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Impact of ultraviolet radiation on *Beta vulgaris* pigments

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Abstract: This research aimed to study the effect of ultraviolet radiation on *Beta vulgaris* pigments. Seeds were exposed to UV radiation at different distances (5,10 and 15 cm) and exposure periods (0, 0.5, 1, 2 and 4 hours). *Beta vulgaris* has been regarded as the unique source of betalains. Red betacyanins and yellow betaxanthins are two categories of betalains, which are nitrogen-containing plant pigments. The results showed that UV radiation significantly affected the content of chlorophyll a, chlorophyll b, the ratio of chl a/chl b, carotenoids, betacyanins, betaxanthins and total betalains depending on the distance and exposure period.

keywords: Ultraviolet radiation, red beet, betalains, chlorophyll a, chlorophyll b

1.Introduction

The non-ionizing area of the electromagnetic spectrum includes ultraviolet (UV) radiation, the wavelengths of which are dominated by the UVA component, which has the longest wavelength, but UVB and UVC components have the shortest wavelengths. The least dangerous type of UV radiation is UVA (3200–4000 Å). Plants frequently respond to UVA light in both stimulatory and inhibitory ways, affecting biomass, accumulation and morphology. Secondary metabolite synthesis and accumulation are extremely complicated processes that are influenced by both internal and external stimuli, such as UV radiation and enzymes. UVB radiation improved the generation of hydroxyl, hydrogen peroxide, superoxide radicals and singlet oxygen [1,2].

Abiotic stresses like drought, a lack of nutrients in the soil and heavy metal contamination have caused the synthesis of secondary metabolites [3.4]. When plants respond to oxidative damage, the superoxide dismutase enzyme and peroxidase, which scavenge the reactive oxygen species (ROS) and

protect lipids, proteins, and nucleic acids, are activated [5,6].

Enzymatic and non-enzymatic antioxidants in plants provide sufficient protection against ROS and free radicals generated as a result of photooxidative damage caused by UVB radiation [6.7]. Additionally, plants produce UVB-absorbing compounds as phenolic and flavonoid compounds to provide protection against damage [8].

Red beet (*Beta vulgaris* L.) is an important source of the natural red-colored food dye betanin (E162). Because of its high oot productivity (50–60 t/ha), pigment yield and environmental adaptability [8], beets cannot be competed with other sources of betanin as prickly pear fruits (*Opuntia vulgaris* Mill.) or red varieties of amaranth (*Amaranthus* L.) [9].

Red beet (*Beta vulgaris*), which contains the two main betalain pigments, red betanin and yellow vulgaxanthin I are thought to be the important sources of betalains. Beetroots are cultivated over the world and consumed widely and continually. Around 275 million metric tons of beetroot were produced globally in 2018, betacyanins make up approximately 75–95% of the pigments in beetroot, with betaxanthins making up the remaining 5–25% [10]. In the category of betalains, betanin (betanidin 5-O-glucoside), a dye derived from beet, predominates (70–95%) [11].

Betalains are plant pigments that contain nitrogen and are characteristic for order Caryophyllales. They fall into two categories: red-violet betacyanins (BC) and yellow-orange betaxanthins (BX), both of which are water-soluble pigments derived from tyrosine. Betanin and iso-betanin represented the majority of BCs, whereas vulgaxanthins (I and II) dominate BXs [12].

The stimulation of BC biosynthesis is regarded to occur in response to ultraviolet radiation, high salinity, low temperatures, mechanical injury or infection by pathogenic fungus [13–14]. Since ROS are produced in response to the same stresses, When plant cells are damaged, BCs work as antioxidants to alleviate oxidative stress. It implies that the accumulation of betalains (especially BCs) is an adaptive survival strategy.

According to the pigment content, the beetroot is among the ten vegetables with the highest antioxidant activity [15]. For the purpose of enhancing human health, the use of beetroot dye combines its coloring impact with its therapeutic benefits.

The biological functions of betalains, which include anti-inflammatory, hepatoprotective, antibacterial, and anticarcinogenic impacts, have attracted the attention of scientists recently, as well as the market trend toward the use of natural food colors [16-17].

Red beet is one of the most powerful vegetables with respect to antioxidant activity [18]. Betacyanins are a group of compounds exhibiting antioxidant and radical-scavenging activities [19].

Antioxidant activity in red beet is linked to antioxidants' role in scavenging free radicals and, as a result, in the prevention of diseases including cancer and cardiovascular disorders [20]. Additionally, betalains which promote resistance to oxidation, have been observed to enrich human low density lipoproteins with antioxidant activity [21].

Because of their various beneficial effects, dietary betalains may be crucial in preserving human health. A. Ferenczi, a Hungarian physician who used beetroot in the early 1950s to treat cancer, was first postulated that it had anticancer activity. The use of betalains in anticancer medications has been granted a patent in the United States [22]. The cell type and exposure time affect the betalains' ability to cause cytotoxicity.

Many studies have demonstrated that betalains and betalain-containing plant extracts are toxic to cancer cell lines [23–24] and that betalains may also hinder the growth of various cell cultures [25].

The aim of the present work was to investigate the effect of UV radiation on photosynthetic pigments (Chlorophyll a, chlorophyll b, carotenoids, and Chlorophyll a / chlorophyll b ratio) and total betalains (betacyanins and betaxanthins) in *Beta vulgaris*.

2. Materials and methods

Experimental design

The experimental plant was *Beta vulgaris* (Family: Amaranthaceae).

The experiment was carried out in the greenhouse of the Botany Department, Faculty of Science Mansoura University to test the effect of exposure to Ultraviolet radiation on photosynthetic pigments of beet root plants grown in clay-sandy soil.

Time course experiment

Beta vulgaris seeds were provided by the Agriculture Research Center, Ministry of Agriculture, Egypt.

Seeds of *Beta vulgaris* were exposed to ultraviolet irradiation for different time periods (0, 0.5, 1, 2 and 4 hours) at different distances from the lamp (5, 10 and 15 cm) according to [26] using a system consisting of fluorescent lamp (Type-C with a wavelength from 2000 to 2800 Å), its power equal to 15 wats. Also, the system was covered with aluminum foil to illuminate the samples from all sides. Then the seeds were surface sterilized by soaking in a solution of 10-3 M $HgCl_2$ for 3 minutes, followed by a sterile water wash. The seeds were then seeded in pots with clay-sandy soil. Seeds were germinated in pots in field and harvested after 1 month.

Estimation of photosynthetic pigments

Photosynthetic pigments (Chl a, Chl b and carotenoids) were estimated using the spectrophotometric method as recommended by [27]. Betaxanthins, betacyanins and total betalains were estimated spectrophotometrically according to [28].

Statistical analysis

In the present study, One-way analysis of variance (ANOVA) with Post Hoc Duncan test was used to statistically analyze all the data that were obtained. Using COSTAT software version 6.3, p value ≤ 0.05 was considered statistically significant.

3. Results and Discussion

Changes in photosynthetic pigments contents

The effect of Ultraviolet radiation on photosynthetic pigments (Chlorophyll a, chlorophyll b, carotenoids, and Chlorophyll a / chlorophyll b ratio) was investigated in leaf samples of cultivated *Beta vulgaris*.

The results in **Table 1** are obtained after exposure of seeds to UV radiation from different distances and different time intervals. At 5 cm : The result showed that there is a significant decrease in Chl a content with increasing time of exposure to the UV source. At 10 cm : The results showed that increasing the time of exposure to UV source led to significant decrease in Chl a content except when exposed for 0.5 h there is a non-significant decrease in Chl a compared to control which was not treated with UV. At 15 cm: The result showed that there is a significant decrease in Chl a content except at 0.5 and 1 hour there is a non-significant decrease.

Table 1: Effect of ultraviolet radiation on chlorophyll a content in *Beta vulgaris*

Time	**Chlorophyll a (mg/g F.wt)**		
	5 cm	10 cm	15 cm
0	1.9^{a}±0.2	1.9^{a}±0.2	1.9^{a}±0.2
0.5 h	1.4^{b}±0.15	1.6^{ab}±0.3	1.8^{ab}±0.45
1 h	1.2^{bc}±0. 1	1.4^{bc}±0.1	1.7^{ab}±0.2
2 h	0.9^{cd}±0. 1	1.2^{cd}±0.25	1.5^{bc}±0.15
4 h	0.6^{d}±0.3	1^{d}±0.30	1.3^{c}±0.1

The results in **Table 2** showed that when seeds were exposed at distance 5 cm from UV source there is a significant decrease in Chl b content except at 0.5 h there is a non-significant decrease but at distances 10 and 15 cm there is a significant decrease in Chl b content except for the time intervals of 0.5 and 1 hour there is a non-significant decrease compared to control.

The treatments at a distance of 5 cm from the UV source cause a non-significant increase in carotenoids content **(Table 3)** except for time exposure 1 hour caused a significant increase compared to the control but all treatments at distances 10 and 15 cm caused a non-significant increase in total carotenoids except 4 h which cause a significant increase in total carotenoids.

Table 2: Effect of ultraviolet radiation on chlorophyll b content in *Beta vulgaris*

Time	**Chlorophyll b (mg/g F.wt)**		
	5 cm	10 cm	15 cm
0	1.3^{a}±0.1	1.3^{a}±0.1	1.3^{a}±0.1
0.5 h	1^{ab}±0.08	1.1^{ab}±0.25	1.2^{a}±0.02
1 h	0.8^{bc}±0.03	1^{ab}±0.05	1.1^{ab}±0.05
2 h	0.6^{cd}±0.2	0.8^{bc}±0.1	$1a^{b}$±0.02
4 h	0.4^{d}±0.15	0.6^{c}±0.2	0.8^{b}±0.3

Table 3: Effect of ultraviolet radiation on carotenoids content in *Beta vulgaris*

Time	**Total carotenoids (mg/g F.wt)**		
	5 cm	10 cm	15 cm
0	0.34^{b}±0.02	0.34^{a}±0.02	0.34^{ab}±0.02
0.5 h	0.37^{ab}±0.01	0.36^{a}±0.03	0.35^{ab}±0.05
1 h	0.39^{a}±0.01	0.37^{a}±0.01	0.36^{a}±0.01
2 h	0.36^{ab}±0.03	0.34^{a}±0.01	0.32^{b}±0.08
4 h	0.34^{b}±0.02	0.30^{b}±0.05	0.28^{c}±0.01

The results in **Table 4** showed that all treatments at distances 5 and 15 cm from UV source caused a significant increase in Chl a/Chl b ratio except at a distance of 10 cm, the exposure time 0.5 h has a non-significant increase compared to the control.

Table 4: Effect of ultraviolet radiation on Chl a/Chl b ratio in *Beta vulgaris*

.Time	**Chl a/Chl b ratio**		
	5 cm	10 cm	15 cm
0	1.46^{b}±0.2	1.46^{c}±0.2	1.46^{d}±0.2
30 min	1.4^{c}±0.11	1.45^{c}±0.17	1.5^{c}±0.03
1 h	1.5^{a}±0.08	1.4^{d}±0.14	1.55^{b}±0.1
2 h	1.5^{a}±0.05	1.5^{b}±0.1	1.5^{c}±0.2
4 h	1.5^{a}±0.15	1.67^{a}±0.1	1.63^{a}±0.08

Plants that exposed to UV radiation showed a significant decrease in chlorophyll-a and chlorophyll-b content, but an increase in the concentration of carotenoids, as reported in the case of the UV radiarion exposed *Arabidopsis* species [29]. Additionally, it has been noted that UV-exposed *Barleria obtusa* and *Vigna unguiculate* plants have a significant decrease in Chl-a and Chl-b concentrations [30]. This was attributed to increased photo-degradation of chlorophylls [31] and lower rates of chlorophyll synthesis as a result of decreasing the expression of genes encoding chlorophyll-binding proteins [32] or to the breakdown of the structural integrity of chloroplasts [33]. Chlorophylls and carotenoids are predominant groups of pigments that can mainly be found in fruits and vegetables, determining the color of fruits and vegetables and providing beneficial impacts on human health [34]. The increase in carotenoid content in response to uvc in harmony with the results of [30] where the concentration of carotenoids has been significantly increased in leaves of *Colophospermum mopana* and *Phylica pubescens*. Since carotenoids aid in the photo-protection of photosynthetic systems by releasing excess excitation energy through the xanthophyll cycle, they were a biochemical response to reduce the effects of UV stress [35]. UV exerts its effect on the enzymes that synthesize pigments such as chlorophylls. In addition, pigments are also degraded by UV light especially chlorophyll b, so exposure to this type of radiation can cause an imbalance in the proportion of pigments.

The present results in **Table 5** showed that the betacyanin content increased significantly when exposed at distances 5,10 and15 cm from UV source except at 5 cm, the exposure period for one hour caused a non-significant increase compared to the control.

Table 5: Effect of ultraviolet radiation on betacyanin content in *beta vulgaris*

Time	**Betacyanin (mg/ g F.wt)**		
	5 cm	10 cm	15 cm
0	$0.21^{b}\pm0.10$	$0.21^{c}\pm0.10$	$0.211^{d}\pm0.10$
0.5 h	$0.39^{a}\pm0.04$	$0.42^{a}\pm0.05$	$0.48^{a}\pm0.10$
1 h	$0.20^{b}\pm0.10$	$0.38^{b}\pm0.02$	$0.41^{b}\pm0.05$
2 h	$0.16^{c}\pm0.03$	$0.19^{d}\pm0.01$	$0.42^{b}\pm0.03$
4 h	$0.13^{d}\pm0.11$	$0.16^{d}\pm0.04$	$0.28^{c}\pm0.05$

Betaxanthin content in the leaves increased in response to UV radiation at all distances and exposure periods compared to control as in **Table 6.**

Table 6: Effect of ultraviolet radiation on betaxanthin content in *beta vulgaris*.

Time	**Betaxanthin (mg/ g F.wt)**		
	5 cm	10 cm	15 cm
0	$0.13^{d}\pm0.02$	$0.13^{d}\pm0.02$	$0.13^{c}\pm0.02$
0.5 h	$0.24^{a}\pm0.08$	$0.27^{a}\pm0.05$	$0.30^{a}\pm0.10$
1 h	$0.21^{b}\pm0.01$	$0.22^{b}\pm0.17$	$0.28^{a}\pm0.10$
2 h	$0.18^{c}\pm0.1$	$0.20^{bc}\pm0.08$	$0.23^{b}\pm0.08$
4 h	$0.17^{c}\pm0.05$	$0.18^{c}\pm0.01$	$0.21^{b}\pm0.05$

The results in **Table 7** showed that at 5 and 10 cm, there is a difference in total betalains content by decreasing or increasing compared to control according to the exposure period to UV source but at 15 cm, all treatments caused a significant increase in total betalains.

Table 7: Effect of ultraviolet radiation on total betalains content in *beta vulgaris.*

Time	**Betalains (mg /g F.wt)**		
	5 Cm	10 Cm	15 Cm
0	$0.35^{b}\pm0.03$	$0.35^{c}\pm0.03$	$0.35^{e}\pm0.03$
0.5 h	$0.33^{bc}\pm0.01$	$0.69^{a}\pm0.08$	$0.78^{a}\pm0.01$
1 h	$0.41^{a}\pm0.01$	$0.51^{b}\pm0.02$	$0.69^{b}\pm0.02$
2 h	$0.34^{bc}\pm0.02$	$0.39^{c}\pm0.02$	$0.64^{c}\pm0.05$
4 h	$0.30^{c}\pm0.05$	$0.35^{c}\pm0.04$	$0.49^{d}\pm0.05$

In plants, increasing the overall amount of betacyanins and betaxanthins may be associated to a potential photo-protective activity that these pigments perform in the presence of UV radiation. Additionally, it was observed in a study on *Amaranthus caudatus* L. that UV radiation induces the production of betacyanins, and that the absence of this sort of radiation inhibits the synthesis of these pigments [36]. According to studies, UV radiation may alter the composition of pigments because plants try to lower the levels of radiation in their tissues, and because the interaction of pigments with other substances, such as co-pigments, may attenuate the oxidative damage caused by UV radiation. Therefore, it has been demonstrated that pigment synthesis in plants is a result of exogenic stress or senescence as well as ecological adaptation to changing conditions like excessive radiation [37]. The degradation of betalains can be attributed to cellular alkalization due to breakdown of the membrane by the presence of ROS and the influx of ions produced. Alkalization may induce the hydrolysis of the aldimine bond in the betalain structure, resulting in the production of a phenolic acids [38]. The accumulation of betalains in UV- treated plant tissues may be caused by their high molar absorption coefficients, which enable them to serve as protectors against UV radiation. Additionally, betalains accumulated in the vacuoles and serve as a potential sink for excess H_2O_2 produced in the chloroplast as a result of UV exposure, reducing the plant damage caused by photo-oxidation [39].

Conclusion

In conclusion, chlorophylls and carotenoids are the predominant group of pigments that play a major role in photosynthesis. Our study approved that at small doses, UV radiation can stimulate various pigments and consequently induce the various biochemical processes thereby inducing a strengthening effect in plant.

4. References

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