

UV-C IRRADIATION EFFECTS ON YOUNG TOMATO PLANTS: PRELIMINARY RESULTS

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Abstract

Ultraviolet-C radiation (UV-C = 100-280 nm) is strongly affected by ozone levels, so that the amount of this radiation reaching the Earth's surface is extremely low. In the future, UV-C radiation is expected to increase as the result of stratospheric ozone depletion due to atmospheric pollution, with strong negative effects on economically important crops. To assess the effect of UV-C irradiation on young tomato plants (*Lycopersicon esculentum* Mill.; cv Cuore di bue), an experiment was conducted in controlled conditions, using a black chamber equipped with an UV-C lamp. Tomato plants were divided in four groups on the basis of UV-C irradiation time (10, 30, 60, and 120 min), and non-irradiated plants were kept as controls. Plant gas exchange, leaf color and morphologic traits were recorded before and after UV-C treatments. The photosynthetic apparatus was influenced by UV-C treatment, as shown by the strong increase in intracellular CO₂, particularly evident in the 120 min treatment (338 $\mu\text{L L}^{-1}$). This was due both to the stomatal and non-stomatal inhibition of the assimilation activity due to UV-C exposure. In 10 and 30-min treated plants, leaf color, measured immediately after the irradiation, did not statistically change, whereas the 60 and 120-min treatments were characterized by a deep senescence with a general stem and leaf yellowing. The results demonstrate that high UV-C doses determined irreversible damages, both at physiological and morphological levels, that lead plants to death, whereas lower irradiations (up to 60 min) allowed plants to partially recovery their normal physiological status.

Introduction

Ultraviolet (UV) radiation is a component of the solar light that naturally reaches the Earth and, depending on its wavelength, it can be divided in three different ranges: UV-A (315-390 nm), UV-B (280-315 nm) and UV-C (100-280 nm). Among these, UV-C is the radiation with the lower wavelength, or rather with the higher associated energy (Katerova *et al.*, 2009). It is known that UV-C has an acute germicidal action on microorganisms in water, on surfaces and in air (Siddiqui *et al.*, 2011). Moreover, it can induce oxidative results and genetic mutations in plants, that in turn have strong negative effects on plant morphology, flowering, pollination, transpiration and photosynthesis (Murali & Saxe, 1984; Booj-James *et al.*, 2000).

Fortunately, UV-C is strongly affected by the ozone layer in the stratosphere, so that the amount of this radiation reaching the Earth's surface, except for high mountains, is extremely low (Häder *et al.*, 2007). Nevertheless, in the last decades, human activities have produced dangerous chemicals, such as chlorofluorocarbons (CFC), which have been released into the atmosphere and have contributed to the depletion of ozone protective layer. Therefore, in the future UV-C radiation could increase as the result of stratospheric ozone depletion due to atmospheric pollution. In fact, the stratospheric ozone layer reduction highlights the ecological implication of increasing UV-C radiation on natural ecosystems and on agricultural productions (Kataria & Guruprasad, 2012). In this scenario, characterized by an increasing trend of UV-C flux on Earth, the study of the effect of this radiation on some crops becomes important.

This work represents a first study on the possible implications of UV-C irradiation on tomato, one of the most economically important crops of the Mediterranean Area. In order to deepen the ecophysiological response of this species to a changing climate.

Materials and Methods

To assess the effect of UV-C irradiation on tomato plants (*Lycopersicon esculentum* Mill. cv 'Cuore di bue'), an experiment was conducted in controlled conditions, using an irradiation chamber (0.82 x 0.52 x 0.68 m) coated with aluminum sheets and equipped with an UV-C lamp (Helios Italquartz, Milan, Italy; model G15T8; characteristics: 15 W, 3.8 J m⁻² at 1 m of distance) (Fig. 1A).

Three seeds were sowed in polypropylene plastic pots having a conical trunk shape (12.0 cm high, 9.5 cm lower diameter, 14.0 cm upper diameter) and a volume of 1.32 cm³. Pots were filled with a substrate (organic compost) additionated of an inorganic mineral base of perlite. Samples of the substrate were air dried at room temperature, and ground to pass a 2.0 mm mesh for the determination of physical-chemical parameters. The pH values were measured using a pH meter (model Jenway 4310; Barloworld Scientific T/As Jenway, Dunmow, Essex, England) on the substrate extract obtained by shaking the soil with double distilled water at 1:2.5 (w/v) substrate:water ratio. Substrate physico-chemical properties were the following: moisture content = 60.0% (w/w), pH = 6.58, total carbon = 34.0% (w/w), total organic nitrogen = 2.14% (w/w), C/N = 14.17.

After 10 days from germination, two seedlings were removed and only the best one was kept alive to undergone to UV-C irradiation. For the whole experiment, except for the irradiation times, plants were maintained under controlled conditions at a constant temperature of 20°C with a 16 h photoperiod and a photosynthetically active radiation (PAR) of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$. On the basis of UV-C irradiation time (0, 10, 30, 60 and 120 min), tomato plants were divided in five groups (treatments) of eight plants ($n = 8$) (Fig. 1B).

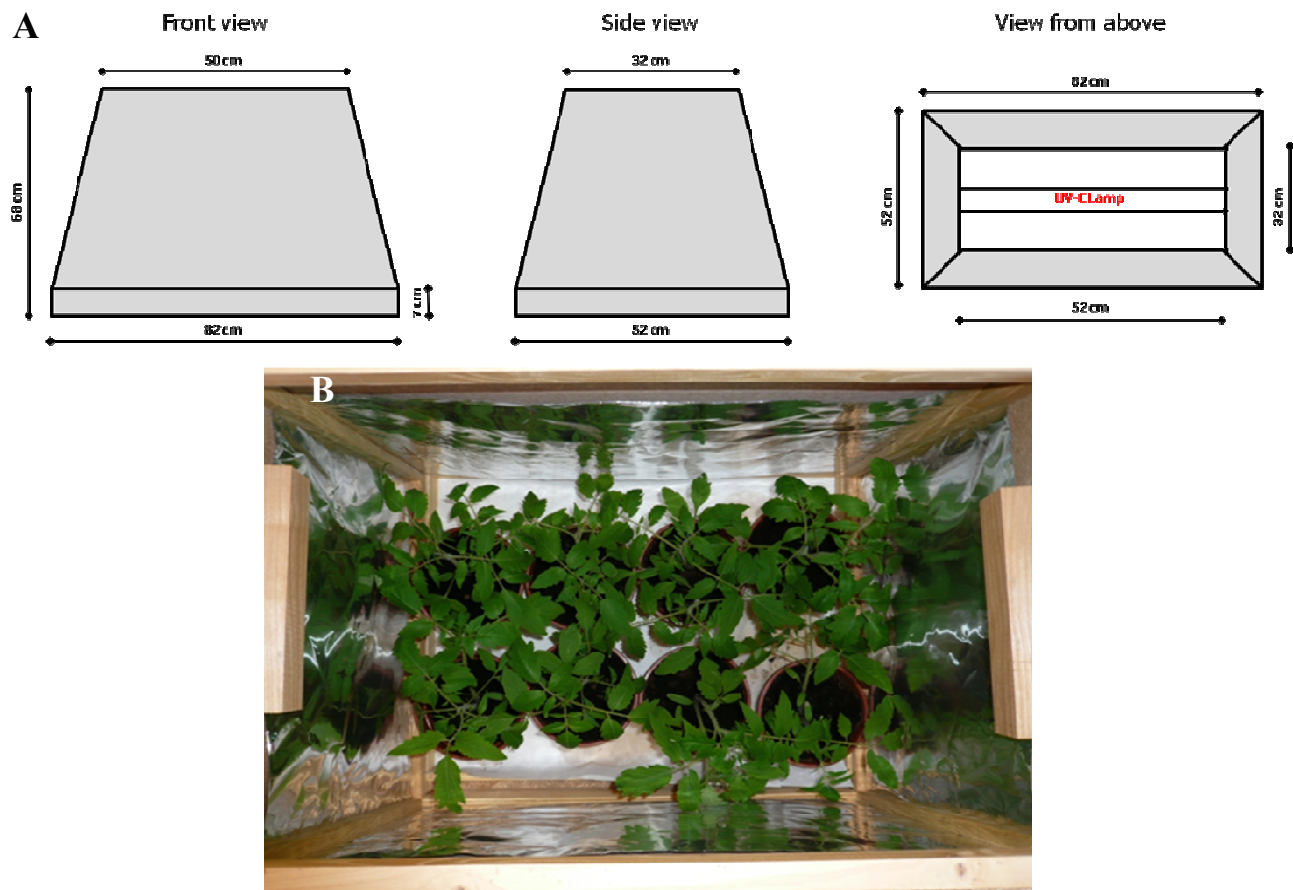


Fig. 1. (A) Characteristics of the irradiation chamber equipped with the UV-C lamp. (B) Tomato plants in the irradiation chamber.

To analyse the possible photosynthetic activity changes induced by exposure to UV-C, instantaneous gas exchange measurements were carried out on tomato plants immediately before the UV-C treatments and 2 h after the treatments (between 10:00 and 12:00 a.m.). Net assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and intracellular CO_2 concentration (C_i) were simultaneously determined using the topmost fully expanded leaf. These measurements were carried out in each of the five treatments by a portable open-gas exchange system (model LiCor-6400; Li Corporation, Lincoln, NE, USA) incorporating infrared CO_2 and water vapour analyzers, using a saturating red light source at $1800 \mu\text{mol m}^{-2} \text{s}^{-2}$ photosynthetic photon flux density (PPFD). The cuvette temperature of this instrument was held at the mean air temperature at noon on the measurement day, and the relative humidity within the cuvette was maintained at 20%.

In order to determinate plants colour change caused by UV-C light, colorimetric leaves measurements have been carried out using a Minolta CR-400 Chroma Meter (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor. Measurements were conducted immediately before the UV-C treatments and 2 h after the treatments. According to Sugar & Dussi (1998), colour change has been evaluated in the CIELAB space system, measuring the colour chromatic coordinates L^* , a^* , and b^* .

Inter-knot distance, plant height and shoot diameter were measured four weeks after the UV-C treatment. Immediately after this time, the root system of each plant

was cleaned and kept in an isotonic water solution to avoid drying. The fresh roots were mounted on slides and observed at different magnifications using a compound optical microscope (model Eclipse 80i; Nikon, Tokyo, Japan) under transmitted light, and then photographed (Digital Camera DS-Fi1; Nikon, Tokyo, Japan). Images were analyzed (NIS-Elements Imaging Software; Nikon, Tokyo, Japan) to compare root morphology and evaluate descriptive parameters. Fresh and dry weights were measured for shoots and roots. The root/shoot ratio and the length per unit root mass (LRM) were so calculated.

Statistical analysis was performed by analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC, USA). Student-Newman-Keuls (SNK) test was performed for the comparison of means at $p \leq 0.05$.

Results and Discussion

There are differences between species as regards to UV radiation sensitivity (Teramura, 1983) but actually there is no information on the effects of UV-C on tomato plants, that is instead considered an important crop in the Mediterranean environments (Albacete *et al.*, 2008).

Net assimilation (A) deeply decreased in tomato plants exposed to UV-C for 60 min ($7.31 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and 120 min ($4.33 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) respect to the control ($16.83 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) (Fig. 2A). Significant decreases in E and g_s were also observed (Fig. 2AB). This latter parameter changed from $0.20 \text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ in control plants to $0.12 \text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ in

the 120-min treatment. Tomato photosynthetic apparatus was affected by UV-C treatment, as shown by the strong increase in C_i , up to $338 \mu\text{L L}^{-1}$, particularly evident in the 120-min treatment. This was likely due both to the stomatal (g_s) and non-stomatal inhibition (A) of the assimilation activity (Fig. 2). The strong effect of UV-C on photosynthesis, particularly on 120-min treatment, reduced the assimilate availability, necessary for plant growth. Indeed, several authors demonstrated that UV-C provokes reduction of carbohydrate content by inactivation of the Rubisco activity in Calvin cycle (Rahimzadeh *et al.*, 2011).

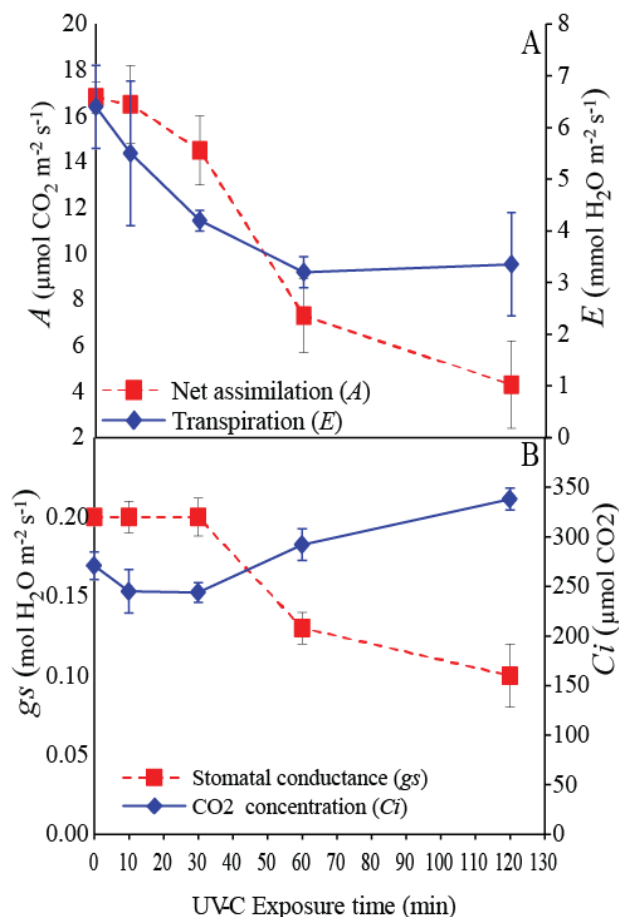


Fig. 2. (A) Trends of net assimilation (A) and transpiration (E) in leaves of tomato plants exposed to UV-C radiation for 0, 10, 30 and 60 minutes, measured two hours after the UV-C treatment. (B) Trends of stomatal conductance (g_s) and intracellular CO_2 concentration (C_i) in leaves of tomato plants exposed to UV-C radiation for 0, 10, 30 and 60 minutes, measured two hours after the UV-C treatment. Data represents means ($n = 8$) \pm standard error.

According to Teramura & Sullivan (1994), the increase of the exposition time to UV-C light caused a photo-inhibition of the assimilation activity, that could be attributed to phytohormone changes, inhibition of essential enzymatic reactions, and decrease in the uptake and partitioning of nutrients.

Najeeb *et al.* (2011) demonstrated that the decrease in photosynthetic performance after UV-C irradiation could be due to the reduction of cell and chloroplast size, accompanied by the disruption of thylakoids and the accumulation of plastoglobuli in chloroplasts.

Biometrical measurements, done 4 weeks after the UV-C exposure, pointed out that a decrease of inter-knot distance was occurred in treated plants (Table 1), in accordance to Lercari *et al.* (2003) and Bertram & Lercari (1996). In fact, this value was equal to 6.0, 5.4, 4.7 and 3.6 cm for the control, 10, 30 and 60-min UV-C exposure, respectively. The same trend was recorded for plants height and stem diameter, ranging respectively from 96.4 cm and 5.8 mm in the control to 34.8 cm and 3.8 mm in the 60-min UV-C exposed plants. No data were recorded for plants exposed for 120 min to UV-C since they died because of the damages caused by irradiation. Similar trends were found by Najeeb *et al.* (2011) in UV-C irradiated *Juncus effusus* plants, that showed a significant reduction in plant growth and biomass. As previous authors reported, in particular for root thickness (Hosseini Sarghein *et al.*, 2011), no changes were observed for root morpho-anatomy after UV-C treatments, if compared to control plants (Fig. 3), supporting the previous hypothesis and excluding a direct action of UV-C on the hypogeeal part.

Colorimetric characterization pointed out that, at 2h from the irradiation, the leaves of UV-C treated plants were characterized by a general color change (Table 2). No differences in colour among plant groups were detected before the UV-C treatments. In particular, in the CIE Lab color space, all the treated theses showed similar values of brightness (L^* parameters), resulting statistically lower respect to control. Regarding a^* (green-red axis) and b^* (blue-yellow axis) parameters, the values of 10, 30 and 60-min UV-C treatments did not differ statistically, but both were statistically lower in the 120-min treatment. Besides, the untreated tomato plants reached the highest values. The same trend was observed for leaf chroma and Hue angle parameters. This colorimetric response was also found by Rozema *et al.* (1997), that observed a reduction in pigment levels due to increasing exposition time to UV-C radiation.

Table 1. Biometrical properties of tomato plants exposed to UV-C radiation for 0, 10, 30 and 60 minutes, measured four weeks after the UV-C treatment. Means ($n = 8$) followed by the same letters on the same column are not significantly different ($p \leq 0.05$), according to SNK Test.

UV-C exposure time (min)	Inter-knot distance (cm)	Plant height (cm)	Stem diameter (mm)
0 (control)	6.0 a	96.4 a	5.8 a
10	5.4 b	88.0 b	5.5 a
30	4.7 c	73.1 c	4.4 b
60	3.6 d	34.8 d	3.8 c

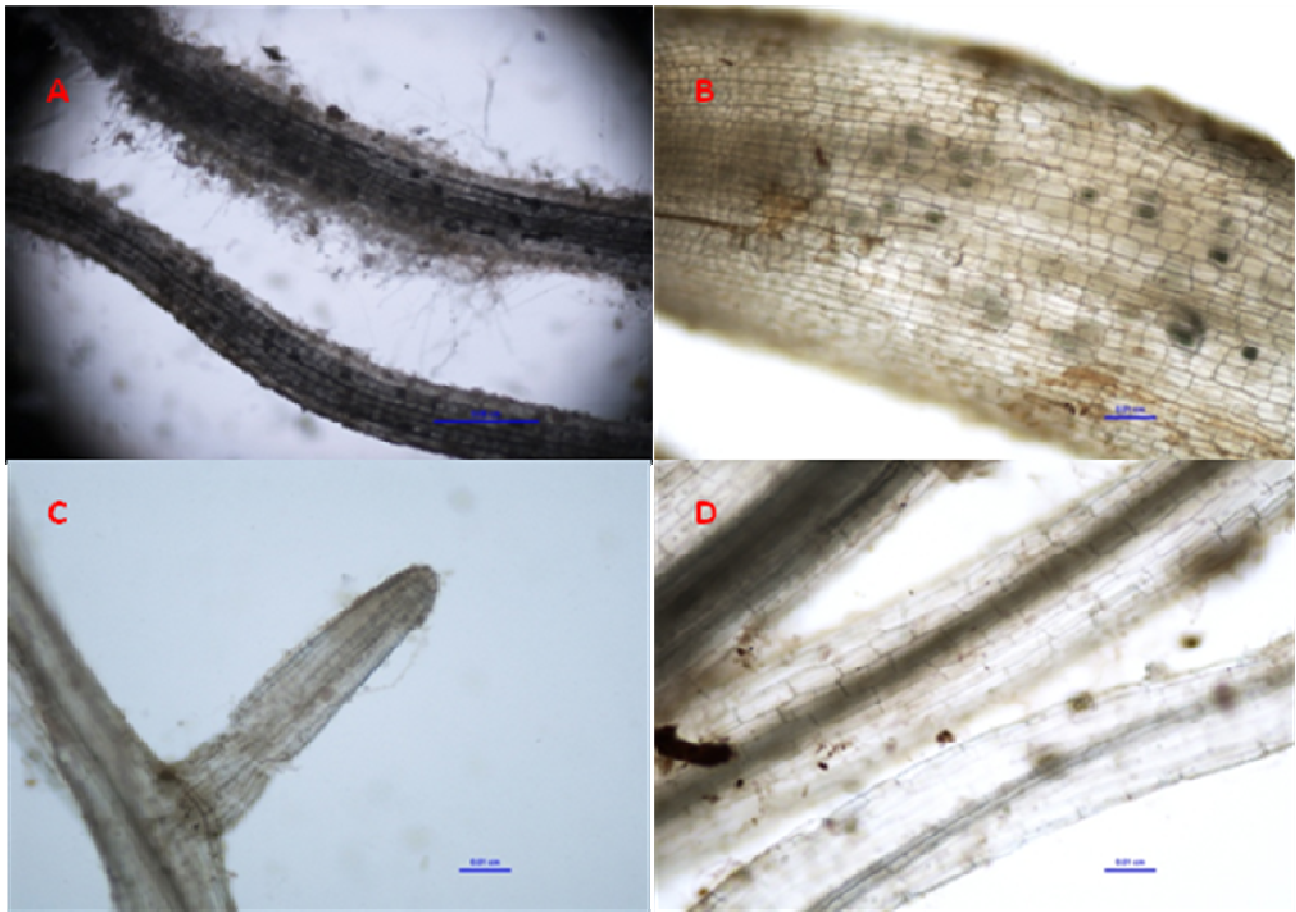


Fig. 3. Root morphology tomato plants exposed to UV-C radiation for 0 (A), 10 (B), 20 (C), 30 and 120 minutes, measured four weeks after the UV-C treatment. The fresh roots were observed at 40x (A) and 100x (B, C, D). Scale bars: 0.05 cm (A); 0.01 cm (B, C, D).

Table 2. Colorimetric characterization of leaves of tomato plants exposed to UV-C radiation for 0, 10, 30 and 60 minutes, measured two hours after the UV-C treatment. Statistic like in Table 1.

UV-C exposure time (min)	L*	a*	b*	Chroma (C)	Hue angle H
0 (control)	44.3 a	-23.3 c	36.5 a	43.3 a	-1.00 b
10	38.7 b	-17.6 a	25.9 c	31.3 c	-0.97 a
30	39.5 b	-17.7 a	25.9 c	31.4 c	-0.97 a
60	40.4 b	-19.6 b	29.5 b	35.5 b	-0.99 ab

Conclusions

This study demonstrated that an exposition of tomato plants to enhanced levels of UV-C radiation can determine important alterations in their growth. Indeed, high UV-C doses (60 and 120 min) determined irreversible damages both at plant physiological and morphological levels, in particular against leaves and shoots, leading the whole plant to death. By contrary, lower irradiations (up to 30 min) allowed plants to partially maintain their normal physiological status. Physiological and structural alterations were observed in shoots of tomato UV-treated plants, that also exhibited a significant colour change, probably due to the photo-oxidation chlorophylls and other pigments. Next steps foresee physiological, genetic and molecular investigations and studies on the possible tolerance mechanisms of tomato plants to face UV-C radiation.

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