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# Moderate UV-A supplementation benefits tomato seed and seedling invigoration: a contribution to the use of UV in seed technology

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ABSTRACT

The production, processing and consumption of *Solanum lycopersicum* L. fruits are increasing worldwide, demanding technologies to improve tomato growth efficiency. Germination is a critical step for intensification of crop production and conditions plants' vigor, a crucial benchmark in plant market. Ultraviolet radiation supplementation is emerging in seed technology as it increases plant growth with no impact on the environment, although its use in crops' nurseries still remains an open field. In the present work, seeds/seedlings of three commercial cultivars ('Oxheart', 'Cherry' and 'Roma') were grown for 15 days under three different ultraviolet (UV)-conditions. The results demonstrated the benefits of supplementing seeds/seedlings with moderate UV-A dose, being evident an acceleration/synchronization of germination rates, higher biomass and cotyledon area, and a stimulation of photosynthetic pigments and anthocyanins in all cultivars analysed. UV-B showed a cultivar dependence effect: 'Cherry' cultivar was in general not affected by the moderate UV-B dose used, but 'Roma' and 'Oxheart' showed a delay in germination and a seedling biomass decrease, in parallel with a decrease in chlorophylls and carotenoids. Both UV-A/B supplements reduced the $H_2O_2$ and MDA seedling levels, but the antioxidant battery was stimulated (e.g., peroxidases that use guaiacol as a substrate (GPX)) as well as the phenol level and the antiradical activity. The Principal Component Analysis (PCA) validates the clear distinction between cultivars and UV-condition effects. These data demonstrate the benefits of UV-A supplementation of tomato seeds pointing out to an "eustress" beneficial of UV-A in seedlings growth and vigor. A possible application of UV-A supplementation to other crops is discussed.

## 1. Introduction

Tomato (*Solanum lycopersicum* L.) is among the most important vegetable crops, being the United States, China, Turkey, Italy and India the top five leading fruit-producing countries. Tomato production reaches ~160 millions of tons year$^{-1}$, of which ~12% is from the European Union (FAO, 2014; Eurofresh Distribution, September 7th of 2016). Tomato production and consumption is predicted to continue to increase and, to address this sector's sustainability, intense research is being undertaken towards the development of varieties with improved agro-traits and the maximization of seed technology (Gerszberg and Hnatuszko-Konka, 2017).

Aligned with the concept of horticulture sustainability, tomato seed-market established for long as a priority the increase of seeds' quality and invigoration using seed technology that may reduce the use of chemicals. The germination of seeds constitutes a critical step in plant's life and is an important factor for the profitability of its producers (Auge et al., 2009). The use of physical treatments on seed's technology may include electromagnetic waves, magnetic fields, ultrasounds or ionizing and non-ionizing radiation. Major advances on physical technologies have been reported to improve seeds' preservation and invigoration, and are emerging as an alternative to the use of chemicals (Araújo et al., 2016a; Paparella et al., 2015).

Effects of ionizing radiation is better studied in seeds while non-ionizing radiation, particularly ultraviolet (UV), remains poorly addressed (Araújo et al., 2016a). Exposure to sunlight (including UV) is necessary to initiate the leaf developmental program, including the evolution of proplastids, or the reprogramming of etioplasts, into chloroplasts (Orozco-Nunnelly et al., 2013). Plants have evolved UV-photoreceptors (Suchar and Robberecht, 2015; Yokawa et al., 2015), which influence multiple physiological aspects of the vegetative and reproductive stage of the plant (Huché-Thélier et al., 2016). Regarding the use of UV-A/B in tomato culture technology, we have recently demonstrated the beneficial impacts of moderate UV supplementation on plants' flowering and fruit ripening, with minimal impacts on photosynthesis, and a controlled stimulation of the phenylpropanoid pathway

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(Ponte et al., 2017). How UV intensity and type modulate the seed biology and germination remains a matter of debate (Araújo et al., 2016a; Nangle et al., 2012; Noble, 2002).

The use of moderate UV-C irradiation of seeds has been studied as an antimicrobial agent (Brown et al., 2001; Guajardo-Flores et al., 2014), has also been reported to increase resistance to abiotic stress (Ouhibi et al., 2014). Also low levels of UV-C have increased seed germination rate and seedling vigor in *Brassica oleracea, Lactuca sativa* and *Arachis hypogaea* (Brown et al., 2001; Ouhibi et al., 2014; Siddiqui et al., 2011). Contrarily to the abundant studies demonstrating that high doses of UV-B rays have negative effects on plants' growth and productivity (Wargent et al., 2009), their effects on seeds germination and seedlings' vigor remain less known. UV-B anticipated the germination in *Vigna mungo* but seedlings became stunted, and with increased oxidative stress (Shaukat et al., 2013). Regarding UV-A supplementation, its cell receptors and modulated pathways are usually assumed to be similar to those of blue light, not being evident how UV-A specifically modulates plant functions, particularly seed germination and seedling vigor (Araújo et al., 2016a). UV-A was reported to stimulate growth, increase leaf size and stem length, fresh and dry mass (Li and Kubota, 2009). *Vigna radiata* seeds exposed to UV-A had improved the germination rate and seedlings' leaf area, root and shoot length and dry weight (Hamid and Jawaid, 2011). These promising data, together with the fact that the blue/UV-A receptors control multiple pathways, constitute a promising field of study to evaluate the viability of introducing UV-A/B supplementation in industrial crop seed technology, to improve germination and/or seedlings vigor.

UV radiation is known as a source of reactive oxygen species (ROS) production, while also activating several antioxidant enzymes to restore the ROS-levels (Kumari et al., 2010). Superoxide Dismutase (SOD) acts in the first line of the scavenging of ROS (namely to $O_2^{\cdot -}$) with the formation of the $H_2O_2$, which is catalyzed by several enzymes including Catalase (CAT), Peroxidases (APX, GPX) (Choudhury et al., 2013; Das and Roychoudhury, 2014). Alternatively, non-enzymatic antioxidant pathways may be stimulated by UV radiation. Non-enzymatic pathways lead to the production of polyphenols (Heijde and Ulm, 2012; Müller-Xing et al., 2014), and may be triggered by photoreceptors for blue/UV-A photons such as phototropins (PHOT), cryptochromes (CRY) and LOV/F-box/Kelch-domain proteins (Yu et al., 2010) and for UV-B light namely the UV resistant locus 8 (UVR8) (Rizzini et al., 2011).

The aim of this work was to functionally understand if moderate supplementation of UV-A or UV-B has beneficial effects on seeds' performance, during germination and first stages of seedling elongation. For that, seeds of three commercial cultivars were daily exposed to two moderate doses of UV-A and of UV-B, and effects on germination rates, seedling morphology, growth and vigor were evaluated together with parameters related to oxidative stress and photosynthesis. This study was focused in three tomato cultivars ('Oxheart', 'Cherry' and 'Roma'), which were chosen based among the most widely produced and consumed cultivars in several European countries (e.g., Portugal, Spain, Italy). This work will contribute to distinguish UV-A and UV-B effects during germination, and identify discriminative characteristics of cultivars in response to irradiation. It will also contribute to the implementation of UV supplementation in seed-technology in nurseries and in protected horticulture, including doses that may have horticultural relevance.

## 2. Material and methods

### 2.1. Plant growth conditions and UV treatments

Seeds of *S. lycopersicum* L. cvs. 'Oxheart', 'Cherry' and 'Roma' (Casa Cesar Santos, Portugal) were pre-treated at 4 °C for two days. Seeds were then sterilized for 5 min with 20% commercial bleach (Neoblanc®, corresponding to ~1% active NaClO), and washed with sterile deionized water. 100 seeds of each cultivar were placed in Petri dish and irrigated with 1/10 strength Hoagland medium (pH 5.7 ± 0.1) and incubated at 20 °C, in the dark during the first 8 h, after which seeds were exposed to the UV irradiation conditions: Control, UV-A 2 h $day^{-1}$ (UV-A 2H) and UV-B 15 min $day^{-1}$ (UV-B 15 min). For each cultivar and each condition five Petri dishes with 100 seeds was used to evaluate the germination rate and for physiological analysis.

Germination and seedlings growth took place in a growth chamber under a 16 h–light/8 h-dark photoperiod and a photosynthetic photon flux density (PPFD) of 200 µmol $m^{-2}$ $s^{-1}$ supplemented by OSRAM L 30W/77 FLUORA lamps. Four lamps with 90 cm of length and 5 cm between them were used per shelf, and each shelf was cover with aluminium foil to separate all treatments. The distance of the lamps to the shelf surface was of 37.5 cm. Relative humidity (RH%) and the temperature were maintained at 45 ± 5% and 23 ± 2 °C, respectively. Daily UV light treatments were applied in the middle of the photoperiod. UV-A supplement was performed, using a 0.45 J $m^{-2}$ $s^{-1}$ UV-A blacklight lamps (F20T12/BLB - 20W T12 (T10)) Fluorescent Blacklight Blue, with a peak wavelength at 368 nm (the intensity of light at wavelengths below 368 nm was close to 0 J $m^{-2}$ $s^{-1}$), for 2 h per day. The UV-A lamps with 60 cm were installed in the middle of the four OSRAM L 30W/77 FLUORA lamps, aligned in the centre at the same height of the other lamps. The exposed seeds and seedlings to UV-A were placed aligned in the middle line of petri dishes under the lamp. UV-B treatment was performed, using six 2.94 J $m^{-2}$ $s^{-1}$ TFP-M/WL 8W lamps with a peak at 312 nm, for 15 min per day. The UV-B lamps were placed at the same height of OSRAM L 30W/77 FLUORA. First exposure was applied 8 h after the imbibition, and the treatment was repeated once a day for 15 days. The same growth PAR conditions were maintained in the control and in the UV-treated groups. UV-A and UV-B light intensity were measured by Sensor Meters PHILP HARRIS (model SEL240) and International Light INC (model IL1400A, USA), respectively. The UV sensor was placed adjacent to Petri dishes, centred with the UV-A or UV-B lamps. The measurement height was of 3 cm, corresponding to the height of the sensor's base.

### 2.2. Germination, seedling growth and vigor

During the first 9 days after imbibition the number of germinated seeds was registered daily. After 15 days of UV exposure the seedlings' morphological characteristics, including shoot length, chlorosis and/or necrotic spots, were evaluated. Cotyledon area, and shoot and root length, Fresh matter (FM) and Dry matter (DM), and water content (WC%) were also determined (Silva et al., 2017). Seedlings' productivity was evaluated at the end of the experiment by using the glutamine synthase (GS) activity as reference (Thomsen et al., 2014). Briefly, samples were prepared and GS activity determined according to Pinto et al. (2014) and expressed as GS nkat/mg total soluble protein (TSP).

### 2.3. Malondialdehyde concentration and cell membrane stability (CMS)

For malondialdehyde (MDA) quantification 0.1 g of fresh cotyledons was macerated in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and then samples were treated according to our standard protocols: MDA levels were calculated as described by Araújo et al. (2016b). For cell membrane stability (CMS) assay, 30 mg of cotyledons with the same age were incubated in deionized water. Then samples were treated according to Araújo et al. (2016b) to assess the ratio of released electrolytes.

### 2.4. $H_2O_2$ content

The $H_2O_2$ in cotyledons was quantified according to Dias et al. (2014). Briefly, 0.1 g of fresh samples was homogenized in 1 mL of 0.1% TCA. The absorbance was measured at 390 nm, and $H_2O_2$ concentration (mmol $g^{-1}$FM) was calculated from a standard curve.