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UV-A and UV-B-induced effects on tomato plant *(Solanum lycopersicum)*

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Abstract
Over the past few decades, there has been a depletion of the stratospheric ozone layer due to emissions of halogen-containing compounds of anthropogenic origin. This has resulted an increase in solar ultraviolet-B radiation. High levels of UV-B radiation are responsible for multiple biologically harmful effects in both plants and animals. Therefore, there is a real interest in a better understanding of the mechanisms set by plants to respond to this stress. The aim of the present work is to study the genetic effects on tomato (Solanum lycopersicum) due to exposure to UV-A of doses 9, 18 and 35 kJ/m^2 and UV-B of doses 6, 12 and 24 kJ/m^2. mRNA was extracted followed by RT-PCR to study the expression of catalase and peroxidase genes. The obtained results indicate an increase in gene expression level of catalase (CAT) and a decrease in activity of peroxidase (POX). Also, the present results arise some components involved in stress-induced signals within the plant, which could help for understanding protective strategies against UV-A and UV-B radiation

Keywords: *POX, CAT, UV-A, UV-B, Solanum lycopersicum, RT-PCR and mRNA*

1. Introduction

The ultraviolet region of the electromagnetic spectrum coming from sun is divided into three bands named UV-A (315- 400nm), UV-B (280-315nm) and UV-C (200-280 nm) [1].

Stratospheric ozone concentrations are decreasing due to human-caused releases of chlorine and bromine-containing substances. Where most CFCs remain in the atmosphere for several decades, keep possession of their ability to destroy ozone. Thus, even if we stop adding CFCs to the atmosphere today, "good" ozone concentrations will remain depressed, and destructive UVB radiation will continue to be enhanced far into the future. Absolutely these decreases could have a substantial impact on human health and agricultural production [2].A detailed understanding the effects of levels of UV-B on crop species is essential to design crops that can produce food, fiber and other raw materials under increasing levels of UV-B radiation [1]. In addition to an environmental change related to an increased risk of skin cancer and with potentially deleterious consequences for plants[3].

Life on earth has adapted itself to the UV stress, mostly UVB stress, for example by forming protective UV-absorbing surface layers, by fixing cell damage or by substituting damaged cells completely. Human skin shows all of these adaptive features. Our eyes are less well adapted, but they are shielded by the brows and by squinting **[4]**.

Nowadays there is great interest in the improvement of the nutritional and antioxidant values of food crops. The protective actions of food crops against vascular diseases and certain kinds of cancer are ascribed to the contemporary presence of carotenoids, flavonoids, and vitamins. The antioxidant properties of tomato fruits are mainly related to carotenoid content, in particular lycopene and β-carotene, the accumulation of which generally increases with the ripening of fruits [5]

Whereas the impact of UV-B radiation on plant growth, development, physiology and morphology has been studied intensively, little is known about its influence on plant genome stability [6]. Also to better understand the processes of UV-B acclimation that result in altered plant morphology and physiology, we investigated gene expression in different organs of tomato at several UV-A and UV-B exposure times.[3]

All of this because no one can deny today that there is important evidence suggesting that increases of UVB can lead to decreased crop yields.[2]

2. Materials & Methods

2.1 Plant material

Tomato seeds were germinated in the green house at ASU. Plants were grown according to normal conditions. The experiment was designed using the control and three treatments according to the time of exposure for ultraviolet UV-A and UV-B.

2.2 Exposure to UV radiation

After three weeks of growth, First group of plants was irradiated with UV-B lamp (EB-180C one 8Watt MW. BLE-8T 312) at 6, 12 and 24 kJ/m^2 doses. another group of plants was irradiated with UV-A lamp (Sunstudio NT296 UV-A 360 nm) at 9, 18 and 35 kJ/m^2 doses. The last group of plants was not exposed to UV-A or UV-B was used as a control. Plants were examined after 1 hour of irradiation. The first and second leaves on the top of the plant were used for analysis.

2.3 Expression profile and total RNA extraction.

Catalase (*CAT*) and cell wall associated peroxidase (*TPX1*) expression profiles were expected to be modified in response to treatment with UV-A and UV-B radiation, either by raise or decline.

RNA extraction was performed using 25 mg of leaf tissue from 3 weeks old UV-A and UV-B treated tomato plants with different doses as well as non-treated control tomato plants, using GeneJET RNA Purification Kit #K0731.

2.4 Reverse transcriptase (RT) PCR technique.

The first strand of cDNA was synthesized as described (BioRT cDNA First Strand Synthesis Kit) by using random hexamers following the producer's instructions. RNA from each treated plant was used for semi quantitative RT-PCR reaction. The specific primer sequences for tomato Catalase (CAT), cell wall associated peroxidase(TPX1) and 18S rRNA which was used as a control for the semi-quantitative RT-PCR were listed in Table 1

Table 1 Sequence of CAT, TPX1 and 18S rRNA primers, accession number of each and the expected fragment size

Name gene	Primer sequence	NCBI Accession number	Expected fragment
Catalase (CAT)	**CATF**, 5'-GAT GAG CAC ACT TTG GAG CA-3' and **CATR**, 5'-TGC CCT TCT ATT GTG GTT CC-3'.	AF112368	145 bp
Peroxidase (TPX1)	**TPX1F**, 5'-TGC AGC ATT GAC AAC ACG TA-3' and **TPX1R**, 5'-TCT TCC CAT TTT CTC CAT CG-3'	L13654	112 bp
18S rRNA	**18SrRNAF**, 5'-AAA CGG CTA CCA CAT CCA AG-3' and **18S rRNAR**, 5'-CCT CCA ATG GAT CCT CGT TA-3'	AY552528	110 bp

The cDNA fragments were amplified by PCR (Master Mix Kit), the denaturing temperature was 95°C for 4 min, annealing temperature is 55°C, extension at 72°C for 30 sec followed by final extension at 72°C for 7 min. These products were visualized on 2% agarose gel against a 1 kb DNA ladder.

3. Results

3.1 Expression profile of antioxidant enzyme genes.

Quantification of *TPX1* and *CAT* gene expression was determined in UV-A and UV-B treated plants as well as, in control plants by RT-PCR using two sets of specific primers and the 18S rRNA as a control for the quantification reaction. The resulted RT-PCR products were visualized and analyzed using Mat lab. The band of control plant in each treated group has been chosen as a reference to which the band's intensities of the other treated plants were compared.

3.2 Gel electrophoresis

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA according to their molecular weight (base pair in case of DNA). So the final products of DNA (PCR product) were visualized on 2% agarose gel against a 1 kb DNA ladder. UV trans illuminator at 302 nm produced an image for each gel. The images of the agarose gels show the specific PCR products for *CAT*, *TPX1* and *18S rRNA* genes in the following figures Figure 1Figure 2Figure 3

3.3 Analysis of gel image using Mat lab

To analysis the gel electrophoresis results image and estimate the concentration of DNA to make a final conclusion of up regulation or down regulation of the genes under study, Mat lab program was used for image processing. The data obtained by Mat lab script was tabulated and shown in the following results. InTable 2, the effect of UV-A doses on genes CAT and TPX1 which draw in Figure 4-10.The result shows an up-regulation in CAT gene due to exposure to UV-A at dose 9 kJ/m^2 (equivalent to 15 min. time of exposure). With increasing the dose to 18 kJ/m^2 (equivalent to 30 min. time of exposure) the percentage increase to 50%. At high dose35 kJ/m^2 (equivalent to 60 min. time of exposure) the percentage in increase of CAT gene in comparing to control (untreated plant) increased to 60%.On the other side, the UV-A cause down regulation of TPX1 gene. At low dose 9 kJ/m^2 there is a slight increase by 10%. With

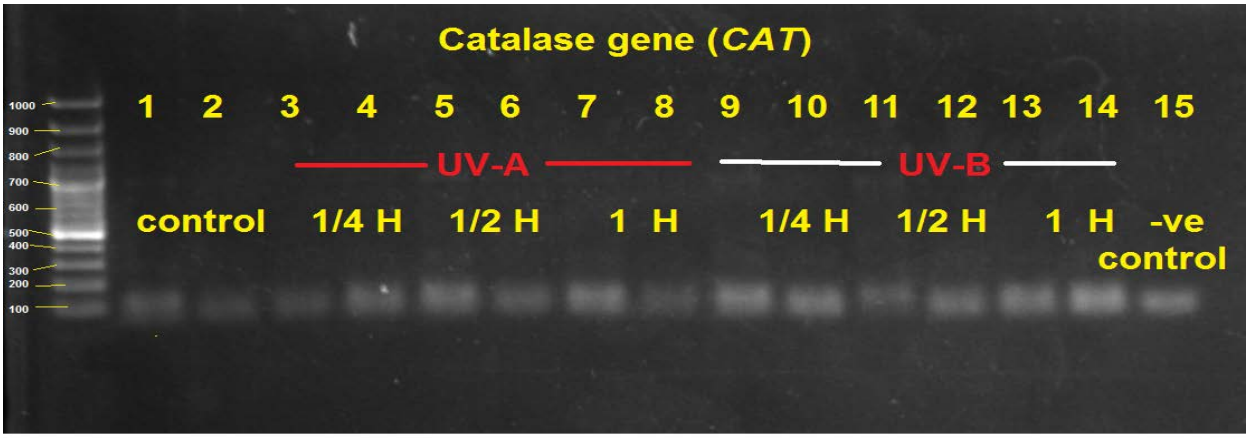


Figure 1: Agarose gel image for CAT gene PCR product.

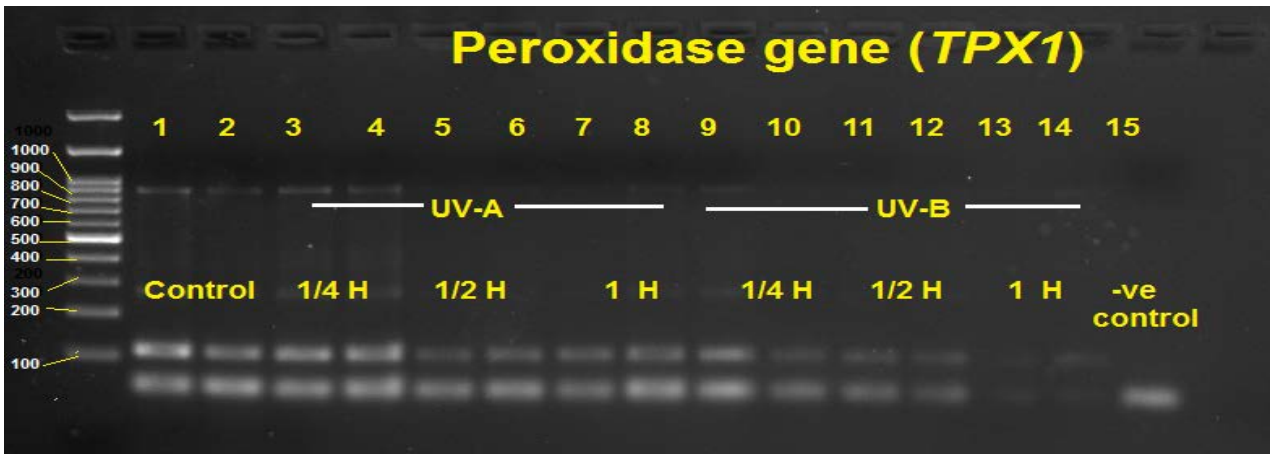


Figure 2: Agarose gel image for TPX1 gene PCR product

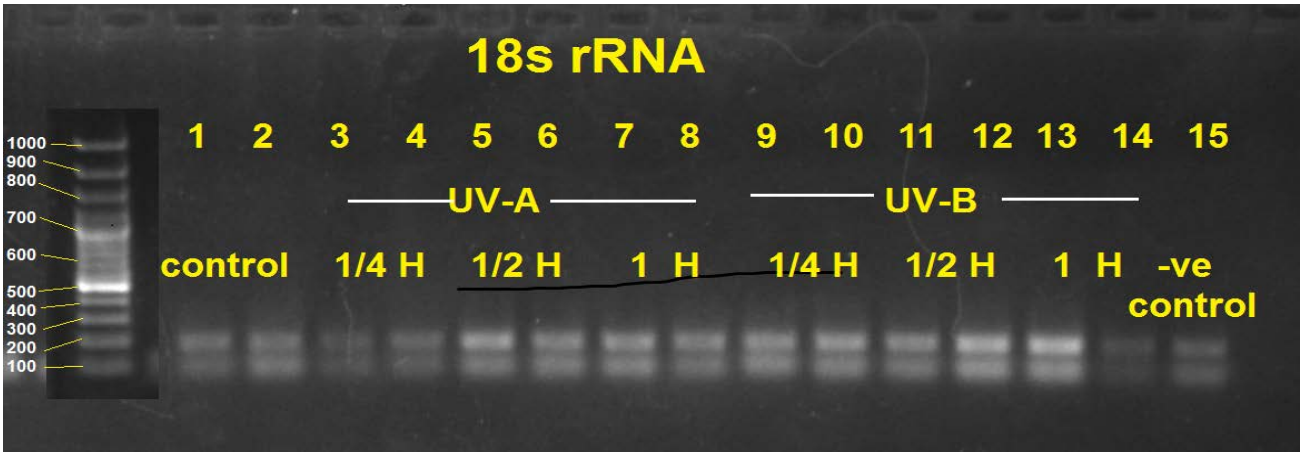


Figure 3: Agarose gel image for 18S rRNA housekeeping gene PCR product

Table 2: Semi quantitative analysis of gel image of CAT and TPX1 genes as a response for UV-A different doses

Exposure time (hr)	Dose (kJ/m^2)	CAT	TPX1
Control	0	1	1
¼	9	1.2	1.1
½	18	1.5	0.4
1	35	1.6	0.5

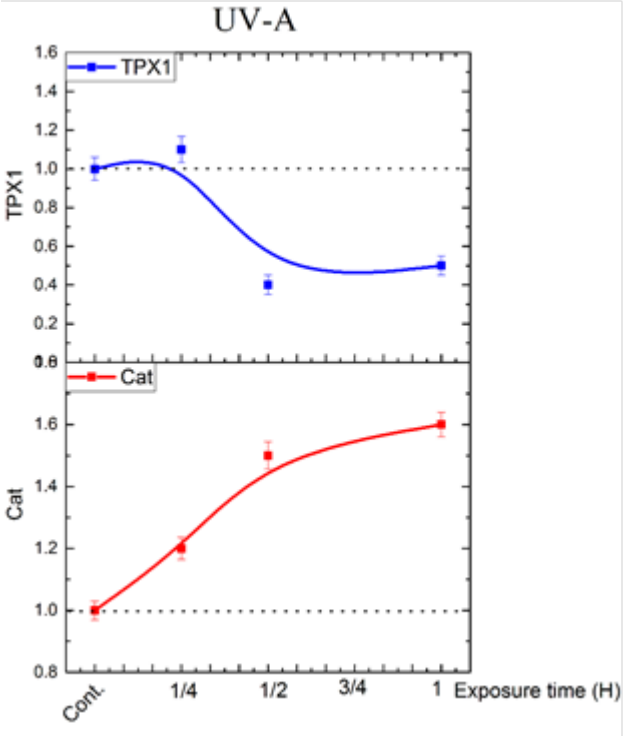


Table 3: semi quantitative analysis of gel image of CAT and TPX1 genes as a response for UV-A different doses

Exposure time (hr)	Dose (kJ/m^2)	CAT	TPX 1
Control	0	1	1
¼	6	2.2	0.5
½	12	1.7	0.2
1	24	2.6	0.1

The effect of UV-B on these two genes (CAT & TPX1) is shown in Table 3 and clearly detailed in Figure 4.The CAT gene is up regulated with large amount at low dose of UV-B 6 kJ/m^2 (equivalent to 15 min. time of exposure) by 120%. This percentage decreased to 70% at dose 12 kJ/m^2 (equivalent to 30 min. time of exposure) and again increased to 160% at high UV-B dose 24 kJ/m^2 (equivalent to 60 min. time of exposure).The harmful effect of UV-B is clearly proved when as shown in Table 3 and corresponding graph Figure 5. This figure show down regulation of TPX1 gene at low and high dose of UV-B. At low dose 6 kJ/m^2 the gene was down-regulated where the amount of transcripts decreased by 50%. The increase of UV-B dose to 12 kJ/m^2 caused an 80% decrease which continued to reach 90% at UV-B dose24 kJ/m^2

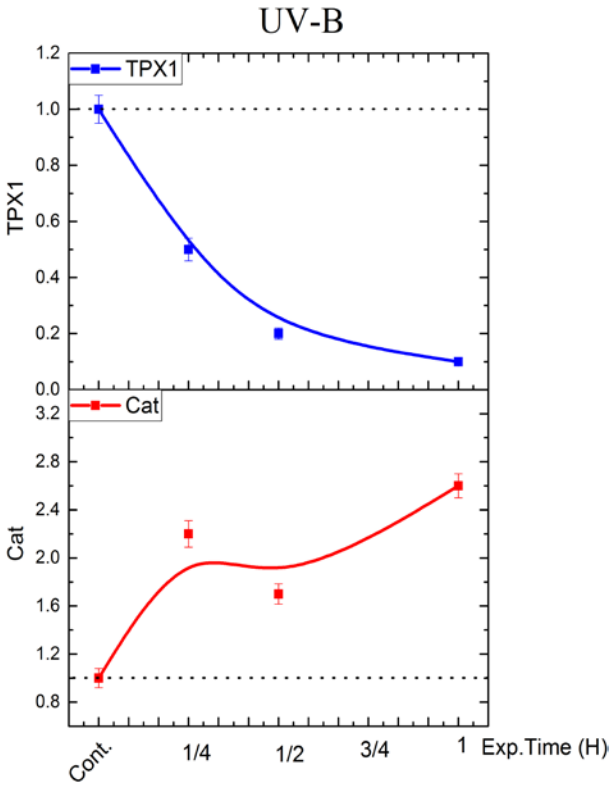


Figure 4: Up regulation & down regulation of CAT and TPX1 genes as a response for UV-B different doses

4. Discussion

Up-regulation of CAT gene expression in response to UV-A and UV-B irradiation was expected as a defense mechanism for plants. Due to its high energy per photon, UV-B has a higher damage effect on the living systems than UV-A. This

clearly appears where the induction of CAT gene expression is higher in case of UV-B than in UV-A. The greatest amount of gene expression was estimated at high dose 24 kJ/m^2 where the percentage of increase became 160% of increase. While the same exposure time with UV-A (1 Hour) but different dose (35 kJ/m^2) induced only an increase in the percentage of CAT gene expression up to 60%. This is a normal behavior for plant to up regulate the genes of the defense system (antioxidant system).
Relatively low levels of ROS can influence signaling and gene expression [7].
UV-B radiation is potentially damaging to plants, impairing gene transcription and translation, as well as photosynthesis. The biological impact of UV-B radiation depends on a number of factors, including the ratio of UV-B and photosynthetically active radiations (PAR), genetic factors, and the exposure history of the plant [8].

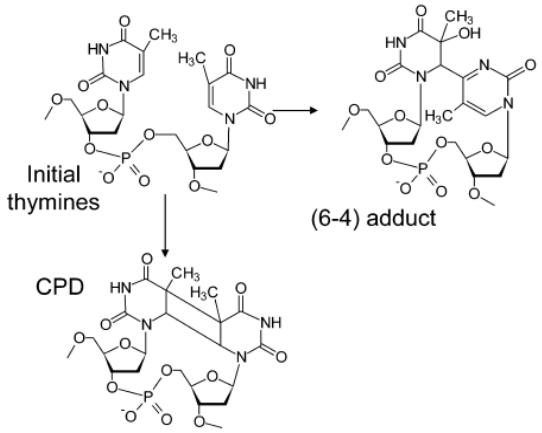
This harmful effect of UV clearly appear on *TPX1* (tomato peroxidase gene) where at low level of UV-B 6 kJ/m^2 down-regulated was noticed while UV-A of low dose 9 kJ/m^2 caused a slightly increase in gene expression as a way of plants to balance the amount of radicals produced due to UV-A stress but by increasing the dose of UV-A to a very high dose 35 kJ/m^2 *TPX1* was down-regulated by 50% but as UV-B again has high energy per photon, it has a large harmful effect than UV-A, as consequence found that TPX1 down regulated by 90% at high dose of UV-B 24 kJ/m^2. The main reason for the large harmful effect of UV-B is the energy per photon where this energy is high enough to form a covalent bond between two adjacent pyrimidines on the DNA template.

Up regulation of antioxidant system and increased expression of genes related to oxidative stress are found in plants grown under lower near-field intensities of UV-B. Under UV-B stress [8]
DNA is a target molecule for UV-B, as it absorbs UV-B powerfully and suffers alteration that leads to the formation of the cyclobutane pyrimidine dimers (CPD) which are formed by covalent bonding of adjacent pyrimidines. These DNA lesions, if not repaired, may interfere with DNA transcription and replication, and can lead to misreading of the genetic code and ultimately cause growth inhibition, mutations and potential death. Levels of UV-B tolerance differ considerably between genera, species, and even closely related cultivars [8].

The major DNA damage caused by UVB radiation is the formation of dimeric photoproducts, cyclobutane pyrimidine dimers (CPDs), and pyrimidine (6-4) pyrimidine photoproducts. The (6-4) photoproducts are also readily isomerized to Dewar photoproducts by UVA radiation. The formation of CPDs is the most common lesion and takes place throughout the DNA molecule. Formation of (6-4) photoproducts, however, takes place in actively transcribed regions of the DNA. Consequently, the presence of (6-4) photoproducts may have more deleterious consequences, especially as they do not appear to be repaired as rapidly as CPDs. Although dimeric DNA lesions form the predominant DNA damage, other lesions can occur, including the formation of monomeric photoproducts, DNA strand breaks and cross linking of DNA to protein. These lesions may still have a significant impact on the biological function of the cell despite being a minor proportion of the DNA damage if the appropriate repair mechanisms are not available. This was shown on *TPX1* gene where it's down-regulated and appears on the enzymatic level by decrease in the activity of peroxidase enzyme.

The various thymine dimers detected by mass spectrometry analysis following UV irradiation is shown in Figure 6. The yield of CPDs in irradiated solutions was found 18 times higher than that of (6-4) adducts [9]

Figure 5: Schematic representation of cyclobutane



dimers (CPDs) and the (6-4) photo adducts

5. Conclusion

Plants possess a number of protective mechanisms against UV induced damage, among these mechanisms, we can mention: protective pigments, production of antioxidants and repair of UV induced lesions in nucleic acids. Increasing dose of UV on tomato plants reduce the pigment content where Chl a was the most affected. Catalase and peroxidase enzyme activity decreased by a small amount as a response to UV-A while UV-B decreased their activity than UV-A. *TPX1* gene was down regulated due to increasing the dose of UV. On the other hand, *CAT* gene was up regulated as a response to UV stress. DNA damage induced by UV-B irradiation typically includes the formation of cyclobutane

pyrimidine dimers (CPD) and 6-4 photoproducts (6-4PP). UV-B can also induce the formation of oxidative compounds that is highly reactive and can cause DNA damage indirectly. UV-A is not readily absorbed by DNA and thus has no direct impact on DNA. UV-A is not readily absorbed by DNA and thus has no direct impact on DNA. Instead, UV-A induces DNA damage indirectly through the absorption of UV-A photons by other cellular structures (chromophores), with the formation of reactive oxygen species (such as hydrogen peroxide [H_2O_2]) that can transfer the UV-A energy to DNA via mutagenic oxidative intermediates such as 8-hydroxydeoxyguanosine (8-OHdG).

6. Acknowledgments

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7. References

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