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Chapter 10

Growth Patterns of Tomato Plants Subjected to Two Non-conventional Abiotic Stresses: UV-C Irradiations and Electric Fields

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1 Introduction

Capabilities for environmental stress perception, signaling, and response of plant species against a broad range of abiotic stressors have a great range of variability.

Ultraviolet (UV) radiation is a component of the solar light and it represents something like 8–9 % of the radiation that naturally reaches the Earth (Frederick 1993). Depending on its wavelength, UV can be divided into three different ranges: UV-A (315–390 nm), UV-B (280–315 nm), and UV-C (100–280 nm). Among them, UV-A represents approximately 6.3 % of the incoming solar radiation and is the least hazardous part of UV radiation; UV-B, even if represents just 1.5 % of the total spectrum, is of particular interest because it can cause a multiplicity of detrimental effects in plants (Hollósy 2002; Jansen and Bornman 2012). Between UV radiations, UV-C is the one with the lower wavelength, or rather with the higher associated energy (Katerova et al. 2009; Nawkar et al. 2013), and it is well known that UV-C has an acute germicidal action on microorganisms in water, on surfaces, and in air (Siddiqui et al. 2011). Indeed, 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 and Saxe 1984; Booij-James et al. 2000).

The stratospheric ozone layer efficiently filters out most of the detrimental UV radiation shorter than 280 nm but it decreases rapidly at wavelength longer of 280 nm reaching zero at about 330 nm (Hollósy 2002; Nawkar et al. 2013). Therefore, UV-B is not completely shielded by the ozone layer and the UV-A are virtually unaffected by the ozone layer. 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).

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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. Indeed, the stratospheric ozone layer reduction highlights the ecological implication of increasing UV-B and UV-C radiations on natural ecosystems and on agricultural productions (Jansen and Bornman 2012; Kataria and Guruprasad 2012). This increasingly worsen condition has led researchers to understand the effects of UV radiation on plants and other organisms. In this view, the primary alarm over ozone depletion is the potential impact on human health and ecosystems due to increased UV exposure. This enhanced exposure, especially to UV-C and UV-B, is potentially detrimental to all living beings. In particular, it can be very harmful to plants due to their obligatory requirement for sunlight for survival and their inability to move. It is known that increased UV exposure has been shown to alter the biotic relationships of higher plants, as demonstrated by the changes in plant disease susceptibility and the balance of competition between plant species. The most frequent UV exposure effects on plants are a reduced growth (plant height, dry weight, leaf area, etc.), photosynthetic activity, and flowering (Teramura et al. 1991; Santos et al. 2004; Jansen and Bornman 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. There are differences between species as regards to UV radiation sensitivity (Teramura 1983) but actually there is very few information on the effects of UV-C on tomato plants, which is instead considered an important crop in the Mediterranean environments (Albacete et al. 2008).

UV-C irradiation on tomato fruit is studied as postharvest treatment for its effects to delay fungal growth or senescence (Liu et al. 2009), to increase ascorbic acid and total phenolic compounds (Jagadeesh et al. 2009), and to increase lycopene content in tomato fruit (Liu et al. 2009). Tomato fruits exposed to a low level of UV-C (3.7 kJ m^{-2}) showed a delay in fruit ripening and senescence, and an increase of photooxidant products, to which plants react by improving their defense antioxidant mechanisms (Ait Barka 2001; Liu et al. 2009). While the impact of UV-C treatment on tomato photosynthetic characteristics was not studied extensively, in other crops, such as wheat (Li et al. 2007) and pea (Li et al. 2006), negative effects of UV-C radiation on gas exchange were observed. Particularly, UV-C-treated pea seedlings showed a reduced activity of the antioxidant enzymes and an increase of membrane peroxidation, resulting in a lower assimilation activity (Li et al. 2006).

The application of electricity can stimulate the growth of plants to a great extent (Wolverton et al. 2000). This little-known technology, called electro-culture, can accelerate growth rates, increase yields, improve crop quality and plant protection against diseases, insects and frost (Ishikawa and Evans 1990). Electro-culture can also reduce the requirements for fertilizers or pesticides (van West et al. 2002; Wang and Wang 2004). The several approaches to electro-culture include: antennas, static electricity, direct and alternating current, magnetism, radio frequencies,

monochrome and intermittent lighting, and sound. The electricity can be applied to the seeds, plants, soil, water, or nutrients. Particularly, the application of an electric field (EF) can affect directly or indirectly the plants exposed to it, inducing a series of physiological and biochemical responses (Scopa et al. 2009; Berghoefler et al. 2012; Vallverdú-Queralt et al. 2013).

Electric fields (EFs) have been tested in several instances with contradictory results, depending on the strength applied, the substrate in which roots grow, and the plant sensitivity. Several experiments of plants subjected to different types and intensities of EFs have been carried out in liquid media (Wolverton et al. 2000), hydroponic conditions or artificial soil (Nechitailo and Gordeev 2004). Scopa et al. (2009) observed that *Arundo donax* seedlings, exposed to a DC EF of 12.0 V m^{-1} showed a significant increase in growing rate of both shoots and roots. The root meristem architecture (Wawrecki and Zagórska-Marek 2007), as well as the development of lateral roots (Hamada et al. 1992), was proved to be affected by EFs. An EF seems to induce changes in cell membrane potential of the root, although the exact nature of these changes is difficult to predict (Ishikawa and Evans 1990; Berghoefler et al. 2012). Chemiosmotic gradient or/and auxin could play a role in the ultimate establishment of the differential growth pattern that various papers underline (Robinson 1985).

On these basis, this chapter is focused on (a) 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; and (b) the estimation of the effect of a DC EF on developing roots of tomato plants grown in a hydroponic floating system under controlled conditions, in consideration of possible applicative outcomes in plant propagation and cultivation of this important cultivated species.

2 Instrumental Equipment for the Study of the Effects of UV-C and EF in Tomato

2.1 UV-C

In order to assess the effect of UV-C irradiation on tomato plants (*Lycopersicon esculentum* Mill.), the experiments are usually conducted in controlled conditions, using irradiation chambers ($0.82 \times 0.52 \times 0.68 \text{ m}$) coated with aluminum sheets and equipped with an UV-C lamp.

Three seeds are sowed in polypropylene plastic pots filled with a substrate containing an inorganic mineral base of perlite. After few days from germination, seedlings are removed and only the best ones are kept alive to undergo UV-C irradiation. For the whole experiment, except for the irradiation times, plants should be maintained under controlled conditions. On the basis of UV-C irradiation times, plants are divided into different groups.

To analyze the possible photosynthetic activity changes induced by exposure to UV-C, instantaneous gas exchange measurements are carried out on plants before and after the UV-C treatments by a portable open-gas exchange system on the topmost fully expanded leaf. In order to estimate plant color change caused by UV-C radiation, colorimetric leaves measurements are carried out before and after the UV-C treatments using a colorimeter. According to Sugar and Dussi (1998), color changes is evaluated in the CIELAB space system, measuring the color chromatic coordinates L^* , a^* , and b^* .

Inter-knot distance, plant height, and shoot diameters are measured some weeks after the UV-C treatment. Subsequently, the root system of each plant are cleaned and kept in an isotonic water solution to avoid drying. The fresh roots are mounted on slides and observed at different magnifications using a compound optical microscope under transmitted light, and then photographed. Images are analyzed to compare root morphology and evaluate descriptive parameters.

2.2 *Electric Field*

To assess the effect of an electric field on tomato plants (*Lycopersicon esculentum* Mill.), seeds are firstly sterilized in a solution of 5 % (v/v) NaOCl, rinsed with 95 % ethanol, washed with distilled water, and then put in an inorganic mineral base of sterile sand as solid substrate. The experiments are usually realized in a floating polystyrene vessel with 96 holes. Plants are grown hydroponically in a nutritive liquid medium. After few days from germination, some seedlings are removed and only the best ones are kept alive. Seedlings should be maintained under controlled conditions. The photosynthetic light source is usually a specific fluorescent lamp. Solution volumes are maintained constant throughout the experiment.

Two parallel stainless steel plates are placed in the medium, and they work as electrodes. After some days from the germination, when root length ranged from 1 to 2 cm, seedlings are exposed to a DC EF of 12.0 V m^{-1} with a current intensity of 10 mA, according to Scopa et al. (2009). The EF is applied continuously directly by a 50 Hz voltage set-up transformer, and monitored by a digital multimeter. Plants not subjected to the EF, grown under the same conditions reported above in another identical polystyrene vessel, are kept as controls.

After 4–10 weeks from EF application, tomato plants are randomly selected next to the positive electrode, in the central area, and next to the negative electrode. For each position, plants are taken from different holes of the polystyrene vessel. The root system of each plant is cleaned and kept in an isotonic water solution to avoid drying. The fresh roots are mounted on slides and observed at different magnifications using a compound optical microscope under transmitted light and then photographed. Images are analyzed to compare root morphology and evaluate descriptive parameters. Root apical meristems and root branching per plant are evaluated. Root mean diameter is also measured at 0.02 cm from the tip. The root/shoot ratio and the length per unit root mass (LRM) are calculated.

3 Growth Patterns and Physiological Effects of UV-C and EF in Tomato

3.1 UV-C

The increase of the exposition time to UV-C radiation causes 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 (Teramura and Sullivan 1994). 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. Net assimilation (A) deeply decreases in tomato plants exposed to UV-C for 60 and 120 min with respect to the control (Fig. 10.1a).

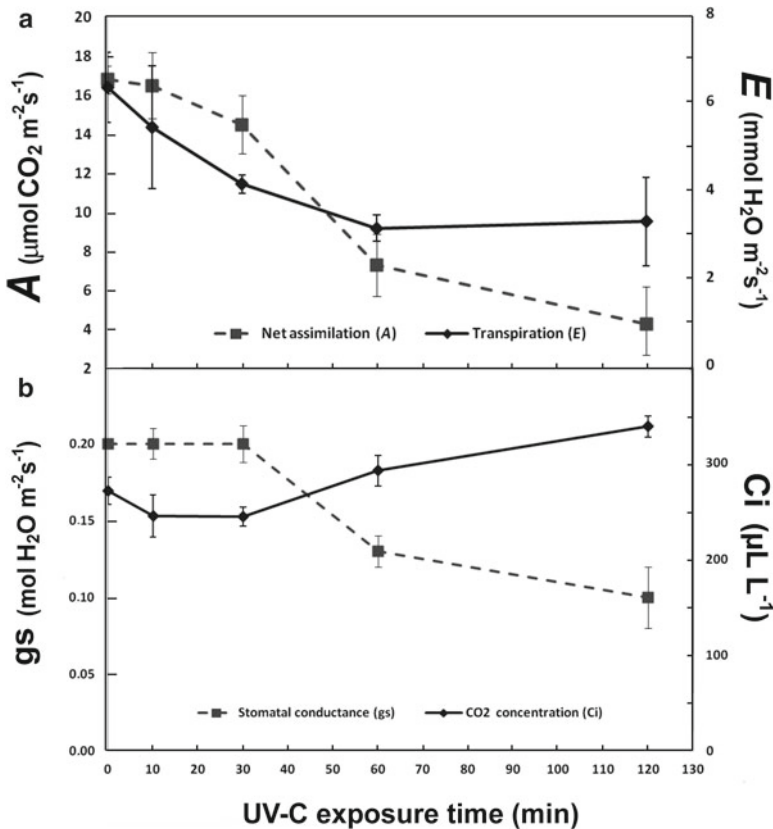


Fig. 10.1 (a) Trends of net assimilation (A) and transpiration (E) in leaves of tomato plants exposed to UV-C radiation for 0, 10, 30, and 60 min, measured 2 h after the UV-C treatment. (b) Trends of net assimilation (A) and transpiration (E) in leaves of tomato plants exposed to UV-C radiation for 0, 10, 30, and 60 min, measured 2 h after the UV-C treatment. Data represents means ($n=8$) \pm standard error

Significant decreases in transpiration (E) and stomatal conductance (g_s) are also observed (Fig. 10.1). Tomato photosynthetic apparatus is affected by UV-C treatment, as demonstrated by the strong increase in intracellular CO_2 (C_i) up to $338 \mu\text{L L}^{-1}$, particularly evident in the 120-min treatment. This is likely due to both the stomatal (g_s) and non-stomatal inhibition (A) of the assimilation activity (Fig. 10.1). The strong effect of UV-C on photosynthesis reduces 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).

Biometrical measurements, done 4 weeks after the UV-C exposure, point out that a decrease of inter-knot distance occurs in treated plants, in accordance with Bertram and Lercari (1996) and Lercari et al. (2003). The same trend is recorded for plants height and stem diameter. Similar results 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 (Hosseini Sarghein et al. 2011), no changes are observed for root morpho-anatomy after UV-C treatments, if compared to control plants, excluding a direct action of UV-C on the hypogeal part of the plants.

Colorimetric characterization demonstrates that, after few hours from the irradiation, the leaves of UV-C-treated plants are characterized by a general color change, while no differences in color among plant groups are detected before the UV-C treatments. In the CIE Lab color space, all the treated plants show similar values of brightness (L^* parameters). Regarding a^* (green–red axis) and b^* (blue–yellow axis) parameters, the values of 10-, 30-, and 60-min UV-C treatments do not differ statistically, but both are statistically lower in the 120-min treatment. Besides, the untreated tomato plants reach the highest values. The same trend is observed for leaf chroma and Hue angle parameters. This colorimetric response was also found by Rozema et al. (1997), who observed a reduction in pigment levels due to increasing exposition time to UV-C radiation.

3.2 Electric Field

Tomato root morphology is strongly affected by the applied EF (Fig. 10.2). Indeed, a significant variation in shoot and root growth rate is observed among the groups of plants grown close to the positive/negative electrode or in the central part of the container (Fig. 10.2). Both the root/shoot ratios of dry weights (R/S) and the LRM of the plants sampled next to the positive electrode show the highest values, if compared to the negative ones. The average length of the main root of the plants ranges from 6 cm next to the negative electrode, to 12 cm in the central part of the container, to 15 cm next to the positive electrode (Fig. 10.2).

The tomato plants grown close to the positive electrode, compared to the plants in the central area and toward the negative one of the container, show pronounced root branching and hair development, and higher root density and length. Under EF

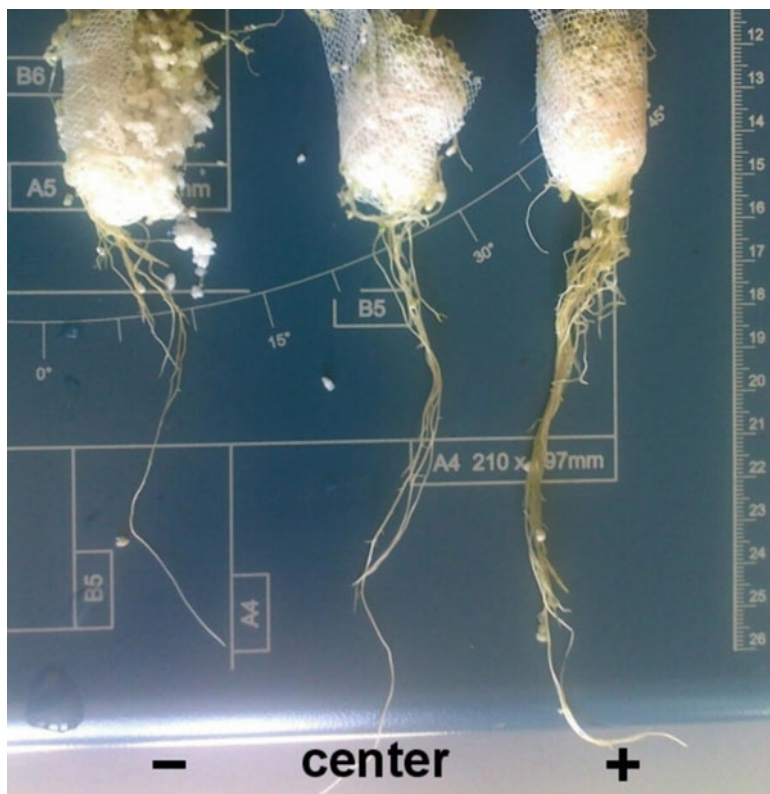
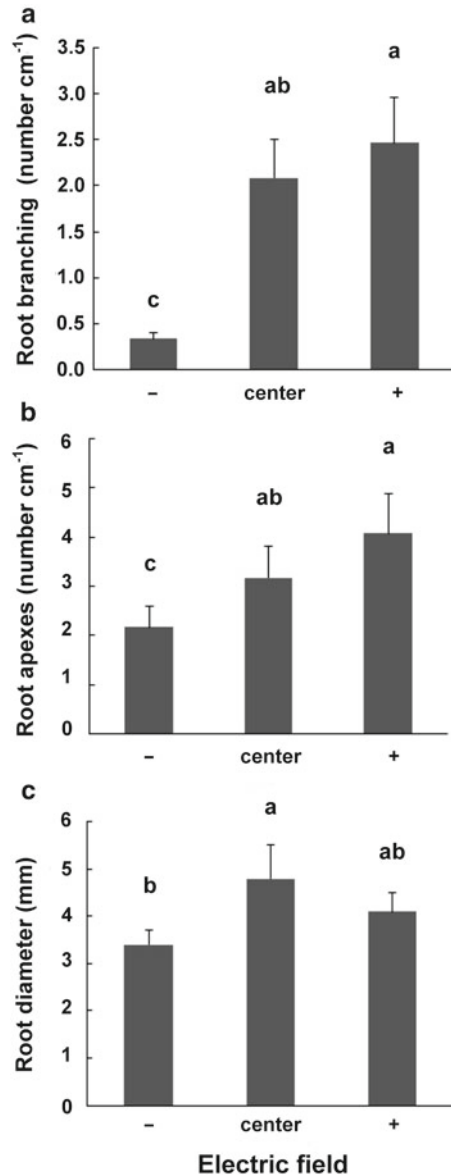


Fig. 10.2 Root morphology of tomato seedlings grown hydroponically under an electric field. Positions: (*left*; -) negative electrode, (*center*) central area, (*right*; +) positive electrode. Scale bars in cm

exposure, root branching increases from 0.3 branches cm^{-1} at the negative electrode to 2.5 branches cm^{-1} at the positive one (Fig. 10.3a). As far as branching is concerned, apical meristems per cm reach the highest counting next to the positive electrode (Fig. 10.3b). The average root diameter of treated plants is less affected by the opposite poles, and it significantly increases in the central area (Fig. 10.3c).

The different growth patterns observed could be related to the different mineral gradients formed by migration of cations and anions in the water solution under the applied EF of 12.0 V m^{-1} . Indeed, chemiosmotic modifications of ion transport, an equivalent of salt influx or salt efflux driven at the expense of an equivalent of electrogenic proton efflux, could occur. The increased ion accumulation seems not to be merely a passive movement under the applied potential, and it is thought that small currents could stimulate active ion pumps or alter the internal distribution of growth-regulating compounds (Black et al. 1971; Robinson 1985). In support of this hypothesis, root morphology, R/S and LRM, and microscopic parameters of all the control plants taken from different vessel position resembles the corresponding

Fig. 10.3 (a) Total root branching, normalized to root main axis length (mean per cm \pm 20 % error), (b) total root apex number, normalized to root main axis length (mean per cm \pm 20 % error), and (c) mean root diameter at 0.02 cm from the tip (\pm standard deviation) of tomato seedlings grown hydroponically under an electric field. Positions as in Fig. 10.2. Means ($n=10$) with different same letters on the columns are significantly different ($P \leq 0.01$) among the positions (-, center, +) of both the groups of plants



parameters of the plants under the EF and taken in the central area of the container, suggesting that ion distribution in the container without EF is uniform, as the EF did not cause ion migration nor physiological changes in the membranes of root cells.

An interesting observation could be related to another hypothesis: the root orientation in the growing medium. In fact, the “root direction” of tomato plants is always well defined in the growing solution, as root-growing direction is usually oriented

toward the positive electrode. This curvature is not present in the control plants. Electrotropic curvature in solutions of low electrolyte concentration was already studied using primary roots of maize (Ishikawa and Evans 1990). When submerged in oxygenated solution across which an EF was applied, the roots curved rapidly and strongly toward the positive electrode (Ishikawa and Evans 1990). These responses are controlled by auxin and auxin transport inhibitors (Goldsworthy and Rathore 1985; Ishikawa and Evans 1990). Therefore, electrotropic curvature is probably due to a particular orientation and distribution of membrane proteins or to a different phytohormonal balance under the EF. In this regard, Brown and Loew (1994) determined that EF-directed locomotion caused the lateral redistribution of plasma membrane glycoproteins in fibroblast cells grown *in vitro*.

4 Conclusion and Future Perspective

An exposition of tomato plants to enhanced levels of UV-C radiation and DC-EF can determine the important and significant alterations in their growth. High UV-C doses (60 and 120 min) determine 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) allow plants to partially maintain their normal physiological status. Physiological and structural alterations are evident in shoots of tomato UV-treated plants that also exhibit a significant color change, probably due to the photo-oxidation of chlorophylls and other pigments, and a reduced growth (Fig. 10.4a). On the other side, the application of DC-EF in tomato causes significant differences in root development, showing a

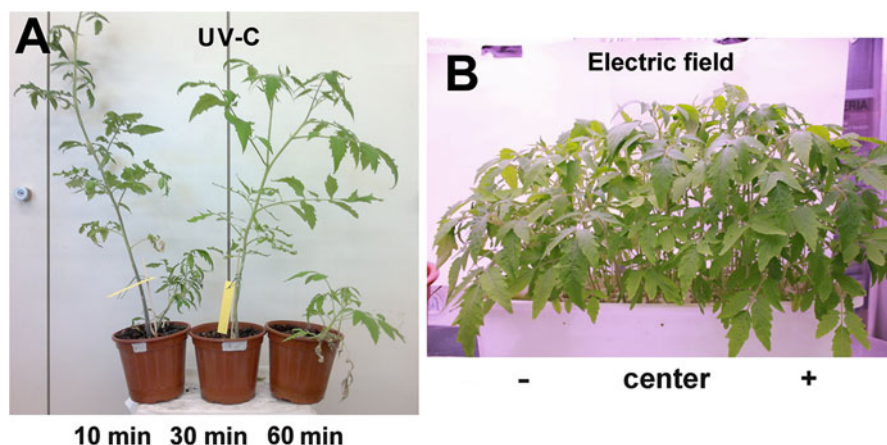


Fig. 10.4 Growth patterns of tomato plants subjected to UV-C radiation (3.8 J m^{-2} at 1 m of distance) and a DC 12.0 V m^{-1} electric field. UV-C exposition times and electric field polarity are indicated in the figure

typical gradient with high developed plants toward the positive electrode (Fig. 10.4b). This plant growth response could be useful in plant nursery techniques. Indeed, a better quality of tomato plants could promote a faster *in vitro* growth and reproduction of micro-propagated plants, increasing also their survival during the following acclimation phase. Next experimentation should investigate the mechanisms by which the application of a DC-EF, varying in current intensity and voltage, cause the morphological effects in tomato.

The future perspectives foresee physiological, genetic, and molecular investigations and studies on the possible tolerance mechanisms of tomato plants to face UV-C radiation. Furthermore, a better knowledge of electro-culture could make possible the practical use of this unusual abiotic stress in plant propagation and cultivation. The protection of tomato plants against UV-C, combined with the growth-promoting effects of electro-culture, could allow farmers to grow bigger and better crops in less time, with less effort, and at a lower cost.

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