurred. But at temperature 100^{o}C the *Azadirachta* DNA got completely damaged which was evidenced by presence of continuous stretch of sheared DNA in the agarose gel. At 50^{o}C, no such remarkable damage was observed. Direct heat exposure to cells causes protein degradation and DNA damage, which can lead to genetic alteration, cell death, apoptosis and loss of cell viability. Also, under dry conditions, DNA degradation begins above 100˚C (Moshe *et al.*, 2013). It is reported that at temperature above 100^{o}C, DNA damage and apoptosis occur in pufferfish (Cheng *et al*., 2018). Moshe et al. had reported that at above 190^{o}C, complete DNA degradation occur under dry condition. But in the present investigation it was found that at 100^{o}C, complete DNA damage occur in *Azadirachta* genome. It is possible that the temperature weakens the chemical bonds between the atoms within the DNA molecule. Another possibility is that the exposure to oxygen in the tube create a pressure, damaged the DNA and made it more sensitive to heat-induced degradation.

5. CONCLUSION

Plants exhibit both positive and negative responses to sunlight and environment temperatures. The ability of the plant to adjust to the UV rays and heat stress varies within regions, species as well as at different developmental stages.Though it is difficult to study the exact mechanism of DNA damage in vivo, however, it can be concluded from the present investigation that UV radiation and high temperature adversely affect the plant genetic material, and thus also affect the expression of some vital genes of plants related to their growth and development. The findings of the study will help to understand the extent and nature of DNA damage which can be done to plants by major environmental parameters.

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