Use of UV-A and UV-B light supplementation in tomato producing: a perspective from plant to fruit

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
O Presidente do Júri,

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Resumo

Solanum lycopersicum L., mais conhecido por tomateiro, representa uma das culturas mais produzidas em todo o mundo e um dos alimentos mais apreciados. A produção de tomate em sistemas de horticultura protegida (ex., estufas) normalmente leva a que este se encontre privado da radiação Ultravioleta (UV), pois as coberturas de vidro ou polipropileno são uma barreira para a sua passagem. Contudo, este tipo de radiação, nomeadamente UV-A e UV-B, em doses moderadas, pode ser um fator importante para a qualidade nutritiva dos frutos e vegetais. Para avaliar se a suplementação de UV pode afetar a planta em diferentes fases do ciclo de vida desta, testou-se o efeito de UV-A e de UV-B durante a fase de germinação e crescimento de plântulas e durante a fase de frutificação. Sementes de 3 cultivares ('Coração de Boi', 'Roma' e 'Cereja') foram expostas a 0,45 J m^{-2} s^{-1} de UV-A e 2,94 J m^{-2} s^{-1} de UV-B, durante 2 horas e 15 minutos, respetivamente por dia, durante 15 dias. Os UV-A foram em geral mais benéficos que os de UV-B, embora se tenha registado diferenças entre as cultivares. UV-A promoveu não só uma aceleração da germinação como também um maior crescimento e vigor das plântulas, e maior acumulação de compostos fenólicos (com função de fotoproteção e antioxidativa).

Posteriormente, usando plantas (100 dias de idade) da cultivar 'MicroTom' já em fase semelhante de floração/maturação e a crescer em condições controladas, estabeleceram-se cinco grupos sujeitos a irradiação durante 30 dias durante a maturação do fruto: o grupo controlo (apenas com iluminação PAR); os grupos UV-A 1h e 4h foram suplementados em 1 e 4 horas por dia, respectivamente, e os grupos UV-B (2 e 5min) foram suplementados em 2 e 5 minutos por dia.

Após este período, a avaliação da componente vegetativa da planta incidiu sobretudo na análise da fotossíntese e de estado oxidativo da planta. A suplementação com UV-A (sobretudo UV-A 4h) foi a que teve um maior impacto na fotossíntese, diminuindo a eficiência máxima do fotossistema II (PSII) (F_V/F_m) e da eficiência efetiva do PSII (ΦPSII). As trocas gasosas foram também afetadas, com uma redução da assimilação do CO_2 (P_N). De realçar que apesar de um aumento da expressão de transcritos da ribulose bisfosfato carboxilase oxigenase (RuBisCO), os níveis desta proteína mantiveram-se, sugerindo algum impacto ao nível funcional, o que também pode justificar o efeito na assimilação de CO_2.

A bateria antioxidante (sobretudo a não enzimática) nas mesmas folhas foi estimulada pela radiação UV-A/B. Este estímulo foi também demonstrado pelo aumento da

expressão do fator de transcrição do *alongamento do hipocótilo (HY5)* e de genes da via dos fenilpropanóides, nomeadamente a *chalcona sintetase* e *flavonoide sintetase* (*chs* e *fls*, respetivamente).

Finalmente a análise de frutificação e maturação dos frutos, mostrou também que o UV-A e o UV-B têm impactos distintos no fruto. Por exemplo, UV-A além de induzir maior aumento do número de frutos e sincronização da maturação, aumentou os níveis de compostos fenólicos e de características fisico-químicas do fruto, algumas com grande importância na agro-indústria (cor, firmeza), sobretudo estimulado pelo UV-A. Numa análise final da potencial aceitação destes frutos pelo consumidor, um painel de provas avaliou e classificou estes frutos face a atributos organolépticos. Mais uma vez, os frutos de radiação UV-A sobressaíram positivamente face a características relevantes como aroma ou sabor.

Com estes resultados contribuímos para uma clara distinção dos efeitos de UV-A face a UV-B, em várias fases de crescimento, e demonstramos o seu valor no melhoramento de frutos como alimentos funcionais. Numa perspectiva aplicada, e considerando o preço e a segurança das radiações UV-A, consideramos que a introdução de suplementação de UV-A nas culturas protegidas, pode representar uma vantagem para o produtor poder manipular a produção além de obter um produto nutricionalmente de potencial melhor qualidade.

Palavras-chave

Fotossíntese; Frutificação; horticultura protegida; *Solanum lycopersicum*; stresse oxidativo; suplementação de ultravioleta, alimentos funcionais,

Abstract

Solanum lycopersicum L., commonly known as tomato, represents one of the most produced and appreciated crops worldwide. Tomato production in protected horticulture systems (e.g., greenhouses) is usually associated with lack of ultraviolet (UV) radiation, as glass or polypropylene roofs work as a barrier to its passage. However, this radiation, namely UV-A and UV-B, in moderate dosage, can be an important factor to fruits' and vegetables' nutritive quality. To evaluate if UV supplementation can affect the plant in its different life cycle phases, we tested the effect of UV-A and UV-B during the germination, seedling growth and fructification phases. Three cultivar seeds ('Oxheart', 'Roma' and 'Cherry') were exposed to 0.45 J m^{-2} $s^{-1}$of UV-A and 2.94 J m^{-2} s^{-1} of UV-B, for 2 hours and 15 minutes, respectively, daily for 15 days. UV-A was generally more beneficial than UV-B, even though differences between cultivars were registered. UV-A promoted not only a germination acceleration but also an increased growth and seedlings vigor, and higher accumulation of phenolic compounds (with photoprotection and antioxidative function).

Posteriorly, 100 days-old 'MicroTom' plants in similar flowering/maturation phases and growing in controlled conditions, were divided in five groups and supplemented for 30 days to different UV conditions: control group (only with PAR illumination); UV-A 1h and 4h groups were supplemented with UV-A for 1 and 4 hours per day, respectively; and UV-B 2min and 5min groups were supplemented with UV-B for 2 and 5 minutes per day.

After this period, the evaluation of the plants' vegetative component was focused essentially in photosynthesis and oxidative status. Supplementation with UV-A (specially UV-A 4h) had the most impact on photosynthesis, decreasing maximum efficacy of photosystem II (PSII) (F_v/F_m) and effective efficiency of PSII (ΦPSII). Gas exchange was also affected, with a CO_2 (P_N) assimilation reduction. It is important to note that, besides an increase in ribulose bisphosphate carboxylase oxygenase (RuBisCO) transcripts expression, the protein levels did not change, suggesting some impact at the functional level, that can also justify the effect in CO_2 assimilation.

The antioxidant battery (essentially non-enzymatic) was stimulated by UV-A/B irradiation. This stimulus was also demonstrated by the increase in *elongated hypocotyl (HY5)* transcription factor expression and phenylpropanoid pathway genes, such as *chalcone synthase (chs)* and *flavonol synthase (fls)*.

Fructification and fruit maturation analysis indicated that UV-A and UV-B have distinct impact on fruits. As an example, UV-A, besides inducing a higher number of fruits and maturation synchronization, increased the levels of phenolic compounds and fruit's physico-chemical characteristics, some with great importance in agro-industry (color, firmness), mostly stimulated by UV-A. In a final analysis of the potential acceptance of these fruits by the consumer, a test panel evaluation with classification of these fruits according to organoleptic attributes was conducted. Once again, fruits of UV-A irradiation stood out positively in relevant characteristics such as aroma or flavor.

With the present results we contribute to a clear distinction of UV-A effects compared to UV-B, in different growth phases, and demonstrate the benefits of irradiating crops on fruit improvement as functional foods. In an applied perspective, and considering the that UV-A lamps are safer and affordable, we consider that introducing UV-A supplementation in protected cultures may represent an advantage to the producer by manipulating fruit production and obtaining a potentially higher quality product in terms of nutrition.

Keywords

Photosynthesis; Fructification; Protected horticulture; *Solanum lycopersicum*; Oxidative stress; Ultraviolet supplementation; Functional foods

Publications/Presentations:

The current dissertation includes work that has been published and/or submitted to publication in the form of scientific communications (oral and poster), or in the form of published/submitted ISI-JCR papers:

ISI-JCR Papers

Mariz-Ponte N, Mendes RJ, Sario S, Melo P, Santos C (2017). Moderate UV-A/B supplementation benefits tomato seed and seedling invigoration: a contribution to the use of UV in seed technology. submitted to Scientia Horticulturae (under revision) (IF: 1.56; Q1; **Chapter 2)**

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Mariz-Ponte N, Mendes RJ, Sario S, Oliveira JMPF, Melo P, Santos C (2017). Tomato plants preferably use non-enzymatic antioxidant pathways to cope with moderate UV-A/B irradiation: a contribution to the use of UV-A/B in horticulture. Journal of Plant Physiology, article in press, doi: 10.1016/j.jplph.2017.11.013 (IF: 3.12; Q1; **Chapter 4**)

Invited oral communications

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Oral communications

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Ponte N*, Mendes R, Gonçalves A, Moutinho-Pereira J, Correia C, Ribeiro C, Melo P and Santos C (2017) Preharvest Ultra-Violet (UV) application enhances tomato fruit quality: parametric analyses. Centro de Congressos de Coimbra (Convento de São Francisco), Coimbra, Portugal. (07/06/2017 - 10/06/2017) page 241st, VIII Congresso Ibérico de Ciências Hortícolas (*speaker) -– **Chapter 4**

Panel communications

N Ponte*, M Couto, I Amorim, P Melo, JPM Oliveira, C Santos (2016) Avaliação da germinação de sementes de três variedades de *Solanum lycopersicum* sob suplementação com radiação UV-A e UV-B. (October 12nd and 13rd of 2016), pg 38, X Jornadas de Biologia, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal. (*presenter)

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Abbreviations

APX – Peroxidase of the ascorbate

BSA – Albumin serum bovine

CAT - Catalase

Chl *a* - Chlorophyll *a*

Chl *b* - Chlorophyll *b*

CRY - Cryptocrome

ETR - Electron transport rate

FM – Fresh matter

F_m – Maximum fluorescence

F'_m - Maximum fluorescence in light

F_v/F_m – Maximum efficiency of photosystem II

F0 – Basal fluorescence

GPX - Guaiacol peroxidase

GR – Glutathione reductase

GSH - Glutathione

GSSG - Glutathione disulfide

gs – Stomatal conductance

H_2O_2 – Hydrogen peroxide

MDA – Malonaldehyde

O_2^- - Superoxide

PAR - Photosynthetically active radiation

PHOT - Phototropins

PN – Net carbon assimilation

PPFD – Photon flux density

PSII - Photosystem II

PVPP - Polyvinylpolypyrrolidone

TF - Transcription factor

qN – Coefficient of non-photochemical extinction

qP - Coefficient of photochemical extinction

ROS – Reactive oxygen species

RuBisCO - Ribulose-1,5-bisphosphate carboxylase/oxygenase

SOD - Dismutase of superoxide

UV – ultraviolet

UVR8 – Ultra-violet resistance locus 8

UV-A/B- ultraviolet-A or ultraviolet-B

VOCs - Volatile organic compounds

I. Chapter 1: Potential use of UV-A and UV-B in horticulture

1. Historical perspective of the UV effects on plants

Solar Ultraviolet (UV) radiation spectrum is composed by three types of wavelength bands: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm) (Williamson *et al*., 2014). This radiation is a natural environmental stress factor for organisms since the pre-Cambrian era (Suchar and Robberecht, 2015). However, while the most dangerous band, UV-C radiation is completely filtered by the atmosphere, significant levels of UV-B and UV-A reach the Earth's surface (Williamson *et al*., 2014).

In the 1970s, Molina and Rowland (1974) proposed that the ozone layer was being destroyed (Bandurska *et al*., 2013; Molina and Rowland, 1974), which was later confirmed in the 1980s. This decrease of the ozone layer implies an increase of the UV-B radiation reaching the Earth's surface. For example, current environmental UV-B levels reaching the Earth's surface may range between 2-12 kJ m^{-2} per day, which represents a 6–14% increase of the measured UV-B radiation during the pre-1980 (Kataria *et al.,* 2014). These facts increased the research on the UV-B potential harmfulness on organisms in the last decades (Hideg *et al*., 2013).

High levels of UV, namely the UV-B light can be absorbed by nucleic acids, proteins and lipids and promote several damages in these molecules compromising metabolic and physiological processes, and ultimately leading to morphological changes (Tian and Yu, 2009) (*Figure I.1*). Part of these effects involve the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cells and tissues (Gill *et al*., 2015) (*Figure I.1*). Mitochondria, chloroplasts and peroxisomes are among the most sensitive organelles to ROS activity, and in particular, the photosynthetic apparatus is highly sensitive to excessive UV-energization, which may compromise the photosynthetic machinery. Namely, excessive UV-B may damage thylakoid membranes, damage the photosystems (particularly Photosystem II (PSII)), reducing chlorophyll levels and reduce fixation of CO_2 (Lindon *et al*., 2012).

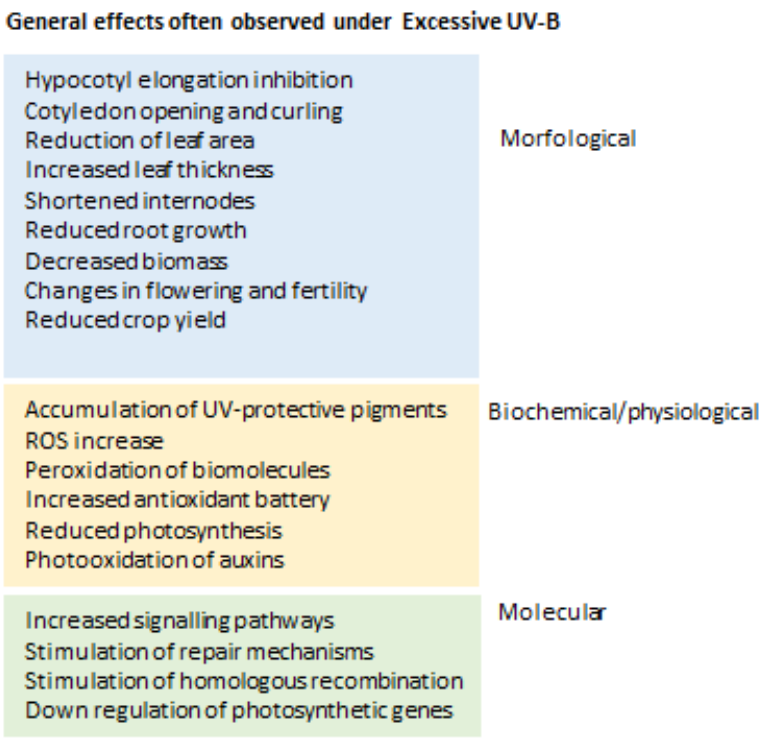

Figure I.1. Some morphological, physiological-biochemical and molecular events often reported when plants are exposed to uncontrolled excessive UV-B radiation (adapted from Kataria *et al*., 2014).

However, this poor reputation of UV is changing, mainly supported by a deeper molecular understanding of UV-response mechanisms and the related production of several metabolites with interest (Kumar and Pandey, 2013; Ncube *et al*., 2012; Pavarini *et al*., 2012). Moderate doses of UV-A/B may contribute to the enrichment of fruits and vegetables, by inducing metabolic shifts in plants without negative consequences. Therefore, the controlled use of low/moderate UV-doses during short periods of exposure has potential in agro-industry, with benefits for improving plant's performance and/or increasing crops' richness in nutritionally valuable compounds (Bian *et al*., 2014; Treutter, 2010).

The use of UVs as a technological tool of modulating crop's yield and nutritional quality thus emerges as a new paradigm in protected horticulture capable of being used to modulate metabolic and physiologic changes in plants (Barnes *et al.,* 2015; Wargent *et al*., 2015).

2. Physiological changes in plants: focus on germination, photosynthesis and oxidative stress

Whilst considering the multiple effects that UV-A/B may have in plants' growth, morphology and physiology, we will focus here on the effects on germination and plant growth, and on photosynthesis and oxidative status. We will describe some current advances in these topics, some of which will be further developed in the following chapters.

2.1. Influence on germination and plant growth

Plants respond to multiple environmental factors, such as the light quality and intensity. This response can include a stimulus or a repression of, for example, germination or biomass production. Seed germination is a critical step in plant production and influences seedlings' invigoration. *Vigna radiata* seeds, exposed to supplementary UV-A light, showed a stimulation of the germination rate and of seedlings vigor, with an increase of leaf area, dry weight, root and shoot length (Hamid and Jawaid, 2011). Also, Victório *et al.* (2010) showed an acceleration of germination in *Senecio cinereia* seeds with UV-A supplementation. The supplementation with other light wavelengths, such as UV-B, has shown a wide range of responses dependent on the species and the intensity of UV-light and photosynthetically active radiation (PAR). For example, Shaukat *et al*. (2013) showed that moderate UV-B supplementation promoted the germination of the *Vigna munga* and Kacharava *et al*. (2009) reported that moderate levels of UV-B promoted the germination, and increased the biomass and height in kidney bean. However, using higher daily UV-B doses, Sugimoto *et al*. (2013) demonstrated a delay in germination in different species (e.g., eggplant, lettuce, pea and spinach).

For UV-A light the biomass production in shoots and roots is dependent on the species and intensity of light, thus promoting a variable response as reported by Verdaguer *et al*. (2016). However, the moderate or low supplementation of UV-A increased the rosette diameter in *Arabidopsis thaliana* (Biswas and Jansen, 2012). In open field with exclusion of the UV-B, the presence of UV-A increased the foliar area and height in *Glycine max* compared to absence of UV-A (Zhang *et al*., 2014), a stimulation that was proposed to be due to an induced stimulatory effect of the photosynthetic status.

2.2. Influence on photosynthesis

The UV-A is far less studied in plants than UV-B and blue light, but has been reported as a stimulator/inhibitor of photosynthetic events. The PSII is a receiver of the UV-A photons and the first structure affected, namely the catalytic Mn cluster of the water-oxidizing complex, and D1/D2 proteins, which can be degraded by excessive UV-A irradiation (Christopher and Mullet, 1994; Tyystjärvi, 2008). Also, the electron transport chain was reported to be damaged in the presence of high doses of UV-A (Nayak *et al*., 2003). Nevertheless, moderate UV-A is also reported as having a stimulatory effect in photosynthesis (Štroch *et al*., 2015). The photosynthetic rates were increased in *Poa annua*, *Sorghum halpense*, *Nerium oleander*, *Pimelea ligustrina* and *Sorghum bicolor* plants under moderate UV-A irradiation (Kataria and Guruprasad, 2012; Mantha *et al*., 2001; Turnbull *et al*., 2013). In particular, it has been proposed that the moderate supplementation of UV-A in leaves may promote an opening of the stomatal aperture, thus increasing the gas exchange (Chen *et al*., 2012).

On the other hand, the excessive UV-B radiation can induce several changes in plant metabolism, physiology and morphology. As has been reported, this radiation promotes a reduction of leaves, as well as its downward curling, also reducing the foliar area (Dotto and Casati, 2017). The leaf surface develops a higher trichome density in plants exposed to high UV-B levels. Seedling growth is also affected by the reduction of the hypocotyl extension and root elongation (Robson *et al*., 2014), thus, promoting a reduction in plant biomass. Additionally, excessive UV-B delayed the flowering time in Arabidopsis, maize, *Phaseolus vulgaris* and *Vigna radiate* (Dotto and Casati, 2017). However, the consequences of UV-B exposure depend on the intensity and duration, and while a high irradiation can promote several structural and physiological damages (Singh *et al*., 2017), a low irradiation may promote photomorphogenic responses without significant damages.

One of the first physiological processes that can be affected is the photosynthesis and photosynthetic apparatus. In fact, excessive UV-B impairs the synthesis of photosynthetic pigments (e.g., enzymes involved in the chlorophyll biosynthetic pathway) while also promoting their degradation, which ultimately leads to a loss of photosynthetic capacity (Kataria *et al.,* 2014). On other hand, the light-harvesting complex of PSII (LCHII) can be damaged, and chlorophylls a/b reduced, allowing the decrease of excessive energization, in a putative photoprotective mechanism (Singh *et al*., 2017). The proteins of the photosystem reaction centre (D1 and D2) are degraded by UV-B exposure (Zlatev *et al*., 2012). In blueberry plants exposed to high UV-B

radiation, the photosynthesis net assimilation (P_N) decreased, as well as, the effective quantum yield (ΦPSII) and electron transport rate (ETR) (Inostroza-Blancheteau *et al*., 2016). Nevertheless, other studies focusing on the expression levels of genes during/after UV-B supplementation showed an increase of the transcripts coding for components involved in LCHII, such as Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) subunits, chl *a*/b-binding proteins and D1 protein (Kataria *et al*., 2014). This reaction is currently being interpreted as an attempt to mitigate the damages provoked by this environmental stressor, but the pathways underlying this regulation are far from being fully unveiled.

Plants have developed different strategies to adapt to these high energetic light rays that act as stressors. Among the adaptative strategies one may cite the accumulation of UV absorbing compounds (e.g., anthocyanins, flavonoids and carotenoids), the thickness increase of the leaf cuticle, the reorganization of the plastids and the increment in its antioxidant batteries (Kataria *et al*., 2014).

2.3. Oxidative stress induced by UV: antioxidant response

An abiotic or biotic stress is described as capable to generate ROS (Soheila, 2000). UV rays are potentially harmful to plant cells, especially when their intensity and exposure time are high and/or uncontrolled. In photosynthesis, high radiation promotes an excess of energy in the PSII. Photosynthetic pigments are converted to an excited state and to return to the steady state, the excess of energy is released to another pigment, or lost as heat or as fluorescence. However, during this process, the excess of energy may increase the rate of electrons that are lost to an O_2 molecule, forming $^1O_2$, a highly reactive and unstable ROS (Anjum *et al*., 2014; Muller and Niyogi, 2001). ROS release may also occur during the electron transport chain, in particular during the plastoquinone photon transfer process (Kataria *et al*., 2014). Thus, the evidence of ROS formation under high UV is verified mainly in the plastidial thylakoid membrane (Anjum *et al*., 2014; Soheila, 2000).

Other ROS are also described as being induced by UV-B radiation during the photosynthetic electron flux, such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) (Anjum *et al*., 2014) and hydroxyl radicals ($^{\bullet}OH$) (Kataria *et al*., 2017). These molecules have as major target proteins, lipids and nucleic acids, affecting firstly the cell membrane redox status, and thus interfering with its integrity (Anjum *et al*., 2014). Under this ROS imbalance, plants respond promoting a stress-defence battery by

activating molecular mechanisms to reduce the high levels of formed ROS (Lindon *et al*., 2012). This antioxidant capacity and the levels of ROS produced by stress can dictate different paths to the survival and productivity of plants (Choudhury *et al*., 2013; Hideg *et al*., 2013). The enzymatic battery is an important strategy to detoxify the excessive ROS formed in stressed plants. The impact of stressors, like excessive UV, is commonly quantified by measuring the activities of antioxidant enzymes that have the capacity to cleave/inactivate ROS (Agarwal, 2007). Several enzymes are involved in a cascade of ROS detoxification, such as dismutase superoxide (SOD) (Abdel-Kader *et al*., 2007; Aksakal *et al*., 2016; Costa *et al*., 2002), catalase (CAT) (Alexieva *et al.,* 2001; Vidovic *et al*., 2015), ascorbate peroxidase (APX) (Aksakal *et al*., 2016; Kargar-Khorrami *et al*., 2014), peroxidases that use guaiacol as substrate (GPX) (Kargar-Khorrami *et al*., 2014) and enzymes of the ascorbate-glutathione cycle (AsA-GSH), such as the previously described APX (Xin-xin *et al*., 2016). SOD enzymes act in a first stage to scavenge ROS, targeting the highly damaging $O_2^{\bullet -}$ to form H_2O_2, which is considered less aggressive to cellular structures. Several enzymes are responsible for the degradation of H_2O_2, including catalases and peroxidases (CAT, APX and GPX).

Besides this enzymatic battery, the non-enzymatic antioxidant defence is also an important complement to the enzymatic battery contributing to the maintenance of the basal levels of ROS (Agati *et al*., 2012). AsA-GSH cycle, in addition to being an important cycle to support the enzymatic battery, is a non-antioxidant promoter which forms e.g., vitamin C and supports the vitamin E formation that has an antioxidant role. An increase of AsA levels (Braizaityte *et al*., 2015) in UV-A irradiated plants has been reported, as well as the increase of the levels of glutathione (GSH) and glutathione disulfide (GSSG) after irradiation with UV-B (Costa *et al*., 2002), suggesting a stimulation of this cycle in response to UV. Also, the production of certain pigments that also have antioxidant properties, such as carotenoids (e.g., lutein and β-carotene), is stimulated by UVs (Bandurska *et al*., 2013; Brazaityte *et al*., 2015). The compounds involved in the phenylalanine pathways, namely phenolic compounds, such as anthocyanins and flavonoids (that have both photoprotective and antioxidant roles) may function as first antioxidant barriers in preventing the UV-induced formation of ROS by absorbing photons, together with their ability to scavenging ROS (Agati *et al*., 2012). Several studies have demonstrated the increase of these type of compounds after exposure to high UV irradiation (Aksakal *et al*., 2016; Alexieva *et al*., 2002; Brazaityte *et al*., 2015; Dai and Mumper*,* 2010).

2.4. Influence on fruit ripening

UV radiation has recently been proposed to have positive effects on controlling diseases and excessive softening during post-harvest and storage (Castagna *et al*., 2014). Thus, controlling and synchronizing fruit ripening is crucial in any horticultural system. However, most available studies on post-harvest effects are conducted with UV-C and UV-B (Castagna *et al*., 2014), and less with blue/UV-A. Moreover, studies of UV impacts during pre-harvest are less abundant than those during post-harvest, of which most are aiming at increasing the fruit shelf life (Castagna *et al*., 2014). Ripening of climacteric fruit such as tomato is highly controlled by ethylene, which triggers cascades of biochemical and physiological processes, ultimately inducing the softening of the fruit. Ethylene is recognized by endoplasmatic receptors, initiating a signalling cascade of metabolic pathways involved in ripening. This process culminates in biochemical/physiological responses including chlorophyll decay, increase of carotenoid levels, softening due to changes in cell walls, and changes in fruit aroma and flavour. Moreover, levels of compounds such as L-ascorbic acid, tocopherols and phenolic compounds are also observed. Liu *et al*. (2011) reported that UV-C induced the expression of defence response genes, signal transduction genes (such as ethylene related genes, IAA receptor protein and calmodulin) and protein metabolism genes. Recent data has demonstrated that UV exposure modulates secondary metabolism in the skin of grapevine berries, affecting fruit ripening related transcripts and phenolic responses (Carbonnell-Bejerano *et al*., 2014). Earlier, Bacci *et al*. (1999) found that UV-B radiation causes early ripening and reduction in the fruit size of two lines of tomato (*Lycopersicon esculentum* Mill.). Castagna *et al*. (2014) have also demonstrated the beneficial effects of UV-B on post-harvest irradiation of tomato fruits, by inducing polyphenol profile and antioxidant activity, increasing its nutraceutical value.

2.5. Recent advances on UV-associated signalization

Light is a limiting factor in plant production. Plants are capable of using different wavelengths to produce different molecular responses and change plant's phenotype. In plant photobiology, photoreceptors are responsible for capturing the specific wavelengths, leading to a direct response. Phytochromes (PHY) are capable of absorbing the red and far-red light. Whilst most studies on the effects of blue/UV-A wavelengths have been mostly performed with the blue region, these two types of

radiation are usually classified as having similar receptors and effects. Blue and UV-A photons are captured by Cryptochrome (CRY), Phototropins (PHOT) families and ZTL/FKF1/LKP2 (ZEITLUPE/FLAVIN-BINDING KELCH REPEAT F-BOX 1/LOV KELCH PROTEIN 2), while UV RESISTANCE LOCUS 8 (UVR8) is a single known photoreceptor of UV-B (Rizzini *et al*., 2011), *Figure I.2*.

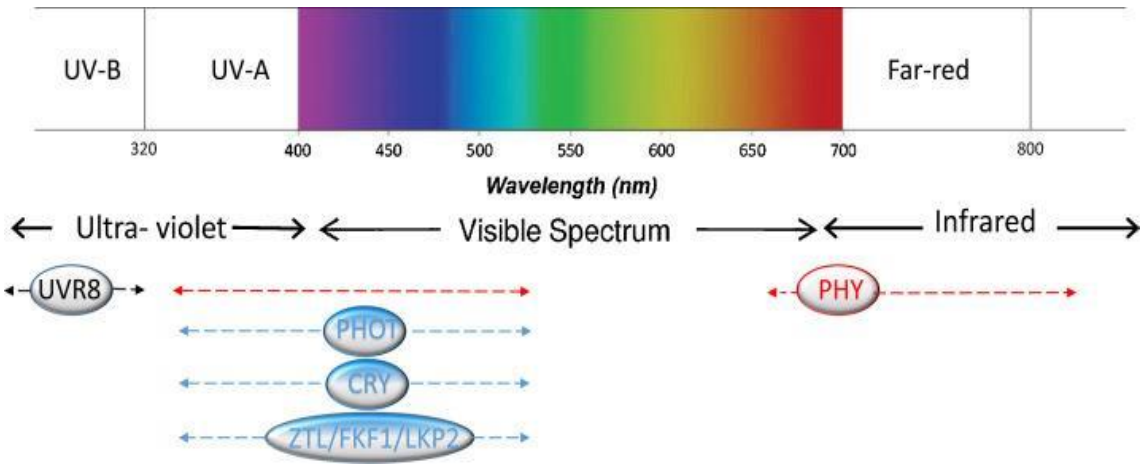

Figure I.2. Infrared, Visible spectrum and Ultra-violet wavelength and their respective photoreceptors. (Adapted from Huché-Thélier *et al*., 2016)

The families of the CRY and PHOT photoreceptors are involved in several processes in plant development, such as control of the circadian clock (Mawphlang and Kharshiing, 2017). Other processes, such as flowering time, plant growing and metabolism of secondary metabolites can be regulated by modulating these photoreceptors in the presence of blue/UV-A light (Huché-Thélier *et al*., 2016). Four CRY genes are expressed in response to UV-A and blue light. There are two types of CRY1 genes (CRY1a and CRY1b), one CRY2 gene and one CRY3 gene. CRY1a controls the photomorphogenesis process in young plants, anthocyanins accumulation and plant development (Facella *et al*., 2016). CRY2 is involved in fruit quality and flowering phase (Kharshiing and Sinha, 2015). Giliberto *et al*. (2005) have shown that CRY2 overexpression increases pigment contents by anthocyanins and chlorophyll overproduction in leaves and flavonoids and lycopene in fruits. CRY3 molecules are localized in mitochondria and chloroplasts having a function of repairing DNA in response to UV damage (Facella *et al*., 2016). PHOTs are involved not only in plant phototropism but also in photosynthetic processes, stomatal opening and mobilization of the chloroplasts in cells (Mawphlang and Kharshiing, 2017). Two types of PHOT are

described (PHOT1 and PHOT2) and its regulation is promoted by UV-A/blue light, which, under low/moderate radiation, can promote growth (Mawphlang and Kharshiing, 2017). Another family of photoreceptors (ZTL/FKF1/LKP2) is also stimulated by absorbing UV-A/blue light. These proteins are involved in the regulation of the circadian clock and their expression may control the flowering time (Huché-Thélier *et al*., 2016; Xue *et al*., 2012).

Regarding UV-B, when plants absorb this radiation a breakdown of the UVR8 dimer into monomers occurs, making this protein active and thus regulating the expression of many genes (Coffey *et al*., 2017). This photoreceptor plays a key role in the transcription of the several genes of the flavonoid pathway synthesis. In some cases, its role can also be stimulated by UV-A (Coffey *et al*., 2017).

Under white light controlled conditions, the light is absorbed by PHY and CRY that control the CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) repressor, which promotes the degradation of the transcription factor (TF) Elongated Hypocotyl 5 (HY5). This is negatively controlled by PHY and CRY. However, under UV-B radiation, the UVR8 dimer divides into two UVR8 monomers and interacts with COP1 stopping the HY5 degradation, and allowing this TF to promote the transcription of several genes involved in UV protection. Some of the proteins coded by these genes include Chalcone Synthase (CHS), Chalcone Isomerase (CHI) and Flavonol Synthase (FLS) that are involved in phenylpropanoid biosynthesis pathway (Heijde and Ulm, 2012). Also, this TF promotes the biosynthesis of chlorophylls, the sucrose metabolism, uptake of nutrients in roots, circadian clock pathways, hormone pathways, while it represses cellular elongation and increases ROS signalization (Gangappa and Botto, 2016).

With UV-A light, the inactivated CRY photoreceptors receive the photon energy and change their conformation thus acquiring an active conformation, and forming a CRY-interacting partner complex capturing the COP1 repressor (Mishra and Khurana, 2017). This will then promote the availability of the nuclear HY5 TF, and thereby stimulate a change of the expression of several genes. CRY1 and CRY2 have the same activation by blue/UV-A light, however, the activated CRY2 can play as a kinase participating in signaling processes or form a complex such as CRY1, but in this time preventing the degradation of CONSTANS (CO), a TF that promotes the transcription of several genes involved in flowering process, and thus stimulating this stage (Yang *et al*., 2017).

3. UV and phytochemical modulation: "healthy foods" and "functional foods"

In recent years, the knowledge of UV irradiation that induces molecular changes in the plant metabolome has shown that UVs may be used as a technological tool to modulate the physico-chemical characteristics and nutritionally enrich fruits and vegetables (Huché-Thélier *et al*., 2016; Schreiner *et al*., 2012). "Functional foods" are known as foods rich in antioxidants that can contribute to healthy eating and disease prevention (Lobo *et al*., 2010). Tomato fruit is also known as a "functional food" by its phytonutritional and nutraceutical composition (Kushi *et al*., 2006). However, tomatoes produced in protected horticultures without UV irradiation may have a lower content in antioxidants (e.g., phenols and flavonoids) since UV is known to increase the pathways of various antioxidants, such as the phenol pathway (Jansen *et al*., 1998; Morales *et al*., 2010). For example, in strawberry fruits, the pre-harvest application of UV-C promoted an increase of phenols' contents, anthocyanins, vitamin C, antioxidant capacity, quercetin and volatile organic compounds (VOCs) (Oliveira *et al*., 2016; Severo *et al*., 2017). In tomato, the use of UV-B in post-harvest stimulated an increase of vitamin C, lutein and lycopene (Castagna *et al*., 2014). Thus, UV-irradiation may specifically regulate several antioxidant pathways promoting the accumulation of antioxidant compounds. Therefore, a new field of research is emerging regarding the use of UV-supplementation in industrial production to modulate/increase "healthy foods" production and quality.

Nevertheless, this innovative approach, whilst well supported by physico-chemical analyses, also has to be validated regarding the product's sensorial attributes. Considering that most VOCs and compounds responsible for taste/aroma attributes are synthesized during the phenylpropanoid pathways, it is interesting to evaluate if these metabolic shifts of UV-irradiated products also have more appellative/strong attributes. For example, Dzakovich *et al*., (2016) exposed tomatoes to a panel of consumers, who differently categorised the fruits, showing some appetence for those irradiated. The consumers preference for these "improved" products represents a new field in agro-food research, since this is the final step for fresh or processed products.

4. Tomato as a model in protected horticulture studies

Solanum lycopersicum L., tomato, is one of the most popular and consumed crop species, having therefore a significant agricultural and economic importance (Žižková

et al., 2015), including in Portugal. In 2014, more than 170 million tonnes of tomato fruit were produced worldwide (FAOSTAT, 2014, www.faostat.org). Its excellent acceptance by the consumers is due to its multiple gastronomic uses and to other features like its taste, colour and high nutritional value (Verma *et al.,* 2015). Tomato fruits are extremely beneficial to human health due to their richness in folate, potassium, vitamin C, carotenoids and flavonoids (Aust *et al.,* 2005; Pérez *et al.,* 2008; Steinmetz and Potter, 1996), and as stated above have been classified as a functional food (Canene-Adams *et al.,* 2005). It is increasingly being demonstrated that carotenoids (e.g., lycopene and β-carotene) and flavonoids present in tomato fruits protect the consumer from various cardiovascular diseases and different types of cancer (Amin *et al.,* 2009; Schreiner *et al.,* 2009; Schreiner *et al.,* 2012; Thies *et al.,* 2017). Tomatoes are the primary source of lycopene in many people's diets. Lycopene is also responsible for the red colour present in tomato (Panthee *et al.,* 2013) and beyond its colour function, this carotenoid is a molecule with antioxidant capacity in $^1O_2$ elimination (Takeoka *et al.,* 2001), and is a precursor of β-carotene.

Initially, MicroTom tomatoes were considered short garden tomatoes because of the small plant's height (around 7 to 10 cm). Furthermore, these miniatures of tomatoes have a short life cycle of 70 to 90 days from sowing to fruit-ripening and a conserved well known genome (Dan *et al.,* 2006), of which several database are available. This cultivar is also an excellent model for further transformation studies. These characteristics, and the fact that it has a largely studied genome, make this cultivar versatile and easy to work with (including to grow in small area conditions) contributed to the dissemination of this variety as a model in several laboratories. Many studies have utilized this tomato cultivar to study the enhanced quality in tomato fruits by antioxidant stimulation with stress factors and pathways of biomolecules, promoting its nutritional value (Gómez *et al.,* 2009; Haroldsen *et al.,* 2011; Zushi *et al.,* 2014).

5. Aims

In face of the current state of art, we hypothesise that the controlled exposure to moderate supplementation of UV-A or UV-B will positively influence the growth and production of crop plants (growing in protected horticultural conditions). To address this hypothesis, the general aim of this work was to evaluate the effects of the intensity and type of UV irradiation on two major stages of tomato development: a) seed germination

and seedling growth; b) vegetative responses and fruit performance during the fruiting stage.

The specific objectives were:

1) to evaluate if moderate UV-A/B supplementation can be a positive factor during seed germination, and increase seedling invigoration. This aim will be addressed in *Chapter 2*.

2) to evaluate if moderate UV-A/B supplementation to plants during flowering/fruiting improve fruit production and/or maturation/synchronization, without compromising morphology and photosynthetic performance. This aim will be addressed in *Chapter 3*.

3) to evaluate the extension of the oxidative disorders induced by moderate UV-A/B supplementation in plants during flowering/fruiting, and if/how plants modulate antioxidant responses to cope with the stress. This aim will be addressed in *Chapter 4*.

4) to evaluate if UV-A/B supplementation to plants during flowering/fruiting promotes changes in ripening and in fruit metabolites, increasing its nutritional value, and consumers' acceptance. This aim will be addressed in *Chapter 5*.

Achieving these aims will contribute to better understand the physiological-biochemical and molecular roles of UV-A and UV-B on crops, but mostly will contribute to elucidate if the moderate supplementation of UV-A/B may be a useful tool in protected production of this and other crops. If confirmed, this work will ultimately contribute to the future validation of this tool by indicating better UV type/intensity, and how different irradiation conditions may reprogram the crop/fruit response, two aspects crucial in agro-food industry. A final general discussion integrating all chapters will be presented in *Chapter 6*, where also future perspectives will be presented.

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II. Chapter 2: Moderate UV-A/B supplementation benefits tomato seed and seedling invigoration: a contribution to the use of UV in seed technology

Abstract

The production, processing and consumption of *Solanum lycopersicon* 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 critical benchmark in plant market. Ultraviolet radiation supplementation is emerging as a 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 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.,GPX) as well as the phenols levels 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 supplementation of tomato seeds pointing out to an "eustress" beneficial of UV-A in seedlings growth and vigor. A possible application of UV supplementation to other crops is discussed.

Keywords

Germination, horticulture, oxidative stress, *Solanum lycopersicum*, Ultraviotet supplementation.

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 consume 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 (Huche-Thelier *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 (Ponte *et al*., 2017; see also Chapters 3 and 4). 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 *et al*., 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 *et al*., 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, 2016). 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 work will contribute to distinguish

UV-A and UV-B specific mechanisms during germination, and identify discriminative characteristics among cultivars. 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 *Solanum lycopersicum* L. cvs. 'Oxheart', 'Cherry' and 'Roma' (Casa Cesar Santos, Portugal) were pre-treated at 4 ºC 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) and incubated at 20ºC, in the dark during the first 8 hours, after which seeds were exposed to the UV irradiation conditions: Control, UV-A 2 hours day^{-1} (UV-A 2H) and UV-B 15 minutes day^{-1} (UV-B 15min). For each cultivar and each condition $\geq$5 Petri dishes were used as replicates.

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. Relative humidity (RH%) and the temperature were maintained at 45±5% and 23±2 ºC, 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 hours per day. 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 minutes per day. First exposure was applied 8 hours 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 was measured by Sensor Meters PHILP HARRIS (model SEL240) and International Light INC (Newbryport, Massachusetts, model 01950, IL1400A), respectively.

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.

2.5. Antioxidant enzyme activities

Total soluble proteins (TSP) were extracted from 0.1 g of frozen cotyledons macerated with 1.5 mL extraction buffer containing 0.1 M phosphate buffer (pH 7.0), 0.5 M disodium ethylenediaminetetraacetate (Na_2EDTA), 1% polyvinylpirrolydone (PVP), 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.2% triton X-100 (v/v) and 2 mM ditiothreitol (DTT). Samples were centrifuged at 8000xg for 15 min at 4 ºC. The supernatant was used to assess TSP and to quantify the activities of catalase (CAT), ascorbate peroxidase (APX) and of peroxidase with affinity to guaiacol (GPX). Protein

content was determined using the Bradford Reagent (Sigma, USA) and bovine serum albumin (Sigma, USA) as standard.

CAT activity was quantified by H_2O_2 degradation during 120 seconds monitored at 240 nm. The activity was determined according to Azevedo *et al.* (2005) using 135 µL extraction buffer, 60 µL of supernatant and 50 µL H_2O_2 (0.083 M). APX activity was determined by following the oxidation rate of ascorbic acid (AsA) at 290 nm during 70 sec (Azevedo *et al.,* 2005). GPX activity determination was also according to Azevedo *et al.* (2005) using 100 µL of plant extract and a reaction solution containing 100 mM phosphate buffer (pH 7.0), 3 mM of H_2O_2 and 15 mM guaiacol. The reaction was monitored for 1 min, at 470 nm.

For SOD analyses, frozen cotyledons were ground in a 100 mM phosphate buffer (pH 7.8) containing 0.5 mM Na_2EDTA, 1% PVP, 1 mM PMSF, 0.2% triton X-100 (v/v), 2 mM DTT. Samples were centrifuged at 15,000xg, for 15 min at 4°C. SOD activity in the SN was measured by the ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT read at 560 nm and the results expressed as nKat mg^{-1} of fresh mass (FM).

2.6. Total phenols and free radical scavenging activity

The quantification of the total hydrosoluble phenol content (TPC) was made according to Dewanto *et al.* (2002) with minor modifications. Briefly, macerated cotyledons (0.1 g) were homogenized in 1.67 mL of deionized water, then paper-filtered and centrifuged at 2500xg (10 min). The reaction medium contained 500 µL of deionized water, 125 µL of extract and 125 µL of the reagent Folin-Ciocalteu. After 6 min, 1250 µL of 7% Na_2CO_3 was added and the final volume adjusted to 1 mL with H_2O. After 90 min, absorbance was measured at 760 nm. A gallic acid (GA, Sigma USA) standard curve (µgGAE.mL^{-1}) was used.

For the antiradical activity of phenols the DPPH assay (Harkat-Madouri *et al*., 2015) was used. Cotyledons (0.1g) were homogenized in methanol and centrifuged at 2500xg (10 min). After reading samples at 517 nm, data were expressed as DPPH SA (%) = (A_C – A_S/A_C) X 100, where A_C and A_S are the control (0%) and sample absorbance values (0%), respectively. IC_{50} values were assayed by the volume of extract to reduce 50% of DPPH.

2.7. Pigment, soluble sugars and starch contents

Pigments and carbohydrates were quantified using 0.1 g of macerated cotyledons from a pool. Pigments were extracted according to Sims and Gamon (2002) with slight modifications of Dias *et al.* (2013). Chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), carotenoids (Car) and anthocyanins contents were quantified reading the absorbance at 470, 537, 647 and 663 nm in a multiplate reader Thermo Fisher Scientific Spectrophotometer (with three technical replicates per sample).

Total soluble sugars (TSS) and starch were quantified by the anthrone method (e.g., Dias *et al.,* 2013), using a multiplate reader Thermo Fisher Scientific Spectrophotometer.

2.8 Statistical analysis

Germination experiments were performed using five replicates of 100 seeds each for 'Oxheart' and 'Roma' cultivars, and 15 replicates of 100 seeds each for 'Cherry'. For biochemical assays, 5-20 plants were used as individual replicates and/or treated as pools, with at least 3 independent technical replicates. Presented values are the mean ± standard deviation. Comparisons between treatments and the control were made using One Way ANOVA test. When data were statistically different, the Dunnett Comparison Test ($p<0.05$) was also applied. Graphpad™ Prism 6 was used. Multivariate analyses for data correlation used Principal Component Analysis and were performed with CANOCO for Windows v4.02 programme.

3. Results

3.1 Germination

'Cherry' seeds' viability was always slightly lower than the one of other cultivars, and was not influenced by none of the UV-A/B conditions (*Figure II.1.*). By day 3, UV-A 2H increased ($p<0.05$) the 'Oxheart' germination rates (from 21% in the control to 47%) and at day 9 the % of germinating seeds also increased ($p<0.05$) from 97% in the control to 99% (*Figure II.1.*). In 'Roma' seeds, UV-A also synchronized the germination, being the effects significant ($p<0.05$) for the first five days (*Figure II.1.*). For example, at day 4 the % of UV-A irradiated and germinated seeds is the double (68%) than those of the control (32%).

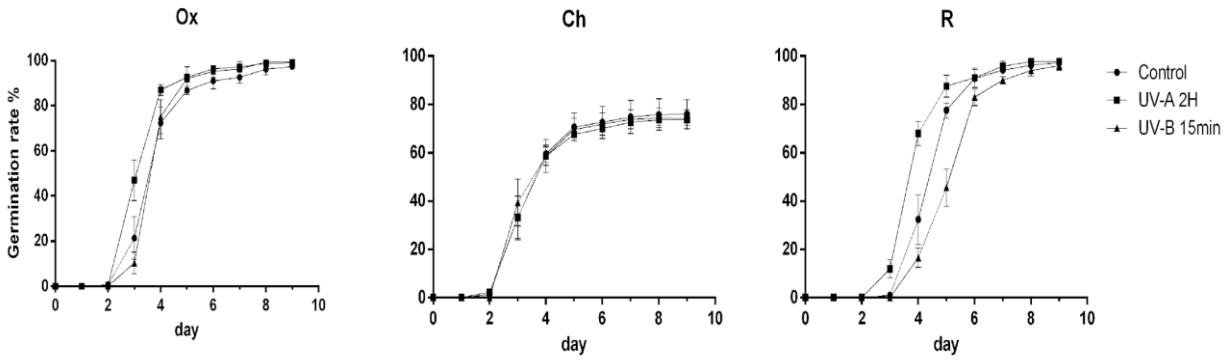


Figure II.1. Influence of UV-A and UV-B in the germination rate of 'Oxheart', 'Cherry' and 'Roma' seeds, 9 days after being exposed to UV-A/B conditions. The percentage of germination is followed for 9 days in Ox ('Oxheart'), Ch ('Cherry') and R ('Roma'). For 'Oxheart' exposed to: UV-A significant differences ($p<0.05$) were observed at days 3, 4 5, 6, 7, 8, 9; UV-B significant differences ($p<0.05$) were seen at days 5, 6, 8 and 9. For 'Cherry' no significant differences were found. For 'Roma' exposed to: UV-A significant differences ($p<0.05$) were shown at days 3, 4 and 5; UV-B significant differences ($p<0.05$) were shown at days 4, 5, 6 and 7.

UV-B 15min induced a delay in both 'Oxheart' and 'Roma' germination in the first days (*Figure II.1.*). In 'Oxheart' only 10% germination was achieved at day 3, while at day 9 values were close to those of UV-A condition. Germination of UV-B irradiated 'Roma' seeds was also delayed until day 7, recovering at day 9 to values close to the control (~96%).

3.2. Biomass and productivity

After 15 days of UV-A/B exposure, seedlings presented different growth responses according to the UV type supplementation and the cultivar. In general, UV-A increased the shoot and root length, and cotyledon area, except for the root length in 'Oxheart' and cotyledon area in 'Roma'. For example, the shoot length and the cotyledon area of 'Oxheart' UV-A irradiated seedlings was, respectively, 17% and 29% higher than those of the control. This increase in length/size was paralleled by an increase of fresh matter in UV-A irradiated seedlings (*Table II.1.*). In response to UV-A, dry matter increased in 'Cherry' and 'Roma' and decreased in 'Oxheart'. The percentage of water content had an opposite behaviour, with a decrease in irradiated 'Cherry' and 'Roma' and an increase in 'Oxheart'.

Table II.1. Growth of 'Oxheart', 'Cherry' and 'Roma' seedlings 15 days after being exposed to UV-A/B conditions. Shoot length (cm), root length (cm), leaf area (mm^2), fresh matter (gFM), dry matter content (g $kgFM^{-1}$) and percentage of water content (WC%) were measured for control, UV-A 2H and UV-B 15min. For the same condition, *, **, * and **** represent significant differences for p≤0.05, 0.01, 0.001 and 0.0001, respectively. Values are expressed as the mean ± standard deviation.**

cv.	Treatment	Shoot length cm	Root length cm	Leaf area mm^2	Fresh matter gFM	Dry matter g.kgFM-1	WC% %
Oxheart	Control	6.49±0.72	2.84±1.31	56.6±7.2	0.18±0.02	69.0±4.6	93.1±0.5
	UV-A 2H	7.61±1.06***	2.45±1.61	73.2±10.2****	0.27±0.04***	58.2±4.6*	94.2±0.5*
	UV-B 15min	5.45±0.80***	1.59±0.82**	32.0±6.5****	0.16±0.01	63.9±9.0	93.6±0.8
Cherry	Control	4.71±0.88	1.64±1.15	42.3±4.2	0.14±0.02	73.8±2.9	92.6±0.3
	UV-A 2H	4.97±1.02	1.72±1.38	47.7±5.9*	0.15±0.03	78.6±4.4	92.2±0.4
	UV-B 15min	4.31 ±0.71	3.87±1.12****	37.4±5.2	0.14±0.02	63.9±4.5	92.2±0.4
Roma	Control	5.53±1.19	2.21 ± 1.66	50.1±8.8	0.18±0.07	63.4±4.3	93.6±0.4
	UV-A 2H	6.22±0.99	2.76 ± 1.41	47.7±6.4	0.20±0.04	69.3±4.6	93.2±1.5
	UV-B 15min	5.46±0.78	3.27 ± 2.09	26.7±3.9****	0.18±0.02	55.9±2.7	94.4±0.3

Seedlings exposed to UV-B showed an overall decrease in shoot and root length, cotyledon area, dry matter and water content. 'Oxheart' shoot length and cotyledon area decreased 16% and 43% regarding the control, and in 'Roma' the cotyledon area was reduced 47%. As exception, the root length of 'Roma' and 'Cherry' was stimulated (48% and 135%, respectively) while in 'Oxheart' it decreased 44%. The soluble proteins contents were not affected while GS activity was in general reduced by UV-A and UV-B supplementation, except for 'Cherry' where the UV-A promoted a significant increase in the activity (*Figure II.2. a,b*).

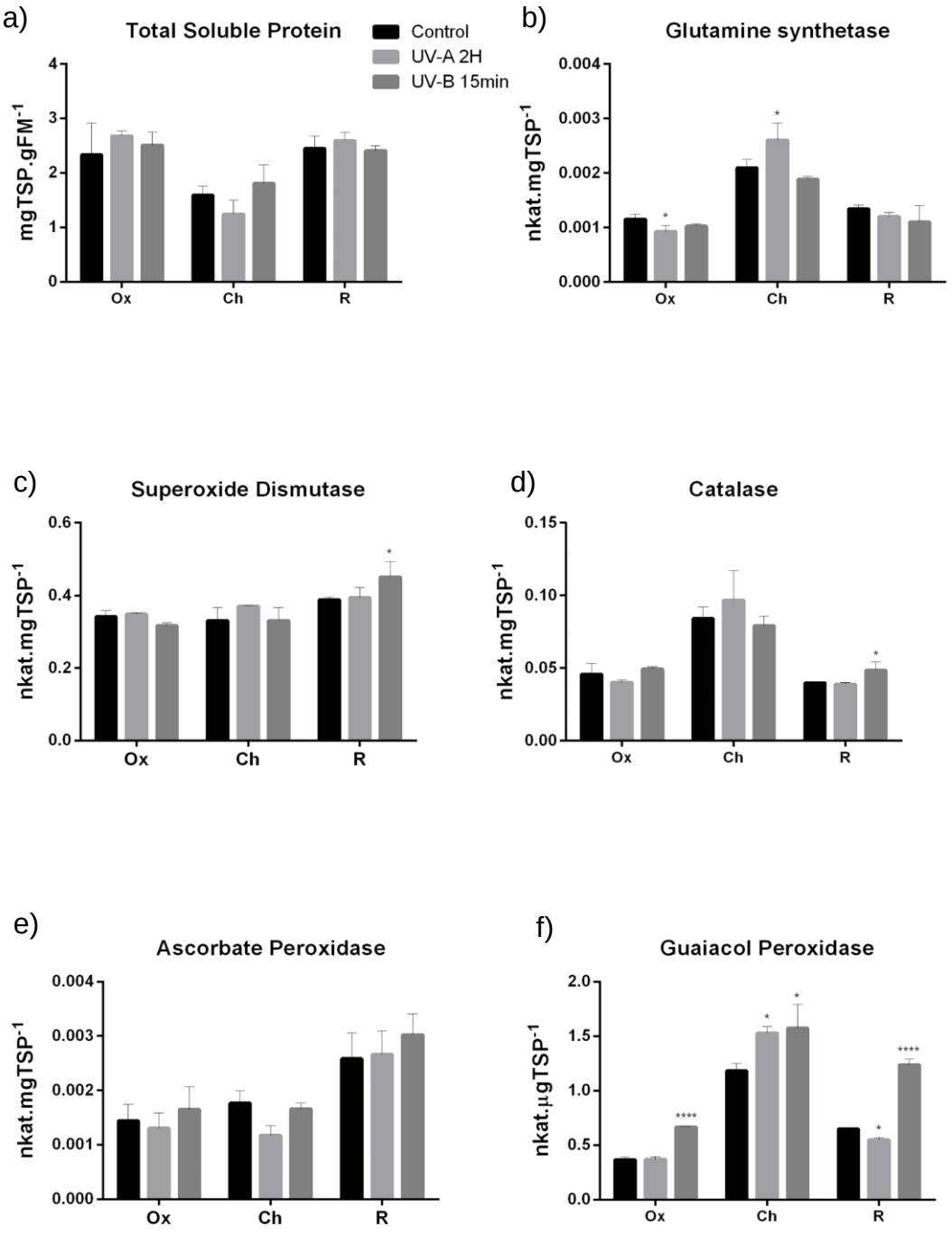


Figure II.2. GS and enzymatic scavenging of ROS activities of 'Oxheart', 'Cherry' and 'Roma' seedlings 15 days after being exposed to UV-A/B conditions. (a) total soluble proteins (TSP); b) glutamine synthetase (GS); (c) superoxide dismutase (SOD); (d) catalase (CAT); (e) ascorbate peroxidase (APX); and (f) guaiacol peroxidase (GPX). FM (Fresh Mass). Compared with the control for each cultivar, * and ** mean significant differences for p≤0.05 and 0.0001 respectively. Results are mean ± SD.**

3.3. Pigments, total soluble sugars and starch

Pigments concentration varies according to the cultivar and the UV-condition. In 'Oxheart' and 'Cherry', UV-A in general does not affect significantly the contents of chl*a,* chl*b*, only decreasing carotenoids in 'Cherry'. Contrarily, UV-B has a general trend to decrease chl*a*, chl*b* and carotenoids in all cultivars ($p<0.05$ for 'Cherry' and 'Oxheart') (*Table II.2.*). Contrarily, in 'Roma', the levels of these pigments were stimulated by UV-A. Anthocyanins, on other hand, show a trend to stabilize, or decrease with both UV-A/B ($p<0.05$ for 'Cherry'). Finally, these cotyledons show in general a high ratio of chl*a*/chl*b*. In spite of the variations of the chlorophylls levels, the ratios chl*a*/chl*b* were maintained stable in both 'Cherry' and 'Oxheart', only increasing in 'Roma' exposed to UV-A (*Table II.2.*).

Table II.2. Pigment levels in 'Oxheart', 'Cherry' and 'Roma' seedlings, 15 days after being exposed to UV-A/B conditions. Chlorophyll a and b (Chl a and Chl b) (mg gFM^{-1}), Chlorophyll ratio a/b (chl *a*/chl *b*), Carotenoids (µmol gFM^{-1}) and Anthocyanins (mg gFM^{-1}) were measured for control, UV-A 2H and UV-B 15min. For the same condition, *, **, * and **** represent significant differences for p≤0.05, 0.01, 0.001 and 0.0001, respectively. Values are expressed as the mean ± standard deviation.**

cv.	Treatment	Chl *a* mg.gFM^{-1}	Chl *b* mg.gFM^{-1}	Chl *a*/Chl *b*	Carotenoids mg.gFM^{-1}	Anthocyanins mg.gFM^{-1}
Oxheart	Control	1.34±0.08	0.113±0.008	11.9±0.6	0.481±0.028	0.295±0.010
	UV-A 2H	1.27±0.18	0.109±0.014	11.7±0.3	0.477±0.017	0.287±0.000
	UV-B 15min	1.09±0.05*	0.095±0.006*	11.5±0.8	0.400±0.017**	0.285±0.014
Cherry	Control	1.40±0.08	0.125±0.008	11.2±0.1	0.536±0.033	0.335±0.008
	UV-A 2H	1.31±0.13	0.118±0.013	11.1±0.2	0.476±0.044*	0.299±0.005***
	UV-B 15min	1.19±0.06*	0.103±0.006*	11.6±0.3	0.435±0.019***	0.316±0.013*
Roma	Control	1.09±0.09	0.098±0.006	11.1±0.3	0.407±0.032	0.305±0.008
	UV-A 2H	1.33±0.11*	0.102±0.004	13.0±0.5***	0.446±0.032	0.300±0.006
	UV-B 15min	1.01±0.02	0.095±0.003	10.7±0.4	0.402±0.008	0.307±0.006

With respect to the control, UV-A irradiation decreased starch levels in all cultivars, particularly in 'Cherry'. The response of soluble sugars was dependent on the cultivar, with a decrease in 'Cherry', an increase in 'Roma', and no changes in 'Oxheart' (*Figure II.3.*).

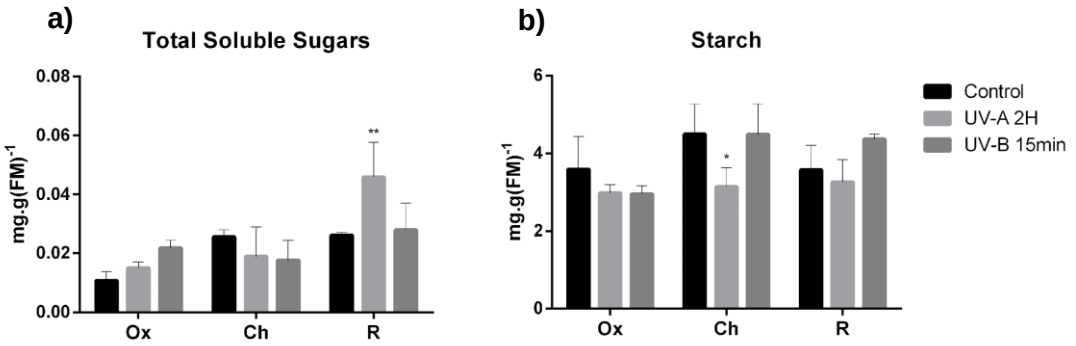


Figure II.3. The content of carbohydrates in 'Oxheart', 'Cherry' and 'Roma' seedlings 15 days after being exposed to UV-A/B conditions. Total soluble sugars (a) and starch (b) contents after exposure to control, UV-A 2H and UV-B 15min. All results are expressed as mg gFM^{-1}. FM (Fresh Matter). For the same condition, * and ** represent significant differences for p≤0.05 and 0.01, respectively. Results are mean ± SD.

3.4. H_2O_2 content, lipid peroxidation and cell membrane stability (CMS)

UV-A exposed 'Oxheart' and 'Cherry' seedlings decreased the levels of H_2O_2 (~20%), while this ROS was not affected in 'Roma'.

Table II.3. Quantification of MDA, H_2O_2 and membrane damage of 'Oxheart', 'Cherry' and 'Roma' seedlings 15 days after being exposed to UV-A/B conditions. Cell membrane stability (CMS) is expressed as percentage of membrane damage (%MD), malondialdehyde (MDA, $nmol.mL^{-1}.mgFM^{-1}$) and H_2O_2 ($mmol.gFM^{-1}$, using a standard curve). For the same condition, * and ** represent significant differences for p≤0.05 and 0.0001, respectively, when compared with the control. Values are expressed as average ± standard deviation.**

cv.	Treatment	H_2O_2 *$mmol.gFM^{-1}$*	MDA *MDA equivalents ($nmol.mL^{-1}.mgFM^{-1}$)*	CMS *%MD*
Oxheart	Control	277.1 ± 14.3	388.0 ± 92.3	3.9 ± 0.94
	UV-A 2H	215.9 ± 16.3****	311.5 ± 28.3	4.5 ± 1.03
	UV-B 15min	76.5 ± 7.4****	192.6 ± 32.7*	10.9 ± 4.69****
Cherry	Control	222.0 ± 41.5	254.7 ± 136.7	5.4 ± 0.70
	UV-A 2H	188.3 ± 9.1*	86.6 ± 25.6	6.8 ± 2.50
	UV-B 15min	195.5 ± 19.6	322.5 ± 57.0	6.9 ± 1.21
Roma	Control	323.3 ± 6.5	250.0 ± 110.0	3.8 ± 0.73
	UV-A 2H	330.4 ± 3.6	157.7 ± 17.2	4.1 ± 0.93
	UV-B 15min	175.8 ± 9.2****	352.4 ± 73.1	12.9 ± 1.19****

The H_2O_2 content was reduced by supplementation of UV-B in all cultivars (from ~15% to ~60%). MDA levels remained stable or showed a trend to decrease in response to both irradiation conditions ($p<0.05$ for UV-B; *Table II.3.*). Finally, CMS was only

negatively affected by UV-B, being effects more evident in 'Oxheart' and in 'Roma' (*Table II.3.*).

3.5. Antioxidant battery

Enzymatic antioxidant responses were dependent on both UV-type and cultivar (*Figure II.2.*). In general, 'Oxheart' and 'Roma' seedlings showed little or no effects in response to UV-A, being evident a decrease in GPX in 'Roma'. On other hand, in UV-A exposed 'Cherry' seedlings there was an increase of GPX ($p<0.05$) and a trend to increase SOD and CAT. All UV-B exposed cultivars showed increases of GPX, while SOD and CAT activities were only stimulated in 'Roma'.

The non-enzymatic battery (phenols and antiradical activity) was sensitive to both UV-A and UV-B. UV-A reduced the phenols levels in 'Cherry' and in 'Oxheart' ($p<0.05$), while UV-B increased their levels in 'Cherry' and 'Roma'. The antiradical activity was stimulated by UV-A and UV-B in all cultivars, with major increases in 'Cherry' and 'Roma' (*Figure II.4.*).

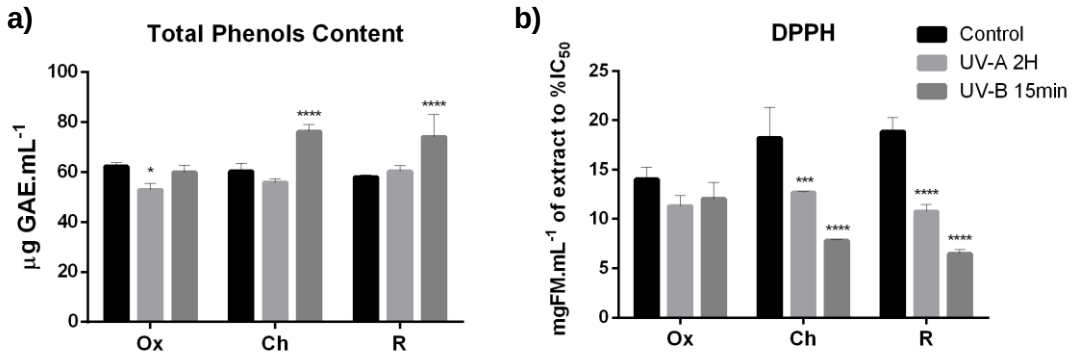


Figure II.4. Non-enzymatic antioxidant capacity of tomato seedlings of 'Oxheart', 'Cherry' and 'Roma' seedlings 15 days after being exposed to UV-A/B conditions. Total phenol content (μgGAE.mL^{-1}) (a), and antiradical activity [mgFM.mL^{-1} (b) of extract to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 50% (%IC50)]. FM (Fresh Mass) and GAE (Gallic Acid Equivalents). For the same condition, *, * and **** mean significant differences for p≤0.05, 0.001 and 0.0001 respectively. Results are mean ± SD.**

3.7. Multivariate analyses

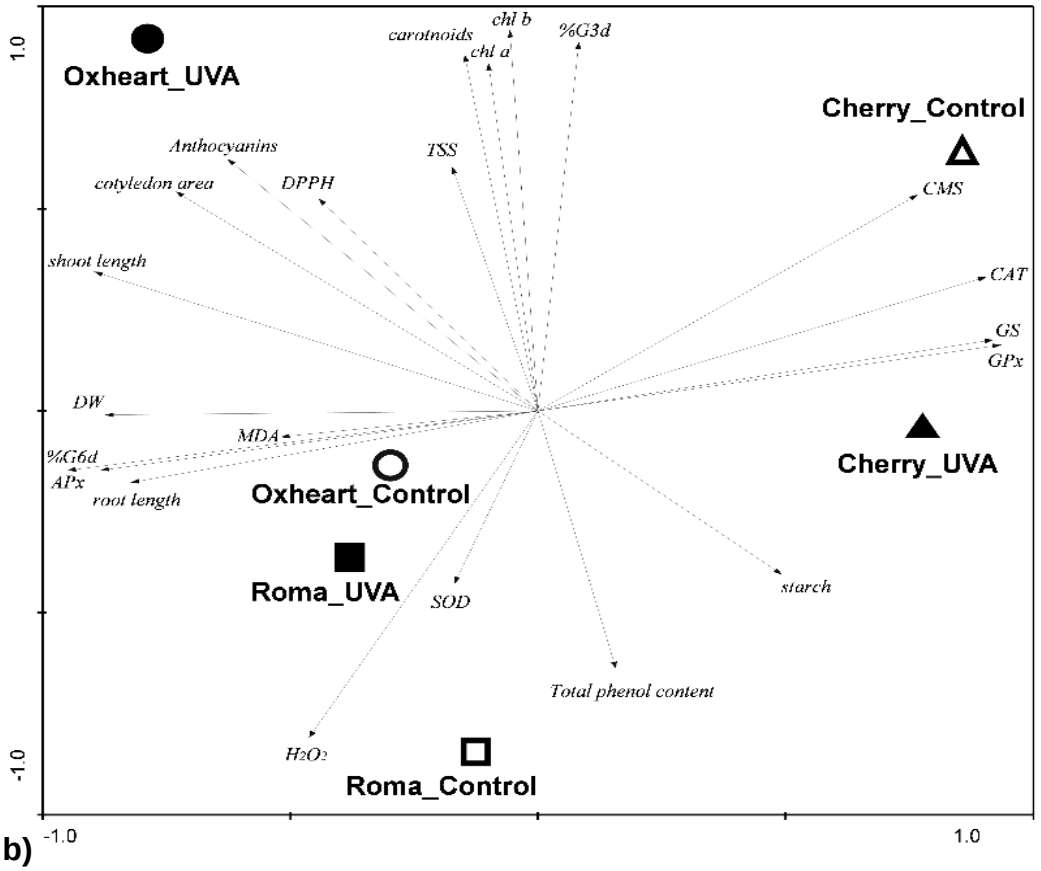
Principle component analysis (PCA) regarding the impacts of UV-A and UV-B on the three cultivars are depicted in *Figure II.5*. For UV-A, there was a clear separation between cultivars (*Figure II.5. a*) and the way they responded to UV-A. In *Figure II.5. a*, PC1 explained 44.2% of the variance and PC2 explained 32.4%. Three distinct groups

can be identified: 1) 'Oxheart' control and UV-A groups are relatively close, although in two different quadrants, being evident that UV-A promoted shoot length, cotyledon area, anthocyanins and antiradical scavenging (DPPH); 2) 'Cherry' control and UV-A groups are also close (right quadrants), but UV-A increased enzymatic (CAT, GPX) and non enzymatic (phenols) antioxidant battery and GS, as well as starch content; 3) 'Roma' control and UV-A treatments are also very close in the same quadrant (left down) being evident that in this cultivar UV-A exposure is correlated with increases of H_2O_2 content and SOD and APX activities, and a stimulation of germination at day 6.

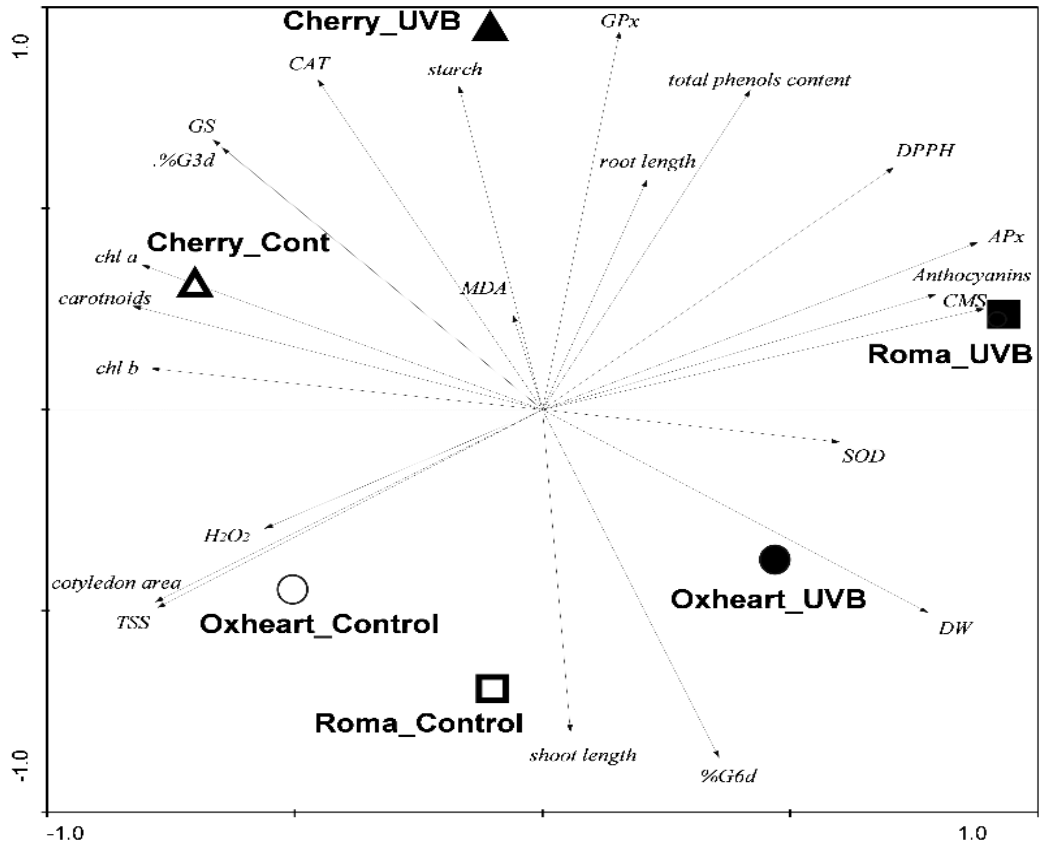
The PCA regarding the impacts of UV-B shows higher differences, being evident that all three controls are closer and placed in the left (group 1, *Figure II.5. b*), and all exposed cultivars show a deviation to the up-right of the *Figure II.5. a* (group 2). In *Figure II.5. b*, PC1 explained 39.3% of the variance and PC2 explained 32.7%. For the group 1 (controls), total soluble sugars, cotyledon area, H_2O_2 content, chl (*a* and *b*), carotenoids, germination at day 3 and GS activity were higher. On the other hand, the group 2 (exposed to UV-B) showed an overall increase of SOD, CAT, APX and GPX activities, and of root length, antiradical scavenging (DPPH), total phenols content, CMS, MDA and dry matter. Interestingly, it is also evident that both 'Cherry' control and exposed populations are close (in the same quadrant), whilst the stimulation of the CAT activity, MDA and starch contents induced by UV-B.

Figure II.5 (next page). PCA analysis of functional responses of three cultivars of tomato seedlings ('Oxheart', 'Cherry' and 'Roma') exposed to UV-A 2H and UV-B 15min during 15 days. a) comparative analysis between the control and UV-A-exposed plants for all cultivars. b) comparative analysis between the control and UV-B-exposed seedlings for all cultivars. The symbols ○, △, □ represent the 'Oxheart', 'Cherry' and 'Roma', respectively, in figure a) black symbols represent the UV-A treatments and for b) represent the UV-B treatments. White symbols represent in all figures the control treatments. Abbreviations: Percentage of germination in 3rd day (%G3d), the percentage of germination in 6th day (%G6d), dry weight by gram of fresh mass (DW). For other abbreviation see text.

a)


Oxheart_UVA
carotnoids
chl b
chl a
%G3d
Cherry_Control
Anthocyanins
TSS
cotyledon area
DPPH
CMS
shoot length
CAT
GS
GPx
DW
MDA
%G6d
APx
root length
Oxheart_Control
Cherry_UVA
Roma_UVA
SOD
starch
Total phenol content
H2O2
Roma_Control
1.0
-1.0
-1.0
1.0

b)


Cherry_UVB
GPx
CAT
starch
total phenols content
GS
%G3d
root length
DPPH
Cherry_Cont
chl a
APx
Anthocyanins
carotnoids
MDA
CMS
Roma_UVB
chl b
SOD
H2O2
cotyledon area
Oxheart_UVB
TSS
Oxheart_Control
DW
Roma_Control
shoot length
%G6d
1.0
-1.0
-1.0
1.0

4. Discussion

Germination and seedlings growth is a critical stage in tomato horticulture. We studied here if moderate doses of both UV-A/B types may have beneficial effects during this stage, not causing significant disorders in the antioxidant capacity and metabolism of the seedlings.

Seeds' responses to UV-A 2H and UV-B 15min depended on the cultivar ('Oxheart', 'Cherry' and 'Roma') and on the UV-type. Overall, UV-A 2H accelerated and synchronized germination namely for 'Oxheart' and 'Roma' seeds. Victório *et al.* (2010) working with *Senecio cinereia* seeds also showed stimulation of both synchronization and acceleration of germination. Nangle *et al.* (2012) showed that seeds of *Poa pratensis* L. daily supplemented with ~1.03 J m^{-2} s^{-1} UV-A also showed higher synchronization. On the other hand, UV-B 15min in general delayed germination, namely for 'Oxheart' and 'Roma' seeds, whilst not compromising the final germination rate in all cultivars. Sugimoto *et al.,* (2013) demonstrated that an irradiation of ~2 J m^{-2} s^{-1} promoted a species' dependent response, with a delay of germination in e.g., eggplant, lettuce, pea and spinach, and an acceleration in buckwheat and carrot. A similar species dependency was reported by Noble *et al.* (2002) in others species (e.g., red Russian kale and cherry bell reddish).

Tomato seedlings demonstrated, in general, an increase of biomass in response to UV-A 2H, shown by an increase in the shoot and root length, cotyledon leaf area, and fresh and dry matter content. These changes promoted the seedlings vigor and are in line with others reports of Brazaityte *et al.* (2010) who showed that UV-A supplementation after transplantation increased the plant height, fresh and dry weight. In other work, Brazaityte *et al.* (2015) demonstrated that the same type of supplementation increased leaf area and fresh weight in microgreens. UV-A stimulated biomass production in *Laurus nobilis* (Bernal *et al.,* 2015) and leaf area in *Crespis japonica* seedlings (Constantino *et al.,* 2017). In tomato, UV-B had an opposite impact on biomass production, as consequence a loss of the shoot length, leaf area and dry matter, and thus loss of vigor. Similar consequences were found in UV-B irradiated soybean that had lower biomass (Hu *et al.,* 2013), and in *Conocarpus lancifolius*, *Picea asperata* and *Monordica charantia* that also showed decreased plant height and leaf area (Han *et al.,* 2009; Mishra *et al.,* 2009; Suleman *et al.,* 2014).

Pigments play important functions namely in photosynthesis (e.g., chl*a*, chl*b* and carotenoids) and in photoprotective systems (e.g., carotenoids and anthocyanins). Tomato seedlings exposed to UV-A and UV-B showed different sensitivities to each

radiation according to the cultivar. In general UV-A showed a tendency to increase ('Roma') or not affect ('Cherry', 'Oxheart') the levels of chlorophylls and carotenoids. A stimulation of pigment levels was also observed for other UV-A irradiated plants, such as microgreens (Brazaityte *et al*., 2015) and lettuce (Caldwell and Britz, 2006). Also for tomato seedlings, Gou and Wang (2010) demonstrated that a supplementation with UV-A increased the content of anthocyanins, suggesting that this wavelength does not play, at moderate levels, a dramatic impairment on the pigments metabolisms and/or their structure or that, by increasing their levels, may stimulate a photoprotective mechanism for the plant. Also, the increase of chl*a*/chl*b* indicates a substantial investment in the pigment chl *a*. This is particularly relevant considering that chl *b* synthesis and degradation involve an intermediate stage of chl *a*, and changes in *chl b* anabolism or catabolism may justify the higher chl*a*/chl*b* ratios in UV-irradiated cotyledons. Interestingly, all cotyledons showed higher ratio of chl*a*/chl*b* than usually reported for plants. High ratios have been reported for cotyledons of other species such as quinoa (González *et al.,* 2009). Also, changes in the chlorophyll content of greening seedlings of Arabidopsis showed a shift in the ratio from 5x to ~10x after 24 hours incubation in the dark (Meguro *et al.,* 2011).

Contrarily to the moderate UV-A supplementation, the moderate UV-B supplementation used here reduced in 'Oxheart' and 'Roma' the levels of photosynthetic and photoprotective pigments, indicating a breakdown in the synthesis of these pigments as reported in other species, such as soybean (Hu *et al.,* 2013), *Picea asperata* (Han *et al.,* 2009) and *Monordica charantia* (Mishra *et al.,* 2009). Also, Khudyakova *et a*l., (2017) reported that in *Arabidopsis thaliana* growing under UV-B, the contents of chl (*a*;*b*) and carotenoids were reduced. This decrease of the photosynthetic pigments may represent a photoprotective mechanism against damages and impairments of photosynthesis caused by UV-B. The lower production of biomass may be a consequence of this susceptibility to the supplemented UV-B dose.

The tendency of UV-A to increase the biomass and photosynthesis is paralleled by an increase of total soluble sugars for 'Oxheart' and 'Roma', which may result from the stimulated photosynthesis and the consumption of starch that is an abundant reserve in few-day-old seedlings. In fact, a decrease of starch content was found, suggesting the normal consumption of this reserve carbohydrate, considering also that cotyledons play a crucial role of promoting the sugars translocation to others tissues/organs (Lemoine *et al.,* 2013). The fact that GS is not compromised by UV-A, and considering this enzyme is crucial to the N mobilization and the balance of glutamine and glutamate in

the plant supports that UV-A dose used here does not compromise the normal metabolism of the seedling.

Excessive UV radiation is well known to promote oxidative stress, but the moderate doses of UV, in particular UV-A, proposed here for seed technology do not promote negative impacts or damages. The H_2O_2 content was reduced by moderate UV-A and UV-B supplementation, demonstrating a possible activation of the antioxidant battery by the moderate doses used. Curiously, the decrease of the H_2O_2 content may be explained by the stimulation of the antioxidant battery in UV-A exposed seedlings, also occurred in UV-B, which is not supported by data of other species, namely, rice (Fedina *et al.,* 2010), lettuce (Aksakal *et al.,* 2016), *Cassia auriculata* (Agrawal, 2007) pea and wheat (Alexieva *et al.,* 2001). It should however be noticed that the UV-B intensity (doses and irradiation period) used here (2.94 J m^{-2} s^{-1} by 15 minutes a day during 15 days) was much lower than the ones used by those authors: 0.8 J m^{-2} s^{-1} by 5 hours day, 3.3 J m^{-2} s^{-1} by 12 hours, 2.60 J m^{-2} s^{-1} (50 and 100 minutes day) and 6.8 J m^{-2} s^{-1} by 2 hours day, respectively.

The decreased levels of H_2O_2 for both UV-A 2H and UV-B 15min support the general decrease of MDA levels in all cultivars, and in UV-B exposed ‘Oxheart’ and the absence of lipid peroxidation increases in the other cultivars. Our data for UV-A support those on *Brassica napus* and *Portulaca oleracea* that showed no changes in MDA after UV-A supplementation (Nasibi, 2005), Peykarestan *et al.* (2012). These results do not support those on lettuce (Aksakal *et al.,* 2016), soybean (Abdel-Kader *et al.,* 2007) and tomato (Balakumar *et al.,* 1997) where MDA increased in response to excessive UV-B, but again the doses used by those authors were much higher than the ones used in this work.

Thus we may conclude that lipid peroxidation and ROS production (H_2O_2) were in general reduced by both UV-A/B doses tested here, which supports an efficient response of the antioxidant batteries activated by ROS-inducing by moderate UV supplementation. However, the increased damages in ‘Oxheart’ and ‘Roma’ in response to UV-B suggest some damages at the cell level, and also demonstrate that ‘Cherry’ is the most resistant cultivar to UV-B.

The enzymatic battery showed, in general, a synchronized response to UV-A without changes for ‘Oxheart’ and ‘Roma’ and an increase in ‘Cherry’. UV-B had a tendency to increase these activities in ‘Oxheart’ and ‘Roma’ and decrease in ‘Cherry’, again showing that the antioxidant battery to UV-B is cultivar dependent. The increase of the enzymatic battery under UV-B supports previous data also on tomato (Balakumar *et*

al., 1997) and cucumber seedlings (Rybus-Zajac and Kubis, 2010). Moreover, as the peroxidases suffer a special stimulation, this may justify the reduction of H_2O_2 content and the low values for MDA. However, the enzymatic antioxidants may not be sufficient *per se* to scavenge the ROS, and the significant increase in UV-A/B of the phenols content, and of antiradical activity indicate a non enzymatic mechanism that also contributes to the reestablishment of plant's performance and vigor. For UV-A, this stimulation to increase the antiradical activity and total phenols content was described in microgreens (Braizaityte *et al.,* 2015) and *Crepis japonica* (Constantino *et al.,* 2017). Similarly, UV-B also increased the non-enzymatic battery in several crops (Agarwal, 2007; Aksakal *et al.*, 2016; Alexieva *et al.,* 2001; Balakumar *et al.*, 1997). Considering the nutritional value of most phenols, our data also support the relevance of applying UV-A/B in edible seedlings, including microgreens.

5. Conclusions

The use of physical treatments are currently emerging as an alternative or complement to chemical ones, usually less environmentally friendly and more prone to be toxic to plants and consumers. This work demonstrates the benefits of using UV supplementation, particularly UV-A, during seed germination and seedling development.

The benefits from using a moderate UV-A dose as the one used here (2H/day) may be seen in the acceleration and/or synchronization of seed germination, and in the increase of seedlings vigor. The fact that only two (in three) cultivars ('Oxheart' and 'Roma') were more responsive to UV-A dose used, supports that responses are cultivar dependent, and that the most efficient dose must be adapted to the cultivar. We also demonstrate that the UV-B 15min showed a delay of the germination and cause an impairment of seedling growth that may affect the photosynthesis. The UV-B supplementation is also a stimulator of the antioxidant activity. Nevertheless, 'Cherry' demonstrated overall to be less susceptible to UV-B light.

Compared to UV-B, the UV-A light supplementation presented here has a higher potential to be used in nurseries, once the plants presented a higher vigor. On the other hand, these UV-A lamps are economically friendly for producers, representing a low-cost investment. These results also provide a valuable contribution to the use of UV-A/B supplementation in "horticultural suitable doses" in line with the new paradigm of sustainable protected horticulture to produce more and better crops with fewer and more environmentally friendly resources.

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III. Chapter 3: Do benefits of UV-A/B exposure during tomato fruiting compensate photosynthetic impacts?: a physiological contribution

Abstract

The supplementation of ultraviolet (UV) radiation in protected horticulture may promote a metabolic shift in crops' performance, putatively improving yield and nutritional/sensorial properties. There is a need to establish the adequate UV light quality and intensity, which may stimulate beneficial traits while not promoting detrimental effects. *Solanum lycopersicum* is one of the most important crops produced in greenhouses, and would benefit from UV supplementation provided by affordable UV-systems. The present work aimed at studying the impact of supplementation with moderate doses of UV-A (1 h and 4 h per day) or UV-B (2 min or 5 min per day) on tomato yield and carbon metabolism during fruiting. After 30 days of daily irradiation, UV-B induced some necrotic spots and structural decreases in the LHC-pigments. The moderate UV-A treatments stimulated flowering and fruiting, paralleled by no visible leaf damages, and the impact on photosynthesis was mostly related with functional changes, in a dose dependent manner. UV-A doses decreased the maximum efficiency of photosystem II (F_v/F_m) and the effective efficiency of photosystem II (Φ_{PSII}), and gas exchange processes, including net carbon assimilation (P_N). Photosystem II (PSII) and RuBisCO related transcripts were highly stimulated by UV, but the maintenance of the RuBisCO protein levels suggests that some RuBisCO is also degraded. End products of photosynthesis (soluble sugars and starch) remained stable. We propose that the decrease of effective efficiency of photosystem II (Φ_{PSII}), may induce lower ATP/NADPH supply to the Calvin cycle, but not sufficient to compromise the yield. Thus, we demonstrate here distinct targets of the photosynthetic machinery to UV-A and to UV-B, and that daily application of UV-A lamps in greenhouses significantly stimulate fruiting, and may become a relevant tool in protected horticulture.

Keywords

Fruiting, horticulture, photosynthesis, *Solanum lycopersicum*, ultraviolet supplementation

1. Introduction

Solanum lycopersicum L., tomato, is one of the most popular and consumed crop species, with major agricultural and economic importance (Žižková *et al*., 2015). In 2014, more than 170 million tonnes of tomato fruit were produced worldwide (www.faostat.org). Its excellent acceptance by the consumers is due to its multiple gastronomic uses and to other features like its taste, colour and high nutritional value (Verma *et al*., 2015).

Nowadays, tomato production includes different models such as open field, greenhouses, glasshouses and vertical horticulture. These production models can have natural light with or without total or partial blocking UV-filter (Tsormpatsidis *et al.,* 2008), solar radiation supplemented with lamps, and lamps as the only source of light (e.g., Wu *et al*., 2014). Tomato production outdoors, which is largely used in pulp industry, allows plants to grow naturally adapted to the solar UV radiation. Contrarily, in protected tomato cultures (mostly dedicated to table tomato), UV-exposure is usually neglected. Further on, while fruits and vegetables can be obtained in greenhouses often out of the season, they are described as having low nutritional and organoleptic attributes (Muñoz *et al*., 2007).

Recently, a new agricultural paradigm is emerging, which considers UV-supplementation as a strategy to improve protected crops' yield and/or quality (Barnes *et al*., 2015; Gil *et al*. 2013). Besides, UV-irradiation systems do not pose legal restrictions, and some (e.g., UV-A) are easily affordable. Controlled UV-supplementation can be a powerful tool not only to control crops' pests and diseases but also, by promoting the synthesis of defence compounds, to increase the nutritional quality of the edible parts (e.g., Nelson and Bugbee, 2014). However, the industrial use of UV-A or UV-B light in horticulture requires that the period and light quality/intensity to be used are optimized according to the crop's requirements not compromising any part of its life cycle.

When plants are exposed to harmful light intensity or quality (PAR and UV), they develop defence mechanisms mediated by molecular receptors that protect them against possible cell damages (Lake *et al*., 2009). Photoreceptors sense and transduce light signals through distinct intracellular signalling pathways to generate a wide range of responses. Most of these responses are triggered by modulating the expression of hundreds of light-regulated genes, which ultimately lead to adaptive changes at the cellular and systemic levels (Yokawa *et al*., 2015).

Plants exposed to UV rays, especially to UV-B, suffer several biological changes, some of which can be observed in the phenotype. Most studies have focused on the exposure to excessive radiation, which leads to multiple effects including decreased growth and productivity, and leaf chlorosis and/or necrosis (Lake *et al.,* 2009). Also, high UV-A/B levels may injure the nucleic acids, increase oxidative damage of macromolecules (Brazaitytė *et al*., 2015) and may decrease (Araújo *et al*., 2016) or not impact (Reyes-Diaz *et al.,* 2016) photosynthesis. Two major approaches are used to ascribe the stress impact on photosynthesis. The first measures the state of the photosystem II (PSII), i.e. the extent to which PSII is using the absorbed energy and the extent of its damage. This is given by the chlorophyll fluorescence in the form of several interrelated fluorescence intensity and quenching parameters (including photochemical and nonphotochemical quenching). The second approach evaluates processes related with the Calvin cycle including gas exchange: transpiration (*E*), intercellular CO_2 concentration (*Ci*), net CO_2 assimilation (P_N) and stomatal conductance (g_s). Also, the quantification of ribulose bisphosphate carboxylase oxygenase (RuBisCO) protein content/activity or its transcripts (e.g., *rbcS* and *rbcL*) is widely used as an indicator of Calvin cycle under stress (e.g. Dias *et al.,* 2013; Dias *et al.,* 2016; Nouri *et al.,* 2015). UV-modulation of RuBisCO may occur at the transcriptional or post-transcriptional levels and, for example, excess UV-B irradiation inhibited RuBisCO activity (Araújo *et al.,* 2016; Viuda-Martos *et al.,* 2014).

For similar doses, UV-B is more deleterious than UV-A, but it was demonstrated that exposure to higher levels of UV-A may lead to responses similar to those induced by lower levels of UV-B (Brazaitytė *et al.,* 2015). However, physiologically tolerable levels of UV-A/B radiation may induce metabolic shifts in plants without negative consequences (Machado *et al.,* 2017), and may potentially be used in agro-industry for improving plants' performance and/or increase crops' richness in nutritionally valuable compounds. Moreover, under the new paradigm of circular economy, the UV-enriched byproduct canopy may be economically used in agricultural industry (Aires *et al.,* 2017; Viuda-Martos *et al.,* 2014).

Currently, there are many gaps in the knowledge of the ideal UV doses (quality and period) necessary to obtain fruits and vegetables with the best quality (reviewed by Huché-Thélier *et al.,* 2016). The collected data is scarce and restricted to empirical approaches, to a low number of species, and to disperse exposure conditions (e.g., time of exposure, plant phase and wavelength). Wargent *et al.* (2015) hypothesised that UV-B can be a new tool of agronomical production by leading to metabolic

changes in plants. However, UV-B lamps are less safe and more expensive than UV-A lamps (e.g., black light, leds). UV-A systems may therefore represent an affordable solution for large scale UV-supplementation in protected horticulture.

We aim at demonstrating the agronomic benefits of using moderate UV radiation during crops growth, stimulating yield without compromising vegetative performance. We'll use tomato as a case study, and will follow how different UV-A/UV-B supplements, applied during flowering/fruiting, will affect photosynthesis and fruit yield. This approach will allow to identify most suitable UV quality and dose, and at what extent UV-A and/or UV-B supplementation may have beneficial effects in fruit production without compromising plant's growth and carbon metabolism.

2. Material and Methods

2.1. Plant material, culture conditions and UV treatments:

Seeds of *Solanum lycopesicum* L. cv. MicroTom (from JustSeed, UK) were germinated in plastic pots (fifty pots with ten plants for each condition) with 0.3L of peat:perlite (2:1) substrate. Plants were grown under controlled conditions, at a photosynthetic photon flux density (PPFD) of 200 µmol m^{-2} s^{-1} of (Fluorescent light by OSRAM L 30W/77 FLUORA lamps), 23±2 ºC, 45±5% relative humidity and 16h:8h light:dark photoperiod. Plants were watered with Hoagland medium with pH adjusted to 5.7±0.05. After 90 days, plants started flowering, reaching maximum synchronization at ~ day 100. From day 100 to day 130, five groups of similar plants (in length and flowering stage) were further supplemented with different UV-radiation: Control Group: plants were maintained under the same irradiation conditions, with no UV supplementation; UV-A 1h Group: plants were exposed for 1 h per day to 0.80 W m^{-2} UV-A supplied by black light lamps (F20T12/BLB - 20W T12 (T10) Fluorescent Blacklight Blue, with a peak wavelength at 368 nm); UV-A 4h Group: plants were exposed for 4 h per day to 0.80 W/m^2 UV-A, supplied by the same lamps; UV-B 2min Group: plants were exposed for 2 min per day to 2.94 W m^{-2} UV-B, supplied by six 312 nm TFP-M/WL 8W lamps; UV-B 5min Group: plants were exposed for 5 min per day to 2.94 W m^{-2} UV-B, supplied by six 312 nm TFP-M/WL 8W lamps. UV-A and UV-B light intensity was measured by sensor Meters PHILP HARRIS (serial number: 4375 model SEL240) and International Light INC (Newbryport, Massachusetts, model: 01950, IL1400A), respectively.

2.2. Plant Growth and Water Status:

The total number of flowers and fruits, the shoot length, the dry mass/fresh mass ratio (DM/FM) and water content [%WC=((FM-DM)/FM)*100] were determined at the end of the experiment. For weight determination, six upper leaves with the same age, from different plants of the same condition were analysed. Also, other morphological aspects (e.g., senescence, chlorosis, necrotic spots) were registered.

2.3. Photosynthetic pigment quantification:

For each condition, three independent leaf pools were used as replicates. Each pool consisted of 10 leaves (from 2^{nd}-3^{rd} upper node) collected from different plants. Pigments were extracted with acetone: 50 mM Tris buffer (80:20, v/v) and centrifuged for 10 minutes at 10000xg at 4ºC. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Car) and anthocyanins contents were quantified by reading the absorbance at 470, 537, 647 and 663 nm in a multiplate reader Thermo Fisher Scientific Spectrophotometer (with three technical replicates per sample) (Dias *et al.*, 2013; Sims and Gamon, 2002).

2.4. Gas exchange and PSII efficiency:

Photophosphorylation pathway was assessed by measuring the minimal fluorescence yield of dark-adapted leaves with all PSII centers open (F_0) in 30 min dark-adapted developed leaves by applying a weak modulated light. Afterwards, by applying a saturating pulse of white light for 0.7 s, the maximum fluorescence (F_m) was assessed. Then, plants were adapted to light, and during 30 s the steady-state fluorescence (F_s) was averaged, followed by exposure to saturating light for 0.7 s to determine the maximal fluorescence (F_m'). The minimal fluorescence (F_0') was determined when actinic light was turned off. The variable fluorescence (F_v) were calculated from the differences of F_m - F_0. Also, maximum efficiency of PSII [$F_v/F_m = (F_m-F_0)/F_m$], maximum efficiency of PSII photochemistry in the light, if all centres were open [$F_v'/ F_m'=F_m'–F_o')/F_m'$], effective efficiency of PSII [$\Phi_{PSII}= (F'_m- F_s)/ F'_m$], photochemical quenching [$qP=(F'_m-F_s)/(F'_m-F'_0)$] and non-photochemical quenching [$NPQ= (F_m-F'_m)/F'_m$] were calculated according to Maxwell and Johnson (2000) and Murchie and Lawson (2013).

For gas exchange analysis, the portable photosynthesis system (LI-COR 6400) was used. Measurements took place under atmospheric CO_2 concentration and under a saturating PPFD (Photosynthetic Photon Flux Density) of 200 µmol m^{-2} s^{-1}. Individual

parameters, such as transpiration rate (E, mol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s), net photosynthetic rate (P_N, µmol m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i, ppm) were determined according to Machado *et al.* (2017). Also, the intrinsic water-use efficiency $\left(iWUE = \frac{PN}{gs}\right)$ was calculated.

Gas exchange and chlorophyll *a* fluorescence parameters were measured on *S. lycopersicum* plants (six plants per treatment) at the middle of the light period, in the youngest and fully developed leaves.

2.5. Carbohydrate content and RuBisCO relative quantification:

Total soluble sugars (TSS) content was measured by using the anthrone method, quantified by a multiplate reader Thermo Fisher Scientific Spectrophotometer (Dias *et al.,* 2013). For carbohydrate quantifications, four replicates of leaf pools from 7-10 plants, were used.

Leaf soluble proteins were extracted and quantified by Bradford method (Sigma-Aldrich, USA). To assess RuBisCO subunits amount, 15 µg of protein was separated by SDS-PAGE and gels were stained with 0.25% of Coomassie Brilliant Blue R250 as described by Li *et al.* (2013). Protein bands were analysed by comparison with a protein molecular weight marker (Fermentas, SM0441). Relative RuBisCO content was performed by isolating the bands of the large and small subunits for each sample and overnight incubation in formamide (2 mL) at 50ºC. Absorbance was measured at 595 nm and the results were expressed as ABS_{RC}/ABS_{TPC}, in which RC is the RuBisCO content and TPC is the total soluble protein content.

2.6. Gene expression:

Total RNA of tomato leaves was isolated using PureZOL™ RNA Isolation protocol (Bio-Rad), following the manufacturer's instructions. For Reverse Transcriptase-PCR, RNA samples were treated with DNAse using Deoxyribonuclease I, Amplification Grade (Invitrogen™). First-strand cDNA was synthesized from 1 µg cleaned total RNA using NZY First-Strand cDNA Synthesis Kit, no oligos, NZYTech™, subsequently treated with 1 µL NZY RNase H, diluted with Milli-Q water and stored at -20 ºC.

Primers of two housekeeping genes (Dzakovich *et al.,* 2016; Løvdal and Lillo, 2014) were used: the elongation factor 1alpha (*ef1*): TGGCCCTACTGGTTTGACAACTG (forward, f) and CACAGTTCACTTCCCCTTCTTCTG (reverse, r) and ubiquitin (*ubi*)

gene: GGACGGACGTACTCTAGCTGAT (f) and AGCTTTCGACCTCAAGGGTA (r). For photosynthetic gene expression, we used genes coding for PSII proteins: D1 (*psbA*): TGGATGGTTTGGTGTTTTGATG (f) and CCGTAAAGTAGAGACCCTGAAAC (r); CP47 (*psbB*): CCTATTCCATCTTAGCGTCCG (f) and TTGCCGAACCATACCACATAG (r). Primers for the two genes encoding RuBisCO subunits were selected: large subunit (*rbcL*): ATCTTGCTCGGGAAGGTAATG (f) and TCTTTCCATACCTCACAAGCAG (r); and small subunit (*rbcS*): TGAGACTGAGCACGGATTTG (f) and TTTAGCCTCTTGAACCTCAGC (r).

The RT-qPCR reactions were conducted in a Bio-Rad (CFX96 Touch™, USA), using 2.5 µL of total first-strand cDNA, 5 µL of enzyme (iTaq™ Universal SYBR® Green Supermix, Bio-Rad), and 2.5 µL of primers, in a total volume of 10 µL. Amplifications were standardized, using the following conditions: 95 °C for 1 minute followed by 60 cycles of 3 seconds at 95 °C and 30 seconds at 60 °C. The melting curve analysis ranged from 10 seconds to 95 °C with increased temperatures by 65 ºC in 5 seconds per cycle.

2.7. Statistical analysis:

Except when mentioned otherwise, experiments used in each condition 7-10 plants, treated as individual samples, or treated as pools (pigment determination and gene expression), each with at least 3 independent technical replicates. Values are presented as mean ± standard deviation. Comparisons between the different treatments and control were made using One Way ANOVA test. When data was statistically different, the Dunnett Comparison Test ($p<0.05$) was also applied. Graphpad™ Prism 6 was used. Multivariate analyses for data correlation used Principal Component Analysis and were performed with CANOCO for Windows v4.02 programme.

3. Results

3.1. Plant growth and water status:

Thirty days after the beginning of the UV treatments, the shoot length of control plants reached 20.6±2.4 cm. Shoot length was decreased by UV, in particular UV-A 4h and UV-B 2min, where the decrease reached around 20% compared with the control (*Table III.1.*). In control and UV-A conditions, plants looked healthy and leaves showed no significant chlorosis or necrosis. UV-B plants looked similar, although with an increased

number of necrotic spots. For UV-A 4h and UV-B 2min conditions, a significant decrease of the %WC was observed (*Table III.1.*), and the dry matter [DM, expressed as mg per g fresh matter (FM)] increased, with significant differences for UV-A 1h and UV-B 5min (*Table III.1.*). Regarding the effects on flowering and fruiting, while there were no significant changes in the total number of flowers, an increase in the number of fruits in plants exposed to both UV-A and UV-B was observed (significant differences for UV-A 1h and UV-B 2min). The total flowers+fruits also increased in both UV-A treatments (*Table III.1.*).

Table III.1. Growing and fruit production of *S. lycopersicum* plants exposed for thirty days to different UV conditions. Plant length (cm), leaf dry matter per unit of fresh matter (g.gFM^{-1}), water content (%), number of flowers, of fruits and of flowers + fruits were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. For the same condition, * and ** represent significant differences for p≤0.05 and 0.01, respectively. Values are expressed as the mean ± standard deviation (n=10).

Treatment	Plant length	WC (%)	Leaf dry matter	Flowers	Fruits	Flowers + Fruits
Control	20.6 ± 2.4	95.07 ± 2.62	0.139 ± 0.061	15.0 ± 6.3	15.7 ± 7.9	30.7 ± 6.4
UV-A 1h	19.4 ± 2.5	93.48 ± 0.95	0.204 ± 0.015*	11.9 ± 5.2	31.3 ± 8.1**	43.2 ± 10.0*
UV-A 4h	16.4 ± 3.8*	91.47 ± 0.95**	0.185 ± 0.023	15.8 ± 7.0	25.8 ± 12.0	41.6 ± 11.9*
UV-B 2m	16.6 ± 2.3*	91.65 ± 0.83**	0.194 ± 0.021	10.2 ± 4.8	30.8 ± 12.5**	41.0 ± 12.7
UV-B 5m	17.7 ± 3.3	93.61 ± 1.10	0.216 ± 0.028**	12.4 ± 6.7	22.3 ± 9.4	34.7 ± 9.0

3.2. Pigment levels and chlorophyll fluorescence:

UV-B irradiation was more effective on increasing the levels of photosynthetic pigments (Chl *a*, Chl *b* and carotenoids) than UV-A, being the increase of UV-B 5 min condition significant ($p<0.01$). The increases of chl *a* were slightly higher than those of chl *b* increasing in general the chl *a*/chl *b* ratio in response to UV, particularly in the plants exposed to UV-B 2min. On the other hand, anthocyanins levels significantly decreased in all conditions (*Table III.2.*).

PSII fluorescence parameters were most affected in the dark-adapted plants exposed to UV-A. Whilst UV-A 1h increased F_0 and slightly decreased the maximum efficiency of photosystem II (F_v/F_m), UV-A 4h had a more severe effect, decreasing F_m and F_v/F_m ($p<0.05$, *Figure III.1. a-c*). On the contrary, UV-B did not induce significant changes in PSII fluorescence. Similarly, the corresponding ratio F_v'/F_m' of the light adapted condition was also affected only by the UV-A (*Figure III.1. d*). Related photochemical quenching parameters (qP), as well as the non-photochemical quenching parameter (NPQ), again were only affected by the UV-A conditions, and followed a quadratic

response, being increased by UV-A 1h and reduced by UV-A 4h (*Figure III.1. e, g*). The Φ_{PSII} decreased also in plants exposed to UV-A 4h (*Figure III.1. f*).

Table III.2. Pigment contents in leaves from plants exposed for thirty days to different UV conditions. Chlorophyll *a* and *b* (Chl *a* and Chl *b*) (mg.gFM^{-1}), chlorophyll ratio a/b (chl *a*/chl *b*), carotenoids (mg.gFM^{-1}) and anthocyanins (µmol.gFM^{-1}) were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. For the same condition, *, **, * and **** represent significant differences for p≤0.05, 0.01, 0.001 and 0.0001, respectively. Values are expressed as the mean ± standard deviation (n=10).**

Treatment	Chl *a*	Chl *b*	Chl *a*/Chl *b*	Carotenoids	Anthocyanins
Control	1.44 ± 0.183	0.80 ± 0.073	1.79 ± 0.077	0.45 ± 0.030	0.050 ± 0.002
UV-A 1h	1.45 ± 0.163	0.80 ± 0.088	1.81 ± 0.006	0.40 ± 0.041	0.046 ± 0.001*****
UV-A 4h	1.40 ± 0.073	0.77 ± 0.030	1.82 ± 0.042	0.39 ± 0.014	0.045 ± 0.001******
UV-B 2min	1.69 ± 0.101	0.89 ± 0.059	1.90 ± 0.012*	0.43 ± 0.031	0.040 ± 0.001********
UV-B 5min	2.04 ± 0.088**	1.10 ± 0.063**	1.86 ± 0.027	0.54 ± 0.033*	0.040 ± 0.001********

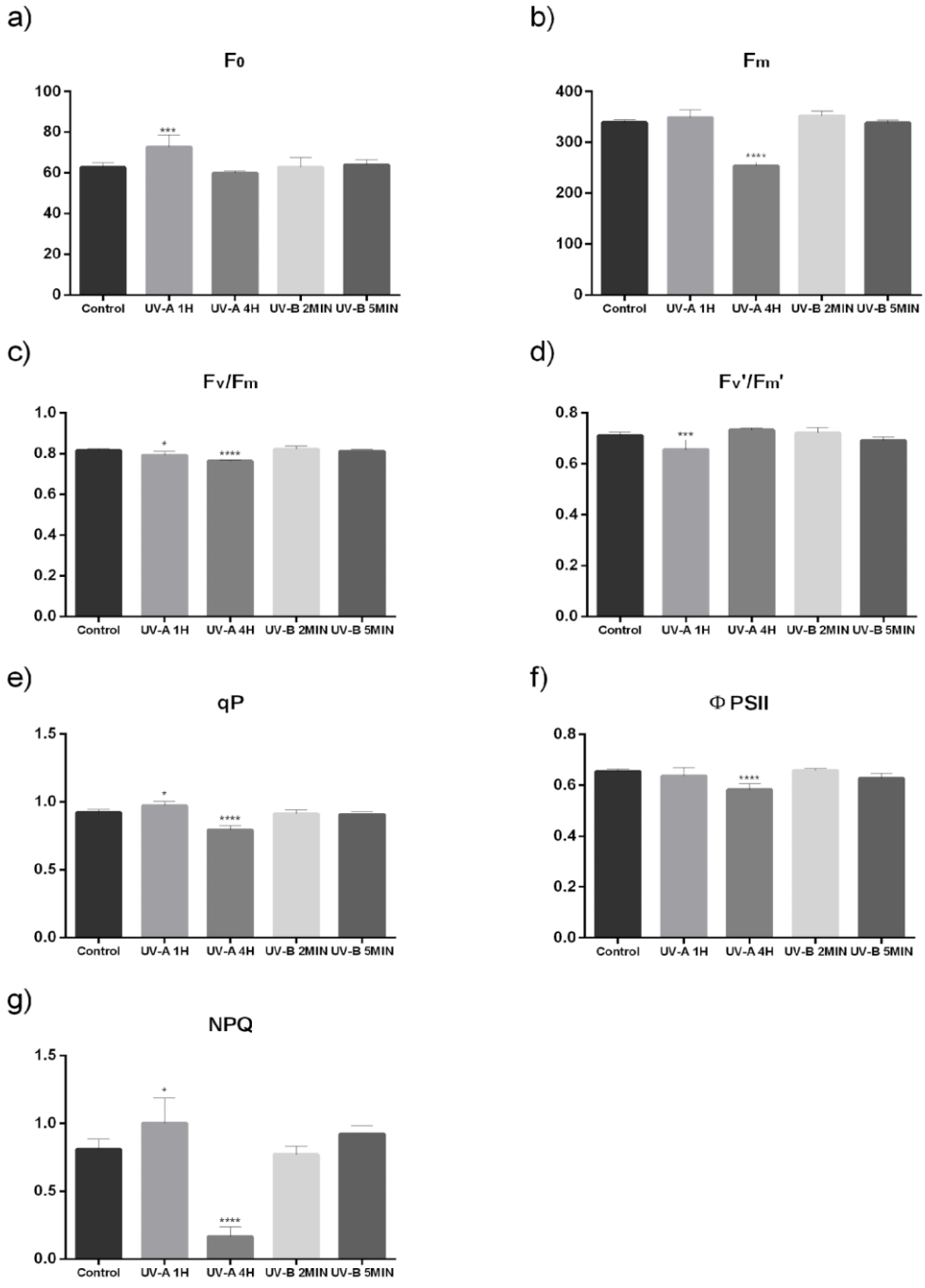

Figure III.1. Fluorescence data of the PSII system for control and UV-A/B treatments. All parameters were measured to control, UV-A 1h, UV-A 4h, UV-B 2min and UV-B 5min. Minimal fluorescence of dark-adapted leaves with all PSII centers closed, F0 (a), maximum fluorescence of dark-adapted leaves with all PSII centers closed, Fm (b), maximum quantum yield of PSII, Fv/Fm (c), maximum fluorescence in saturating light if all reaction centers are open, Fv'/Fm' (d), photochemical quenching, qP (e), effective quantum yield of PSII, ΦPSII (f) and non-photochemical quenching NPQ (g). For the same condition, *, **, * and **** mean significant differences for p≤0.05, 0.01, 0.001 and 0.0001 respectively. Values are expressed as the mean ± standard deviation (n=6).**

3.3. Gas exchange:

Similarly to fluorescence, overall, the gas exchange data were more responsive to UV-A. Net CO_2 assimilation rate (P_N) was only affected by UV-A conditions (*Figure III.2. a*), but the internal concentration of CO_2 (Ci) remained constant in all conditions (*Figure 2. b*). Also, the exposure to UV-A 4h and UV-B significantly decreased the stomatal conductance (gs) (*Figure III.2. c*).

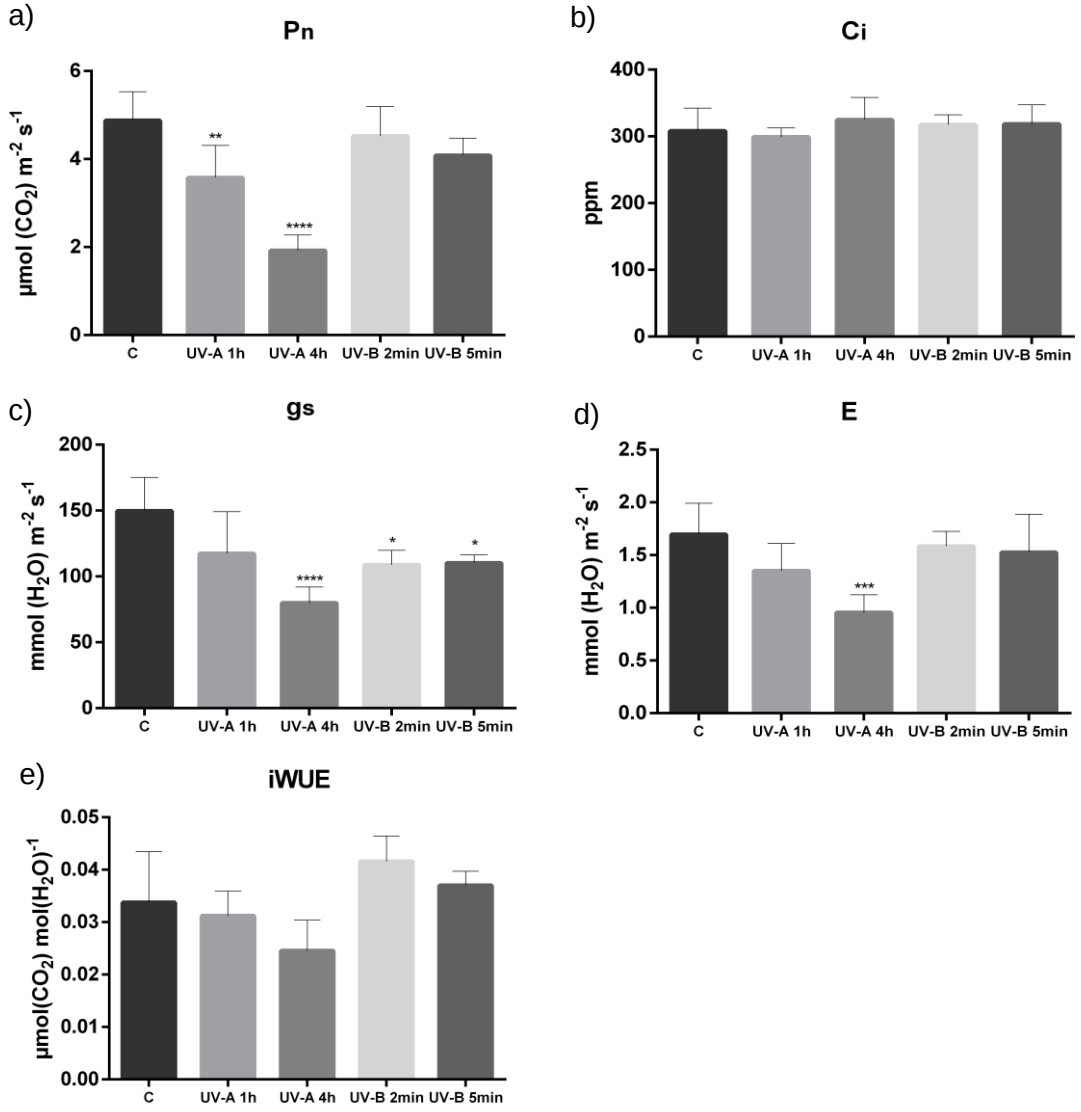

Figure III.2. Leaf gas-exchange after thirty days of exposure to different UV conditions. All parameters were measured to control, UV-A 1h, UV-A 4h, UV-B 2min and UV-B 5min. Net photosynthetic rate, P_N (a), intercellular CO_2 concentration, C_i (b) stomatal conductance, gs (c), transpiration rate, E (d), intrinsic water-use efficiency and iWUE (P_N/gs) (f). For the same condition, *, **, * and **** represent significant differences for p≤0.05, 0.01, 0.001 and 0.0001 respectively. Values are expressed as the mean ± standard deviation (n=6).**

Despite the similar transpiration (E) profiles in the different treatments, significant changes were seen at UV-A 4h (*Figure III.2. d*). The instantaneous water use efficiency (iWUE), whilst showing a tendency to decrease at UV-A 4h, were not significantly affected by any condition (*Figure III.2. e*).

3.4. Carbohydrates levels and RuBisCO relative quantification:

After 30 days of irradiation, there was an increase of total soluble sugars contents in the UV-A 1h treatment, while the starch content showed a trend ($p>0.05$) to decrease in UV-A treatment and increase in UV-B 5min (*Figure III.3. a,b*). The relative amount of RuBisCO was not significantly affected by UV supplementation despite the observed trend to increase under those conditions (*Figure III.3. c*).

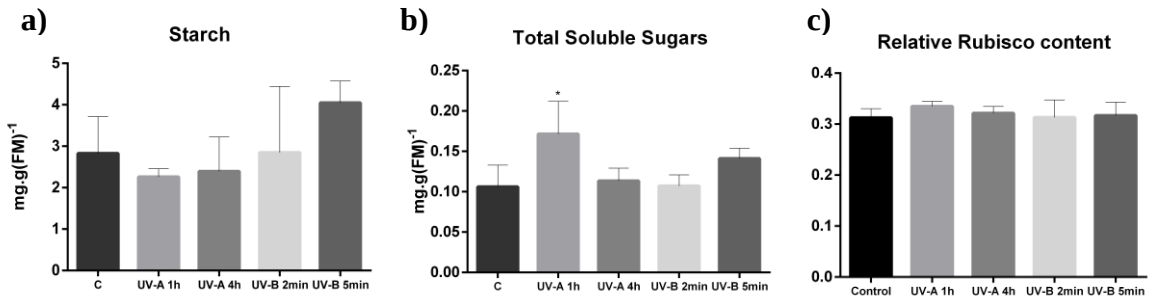

Figure III.3. Carbon fixation in plants exposed thirty days to UV-A and UV-B. Starch (µmol.gFM^{-1}), Total Soluble Sugars (TSS, µmol.gFM^{-1}), and Relative RuBisCO content ($ABS_{RC}.ABS_{TPC}^{-1}$) were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. FM (Fresh Matter). For the same condition, * represent significant differences for $p\leq0.05$. Vertical bars mean standard deviation. (n between 7-10).

3.5. Gene expression for RuBisCO and PSII:

UV-A 4h and UV-B 5min treatments induced an upregulation of the two genes coding for the components of RuBisCO (large and small subunits) *rbcL* and *rbcS*. Also, the genes coding for protein subunits of the PSII, D1 protein (*psbA*) and CP47 (*psbB*) showed an upregulation in all UV exposures. Except for *rbcS*, higher increases were observed in UV-A 4h exposed plants (*Figure III.4. a-d*).

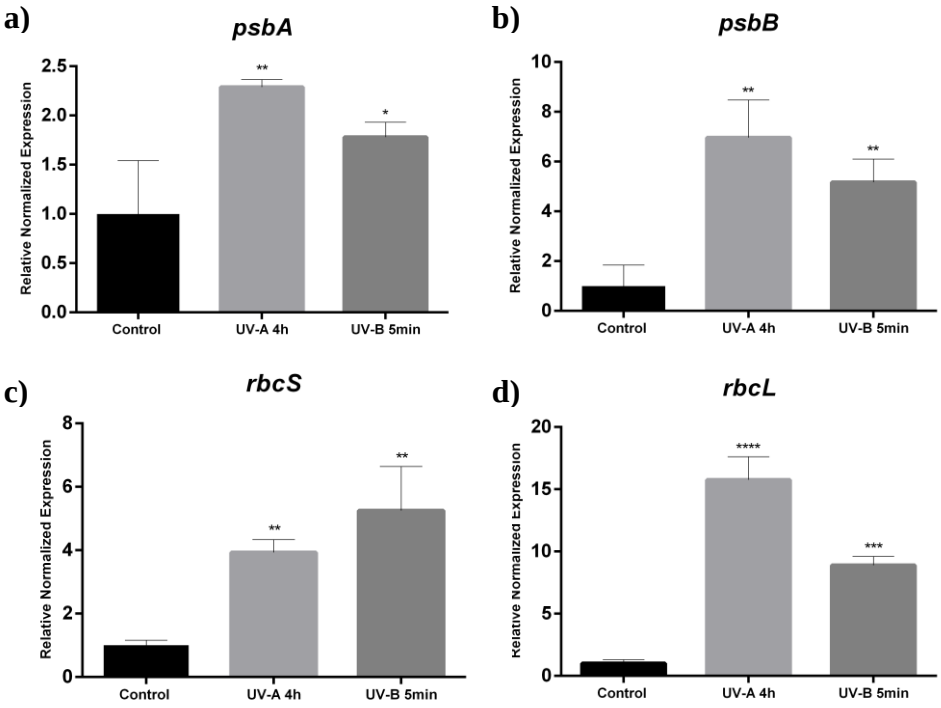

Figure III.4. Genes of photosynthesis components are regulated by UV light conditions. All parameters were measured in control, UV-A 4h and UV-B 5min (the last two are the higher exposition times for each radiation). The relative expression of the photosynthetic components was assessed for *psbA* (a) and *psbB* (b) which encodes the D1 protein and CP47, respectively. At the same time, the relative expression of the genes to two subunits of RuBisCO was also assessed: *rbcS* (c) and *rbcL* (d) for small and large subunits, respectively. For the same condition, *, **, * and **** represent significant differences for p≤0.05, 0.01, 0.001 and 0.0001 respectively. Values are expressed as the mean ± standard deviation. (n between 7-10).**

3.6. Multivariate approach:

Principle component analysis showed a clear separation between control and UV-B treatments (*Figure III.5.*). PC1 explained 43% of the variance and PC2 28% of the variance. Regarding the control (the most centered population, top-left quadrant), both UV-B 2min and UV-B 5min scores are quite similar and both located at the down-left quadrant, being mostly associated with higher levels of photosynthetic pigments, iWUE and starch. Contrarily, the scores for UV-A 1h and UV-A 4h show that these two populations have different profiles, and both are highly different from the control and the UV-B scores. UV-A 1h score is located at the the central-top and associated with increases of anthocyanins, F_0, TSS, RuBisCO, while UV-A 4h score is positioned at the right center and relates with fruits and fruits+flowers.

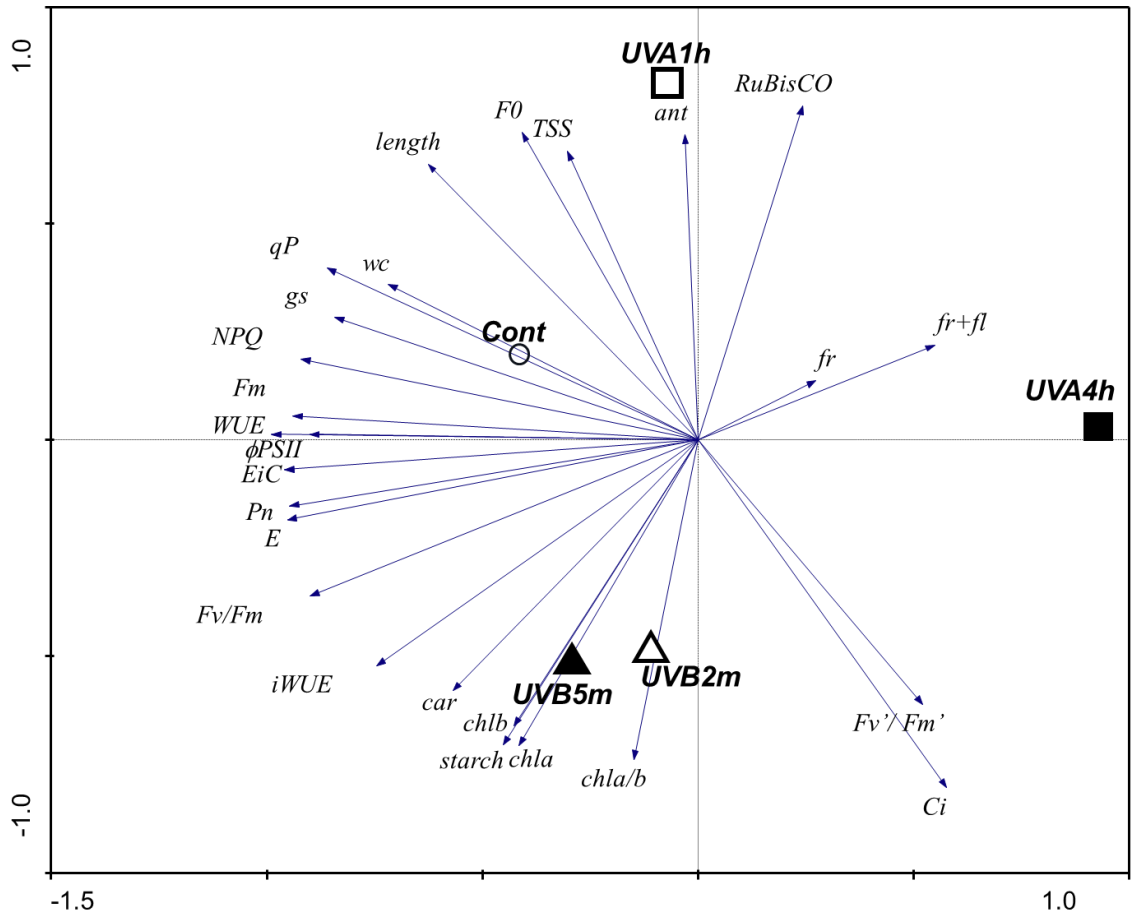

Figure III.5. PCA analyses of functional responses of tomato fruiting plants exposed to UV-A (1 and 4h) and to UV-B (2 and 5min) for 30 days.

4. Discussion

With the paradigm of producing "more with less" in indoors soilless controlled systems, the massive use of protected cultures represents a new era of modern agriculture. However, it faces the challenge of mimicking natural conditions, including the natural solar light. Whilst greenhouses create an ideal environment for crop production, the use of UV-absorbing greenhouse covering materials impair all benefits that moderate UV radiation (UV-A and UV-B) may induce on crops along their life cycle. Compared with their outdoor growing counterparts, crops growing indoors may have repressed metabolic pathways that are triggered by UV-A and UV-B sensors (Wargent, 2016). Both UV-A and UV-B doses daily used in the present work were beneficial to the fruiting process (number and maturation), and had little impact on shoot length/branching, but UV-B irradiation was more deleterious to the plant, inducing

necrotic spots. These data along with the comparative cost of the UV-A (dark light) and UV-B lamps supports that UV-A may be a better solution for tomato horticulture.

The plant length reduction observed in UV-A 4h and UV-B 2min exposed plants supports previously literature reporting that UV-B reduced shoot growth and/or leaf area (Bandurska *et al.,* 2013) due to a decline in cell division and cell expansion. Cell expansion can be controlled by changing leaf water content and turgor pressure, and cell extensibility. This is in line with the correlated decrease in plant length and water content shown herein (which are in opposite sides regarding UV-A 4h and UV-B 2min, *Figure III.5.*) and suggests an adjustment of the tomato plants metabolism under the UV doses tested, also supported by the reduced stomata aperture (*Figure III.5.*).

The increase in fruit number observed under UV radiation is in accordance with the reported action of UV-A on the leaf blue/UV-A photoreceptor, CRYPTOCHROME (CRY1 and CRY2), which plays a pivotal role in plant growth and development, including stem growth, flowering time, stomatal opening, circadian clock, and other light responses (Wang *et al.,* 2013). This is also in accordance with previously reported stimulation of cry2 by moderate levels of UV-A increasing flowering (Kharshiing and Sinha, 2015). UVR8 (UV Resistance Locus 8), an ultraviolet-B (UV-B; 280-315nm) light receptor is also involved in the regulation of many aspects of plant growth and development, and it was shown that UV irradiation can increase flowering and fruiting (Zhao *et al.,* 2016), supporting our data.

Photosynthetic activity may be transiently reduced during the acclimation to UV-B, and thus shifting carbon sink, which may compete with sugar storage in fruits (Martinez-Luscher *et al.,* 2015). This may be paralleled by a shift of the secondary metabolism, with crops increasing their levels of valuable secondary compounds like flavonoids/phenolics, etc (Guidi *et al.,* 2016; Krizek, 2004; Machado *et al.,* 2017). Regarding pigments, UV-B decreased anthocyanins that are protective non photosynthetic pigments known to be important in UV screening (Guo and Wang, 2010). The differences in photosynthetic pigments and anthocyanins responses to UV are dose and species dependent (Brazaityte *et al*., 2015; Wenke and Qichang, 2012). While in our case there was a general decrease, as occurred in UV-A exposed pea plants (Wenke and Qichang, 2012), in wheat leaves UV-B increased their levels (Chatuvrdi *et al.,* 1998). In tomato plants, the slight increase of Chl *a*:Chl *b* reported in the present work supports a higher susceptibility of Chl *b*, and its putative conversion into Chl *a*. This is supported by their strong positive correlation with UV-B conditions

(*Figure III.5.*). Contrarily, carotenoids decreased in Arabidopsis exposed to high UV-B levels (Khudyakova *et al.,* 2017).

As can be seen from the data and PCA analyses (*Figure III.5.*), our results show that the moderate UV levels differently affects tomato photosynthesis. While UV-B has major impacts on pigment levels, UV-A 4h reduces preferably the PSII function. The UV-A 4h has shown the most severe effects, decreasing chlorophyll fluorescence parameters (e.g., F_m, F_v/F_m), which provide information about the efficiency of PSII photochemistry. The decrease of F_v/F_m supports a slight negative impact in the maximum potential quantum efficiency of PSII, which however may not compromise the plant's performance due to the maintenance of F_v/F_m values close to 0.8. F_v/F_m is widely used as an indicator of photoinhibition or other injuries at the PSII complexes (Hou *et al.,* 2017), and under non stressful conditions remains around ~0.8, decreasing under stress (Maxwell and Johnson, 2000).

The quadratic profile shown by NPQ in response to UV-A (a stimulation for lower doses followed by a drastic decrease) suggests an hormetic effect of this radiation, meaning that low doses of UV-A promote heat dissipation (which includes photo-protective mechanisms), while at higher doses this strategy is compromised. Non-photochemical quenching (NPQ) of chlorophyll fluorescence is an indicative of the level of non-radiative energy dissipation in the LHC II of PSII, which is ascribed to prevent overreduction of the electron transfer chain and thus preventing photodamage. The NPQ decrease observed at UV-A 4h may be supported by the decrease of the light-harvesting antenna size (lowered by F_m, positioned at the opposite side of UV-A4h (*Figure III.5.*) and/or by other causes of PSII inactivation (Hou *et al.,* 2017).

The UV-A effect on the energy/centers functionally involved in the photochemical quenching (qP) is strongly dependent on the dose. While lower UV-A increased energy (qP) flow and/or increased the number of centers functionally involved in the photochemical quenching, higher UV-A doses decrease this availability. The observed reduction of the quantum photosynthetic yield (Φ_{PSII}), i.e., the light adapted quantum yield of PSII, is associated with several stressful conditions, supporting our data for UV-A 4h. Contrarily, UV-B did not induce stress on the fluorescence/ quenching parameters, suggesting rather changes in the LHC-pigments. On the other hand, Φ_{PSII} is often reported as more sensitive to stress than F_v/F_m, and its decrease also indicates that plants at UV-A 4h had a putative restriction of NADPH and ATP to the Calvin cycle. Despite the multiple variables involved (e.g., respiration), this assumption is

supported by the PCA analysis, which show a positive correlation between the Φ_{PSII} the CO_2 data regarding P_N (*Figure III.5.*).

Finally, the complexity of this process is shown by the increased transcripts of *psbB* coding for CP47. This protein is located at the antenna pigment complex CP43-47 and binds to chlorophylls and carotenoids, acting in the transfer of energy from the peripheral antenna to the photochemical reaction centre. A similar behaviour was found for the transcript *psbA* coding for the protein D1, a protein involved in receiving electrons in the PSII. Two hypothesis may underlie these transcript increases under UV-radiation, either there is a light-induced degradation of these proteins in the PSII, compensated by an increase of transcription to synthesize new proteins, or in an adaptive process to UV radiation, new PSII centres may be under generation to compensate the lower Φ_{PSII}. Current data support preferably the first hypothesis with demonstrated susceptibility of the D1 protein to light, including UV (e.g., Kiss *et al.,* 2012, Nouri *et al.,* 2015). On other hand, Zheng *et al.* (2016) showed that UV-A induced in *Taxus*, an increase of several proteins of the PSII.

UV-A 4h conditions induced the most significant changes in gas exchange parameters, also supported by the PCA analysis. These effects were most evident in the decreases of net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E). In the UV-A treated plants, while stomatal closure (less stomatal *aperture*) occurred and P_N decreased, the levels of internal CO_2 (Ci) remained unchanged which indicate less activity of the Calvin cycle. This suggests that P_N reduction can be related to biochemical impairments (e.g. Calvin cycle enzymes). Also, Calvin cycle is highly dependent on the photochemical reactions, and its lower activity may be related with lower ATP/NADPH availability (decreased levels of Φ_{PSII}). The observed increase of the RuBisCO transcriptional levels support an investment on more protein. RuBisCO has been for long used as an indicator of Calvin cycle dynamics and CO_2 fixation (e.g., Dias *et al.* 2013), and the transcripts of the larger and small units (*rbcL* and *rbcS*) may indicate if new RuBisCO proteins are being synthesized under a specific stress. The increase of both *rbcL* and *rbcS* transcripts also suggests an UV-induced inactivation/degradation of RuBisCO, as suggested earlier (Araújo *et al.,* 2016), which is being compensated by an increase of new transcription to synthesize new RuBisCO peptides. Our further findings that no significant changes are observed on the relative content of RuBisCO, strongly supports the hypothesis of UV-induced RuBisCO degradation, which is reset by new protein, so not jeopardizing the Calvin cycle. In fact, despite the impacts on photosynthesis, the final content of leaf soluble sugars show no

significant changes, including an increase of starch was observed (UV-A 1h, *Figure III.3.*).

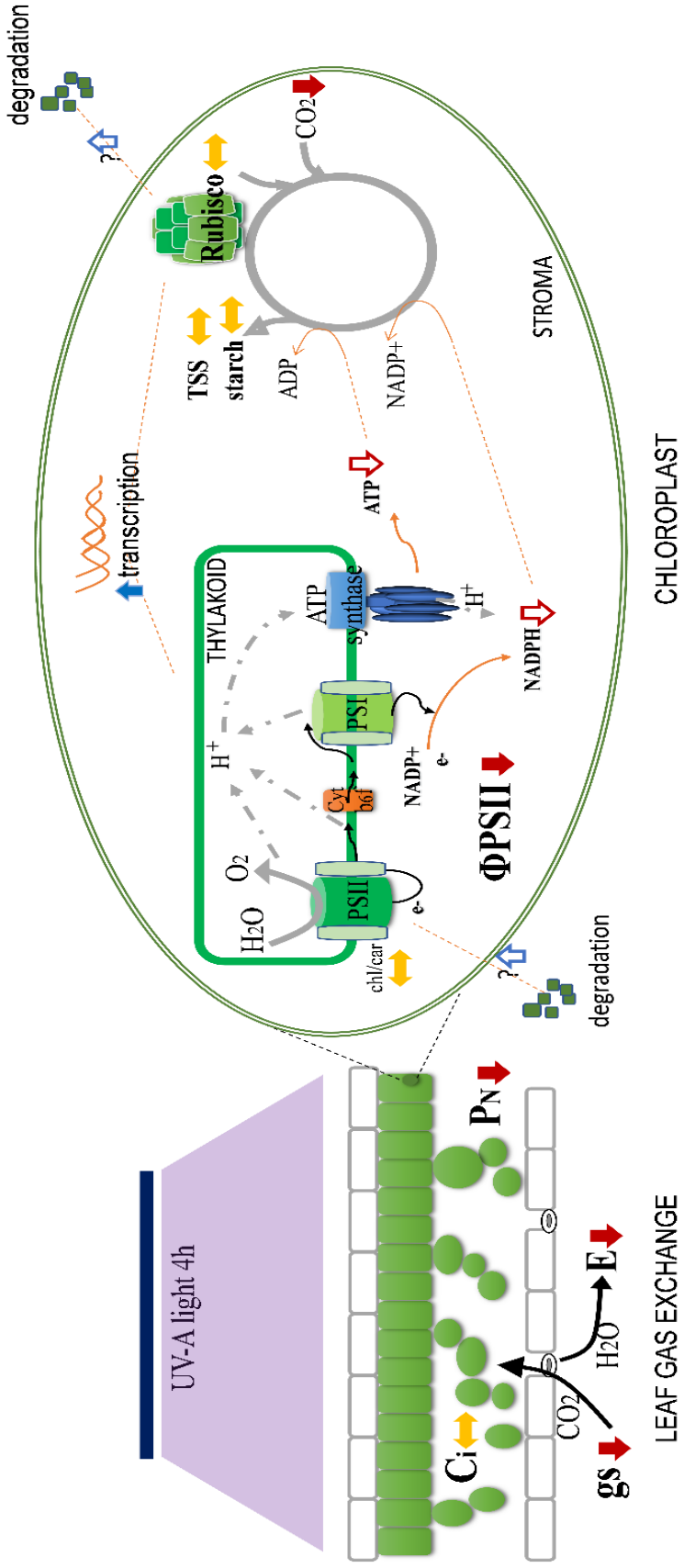

Figure III. 6. Major photosynthetic impacts and changes induced by moderate UV-A 4h/day, during 30 days, in tomato flowering plants. Overall, the photosystem II (PSII) fluorescence is affected with a decreased efficiency of Φ_{PSII}, although LHC-pigments (chl/car=chlorophyll/carotenoids) levels are not affected. This leads to less electrons being transported and thus promoting a reduction of NADPH and ATP production and availability for the Calvin cycle. This decrease is related with the decrease of the net photosynthetic rate (P_N), meaning that internal CO_2 concentration (C_i) is not so depleted and the stomatal conductance (gs) may decrease, therefore decreasing transpiration rate (E). Simultaneously, a degradation of RuBisCO may occur, but it can be replaced by new protein due to the stimulated accumulation of its transcripts (and increase its transcription), which overall may reset the negative impacts on the Calvin cycle, thus not having negative impacts on total amounts of soluble sugars and starch. Solid red arrows mean a decrease and solid blue arrows to increase. Dashed red and blue arrows mean a putative decrease and increase, respectively.

5. Conclusions

In conclusion, our results indicate that the use of UV light (particularly UV-A) treatments positively influence yield and, despite impairments in photosynthetic pathways, the final byproducts are not compromised. Our data also demonstrate that UV-B and UV-A differently interfere with plants fruiting and photosynthesis in greenhouses, and that the effects are also dose-dependent, particularly regarding the UV-A doses tested. Despite the different impacts of UV-B being more evident in the amount of chlorophylls/photochemical structure, rather than in the photochemical process per se, the necrotic spots observed mostly in the highest dose suggest more profound effects of UV-B. Also, considering the highest price of the UV-B lamps regarding the dark-light ones, suggest that UV-A may be a better choice for producers. Both UV-A doses tested might be used, and we suggest that whilst some photosynthetic and photochemical processes may be negatively affected, the plant develops adaptation mechanisms (including increased transcription of PSII peptides and RuBisCO) not compromising the final carbohydrate balances (*Figure III.6.*). Furthermore, the increase of flowers' and fruits' number can be an interesting reason to apply this light in protected horticultures.
In summary, also considering the stimulation of fruit maturation under both UV-A conditions, we suggest that tomato plants growing indoors benefit from daily radiation of UV-A, even at the expenses of some photosynthetic limitations.

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IV. Charper 4: Tomato plants preferably use non-enzymatic antioxidant pathways to cope with moderate UV-A/B irradiation: a contribution to the use of UV-A/B in horticulture

Abstract

Plants developed receptors for solar UV-A/B radiation, which regulate a complex network of functions through the plant's life cycle. However, greenhouse grown crops, like tomato, are exposed to strongly reduced UV radiation, contrarily to their open-field counterparts. A new paradigm of modern horticulture is to supplement adequate levels of UV to greenhouse cultures, inducing a positive mild stress necessary to stimulate oxidative stress pathways and antioxidant mechanisms. Protected cultures of *Solanum lycopersicum* (cv. MicroTom) were supplemented with moderate UV-A (1h and 4h) and UV-B (1min and 5min) doses during the flowering/fruiting period. After 30 days, flowering/fruit ripening synchronization were enhanced, paralleled by the upregulation of blue/UV-A and UV-B receptors' genes *cry1a* and *uvr8*. UV-B caused moreover an increase in the expression of *hy5*, of HY5 repressor *cop1* and of a repressor of COP1, *uvr8.* While all UV-A/B conditions increased SOD activity, increases of the generated H_2O_2, as well as lipid peroxidation and cell membrane disruption, were minimal. However, the activity of antioxidant enzymes downstream from SOD (CAT, APX, GPX) was not significant. These results suggest that the major antioxidant pathways involve phenylpropanoid compounds, which also have an important role in UV screening. This hypothesis was confirmed by the increase of phenolic compounds and by the upregulation of *chs* and *fls,* coding for CHS and FLS enzymes involved in the phenylpropanoid synthesis. Overall, all doses of UV-A or UV-B were beneficial to flowering/fruiting but lower UV-A/B doses induced lower redox disorders and were more effective in the fruiting process/synchronization. Considering the benefits observed on flowering/fruiting, with minimal impacts in the vegetative part, we demonstrate that both UV-A/B could be used in protected tomato horticulture systems.

Keywords

Fruiting, horticulture, oxidative stress, *Solanum lycopersicum*, ultraviotet supplementation

1. Introduction

Solanum lycopersicum L., tomato, is among the crops most widely produced and consumed. Nowadays, the production of this crop is diversified, ranging from open-field to protected horticulture (Martínez-Blanco *et al*., 2011). In protected horticultural systems (which include glass/plastic greenhouses) it is possible to produce in season and off-season with or without supplemental light (Bian *et al*., 2014). However, crops produced off-season often have an inferior reputation regarding sensorial attributes and chemical composition, when compared to open field products (Muñoz *et al*., 2007).

Solar ultraviolet (UV) radiation, namely the UV-A and UV-B, is a natural environmental stressor and plants have evolved UV-photoreceptors and adaptive mechanisms to cope with UV-stress (Lin and Todo, 2005; Suchar and Robberecht, 2015; Yokawa *et al*., 2015). On the one hand, most plant studies in the last decades have been focused on the harmful impacts of excessive UV radiation, which include damages on cell structures and metabolism e.g., photosynthesis and increased oxidative stress, that ultimately may compromise plants' productivity and lifespan (Nawkar *et al*., 2013). On the one other hand, protected horticulture is an example of how UV-deficiency may have detrimental impacts on crops performance and productivity (Wargent and Jordan, 2013). Crops growing in protected systems are not exposed to natural doses of UV-radiation (Kumar and Poehling, 2006) thus not benefiting from the impacts that moderate UV-radiation may have on fruit production, sensorial attributes and chemical quality (Carvalho *et al*., 2016; Kasim and Kasim, 2015). So, it is crucial to establish a compromise between the UV-intensity and duration of exposure to get a positive mild stress - "eustress" – which may increase yield and/or fruits nutritional value, and may therefore be useful in agro-industry (Hideg *et al*., 2013).

Photoreceptors modulate the expression of hundreds of light-regulated genes, which leads to adaptive changes at the cellular and systemic levels (Major *et al*., 2017). Blue light (400-500 nm) and UV-A radiation (315-400 nm) are perceived by phototropins (PHOT), cryptochromes (CRY) and LOV/F-box/Kelch-domain proteins (Yu *et al.,* 2010). Besides CRY, UV resistant locus 8 (UVR8) is also an important receptor to lower wavelength UV-A and to UV-B (280-315 nm) (Rizzini *et al*., 2011). Four *CRY* genes

expressed in response to UV-A and blue light were identified in tomato cultivars such as “Moneymaker” (Facella *et al.*, 2016). These genes unleash multiple responses during the different plant developmental stages (Liu *et al*., 2011). There are two types of *CRY1* genes (*CRY1a* and *CRY1b*), one *CRY2* gene and one *CRY3* gene. *CRY1* mostly controls photomorphogenesis in young plants, anthocyanins pathways and plant development (Facella *et al.*, 2016). *CRY2* is involved in flowering and fruit quality (Kharshiing and Sinha, 2015). Giliberto *et al.* (2005) showed that *CRY2* overexpression increases pigment contents, stimulating an overproduction of anthocyanins and chlorophylls in leaves and of flavonoids and lycopene in fruits. *CRY3* has a DNA repair and protective role, occurring mostly in mitochondria and chloroplasts (Facella *et al.*, 2016).

PHY and CRY control the Constitutive Photomorphogenic 1 (COP1) repressor, which promotes the degradation of the transcription factor (TF) Long Hypocotyl5 (HY5) (Heijde and Ulm, 2012). Most data refer to blue or UV-B effects and little is known about UV-A modulation, being assumed it is similar to the blue one. UV-B radiation promotes the separation of the UVR8 dimer and the resulting UVR8 monomers interact with COP1 blocking HY5 proteasomal degradation, and allowing this TF to promote the transcription of several genes involved in protection against UV. Some of the proteins coded by these genes include Chalcone Synthase (*CHS*), Chalcone Isomerase (*CHI*) and Flavonol Synthase (*FLS*) that are involved in phenylpropanoid biosynthesis (Heijde and Ulm, 2012).

Phenols resulting from the phenylpropanoid pathway are important antioxidants, pointed out as contributing to the efficient control of reactive oxygen species (ROS) (Agati *et al*., 2012; Martinez *et al*., 2016). ROS are free radicals, a typical by-product of the photo-excitation in thylakoidal photosystems I and II compounds (Anjum *et al*., 2014). ROS levels are commonly increased by biotic and abiotic factors (including UV radiation), changing the redox-homeostasis necessary for the regulation of cellular bioactivity (Yokawa *et al.*, 2015). As reported above, blue/UV-A radiation leads to an overexpression of CRY and PHOT proteins. This increase affects gene transcription and triggers molecular responses that include changes in the biosynthesis of secondary metabolites, including polyphenols (Müller-Xing *et al.*, 2014). Several polyphenols, of which flavonoids (e.g., anthocyanins, flavonols) represent a major family, result from the phenylpropanoid pathway, and not only may scavenge and/or inhibit the generation of ROS (Brunetti *et al.*, 2013; Zoratti *et al.,* 2014) but also may selectively absorb UV-A and UV-B wavelengths (Agati *et al*., 2010).

UV radiation also enhanced the transcription, translation and activity of antioxidant enzymes (Kumari *et al*., 2010). These enzymes are responsible for scavenging the excess of ROS molecules, such as $O2^{\cdot -}$, H_2O_2, $^1O_2$, $HO_2^{\cdot -}$, $OH^{\cdot}$, ROOH, $ROO^{\cdot}$, and $RO^{\cdot}$. Superoxide Dismutase (SOD) family acts in the first step of ROS scavenging by catalyzing the $O_2^{\cdot -}$ dismutation to H_2O_2 and O_2. The following step involves the decomposition of H_2O_2 catalyzed by various enzymes, e.g., catalase or peroxidases such as Catalase (CAT), Ascorbate Peroxidase (APx) and Peroxidases that use guaiacol as substrate (GPX) (Choudhury *et al*., 2013; Das and Roychoudhury, 2016). While it is well described that UV-rays are perceived by photoreceptors and also increase oxidative stress, several aspects remain to unveil related with the distinctive modulation of UV-A vs UV-B, and the pathways involved in the stimulation of antioxidant enzymes as well as their contribution through exposure time. For example, it was demonstrated that nitric oxide is involved in the signaling pathway that up-regulates specific isoforms of antioxidant enzymes protecting against UV-B-induced oxidative stress (Santa-Cruz *et al*., 2014). Also, Kumari *et al.* (2010) demonstrated in *Acorus calamus*, that UV-B stimulation of antioxidant enzymes activities (SOD, CAT, APX, GR) was observed at initial growth period but CAT and SOD activities decreased at later age of sampling.

The aim of this work is to functionally understand how moderate supplementation of UV-A or UV-B on protected tomato cultures increases oxidative eustress, which defense mechanisms are activated, and if this supplementation may improve protected cultured tomato yield, and favour agronomic traits. With this work, we will also be able to distinguish UV-A and UV-B specific mechanisms of oxidative stress and defense strategies.

2. Material and Methods

2.1. Plant growth conditions and UV treatments

Seeds of *Solanum lycopersicum* L. cv. MicroTom (Just Seed, UK) were soaked in distilled water and germinated on 0.3 L plastic pots with Peat:Perlite (2:1) substrate. Germinated plants were grown in a growth chamber with a photosynthetic photon flux density (PPFD) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by fluorescent light lamps (OSRAM L 30W/77 FLUORA) and a photoperiod of 16h:8h light:dark. Relative humidity (RH) and temperature were maintained at 45 ± 5% and 23 ± 2 ºC, respectively. Pots were irrigated twice a week with Hoagland medium (Sigma, USA), with pH adjusted to 5.70 ±

0.05. At the 90th day, the first flower buds emerged and after 10 days (100-day-old plants) a high synchronization in flowering was observed. Between days 100 and 130 (i.e., during fruiting and fruit ripening), plants were randomly divided in five groups, and each group exposed to a different UV condition: *Control Group* (C): plants were maintained under the same irradiation conditions, with no UV supplementation; *UV-A 1h Group*: plants were exposed for 1 h per day to 0.8 J/m^2 UV-A supplied by black light lamps (F20T12/BLB - 20W T12 (T10)) Fluorescent Blacklight Blue (Supra Life®, Italy), with a peak wavelength at 368 nm (the intensity of light at wavelengths below 368 nm was close to 0 W/m^2); *UV-A 4h Group*: plants were exposed for 4 h per day to 0.8 J/m^2 UV-A, supplied by the same blacklight lamps; *UV-B 2min Group*: plants were exposed for 2 min per day to 2.94 J/m^2 UV-B, supplied by six 312 nm TFP-M/WL 8W lamps (Vilber, Germany), which have an irradiation of wavelengths below 312 nm close to 0 W/m^2); *UV-B 5min Group*: plants were exposed for 5 min per day to 2.94 kJ/m^2 UV-B, supplied by the same UV-B lamps. UV-A and UV-B irradiance was measured by Sensor Meters Philip Harris (serial number: 4375 model SEL240) and International Light INC (Newbryport, Massachusetts, model: 01950, IL1400A), respectively. Irradiation values are the mean of the irradiance measured at the top mature-leaves in the first and last days of exposure. These leaves receiving the measured irradiance (and with similar age and size) were sampled for the biochemical and transcriptional analyses.

2.2. Plant morphology and productivity evaluation

After 30 days of UV exposure plants' morphological characteristics, including shoot length, leaf chlorosis, necrosis, were evaluated. The carbon metabolism efficiency did not suffer major effects, as described elsewhere (Ponte *et al.,* 2017; see also *Chapter 3*). The plants' productivity and nitrogen metabolism was evaluated according to Thomsen *et al*. (2014) using the glutamine synthase (GS) activity. For GS determination, samples were treated and GS assay was determined according to Pinto *et al.* (2014). Briefly, 0.1 g of leaf samples were homogenized in 1.5 mL containing 0.1 M phosphate buffer (pH at 7.0), 0.5 M of ethylenediaminatetracetic acid disodium salt (Na_2EDTA), 1% polyvinylpyrrolidone (PVP), phenylmethylsulphonyl fluoride (PMSF) 1 mM, 0.2% triton X-100 (v/v) and 2 mM of dithiothreitol (DTT). The homogenate was centrifuged at 12000xg for 15 min. GS activity was measured after 30 min of reaction in a mixture containing the supernatant (SN), sodium arsenate and activity solution. After

adding the stop solution, the change of colour was measured at 500 nm and the protein concentration was presented as a unit by mg of total soluble protein (TSP).

2.3. Fruiting and fruit ripening

During the experiment, the number of fruits was quantified and distributed by 4 developmental categories. The development stages were according to the scale in Yin *et al*. (2009). Immature green, mature green, yellow and red fruits were selected by age and mature stage.

2.4. Antioxidant enzyme activities

Total soluble proteins (TSP) were extracted from frozen leaves (100 mg) ground with liquid N_2 and with 1.5 mL extraction buffer containing 0.1 M phosphate buffer (pH 7), 0.5 M Na_2EDTA, 1% PVP, 1 mM PMSF, 0.2% triton X-100 (v/v) and 2 mM DTT. The samples were centrifuged at 8000xg for 15 min at 4 ºC. Protein concentration was determined using the Bradford Reagent (Sigma, USA) and bovine serum albumin (Sigma, USA) as standard. The supernatant obtained for the TSP assay was used to quantify CAT, APX, GPX activities.

CAT activity was assayed by following the initial rate of H_2O_2 degradation for 120 seconds (recording 10 in 10 seconds), monitored at 240 nm. Enzyme activity was determined according to Azevedo *et al.* (2005) with some modifications, using 135 µL extraction buffer, 60 µL of supernatant and 50 µL H_2O_2 (0.083 M) mixture, and considering the extinction coefficient (39.4 $mM^{-1}cm^{-1}$) for H_2O_2.

APX was determined by spectrophotometry according to the oxidation rate of AsA at 290 nm during 70 sec. (Azevedo *et al.,* 2005) and using the extinction coefficient 2.8/mM/cm for AsA.

GPX activity determination was according to Azevedo *et al.* (2005) with some modifications, the reaction solution contained the enzyme extract (100 µL), 100 mM phosphate buffer (pH 7.0), 3 mM of H_2O_2 and 15 mM guaiacol. The increase in absorbance at 470 nm resulted by guaiacol oxidation was followed by 1 minute. The activity was calculated using the extinction coefficient of the tetraguaicol (26600 $M^{-1}cm^{-1}$).

For SOD analyses, frozen leaves were ground with liquid N_2 and extracted with a buffer containing 100 mM phosphate buffer (pH 7.8), 0.5 mM Na_2EDTA, 1% PVP, 1 mM PMSF, 0.2% triton X-100 (v/v) and 2 mM DTT. The homogenate was centrifuged at 15,000xg, for 15 min at 4°C. The SN was used to quantify SOD activity by measuring

its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT read at 560 nm and the results expressed as nKat.mg^{-1} of fresh mass (FM).

2.5. Gene expression

Leaf total RNA was isolated using PureZOL™ RNA Isolation (Bio-Rad), following the manufacturer's instructions. For Reverse Transcriptase-PCR, total RNA samples were treated with DNAse I, Amplification Grade (Invitrogen™); then, first-strand cDNA was synthesized from 1 µg total RNA using NZY First-Strand cDNA Synthesis Kit, no oligos (NZYTech™), using random hexamers (NZYTech™) and subsequently treated with 1 µL NZY RNase H, diluted with Milli-Q water and stored at -20 ºC. To normalize gene expression data, two previously described primers (Dzakovich *et al.,* 2016; Løvdal and Lillo, 2014) specific for the housekeeping genes elongation factor 1alpha (*ef1*) and ubiquitin (*ubi*) were used (*Table IV.1.*).

Table IV. 1. Primers used for quantification of relative expression after 1 month of exposure to moderate UV-A and UV-B supplement. Housekeeping genes used: elongation factor 1alpha (ef1) and ubiquitin (ubi). The following primers were used for molecular analysis of light and antioxidant responses: uv-b resistance 8 (uvr8), constitutive photomorphogenic 1 (cop1), elongated hypocotyl 5 (hy5), cryptochrome 1a (cry1a), catalase 1 (cat1), glutathione reductase of cytosol (grcyt), flavonol synthase (fls) and chalcone synthase 1 (chs1). Forward primer (F) and reverse primer (R).

gene	Primer (5' → 3')
ubi	F: GGACGGACGTACTCTAGCTGAT R: AGCTTTCGACCTCAAGGGTA
ef1	F: TGGCCCTACTGGTTTGACAACTG R: TGGCCCTACTGGTTTGACAACTG
uvr8	F: CTGCTATGGTCAAGCGGCTA R: AGCATGCATCAGTCAGCACT
cop1	F: ACGGGCTTGGAGTGTTGATT R: CCTGCTTCGTGCACCAAACT
hy5	F: AAGCAAGGGTGAAGGAATTG R: ACAATCCACCCGAAACTAGC
cry1a	F: TCGAACCAATGCTACCCCAC R: TCGAACCAATGCTACCCCAC
cat1	F: GTTGGAACCTGAATAAGTTCACAG R: TCTTCAAGCACCAAAGTGAACA
grcyt	F: GCAAAGAATTATGGATGGGA R: CACAGCACGCTTTGGTAA
fls	F: ATAGCTCCACAACCAGGTGC R: TCCATTTGGCCTCACCACTC
chs1	F: ACCAACAAGGTTGCTTTGCC R: GAGATTCACTGGGTCCACGG

Gene expression analysis of photoreceptors, oxidative stress enzymes and defence signal compounds production was evaluated using the following genes: uv-b resistance 8 (*uvr8*), constitutive photomorphogenic 1 (*cop1*), elongated hypocotyl 5 (*hy5*), cryptochrome 1a (*cry1a*), catalase 1 (*cat1*), glutathione reductase of cytosol (*grcyt*), flavonol synthase (*fls*) and chalcone synthase (*chs1*) (*Table IV.1.*). The RT-qPCR reactions were performed using CFX96^{TM} Real-Time PCR Detection System (BioRad, USA) and iTaq Universal SYBR Green Supermix (BioRad, USA), according to manufacturer. The amplification conditions were as follows: 95 °C for 1 minute followed by 60 cycles of 3 seconds at 95 °C and 30 seconds at 60 °C and melting curve generation. iQ5 Optical System Software was used for calculation of the cycle threshold (CT) and primers efficiency.

2.6. Cell membrane stability (CMS)

Leaves of similar age and fresh weight (FW~100 mg) were collected. Each leaf was incubated in 10 mL of deionized ultrapure water at 25 ºC with slight agitation. After 24h, the water electric conductivity (L1) was measured. The samples were autoclaved for 10 min at 120 ºC, and then the electric conductivity (L2) was measured again. Results were presented as a percentage of membrane damage, %MD = (L1/L2) x 100 (Araújo *et al.,* 2016).

2.7. Concentration of malondialdehyde (MDA)

For malondyaldehyde (MDA) quantification, fresh leaf samples (100 mg) were macerated in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The samples were centrifuged for 10 min at 10000xg in 4 ºC. Negative and positive reaction tubes were prepared with 1 mL of 20%TCA and with 1 mL of 20% TCA + 0.5% thiobarbituric acid (TBA), respectively. A volume of 250 µL of SN was added to each tube, these were incubated for 30 min at 95 ºC and quickly placed on ice for 10 min. After centrifugation at 10000xg for 10 min at 4 ºC, the SN absorbance was determined at 532 and 600 nm (Araújo *et al.,* 2016). MDA concentration was calculated from the difference of the absorbance between Abs^{+} ($Abs\ 532^{+}$ - $Abs\ 600^{+}$) and Abs^{-} ($Abs\ 532^{-}$ - $Abs\ 600^{-}$). MDA equivalents ($nmol.mg^{-1}FM$) were calculated as (Abs^{+} - Abs^{-}) / 157000) x 10^{9}.

2.8. H_2O_2 content

The H_2O_2 concentration in leaves was measured according to Dias *et al.* (2014). Fresh samples (100 mg) were homogenized with 1 mL of 0.1% (w/v) TCA. The homogenates were vortexed and centrifuged at 12000xg for 15 min at 4°C. To determine the H_2O_2 content, 500 µL of the supernatant was added to 500 µL phosphate buffer 0.1 M (pH~7.0) and 1 mL of KI 1 M. After 1h of incubation in dark, absorbance was measured at 390 nm. H_2O_2 concentration (mmol.g^{-1}FM) was calculated from of a standard curve.

2.9. Total phenols and free radical scavenging activity

Total hydrosoluble phenol content (TPC) was quantified according to the methodology reported by Dewanto *et al.* (2002) with some modifications. Fresh sample leaves (100 mg) were homogenized in 1.67 mL of deionized water, then filtered (by membrane filters, Whatman®) and centrifuged at 2500 rpm for 10 min. The reaction solution contained 500 µL of deionized water, 125 µL of extract and 125 µL of Folin–Ciocalteu reagent. After 6 min, 1250 µL of 7% Na_2CO_3 was added and the final volume adjusted to 1 mL with deionized water. After 90 min of reaction, the samples were measured by reading the absorbance of 760 nm. A standard curve was made using gallic acid (GA). TPC was expressed as gallic acid equivalents per mL of volume solution (µgGAE.mL^{-1}).

The antiradical activity of phenols was measured according to Harkat-Madouri *et al*. (2015) with some modifications. Fresh leaves (100 mg) were homogenized in pure methanol (1.67 mL). The homogenate was centrifuged at 2500xg for 10 min. A solution reaction was made with different dilutions of the extract (0, 4%, 8%, 10% and 30%) for 250 µL and 1.250 mL of DPPH 0.1 mM and after 30 min read at absorbance 517 nm. Data were expressed as DPPH SA (%) = $[(A_C - A_S)/A_C] \times 100$, where A_C and A_S are the control (0%) and sample absorbances, respectively. IC_{50} values were determined by the volume of extract necessary to cause 50% reduction of DPPH.

2.10 Statistical analysis

Experiments used ~7-10 top mature leaves (with similar age) from different plants, which were treated as biological replicates and/or as pools, with at least 3 independent technical replicates. Presented values are the mean ± standard deviation. Comparisons between all treatments and the control were made using One Way ANOVA test. When data was statistically different, the Dunnett Comparison Test

(p<0.05) was also applied. Graphpad™ Prism 6 was used. Multivariate analyses for data correlation used Principal Component Analysis and were performed with CANOCO for Windows v4.02 programme.

3. Results

At the end of the UV-exposures (30 days), UV-A irradiated plants showed (more evidently at the lowest dose) growth and morphology similar to those of the control.

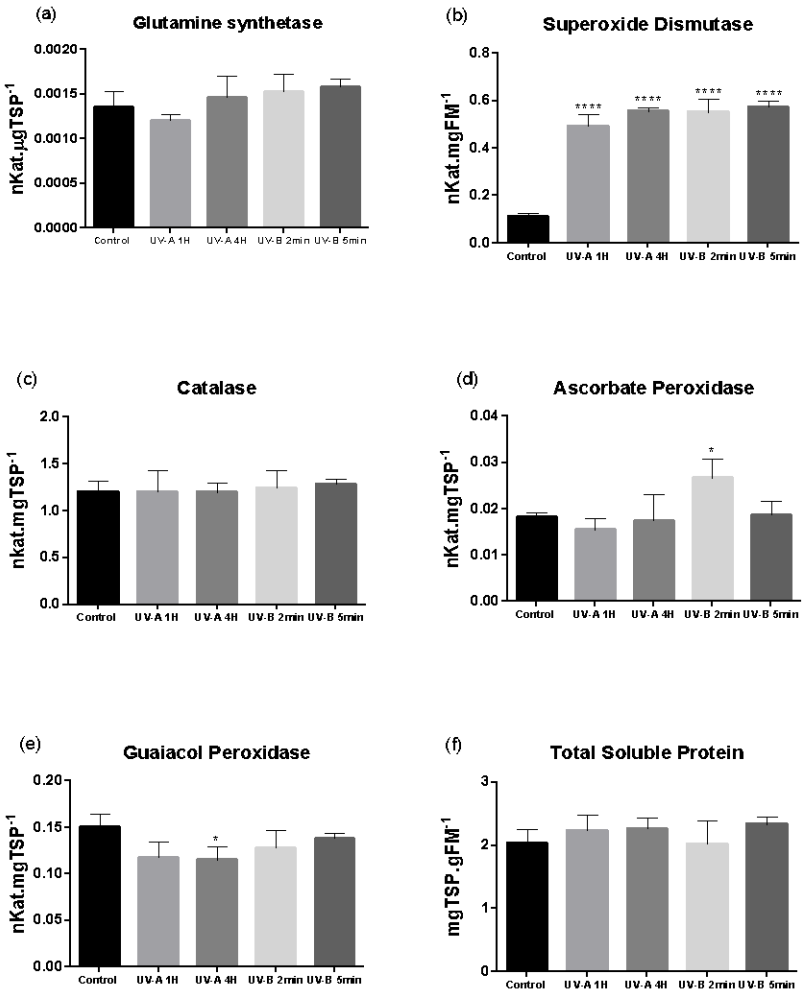

Figure IV.1. GS and enzymatic scavenging of ROS activities, after 1 month of exposure to moderate UV-A and UV-B supplementation. Activities of several enzymes were measured: (a) glutamine synthetase (GS, µmol.µgTSP-1); (b) superoxide dismutase (SOD, U.mgFM-1); (c) catalase (CAT, nkat.mgTSP-1); (d) ascorbate peroxidase (APX, U.mgTSP-1); and (e) guaiacol peroxidase (GPX, U.mgTSP-1). Total soluble protein (mgTSP.gFM-1) was also measured. Compared with the control, in each UV condition, * and ** mean significant differences for p≤0.05 and 0.0001 respectively. The bars represent the standard deviation.**

These plants looked healthy and leaves showed no significant chlorosis or necrotic spots. UV-B plants presented a slight increase in the number of necrotic spots. Similarly to what was described elsewhere on the negligible effects of UV on carbon metabolism (Ponte *et al.,* 2017; see also *Chapter 3*), also all UV-A and UV-B irradiation doses had no negative impacts in GS activity, a parameter often used to assess the status of plant nitrogen metabolism and productivity, and also related with stress (*Figure IV.1. a*).

3.1. Leaf enzymatic antioxidant mechanism to UV exposure

Plants exposed to UV-A or UV-B for 30 days, in general, had stimulated their first antioxidant enzymatic battery (*Figure IV.1. b-e*). In particular, SOD activity increased from of 431 to 503% compared to the control (*Figure IV.1. b*). Contrarily the CAT activity was not significantly affected by the UV-A/B irradiation (*Figure IV.1. c*), while APX activity only increased in leaves exposed to the lowest UV-B dose (2min; *Figure IV.1. d*). The GPX activity had, in general, a trend to be reduced by UV-A/B moderate treatments compared to control, a trend that was significant for UV-A 4h (*Figure IV.1. e*). TSP did not show significant differences, but its amount tendentially increased in plants supplemented with UV-A/B (*Figure IV.1. f*).

A relative expression of *cat1* gene showed that despite an increase of the transcript was observed in UV-A 4h and UV-B 5min treatments compared to control, this was not significant (*Figure IV.2. a*). Contrarily, the transcript level of the *grcyt* gene increased for UV-supplementation conditions, particularly at UV-B 5min (*Figure IV.2. b*).

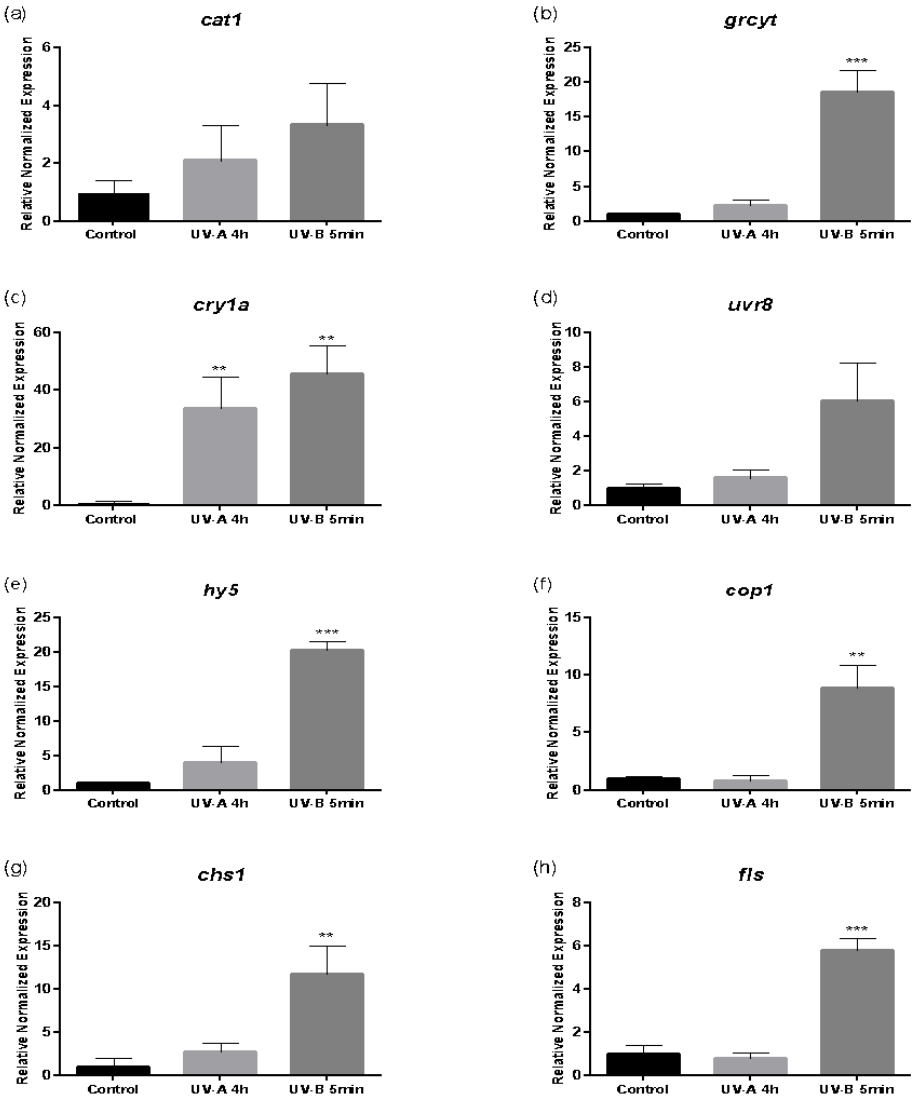

Figure IV.2. Transcripts of enzymatic/non-enzymatic battery and polyphenol biosynthesis after 1 month of exposure to moderate UV-A and UV-B supplementation. The relative normalized expression regarding the control is presented: transcripts related with oxidative stress: (a) catalase 1 (cat1); (b) glutathione reductase of cytosol (grcyt). Transcripts related with non-enzymatic antioxidant pathways: (c) cryptochrome 1a (cry1a) and (d) uv-b resistance 8 (uvr8); transcriptor factor: (e) elongated hypocotyl 5 (hy5) and repressor: (f) constitutive photomorphogenic 1 (cop1). Transcripts related to polyphenol biosynthesis: (g) chalcone synthase 1 (chs1) and (h) flavonol synthase (fls). Transcripts were assessed for control, UV-A 4h and UV-B 5min conditions. For the same control condition, ** and * mean significant differences for p≤0.01 and 0.001 respectively. The bars represent the standard deviation.**

3.2. Leaf non-enzymatic antioxidant mechanism to UV exposure

The TPC showed a trend to increase with UV-supplementation, with significant changes for UV-A1h, UV-B 2min and UV-B 5min (*Figure IV.3. a*). On other hand, radical scavenging activity of phenols in UV-treated leaves was stronger, as the volume

of sample extract necessary to reduce 50% of DPPH significantly decreased in those leaves (*Figure IV.3. b*).

A relative expression of *cry1a* gene showed a significant increase of this transcript in response to UV-A 4h and mostly to UV-B 5min (*Figure IV.2. c*). The *urv8* photoreceptor and *hy5* transcripts increased in both conditions, particularly for UV-B condition (*Figure IV.2. d-e*). The gene of c*op1* was upregulated also by UV-B. Similarly, the relative expression of *chs1* and *fls* increased mostly at UV-B 5min (*Figure IV.2. f-h*).

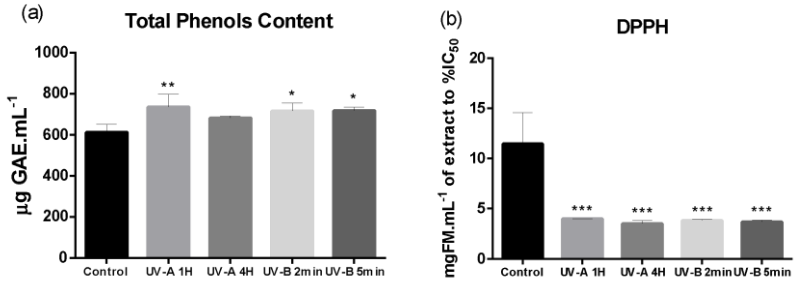

Figure IV.3. Non-enzymatic antioxidant capacity after 1 month of exposure to moderate UV-A and UV-B supplementation. Total phenol content (µgGAE.mL-1) and antiradical activity [mgFM.mL-1 of extract to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 50% (%IC50)] after 30 day of UV-A and UV-B supplementation. FM (Fresh Matter) and GAE (Equivalents of Galic Acid). All parameters were measured to control, UV-A 1h, UV-A 4h, UV-B 2min and UV-B 5min. For the same control condition, *, ** and * mean significant differences for p≤0.05, 0.01 and 0.001 respectively. The bars represent the standard deviation.**

3.3. H_2O_2 content and stability of membranes

The amounts of H_2O_2 in leaves supplemented with UV-A 1h and UV-B 2m remained statistically unchanged compared with those of the control, and increased in both UV-A/B higher exposures being statistically significant for UV-B 5min (*Table IV.2.*). MDA levels overall did not change significantly with any UV supplementation (*Table IV.2.*). Similarly, cell membrane stability was not affected, i.e. no increase in electrolyte leakage was found (*Table IV.2.*).

Table IV.2. Quantification of membrane damage, MDA and H2O2 after 1 month of exposure to moderate UV-A and UV-B supplementation. Leaves of tomato plants were used to quantify the percentage (%) of membrane damage (MD). Malondialdehyde (MDA) was measured (MDA equivalents) to assess the lipid peroxidation and the hydrogen peroxide (H2O2) was quantified. * represents significant differences for p≤0.05, when compared with the control, in each condition. Values are expressed as mean ± standard deviation.

Treatment	H_2O_2	MDA	CMS
	mmol.gFM^{-1}	*MDA equivalents (nmol.mL^{-1}.mgFM^{-1})*	*%MD*
control	1.925 ± 0.200	270.446 ± 40.109	5.154 ± 0.517
UV-A 1h	1.178 ± 0.203	262.420 ± 27.154	3.902 ± 0.676
UV-A 4h	2.154 ± 0.432	233.631 ± 20.636	5.790 ± 0.884
UV-B 2min	1.639 ± 0.265	242.548 ± 14.967	3.362 ± 0.698
UV-B 5min	2.908± 0.252 *	238.089 ± 21.237	5.224 ± 0.400

3.4. Fruiting and productivity

The production of total fruits, and their distribution considering four different ripening categories (immature green, mature green, yellow and red fruits), was significantly influenced by the UV-A/B supplementation. Overall, while all treatments showed a tendency to increase all classes of ripening, the effects were more evident for UV-A 4h and UV-B 2min in immature green fruits, and for UV-A 1h to red fruits (*Table IV.3.*).

Table IV.3. Production and maturation of fruits after 1 month of exposure to moderate UV-A and UV-B supplementation. The number of fruits in each plant of each condition was verified by four different development stages (immature green, mature green, yellow and red). The percentage (%) of differences to control situation were calculated, positive (+) and negative (-). All data were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. * and ** represent significant differences for p≤0.05 and 0.01, respectively, when compared with the control, in each condition. Values are expressed as mean + standard deviation. The percentage (%) of variation in relation to the control is expressed in brackets.

Treatment	Immature green	Mature green	Yellow	Red
control	4.3 ± 4.22	3.1 ± 2.99	1.0 ± 1.25	6.2 ± 3.19
UV-A 1h	11.7 ± 6.72 (+155.8)	3.9 ± 3.57 (+25.8)	2.9 ± 2.38 (+190.0)	12.8 ± 3.55**(+106.5)
UV-A 4h	13.1 ± 9.99* (+204.7)	1.7 ± 1.42 (-45.2)	1.3 ± 1.49 (+30.0)	9.7 ± 2.83 (+56.5)
UV-B 2min	14.2 ± 9.31* (+230.2)	3.8 ± 2.25 (+26.7)	2.5 ± 2.72 (+150.0)	10.3 ± 6.02 (+66.1)
UV-B 5min	7.7 ± 5.64 (+79.1)	3.7 ± 2.00 (+19.4)	1.2 ± 0.92 (+20.0)	9.7 ± 3.83 (56.5)

3.5. Multivariate approach

Principle component analysis showed a clear separation between control and UV-B treatments (*Figure IV.4.*). PC1 explained 41.9% of the variance and PC2 28.5% of the variance. Regarding the PCA analysis, three distinct populations are identified: 1) the control (the only population positioned in the right half) associated with GPX; 2) the two

populations of UV-A 1h and UV- B 2min, both at the left upper quadrant, scoring associated for fruit ripening; and the two higher UV-A/B doses (UV-A 4h and UV-B 5 min) positioned at the left lower quadrant with similar scores regarding the oxidative impact and antioxidant responses (particularly H_2O_2 and CAT). All UV groups positioned at the left half also correlated with SOD and total phenolic compounds.

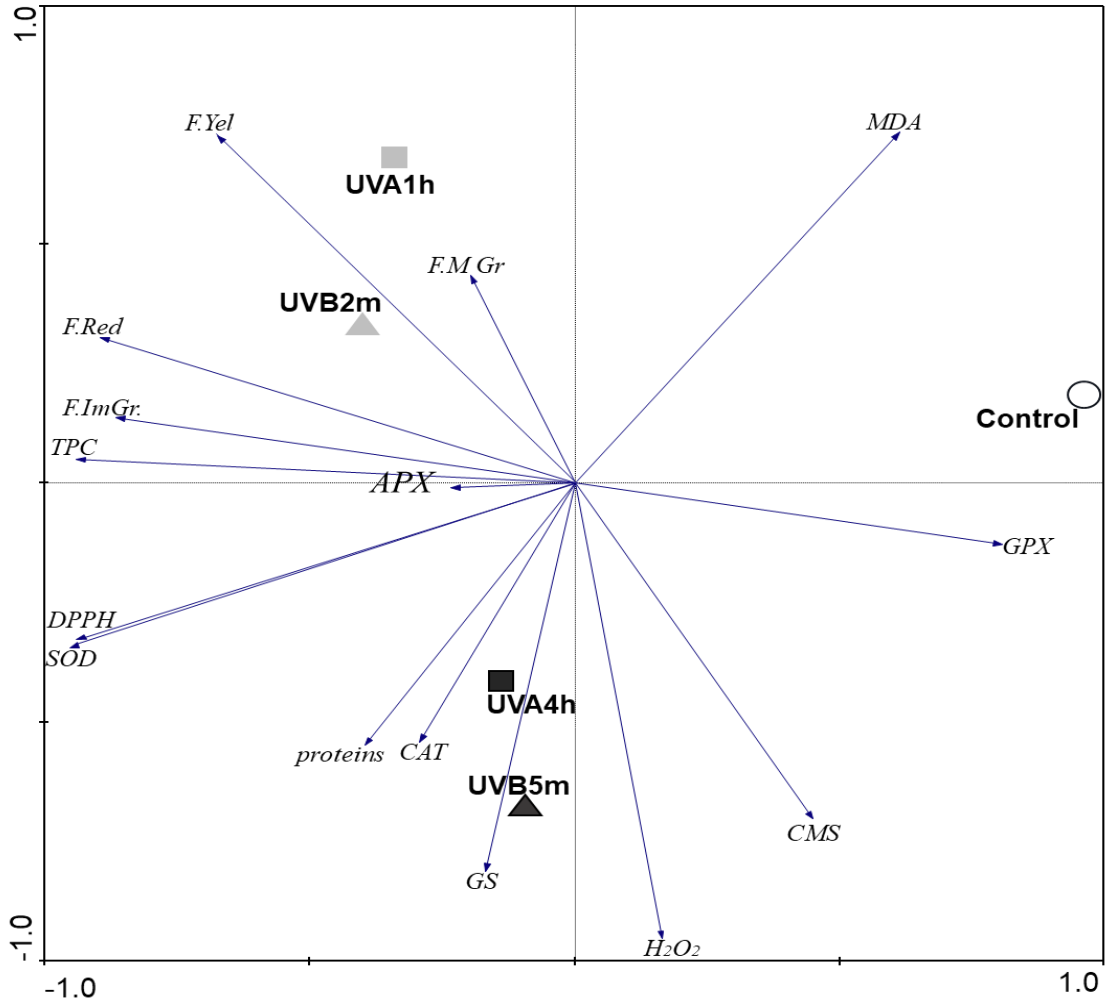

Figure IV.4. PCA analyses of functional responses of tomato fruiting plants exposed to UV-A (1 and 4h) and to UV-B (2 and 5min) for 30 days. Abbreviations: F.Yel (Yellow Fruit); F.Red (Red Fruit); F.ImGr (Immature Green Fruit); F.M Gr (Mature Green Fruit).

4. Discussion

To advise horticulture producers regarding the best UV-lamp sources and irradiation programs, there is a need to ensure that the applied UV-A/B dose does not induce deleterious impacts on the cultures (e.g., necrosis, oxidative stress disorders and compromised photosynthesis), while leading to beneficial impacts (e.g., increased

flowering/fruiting synchronization, maturation, nutritional value) (Bernal *et al*., 2015; Brazaityte *et al*., 2015; Mewis *et al*., 2012; Perez *et al*., 2009; Sakalauskaitė *et al*., 2013). We have recently demonstrated in MicroTom cultures that moderate UV-A and UV-B increased fruit phenols' profiles (data not shown) and stimulated plant yield, while inducing minor/negligible impairments in carbon metabolism (Ponte *et al*., 2017; see also *Chapter 3*). These data are in line with the stimulation of fruit production and maturation, paralleled by an increased synchronization of total flowers and fruits, observed in the MicroTom irradiated plants, particularly for the lower UV-A and UV-B doses as demonstrated by the PCA analyses (*Figure IV.4.*). This UV-control of fruit maturation/synchronization represents an additional tool for producers to better schedule their harvest campaigns in order to place in the market the fruits at an optimal maturation stage and richer in nutritionally valuable compounds.

The observed UV-A/B control on flowering/fruiting may be an ultimate result of the cascade of events triggered by blue/UV-A or UV-B photoreceptors. The fruit synchronization observed in the UV-B irradiated MicroTom plants may ultimately result from the photoreceptor UVR8. However, the results of physiological response to UVR8/UV-B seems not consensual, depending on species and UV-B dose (reviewed by Huché-Thélier *et al*., 2016). For example, in *Limnanthes alba* and in *Phacelia campanularia* exposed for 5h/day to high UV-B doses ranging from 3-15 kJ/m^2 (much higher than the doses used in the present study) *L. alba* plants showed a decrease in flowering and *P. campanularia* delayed flowering (Sampson and Cane, 1999). In other experiments, *Malcomina maritima* plants growing under solar light with blocked UV-B irradiation had impairments in flowering/fruiting compared to plants growing with unfiltered solar radiation (Petropoulou *et al.,* 1995). The results of beneficial effects of blue/UV-A light seems more consensual and may ultimately derive from the cascade of events triggered by the photoreceptors for blue/UV-A that include the FLAVIN BINDING KELCH REPEAT, F-BOX 1 (KF1) (Song *et al.,* 2012), which together with cryptochromes (CRY1 and CRY2), is deeply involved in flowering, and consequently determine fruit production (reviewed by Huché-Thélier *et al*., 2016).

UV rays are also known to induce changes in the ROS levels/profiles and therefore influence the cell redox balance, affecting cell functional changes. The cell will respond in order to regain its normal homeostatic balance and function, reducing ROS levels through enzymatic and non-enzymatic mechanisms (Gill and Tuteja, 2010; Hasanuzzaman *et al.*, 2012). It is well documented that the leaf antioxidant enzymatic battery (eg., SOD, APX, CAT and GPX) is increased by solar and artificial exposure to

UV rays (Abdel-Kader *et al* 2007; Alexieva *et al*., 2001; Inostroza-Blancheteau *et al.,* 2016; Mishra *et al.,* 2009). In the MicroTom irradiated leaves, we demonstrate that the activity of SOD is highly stimulated by both moderate UV-A/B supplementation, which indicates that there is a high imbalance on ROS homeostasis particularly with the increase of superoxide, which is converted into H_2O_2 by SOD. The PCA analysis clearly demonstrates that these changes in the redox status are particularly evident for the higher UV-A and UV-B doses. Thus, even considering an acclimation period and that despite protective mechanisms the UV reaching the mesophyll, the UV-reaching the mesophyll cells was sufficient to induce some oxidative stress.

Interestingly, for other UV-exposures and species (Costa *et al.,* 2002) the formed H_2O_2 is removed by CAT, APX and GPX, but for the UV-A/B doses used in MicroTom, these enzymes showed a modest stimulation The analysis of transcripts, namely *cat1*, also validates that this enzymatic battery does not play a major role in MicroTom response to UV radiation. On other hand, the increase of *grcyt* in UV-B exposed leaves (contrarily to UV-A irradiated ones) also suggests differences in the cell responses to different UV wavelengths, probably with a higher recruitment of the GR enzyme in the UV-B exposed leaves. Younis *et al.* (2010) also showed that both UV-A and UV-C stimulated GR activity (together with other enzymes) in bean, as well as, Costa *et al.* (2002) in sunflower seedlings.

The modest contribution of the enzymatic antioxidant battery, and the low increments on the H_2O_2 produced by the high increments of SOD suggest that for this moderate UVA/B supplementation, the plant responds triggering an alternative efficient antioxidant capacity to degrade the formed H_2O_2. To address this hypothesis, we evaluated the phenol content and the antiradical activity. Phenol compounds (e.g., flavonoids) have a capacity to scavenge H_2O_2, $^1O_2$ and/or OH^-, in cytosol and vacuoles (Agati *et al*., 2013; Das and Roychoudhury, 2016). Phenolic compounds result from plant secondary metabolism, and play a major role in antioxidant activities, being able to act as the first barrier in ROS scavenging or suppress/complement the antioxidant-enzymatic battery to control excessive ROS (Agati *et al*., 2012). The overall increases of antiradical activity and TPC under both UV-A/B conditions (evident in the PCA analysis) and demonstrate that phenols play a major antioxidant protective role in UV-irradiated MicroTom leaves. This is in line with the findings of Brazaityte *et al.* (2015) who showed that microgreens exposed to moderate UV-A (1.26 and 2.49 J/m^2) increased their antiradical activities and phenol content. The same increase was

demonstrated by Ghasemzadeh et al. (2016) in *Ocimum basilicum* growing under three moderated UV-B doses (2.3, 3.6 and 4.8 J/m^2) for 4-10 h.

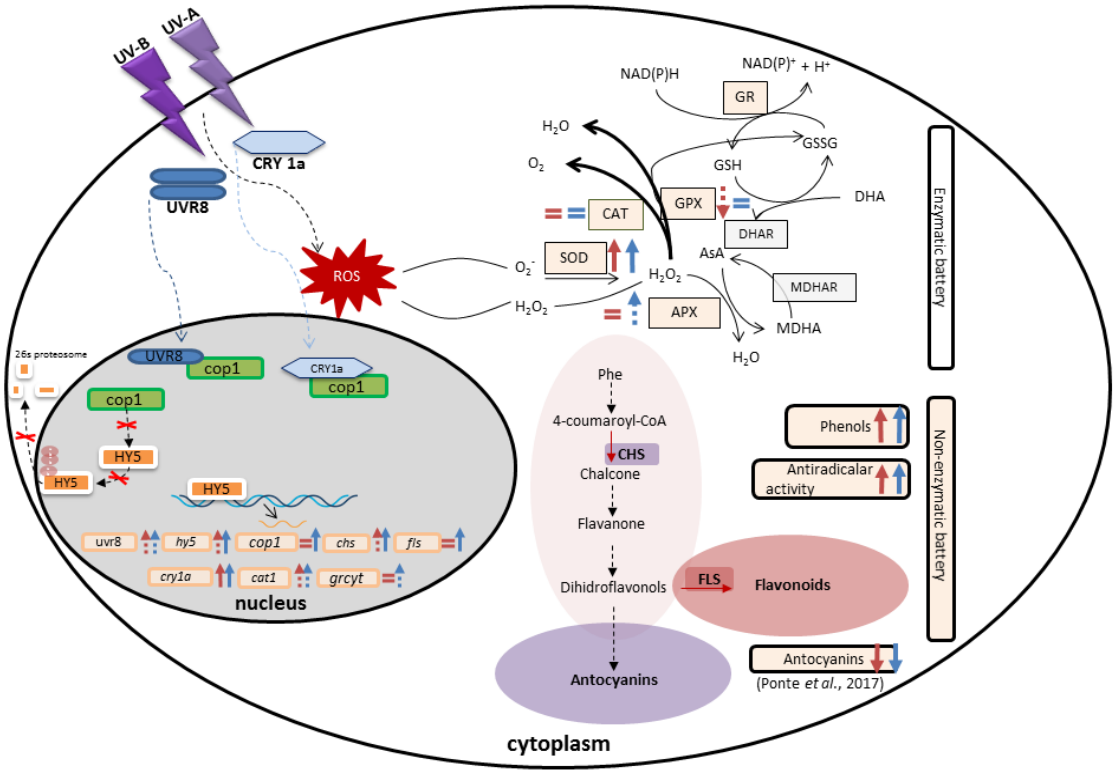

Figure IV.5. An integrative response of the enzymatic and non-enzymatic battery to ROS-induced by UV-A and UV-B, and molecular response. Cellular response after 1 month of supplementary UV application in fructification phase of *Solanum lycopersicum*. UV-A and UV-B promote an imbalance in ROS levels which promote an increased enzyme activity (especially SOD activity) and a stimulus for the transcription of some genes of this antioxidant machinery (*cat1* and grc*yt*). CRY1a and UVR8 photoreceptors, when stimulated by UV-A and UV-B respectively, will bind to the HY5-repressor COP1 in the nucleus, preventing the degradation of HY5 transcription factor (marked as "red x"). Consequently, HY5 can bind to its link site in DNA and promote the transcription of several genes involved in phenol biosynthesis (such as, *chs1* asn *fls*). Non-enzymatic antioxidant battery (phenol content and antiradical activity) was increased by UV-A and UV-B, helping the cell with ROS detoxification. Red arrows represent the UV-A related increase (up arrow) or decrease (down arrow) and blue arrows the changes promoted by UV-B, increase (up arrow) or decrease (down arrow). Dashed arrows represent a trend influenced by UV-A (red dashed arrow) and UV-B (blue dashed arrow). The equal sign represents no alteration compared to control. Abbreviations: UV-A/B (ultraviolet A/B); ROS (reactive oxygen species); UVR8/ *uvr8* (uv-b resistance 8 protein/ gene); CRY1a/ *cry1a* (cryptochrome 1a protein/ gene); COP1/ *cop1* (constitutive photomorphogenic 1 protein/ gene); HY5/ *hy5* (elongated hypocotyl 5 protein/ gene); *cat1* (catalase 1 *gene*); *grcyt* (cytosolic glutathione reductase gene); *fls* (flavonol synthase gene); *chs1* (chalcone synthase 1 gene); SOD (superoxide dismutase); CAT (catalase); APX (ascorbate peroxidase); GPX (guaiacol peroxidase); GR (glutathione reductase).

The phenylpropanoid pathway is among the multiple pathways influenced by photoreceptors blue/UV-A and UV-B photoreceptors. Transcriptional analysis of some related genes, namely the photoreceptors *cry1a* and *uvr8*, the transcription factor *hy5*

and its repressor *cop1* support the functional and biochemical changes observed in irradiate MicroTom plants, and their consequent cascade of events is proposed in *Figure IV.5*. These genes have been associated with upregulation of genes associated to UV light, promoting a photoprotective mechanism, namely by inducing a largely family of polyphenol through increase on the phenylalanine pathway, particularly the flavonoid pathway (Gruber *et al*., 2010; Singh *et al*., 2014). In MicroTom, the stimulus of this polyphenols pathway was confirmed by the upregulation of *chs* and *fls*, respectively upstream and downstream the flavonoids pathway. This increase in relative gene expression strongly supports the increase of TPC and antiradical activities of the same irradiated plants. Other studies showed that *chs* (Favory *et al.,* 2009; Jenkins and Brown, 2007) and *fls* (Liu *et al.,* 2014) were upregulated after UV-A or UV-B exposure (Müller-Xing *et al.,* 2014). Considering the results of the enzymatic pathway, one may propose that besides their antioxidant role, another major function of phenylpropanoid compounds could have been screening of UV radiation.

As we demonstrated previously (Ponte *et al.,* 2017; see also *Chapter 3*), the UV-doses used here do not induce significant morphological changes, except for an occasional increase of some necrotic spots in UV-B irradiated leaves. This fact, together with the activation of the antioxidant non-enzymatic machinery and the low levels of H_2O_2, support the lack of significant increases of cell damage measured by the CMS. The membrane is a major target of the increase of free radicals and uncontrolled increase of oxidative stress (Anjum *et al*., 2014). The tropical species *Moringa oleifera* exposed to supplemental UV-B, showed an increase of MDA and CMS but these increases were reverted after a few days recovery period, supporting also that plants may trigger recovery strategies to repair oxidative damages.

5. Conclusion

In conclusion, while the vegetative growth was not impaired (shown by the morphological analyses and by the normal GS activity) by the supplementation of moderate UV-A/B irradiation, plants' flowering and fruit ripening synchronization was enhanced. An integrative functional model, comparing the effects induced by UV-A and UV-B regarding the antioxidant enzymatic vs non enzymatic strategies is outlined in *Figure 5*. From this functional model, and from the PCA analyses it is evident that UVA/B-irradiation increased ROS production (most probably superoxide, converted to H_2O_2 by SOD), then neutralized by phenylpropanoid pathways. Interestingly, and as

demonstrated by the PCA analyses the minimum and maximum doses of UV-A paralleled the minimum and maximum effects of UV-B respectively. As clearly shown by the PCA, the lower doses of UV-A or UV-B induced the best effects on flowering/fruiting with lowest redox disorders, thus fitting the requirements for an "eustress" condition. Finally, considering the benefits observed on flowering/fruiting, with minimal impacts in the vegetative part, we demonstrate that both UV-A/B could be used in protected tomato horticulture systems, making these systems closure to the open field UV radiation often observed in e.g., Mediterranean open fields. Considering the overall benefits here demonstrated, the economic aspects for the producer (e.g., the cost of UV-A and UV-B lamps) and the risks to the operator inherent to UV-B radiation, we suggest that UV-A would be more suitable in large scale production.

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V. Chapter 5: The potential use of UV-A and UV-B to improve tomato quality and preference for consumers

Abstract

Solanum lycopersicum L., considered a “functional food”, is one of the most worldwide consumed greenhouse-grown crops. Greenhouses and off-season productions have poor reputation when compared to in-season and field productions, mostly because they do not allow UV rays to reach plants. We hypothesise that controlled moderate UV-A and UV-B irradiation during fruit ripening is capable to shift antioxidant and phenols-related pathways and increase fruit nutritional value. We exposed ‘MicroTom’ fruiting plants to two daily doses of UV-A (1 and 4 hours) and UV-B (2 and 5 minutes) for 30 days as supplementation to photosynthetic active radiation. Overall, all UV-A/B conditions increased fruit production. UV-A irradiation stimulated the antioxidant capacity in fruits, antiradical activity and the accumulation of phenolic compounds, ortho-diphenols, and flavonoids. Moreover, UV-A 4h increased the fruit firmness, an important parameter for shelf life. Tomatoes were also evaluated by a consumer's’ panel, who pointed UV-A as the preferred fruits. This preference supported that UV-A was particularly effective in increasing the nutritional value in fruits and also increasing their aroma and flavour making them more appealing to consumers.

In conclusion, the use of UV supplementation, particularly UV-A, can be a preharvest tool to modulate in a controlled manner crops/fruits nutritional quality (increasing antioxidant and phenols) and promoting an increase of this “healthy food”.

Keywords

Antioxidants; Functional foods; Metabolism modulation; Nutritional value; Phenolic compounds; *Solanum lycopersicum*; Ultraviolet supplementation

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular and appreciated fruits, having a significant agricultural and economic importance (Žižková *et al*., 2015). Its

excellent acceptance by the consumers is due to its multiple gastronomic uses and to other features like its taste, colour and high nutritional value (Verma *et al.*, 2015). Fresh tomato fruits are extremely beneficial to human health due to their richness in folate, potassium, vitamin C, carotenoids and flavonoids (Aust *et al.*, 2005; Panthee *et al.*, 2013; Pérez *et al., 200*8). Carotenoids (e.g., the reddish lycopene) and flavonoids present in tomato fruits protect the consumer from various cardiovascular diseases, different types of cancer and neurodegenerative disorders (Figueira *et al*., 2017; Thies *et al.*, 2017). This richness in phytocompounds of interest, which can have a beneficial role in human health, set the tomato as a "functional and healthy food" (Canene-Adams *et al*., 2005; Kushi *et al*., 2006).

This crop can be produced in open-field or protected horticulture (e.g., greenhouses). Modern greenhouses usually use polycarbonate covers, which present several advantages and are more affordable than glass covers. These covers also avoid the transmittance of ultra-violet (UV) rays, thus allowing that only wavelengths >400 nm reach the plant and fruit (Kwon *et al.,* 2017). On the other hand, vegetables and fruits produced in greenhouses have several differences when compared to open-field productions. Whilst the production may be extended to off-season periods, the depletion of solar UV in protected cultures compromises the sensorial/nutritional quality of the fruit, compared with their open-field counterparts. Moreover, this loss of quality may be aggravated in off-season compared to the in-season production. Overall, off-season tomato fruits present a poor reputation (e.g., taste and flavour) compared to in-season and, even less, to open-field productions (Gruda *et al*., 2005, Muñoz *et al*., 2007).

UV radiation is involved in several changes in the plant metabolome. Studies have addressed plant responses to UV-B, and less to blue/UV-A (Coffey *et al*., 2017; Facella *et al*., 2016; Heijde and Ulm, 2012). Recent research has demonstrated an important role of the photoreceptors in the modulation of several molecular changes that are involved in many specific metabolic pathways. Besides its direct effects mediated by photoreceptors, UV light also increases ROS production and stimulates several antioxidant compounds, as a protection mechanism for plants (Ilic and Fallik, 2017). Specifically, phenolic compounds (e.g., flavonoids) have an important role in plant photoprotection due to both UV-A/UV-B screening and antioxidant roles (Agati *et al.*, 2012; Lobo *et al*., 2010). Under UV, light several pathways of these compounds are increased, namely the phenylalanine ammonia-lyase (PAL) (Oliveira *et al*., 2016), the chalcone synthase (CHS) and flavonol synthase (FLS) pathways, eventually also

increasing the proportion and levels of different polyphenols in the fruit/crop (Heijde and Ulm, 2012). Therewith, the inherent rise of the antioxidant capacity will potentially increase the nutritional benefits to consumer's health (Lobo *et al.,* 2010). Moreover, several volatile organic compounds (VOCs) that have an important contribution to fruit flavour and taste experience, are synthesized in the flavonoids and carotenoids pathways, thus having an important contribution to fruit flavour and taste experience (Severo *et al*., 2017).

The application of UV-A/UV-B rays in vegetable and/or fruits production, namely during the pre-harvest phase has much to be explored. Industries are starting to use mostly UV-C ionizing radiation in post-harvest and processed foods, particularly focused on antimicrobial effects (Manzocco *et al*., 2016; Sivakumar and Bautista-Baños, 2014). However, additional potential benefits of UV's by changing the quality of fruits/vegetables (e.g., antioxidant properties) and increasing their shelf-life are being increasingly demonstrated (Urban *et al*., 2016). Application of UV-A/UV-B in pre-harvest systems is less explored, but can be a way of also modulating the quality of protected vegetable products, making them more appealing to consumers (Bian *et al*., 2014). Brazaityte *et al*., (2015) showed that moderate doses of UV-A light can increase anthocyanins content in microgreens. Lee *et al*. (2013) using UV-A and UV-B supplementation in sowthistle also demonstrated a modulation of phenolic compounds production. In tomato production, Dzakovich *et al*. (2016) showed that use of UV-B promoted the fruit quality by strongly increasing some genes expression of carotenoids and polyphenols pathways. Finally, the use of UV-C during pre-harvest in strawberry fruits also improved the quality of the fruits (Oliveira *et al*., 2016; Xie *et al*., 2015; Xie *et al*., 2016; Xu *et al*., 2017).

Using moderate UVs to promote fruit antioxidant properties and stimulate specific cell pathways to increase the levels of certain secondary metabolites is already supported by some literature. Our aim in this work is to increase the quality of tomato fruits through the use of UV-A and UV-B supplemental light to modulate the increase of phytocompounds' production and evaluate the acceptability of consumers to the product.

2. Material and Methods

2.1. Plant growth conditions and UV treatments

Plants of *Solanum lycopersicum* L. cultivar 'MicroTom' (Just Seed, UK) were germinated on peat:perlite (2:1) in 0.3 L pots. Plants were grown in a climate chamber with a photoperiod of 16h:8h (light:dark) and 200 µmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) supplied by OSRAM L 30W/77 FLUORA fluorescent lamps. Temperature was adjusted to 23 ± 2 ºC and relative humidity (%RH) was 45 ± 5%. Twice a week, plants were irrigated with Hoagland medium (Sigma, USA) with a pH~5.70. First flowering buds emerged by the 90th day, and 10 days after (i.e., 100-day-old plants) all plants looked similar and had a highly synchronized flowering. During fruiting and fruit ripening period (corresponding to the period between day 100 and day 130), plants were randomly divided in five groups, for exposure to UV. In Group 1, plants were irradiated with the same PAR condition and were not supplemented with UV (*Control, C*). In Group 2 (*UV-A1h*) plants were supplemented daily for 1 h with 0.8 J/m^2 UV-A supplied by Fluorescent Blacklight [F20T12/BLB - 20W T12 (T10)] lamps with a maximum peak emission at 368 nm (irradiation of l<368 nm was ~0 W/m^2). In Group 3 (*UV-A4h*), plants were daily exposed to 0.8 J/m^2 UV-A during 4 h (same lamps used in *Group UV-A1h*). In Group 4 (*UV-B 2min*) plants were daily exposed to 2.94 J/m^2 UV-B for 2 min (light was supplied by six 8W lamps 312 nm TFP-M/WL, having no detectable emission of l<312 nm). Finally, in Group 5 (*UV-B 5min*) plants were exposed to the same conditions of the Group *UV-B 2min* but for 5 minutes. UV-A and UV-B intensity was quantified, respectively, by Sensor Meters Philip Harris (4375 model SEL240) and International Light INC (Newbryport, Massachusetts, 01950, IL1400A).

After 30 days of UV supplementation, the number of total fruits per plant in each condition was registered. Also, the stage of fruit ripening in fruits with the same age was determined, and fruits were used in the physicochemical and sensorial assays.

2.2. Fruit biometric and total soluble solids analyses

Fruit fresh matter (FM) was measured in a precision balance (0.001 g) (LPW-213i, VWR). Dry matter (DM) was also quantified after six days at 70 ºC. Water content (WC%) was quantified by FM and DM differences. The calibre of fruits (length, small

diameter and larger diameter) was measured with an electronic digital caliper, and expressed in mm.

Total soluble solids were measured in tomato juice of 18 ripe red tomatoes from 10 different plants per treatment. To measure the total soluble solids values a refractometer (Atago PR-101, Japan) was used at 20 ºC. Results are expressed in ºBrix content.

2.3. Colour

Fruit colour was monitored with a colorimeter Minolta CR-300 (USA). Measurements were made in the middle zone of fruit and the CIELab chromatic system was applied to analyse the colour. Results were expressed by luminosity coordinates (L*), chroma (C*) and tonality (hº). Luminosity can change between 0 (black) and 100 (white). Chroma values represent the colour saturation, and tonality is expressed in degrees (hº), where values near to 0º are red tonalities, around 90º are yellow tonalities, near to 180º are green, values around 270º are for blue (Pathare *et al*., 2013). For each treatment, eight ripe tomatoes, from different plants, were used. The following equations were used to calculate C* (a) and hº (b):

a) $C^* = \sqrt{a^2 + b^2}$

b) $\mathrm{h^o} = \frac{(\mathrm{arctg}\,\frac{\mathrm{b*}}{\mathrm{a*}})}{6.2832} \times 360$

2.4. Texture

Tomato fruit texture was measured through its firmness by applying a Stable Micro Systems coupled with a P75 plate (with 75 mm of diameter) and a charge of 5 kg. Eight ripe tomatoes in the same state per group condition were collected and used to assess the firmness. The charge was applied to the fruit with a velocity of 1 mm s^{-1} until 5 mm of distance was achieved. The firmness was assessed in the equatorial zone of the whole tomatoes and the results are expressed in Newton (N).

2.5. Antioxidant activity

Frozen tomato dried mass (1 g) was extracted in 30 mL of methanol:deionized water (1:1) and incubated 30 minutes at room temperature with smooth agitation. The extract

was centrifuged for 10 min at 10000 g (3K30, Sigma, Germany). The supernatant (SN) was collected and the volume adjusted to 30 mL with extraction solution, being this step repeated twice. At the end, the volume of the SN was adjusted to 150 mL.

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method (with some modifications) was used to assess the antioxidant capacity (Ozgen *et al*., 2006). Briefly, the reduction capacity of the ABTS was quantified as percentage of inhibition, by measuring the absorbance at 734 nm, and using a standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma). Results were expressed in mg of trolox equivalents per g of DM.

$$inhibition\ \% = \left[\frac{(blancABS_{734\,nm} - sampleABS_{734\,nm})}{blancABS_{734\,nm}}\right] \times 100$$

Total phenols in the same supernatant, were quantified according to Marinova *et al*., (2005). For the reaction, 200 µL of SN were added to 1 mL of Folin-Ciocalteu reagent and to 800 µL of Na_2CO_3 (7.5%, w/v). Samples were homogenised, incubated at room temperature for 30 minutes and were read in a spectrophotometer at 750 nm. A standard curve was made with known concentrations of gallic acid, and results were presented as mg of gallic acid equivalents (GAE) per g of DM.

Ortho-diphenols (o-diphenols) of tomato fruits were measured using the molybdate assay. Briefly, to 4 mL of SN, 1 mL of sodium molybdate solution (5%) was added. The sample was mixed and the reaction occurred for 15 min at ~20ºC. The samples' absorbance was read at 370 nm (Giertych *et al*., 1999), and the results were expressed as GAE, using a standard curve of gallic acid (Sigma, USA).

Total flavonoids were measured according to Silva-Beltrán *et al*. (2015) with some modifications. For the reaction, 250 µL of the SN, 1.25 mL of deionized water and 75 µL of $NaNO_2$ (5%) were mixed. After 5 minutes, 150 µL of $AlCl_3$ (10%) was added and after 6 min of incubation, 500 µL of NaOH (1M) and 250 µL of deionized water were added. The absorbance was read at 510 nm. A standard curve of catechin (Sigma USA) was used, and results were expressed in equivalents of catechin.

2.6. Consumer tasting

A panel of consumers assessed the sensorial fruit quality of the different groups, and general recommendations of Harry *et al*., (2010) and/or of Morten *et al*., (2016) were

followed. The panel consisted of a total of 19 adult volunteers, with balanced gender distribution. The taste room had individualised clean and ventilated cabinets, with a temperature of 20 ± 1 ºC. Supplied light was artificial and its intensity and orientation was similar in all cabinets. Fresh fruits from all conditions (supplied with the anonymous code of A, B, C, D and E) were available to consumers in the moment of evaluation. Water was supplied to be used between samples. Consumers responded to a questionnaire divided in two sections of sensory analyses: Visual/Touch and Gustation/Olfaction. Each section of sensory parameters was divided in two sub-sections: color and surface for Visual/Touch; and taste and aroma for Olfaction/Gustation. All parameters were evaluated using a semiquantitative scale from 1 to 9. For the colour parameter, the panel was asked regarding the fruit colour intensity and homogeneity, peel colour intensity and pulp colour homogeneity (using an observation of the 3 transversely sectioned tomatoes and 3 tomato peels). In colour intensity, 1 represented a "yellowish colour" and 9 a "dark red". For other colour parameters, 1 is "lowest" and 9 is "highest". For surface analysis, the panel analysed the presence of stains [1 (none) and 9 (many)], texture [1 (smoothest) and 9 (roughest)], solidity to touch [1 (softest) and 9 (harshest)], general texture appearance [1 (most brittle) and 9 (most robust)] and deterioration [1 (lowest) and 9 (highest)]. In flavour and taste parameters, the panel evaluated the succulence degree [1 (lowest) and 9 (plenty)], pulp homogeneity [1 (lowest) and 9 (highest)], fresh flavour, common flavour, sweet, salty, balanced, acid, intensity, sour taste [1 (lowest) and 9 (plenty)] and remaining flavour [1 (lowest) and 9 (plenty)]. For the aroma parameter, the panel analysed the balance, acidity, intensity, fruity and matured aroma [1 (lowest) and 9 (plenty)].

To complete the sensorial analyses, the consumers were asked to rank the tomato fruits according to the questions: "*As a consumer, how do you evaluate, visually, each fruit (rank from the most to the least appellative of the fruits A, B, C, D and E)*" and "*As a consumer, how do you evaluate the aroma and the taste of each fruit (rank from the most to the least appellative of the fruits A, B, C, D and E)*. For each corresponding position order (1^{th}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th}) a corresponding inverse score (5, 4, 3, 2, 1) was attributed.

2.7 Statistical analysis

Depending on the parameter, assays used 8-18 plants/fruits, as individual replicates and/or treated as pools, with at least 3 independent technical replicates. Presented values are the mean ± standard deviation. Comparisons between all treatments and the control were made using One Way ANOVA test. When data were statistically different, the Dunnett Comparison Test (p<0.05) was also applied. Graphpad™ Prism 6 was used. Multivariate analyses for data correlation used Principal Component Analysis and were performed with CANOCO for Windows v4.02 programme.

3. Results

After different UV-A (1h and 4h) or UV-B (2min and 5 min) supplementation during 30 days, the fruit production, biometric and analytical characteristics, and sensorial preference were evaluated.

3.1. Fruit production

At the last day of UV supplementation, the number of total fruits was higher in all UV-treatments, with significant differences (p<0.05) in UV-A 1h and UV-B 2min (Table 1). Fruit length was lowest (p<0.05) in UV-A 1h, UV-B 2min/5min. Small diameter decreased in UV-A 1h and large diameter also decreased in UV-A 1h and UV-B 2min (p<0.05).

Table V.1. Biometric analyses of the *Solanum lycopersicum* fruits growing during 1 month with UV-A and UV-B irradiation. The number of total fruits per plant was quantified; at the end of the experiment, fruits were measured regarding Fresh Matter (FM, g), relative Dry Matter (DM, mg.gFM^{-1}), Length (mm), Large Diameter, Small Diameter, ºBrix (%) and Water Content (WC%). Measurements were made in groups of Control, UV-A (1 and 4h) and UV-B (2 and 5min). The symbols *, ** and * represent significant differences for p≤0.05 and 0.01 and 0.001, respectively, when compared with the control, in each condition. Values are expressed as mean ± standard deviation (for n>8).**

Treatment	Number of Fruits	Fresh Matter	Dry Matter	Length	Large Diameter	Small Diameter	ºBrix	WC%
		g	*mg.gFM^{-1}*	*mm*	*mm*	*mm*	*%*	*%*
Control	15.7 ± 7.9	12.02 ± 1.62	74.78 ± 9.63	22.41 ± 1.95	22.58 ± 0.97	20.88 ± 0.62	4.99 ± 0.87	92.52 ± 0.96
UV-A 1h	31.3 ± 8.1**	7.92 ± 1.26***	76.80 ± 8.11	19.06 ± 0.78***	19.82 ± 1.36*	18.59 ± 1.23**	4.63 ± 0.71	92.32 ± 0.81
UV-A 4h	25.8 ± 12.0	10.42 ± 1.62	79.04 ± 10.38	20.83 ± 1.35	22.39 ± 1.32	20.83 ± 1.55	4.22 ± 0.85*	92.08 ± 1.04
UV-B 2min	30.8 ± 12.5**	8.52 ± 1.84***	79.16 ± 4.16	19.26 ± 1.52***	19.20 ± 3.69**	19.65 ± 1.62	4.48 ± 0.58	92.08 ± 0.42
UV-B 5min	22.3 ± 9.4	8.76 ± 1.52***	82.36 ± 11.56	19.33 ± 1.30***	20.94 ± 1.26	19.84 ± 1.43	4.55 ± 0.62	91.76 ± 1.16

Fruit FM was lower in UV-A 1h, UV-B 2min/5min, whilst the relative DM/g FM had a tendency to increase in UV-A 4h, UV-B 2min/5min. Fruit WC% was not affected by these moderate UV conditions. The amount of soluble solids (ºBrix) showed a tendency to decrease with UV, except in plants exposed to UV-A 4h that showed a significant reduction (*Table V.1.*).

3.2. Colorimetric analyses and texture

UV light treatments changed the fruits' colour parameters and texture. UV-A 4h and UV-B 2min showed a tendency to increase the luminosity of the fruits (*Table V.2.*). Tonality of the tomatoes was changed to more yellow, once the degree of hº increased to UV-A 1h/4h and UV-B 2min, a change that was significant for UV-B 2min. The fruit chroma (colour saturation) was not changed by UV supplementation (*Table V.2*.). Firmness of tomato fruits was increased by UV-A and UV-B supplementation, with a significant increase in UV-A 4h (*Table V.2.*). The firmness was analysed in all fruits that did not broken during the measurement process. Interestingly, the percentage of broken tomatoes during the test was 50% in the control and UV-B 5min, 37.5% to UV-A 1h, 25% for UV-A 4h and UV-B 2min.

Table V.2. Color and texture analysis. Mature fruits (ripe red tomatoes) were measured regarding color and firmness parameters: L-luminosity coordinates; C-chroma, and hº- tonality, and firmness (expressed in Newton). All data were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. The symbol * represent significant differences for p≤0.05, when compared with the control, in each condition. Values are expressed as mean ± standard deviation (for n=8).

Treatment	L	hº	C	Firmness (N)
Control	44.99 ± 1.12	45.24 ± 2.37	52.50 ± 2.85	10.25 ± 1.90
UV-A 1h	44.87 ± 1.22	46.80 ± 2.57	52.93 ± 3.40	12.47 ± 1.60
UV-A 4h	46.45 ± 1.78	47.10 ± 2.02	53.66 ± 2.19	16.48 ± 4.89*
UV-B 2min	46.46 ± 1.22	48.84 ± 1.60*	54.10 ± 2.94	12.63 ± 2.46
UV-B 5min	44.42 ± 1.80	45.06 ± 2.58	51.94 ± 2.39	11.07 ± 2.63

3.3. Phenol and Antioxidant content

The concentration of antioxidant compounds was stimulated particularly by UV-A supplemental light after 30 days of exposure in fruiting phase. ABTS assay showed an increase of antiradical activity in UV-A 1h/4h and UV-B 2min, with a significant increase in the UV-A groups (*Figure V.1*.). Total phenol content (TPC) were significantly increased in all UV-A conditions and had a stimuli in UV-B 2min. The amount of o-

phenols was stimulated by UV-A with a significant increase for UV-A 1h. Flavonoids content was also stimulated (p<0.05) by both UV-A conditions (*Figure V.1.*).

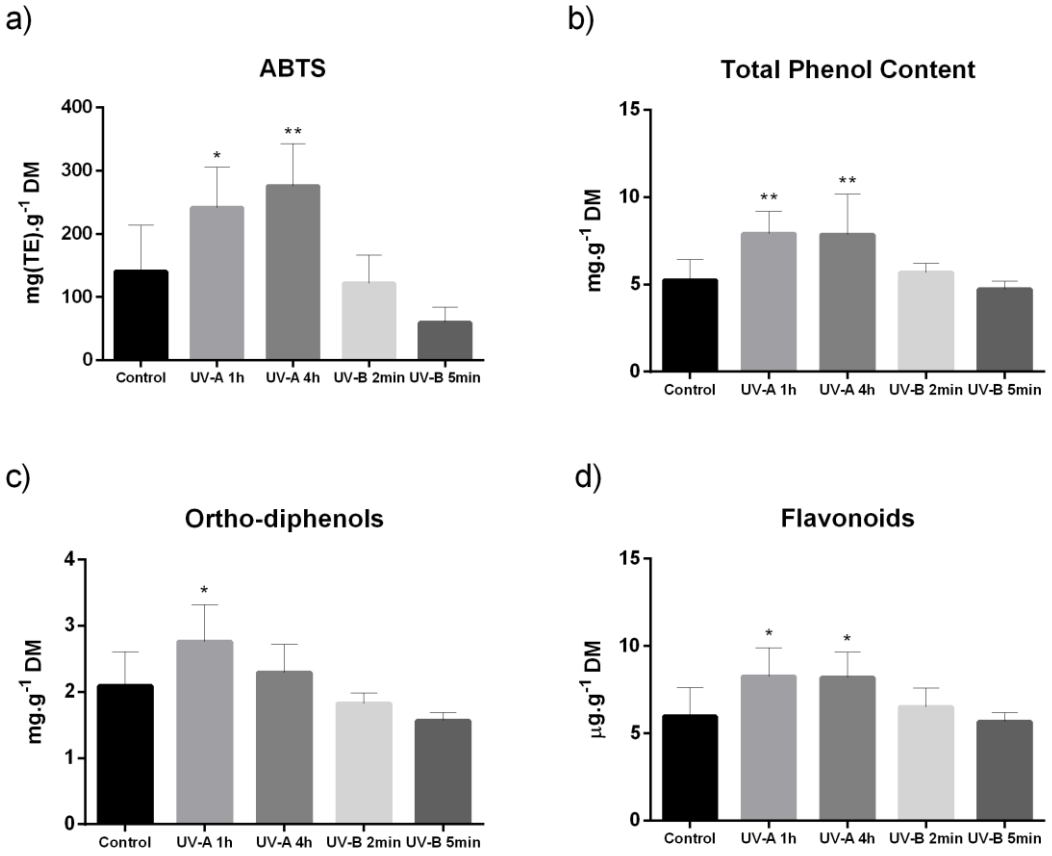

Figure V.1. Antioxidant activity and Phenol, ortho-diphenol and flavonoids contents. After 30 days of UV-A and UV-B supplementation in tomato plants during fruiting phase, the antiradical activity was measured with ABTS method (a) and expressed as mg g^{-1}(DM) for ripe red fruits. For the same fruits, the total phenol content [mg g^{-1}(DM)] (b), ortho-diphenol [mg g^{-1}(DM)] (c) and flavonoids [µg g^{-1}(DM)] (d) were also measured. All data were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. The symbol * represent significant differences for p≤0.05, when compared with the control, in each condition. Values are expressed as mean ± standard deviation (n=3).

3.4. Sensorial analysis

A group of consumers evaluated all 5 groups of treated tomatoes and denoted a deviation (p<0.05) of the fruit colour intensity (more yellowish) in UV-A 4h compared to the control and to the other UV treatments. Also, tomatoes from UV-A 4h treatment showed the lowest (p<0.05) color homogeneity (*Figure V.2.*). Peel and pulp colour homogeneity was considered lower (p<0.05) in UV-B 2min compared to control and the other UV treatments (*Figure V.2.*). On the other hand, this sensorial test showed some

trends, such as an increased solidity to touch of UV-A 4h fruits, higher acid aroma in UV-A 4h and UV-B 5min irradiated fruits, and higher acidity in the taste for UV-A 4h and UV-B 2min/5min. There was also a tendency of decrease of the sour taste in UV-A 1h, and of the pulp homogeneity and balance in fruits supplemented with UV-B 2min (*Figure V.2.*).

In the preference of the consumers by decreasing ordination, for the visual and surface parameters of the tomatoes, they considered the UV-A 4h and UV-B 2min fruits as being less appellative ($p<0.05$, *Figure V.3.*). The preferential order for this parameter by average of rating was as follows: Control > UV-A 1h > UV-B 2min > UV-B 5min > UV-A 4h. However, the response to the second question relative to ordering the preferential tomato groups by its more appellative aroma/taste denoted a trend to choose the UV-A tomato groups, where the order was as follows: UV-A 1h > UV-A 4h > Control > UV-B 5min > UV-B 2min (*Figure V.3.*).

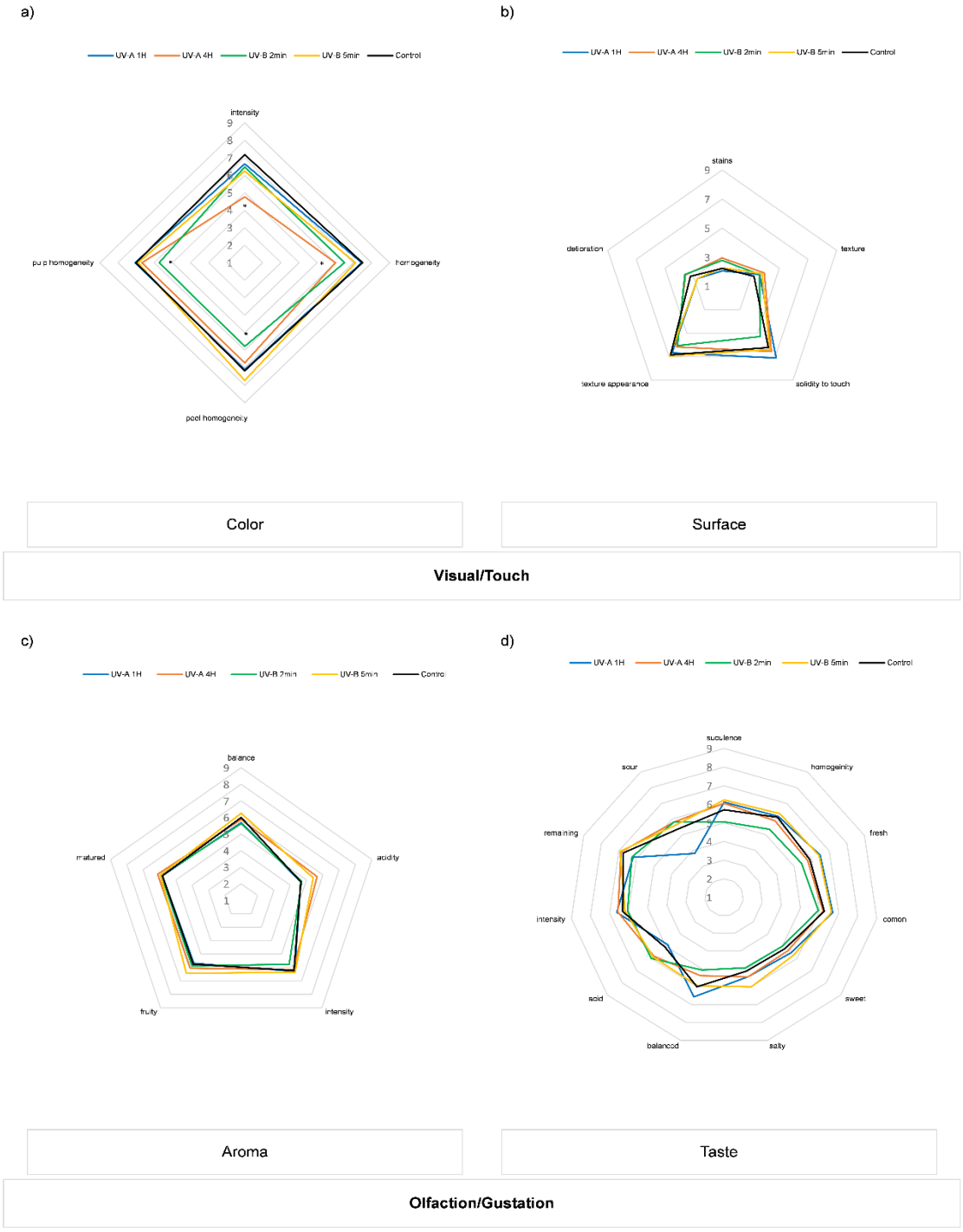

Figure V.2. Sensorial evaluated by a consumer panel. After the 30 days of treatment with UV supplementation, ripe red tomatoes were used to sensorial analyses. The consumers' evaluations were made regarding visual/touch parameters, where colour (a) and surface (b) analyses were included. Other group of sensorial parameters, namely olfaction/gustation were also evaluated for aroma (c) and taste (d). All data were measured for control, UV-A 1 and 4h and UV-B 2 and 5min. The symbol * represents significant differences for p≤0.05, when compared with the control, in each condition. Values are expressed as mean of classifications for different parameters (see methods).

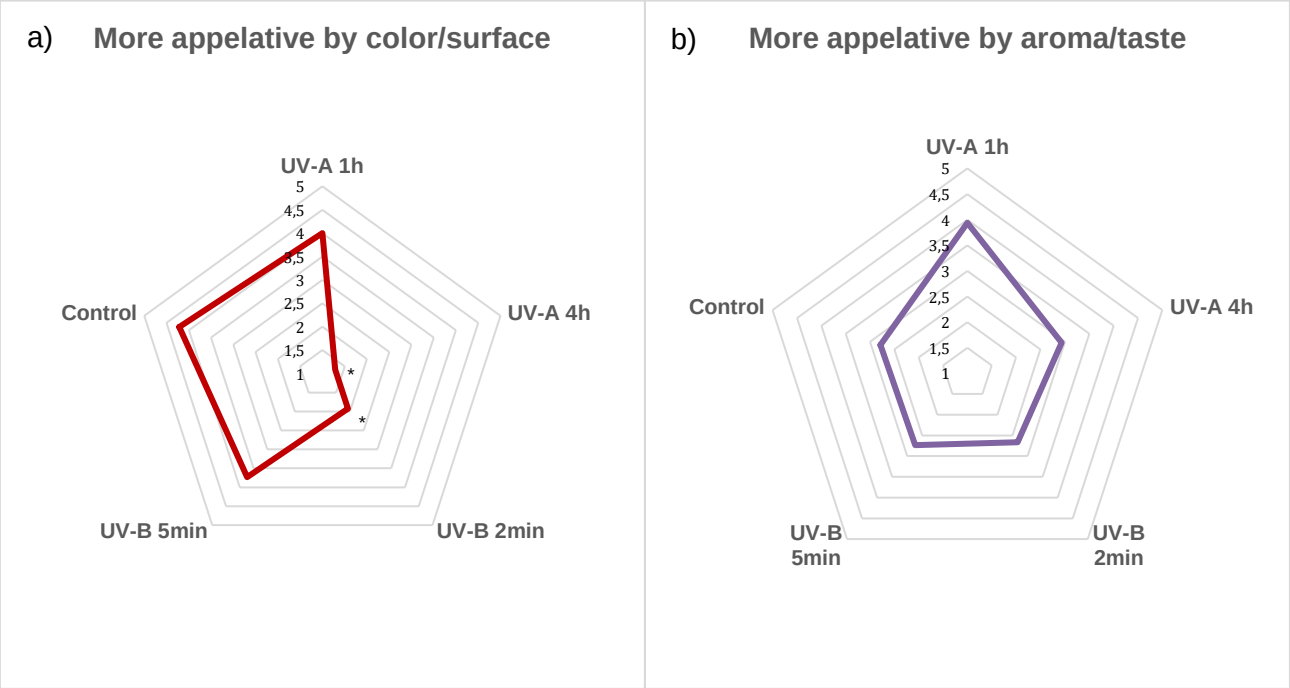

Figure V.3. Preferential order of the tomatoes for consumers to visual/surface and aroma/taste. After sensorial analyses the consumers were asked to rank the fruits according to a decreasing scale of preference, which was converted in a scale from 1-5 (from less to most appellative). The consumers evaluated by visual/touch (a) and aroma/taste (b) preference for control, UV-A (1 and 4h) and UV-B (2 and 5min). The symbol * represent significant differences for p≤0.05, when compared with the control, in each condition. Values are expressed as mean of classifications for different parameters, see in methods section.

3.5. Multivariance analyses

Principle component analysis (PCA) regarding the impacts of UV-A and UV-B on the fruit biochemical attributes are deciphered in *Figure V.4. a*. PC1 explained 42.0% of the variance and PC2 explained 29.7%. Three distinct groups can be identified: 1) control is located at the low right quadrant only positively related with diameter and FM; 2) a second group at the left quadrant ranks for UV-A 1h/4h, strongly correlated with firmness, flavonoids, TPC, o-diphenols and ABTS; 3) a final group of UV-B 1min/5min ranks in the top half only correlated with DM, whilst negatively related with firmness, flavonoids, TPC, o-diphenols and ABTS.

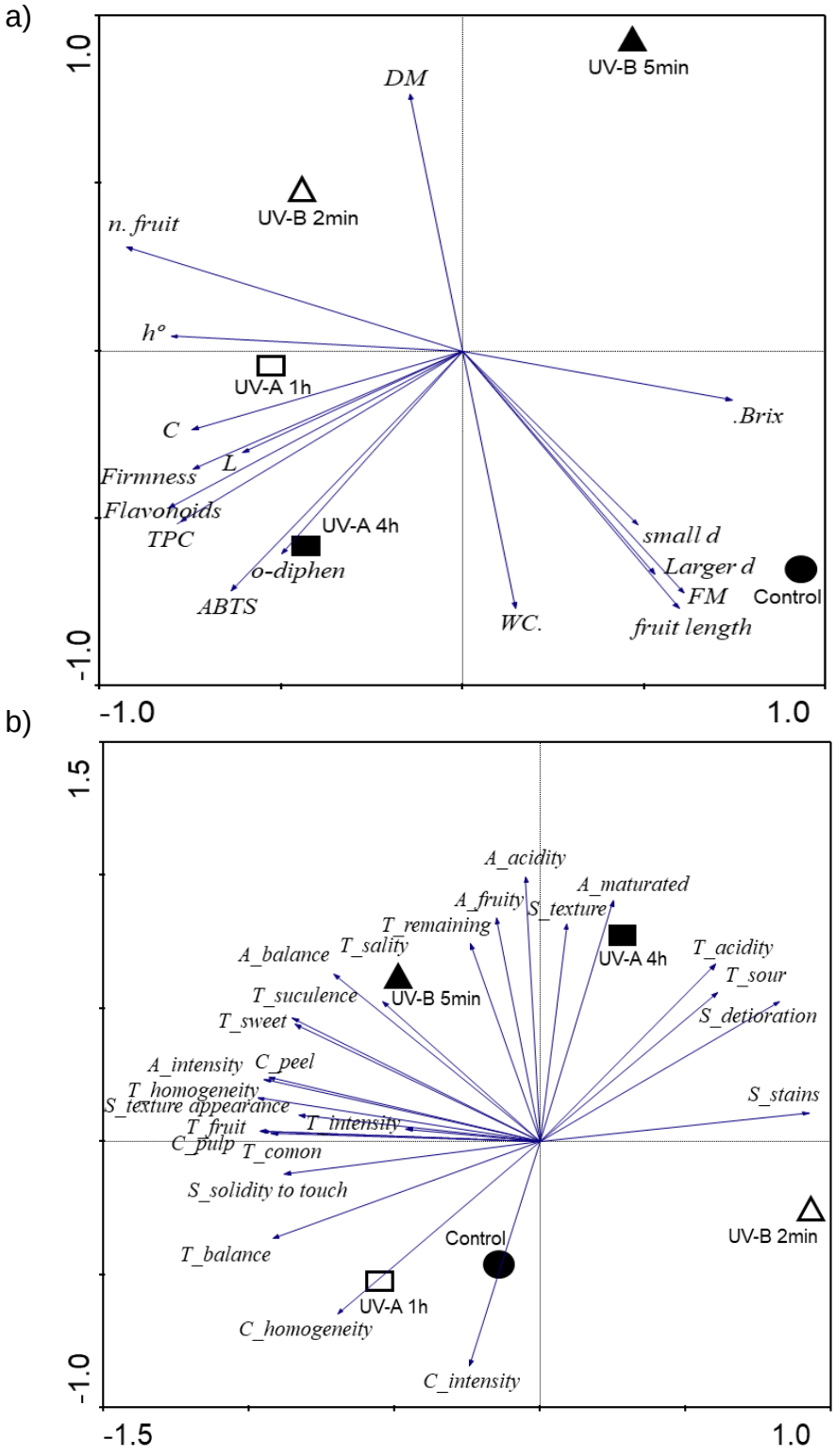

Figure V.4. PCA analysis of functional responses of tomato fruit (a) and consumer's evaluation (b) for Control, UV-A (1 and 4h) and UV-B (2 and 5min). Abbreviations: a) Number of total fruits (n.fruit); small and larger diameter (small d, larger d); Total phenol content (TPC). b) The initial words (C, S, A and T) represent the color, surface, aroma and taste, respectively. Other abbreviations are described in methodologies.

PCA analyses of the sensorial attribute is depicted in *Figure V.4. b*. Overall, PC1 explained 52.4% of the variance and PC2 explained 29.2%. Control ranks at the down center correlating with color intensity, and close to UV-A1h that correlates with taste balance, solidity to taste, color homogeneity. Contrarily, the UV-A4h ranked at the top right quadrant and correlated with more attributes of aroma and taste (*Figure V.4. b*). Both UV-B 5min was positioned at the left top quadrant correlating with taste and aroma attributes (e.g., salty), and negatively correlated with the UV-B 2min.

4. Discussion

For long, there has been an apprehension of the negative impacts that high uncontrolled environmental UV irradiation may have in plants/crops. However, after the discovery of the role of UV signalling in plant cells, the scientific community is looking at UV as an opportunity to artificially modulate the seed/seedling performance, plant development, flowering, and fruit production (Huché-Thélier *et al*., 2016; Hiramatsu *et al*., 2014; Noble *et al*., 2002). Thus, a new paradigm in agro-food industry is emerging, related to the benefits that UV supplementation in crop production may bring to improve its nutritional value. Currently, the few studies that demonstrate that UV irradiation may improve the nutritional value of some crops, thus increasing their value as “functional foods”, are promising, raising the use of UV in agro-food industry to a novel area. In particular, the potential of using UV to increase the levels of phytocompounds and/or antioxidants and secondary metabolites in crops/fruits has already been demonstrated (Brazaityte *et al*., 2015; Schreiner *et al*., 2012). Besides its intrinsic value to the consumer’s health, this UV-modulation of fruit quality can also promote the appetence of the consumer to better appreciate and preferentially choose these UV-improved products.

UV-A and UV-B supplementation to tomato plants during fruiting/ripening changed the plant’s productivity and synchronization with an increase tomato fruits amount for plants with the same age. We have previously shown that these irradiated plants also had stimulated a mild oxidative stress in leaves, without compromising yield, suggesting that an “eustress” phenomena occurred particularly in UV-A irradiated plants (Mariz-Ponte *et al*., 2017; see also *Chapter 4*). Fruiting stimulation was accompanied by decreases of the calibre parameter (e.g., large/small diameters and length) and of FM in UV irradiated groups. Bacci *et al*. 1999 showed that UV-B irradiated tomato plants had a non-significant increase of the number of fruits, which

was accompanied by a reduction of the fruits' calibre. This reduction of fruit size may be a consequence of the increase in the number of fruits. Xu *et al*. (2017) showed that UV-C irradiation during pre-harvest phase, increased the number of flowers in strawberry plants, supporting that controlled doses of UV-irradiation can modulate/stimulate flowering and/or fruit production.

UV radiation can also modulate the fruit ºBrix and other physicochemical quality attributes. Whilst UV-A 4h reduced ºBrix, UV-B did not affect this parameter, supporting previous data reported for this irradiation (Bacci *et al*., 1999). Fruit luminosity was stimulated by UV supplementation, which is positively correlated with the increase of fruit tonality, and a visible increase of yellowish hue, especially in UV-A tomatoes (*Figure V.4 a*). This increase of the luminosity and tonality in tomatoes is in accordance with Xie *et al*. (2015; 2016) who irradiated strawberry plants with UV-C during the pre-harvest phase and observed an increase in yellowish colour.

Our data also shows that controlled supplementation of UV-A/B can be used to increase fruits' firmness. This increase of firmness was also detected by the panel mostly by noting an increase of the surface-texture in UV-A irradiated fruits (*Figure V.4. a,b*). Also, strawberry fruits supplemented by UV-C in pre-harvest showed higher firmness (Xie *et al*., 2015; Xie *et al.,* 2016; Xu *et al*., 2017). This data is particularly relevant because the increase of fruit firmness can lead to increased shelf life of foods, and increase the resistance to fungi attacks that promote the rapid deterioration of fresh foods.

This work also demonstrated that controlled UV-irradiation may be used to increase the functional properties of fruits, namely regarding antioxidant properties and richness in polyphenols (*Figure V.4. a*). Fruits with major contents in antioxidants are a trend in consumers' preferences, mostly because they present several health benefits when accompanied by a balanced diet. In this case, UV-A 1h/4h irradiated tomatoes had higher levels of antioxidant compounds such as phenols, o-diphenols and flavonoids (*Figure V.4. a*), which increase the antioxidant capacity of this "functional food".

Use of UV-B supplementation during pre-harvest is scarcely studied (Dzakovich *et al*., 2016), and even less studied are the impacts of UV-A on fruit production/quality. Our results demonstrate the potentiality of moderate UVs in promoting fruit antioxidant properties and the levels of certain secondary metabolites, which supports some literature. For example, Helsper *et al*. (2003) showed an increase of the antioxidant content in *Rosa hybrida* and *Fuchia hybrida* growing under UV-A supplementation. The same increase, but for UV-B supplementation, was demonstrated in *Tropaeolum majus*

L. (Schreiner *et al*., 2009). In lettuce, the exclusion of solar UV-A/UV-B showed a decrease of anthocyanins, flavonoids and phenols compared to UV-irradiated plants (Tsormpastsidis *et al*., 2008). Guerrero *et al*. (2016) showed an increase of antioxidants, such as resveratrol in UV-C irradiated grapevine plants. UV-C irradiated strawberries also had increased levels of antioxidant contents (Oliveira *et al*., 2016; Severo *et al*., 2017; Xie *et al*., 2015). On the other hand, Dzakovich *et al*. (2016) showed that supplemental UV-B did not significantly affect tomato fruits levels of antioxidant compounds.

Moreover, post-harvest studies also demonstrated that UV irradiation changes the metabolome of plants and fruits, namely increasing the production of volatile organic compounds (VOCs) that have an important role in the attractiveness for the consumer and fruit degustation (Severo *et al*., 2017). For example, Gasemzadeh *et al*. (2016) demonstrated that UV-B irradiation stimulated the antioxidant battery in *Ocimum basilicum* L., thus promoting the pharmaceutical properties of this herb. Castagna *et al*. (2013) irradiated post-harvested tomatoes with UV-B and reported a change in tomato metabolome with an increase of some antioxidant compounds, such as ascorbate, lutein and lycopene. Thus, in recent years, increasing evidence points to the powerful use of UV-irradiation to modulate, in a controlled manner, different pathways involved in antioxidant properties and in bioactive phytocompounds, and thus promoting the nutritional value of crops/fruits.

The perception of the consumers is an important step to evaluate the potential market value of new products or new concepts for traditional products. Tomato is a well-known and a well-accepted product in the global market, thus novel strategies capable to induce fruit quality parameters (e.g., increased aroma and flavour) in greenhouse and/or off-season productions have to be recognized by consumers as being able to improve organoleptic attributes of the fruits. The consumers' panel noticed decreases in the tomato colour (intensity and homogeneity) mostly for UV-A 4h (also supported by the PCA analyses, *Figure V.4. b*). This perception pointing to the loss of reddish colour, which was also corroborated by physical analysis, with UV-A irradiated fruits showing an increase of the yellowish tone, suggests a shift in the carotenoids synthesis, with increased levels of carotenoids other than lycopene (Castagna *et al*., 2014). For aroma, it was verified a tendency to acidity in UV-A 4h and UV-B 5min, such as more acidic flavour, which supports the increased levels of phenols and antioxidant compounds/activity observed in these conditions (*Figure V.4. a*). Interestingly, the reduction of reddish intensity by UV-A was determined by both the panel and the

biochemical characterization, and the aroma and flavour (namely acidity) of these fruits was incremented for UV-A (*Figure V.4. a,b*).

In the present study several similarities are presented between the sensorial perception of consumers and the biochemical characterization, pointing to the fact that UV-A and UV-B are capable to promote specific metabolic shifts in fruit metabolome, mainly to increase the antioxidant pathways and its concentration in fresh fruits and may increase the VOCs related with these pathways. The preferential choice of the consumers according to better aroma and flavour of the tomatoes were UV-A conditions (1h/4h) (*Figure V.4. b*). These results are in accordance with data from Dzakovich *et al*. (2016) where a sensorial panel also selected tomatoes growing under UV-A as "overall approval" for color, aroma and flavour parameters when compared to tomatoes grown under PAR radiation, PAR supplemented with UV-A plus UV-B and outdoor situations.

5. Conclusions

In conclusion, the use of UV-A and UV-B can be a tool of modulation in fruit content and production of "functional foods", increasing the potential of the healthy food. UV-A doses tested here are more promising when compared to UV-B doses (*Figure V.4. a,b*), as it promotes better firmness (relevant to shelf life), stimulate the accumulation of antioxidant compounds (relevant for healthy eating). Moreover, whilst UV-A 1h and UV-A 4h fruits ranked in opposite sides of the PCA analysis, they were both the most appreciated by the panel regarding the fruit aroma and flavour. Furthermore, UV-A lamps represent a low-cost investment to producers compared with UV-B/C lamps. The industrial use of this artificial UV sources may promote an increase of products' quality in the market and even increase the preference of the consumers.

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VI. Chapter 6: General Discussion and Conclusions

The interaction of plants-UV radiation is currently regarded in two perspectives: a) as a putative distress situation, when regarded in the environmental perspective of climate changes, where it is expected an increase of plant disorders due to the exposure to high uncontrolled UV-B doses; b) as a technological strategy to improve yield and food quality in agriculture, benefiting from the putative eustress that moderate and controlled UV doses may cause in the plant.

The use of moderate UV-A and UV-B irradiation in agriculture as supplementary light has been discussed in the recent years (Jansen *et al*., 2012; Lee *et al*., 2013; Suchar and Robberecht., 2016; Wang *et al*., 2012) aligned with the new paradigm of using the known UV-receptors interactions and subsequent effects to modulate plants' biochemistry, metabolism and physiology. These first approaches to characterize impacts, doses etc, have used protected horticulture model species, e.g., lettuce, microgreens, and tomato (Brazaityte *et al*., 2015; Dzakovich *et al*., 2016; Tsormpatsidis *et al*., 2008). However, it should be stressed that physiological and morphological changes induced by UV depend on the species and cultivar, among other variables. For example, the final product (e.g., leaves, fruits, etc.) to be launched in the market may condition the period and type of UV supplementation. Also, the selected physiological, biochemical or molecular endpoints (not being yet standardized) may influence the measurement of the extension of the eustress/distress that the UV-radiation may impose, and thus may compromise final recommendations regarding best doses, most resistant cultivars, etc. Therefore, the use of a large battery of endpoints targeting multiple physiological (germination, growth, fruiting, ripening, etc.), metabolic (e.g., photosynthesis, oxidative stress) and molecular responses will provide a broad and potentially more accurate "picture" of the plant's response.

Moreover, in the perspective of the consumer, UV supplementation can be important to enhance the production of several phytocompounds beneficial to health (producing "healthy foods"), but also increase the vegetables and fruits sensorial attributes (e.g., aroma and taste) and thus benefit from the consumer's preference, as recently also highlighted by Dzakovich *et al*. (2016).

This work explored the UV-A and UV-B supplementation (2H and 15min day^{-1}, respectively) in different phases (seed germination and flowering/fruiting) of the tomato life cycle aiming at improving plants' production. When comparing UV-A *versus* UV-B, in different cultivars, the most promising irradiation system to improve seed germination was achieved with UV-A (2H), which led to highest germination rate and synchronization in the early days. However, as demonstrated by other authors for others species (Nangle *et al*., 2012; Sugimoto *et al*., 2013; Victório *et al*., 2010) it is clear the influence of the genotype in the germination response and further seedling invigoration. On the other hand, and contrary to UV-A, UV-B supplementation functioned as a growth inhibitor where seedlings presented a short hypocotyl and less cotyledons area. This growth response was supported by biochemical changes, namely an increase of oxidative disorders. For example, 'Oxheart' and 'Roma' exposed to UV-B irradiation had a decrease of the cell membrane stability, often associated to putative disorders of the oxidative status. The antioxidant enzymatic battery, however was not significantly changed, while the non-enzymatic pathway was stimulated. Our data clearly show that the UV-A dose induced a response dependent of the cultivar, whilst the cultivars when exposed to the moderate UV-B showed more similar shifts. These different responses to UV-A *versus* UV-B are supported by data of other species exposed to moderate UV-A/B supplementation (Noble *et al*., 2002; Sugimoto *et al*., 2013). Nevertheless, we suggest that our developed protocol for UV-A irradiation during germination, uses physiologically valuable doses for tomato, and may be transferred in a near future to nurseries of this or other species.

The moderate UV-A/B intensity used during germination was also selected to study the plants' response during the flowering/fruiting stage, and in general they showed no/little damages in plant's fruiting. This was particularly relevant for UV-A that induced some benefic impacts (e.g., increase of biomass) together with metabolic shifts (e.g., phenolics accumulation). During the fruiting stage, UV-A increased fruit yield, and stimulated the ripening synchronization. However, this positive yield effect was achieved at expenses of some decreases in the vegetative part of the plant. Similarly to seedlings, where a positive effect on seedling invigoration was observed, also during this reproductive phase, UV-A showed a stimulation in the fruit yield.

Interestingly, the pigments content were reduced mostly by UV-B, and anthocyanins were reduced in some cultivars by UV-A and UV-B. This decrease of photoprotective and antioxidant pigments might suggest that plants became less protected against this UV irradiation. However, considering the phenylalanine pathway, this may represent a

shift in the upstream pathway of the anthocyanins to favour the production of other phenols that may have more effective roles in photoprotection or in scavenging ROS. This hypothesis is supported by the increase of phenols and antioxidant capacity in both seedlings and leaves from fruiting plants. This is also supported by our data reporting the increase of the *fls* and *chs* transcripts coding for enzymes involved in the phenylpropanoid metabolism. On other hand, and contrarily to our initial hypothesis, the enzymatic antioxidant pathways seem to play more modest roles in the plant protection against these moderate UV-levels, most probably due to a major role of the non enzymatic antioxidant battery. These data also raise the hypothesis that similar events of protection may take place in the fruit, as proposed earlier for tomato (Dzakovich *et al*., 2016) and for others species (e.g., Inostroza-Blancheteau *et al*., 2016). Furthermore, considering also the nutritional implications that this topic may have to the final quality of the fruit, this hypothesis deserves further studies.

Any irradiation condition has as primary potential target photosynthetic pigments (particularly the LHCII), and consequently its impact on the photosynthetic apparatus has to be evaluated. In these tomato plants, the maximum efficiency of the photosystem (F_v/F_m) and the efficiency of photosynthesis (Φ_{PSII}) were affected by UV-A. These results suggest that there was a reduction in the electrons flow through electronic chain, and are probably due to an inactivation and/or degradation of some components of this apparatus induced by UV-A. On the other hand, the CO_2 assimilation (P_N) was decreased by UV-A, which may be a consequence of the reduction in the stomatal conductance (g_s) or an inactivation and/or degradation of the RuBisCO by this wavelength. Interestingly, our data show a clear stimulation of RuBisCO associated transcripts, whilst the levels of the protein tend to remain constant. These data suggest that an inactivation of RuBisCO may occur due to UV-A, as early suggested by Bischof *et al*. (2000) and Kataria *et al*. (2014), and it would be interesting to evaluate if these effects may explain (at least in some extension) the observed decrease in carbon assimilation. As summary, considering the benefits observed in fruit formation and ripening, it is clear that UV-A was much more beneficial than UV-B. It is also evident, that UV-A and UV-B play different roles and have different targets in the photosynthesis. Also, it is highlighted here that, although UV-A may induce some putative damages in the photosynthetic apparatus, our data support a compensatory molecular capacity recovery. Finally, our data contribute to elucidate discriminative effects of the UV-A *vs.* UV-B mostly regarding the photosynthetic and antioxidant pathways, which is extremely relevant, considering the lack of information

regarding the effects of UV-A *per se*, instead of being considered as having similar impacts as blue light (Verdaguer *et al*., 2016).

Also, and compared with UV-B, UV-A was a more effective in increasing the antioxidant contents in fruits. These differences support that UV-A modulates differently phenylpropanoid pathways, and can be used in a controlled manner to promote phenolic compounds accumulation in leaves and/or fruits. On the other hand, UV-A 4h also increased the firmness of the fruits, a characteristic that is important for fruit storage. Besides, another subject deserving further research would be to investigate if these UV-A doses may contribute to increase the fruit resistance to microbial infections, thus increasing the fruit shelf life. Moreover, the influence of this irradiation in the transcription of ripening associated genes, and on the expression of genes related to the synthesis of hormones (e.g., ethylene) needs to be further unveiled.

The evaluation of the consumer's panel confirmed several physicochemical data, and overall confirmed a general preference of the panel for UV-A irradiated fruits. This organoleptic and sensorial acceptance was aligned with the increase of the nutraceutical properties (e.g., increase antioxidant levels, and phenolics). This preference allied with the increase of phytocompounds of interest, and an increase of the number of total fruits, strongly support that UV-A negative impacts observed in photosynthesis were compensated by the production of a "higher quality" fruit. Moreover, whilst the range of UV-A doses reported here seem to function adequately in tomato, it would be relevant to explore other intermediate doses considering the plant developmental stage, the profile of the desired improvement (e.g., higher richness in phenolics) and the targeted cultivar or species. On other hand, UV-B supplementation, although not completely discouraged, we recommend that further studies use lower doses or periods. In conclusion, when compared to UV-B, UV-A supplementation is more interesting, as it promotes more positive shifts in the plant metabolites with no/low negative impacts in plant morphology/physiology. Thus, it can be used as a technological tool to promote desired shifts in the metabolism, increasing the production of several phytocompounds that may improve aroma and flavour, with interest for the market.

It should also be stressed that precision agriculture is currently one of the most explored lines of investigation, including the development of precision light approaches (Ilic and Fallik, 2017; Kipp *et al*., 2014; McBratney *et al*., 2005). Whilst the UV-A lamps used here represent a safer and "economically friendly" alternative for producers

(particularly when compared with UV-B lamps), it would also be interesting to assess if the use of UV light-emitting diodes (LEDs) systems may provide similar results.

Besides the topics reported above as deserving further investigation, it would also be important to study in more detail other issues, such as the pathways involved in the distinctive responses to UV-A *versus* UV-B in the fruits through the analysis of the transcripts and metabolomic profiles (ongoing), and evaluate if the UV-A impacts on the photosynthesis may compromise the carbohydrate translocation from the source to the fruit sink.

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