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Study of morphological and histological changes in melon plants grown from seeds irradiated with UV-B

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Abstract

It is well known that exposure of plant seedlings or plants to UV-B radiation induces changes in gene expression resulting in biochemical and morphological modifications. However, there is little information on the effects and potential utility of irradiation of seeds with UV-B. The aim of this study was to apply UV-B radiation in melon seeds using various irradiation times and then assess the effect on growth and morphology of the plant. Seeds of cultivar 'Topmark' were exposed to UV irradiation with wavelength centered at 302 nm, for periods of 0, 15, 30 and 45 minutes (UV dosages of 0, 99, 198 and 297 mJ cm^{-2}, respectively). The irradiated seeds were seeded in a mixture of peat moss and perlite for greenhouse germination. Morphological parameters such as plant height, stem diameter, number of leaves, leaf area, fresh and dry weights were evaluated. Stomatal frequency, stomatal index, and length and width of stomata were studied. Histological analyses were conducted to determine the areas of the stem vascular bundle and xylem vessels, width and length of vascular bundles, and the area of the lumen of xylem vessels. The analysis of variance indicated significant differences between treatments, with the treatment of 15 minutes (99 mJ cm^{-2}) of seed exposure to UV-B radiation generating 24.87 and 32.42 % more fresh and dry weight of the plants, respectively. Stomatal index was augmented on the adaxial surface by 52.26, 7.14 and 13.55 %, in the treatments of 99, 198 and 297 mJ cm^{-2}, respectively, in contrast with the control treatment, while the length of stomata was increased by 6.99% in the treatment with 30 minutes exposure time (198 mJ cm^{-2}). Stomatal frequency was unchanged by exposure to radiation. The irradiation of the seeds caused decrease in P, Ca and Na in the leaves of plants.

Key words: Irradiance, photoreceptors, signaling, seed irradiation, seed priming, *Cucumis melo*.

Introduction

Ultraviolet-B (UVB: 280-320 nm) radiation is a relatively minor component of sunlight, but it can induce physiological processes related to stress or photomorphogenic responses in plants (Li *et al.*, 2013). The type of responses that are induced by UV-B radiation are mainly determined by the exposure dose and also depend on whether the plants are acclimatized by previous exposure to UV-B radiation (Heijde and Ulm, 2012). Non-harmful levels of UV-B radiation can induce transcriptional changes that result in the alteration of expression of genes encoding enzymes, membrane proteins, transcription factors, signaling components and several proteins involved in different cellular processes, including photosynthesis, primary metabolism and secondary cell wall biosynthesis, protection from stress, DNA release processes, processing of ribonucleic acid (RNA), and translation and proteolysis (Berli, 2011).

A moderate exposure to UV-B radiation can induce active acclimation responses, while more severe conditions can cause metabolic disorders. The response of a plant acclimated to a variety of environmental stresses is the accumulation of secondary metabolites and antioxidants (Carrasco, 2009). Similarly, the UV-B modifies the levels of a wide range of metabolites, including phenolic compounds, terpenoids, alkaloids and flavonoids (Eichholz *et al.*, 2012). It has been found that the concentrations of some of these metabolites augment after exposure to UV-B, while others suffers attenuation or transient change. In the last case the direction of the changes is dependent on whether the dose of radiation is high or low (Jansen *et al.*, 2008). Exposure to UV-B promotes various changes in morphology and growth of plants, including inhibition of the extension of the stem and reduced internode elongation, reduction in leaf area, increase in the thickness of the leaf lamina and in the number of epidermal cell layers, epicuticular waxes and pubescence (Jansen *et al.*, 1998; Pinto *et al.*, 2000; Frohnmeyer and Staiger, 2003). Studies of leaf growth in *Arabidopsis thaliana* indicated that the epidermal cells and stomatal density were higher in plants treated with UV-B (Wargent *et al.*, 2009).

Most of the aforementioned morphological changes were observed following exposure of seedlings of different species to UV-B radiation (Singh *et al.*, 2011; Zuk-Golaszewska *et al.*, 2003). Sugimoto (2013) noted that the information about the reaction of plants whose seeds were exposed to UV-B might offer a solution on how to deal with the recent poor climate conditions hampering crop production. However, there are few reports on the effects of UV-B radiation on seeds and their subsequent impact on the behavior of plants. Therefore, the aim of this study was to determine the effects of the UV-B irradiation of seeds on the growth, stomatal morphology, stem anatomy and mineral content of melon plants (*Cucumis melo* L.). The hypothesis was that the active UV-B receptors in the seed cells can induce changes in the program of development of the plant embryos, such that a series of morphological and biochemical differences manifest in a trans-developmental manner (Magliano and Casal, 2004) in later stages of plant growth.

Materials and methods

The experiment was conducted at the Universidad Autónoma Agraria Antonio Narro, located in Saltillo, Coahuila, Mexico, at latitude 25°23'N and longitude 101°02'W, with an altitude of 1,743 meters.

Cantaloupe type melon seeds of cultivar 'Topmark' were used. Four different exposure times: 0, 15, 30 and 45 minutes were applied to the seed, corresponding to UV dosages of 0, 99, 198 and 297 mJ/cm^{-2}, respectively, with irradiation intensity of 1.1 J m^{-2} s^{-1} using a UV-B radiation lamp (Lamp 3UV, model 3UV-36) with wavelength centered at 302 nm. The irradiance was kept constant at 1.1 W m^{-2} and the applied doses were obtained by varying the exposure time at a fixed distance using the expression:

$$D=\frac{I - t}{1000}$$

where: D = applied radiation dose (kJ m^{-2}), I = Irradiance (W m^{-2}) and t = exposure time (in seconds).

Planting was done in a greenhouse 24 hours after irradiation of the seed in polyethylene pots of 1 L, using peat moss and perlite in the ratio (70:30 v/v) as substrate for germination. The periods of irradiation of the seeds were the treatments; each treatment had six replicates with 10 seeds per repetition. Steiner nutrient solution (Steiner, 1961) was used for irrigation of plants every other day with concentration of 30 and 50% according to the plant growth. The first application was made 30 days after planting (DAP) and the last 80 DAP.

Morphological variables: Evaluations were performed at 56, 67 and 81 DAP, determining plant height (PH) from the base of the stem to the stem apex using a tape measure; stem diameter (SD) was obtained with a digital vernier (AutoTec) and was measured in the middle part of the stem; total number of leaves (TNL) of 6 randomly selected plants from each treatment were counted; and leaf area (LA) using a leaf area meter (LI-COR 3100) (Dai *et al.*, 1995). Fresh weight (FW) and dry weight (DW) of the whole plant were determined. The aerial part was cut from the base of the stem, the root thoroughly washed to remove the adhered soil. Samples were taken to the laboratory where they were weighed on a digital balance (Precisa BJ 610C), then placed in paper bags in an drying oven (Riosa H - 48) at 65 °C for 72 h until constant weight was achieved and then weighed on a digital scale with 0.01 g precision.

Stomatal study: The stomata study was conducted on a sample obtained 71 DAP. Three plants of each treatment were randomly selected and a leaf lamina having physiological maturity and an east-facing orientation was taken from each plant. Two foliar impressions were taken from both adaxial and abaxial surfaces. A coating of transparent varnish was used on the samples, whereby a thin layer over the epidermal surface of the adaxial and abaxial leaf surfaces was placed over an area of about 3 cm^2 in the middle of the leaf blade, allowed to dry and then the film was carefully removed with the aid of transparent adhesive tape placed over the layer of varnish. The ribbon with the leaf print was placed on a clean microscope slide, previously labeled.

For each print two random fields were observed under a microscopic at 40X magnification in order to analyze the adaxial and abaxial surfaces. A photomicrograph of each field was taken, in which the number of stomata and epidermal cells were counted, stomatal width (SW) and length (SL) were measured for five stomatal guard cells in each of the fields of the microscope so that 30 adaxial surfaces and 30 stomata abaxial surfaces of each treatment were measured. The stomatal density (SD) was obtained as follows:

$$SD=\frac{Number\ of\ stomata}{0.02479}$$

where SD = number of stomata mm^{-2}, 0.02479 mm^2 = area of the photographed image. To determine the stomatal index (SI) the following formula (Wilkinson, 1979) was used:

$$SI=\frac{Number\ of\ stomata}{Number\ of\ epidermal\ cells}\text{x } 100$$

The abaxial SI could not be determined, because the impression did not permit a clear determination of the number of epidermal cells. A compound microscope (Carl Zeiss) with digital camera (Pixera Winder Pro) and measurement software (AxionVision Rel. 4.8) were used.

Analysis of the histological structure of vascular bundles: In order to study the vascular bundles of the stem four plants of each treatment were collected at 44 DAP, from which stem samples of the first and fourth internodes were taken. They were cut with a razor and 2 pieces of approximately 2.5 cm in length of each plant were obtained, thus having a total of 8 samples per treatment. The stems were fixed with formalin-acetic-alcohol (FAA) mixture (40% formaldehyde 5 mL, glacial acetic acid 5 mL, 70% alcohol 90 mL) and were subsequently embedded in histological paraffin sections, made on a microtome (American Optical 820) for sectioning these blocks with a thickness of 18 µ. The sections were stained with safranin 1% and Fast Green 0.05% (Cañizares *et al.*, 2005; Hernández, 1990).

From each plant, cuts from the first and fourth internodes were photographed using a compound microscope (Carl Zeiss) with digital camera (Pixera Winder Pro). Area measurements were taken of the vascular bundle (AVB), and xylem vessels (AXV). Also were checked the widths (WVB) and lengths (LVB) of the middle part of 4 vascular bundles taken randomly, measuring the vascular bundle full xylem and phloem viewed at magnification 5X. In addition, the average lumen area of the xylem vessels (LAX) was measured in five small-sized, five medium-sized and five large-sized xylem vessels of four vascular bundles in each plant. The number of xylem vessels (NXV) in four vascular bundles in four different plants per treatment was counted. The measurements were carried out using the software AxionVision Rel. 4.8.

Analysis of mineral content: The mineral content was determined from samples collected 81 DAP in four plants from each treatment. The leaves were placed in a paper bag and dried in a drying oven (Riosa H-48) at 65 °C for 72 h, and then ground in a mortar. This material was digested using a solution of perchloric acid and nitric acid in a 1:3 ratio. The contents of K, Ca, Mg, Na, Fe, Mn, Cu, and Z were determined by an atomic absorption spectrophotometer (Varian AA-1275) (Fick *et al.*, 1976). For the determination of total N the micro Kjelhdal method (AOAC, 1990) was used whereas for P the colorimetric method (AOAC, 1980) was used.

Statistical analysis: The experimental design used for the morphological analysis was a randomized block. Analysis of variance (ANOVA) and least significant difference (LSD) tests ($\alpha = 0.05$) were performed. For the variables related to stomatal characters, anatomy of the vascular bundles of the stem, and mineral content, a completely randomized layout was employed, with analysis of variance (ANOVA) and LSD tests ($\alpha = 0.05$).

Results and discussion

Morphological analysis of seedlings: The morphological parameters evaluated showed statistical differences following exposure of seed to UV-B. Fresh weight in control plants was less than those of irradiated plants, the latter showing a greater height and larger leaflets, as is clear from the fact that the control had a higher number of leaves, but lower leaf area (Table 1). Similarly, treatment with doses of 99 and 297 mJ cm^{-2} exceeded the control in dry weight, leaf area, and stem diameter. These results differ from those reported by Zuk *et al.* (2003), who indicated a negative impact of irradiation with UV-B on the fresh weight and height of seedlings of *Avena fatua*. The difference may be explained by the fact that the aforementioned researchers irradiated the seedlings while in the present study the seeds were irradiated.

Figure 1 shows the increase in fresh weight by considering the different samples. The positive results of the irradiation of seed weight on seedling in the first sampling, and no differences in subsequent samplings, are in contrast to those reported by Shaukat *et al.* (2013), when they irradiated hydrated seeds of *Vigna mungo* (L.) Hepper with UV-B (centered on 280-312 nm) for 10, 20, 30 and 40 minutes, achieving reduction in fresh weight of radicle and shoot of seedlings. We assume that the hydration of the seeds changes their response to the irradiation, being similar to the negative response described by Zuk *et al.* (2003) in irradiated seedlings.

With respect to leaf area no differences among treatments was detected (Table 1). Dai *et al.* (1995) reported no changes in leaf area when seedlings of *Oryza sativa* L. were irradiated for four weeks with 1.94 W m^{-2} of UV-B. The authors noted that it was possible that the irradiance used was not sufficient to induce an alteration in leaf area.

Stomatal study: Analysis of variance showed no differences in any of the evaluated variables in abaxial leaf surface. However, significant differences ($P \leq 0.005$) for stomatal index variables and length of stomata on the adaxial surface were detected (Table 2). The adaxial stomatal index was greater with 99 mJ cm^{-2} (Fig. 2), with no difference effects for all other variables. Similar results were reported by Dai *et al.* (1995) with rice seedlings. These authors explain that the effect of only modifications in the adaxial side may be because this area of the plant epidermis is more susceptible to the effects of UV-B irradiation.

Wargent *et al.* (2009) assessed some aspects of cell development in leaves of *Arabidopsis thaliana* irradiated with UV-B at doses in the range 0 and 25 kJ m^{-2} d^{-1}. They observed that upon irradiation of plants the number of epidermal cells decreased, increasing the area of the external surface of the cells. It is possible that this reaction appears in our results on exposing the seed to a dose of 198 mJ/cm^{-2} where SI is less, but greater SL and SW were observed. This effect may be the phenotypic expression of the receptors of the seed and the issuing a signaling response subsequent to the irradiation with UV-B.

Histological analysis of the vascular bundles of the stem: There were significant effects in samples obtained from the first internode (Table 3) with respect to variables of AXV, LHV, and NXV. The increase in the number of xylem vessels was associated with environmental stress (Schmitz *et al.*, 2006). However in our results a direct relationship between dose and number of vessels was not observed, rather the maximum response was observed with intermediate dose and the response decreased in the high dose in both internodes.

The data obtained from the first internode of the plant, showed that treatment with 99 and 198 mJ/cm^{-2} produced an increase in the area of xylem besides showing greater vascular bundle width (transverse length) (Table 3 and Fig. 3). Treatment with 297 mJ cm^{-2} resulted in lower values of xylem area, length and width of vascular bundle, and fewer xylem vessels. Ramos *et al.* (2004) also found that high concentrations of NaCl (18 mS/cm) in

Table 1. Effect of irradiation of melon seeds with UV-B on plant morphological variables

Treatment mJ/cm^{-2}	FW (g)	DW (g)	LA (cm^{-2})	TNL	pH	ADS (mm)
0	27.50b	3.27b	400.88b	12.28a	37.88c	3.88b
99	34.34a	4.33a	469.17a	12.22a	42.18bc	4.22a
198	30.82ab	3.75b	440.59ab	10.78b	45.44ab	4.07ab
297	30.77ab	3.72b	458.88a	11.83ab	48.46a	4.11ab
$\bar{X}$	30.86	3.77	442.38	11.78	43.49	4.07
LSD	3.72	0.50	46.40	1.31	5.36	0.33

Means with the same label in each column are equal according to the LSD test ($P \leq 0.05$). FW = Fresh weight; DW = dry weight; LA = Leaf area; TNL = total number of leaves; PH = Plant height; ADS = average diameter of the stem.

Table 2. Mean values of variables of foliar epidermal morphology of plants grown from seeds irradiated with different doses of UV-B.

Treatment mJ/cm^{-2}	Abaxial[§]			Adaxial			
	SD	SL (µm)	SW (µm)	SD	SI	SL (µm)	SW (µm)
0	500.00a	19.99a	13.57a	256.9a	25.83b	21.86b	14.63a
99	541.67a	21.97a	14.62a	312.50a	39.33a	20.38c	13.77a
198	416.67a	21.10a	14.09a	256.94a	27.83b	23.39a	17.90a
297	486.11a	20.74a	14.53a	229.17a	29.33b	21.56bc	12.91a
$\bar{X}$	486.11	20.95	14.21	263.89	30.58	21.78	14.80
LSD	127.61	2.03	1.24	87.40	8.32	1.41	5.26

[§]The abaxial SI could not be determined, because the impression did not permit a clear determination of the number of epidermal cells. Means with the same label in each column are equal according to the LSD test ($P \leq 0.05$). SD = stomatal density; SL = stomatal length; SW = stomatal width; SI = stomatal index.

Pappophorum philippianum plants induced a diminished diameter of xylem vessels in the root. This may represent a strategy for dealing with stress, possibly to reduce the possibility of embolism in the xylem under conditions of water deficit caused by high salinity or high irradiance.

The analysis of variance of the measurements made in the fourth internode samples indicated that there were highly significant differences for variables of xylem area, vascular bundle width and changes in the area of the lumen xylem vessels, plus significant differences in the number of xylem vessels.

In the fourth internode the dose of 99 mJ cm^{-2} produced an increase in the AVB, AXV and NXV (Fig. 4). These results differ from those reported by Torres-Boeger and Poulson (2006), who found no significant differences in structural vascular bundles of leaves from *Arabidopsis thaliana* (L.) using a dose of 6 kJ m^{-2} d^{-1}, which is 6.06 times the dose of 99 mJ cm^{-2} that we used. In our study, treatment without irradiation resulted in overall lower values for the vascular bundle area, area of the lumen xylem vessels, and vascular bundle width. The fact that the irradiation of the seed causes this adjustment response in the vasculature of both the first and fourth internodes of seedlings may suggest an effect of trans-developmental signalling as described by Magliano and Casal (2004).

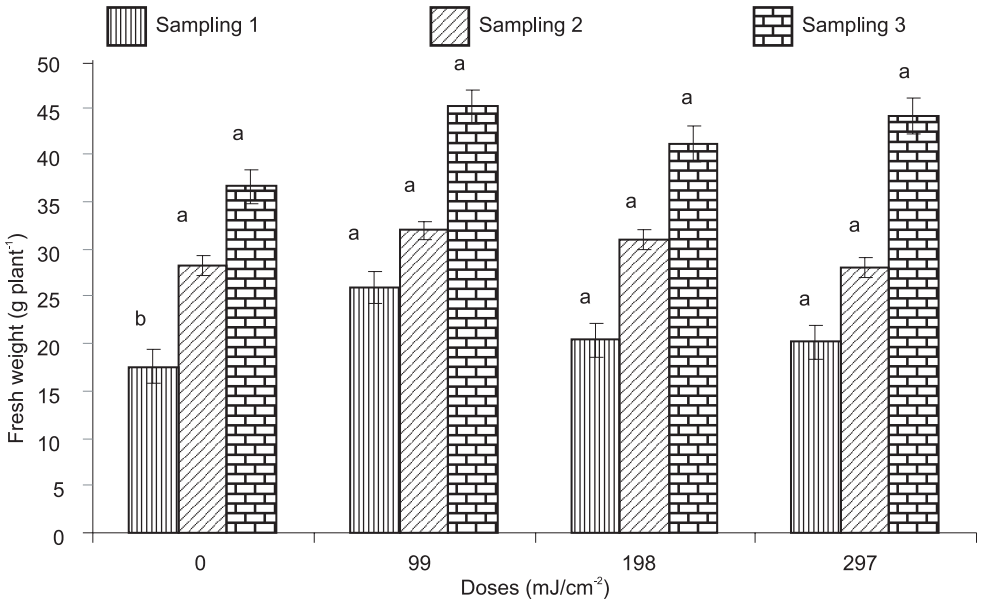

Fig. 1. Fresh biomass of melon plants grown from seeds irradiated with different doses of UV-B; results from three samples. Bars indicate standard error range.

Mineral content: There were significant effects ($P \leq 0.05$) in foliar concentrations of P, Ca and Na (Table 4). However N, K, Mg, Cu, Zn, Fe and Mn contents were not affected by irradiation dose. The differences in mineral concentration were also reported by Yao *et al.* (2013) when irradiating wheat seedlings with doses of 2.54 kJ m^{-2} d^{-1} and noting that the contents of N, Fe and Cu in the grain were lower in irradiated plants. Correia

Table 3. Characteristics of the histological structure of the vascular bundles of the stem of plants grown from seeds irradiated with different doses of UV-B. Samples were obtained from the first and fourth internodes

Treatment mJ/cm^{-2}	AVB (μm^2)	AXV (μm^2)	LAX (μm^2)	LHV (μm)	WVB (μm)	NXV
	First internode					
0	774,030.00a	148,700.00ab	2,167a	1,069.59ab	485.63ab	74.00ab
99	456,313.00a	161,493.00a	2,326a	1,140.09a	478.31ab	73.06bc
198	550,966.00a	178,458.00a	2,277a	1,125.35a	524.03a	85.50a
297	426,468.00a	114,841.00b	6,785a	964.54b	428.99b	62.00c
$\bar{X}$	551,944.20	150,873.40	3,388.58	1,074.89	479.24	73.64
LSD	451,400	35,265	6,528	133.93	75.61	11.655
	Fourth internode					
0	226,605.00b	55,558.00b	927.75b	737.79a	276.45b	29.625ab
99	285,462.00a	83,426.00a	1,226.75a	788.10a	341.90a	37.750a
198	261,188.00ab	60,314.00b	1,266.09a	786.26a	330.59a	30.313ab
297	258,873.00ab	51,168.00b	1,208.92a	726.91a	312.60ab	25.563b
$\bar{X}$	258,032.20	62,616.33	1,157.38	759.77	315.38	30.81
LSD	49,501.00	15,606.00	147.51	96.512	38.023	8.677

Means with the same letter within each column of factors are equal according to the LSD test ($P \leq 0.05$). AVB = area of vascular bundle; AXV = area of xylem vessels; LAX = average lumen area of xylem vessels; LHV = length of the vascular bundle; WVB = width of vascular bundle; NXV = number of xylem vessels.

Table 4. Averaged foliar mineral concentrations in melon plants grown from seeds irradiated with different doses of UV-B

Treatment mJ/ cm^{-2}	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
0	0.10a	0.26a	3.27a	4.40a	0.18a	0.24a	6.67a	13.33a	100.33a	11.33a
99	0.11a	0.22ab	3.25a	3.54b	0.17a	0.15b	6.00a	3.33a	101.00a	14.33a
198	0.10a	0.21ab	3.31a	4.09ab	0.08a	0.15b	5.33a	9.33a	101.00a	13.67a
297	0.09a	0.18b	3.13a	4.34a	0.03a	0.12b	5.33a	9.67a	86.00a	16.33a
$\bar{X}$	0.10	0.22	3.24	4.09	0.12	0.16	5.83	8.92	96.42	13.92
LSD	0.02	0.07	0.77	0.68	0.17	0.09	2.31	12.68	49.44	13.42

Means with the same letter within each column of factors are equal according to the LSD test ($P \leq 0.05$).

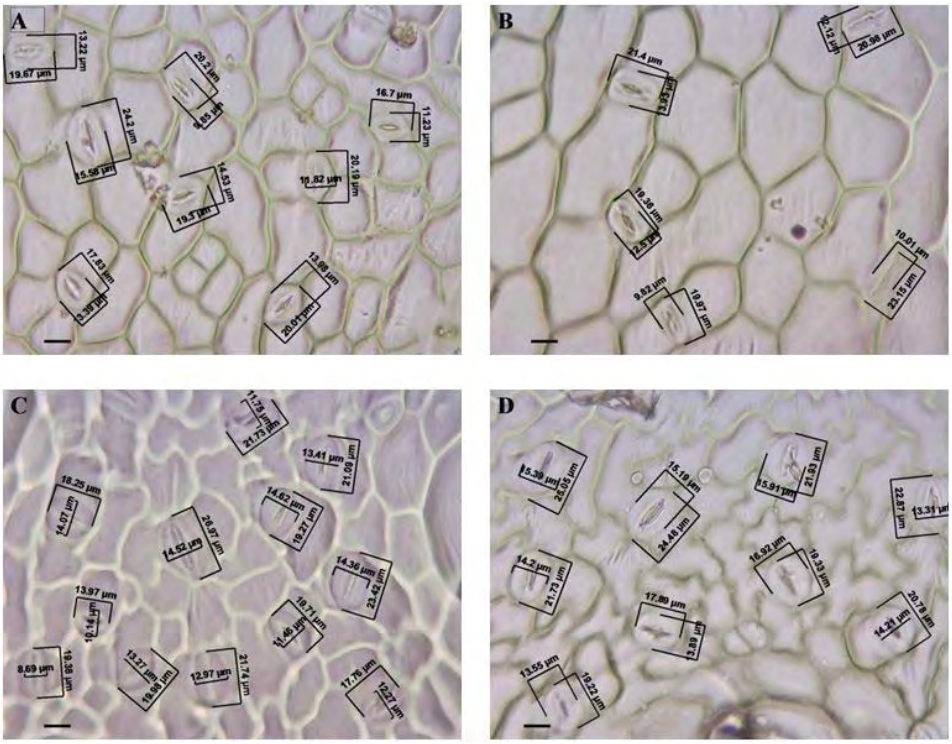

Fig. 2. Adaxial epidermal impressions of plants grown from seeds irradiated with UV-B dose of 99 mJ cm^{-2} (A) and 297 mJ cm^{-2} (B). Epidermal abaxial leaf imprints from plants grown from seed exposed to UV-B dose of 99 mJ cm^{-2} (C) and 297 mJ cm^{-2} (D). 40X objective. Bar = 10 µm.

et al. (2012) reported that irradiation with UV-B increased the content of Zn and Cu and decreased concentration of Mn in organs of mature corn. Previous reports showed that different plant species respond differently to the UV-B exposure. In our study seed irradiation also modified the mineral content in the leaves, indicating similarities in responses to irradiation in seeds and seedlings.

Conclusion

Exposure of melon seeds to UV -B radiation induced morphological changes reflected as increased plant height and weight, more leaf area and stem diameter. Additionally histological changes were observed as increased number and areas of conducting tissues. The content of P, Ca and Na in leaves decreased in irradiated treatments compared to the control.

Acknowledgement

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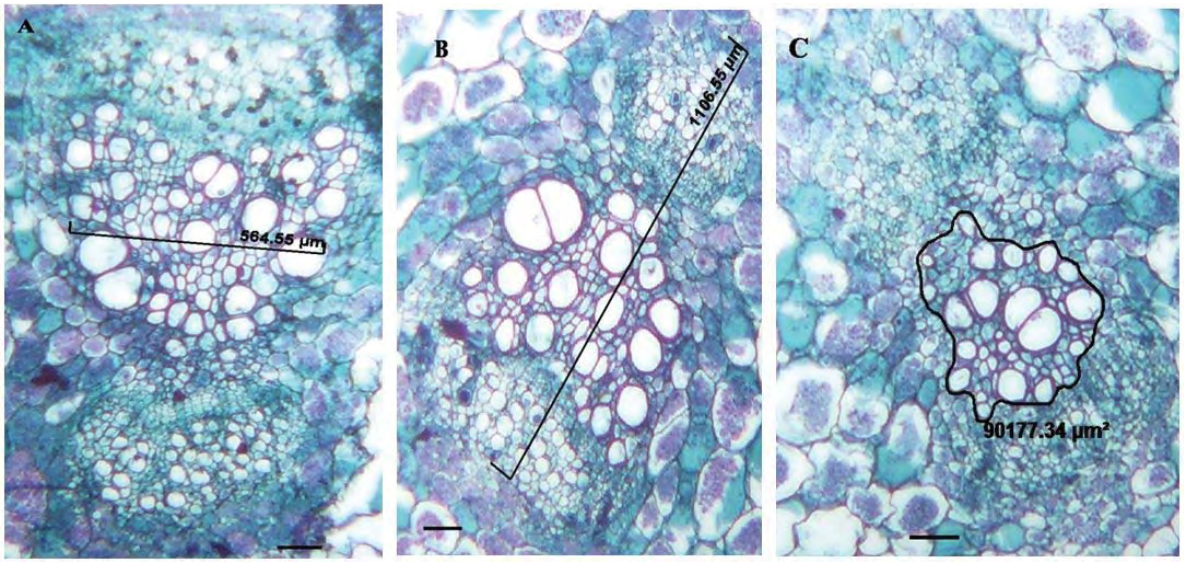

Fig. 3. Vascular bundles of the first internode of plants grown from seeds irradiated with UV-B. A) Dose 198 mJ cm^{-2}. B and C) Dose 297 mJ cm^{-2}. 5X objective. Bar 100 µm.

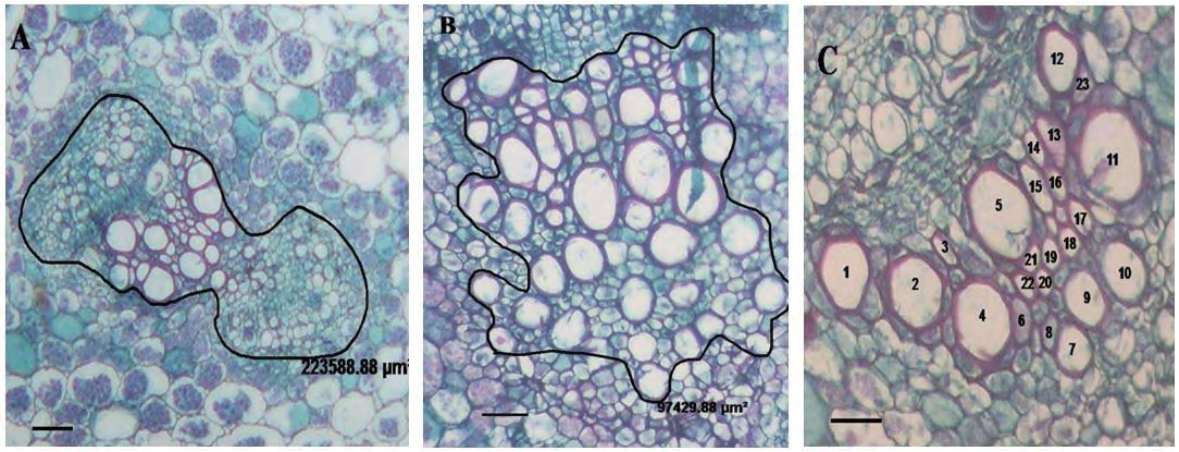

Fig. 4. Vascular bundles of the fourth internode of plants obtained from UV-B irradiated seeds. A) Dose 0 mJ cm^{-2}. Bar 100 µm, 5X objective. B) Dose 99 mJ cm^{-2}. Bar 50 µm, 10X objective. C) Number of xylem vessels treated with 297 mJ cm^{-2}. Bar 50 µm, 10X objective.

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