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ORIGINAL ARTICLE

Alterations of protein and DNA profiles of *Zea mays* L. under UV- B radiation

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UV radiation is an important stress factor for plants which may result in damage to genetic system and cell membranes and several metabolic processes. UV-B has greater damaging effects on plants because the cell macromolecules such as DNA and protein having strong absorption at 280-320 nm. In the present study, UV-B stress was given to the seeds of *Zea mays* L. at two different time intervals (30 and 60 min) and that stressed seeds were grown under normal environment condition. The leaves of 10th and 20th day seedlings were collected for the analysis of protein and DNA profiles. Protein was analyzed by SDS-PAGE and DNA was analyzed by Restriction enzymes. When compared with control plants, increased numbers of protein and DNA bands were observed in UV-B treated plants. The present study concluded that the plant synthesis new proteins and DNA under UV treatment for the adaptation to the environmental conditions. These stressed proteins could be used as biomarkers for identification of stressed plant. Identification of quantitative trait loci for UV stress resistance may well be an effective analytical tool. This approach is promising, considering that saturated DNA marker maps are now available for both genetic model plants and crop plants.

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The UV spectrum is generally divided into three regions: the UV-C region (220- 280 nm), the UV-B region (280 - 320 nm) and the UV-A region (320 - 400 nm). UV-B radiation is the most energetic component of sunlight reaching the earth's surface, and this has increased due to ozone depletion (Strid *et al.*, 1994). All of the most damaging UV-C radiation and most of the UV-B are filtered out by atmospheric ozone before it reaches the earth's surface. However, UV influence rates at the earth's surface have been increasing recently as levels of atmospheric ozone have been decreasing. This

progressively worsening situation has led to renewed impetus in efforts to understand the effects of UV radiation on plants and other organisms. The impact of increased UV-B radiation on the biosphere, as a result of stratospheric ozone depletion, has heightened awareness of the cytotoxic, mutagenic, and carcinogenic consequences of UV-irradiation (Longstreth *et al.,* 1995), and increased interest in the mechanisms by which cells repair and/or tolerate UV-induced damage to DNA (Britt, 1996; Sancar and Tang, 1993; Taylor *et al.,* 1997).

Biological systems are vulnerable to wavelengths in the transitional range of 280 - 320 nm and are thus greatly affected by ozone losses (Caldwell *et al.,* 1989; Rozema *et al.,* 1997). Any perturbation that leads to an increase in UV-B radiation demands careful consideration of the possible consequences. As a result, the UV-B region of the ultraviolet spectrum has gained importance over other environmental factors (Jordan 2002; Rozema *et al.,* 2002). This enhanced exposure to UVB is potentially detrimental to all living things but is particularly harmful to plants due to their obligatory requirement for sunlight for survival and their inability to move (Strid *et al.,* 1994).

Studies have shown that plants exhibit a tremendous variability in their sensitivity to UV-B radiation (Musil *et al.,* 2002; Zuk-Golaszweska *et al.,* 2003). Responses that occur include changes in leaf secondary chemistry (flavonoid accumulation), alterations in leaf anatomy and morphology, reductions in net carbon assimilation capacity (photosynthesis) and changes in biomass allocation and growth (Musil, 1996). The direct UV-B action on plants that results in changes in form or function of plants appears to occur more often through altered gene activity rather than non-specific damage to DNA (Britt, 1997).

In absorption spectrum, DNA is a major and long studied target of UV-B damage, but UV-B radiation can also directly damage proteins and lipids; that UV-B radiation cross-links RNA to particular ribosomal proteins with a concomitant decrease in translation and conclude that RNA is also a major target of UV-B radiation (Casati and Walbot, 2004). UV damage-specific binding proteins are considered to play important roles in early responses of cells irradiated with UV, including damage recognition in the DNA repair process. UV-B was found to cause a decline in total RNA, enzyme activity and protein levels of several key photosynthetic proteins including RUBISCO (Jordan *et al.,* 1992), Dl polypeptide of photosystem II, chlorophyll a/6-binding protein and the ATPase complex (Zhang *et al.,* 1994). From this context, in the present investigation alteration of Protein and DNA profiles of ***Zea mays*** L. was carried out due to the VU – B radiation.

MATERIALS AND METHODS

The ***Zea mays*** L. seeds were collected from seed testing centre, Tirunelveli. They were surface sterilized by immersing the seeds in 0.1% Mercury Chloride for 3 minutes and rinsed with distilled water. The seeds were soaked for 12hrs in distilled water.

After soaking, the seeds were kept in UV chambers equipped with two UV-B tubes (Philips TL/12 20W) at constant temperature. Two sets of seeds were irradiated under UV-B tubes for 30 minute and 60 minute. A set of control seeds was maintained for comparison in experiment. After irradiation, the seeds were collected and sown in a tray containing equal amount of sandy soil and garden soil. The soils were mixed completely and watering. The trays were labeled and arranged randomly at regular intervals to ensure uniform

environmental conditions on the plants. Watering was done at regular intervals.

Isolation of protein

The isolation of protein was performed by modified method of Lowry *et al.,* (1951). Proteins were separated in 10% Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) following the procedure given by Laemmli (1970).

DNA Isolation

Genomic DNA was isolated from the young leaves of the stressed plants taken for experiments. The DNA was isolated by the standard protocol of Doyle and Doyle (1987) and quantified by Sambrook and Russell (2000) method.

Restriction Enzyme Digestion

Restriction digestion performed by two restriction enzymes *EcoRI* and *HindIII*. The restriction digestion mixture was prepared by sterile double distilled water (13 µl), 10X restriction buffer (2 µl), DNA sample (3 µl) and restriction enzyme (2 µl) in a micro centrifuge tube. The mixture was gently mixed by repeated pipetting or tapping and incubated at 37^0 C. The reaction was stopped by incubating the mixture at 60^0 C for 2 minutes. The reaction product was checked in the Agarose Gel (1%) Electrophoresis.

Determination of Molecular Weight

The molecular weight of the protein and DNA was compared by standard molecular ruler. Standard proteins with molecular weights of 205.0, 97.4, 66.0, 43.0, 29.0, 20.1, 14.8, 6.5, 3.5 and 2.8 kilo Dalton (kDa) were used as markers. 100 base pairs (bp) molecular marker was used as DNA standard ruler.

Table 1 Molecular weight of the different proteins observed in *Zea mays* L.

Band No.	10th Day			20th Day		
	Control	**30 min**	**60 min**	**Control**	**30 min**	**60 min**
1	-	89.12	-	-	-	-
2	79.43	79.43	-	-	-	-
3	-	-	70.79	70.79	-	70.79
4	63.09	-	-	-	-	-
5	-	-	50.12	-	50.12	-
6	-	-	-	-	-	44.67
7	-	31.62	-	-	-	-
8	-	-	28.18	-	28.18	-
9	-	-	-	25.58	-	-
10	25.12	25.12	-	-	-	-
11	-	-	-	22.12	-	22.12
12	-	-	19.95	-	-	19.95
13	15.85	-	-	-	15.85	15.85
14	-	-	-	-	-	11.22
15	-	-	10	10	-	-
16	-	-	-	-	8.91	-
17	-	7.94	-	-	-	-
18	-	6.31	-	-	-	-
19	-	-	5.01	-	5.01	5.01
20	-	4.47	-	-	-	-
21	3.55	3.16	-	-	-	-
Total	5	8	6	4	5	7

RESULT AND DISCUSSION

Ultraviolet radiation is the part of the non-ionizing region of electromagnetic spectrum which comprises approximately 8-9% of the total solar radiation. The increase in UV-B levels is due to an accelerating depletion of the stratospheric ozone shield and is caused by man-made air-pollutants such as chlorofluorocarbons (CFCs). UV-B has greater damaging effects on living organisms because the cell macromolecules such as DNA and protein have strong absorption at 280 - 320 nm.

Alteration of Protein pattern of leaf under UV-B stress:

The protein pattern was investigated in UV-B stressed plant of *Zea mays* L. at different day (10 and 20th day) and time intervals (30 and 60 min). Protein pattern differed under both control and stressed condition (Fig.1). Protein analysis revealed the presence of about 5-8 bands in *Z. mays*. On the 10th day UV-B treatment, more number of protein bands were observed in plant treated with UV for 30 min and the number were slightly decreased in 60 min. But on the 20th day, slightly increase number of protein bands were observed in plant treated with UV for 30 and 60 min. (Table 1)

10th day:

On the 10th day in the control plants, a total number of 5 bands of 79, 63, 25, 15 and 3 kDa molecular weight were observed. On the 10th day, 8 bands (89, 79, 31, 25, 7, 6, 4 and 3 kDa) were observed in 30 min treated plants and 6 bands (70, 50, 28, 19, 10 and 5 kDa) in 60 min treated plants. In plants treated UV-B for 30 min, four new proteins of 89, 31, 7, 6 and 4 kDa molecular weight was observed. The protein pattern in the 60 min was totally different when compared with control.

Table 2 : Molecular weight for *EcoR*I and *Hind III* digestion in UV-B treated 20th Day plant of *Zea mays* L.

Band No.	*EcoRI (bp)*			*Hind III (bp)*		
	Control (Lane-1)	**30 min (Lane-2)**	**60 min (Lane-3)**	**Control (Lane-1)**	**30 min (Lane-2)**	**60 min (Lane-3)**
1	2818	2818	2818	-	-	2818
2	-	-	-	-	2511	-
3	-	1995	1995	1995	-	-
4	-	-	-	-	-	1584
5	-	-	1412	-	-	-
6	-	-	-	-	1258	-
7	1122	1122	1122	1122	1122	1122

Earlier studies have shown that the UV-B radiation damages ribosomes by crosslinking cytosolic ribosomal proteins S14, L23a, and L32, and chloroplast ribosomal protein L29 to RNA. In Maize leaves, synthesis of some ribosomal proteins is increased after 6 h of UV-B exposure (Paula and Virginia, 2004). Casati *et al.,* (2005) identified changes in protein accumulation in response to UV-B radiation in Maize leaves.

20th day:

On the 20th day in the control plants, only 4 bands (70, 25, 22 and 10 kDa) was observed, 5 bands (50, 28, 15, 8 and 5 kDa) were observed in 30 min treated plants and 7 bands (70, 44, 22, 19, 15, 11 and 5 kDa) were observed in 60 min treated plants. On 30 min UV-B treated plants, total of 5 bands (50, 28, 15, 8 and 5 kDa) were newly observed and on 60 min, 5 bands (44, 19, 15, 11 and

5 kDa) were newly observed when compared with control.

In our studies, 5 protein bands were newly observed. Similar work has been done by Charles *et al.,* (2008). They also reported the synthesis of 5 new proteins under UV-C stress. UV damage-specific binding proteins are considered to play important roles in early responses of cells irradiated with UV, including damage recognition in the DNA repair process. UV-B was found to cause a decline in total RNA, enzyme activity and protein levels of several key photosynthetic proteins including RUBISCO (Jordan *et al.,* 1992), Dl polypeptide of photosystem II, chlorophyll a/6-binding protein and the ATPase complex (Zhang *et al*., 1994).

DNA alteration detected by Restriction Enzymes:

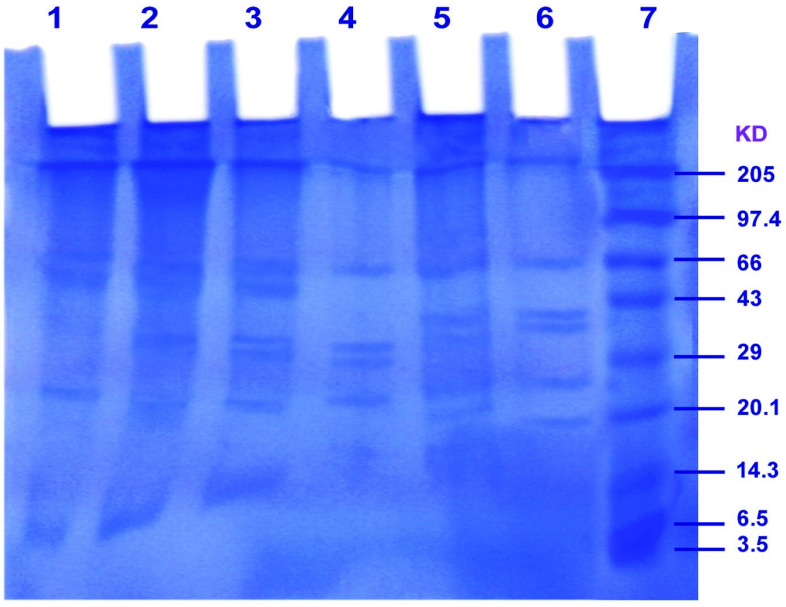
If the DNA is affected by UV-B radiation, the restriction site is also affected. With the help of restriction enzyme, we can find out whether the DNA is affected or not. The genomic DNA of control and treated plant was subjected to two restriction enzymes (*EcoRI* and *HindIII*) and the banding patterns were observed (Fig.2).

***EcoRI* digestion in UV-B treated 20th Day plant:**

On 20th day *Zea mays* L. control plants, a total of 2 bands (2818 and1122 bp) were observed. On 30 min treated plants 2 bands (2818 and 1995 bp) and 4 bands (2818, 1995, 1412 and 1122 bp) were observed in 60 min. On *EcoRI* digestion, 4 bands were observed in *Z. mays* L. at 60 min treated plants (Table 3).

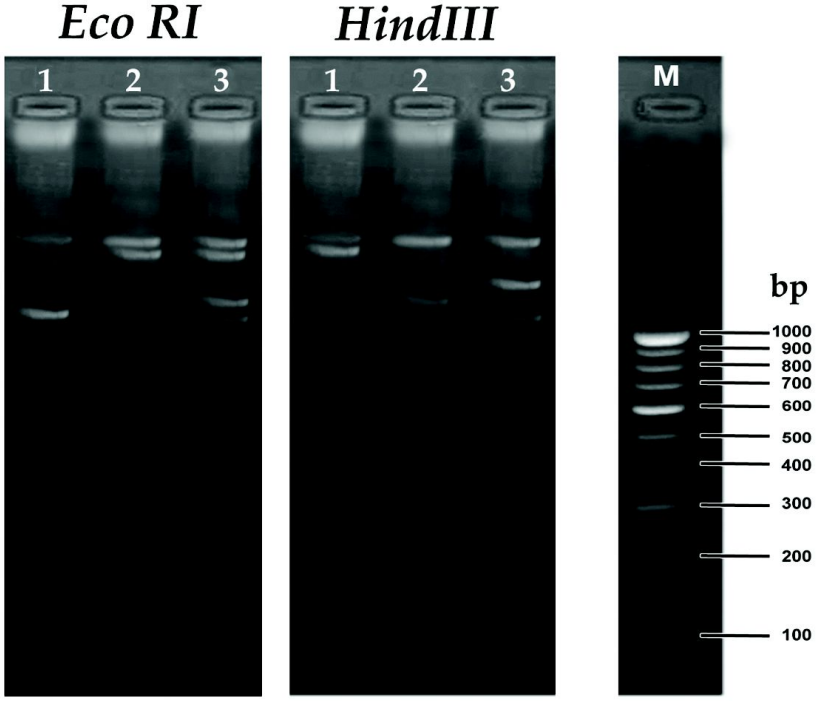
As explained by Mpoloka, (2008 (b)) UV radiation affects adjacent thymine bases of DNA, linking them together to form dimers which block restriction endonuclease recognition or cleavage for those enzymes that recognize sites containing adjacent thymines

Fig.1. Effect of UV stress on Protein in Zea mays L.



1. 10th Day control 2. 10th Day 30 min. 3. 10th Day 60 min.
4. 20th Day control 5. 20th Day 30 min. 6. 20th Day 60 min.
7. Marker protein.

Fig. 2. Restriction Digestion banding pattern on 20th day UV treated *Zea mays* L.



1. 20th Day control 2. 20th Day 30 min.
3. 20th Day 60 min. 4. Marker DNA.

***HindIII* digestion in UV-B treated 20th Day plants:**

On the 20th day in *Zea mays* L. 2 bands (1995 and 1122 bp) were observed in the control plants. Then, 3 bands (2511, 1258 and 1122 bp) were observed in 30 min and 3 bands (2818, 1584 and 1122 bp) were observed in 60 min. (Table 3).

Different molecular weight of DNA band was observed in both restriction enzymes. This is due to the fact that restriction site of DNA is affected during UV-B stress. UV-B wavelength of the electromagnetic spectrum causes two major types of DNA-damage: the formation of cyclobutane pyrimidine dimmers (CPDs) and pyrimidine (6-4) pyrimidine dimers (6-4 photoproducts) (Mitchell and Nairn, 1989). Similar work has been done by Gerhard *et al.,* (2000) and they showed increased the frequency of somatic homologous DNA rearrangements in *Arabidopsis* and Tobacco plants under solar UV-B dose.

CONCLUSION

These stressed proteins could be used as biomarkers for identification of stressed plant. Identification of quantitative trait loci for UV stress resistance may well be an effective analytical tool. This approach is promising, considering that saturated DNA marker maps are now available for both genetic model plants and crop plants. The use of novel approaches combining genetic, physiological and molecular techniques should provide excellent results in the near future.

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