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Elucidating the Dynamic Effects of UV on *Mentha spicata* L.: From the Laboratory to Innovative Horticulture

Thesis presented by

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for the degree of

Doctor of Philosophy

University College Cork

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2024

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.



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Abstract

Indoor farming provides a novel opportunity to manipulate crops in order to improve their architecture, nutritional value, and stress resistance. Moreover, indoor farming allows the control of key environmental parameters such as light, temperature, and water availability, which normally depend on the local season. Light quantity and quality are particularly critical parameters as these play a major role in determining the yield, architecture, and quality of produce. In particular, low doses of UV radiation can induce small, regulatory adjustments in plant physiology and morphology, which in many cases can be valuable from a horticultural perspective. The general aim of this thesis was to conduct a comprehensive study of UV effects on *Mentha spicata* L., a valuable commercial crop. This was achieved by exploring the effects of broadband and narrowband UV radiation on mint plants, with a focus on morphological responses, UV-priming, resistance against stressors, and variations in secondary metabolites. The accumulation of these metabolites is crucial for the plant's acclimatory response and holds significance for human health. Investigating the metabolic responses following UV exposure revealed a strategy to enhance plant quality and resistance. Additionally, the study delves into the content and composition of essential oils, particularly monoterpene and sesquiterpene content, under different narrowband UV wavelengths. Based on these findings, it is proposed that UV LEDs could play a significant role in shaping the future of agriculture for a more efficient, sustainable way to produce quality food.

Chapter 1

General Introduction

This chapter will be submitted as part of a literature review.

1.1 UV: an overview from the past to nowadays

Scientific interest in the impact of UV radiation on organisms and materials was first triggered following the discovery of the hole in the stratospheric ozone layer, and the measurement of consequent increases in UV-B penetration into the biosphere, in the mid-1980s. The ozone layer, in conjunction with oxygen, water vapour, and carbon dioxide, plays a vital role in protecting the Earth from excess UV radiation (Barnes et al., 2022). The ozone layer acts like an invisible shield that fully blocks UV-C (100 nm – 280 nm) radiation and a substantial part of the UV-B wavelengths (280 nm – 315 nm). Longer UV wavelengths, ranging between 315 nm – 400 nm and defined as UV-A, are minimally affected by stratospheric ozone (Paul, 2000).

In order to address the problem of the thinning of the stratospheric ozone layer, the Montreal Protocol was signed in 1989 by over 200 Countries. This international agreement strictly regulates the use of a wide number of Ozone Depleting Substances (ODSs) such as chlorofluorocarbons (CFCs) emitted, for example, by spray cans and refrigerators. The danger of CFCs was highlighted by Molina & Rowland (1974) who demonstrated that these compounds, when exposed to UV radiation, produce free chlorine that interacts with ozone causing its destruction. The effectiveness of the Montreal Protocol is primarily a strong reduction in the emission of CFCs and other ozone-depleting substances, as well as an initial recovery of the stratospheric ozone layer, seen as a progressive decrease in the ozone hole located over the South Pole. There is also tentative evidence of a decrease in UV levels. Without the Montreal Protocol, a possible increase in UV radiation would have affected, amongst others, the plant's capacity to efficiently use carbon dioxide through photosynthesis with repercussions for food supply but also carbon dioxide capture by plants and, therefore, global warming (Young et al., 2021). Since many ODSs are long-lived molecules,

modelled predictions suggest that the ozone will realistically only recover completely by the second half of the 21st Century (Barnes et al., 2019). Despite this, since the protocol was signed fluctuations in the level of UV have been recorded in different locations in the South Hemisphere and Northern Hemisphere as reported in the last UNEP report (UNEP, EEAP, 2022) (Figure 1.1). These variations depend not only on anthropogenic activities but also on the natural fluctuation in the level of atmospheric ozone, latitude, longitude, atmospheric aerosols, solar cycles, volcanic activities, meteorological activities and ultimately climate change (Barnes et al., 2019). Moreover, ozone layer depletion has also been linked to climate change. Ozone layer depletion is not the cause of climate change, but both phenomena are closely linked (WMO, 2022). Indeed, most ODSs are also potent greenhouse gases (GHGs) that contribute to global warming. In this context, another important agreement, the Kigali Amendment to the Montreal Protocol, was signed off in 2016. New limitations were proposed to counter the negative effects of a range of further ODSs that are also potent GHGs. It is estimated that the Montreal treaty together with the Kigali agreement will help to avoid an estimated global warming of 0.3 – 0.5 °C by 2100 through the limitation of emissions of hydrofluorocarbons (HFCs) (WMO, 2022). These measures aim to mitigate the deleterious effects of climate change, avoid stratospheric ozone depletion, as well as help prevent unexpected interactive effects of these two phenomena.

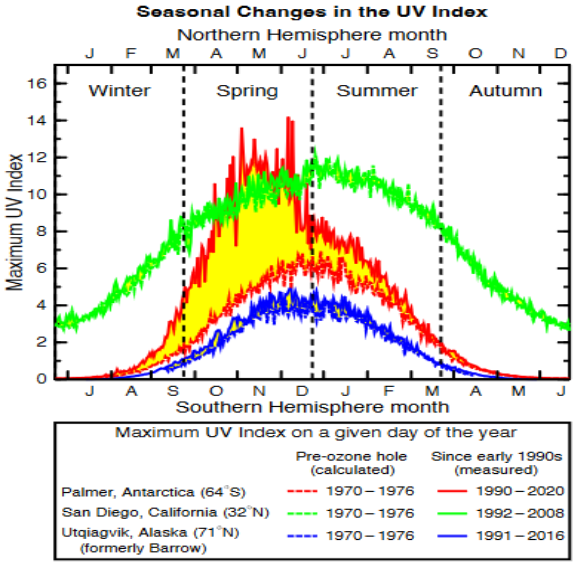


Figure 1.1. *Seasonal fluctuation in the UV index measured in different locations. Red, green, and blue dashed lines represent measurements taken in the period between 1970 and 1976 respectively in Palmer, San Diego, and Barrow. The solid line represents the measurement taken in the same location between 1990 and 2020. Source: UNEP Assessment Report 2022 pp. 63.*

1.1.1 Definition of UV-A, UV-B and UV-C

From a biological perspective, different UV wavelengths have different effects, with the shorter wavelengths being more harmful. Thus, it has long been known that energy-rich UV-C (215 nm – 280 nm) wavelengths are highly damaging to organisms, and as a consequence, such wavelengths are widely used for disinfection purposes in a variety of settings ranging from hospitals to water treatment plants (Chatzisymeon et al., 2011; Demeersseman et al., 2023). In horticulture, UV-C radiation has been used to control powdery mildew (*Podosphaera xanthii*) on crops such as cucumber (*Cucumis sativus* L.) and strawberry (*Fragaria x ananassa*) (Patel et al., 2020; Mello et al., 2022), although the application of UV-C can also result in damage to crops. The portion of UV-B (280 nm – 315 nm) that goes through the ozone layer is very small but it may potentially have damaging effects on organisms and ecosystems (Bais et al., 2019). UV-A (315 nm – 400 nm) is less damaging despite accounting for the majority of the UV reaching the Earth (Bais et al., 2019). The level of potential damage caused by different UV wavelengths is measured with the Biologically Effective Dose (Aphalo et al., 2012). This parameter estimates the biological impact of UV on

different organisms, including plants, by integrating the UV intensity and spectrum (Aphalo et al., 2012).

For plants, both UV-B and UV-A can be considered stressors as well as regulators. Noteworthy, UV effects include the upregulation of antioxidant defence, the alteration of plant architecture and the production of defence compounds. The type of response depends on different factors, including the intensity of UV applied, the duration, but also the target plant species (See Box 1). The definition of what is considered a 'high' or 'low' UV dose varies considerably in the plant literature (Hideg et al., 2013). However, low doses of UV radiation are typically more likely to cause eustress, and induce small, regulatory adjustments in plant physiology and morphology, which in many cases can be valuable from a horticultural perspective. On the other hand, high doses of UV are more likely to cause distress and can have a negative impact on the plants, causing DNA and cellular damage and impacting the efficiency of the photosynthesis and increasing the production of Reactive Oxygen Species (ROS) (Meyer et al., 2021).

Regulatory responses involve a cascade of internal signalling events that result, amongst others, in alterations in plant architecture, accumulation of a range of phytochemicals, and resistance against environmental stressors such as drought. These regulatory responses are controlled through dedicated UV-B (UVR8) and UV-A (phototropins & cryptochromes) photoreceptors in the plants, which represent the plant's ability to sense UV radiation. Thus, like many insects, rodents, and some birds (but not humans), plants can not only perceive UV wavelengths but also respond accordingly.

1.2 UV signaling: UVR8-mediated response

Plants have a specific UV photoreceptor UV RESISTANCE LOCUS 8 (UVR8), that works in the UV-B part of the spectrum, as well as in the UV-A range for wavelengths up to 350 nm (Rai et al., 2020). The signalling of UV is initiated by the monomerization of UVR8 dimers and the subsequent binding of the monomer to E3 ligase of Constitutively Photomorphogenic 1 (COP1) (Tilbrook et al., 2013). The UVR8-COP1 complex comprises the active form of UVR8 that triggers the UV signalling pathway. UVR8 can, however, be re-dimerised by the action of repressors of UV-B morphogenesis RUP1 and RUP2 to the homodimeric inactive form (Binkert & Ulm, 2017). In the signalling cascade triggered by UVR8-COP1, elongated hypocotyl 5 (HY5) and far-red elongated hypocotyl 3 (FHY3) transcription factors regulate the expression of different genes involved in the activation of DNA repair, accumulation of flavonoids, antioxidant defences, and induction of morphological changes (Jenkins, 2014; Binkert & Ulm, 2017) and thus, the plant's phenotypic response to UV.

In parallel to the UVR8-mediated pathway, plants operate non-specific UV signalling pathways that involve DNA damage and ROS production as well as general defence signalling mediated by the production of specific hormones such as ethylene, jasmonic acid and salicylic acid (Jenkins, 2009).

1.3 UV: Between protection, damage, and repair

Plant reactions to UV include a complex range of responses that comprise damaging effects as well as protection mechanisms that minimise the potential damage. As a stressor, UV can directly affect the integrity of the lipidic membrane, of proteins such as the D1 and D2 proteins of photosystem II, and especially of DNA, resulting in the

accumulation of DNA dimers (Jansen et al., 1998). Moreover, UV can induce oxidative damage through the production of Reactive Oxygen Species that promptly initiate a rapid defence response through an increase in antioxidant content (Sharma et al., 2012). As a regulator, UV induces a range of efficient strategies to cope with UV including morphological responses, the production of secondary metabolites such as UV-screening pigments and antioxidants (Yeshi et al., 2022).

1.3.1 Damaging effect of UV and ROS production

UV radiation can induce potential damage to different macromolecules, including DNA. UV-B is directly absorbed by DNA molecules leading to the formation of cyclobutene pyrimidine dimers (CPDs) as well as pyrimidine (6-4) pyrimidone dimers (6-4PPs) (Gill et al., 2015). Pyrimidine dimers are crosslinks formed between adjacent pyrimidine bases on the same DNA strand (Banaś et al., 2020).

DNA dimers block both DNA transcription and DNA replication and can lead to cell death. Yet, these DNA alterations are removed by efficient repair systems that restore the original, undamaged state of the DNA. This includes both light-dependent and light-independent DNA repair mechanisms (Bray & West, 2005). The light-dependent DNA repair, also named photorepair, is driven by photolyases in the presence of blue and/or UV-A light. These photolyases efficiently monomerise the dimers. The light-independent mechanism occurs in the dark and results in either base or nucleotide excision repair (Bray & West, 2005).

ROS production potentially can lead to oxidative stress causing damage to different macromolecules including proteins, lipids and DNA (Huang et al., 2019). UV-A mediated damage occurs almost exclusively through the production of ROS since UV-A is not absorbed by DNA (Gill et al., 2015). ROS also play a role in physiological

processes within plants, serving as signalling molecules when produced in low concentrations. The production of ROS increases in response to different biotic or abiotic stressors as part of the plant's defence mechanisms (Sharma et al., 2012). Under optimal conditions, ROS production is countered by an efficient detoxification system made up of antioxidants. Yet, when the physiological level of ROS exceeds detoxification capacity, ROS can cause oxidative stress.

The antioxidant defence comprises both enzymatic antioxidants and non-enzymatic antioxidants that remove excess ROS and thus maintain the redox balance. The former include, amongst others, superoxide dismutase (SOD), catalase (CAT), peroxidases (POX), glutathione peroxidase (GPX), and glutathione reductase (GR) (Rajput et al., 2021). The latter include different metabolites such as ascorbate, glutathione, free amino acids, flavonoids, tocopherols and carotenoids, that plants also produce in response to stress (Rajput et al., 2021).

1.3.2 Effects of UV on Plant Architecture

UV-B induces changes in plant morphology, particularly 'dwarfing'. The typical morphology induced by UV-B radiation, as studied across numerous species, is a more compact plant with shorter stems and petioles, and smaller and thicker leaves with an increase in epidermal trichomes (Jansen et al., 2012; Robson, et al., 2015B). Leaf thickening and trichomes seem to have a protective role that reduces the penetration of UV to deeper layers (Verdaguer et al., 2012). Also, in some species the decrease in stem elongation is matched by increased branching, resulting in a more compact phenotype. For example, it was found that UV-B exposed mint (*Mentha spicata* L.) the number of axillary side shoots increased significantly following eight days of UV-B exposure (Crestani, et al., 2023A) (Figure 1.2). These morphological effects are

transient in some species, and the plant will outgrow the phenotype after UV exposure has ceased, while in other species, the effects of UV-B are long-lasting (Robson, et al., 2015A). UV-A can have quite a different effect on plant architecture compared to UV-B, including an increase in leaf size, and to some extent, an increase in biomass (Verdaguer et al., 2017). Thus, UV-A exposure is potentially of interest in the case of the cultivation of leafy greens where larger leaves and added biomass are an advantage.

Accurate control of plant architecture is of critical importance for the horticultural industry. In particular, excess stem elongation can be problematic and decrease the commercial value of crops, impede efficient transport from the nursery to shops in stacked containers, making plants prone to mechanical damage, and diminishing the ornamental value. A common strategy to reduce stem elongation is to spray plants with mixtures of plant growth regulators (PGRs) such as hormones to re-direct growth (Gaba, 2005). However, an increasingly strict regulatory system, the public dislike of chemical treatments, and the need to minimise the risk of human consumers ingesting PGRs residues (*Regulation (EC) No 1107/2009*; Czaja et al., 2015) necessitates the development of alternative strategies to control plant architecture. Potentially, UV can be used as a tool to alter plant architecture for the horticultural industry.

1.3.3 UV effects on hormones

UV-B radiation influences the levels and activities of various plant hormones, contributing to plant responses to different stressors and modulating growth and development. Some hormones including auxins, cytokinins (CKs), gibberellins (GAs), brassinosteroids play an important role in plant growth while others including abscisic acid (ABA) and salicylic acid (SA), jasmonic acid (JA) mainly contribute to plant

defence against biotic and abiotic factors (Gilroy & Breen, 2022). Nevertheless, the interplay among phytohormones diminishes this clear division between effects of individual hormones, and in reality many hormones are part of complex signalling networks (Gilroy & Breen, 2022).

UV-B, perceived by UVR8 photoreceptor, interferes with both auxins and GAs synthesis by increasing the level of elongated hypocotyl 5 (HY5) and HY5 homologue (HYH), two transcription factors that when expressed, suppress hypocotyl elongation (Sharma et al., 2023). Additionally, UV-B promotes the stabilization of DELLA proteins and causes the degradation of Phytochrome Interaction Factors (PIFs) involved in the GAs synthesis pathway (Vanhaelewyn et al., 2016). The dwarfed UV-B induced phenotype is dependent on the breakdown of DELLA proteins (Davière & Achard, 2016) as well as alterations in auxin signalling (Jansen et al., 2012). This UVR8-mediated response antagonises the shading response caused by exposure to a reduced red: far-red ratio, that promotes plant elongation (Hayes et al., 2014).

Cytokinins are important in alleviating the damaging effects of UV by activating both antioxidant defences as well as morphological responses (Kataria & Guruprasad, 2018). The distribution and concentration of various cytokinins in different tissues are intricately linked to the functionality of the cytokinin oxidase (CKX) enzyme, which induces the degradation of cytokinins, leading to a swift turnover of this hormone (Vaseva-Gemisheva et al., 2004; Avalbaev et al., 2012). The activity of cytokinin oxidase is hindered by UV-B light, potentially contributing to the compact and dwarfed phenotype observed in UV-B-exposed plants (Kataria & Guruprasad, 2018).

ABA is also activated in the defence against UV radiation. Indeed, ABA enhances the biosynthesis of flavonoids and phenylpropanoids that act as antioxidants and screen UV-B penetration (Berli et al., 2011). Moreover, ABA via the synthesis of nitric oxide

(NO), protects the plants against reactive oxygen species produced due to UV exposure, maintaining a normal redox state at the cellular level (Tossi et al., 2012).

1.3.4 Effect of UV on plants' secondary metabolites

For survival, plants produce groups of secondary metabolites which mediate plant-environment interactions. Secondary metabolites provide protection against biotic (e.g., fungi, bacteria, insects, viruses) and abiotic (e.g., cold, heat, drought, salinity, UV) (Takshak & Agrawal, 2019) factors. UV-B and UV-A radiation are known to activate the accumulation of different species-specific metabolites including phenolic acids, flavonoids, carotenoids, terpenoids, glucosinolates, saponins, and phytosterols (Schreiner et al., 2012). The most common response to UV is an increase in UV-absorbing, photoprotective metabolites, particularly phenolic acids and flavonoids. These compounds not only serve as antioxidants, but they also act as physical barriers, accumulating in the leaf epidermis and preventing the penetration of UV into the deeper layers of the leaf. The accumulation of specific plant metabolites will vary depending on the plant species but also on the type of UV applied. Indeed, different sources of UV with different emission spectra generate different effects on plants secondary metabolism.

Secondary metabolites are key determinants of horticultural traits such as flavour, colour, taste, as well as nutritional value (Sanchez-Ballesta et al., 2022). For example, β-carotene is responsible for the orange colouration, as well as taste, of some fruits and vegetables. Consequently, control of the secondary metabolite profile is of critical importance for the horticultural industry. An interesting study conducted by Castro-Alves et al., (2021) demonstrated how supplementing the growth light of dill plants (*Anethum graveolens* L.) with either UV-A or UV-B increased the content of different

compounds, and human tasting panels noted this change as altered dill flavour. Similar results were found in tomato plants (*Solanum lycopersicum* L.) grown in glasshouses where the addition of UV-B increased the flavour and taste of the fruits (Dzakovich et al., 2016) (Figure 1.2). Secondary metabolites are not only responsible for colour and flavour but they also have potential benefits for human health (Schreiner et al., 2012). These compounds can reduce the risk of chronic diseases. Here, the most important classes of UV-regulated secondary metabolites, their role in plants, and possible benefits for human health are summarised.

1.3.4.1 Phenolics and flavonoids

Phenolic acids and flavonoids are a class of compounds that share the presence of both a hydroxyl group and an aromatic ring (Tungmunnithum et al., 2018). In UV-exposed plants, one of the first responses is an increase in UV-absorbing, photoprotective phenolic acids and flavonoids. Both UV-A and UV-B radiation have been extensively used to increase the content of phenolic acids and flavonoids. For example, pre-harvest exposure of basil plants (*Ocimum basilicum* L.) to 1 hour of UV-B radiation for 2 consecutive days resulted in an elevation of both phenolic and flavonoid content, while maintaining the plant biomass yield (Dou et al., 2019). Mariz-Ponte et al. (2019) observed that subjecting tomato fruits (*Solanum lycopersicum* L.) to 1 hour of UV-A daily for 30 days also led to an increase in the overall phenol and flavonoid content. Interestingly, the taste of the UV-A treated fruits was found to be superior in comparison to fruits exposed to other wavelengths and exposure durations. Another study demonstrated that the use of UV-A radiation facilitated the ripening of tomato fruits and boosted their antioxidant levels (Escobar-Bravo et al., 2019).

1.3.4.2 Terpenoids

Terpenoids represent a broad class of secondary metabolites that derive from the condensation of isoprene units to generate monoterpenes, sesquiterpenes and ultimately carotenoids. Similarly to phenolic compounds, monoterpenes, sesquiterpenes and carotenoids all contribute to the plant's antioxidant defence (Kasote et al.,2015). Furthermore, mono and sesquiterpenes contribute to the flavour and aroma of fruits and vegetables while carotenoids are responsible for the typical colouration of different plant organs and fruits and vegetables. These organoleptic characteristics can be improved through the application of UV radiation. For example, the pre-harvest exposure of grapevine (*Vitis vinifera* cv. Malbec) to low UV-B increased significantly the content of different monoterpenes such as limonene and geraniol (Gil et al., 2013). It was also found that moderate doses of UV-B increased the accumulation of β-carotene and lycopene in tomato plants (*Solanum lycopersicum* L.) when applied a few days before harvest (Pérez et al., 2009).

Besides the potential of UV to enhance the flavour and the taste of plant-derived products, terpenoids are also known for their health-related properties. In particular, carotenoids are an important source of vitamin A, fundamental for human vision (Jansen et al., 2008). Carotenoids also contribute to preventing chronic and cardiovascular diseases (Rao & Rao, 2007). Given the important beneficial properties of secondary metabolites for human consumers, the application of UV can be exploited to increase the nutraceutical value of food as well as taste, in a cost-effective way (Loconsole & Santamaria, 2021).

1.3.5 Spectral manipulation for enhanced crop quality and nutritional content/value

A recent report by the Food and Agriculture Organisation of the United Nations (FAO) revealed that populations in more than 45 countries suffer lack of access to food (FAO, 2023). Such food insecurity can be caused by a myriad of factors, ranging from extreme weather that diminish crop yields, economic factors, war, and other conflicts. The estimated increase in the world population to 9.7 billion by 2050 (United Nations, 2022) and the increased frequency of extreme weather events caused by climate change emphasise the need to optimise indoor agricultural techniques on a large scale. Apart from the struggle to satisfy the daily minimum caloric intake, in many cases, the populations of poorer countries also suffer from further nutritional insecurities due to poor dietary diversity (FAO, IFAD, UNICEF, WFP and WHO, 2023). However, the populations of many Western countries also suffer from nutritional deficiencies due to the sub-optimal consumption of, amongst others, fruits and vegetables (Clemente-Suárez et al., 2023). The association between the incidence of chronic diseases and low fruits and vegetable intake is related to low intake of various health-promoting secondary metabolites present in fruits and vegetables (Sreenivasulu & Fernie, 2022). It has been argued that increased intake of these health-promoting plant metabolites could be achieved by increasing their levels within the plant, and this may be obtained through the development of novel cultivation methods (Schreiner et al., 2012).

Indoor farming provides a novel opportunity to manipulate crops in order to improve their architecture, nutritional value, and stress resistance. Indoor farming allows control of key environmental parameters such as light, temperature, and water availability, which normally depend on the local season (Wong et al., 2020). Light quantity and quality are particularly critical parameters as these play a major role in

determining the yield, architecture and quality of produce (Paradiso & Proietti, 2022). The light quantity refers to the total amount of light, measured as either energy or photons, per surface area per unit of time, and it is typically measured across the visible wavelengths that drive photosynthesis (400 nm – 700 nm) . In contrast, light quality refers to a broader spectrum and encompasses the contributions of all wavelengths, ranging from UV-B to UV-A, to blue, to yellow/green, to red and far-red. Plants display distinct responses to different wavelengths and combinations of wavelengths, and this creates new opportunities for 'spectral manipulation' of crop quality. While there is extensive information available concerning the manipulation of visible wavelengths and the consequent effects on growth and crop quality (Cammarisano et al., 2020), relatively little is reported about the novel opportunities to exploit UV wavelengths for crop manipulation.

1.3.6 UV mediates the plant-pest and pathogens interaction

Pests and pathogens represent a very significant threat to horticultural productivity, potentially reducing yields, reducing crop quality, negatively affecting post-harvest storage of produce and ultimately, causing financial losses. While pesticides have been a key tool for pest control in past decades, a lack of new and effective pesticides, pesticide resistance, the cost of pesticides and the general preference of consumers for pesticide-free produce drive a search for alternatives . Promisingly, it has been shown that the application of a low dose of UV can modify the interactions of pests and pathogens with plants and it has been argued that UV can provide growers with a valid alternative to pesticides (Lagunas-Solar et al., 2006). UV mediates the plant-host interaction by both initiating a morpho-physiological plant response and simultaneously directly affecting pests and pathogens. The former effect is mediated

by alterations in plant morphology that act as a physical barrier (e.g., leaf thickening, trichomes, and waxes) as well as by the accumulation of secondary metabolites which act as deterrents (Escobar-Bravo et al., 2017). For example, Mewis et al. (2012) found that UV-B increased the content of glucosinolates, flavonoids, and other defence metabolites in broccoli sprouts (*Brassica oleracea* var. *italica*) negatively affecting the population of green aphids (*Myzus persicae* Sulzer) as well as the feeding rate of caterpillars (*Pieris brassicae* L.). This UV-induced response in plants is potentially accompanied by the direct effect that UV can have on the pest, for instance UV is important for the visual orientation of many insects but also influences their feeding behaviours and their reproduction cycle (Raviv & Antignus, 2004; Prieto-Ruiz et al., 2019). Hypothetically, the absence of UV could interfere with the insect's capacity to detect the plant, yet the application of UV in most cases acts as a deterrent for the herbivorous insect due to the accumulation of different metabolites in the target plant (Mazza et al., 2013). UV has also been demonstrated to be effective against fungal infection. For example, UV-B significantly reduced the sporulation of grey mould (*Botrytis cinerea* Pers.) across numerous plants cultivated in the glasshouse including tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), and basil (*Ocimum basilicum* L.) (Raviv & Antignus, 2004) (Figure 1.2).



Figure 1.2. *Summary of the general effects of UV (on the right of each square) compared with a control exposed to visible light only (on the left of each square) on morphology, colour/flavour, and pest control.*

1.4 UV-Induced Stress Resistance

The application of UV can enhance plant resistance to various environmental stressors by triggering a combination of aforementioned morpho-physiological responses. It has been demonstrated that priming plants with UV enhances the plant's resistance to subsequent exposure to other stressors (Dhanya Thomas, 2020; Sáenz-de la O et al., 2021). A relevant stressor in a modern horticultural setting is transplanting plantlets to a different substrate which represents a composite stressor that incorporates elements of drought and mechanical stress. It has been shown that lettuce (*Lactuca sativa* L.) pre-grown in a glasshouse in the presence of UV-B coped better when transplanted to field conditions compared to control plants that had not been UV-exposed (Wargent et al., 2011). Thus, UV-B radiation pre-treatment yielded protection against transplantation stress and the changed environmental conditions in the field. A recent meta-analysis of 52 published studies revealed similar physiological responses induced by drought and UV, indicating that the impact of drought can be lessened by UV exposure (Jansen et al., 2022). However, there is considerable variation across individual plant species. For example, pre-treatment of rice (*Oryza sativa* L.), silver

birch (*Betula pendula* Roth.), and tobacco (*Nicotiana tabacum* L.) plants with UV-B helps plants cope better with the subsequent application of drought (Dhanya Thomas, 2020; Robson, et al., 2015a; Mátai et al., 2019) but this was not seen for other plants such as chilli peppers (*Capsicum annum* L.), rosemary (*Rosmarinus officinalis* L.) and lavender (*Lavandula stoechas* L.) (Rodríguez-Calzada et al., 2019; Nogués & Baker, 2000).

The variable UV response might not be solely dependent on the specific plant species but can also be influenced by the balance between applied UV and PAR (Photosynthetically Active Radiation) wavelengths, as detailed in box 1. Nevertheless, the application of UV radiation during plant growth can represent an innovative strategy to mitigate other stressors that can negatively affect the yield and, in extreme cases, plant survival. Indeed, this approach could be used in protected cropping where the exposure of plants to abiotic stressors is mostly avoided due to good plant husbandry. Yet, exposure to stressors will occur due to plant handling and this includes, for example, dehydration, mechanical and temperature stresses during transport from nursery to shops, transplanting stress when plants transition from tissue culture containers to soil cultivation, and water or nutrient-supply stress associated with failures of electronic control systems.

An example of the application of UV radiation to avoid transplanting stress during *in vitro* (tissue) culture is a study in which mint plants were UV exposed resulting in the minimising of the deleterious impacts of transplanting to soil conditions (Crestani et al., 2023B).

Box 1. Which factors influence the UV – response?

The morpho-physiological response of plants is complex and depends on the simultaneous presence of different variables classified as light-dependent, environmental-dependent, and plant-dependent factors. Light-dependent factors include not just the UV wavelengths, but the entire combination of different wavelengths including visible light. Environmental factors include all the biotic and abiotic stressors that can possibly interfere with the UV effects in a synergic, antagonistic, or additive way. Plant-dependent factors include the species-specific response, age, and organs of the plants we want to expose to UV. All these factors contribute to increase the variability in plant response.

Light-dependent factors

- UV wavelengths
- Dose / duration of the exposure
- Combination of different UV wavelengths
- Diurnal/ nocturnal application of UV
- Presence of visible light
- Composition and dose of the visible light (red: blue ratio)

Environmental-dependent factors

- Presence of biotic stressors (e.g., viruses, bacteria, fungi & insects)
- Presence of abiotic stressors (e.g., temperature, humidity, pH)
- Composition of the soil/ nutrient solutions

Plant-dependent factors

- Species and genotype
- Developmental stage of the plant (from germination to fruit/seed production)
- Exposed plant organ
- Plant architecture

1.5 Manipulation of UV in Protected Horticulture

Protected cropping involves a wide range of different structures, albeit with the common aim of protecting plants from adverse environmental conditions. At its most basic, protected cropping comprises semi-permanent plastic covers such as polytunnels. Plastic cladding materials typically contain UV-absorbing stabilisers, which severely limit UV penetration into the protected structure, and therefore plants are grown in the virtual absence of UV radiation. Similarly, most commercially used glasses for greenhouses block UV-B and partly UV-A transmission (Lycoskoufis et al., 2022). However, this does not necessarily need to be the case, UV-transmitting plastics as well as glass have been developed and these facilitate penetration of solar UV-A radiation or penetration of both solar UV-A and UV-B radiation. Such UV-B transmitting plastics have been used to increase the content of the flavonoids luteolin and quercetin in rocket (*Eruca vesicaria*) in greenhouse conditions (Mormile et al., 2019). Similarly, Behn et al. (2010) found that the use of UV-B transmitting covers increased the flavonoid content in lettuce (*Lactuca sativa* L.). Rather than altering the UV-transmitting properties of plastic cladding or glass, artificial UV lighting can also be used to supplement the UV-depleted solar spectrum inside a protected structure. An extreme example of this approach is in indoor farming, the growing of crops inside a building or underground, which necessitates the development of a full artificial lighting system. At present, many such systems do not include UV wavelengths in the lighting system (Nájera et al., 2023), despite the obvious benefits of UV for crop quality.

1.6 UV Sources for Protected Horticulture

Artificial sources of UV radiation can be categorised as either traditional sources or novel sources of UV. Traditional sources comprise mainly fluorescent tubes and incandescent lights that mostly emit a broadband spectrum including UV wavelengths. In contrast to traditional UV sources, UV light-emitting diodes (UV-LEDs) are now emerging as a new and viable, mercury-free and environmentally friendly source of UV radiation, which can be used for precision manipulation of spectra (Darko et al., 2014). (See Figure 1.3).

Traditional sources of UV, which emit UV-A and/or UV-B wavelengths, are extensively used both for research and a wide array of applications (Jordan et al., 1992; Neugart et al., 2014; Qian et al., 2020). A disadvantage of most fluorescent tubes is their relatively broad emission spectrum, thus desirable wavelengths are accompanied by wavelengths that are either not required, or even cause deleterious effects. For example, the widely used Philips TL12 tube emits a small amount of highly damaging UV-C radiation, as well as UV-A wavelengths (Mairinger, 2000). A second disadvantage of these tubes is the limited choice of wavelengths, making precision manipulation of the spectrum problematic. To resolve this problem, the use of traditional UV sources is often combined with the use of specific UV-blocking or transmitting filters (See Figure 1.4). The aim of using these filters is to obtain the desired wavelength by interposing the filters between the UV source and the plants. Usually, filters absorb the unwanted wavelengths, transmitting only the desired ones (Aphalo et al., 2012). Using filters it is possible through the use of cut off (long-pass or short-pass) filters to remove undesired UV wavelengths from the spectrum (Aphalo et al., 2012). However, in practice this is often not easy, and it can be expensive. Filters are generally made of glass (e.g., Corning or Schott) or plastic such as cellulose acetate

or polyester (e.g., Mylar). Glass filters are more expensive and last longer while plastic filters have a limited lifespan and need to be replaced frequently because the heat produced by the lamps degrades the plastic of the filters altering their optical properties. For example, cellulose acetate filters are used to remove the UV-C emitted by UV-B tubes and have a useful lifespan of ~20 hours after which its blocking properties are progressively reduced. In this context, it should also be noted that changing the filters can be time-consuming, especially for large-scale setups. Furthermore, in the case of fluorescent tubes, there is the risk of releasing mercury when tubes are broken (Johnson et al., 2008), as mercury is hazardous for humans and the environment (Teng & Altaf, 2022). For this reason, with the directive 'Restriction of Hazardous Substances in Electrical and Electronic Equipment (RoHS)', the European Commission is now limiting the commercialization of fluorescence lights containing mercury for both safety and disposal reasons (Directorate-General for Environment (European Commission) et al., 2021).

UV-LEDs are promising alternatives that can replace traditional UV sources due to increased service lifetime, better energy efficiency, faster responses, and a compact structure (Liang & Sun, 2022). LEDs emit a narrow UV spectrum, whilst there is a rapidly expanding range of different UV wavelengths available. Thus, precision manipulation of the spectrum is possible. Multiple UV-LEDs can be combined to emit a broader UV wavelength band (Paradiso & Proietti, 2022) and avoid the use of filters.

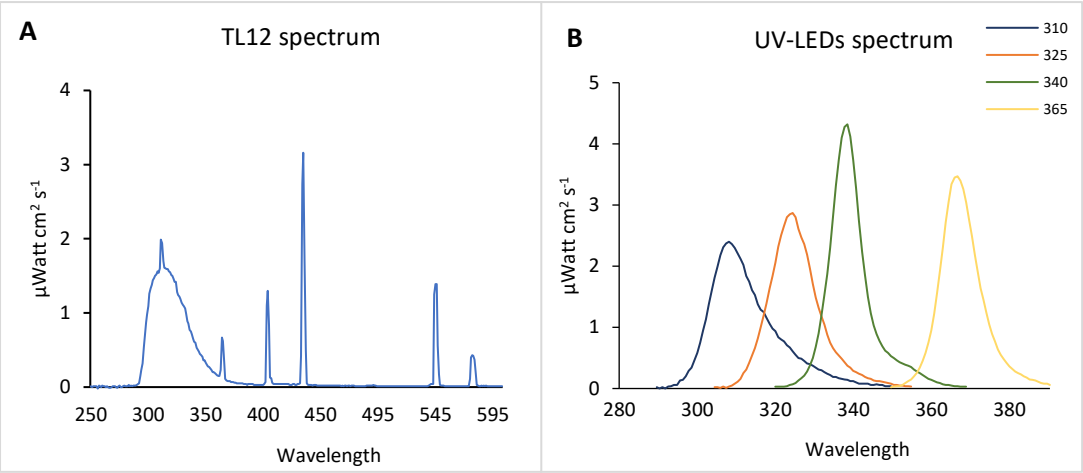


Figure 1.3. *Spectra emitted by broadband Philips TL 40W 12 (**A**) and by different narrowband UV LEDs (**B**) used for the experiments of this thesis. UV intensity is reported in µWatt cm^{-2} s^{-1}.*

The use of UV-LEDs in plant research and, consequently, their application in horticultural practises is currently still limited due to the high price of the UV-LEDs compared to red and blue ones (Wargent, 2016). Yet, in the past few years, the price has dramatically dropped, offering the opportunity to replace old lamps with more energy-efficient LEDs and develop large-scale applications. Current developments in LED technology enable exposure to a range of specific, narrow wavelength ranges of either UV-B or UV-A, enabling precision manipulation of crops.

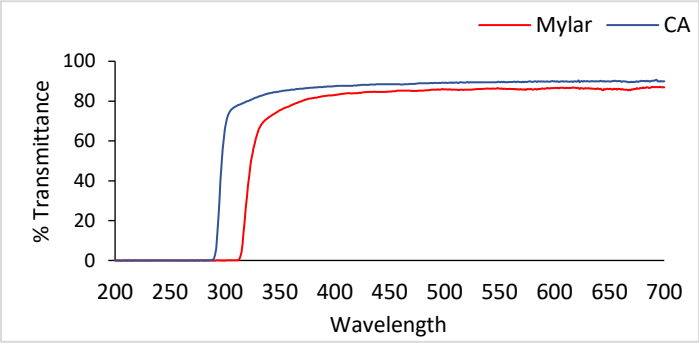


Figure 1.4. *Transmittance spectra (%) of UV-blocking filter, Mylar (red) and UV- transmitting filter, cellulose acetate (CA)(blue).*

1.7 Aims and objectives

The central aim of this thesis was to conduct a comprehensive study of UV effects on *Mentha spicata* L., a valuable commercial crop. This work goes beyond the model plant *Arabidopsis thaliana* and delves into the complex UV-mediated changes in a valuable, understudied plant. Underpinning this work is a need to enhance the quality of the crop by improving the nutritional value and enhancing the resistance against different stressors. Both traditional and new UV sources were used (See Table 1).

We explored different objectives, which are explained in detail below:

- **In Chapter 2,** the effect of broadband UV-B radiation on plant architecture and hormone distribution will be explored in *Mentha spicata* plants grown *in vitro*. Hormones are one of the factors that mediate the UV- induced phenotype. In this chapter, plant morphology including both shoot and root parameters as well as the content of different hormones across leaves, stems and roots will be quantified immediately after UV exposure and after a period of recovery on soil.

- *In vitro* cultures often experience acute stress during transplanting to soil due to a drastic drop in relative humidity. **In Chapter 3**, it will be explored if UV-priming can boost plant protection during transplanting from *in vitro* to soil conditions. Parameters such as antioxidant activity, stomatal opening, leaf area growth, necrosis, root development and photosynthetic efficiency will be monitored in *Mentha spicata* plants after UV exposure and after a period of recovery on soil without UV. A practical application of UV as a tool to increase plant resilience in horticulture will be proposed.

- **Chapter 4** aims to explore how different classes of metabolites are affected by UV in *Mentha spicata* plants grown *in vitro*. The association between different metabolic responses, with particular attention paid to the relationships between different classes of secondary metabolites including, phenolics, flavonoids, monoterpenes and sesquiterpenes, carotenoids, tocopherols and phytosterols will be explored. Metabolite content will be quantified both immediately after the UV exposure and seven days after 'recovery' under visible light. The introduction of UV to an *in vitro* growth system has the potential to enhance the quality and content of valuable metabolic products in crops, offering the industry new opportunities to optimise and manipulate plants' metabolites on a large scale.

- Based on the results of Chapter 4, where the effectiveness of UV to modulate the terpenoid pathway is demonstrated, **in Chapter 5**, it will be explored the effect of different UV-wavelengths emitted by narrowband LEDs. LED devices with emission peaks at 310 nm, 325 nm, 340 nm, and 365 nm will be trialled for their effects on terpenoid content. In parallel, it has been explored how different UV-wavelengths will affect the expression of key genes involved in the mono and sesquiterpenes pathway. This approach can be used to manipulate the production of secondary metabolites through the application of narrow UV wavelengths, leading to a new possible future approach for the indoor and farming sectors.

Table 1.1. *Comparison between the traditional source of UV (Broadband system) and the new source of UV (LEDs lamps) used in this thesis and the experimental conditions applied during the experiments.*

		Broadband system	**LEDs lamps**
PAR	*Source*	Valoya AP673L	Red and blue LEDs (Roithner Electronic)
	Red: blue ratio	8	0.8
	Intensity	~180 μmol m^{-2} s^{-1}	~33 μmol m^{-2} s^{-1}
	Far-red	Yes (Red: far-red ratio 1.2, 6:00 to 22:00)	No far-red
	Light cycle	14 h light / 10 h dark (7:00 to 21:00)	14 h light / 10 h dark (7:00 to 21:00)
UV	*Type*	Broadband	Narrowband
	Spectrum	UV-B + UV-A	Single wavelengths with peaks at 310 nm, 325 nm, 340 nm 365 nm
	Source	TL 40 W 12 (Philips)	UV-A and UV-B LEDs (Roithner Electronic)
	Intensity	~0.3661 W m^{-2} UV-A and ~0.2225 W m^{-2} UV-B	~0.46 W cm^{-2}
	Biologically active dose	0.283 kJ m^{-2} UV-A and 0.252 kJ m^{-2} UV-B (CA), 0.179 kJ m^{-2} UV-A and 0.145 kJ m^{-2} UV-B (Mylar)	1.07 kJ m^{-2} (310 nm), 0.151 kJ m^{-2} (325 nm), 0.149 kJ m^{-2} (340 nm) , 0.107 kJ m^{-2} (365 nm)
	Use of filters	Yes (Cellulose acetate or Mylar)	No
	Duration	6 h (10:00 – 16:00)	6 h (10:00 – 16:00)

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Chapter 2

UV-B radiation as a novel tool to modulate the architecture of in vitro grown *Mentha spicata* L.

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Abstract

In vitro culturing can generate plants with a distorted morphology. Some distortions affect the plant's survival after transfer to an *ex vitro* environment, while others can affect the aesthetic value. Therefore, exogenous hormones are often applied in *in vitro* cultures to modulate plant architecture. In this study, it was hypothesized that regulatory effects of UV-B radiation on plant morphology can be exploited under *in vitro* conditions and that UV exposure will result in sturdier, less elongated plants with more branches and smaller leaves, mediated by changes in plant hormones. Plants were grown in tissue-culture containers and exposed to ~0.22 W m^{-2} UV-B for eight days. Subsequently, plants were transferred to soil and monitored for a further seven days. Results show that UV induced a marked change in architecture with a significant increase in axillary branches, and reductions in leaf area, plant height and root weight. These changes were associated with significant alterations in concentrations of hormones, including IAA, GA7, GA3 and iP9G. Changes in hormone concentrations suggest a regulatory, rather than a stress response to UV-B. Therefore, it is proposed that the application of UV in *in vitro* culture can be an innovative approach to manipulate plant architecture.

2.1 Introduction

Since its inception in the early 1900s, plant tissue culture or plant *in vitro* culture has developed into a commercially important propagation method that is widely applied by the horticultural industry (Thorpe, 2007). *In vitro* propagation may refer to the cultivation of protoplasts, suspensions of individual cells, calluses, and explants from various plant organs, embryos, or seeds. The commonality between these distinct cultures is the controlled environment, which is typically aseptic and characterised by high relative humidity, constant temperature, and media enriched with nutrients and/or plant growth regulators (Teixeira da Silva et al., 2017). The use of *in vitro* techniques facilitates rapid clonal propagation, with a high success rate, which in many cases cannot be achieved under less controlled conditions and/or on soil (Pence, 2010). However, as a consequence of imposing these controlled and unnatural environmental conditions, *in vitro* raised plants are characterised by physiological, anatomical and morphological adjustments, that differentiate these plants from those grown using non-*in vitro* conditions (Hazarika, 2006; Rodrigues et al., 2014). Generally, in *in vitro* generated plants, leaves are thinner, stomatal opening and epicuticular wax deposition are altered, the photosynthetic apparatus is not well developed, and the vascular system is not efficient in transferring water and assimilates. Some of these alterations make plants more vulnerable to stress when they are transferred to an *ex vitro* environment (Chandra et al., 2010). To stimulate the development of a less aberrant morphology, media have been supplemented by adding exogenous plant hormones (plant growth regulators) such as 2,4-Dichlorophenoxyacetic acid (2,4-D) and/or 6-benzyl adenine (BA) (Jiménez, 2005; Daffalla et al., 2011; Phillips & Garda, 2019). Furthermore, for the same reason, it has also been attempted to modulate the *in vitro* environment through alterations in temperature, humidity, and/or light (McClelland

et al., 1990; Hazarika, 2006; George et al., 2007). Light in particular has a strong influence on the physiology and morphology of *in vitro* raised plants, and this effect depends both on light quantity and quality (i.e., spectrum). Light can affect a variety of physiological processes such as the development of photosynthetic functioning, accumulation of secondary metabolites, cell wall development, and plant and leaf morphology (Batista et al., 2018). While the importance of the light spectrum for *in vitro* culture is well established (Batista et al., 2018; Barceló-Muñoz et al., 2021) knowledge is largely limited to Photosynthetically Active Radiation (400 nm – 700 nm). In contrast, very limited information is available on the influence of ultraviolet A (315 nm – 400 nm) and B (280 nm – 315 nm) wavelengths. These wavelengths are part of the natural solar spectrum, and plants have evolved to sense and respond to such wavelengths using dedicated photoreceptors and signalling cascades (Podolec et al., 2021). For example, UV-B wavelengths are sensed through the UVR8 photoreceptor, while both phototropins and cryptochromes photoreceptors are active in the UV-A part of the spectrum. UV-B wavelengths in particular can trigger a wide array of plant responses, including the accumulation of various metabolites, increases in antioxidant defences and changes in plant morphology (Jansen et al., 1998; Hayes et al., 2014; Köhler et al., 2017). Indeed, it has been argued that UV-B radiation can be used as a method to control plant morphological parameters (Robson, et al., 2015). Noted alterations in the architecture of UV-B exposed plants include leaf thickening and a reduction in leaf area, shortening of petioles, a reduction in stem length, increased axillary branching and alterations in root morphology (Jansen, 2002; Wargent et al., 2009; Kataria et al., 2014; Robson et al., 2015; Qian et al., 2021). While the mechanisms underlying such morphological responses are not fully understood, it is accepted that the photoreceptor UVR8 can play a role in this process. UVR8-

mediated signalling, can affect hormone-related signalling, ultimately impacting on organ differentiation and causing UV-induced morphological changes (Vanhaelewyn et al., 2016). UV-B affects hormonal pathways through two possible mechanisms. The first one involves UVR8-mediated effects on growth-related hormones such as auxins, cytokinins (CKs) and gibberellins (GAs). The second pathway involves a more generic UV-induced stress response, altering concentrations of defence hormones such as abscisic acid (ABA) (Hronková et al., 2003; Vanhaelewyn et al., 2016).

This study explored whether UV radiation can be exploited as a tool for the *in vitro* culture of plants. The question arises whether UV radiation can be used to modulate plant morphology under sterile, tissue culture conditions. To develop such an innovative application of UV radiation we used UV-transmitting containers and used sterile, seed-based culture of mint (*Mentha spicata* L.) as a model system. It was hypothesised that the well-documented effect of UV-B radiation on plant morphology can be exploited under *in vitro* conditions and that UV exposure would result in a sturdier, less elongated plant with more branches and smaller leaves, mediated by changes in plant hormones.

2.2 Materials and Methods

2.2.1 Plant material and germination stage

Mint (*Mentha spicata* L.) seeds were obtained from a commercial grower (Moles Seeds Ltd., Stanway, U.K.). Seeds were surface sterilised by incubating in 0.05% Triton X-100 for 5 minutes, followed by 10 minutes in 50% sodium hypochlorite in 0.05% Triton X-100. Afterwards, the seeds were rinsed in distilled water.

Sterilised seeds were incubated for 3 days at 4 °C, then 20 seeds per box (RA40 plastic micro boxes, Sac 0_2, Deize, Belgium) were sown in solid Murashige and Skoog medium (MS) (pH 5.7) without added sucrose nor hormones. Seeds germinated

around 14 days after sowing, and seedlings were grown for a further 16 days in a growth room under 180 µmol m^{-2} s^{-1} PAR, until six leaves were formed.

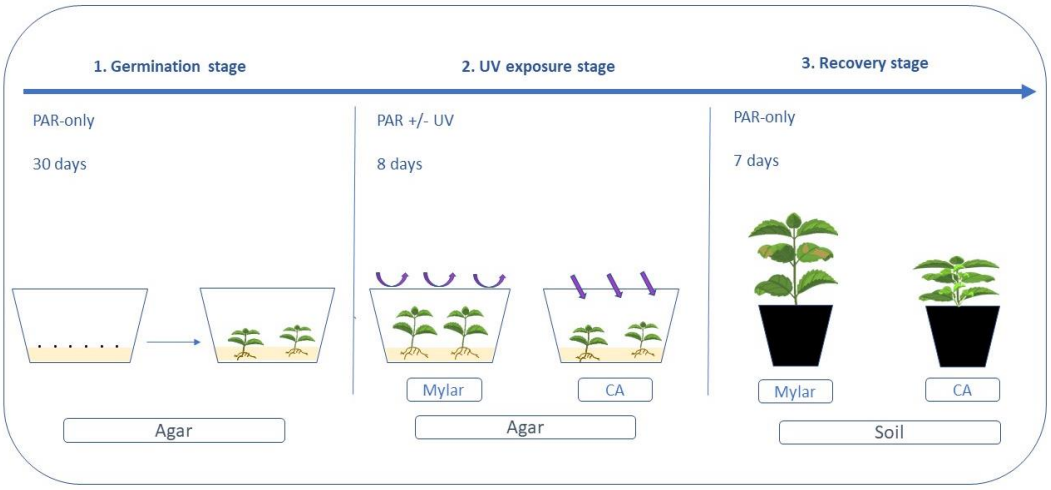


Figure 2.1. *A schematic of the experimental design. Plants were grown for 30-days under PAR light (germination stage), then the lid was replaced with either Mylar or CA and exposed to UV and PAR for eight days (UV exposure stage) and finally transplanted on soil for seven days under PAR light (recovery stage). Measurements were taken in between each step.*

2.2.2 UV – exposure stage

Prior to UV treatment, the aforementioned boxes were randomly divided into two groups and the lids were replaced with either a UV-B blocking filter Mylar (125µm thickness, Polyester film, Tocana Ltd., Ballymount, Ireland) or a UV-B transmitting Cellulose Acetate (CA) filter (95µm thickness; Kunststoff-Folien-Vertrieb GmbH, Hamburg, Germany). Furthermore, the external sides of the boxes were covered with Mylar or CA. Filters were previously sterilised with 70% ethanol and changed after every 20 h of UV exposure (Figure 2.1). At least six plastic boxes per treatment (Mylar or CA) were used for individual replicates. Six plants from each treatment were analysed for morphological parameters immediately after the UV exposure and six plants were transplanted on soil. The remaining plants were frozen in liquid nitrogen

and used for the hormone analysis. Results were obtained as the average of five independent biological replicates for the biometric analysis while root morphology was obtained from three independent biological replicates.

2.2.3 Recovery stage

After eight days of UV exposure, a random subset of the plants was harvested, while another subset was transplanted to John Innes II compost (William Sinclair Horticulture Ltd., Lincoln, United Kingdom) and moved to a growth room. Conditions in the growth room (i.e., PAR, temperature, and relative humidity) were similar to those applied during the seedling growth and UV exposure phases. Plants were harvested for analysis seven days after transplantation to soil conditions (Figure 2.1).

2.2.4 Light, humidity, and temperature conditions

Plantlets were exposed to ~180 μmol m^{-2} s^{-1} PAR background provided by LED lamps (AP673L, Valoya, Finland), at a temperature of 20 ± 2 °C (over 24 hours) and ~50% relative humidity. Far-red LED tubes (L18C, Valoya, Finland) were used to achieve the red: far-red ratio of 1.6. The light regime was set up for 14 h light / 10 h dark for PAR (7:00 to 21:00) and 16 h light / 8 h dark (06:00 to 22:00) for far-red. UV-B radiation was provided by fluorescence tubes (TL40W/12 Philips, Germany) wrapped with a single layer of CA to block the UV-C radiation. Each sheet of CA was changed after 20 h of UV exposure. Boxes were exposed to ~0.5886 W m^{-2} total UV (with 0.3661 W m^{-2} UV-A and 0.2225 W m^{-2} UV-B) under the CA filter and ~0.2498 W m^{-2} total UV (with 0.2464 W m^{-2} UV-A and 0.004 W m^{-2} UV-B) under Mylar for 4 h around mid-day (12:00 to 16:00). The daily biologically effective UV-dose was equivalent to 2.7937 kJ/m^2 (0.2830 kJ/m^2 for UV-A and 2.5258 kJ/m^2 for UV-B) under CA and 0.3250 kJ/m^2 (0.1797 kJ/m^2 for UV-A and 0.1453 kJ/m^2 for UV-B) under

Mylar (Flint & Caldwell, 2003). Mint plants were exposed to the UV treatment for eight days, then half of the plants were used for morphological measurements and the other half were transplanted on soil (Figure 2.1).

The light spectra, the UV intensity and the red: far-red ratio were determined using an optical fibre spectroradiometer Flame-S equipped with a cosine corrector (Ocean Optics, Duiven, The Netherland) using the manufacturer's software, Oceanview (version 1.6.7). PAR intensity was measured using a PAR meter (PAR special sensor, Skye Instrument Ltd, Powys, United Kingdom). The red: far-red ratio was calculated as photon irradiance between 655 and 665 nm/photon irradiance between 725 nm and 735 nm (Franklin & Whitelam, 2005).

A portable data logger (EL-USB-2, Lascar Electronics, Whiteparish, United Kingdom) was used to determine and monitor the temperature and humidity.

2.2.5 Biometric analysis

All morphological parameters were measured at two different stages: (1) immediately after the UV exposure stage and (2) after the recovery stage. For each stage, six plants per treatment were evaluated. During the measurements leaf 1 (L1) is the oldest leaf and progressively leaf 6 (L6) is the youngest leaf. Parameters measured included the total number of leaves, number of branches, number of leaves on branches, stem thickness, plant height, internode length, leaf area, dry weight, and specific leaf area. Stem thickness was measured using a calipers at the soil level, while plant internode length was calculated as plant height divided by the number of leaf pairs on the main stem. Total leaf area (TLA) measurements were based on photographs of the first six leaves and processed using ImageJ software (version 1.52a) (Wayne Rasband, National Institute of Health, United States). Leaf dry weight (LDW) for the oldest six

leaves was recorded after drying leaves at 60 °C for five days. Specific Leaf Area (SLA) for the oldest six leaves was calculated as a ratio between leaf area and leaf dry weight (LA / LDW). Leaf area, leaf dry weight and leaf specific area were also calculated for individual leaves, albeit pooling together L1 – L2, L3 – L4 and L5 – L6. The results were obtained from five independent replicates.

2.2.6 Root Morphology

Root morphology refers here to the number and length of the primary roots and the number and length of the secondary roots. These were measured with the help of SmartRoot (Lobet et al., 2011) a plugin of ImageJ software (version 1.52a) (Wayne Rasband, National Institute of Health, United States). Root fresh weight was recorded at the end of each experimental stage, after the removal of the stems and agar or soil residuals. Root dry weight was recorded after drying roots at 60 °C for five days. Analyses were conducted directly after the UV exposure stage and once more at the end of the recovery stage on six samples for each stage and on three biological replicates.

2.2.7 Hormone analysis

Hormone concentrations were measured in different plant organs: leaves, stems and roots. For each replicate, material from different plants was pooled to reach the necessary amount of 100 mg per organ. Each measurement was independently replicated (i.e., independent plants, SPE extraction and chromatographic quantification for each aliquot) at least five times. After collection, plant material was immediately frozen in liquid nitrogen and stored at –80 °C until the extraction. Data were collected after the UV-exposure stage and after the recovery stage. The extraction procedure followed Qian et al. (2021) with some modifications. Approximately 100

mg of frozen and homogenized plant material was mixed with 1 mL extraction solvent (80% (v/v) methanol, 20% 0.8 mM formic acid) and extracted overnight at –20 °C. The following internal standards were added: 30 pmol 3-[phenyl-$^{13}C_6$] indolylacetic acid (Cambridge Isotopes, Tewksbury, MA, USA), 30 pmol [$^2H_6$](+)-cis,trans-abscisic acid, ([(S)-5-[2H6](1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methyl-(2Z,4E)-pentadienoicacid] (Olchemim Ltd., Olomouc, Czech Republic), 30 pmol for each GA ($^2D_4$-Gibberellin A1, $^2D_4$-Gibberellin A4, $^2D_2$-Gibberellin A8, $^2D_2$-Gibberellin A9, $^2D_2$-Gibberellin A15, $^2D_2$-Gibberellin A19, $^2D_2$-Gibberellin A20 (Olchemim Ltd.)), and 40 pmol each for the cytokinins ([$^2H_3$]dihydrozeatin, [$^2H_3$] dihydrozeatin riboside, [$^2H_5$]*trans*-zeatin-*7*-glucoside, [$^2H_5$]*trans*-zeatin-*9*-glucoside, [$^2H_6$]N^6-isopentenyladenine, [$^2H_6$]*trans*-zeatin-*O*-glucoside, [$^2H_6$]*trans*-zeatin-*O*-glucoside riboside, [$^2H_6$]N^6-isopentenyladenosine, [$^2H_6$]N^6-isopentenyladenine-*7*-glucoside, [$^2H_6$]N^6-isopentenyladenine-*9*-glucoside, (Olchemim Ltd.)), ([$^2H_7$]N^6-benzyladenine (D-BA), [$^2H_7$]N^6-benzyladenosine (D-BAR), [$^2H_7$]N^6-benzyladenine-*9*-glucoside (D-BA9G), ([$^{15}H_4$]*meta*-topolin (15N-mT), [^{15}N4]*ortho*-topolin (15N-oT), Olchemim Ltd.)). After overnight extraction, Oasis® HLB (10 mg, Waters, Deerfield, IL, USA) was added in batches to bind pigments. After centrifugation at 14000 rpm, 4 °C, for 15 min to remove cell debris and Oasis sorbent, the extract was split with separate aliquots for the analysis of cytokinins (aliquot 1), indole-3-acetic acid, abscisic acid, gibberellins (aliquot 2), and IAA-conjugates (aliquot 3).

Aliquot 1 was filtered through a Chromafil® AO-20/3 filter (nylon, pore size 0.20µm, diameter 3 mm; Macherey-Nagel, Düren, Germany). The filtrate was collected in an LC-MS vial and kept at 4 °C. Isoprenoid cytokinins were analysed in aliquot 1 by ACQUITY UPLC coupled ES(+) TQD Tandem Quadrupole UPLC/MS/MS (Waters, Deerfield, IL, USA) in multiple reactions monitoring (MRM) mode using a BEH C18

Column (130Å, 1.7 µm, 2.1 mm x 50 mm) coupled to a BEH C18 VanGuard Pre-column (130Å, 1.7 µm, 2.1 mm x 5 mm), in combination with the following linear gradient: 95:5 A: B with A 0.1 M ammonium acetate in water and B methanol, isocratic 95:5 A: B for 0.4 min, 95:5 A: B to 76:24 A: B in 3 minutes, isocratic 76:24 A: B for 2 minutes, flow 0.39 µL min^{-1}, column temperature 50 °C, injection volume 6 µL. Aromatic cytokinins were analysed in a separate UPLC-TQD MS/MS run with the same analytical column at: 95:5 A: B with A 0.1 M ammonium acetate in water and B methanol, 99.9:0.1 A: B to 95:5 A: B to 72:28 A: B in 5 minutes, isocratic 72:28 A: B for 0.5 minutes, linear gradient 72:28 A: B to 0.1:99.9 A: B in 0.5 minutes, flow 0.4 µL min^{-1}, column temperature 48 °C, injection volume 6 µL.

Aliquot 2 was acidified with 5 6% formic acid (FA) and loaded on a pre-conditioned Bond Elut C18 solid phase extraction cartridge (Agilent, Santa Clara, CA, USA). Compounds of interest were eluted with 4 mL of diethyl ether. The eluent was dried under a nitrogen stream, dissolved in 60 µL methanol, derivatized with N-(3-Dimethylaminopropyl)-N′-ethyl carbodiimide, and kept at 4 °C until analysis. IAA, ABA and GA were analysed by ES(+)-UPLC-MS/MS in MRM mode, using the same combination of column and guard detailed for aliquot 1, and using the solvent gradient: 92:8 A: B with A 0.1% (v/v) FA in water and B 0.1% (v/v) FA in acetonitrile to A: B 60:40 for 5 minutes under a linear gradient, followed by a linear gradient from 60:40 to 10:90 in 0.5 minutes, flow rate 0.420 µL min^{-1}, column temperature 40 °C.

Aliquot 3 was mixed with an equal volume of 12N NaOH, flushed with a water saturated nitrogen stream for 20 minutes and hydrolyzed for 180 minutes at 100 °C in a Pierce Reacti-Therm III. After alkaline hydrolysis, the sample was titrated with 2M HCl and diluted to a final volume of 10 mL with deionized water. Further solid phase extraction and derivatization are as described for aliquot 2. IAA conjugates were

analysed as IAA-EDC using the chromatographic conditions described for IAA. All data are expressed in picomoles per gram fresh weight (pmol g^{-1} FW).

2.2.8 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics v28 (Armonk, New York, US). Two tails t-test or Mann-Whitney test were used to determine the difference between –UV and +UV after the UV-exposure stage or after the recovery stage. Data are shown as mean ± SE (Standard Error).

2.3 Results

2.3.1 Plant morphology

M. spicata plants were grown in closed containers, then morphological parameters and biomass were measured immediately after eight days of UV exposure and again seven days after transplanting to soil.

The total number of leaves, which includes the leaves on branches, showed a significant UV-induced increase, both after the UV exposure phase ($p = 0.02$) and at the end of the transplanting phase ($p < 0.001$) (Figure 2.2 A). A highly significant change in the number of branches and the number of leaves on branches was also observed. Thus, UV-treated plants developed a higher number of branches during UV exposure ($p < 0.001$) and this number further increased significantly during the following seven days when the plants were not exposed to UV ($p < 0.001$) (Figure 2.2 B & 2.2 C). In addition, the transplanting effect was more pronounced in the –UV plants, resulting in a 4-fold increase in both the number of branches and the number of leaves on branches. In contrast, the number of branches and leaves on branches increased by 1.40 and 1.48 times respectively in +UV plants (Supplementary Table 2.4).

Moreover, the stem thickness decreased due to UV radiation exposure. Stems of the UV-exposed plants were significantly thinner after both UV exposure and recovery stages ($p < 0.001$) compared to the - UV plants (Figure 2.2 D).

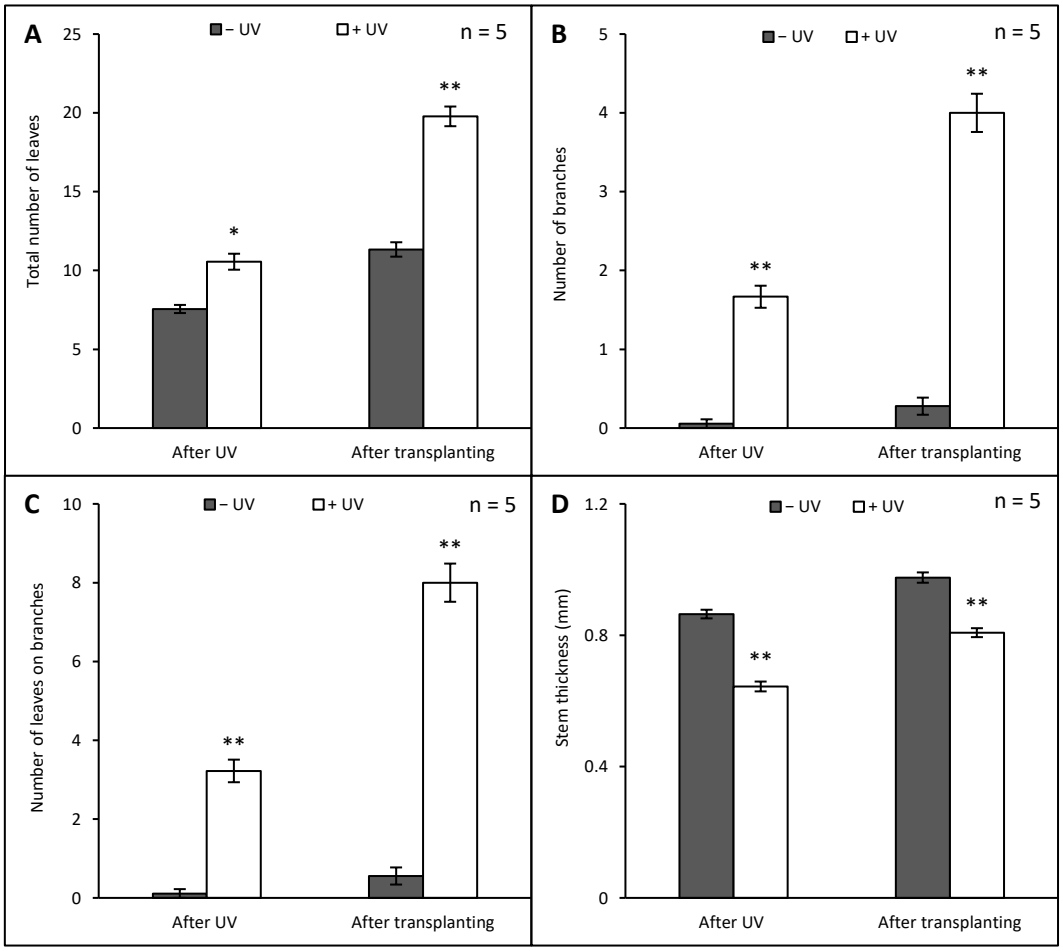


Figure 2.2*. Parameters measured were the total number of leaves (**A**), number of branches (**B**), number of leaves on branches (**C**) and stem thickness (mm) (**D**). Before the UV exposure stage, the mean of the total number of leaves was equal to 6, the number of branches and the number of leaves on branches was equal to 0, while the stem thickness was 0.558 mm. Asterisks indicate a significant difference between UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

UV exposure led to a reduction in plant size (Figure 2.3 A & B), indeed plants exposed to UV were significantly shorter than plants grown under the UV-blocking filter after both UV exposure stage and the recovery stage ($p < 0.001$) (Figure 2.4 A).

UV also affected the internode length causing a reduction both immediately after UV exposure ($p < 0.001$) and after transplanting ($p < 0.001$) (Figure 2.4 B).



Figure 2.3. *The effect of UV on mint morphology. Change in plant size and branching (**A**) and leaf area and petiole length (**B**) between control plants and plants exposed to UV.*

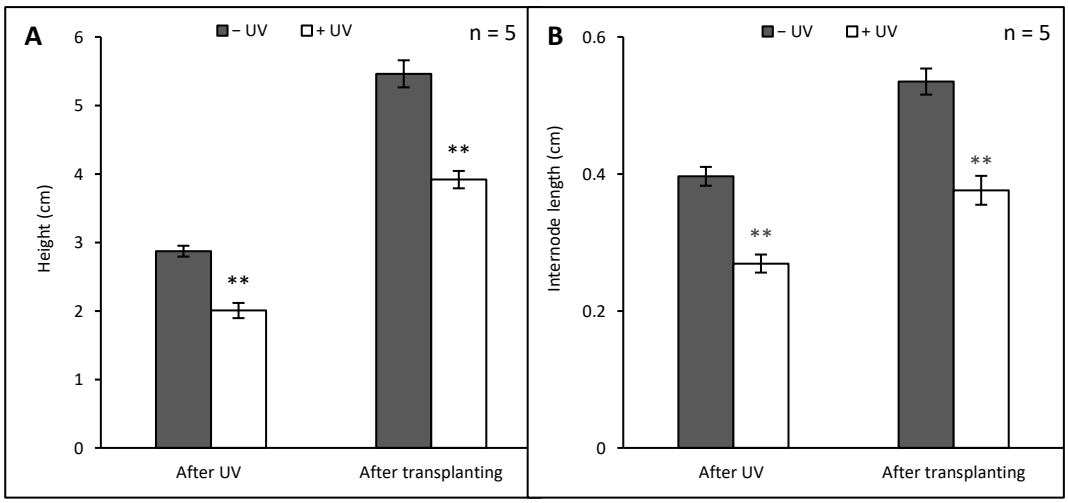


Figure 2.4. *Mint plants were covered with UV-blocking filter Mylar (grey) or UV-transmitting filter, cellulose acetate (white) sampled immediately after UV or seven days after transplanting. Parameters measured were plant height (cm) (**A**), internode length (cm) (**B**) Asterisks indicate a significant difference between the control and the treatment with $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

The total leaf area across all the main leaves on the plant showed a significant decrease for the UV-treated plants compared to –UV immediately after the UV exposure phase ($p < 0.001$). A significant decrease in total leaf area was also measured after transplanting to soil ($p < 0.001$) (Figure 2.5 A). The same trend was recorded for leaf dry weight where the change between the UV-exposed plants and the non-UV exposed plants was both significant immediately after the UV exposure stage and after transplanting ($p = 0.033$ & $p = 0.001$) (Figure 2.5 B). On the contrary, the SLA did not show any significant variance after the UV-exposure stage, but the difference became both substantial and significant after the recovery stage ($p = 0.001$) (Figure 2.5 C).

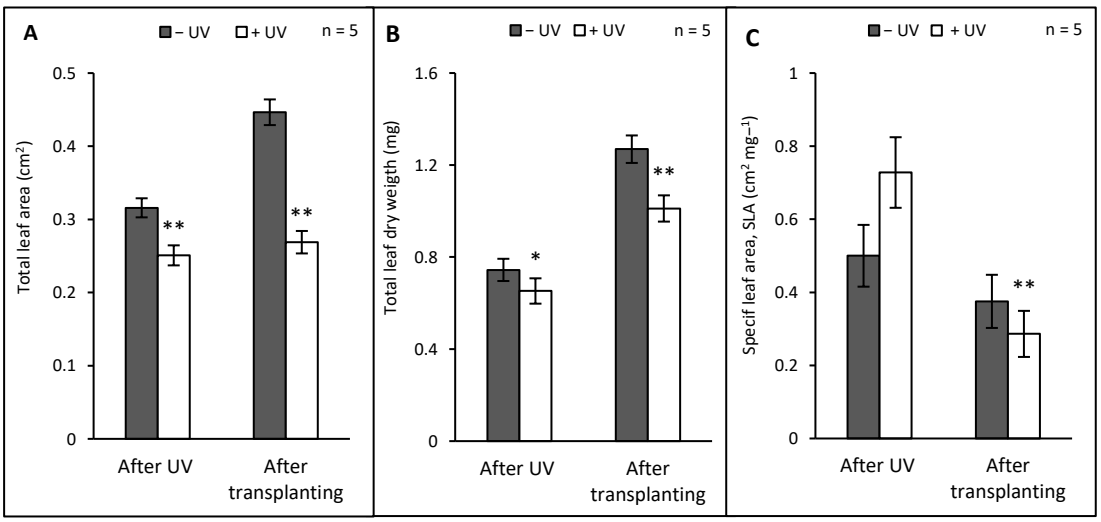


Figure 2.5. *Parameters measured were leaf area (cm^2) (**A**), leaf dry weight (LDW, mg) (**B**) and specific leaf area (SLA, $cm^2\ mg^{-1}$) (**C**). Before the UV exposure stage, the mean of the total leaf area was 0.216 cm^2, the LDW was 0.638 mg, and the SLA was 0.451 $cm^2\ mg^{-1}$. Asterisks indicate a significant difference between UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

Leaf area, leaf dry weight and specific leaf area were also analysed for individual pairs of leaves, i.e., L1 – L2, L3 – L4, L5 – L6. The leaf area of the oldest pair of leaves, L1 - L2, was relatively similar for UV-treated and non-UV treated plants immediately

after the UV-exposure stage. In contrast, there was a significant decrease in the area of L2 - L3 ($p < 0.001$) and L4 - L5 ($p = 0.023$) in UV-exposed plants (Figure 2.6 A). A comparison of leaves analysed after UV exposure, and those measured after recovery, showed that leaves did continue growing during the transplanting phase. After the recovery, the leaf area was significantly smaller among all the leaves that had previously been exposed to UV ($p < 0.001$, $p < 0.001$ & $p < 0.001$) (Figure 2.6 B).

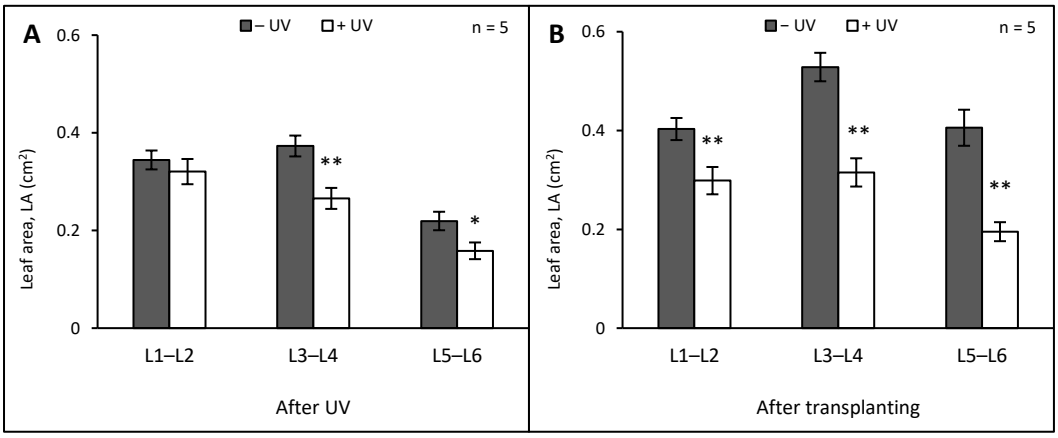


Figure 2.6. *The parameter measured was the leaf area (cm^2) across leaf L1 - L2, L3 - L4, and L5 - L6 after the UV (**A**) or after transplanting (**B**). Before the UV exposure stage, the mean of the leaf area for L1 - L2 was 0.274 cm^2, for L3 – L4 was 0.264 cm^2 and for L5 – L6 was 0.113 cm^2. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

In contrast, UV did not affect the leaf dry weight after the UV exposure stage, remaining relatively similar for L1 – L2, and not significantly decreasing for L3 – L4 and L5 – L6 (Figure 2.7 A). The same trend was recorded after transplanting with the same relative leaf dry weight for the first pair of leaves, irrespective of previous UV-exposure. However, a UV-induced decrease in leaf dry weight was noted for the second and third pair of leaves following recovery. This reduction was significant for L3 - L4 ($p = 0.001$) but not significant for L5 – L6 (Figure 2.7 B).

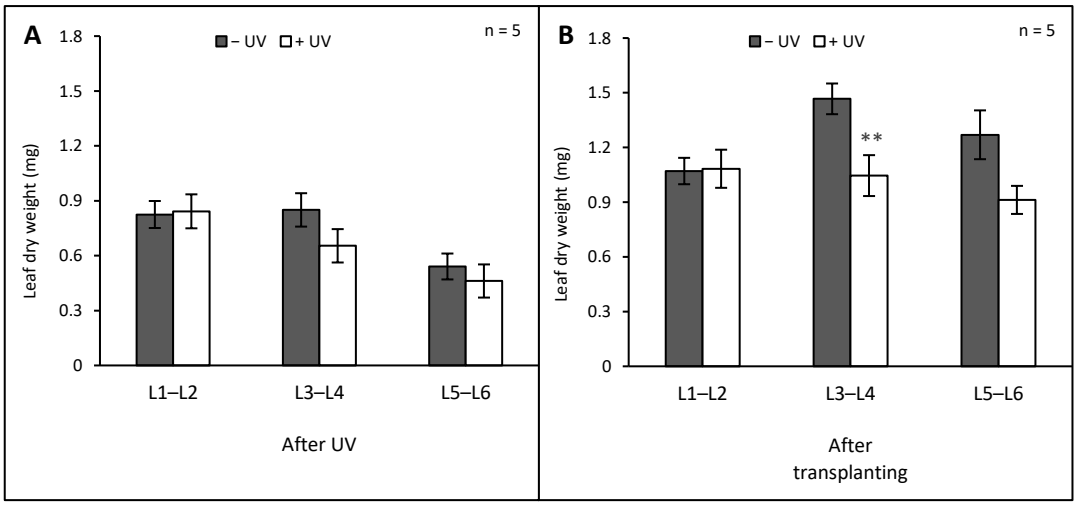


Figure 2.7. *The parameter measured was the leaf dry weight (LDW, mg) across leaf L1 - L2, L3 - L4, and L5 - L6 after the UV (**A**) or after transplanting (**B**). Before the UV exposure stage, the mean of the LDW for L1 - L2 was 0.876 mg for L3 – L4 was 0.708 mg and for L5 – L6 was 0.170 mg. Asterisks indicate a significant difference between the UV treatments with $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

Specific leaf area rose with UV exposure for all pairs of leaves with a progressive increase from the oldest to the youngest leaves, but the change was not significant at the end of the UV exposure stage (Fig. 2.8 A). After transplanting, the SLA recorded for previously UV-exposed plants tended to be lower than that of controls, decreasing from L1 to L6. This reduction was significant only for the youngest leaves L5 – L6 (p = 0.006) (Figure 2.8 B).

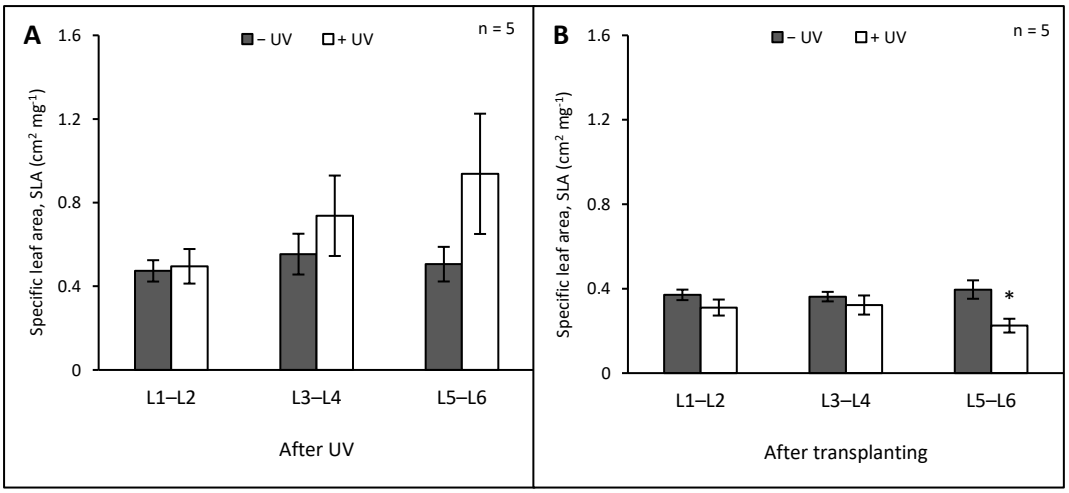


Figure 2.8. *The parameters measured were the leaf specific area (SLA, $cm^2\ mg^{-1}$) across leaf L1 - L2, L3 - L4, and L5 - L6 after the UV (**A**) or after transplanting (**B**). Before the UV exposure stage, the mean of the SLA for L1 - L2 was 0.406 $cm^2\ mg^{-1}$, for L3 – L4 was 0.470 $cm^2\ mg^{-1}$ and for L5 – L6 was 0.493 $cm^2\ mg^{-1}$. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

2.3.2 Root morphology

Root fresh weight was significantly lower after UV exposure (p = 0.031). The difference was most marked after transplanting when the control roots were much heavier than the UV-treated roots ($p < 0.001$). In general, the increase in fresh weight change was smaller in non-UV exposed plants compared to controls when comparing plants immediately after UV exposure and plants transplanted to soil (Figure 2.9 A). Root dry weight was similar across the treatments immediately after the UV exposure stage. However, the difference in dry weight became significant after the recovery stage with higher root biomass in non-UV exposed plants (p = 0.003) (Figure 2.9 B).

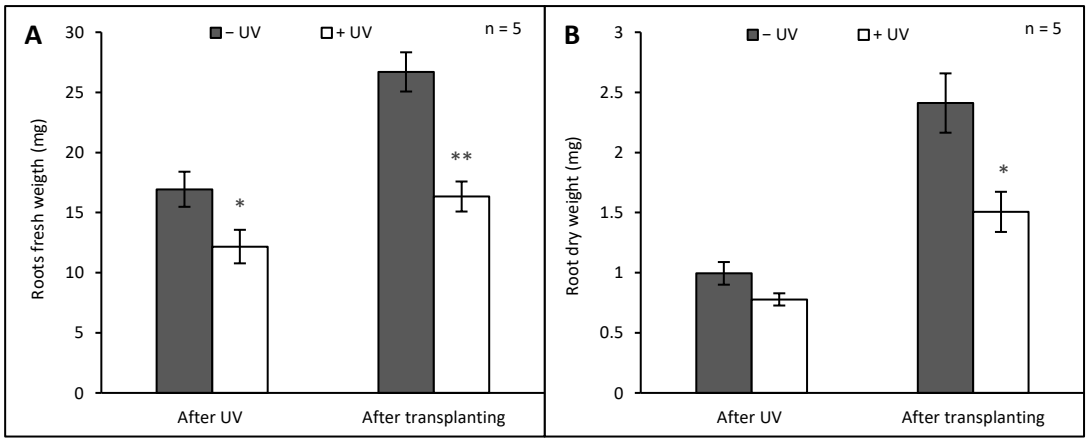


Figure 2.9. *Parameters measured were root fresh weight (mg) (**A**) and root dry weight (mg) (**B**). Before the UV, the mean of the root fresh weight was 8.00 mg while the mean of the root dry weight was 0.54 mg. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

The number of primary and secondary roots is not affected by UV exposure. Indeed, no differences in root numbers were recorded between UV-exposed and non-UV exposed plants at either stage. An increase in secondary roots was noted after transplanting, but this decrease was registered for both sets of plants (Figure 2.10 A & 2.10 B). Immediately after the UV exposure stage, the primary roots were significantly shorter in UV-treated plants compared to non-UV treated plants ($p = 0.011$). During the recovery stage, the primary roots of UV-exposed plants kept growing while the primary roots of non-UV exposed plants did not elongate further. As result, there was no significant difference in primary root length between the UV-treated and non-UV treated plants (Figure 2.10 C). UV also affected the length of the secondary root, and these were significantly shorter in UV-exposed plants ($p = 0.006$). In contrast to the main roots, secondary roots from non-exposed plants grew strongly after transplanting, while the UV-exposed ones did not grow when transplanted into the soil.

The analysis revealed that this change was not significant after the recovery stage (Figure 2.10 D).

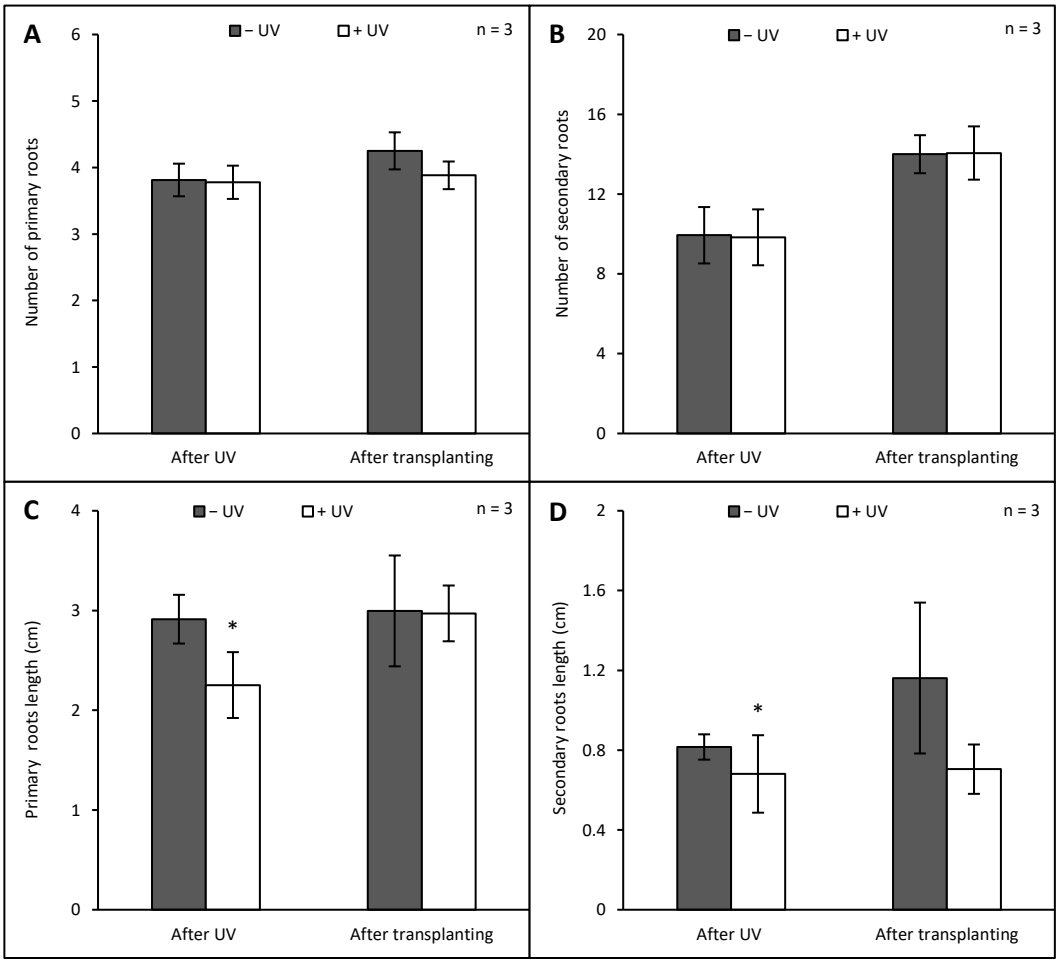


Figure 2.10. *Parameters measured were the number of primary roots (**A**), the number of secondary roots (**B**), primary roots length (cm) (**C**) and secondary roots length (cm) (**D**). Before the UV exposure stage, the mean number of primary roots was 3.8 cm, the mean of the number of secondary roots was 6.85 cm while the mean length of the primary roots was 2.47 cm, and the mean length of the secondary roots was 0.80 cm. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*). Fold change between after UV and after transplanting is reported in Supplementary Table 2.4.*

2.3.3 Hormone profile

2.3.3.1 Leaves

In order to determine the mechanism underlying the effect of UV-B on different plant organs, the concentration of different hormones was evaluated in leaves, stems and roots both immediately after UV exposure and seven days after transplanting. Cytokinins including BAP, BA9G, iPA, iP9G, abscisic acid, auxins including IAA and its conjugated form and gibberellins including GA3, GA19, GA7 and GA15 were analysed.

Most of the hormones measured immediately after UV exposure showed a decreasing trend, their content was lower in UV-exposed leaves. In detail, relative to the untreated plants, concentrations of BAP (-28%) (Supplementary Table 2.1), BAG9 (-61%) (Supplementary Table 2.1), iPG9 (-56% , $p < 0.001$) (Figure 2.11 A), ABA (-45%) (Figure 2.11 D), IAA (-69%) (Figure 2.11 G), GA3 (-30%) (Figure 2.11 L), GA7 (-43%) (Supplementary Table 2.1), were all decreased following UV treatment. Especially, the IAA–C concentration significantly diminished in the UV-exposed plants (-99%, $p = 0.016$) (Supplementary Table 2.1). Concentrations of iPA, GA19 and GA15 increased, but this was not significant (Supplementary Table 2.1).

At the end of the recovery stage, similar trends were recorded immediately after UV exposure. The content of BA9G and iPA in UV-exposed leaves was even below the detection limit. Decreased concentrations in UV-exposed leaves were measured for BAP (-25%) (Supplementary Table 2.1), ABA (-78%) (Figure 2.11 D), IAA-C (-74%) (Supplementary Table 2.1), and GA19 (Supplementary Table 2.1), but the difference is statistically different only for iPG9 (-51%, $p = 0.038$) (Figure 2.11 A) and GA3 (-63%, $p = 0.009$) (Figure 2.11 L). IAA (Figure 2.11 G) and GA7 slightly increased respectively by 10% and 34% (Supplementary Table 2.1).

2.3.3.2 Stems

The concentrations of most hormones measured in the stem compartment tended to decrease in plants exposed to UV, immediately after the end of the UV treatment. As for the leaves, the content of iPG9 (-20%) (Figure 2.11 B), ABA (-82%) (Figure 2.11E), IAA (-77%) (Figure 2.11 G), IAA-C (-96%) (Supplementary Table 2.2), GA3 (-5%) (Figure 2.11 M) were lower in stems of UV-exposed plants but not in a significant way. GA19 concentrations also slightly decreased (-2%) in stems, unlike leaves where a strong increase in concentration was observed (Supplementary Table 2.2). Concentrations of BAP (+22%), BAG9 (+15%) iPA (+545%) and GA19 (+28%) slightly increased and GA7 significantly increased (+79%, $p = 0.016$) in the stems (Supplementary Table 2.2).

After the recovery stage, concentrations of BAP (-6%) (Supplementary Table 2.2), iPG9 (-28%) (Figure 2.11 B), ABA (-62%) (Figure 2.11 E), IAA-C (-35%) (Supplementary Table 2.2), GA3 (-15%) (Figure 2.11 M) BAG9 (-23%) and iPA (-20%) (Supplementary Table 2.2), were found to decrease in the stems of the plants previously exposed to UV. However, IAA (+11%) (Figure 2.11 H) and the content of the majority of gibberellins (GA7 (+14%), GA15 (+28%), GA19 (+8%)) measured increased in +UV plants (Supplementary Table 2.2).

2.3.3.3 Roots

The effects of UV on hormone concentrations were also determined for the below-ground organs. As per the leaves and the stems, UV negatively influenced the content of some hormones such as iPG9 (-50%) (Figure 2.11 C), ABA (-51%) (Figure 2.11 F), GA3 (-19%) (Figure 2.11 N), BAP (-35%) (Supplementary Table 2.3) and GA15 (-39%) (Supplementary Table 2.3) in the roots. This effect was significant for both

IAA (-91%, p = 0.032) (Figure 2.11 I) and IAA-C (-96%, p = 0.008) (Supplementary Table 2.3) when measured immediately after the UV exposure. In contrast, the content of BAG9 (+102%), GA19 (+11%) and GA7 (+1%) increased in the roots of UV-exposed plants (Supplementary Table 2.3).

Measurements on roots seven days after transplanting confirmed a reduction in GA3 (-26%, p = 0.026) (Figure 2.11 N), GA15 (-6%) (Supplementary Table 2.3), BAP (-26%) (Supplementary Table 2.3), iPA (-35%) (Supplementary Table 2.3) and iPG9 (-19%) (Figure 2.11 C), ABA (-51%) (Figure 2.11 F), IAA-C (-70%) (Supplementary Table 2.3) in roots of plants exposed to UV-B. The trend was similar to that seen in UV-exposed leaves and stems. A parallel increase in IAA (+34%) (Figure 2.11 I) and GA7 (+6%) concentration was detected (Supplementary Table 2.3).

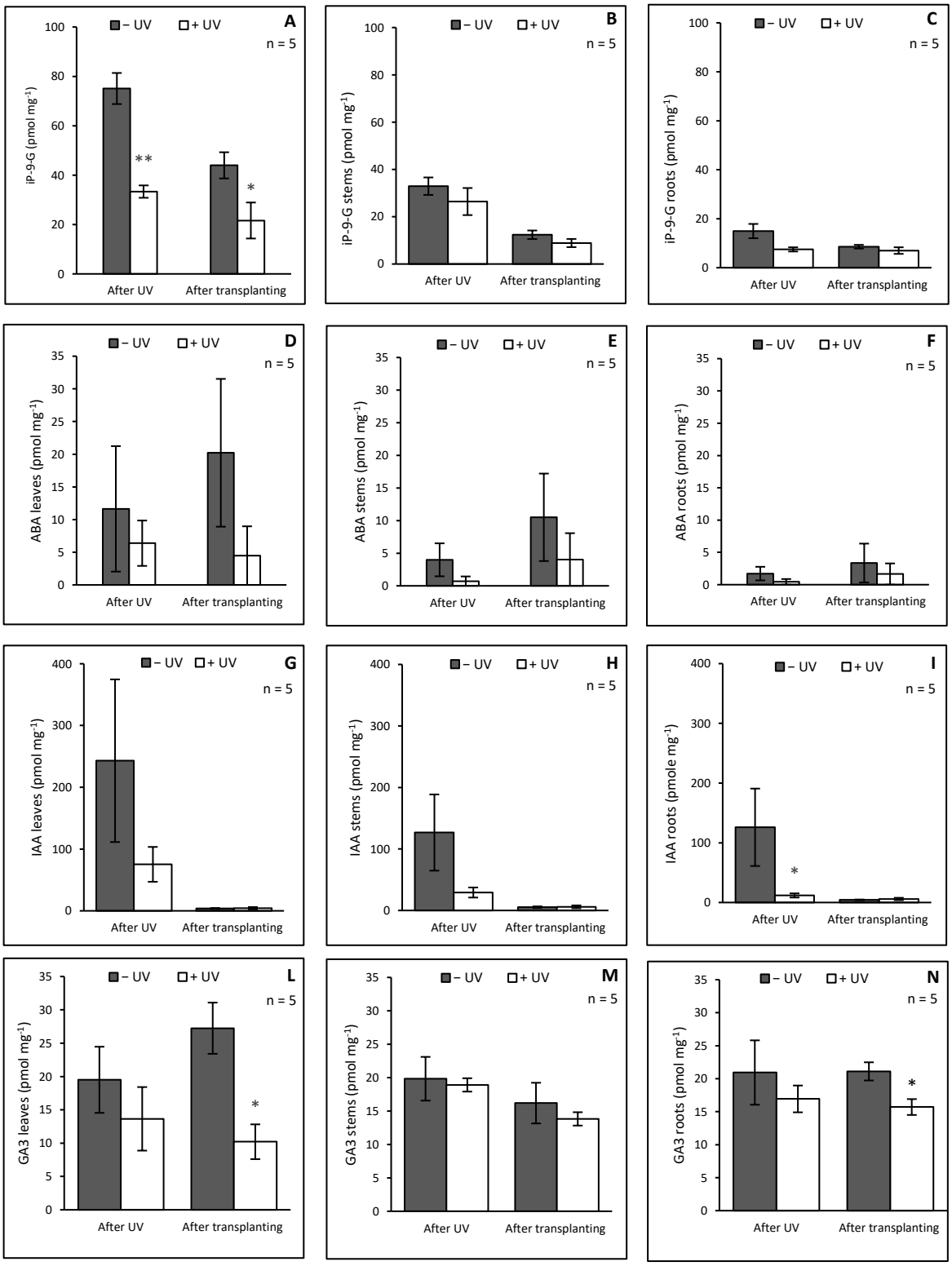


Figure 2.11. *In the graphs, level of hormones IPG9 (**A, B, C**), ABA (**D, E, F**), IAA (**G, H, I**) and GA3(**L, M, N**) (pmol mg^{-1}) are represented. Hormones were measured respectively in the leaves (graphs on the left), in the stems (graphs in the middle) and in the roots (graphs on the right). Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) and $p < 0.01$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 2.1, 2.2 and 2.3.*

2.4 Discussion

In this study, we found that UV induces morphological adjustments in plants exposed to UV. This adjustment persists for some time, even when the UV exposure is ended, it is associated with changes in the concentrations of several plant hormones.

2.4.1 UV-induced changes in morphology and possible applications for *in vitro* culture

UV-B has been reported to induce morphological responses in plants (Robson et al., 2015). For example, in cucumber a dwarfed phenotype has been reported, with a reduction in leaf area, petiole length and stem elongation, following UV exposure (Qian et al., 2021). Here, it was observed that similar morphological responses can be induced under *in vitro* conditions in *Mentha spicata* L. The results show that the oldest leaves respond weakly to UV, while younger leaves are strongly affected (Figure 2.6). This may occur through the activation of a specific signalling cascade initiated by the UVR8 photoreceptor (Yadav et al., 2020). Alternatively, the response can be a generic, so-called stress-induced morphogenic response (SIMR) (Potters et al., 2007). SIMRs have been characterised as redistribution, rather than cessation of growth, and this can be seen in the observed phenotype. On one side, UV promotes plant growth seen as an increase in axillary branching, on the other side, UV acts as a growth inhibitor seen as the reduction of stem elongation and leaf elongation (Potters et al., 2007). This phenotype is recognised as typical for plants acclimated to low doses of UV radiation (Jansen et al., 1998; Robson et al., 2015). Thus, it is concluded that environmentally relevant UV doses can be used under *in vitro* conditions to similarly adjust plant morphology.

Overall, the UV-induced plant architecture may not only make for a commercially more attractive plant but may also contribute to a better acclimation to the new growth

conditions, for example under *ex vitro* conditions (Hazarika, 2006). Previous studies have shown that the UV-induced phenotype may either be transient or permanent. The data observed in this study, are consistent with work by Metwally et al. (2019) who showed that a daily UV exposure (15 – 45 minutes) of *in vitro* Spathiphyllum explants not only increased the survival rate of the explants, but also contributed to the reduction of plant elongation.

Here, it was noted that UV-induced increases in leaf number and stem branching, and UV-induced decreases in leaf area, stem height and root weight persist at the end of the seven-day recovery phase. In contrast, UV effects on primary and secondary root length appear to have largely been lost, suggesting that following cessation of UV exposure, the architecture of a growing plant slowly re-adjusts. This interpretation matches that by Qian et al. (2021) who demonstrated that marked UV-induced morphological changes in cucumber seedlings had no impact on the morphology, and indeed the fruit yield, of mature plants. Thus, the observed UV-induced reductions in plant elongation are not limited to mint but seem to apply to a wider range of species. A more compact phenotype can be directly beneficial for ornamental plants yet can also help to reduce transport costs for a wider variety of crops.

2.4.2 The link between hormones and morphological changes

In this study, architectural changes were accompanied by marked changes in the content of several phytohormones. Although the link between changes in hormone content and specific architectural adjustments is not conclusive, data give an insight into potential underlying mechanisms of UV-driven architectural adjustment. For example, it is noted that increased axillary branching is matched by increased concentration of cytokinins (BAP, BA9G and iPA) in the stem together with strongly

decreased concentrations of auxin (IAA). This observation matches the long-established antagonistic relationship of auxin and cytokinins in controlling axillary buds (Müller & Leyser, 2011). Typically, endogenous cytokinins concentration tend to decrease when a plant is exposed to abiotic stress (Zwack & Rashotte, 2015) thus the slight increase in cytokinins in the stem implies that the low doses of UV used in this study have a regulatory rather than a stress-inducing effect. Decreases in BAP in other organs may be linked with the quenching of oxyradicals by cytokines in response to UV-B exposure (Kataria & Guruprasad, 2018). The observed changes in auxin are consistent with the observations of Hectors et al. (2012), who reported a decreased IAA concentration in the leaf compartment after UV exposure. Here, results showed a significant decrease in both free and conjugated IAA. The decrease in auxin is not limited to the leaf but also is noted in the stem, where it may be associated with increased axillary branching, and the root where it may be associated with decreased root elongation (Ivanchenko et al., 2010). The changes in auxin concentration are likely to be linked to the fundamental mechanism of UV-B responses. UV-B perceived via UVR8 and mediated via the HY5/HYH dependent pathway alters auxin synthesis (e.g. PIFs), transport (e.g. PIN proteins) and signalling (e.g. AXR2/IAA7) (Vanhaelewyn et al., 2016). In this experiment, the altered auxin distribution after UV exposure is also paralleled by decreases in leaf area and stem elongation. Effects of auxin on leaf area are consistent with the role of this hormone in leaf development (Xiong & Jiao, 2019), while effects on stem length are well documented, particularly in the context of the shade-avoidance response (Ma & Li, 2019). Simultaneous, UV-mediated decreases in GA3 are likely to have further slowed down the elongation of leaf, stem, and root. Finally, the amount of ABA in mint plants is reduced but is not significantly affected by the treatment across all the organs. A possible explanation

can be found in the *in vitro* environment to which the plants were exposed. As reported by Hronková et al. (2003), the closed container and consequently the high relative humidity can limit the production of ABA. ABA concentration is often reported to increase under conditions of plant stress, where ABA can play a key role as regulator (Bulgakov et al., 2019). The observation that UV does not induce increases in ABA, re-confirms the hypothesis that UV-B is a regulator rather than a stressor. Overall, it is concluded that subtle, regulatory differences in concentrations of key hormones such as cytokinin, gibberellic acid and auxin occur in UV-B exposed plants, and it is hypothesised that these lead to a re-adjustment of the plant architecture under *in vitro* conditions. Although the link between changes in hormone concentrations and specific architectural adjustments is not conclusive, it is recognised that phenotypic changes may be associated with local changes in hormone concentrations, and that some of these changes will remain undetected where whole plants are analysed.

2.5 Conclusion

In this paper, it is shown that UV supplementation during *in vitro* culture led to an alteration of plant morphology, characterized by a more compact phenotype and an increase in axillary branching. Thus, it is shown that UV-B supplementation is an alternative and innovative approach to alter plant morphology and obtain the desired plant architecture under *in vitro* conditions, without the application of exogenous PGRs.

2.6 Supplementary information

2.6.1 Hormones in leaves

Supplementary Table 2.1. *Mint plants were covered with a UV-blocking filter Mylar (–UV) or UV-transmitting filter, cellulose acetate (+UV). Main plant hormones (pmol mg^{-1}) were measured on leaves collected immediately after UV or seven days after transplanting. Arrows indicate the percentage change between –UV and +UV. One arrow (↑/↓) has been used for differences < ± 40%, two arrows (↑↑/↓↓) have been used for values≥ ± 40% and ≤ ± 60%, and three arrows (↑↑↑/↓↓↓) have been used for differences > ± 60%. Significant results were reported when the p-value was < 0.05. Fold change indicates the difference between plants sampled after the UV and plants sampled after transplanting both in –UV and + UV treatments.*

Hormone	*–UV after UV*	*+UV after UV*	*Trend*	*–UV after transplanting*	*+UV after transplanting*	*Trend*	*Fold change –UV*	*Fold change +UV*
BAP	8.71 ± 0.92	6.27 ± 0.96	↓	11.68 ± 2.79	8.71 ± 0.98	↓	0.34	0.39
BA9G	3.05 ± 2.06	1.18 ± 0.80	↓↓↓	0.31 ± 0.31	0.00 ± 0.00	-	-0.90	-1.00
iPA	0.09 ± 0.09	1.00 ± 0.27	↑↑↑	0.24 ± 0.24	0.00 ± 0.00	-	-0.41	-0.35
iP9G	75.09 ± 6.29	33.33 ± 2.50	Significant ↓↓	43.96 ± 5.31	21.62 ± 7.29	Significant ↓↓	1.56	-1.00
ABA	11.64 ± 9.60	6.38 ± 3.47	↓↓	20.22 ± 11.31	4.48 ± 4.48	↓↓↓	0.74	-0.30
IAA	243.00 ± 131.66	75.24 ± 28.20	↓↓↓	3.90 ± 0.69	4.29 ± 1.82	↑	-0.98	-0.94
IAA-C	380.64 ± 237.33	5.59 ± 2.99	Significant ↓↓↓	26.66 ± 12.10	6.96 ± 3.83	↓↓↓	-0.93	0.24
GA3	19.50 ± 4.96	13.65 ± 4.77	↓	27.24 ± 3.84	10.21 ± 2.61	Significant ↓↓↓	0.40	-0.25
GA19	9.98 ± 0.89	37.51 ± 31.17	↑↑↑	22.62 ± 8.82	9.87 ± 1.37	↓↓↓	1.27	-0.74
GA7	390.46 ±52.33	221.4 ± 57.00	↓↓	279.63 ± 71.67	374.31 ± 48.52	↑	-0.28	0.69
GA15	260.57 ± 37.11	358.28 ± 143.7	↑	313.05 ± 31.62	1015.40 ± 716.26	↑↑↑	0.20	1.83

2.6.2 Hormones in stems

Supplementary Table 2.2. *Mint plants were covered with a UV-blocking filter Mylar (–UV) or UV-transmitting filter, cellulose acetate (+UV). Main plant hormones (pmol mg^{-1}) were measured on stems collected immediately after UV or seven days after transplanting. Arrows indicate the percentage change between –UV and +UV. One arrow (↑/↓) has been used for differences < ± 40%, two arrows (↑↑/↓↓) have been used for values≥ ± 40% and ≤ ± 60%, and three arrows (↑↑↑/↓↓↓) have been used for differences> ± 60%. Significant results were reported when the p-value was < 0.05. Fold change indicates the difference between plants sampled after the UV and plants sampled after transplanting both in –UV and + UV treatments.*

Hormone	*–UV after UV*	*+UV after UV*	*Trend*	*–UV after transplanting*	*+UV after transplanting*	*Trend*	*Fold change –UV*	*Fold change +UV*
BAP	6.74 ± 0.13	8.21 ± 1.34	↑	7.31 ± 1.39	6.89 ± 1.01	↓	0.09	-0.16
BA9G	5.73 ± 2.39	6.56 ± 4.06	↑	1.42 ± 1.00	1.10 ± 0.47	↓	-0.75	-0.83
iPA	0.86 ± 0.45	5.56 ± 2.28	↑↑↑	1.27 ± 0.13	1.01 ± 0.08	↓	0.47	-0.82
iP9G	32.92 ± 3.69	26.41 ± 5.72	↓	12.38 ± 1.81	8.87 ± 1.73	↓	-0.62	-0.66
ABA	3.99 ± 2.53	0.72 ± 0.72	↓↓↓	10.50 ± 6.70	4.04 ± 4.04	↓↓↓	1.63	4.60
IAA	126.70 ± 61.95	29.18 ± 8.18	↓↓↓	5.35 ± 1.31	5.95 ± 2.30	↑	-0.96	-0.80
IAA-C	277.26 ± 148.18	10.52 ± 5.19	↓↓↓	7.07 ± 2.36	4.63 ± 2.27	↓↓	-0.97	-0.56
GA3	19.84 ± 3.27	18.91 ± 3.33	↓	16.19 ± 3.04	13.83 ± 1.51	↓	-0.18	-0.27
GA19	30.72 ± 2.51	39.32 ± 11.67	↑	56.49 ± 3.64	61.25 ± 5.26	↑	0.84	0.56
GA7	160.33 ± 20.05	287.22 ± 36.86	Significant ↑↑↑	245.46 ± 18.25	279.1 ± 21.92	↑	0.53	-0.03
GA15	833.21 ± 523.03	818.93 ± 433.58	↓	620.83 ± 158.67	794.17± 300.17	↑	-0.25	-0.03

2.6.3 Hormones in roots

Supplementary table 2.3. *Mint plants were covered with a UV-blocking filter Mylar (–UV) or UV-transmitting filter, cellulose acetate (+UV). Main plant hormones (pmol mg^{-1}) were measured on roots collected immediately after UV or seven days after transplanting. Arrows indicate the percentage change between –UV and +UV. One arrow (↑/↓) has been used for differences < ± 40%, two arrows (↑↑/↓↓) have been used for values≥ ± 40% and ≤ ± 60%, and three arrows (↑↑↑/↓↓↓) have been used for differences> ± 60%. Significant results were reported when the p-value was < 0.05. Fold change indicates the difference between plants sampled after the UV and plants sampled after transplanting both in –UV and + UV treatments.*

Hormone	***–UV after UV***	***+UV after UV***	***Trend***	***–UV after transplanting***	***+UV after transplanting***	***Trend***	*Fold change –UV*	*Fold change +UV*
BAP	8.07 ± 1.66	5.28 ± 1.25	↓	11.51 ± 2.31	8.51 ± 1.08	↓	0.43	0.61
BA9G	81.01 ± 45.82	163.80 ± 107.10	↑↑↑	74.70 ± 29.34	142.48 ± 50.91	↑↑↑	-0.08	-0.13
iPA	0.00 ± 0.00	0.35 ± 0.25	-	1.50 ± 0.40	0.97 ± 0.06	↓	-	1.74
iP9G	14.96 ± 2.92	7.48 ± 0.84	↓↓	8.58 ± 0.75	6.99 ± 1.34	↓	-0.43	-0.07
ABA	1.71 ± 1.05	0.44 ± 0.44	↓↓↓	3.36 ± 3.00	1.65 ± 1.65	↓↓↓	0.96	2.76
IAA	125.96 ± 64.80	11.81 ± 3.43	Significant ↓↓↓	4.44 ± 0.61	5.93 ± 1.95	↑	-0.96	-0.50
IAA-C	99.00 ± 62.44	3.55 ± 0.84	Significant ↓↓↓	3.25 ± 0.40	0.98 ± 2.05	↓	-0.97	-0.72
GA3	20.93 ± 4.88	16.93 ± 2.03	↓	21.11 ± 1.38	15.71 ± 1.20	Significant ↓	0.001	-0.07
GA19	4.41 ± 0.48	4.88 ± 0.86	↑	3.89 ± 0.48	4.13 ± 0.77	↑	-0.12	-0.15
GA7	61.67 ± 8.70	61.99 ± 12.37	↑	145.22 ± 13.80	153.66 ± 16.47	↑	1.35	1.48
GA15	673.39 ± 406.89	407.90 ± 98.93	↓↓↓	358.73 ± 63.08	337.67 ± 72.43	↓	-0.47	-0.17

2.6.4 Change between after UV and after recovery

Supplementary Table 2.4. *. Fold change of morphological parameters measured after the UV and after transplanting both in –UV and +UV treatments.*

Parameter	–UV After UV	–UV After transplanting	Fold change	+UV After UV	+UV After transplanting	Fold change
Total number of leaves	7.56	11.33	**0.50**	10.56	19.78	**0.87**
Number of branches	0.06	0.28	**4.00**	1.67	4.00	**1.40**
Number of leaves on branches	0.11	0.56	**4.00**	3.22	8.00	**1.48**
Stem thickness (cm)	0.86	0.98	**0.13**	0.64	0.81	**0.25**
Height (cm)	2.87	5.46	**0.90**	2.01	3.92	**0.95**
Internode (cm)	0.40	0.53	**0.35**	0.27	0.38	**0.40**
Total Leaf Area (cm^2)	0.32	0.45	**0.41**	0.25	0.27	**0.07**
Total Dry weight (mg)	0.74	1.27	**0.71**	0.65	1.01	**0.55**
Specific Leaf Area ($cm^2\ mg^{-1}$)	0.51	0.38	**-0.26**	0.72	0.29	**-0.60**
Leaf Area L1-L2 (cm^2)	0.34	0.40	**0.17**	0.32	0.30	**-0.07**
Leaf Area L3-L4 (cm^2)	0.37	0.53	**0.42**	0.27	0.32	**0.19**
Leaf Area L5-L6 (cm^2)	0.22	0.41	**0.85**	0.16	0.20	**0.23**
Dry weight L1-L2 (mg)	0.83	1.07	**0.30**	0.84	1.08	**0.29**
Dry weight L3-L4 (mg)	0.85	1.47	**0.73**	0.65	1.05	**0.60**
Dry weight L5-L6 (mg)	0.54	1.27	**1.35**	0.46	0.91	**0.98**
Specific Leaf Area L1-L2 ($cm^2\ mg^{-1}$)	0.47	0.37	**-0.22**	0.50	0.31	**-0.37**
Specific Leaf Area L3-L4 ($cm^2\ mg^{-1}$)	0.55	0.36	**-0.35**	0.74	0.32	**-0.56**
Specific Leaf Area L5-L6 ($cm^2\ mg^{-1}$)	0.51	0.40	**-0.22**	0.94	0.22	**-0.76**
Root fresh weight (mg)	16.94	26.70	**0.58**	12.17	16.34	**0.34**
Roots Dry weight (mg)	0.99	2.41	**1.42**	0.78	1.51	**0.94**
Number of primary roots	3.81	4.25	**0.11**	3.78	3.88	**0.03**
Number of secondary roots	9.94	14.00	**0.41**	9.83	14.06	**0.43**
Primary roots length (cm)	2.91	3.00	**0.03**	2.25	2.97	**0.32**
Secondary roots length	0.82	1.16	**0.42**	0.68	0.70	**0.04**

2.7 References

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Chapter 3

From stressor to protector, UV-induced abiotic stress resistance

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Abstract

Plants are continuously exposed to combinations of abiotic and biotic stressors. While much is known about responses to individual stressors, understanding of plant responses to combinations of stressors is limited. The effects of combined exposure to drought and UV radiation are particularly relevant in the context of climate change. In this study, it was explored whether UV exposure can be used as a tool to prime stress-resistance in plants grown under highly protected culture conditions. It was hypothesised that priming mint plantlets (*Mentha spicata* L.) with a low dose of UV irradiance can alleviate the drought effect caused by a change in humidity upon transplanting. Plants were grown for 30 days on agar in sealed tissue culture containers. During this period, plants were exposed to ~0.22 W m^{-2} UV-B for eight days, using either UV-blocking or UV-transmitting filters. Plants were then transplanted to soil and monitored for a further seven days. It was found that non-UV exposed mint plants developed necrotic spots on leaves, following transfer to soil, but this was not the case for plants primed with UV. Results showed that UV induced stress resistance is associated with an increase in antioxidant capacity, as well as a decrease in leaf area. UV-induced stress resistance can be beneficial in a horticultural setting, where priming plants with UV-B can be used as a tool in the production of commercial crops.

3.1 Introduction

Complex interactions between the stratospheric ozone layer and escalating changes in weather patterns, simultaneously affect the climate as well as the intensity of UV radiation reaching the Earth's surface (UNEP EEAP, 2019). Historically, changes in UV irradiance have been strongly linked with the state of the stratospheric ozone layer, however, evidence is mounting that future alterations in UV irradiance will be more strongly associated with climate change effects namely altered cloud cover, surface reflectivity, aerosol concentrations, and solar energy penetration (Bais et al., 2014; Bornman et al., 2019). Conversely, changes in stratospheric ozone can impact the climate, for example, the 'hole' in the Antarctic stratosphere is associated with changes in atmospheric air circulation and the local climate (UNEP EEAP, 2019). These interdependencies result in cascading effects that have potentially serious consequences for biodiversity, the economy, and human well-being (Hansen et al., 2013). Moreover, these interdependent effects expose plants to new combinations of UV radiation and other environmental change factors such as temperature and water availability. Alterations in the availability of water, derived from a shift in precipitation patterns, cause heavy rains in some areas while other areas are affected by aridity (UNEP EEAP, 2019). In the latter case, water scarcity can potentially cause an alteration in plant growth and impact crop production and quality (Pareek et al., 2020). Furthermore, aridity caused by decreases in cloud cover will potentially be accompanied by increased local UV-irradiance, exposing plants to new combinations of enhanced UV and drought (UNEP EEAP, 2019). Areas that are predicted to experience more frequent dry periods include the Mediterranean, western North America, southwestern South America, South Africa, and southwestern Australia (IPCC, 2022).

Plants, like other organisms, are continuously exposed to combinations of abiotic and biotic stressors (Kovács et al., 2014). While much is known about responses to individual stressors, understanding of plant responses to combinations of stressors is limited (Zandalinas et al., 2021). Furthermore, it appears that the response of plants to each combination of specific stressors is unique making predictions of plant responses problematic (Zandalinas et al., 2021). In this context, the effects of combined exposure to drought and UV radiation are particularly relevant in relation to climate change (UNEP EEAP, 2019). Previous studies showed that both UV and drought have similar morpho-physiological effects in plants such as a reduction in leaf area and plant height, but increases in root biomass and leaf thickness (Comont et al., 2012; Robson et al., 2015B; Jansen et al., 2022). For example, Comont et al. (2012) reported acclimatory responses including reductions in leaf area and specific leaf area in *Arabidopsis thaliana* plants after exposure to UV, drought and especially a combination of UV and drought. UV and drought exposures have also been associated with an increase in antioxidants, and metabolites such as flavonoids, terpenoids and carotenoids (Jansen et al., 2022). In particular, an increased accumulation of the amino acid proline was reported, and this is associated with increases in plant stress resistance mediated through enhanced oxidative defence and plant signalling (Hayat et al., 2012). Proline metabolism under stress conditions is, in part, dependent on the hormone abscisic acid (ABA) (Bhaskara et al., 2015). Indeed, it is noted that both UV and drought interact with ABA metabolism, increasing ABA accumulation as part of a stress-response strategy (Vanhaelewyn et al., 2016). An increase in ABA also mediates stomatal closure, decreasing transpiration under drought conditions (Lim et al., 2015). The combination of different stressors may lead to the activation of multiple stress response-related pathways the effects of which may be additive, synergistic or

antagonistic (Kovács et al., 2014; Jansen et al., 2022). A review of the relevant literature summarised that the simultaneous exposure of plants to drought and UV generates an acclimatory response that is not quite cumulative, while simultaneously negative stress effects are also less than cumulative (Jansen et al., 2022). For example, Cechin et al. (2008) found that stomatal conductance was reduced both under UV and drought, although the reduction in plants exposed to combinations of the two stressors was not synergistic.

A special form of interaction between two stress-responses is when exposure to one stressor precedes that of a second stressor. It has been found that a UV pre-treatment can alleviate the effects caused by drought and *vice versa*, indicating a cross-resistance between the two factors (Comont et al., 2012). UV-induced priming can potentially have a wide range of applications within the agricultural sector and can help plants harden and enhance stress resistance (Dhanya Thomas et al., 2020). This specifically applies to plants pre-cultured in protected environments such as glasshouses. For example, Wargent et al. (2011) showed that a pre-treatment with UV-B during the glasshouse growing stage of lettuce seedlings resulted in higher-yielding plants under field conditions. It was hypothesised that UV pre-treatment reduced the transplantation stress experienced when plants are transferred to outdoor conditions. An extreme example of a protected environment is *in vitro* conditions, whereby plants are raised under a high relative humidity, constant temperature, and an aseptic environment (Teixeira da Silva et al., 2017). Upon transfer of *in vitro* plants to an *ex vitro* environment, plants are vulnerable to drastic changes in conditions, potentially affecting fitness and even survival (Kadleček et al., 2001). In this study, it was explored whether UV exposure can be used as a tool to prime plants grown under highly protected culture conditions, to induce a degree of stress resistance. It was

hypothesised that priming mint plantlets (*Mentha spicata* L.) with a low dose of UV irradiance can alleviate the drought effect caused by a change in humidity upon transplanting. It was also hypothesised that the increase in plant resistance would be accompanied by measurable changes in traits associated with plant defences, including stomatal opening, leaf area, and root development.

3.2 Materials and Methods

3.2.1 Plant material and germination stage

Mint (*Mentha spicata* L.) seeds were commercially sourced (Moles Seeds Ltd., Stanway, U.K.), and sterilised as described in Crestani et al. (2023). Briefly, approximately 20 seeds per box were sown on half-strength Murashige and Skoog medium without sucrose (MS) and grown for 30 days (from sowing day) in plastic boxes (RA40 plastic micro boxes, Sac $0_{2,}$ Deize, Belgium). Germination was noticed around 14 days after sowing. Seedlings were grown under ~180 μmol $m^{-2}s^{-1}$PAR provided by an LED lamp (AP673L, Valoya, Finland), the output of which was measured with a PAR meter (PAR special sensor, Skye Instrument Ltd, Powys, United Kingdom). The day/night regime was 14 h light (7:00 to 21:00) and 10 h dark, at a temperature of 20 ± 2 °C and relative humidity (RH) of ~98% inside the tissue culture boxes. Relative humidity and temperature were measured with a portable data logger every 10 minutes (EL-USB-2, Lascar Electronics, Whiteparish, United Kingdom). At least six plastic boxes per treatment (Mylar or CA) were used for each individual replicate. A minimum of six plants were analysed from each treatment, for each stage (after UV or after transplanting). Results were obtained as the average of three independent replicates for the necrose and stomata analysis and humidity experiment, while proline, antioxidant capacity and chlorophyll *a* fluorescence were obtained from five independent biological replicates.

3.2.2 UV–exposure stage

After 30 days from germination , seedlings were exposed to a UV treatment for eight days. Prior to UV treatment, the plastic containers were wrapped in either a UV-B blocking filter, Mylar (125 µm thickness, Polyester film, Tocana Ltd., Ballymount, Ireland) or a UV-B transmitting filter, Cellulose Acetate (CA) (95µm thickness; Kunststoff-Folien-Vertrieb GmbH, Hamburg, Germany). Similarly, the lids of containers were replaced by either a Mylar or CA cover. Filters were swabbed with 70% ethanol and replaced after 20 hours of UV exposure (Figure 3.1). UV-B radiation was provided by fluorescence tubes (TL40W/12 Philips, Germany) wrapped with a single layer of CA to block UV-C radiation. Plants were exposed to UV for 4 h at mid-day (12:00 to 16:00). Under the CA filter, plants were exposed to ~0.5886 W m^{-2} total UV (comprised of 0.3661 W m^{-2} UV-A and ~0.2225 W m^{-2} UV-B) and this treatment is labelled as "+UV". Under the Mylar filter, plants were exposed to 0.2498 W m^{-2} total UV (comprised of 0.2464 W m^{-2} UV-A and 0.004 W m^{-2} UV-B) and this treatment is labelled as "- UV". The daily biologically effective UV dose was equivalent to 2.7937 kJ m^{-2} (0.2830 kJ m^{-2} for UV-A and 2.5258 kJ m^{-2} for UV-B) under CA and 0.3250 kJ m^{-2} (0.1797 kJ m^{-2} for UV-A and 0.1453 kJ m^{-2} for UV-B) under Mylar (Flint & Caldwell, 2003). Plants were exposed to ~180 µmol m^{-2} s^{-1} PAR as detailed for the seed germination stage. PAR was supplemented by far-red LEDs tubes (L18C, Valoya, Finland) to achieve a red: far-red (R: FR) ratio of 1.6. The R: FR ratio was calculated as: photon irradiance between 655 and 665 nm / photon irradiance between 725 and 735 nm (Franklin & Whitelam, 2005).

Both the UV irradiance and the R: FR ratio were mapped using an optical fibre spectroradiometer Flame-S equipped with a cosine corrector (Ocean Optics, Duiven, The Netherlands) using the manufacturer's software, Oceanview (version 1.6.7).

All other growth room conditions were as specified for the seed germination stage (Figure 3.1).

3.2.3 Recovery stage

Six plants per box were sampled immediately after eight days of UV exposure while the other six were transplanted on soil and monitored for further seven days (Figure 3.1). The latter plants were transplanted on John Innes II compost (William Sinclair Horticulture Ltd., Lincoln, United Kingdom) with one plant per pot (7 x 7 x 8 cm, Walz, Effeltrich, Germany), and grown under the same conditions as reported for the germination stage but under the growth room relative humidity of ~60%. Potted plants were watered once during the recovery stage. Seven days after transplanting to soil, plants were harvested for analysis (Figure 3.1).

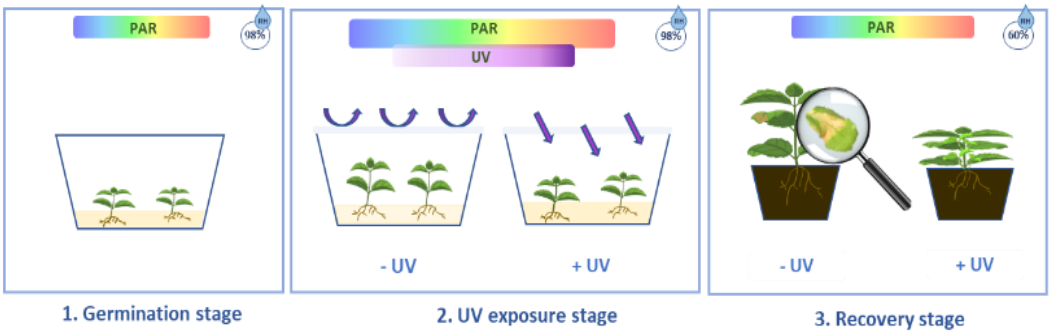


Figure 3.1. *Plants were grown in tissue culture boxes for 30 days under PAR light (germination stage). Then, half of the plants were covered with UV-blocking filter and half with UV-transmitting filter and exposed for eight days to UV and PAR (UV-exposure stage). Finally, plants were transferred to soil for further seven days (Recovery stage). Relative humidity inside the tissue culture boxes was around 98% while the relative humidity recorded during the recovery stage was around 60%.*

3.2.4 Necrotic areas

Leaf necrotic spots were noted on some plants at the end of the recovery stage. Necrotic areas (NA) were quantified from photographs of leaves using the software

ImageJ (version 1.52a) (Wayne Rasband, National Institute of Health, United States). A necrotic spot was any dry and brownish area present on the adaxial leaf surface. The total leaf surface area covered by necrotic spots was quantified, and this was expressed as a ratio between necrotic area and total leaf area (NA/LA). For all measurements, leaf one (L1) is defined as the oldest leaf and leaf six (L6) is defined as the youngest leaf. To assess the effect of the relative humidity on the formation of necrotic spots, plants were exposed to different humidities during the recovery stage. After being exposed to UV, both non-UV and UV-exposed plants were transplanted on soil and randomly divided into three groups, for exposure to low (~40%), normal (~60%), and high humidity (~90%). Plants were placed in seed propagator trays for the whole duration of the experiment. In order to define the humidity level and to monitor the humidity over time, a portable data logger was used.

Low humidity was obtained using silica pearls (drying pearl orange, Sigma Aldrich) that were spread on the bottom of a closed seed propagator tray and replaced every 48 h. High humidity was obtained by connecting the plant tray to a humidifier filled with distilled water. The extent of necrotic areas was recorded at the end of the recovery stage for three independent replicates, each comprising six plants from each UV treatment. The evaluation was carried at the end of the recovery stage, on six plants per humidity level, and pre-treated or not with UV. There were three independent replicates.

3.2.5 Proline content

Proline was extracted according to Alaiz et al. (1992) with some modifications. A total of 100 mg of leaves was obtained by pooling together material from different plants and from different boxes, previously frozen in liquid nitrogen. Leaves were incubated

for 24 h with 1 mL of 6 M HCl at 110 °C. Samples were filtered using 0.22 µm filters (Millipore, Bedford, USA). Extract (200 µL) was taken and evaporated under vacuum at 2000 rpm at 60 °C. Pellets were resuspended in 1 mL of 1 M borate buffer, and afterwards samples and standards were incubated with 2 µL of N,N′-Dimethylethylenediamine (DEEM) at 50 °C for 50 minutes. After incubation, 200 µL of the samples was transferred into HPLC vials. A calibration curve was constructed using different dilutions of a standard amino acid mixture (Merck Life Science Limited, Arklow, Ireland) at different concentrations, namely: 0.0125, 0.025, 0.0437, 0.625, 0.125, 0.1875 µM. The HPLC used was Agilent 1290 Infinity II LC (Agilent Technologies, Santa Clara, CA, USA) equipped with a column InfinityLab Poroshel 120 EC – C18, 2.1 x 50 mm, 1.9 µm (Agilent Technologies, Santa Clara, CA, USA), detector DAD UV 280 nm, autosampler temperature 10 °C, column compartment temperature 18°C and 0.613 mL min^{-1} flow rate. The gradient program uses acetonitrile (solvent A), and sodium acetate 25 mM (solvent B) as reported in table 3.1 below.

Table 3.1. *HPLC operating conditions used for the extraction of proline including the time (min), the gradient of solvent A (%) and solvent B (%) and the flow (mL/min).*

Time [min]	A [%]	B [%]	Flow [mL/min]
0.30	12.00	88.00	0.613
0.60	14.00	86.00	0.613
1.30	14.00	86.00	0.613
2.20	21.00	79.00	0.613
3.50	31.00	69.00	0.613
5.10	8.00	92.00	0.613

Proline content was quantified after the UV exposure stage and again at the end of the recovery stage. Measurements were obtained from five independent experiments. Proline content is reported in µg g^{-1} of fresh weight (FW).

3.2.6 Stomata

To quantify stomatal density and stomatal pore aperture on adaxial and abaxial surfaces, 12 leaves were picked randomly from amongst the first six (i.e., older) leaves of separate plants, collected from each UV treatment, either immediately after UV-exposure or after transplanting to soil. Additionally, stomatal density and pore aperture were also measured on leaves of plants before UV exposure. Six leaves were used to study the adaxial and abaxial leaf surface, respectively. For each leaf, measurements were taken at two locations, one near the leaf base and one near the leaf tip. Stomatal impressions were prepared following (Wu & Zhao, 2017). Leaves were detached from the plants and immediately painted with clear nail varnish. When dry, the leaf imprint was transferred on to a microscope slide, using a piece of clear tape. Stomata were identified and apertures were quantified using a Leica DM500 microscope (Leica Microsystems, Wetzlar, Germany) integrated with a camera with an objective magnification of 20X and total magnification 200X. Stomatal density and aperture were quantified in three different areas on each impression, each of which covered an area of 509 µm x 457 µm. The images were saved using Leica DM500 software (Leica Microsystems, Wetzlar, Germany) and then processed using ImageJ software (version 1.52a) (Wayne Rasband, National Institute of Health, United States). Stomatal opening was calculated as the distance between the two adjacent guard cells (width) (Jansen & Van Den Noort, 2000). A total of three independent replicates were carried out for each stage. The presence of some round microstructures between the guard

cells in figure 3.7 represents an artefact of the tape used for making the epidermal impression.

3.2.7 Antioxidant capacity

3.2.7.1 Extraction of plant material

Mint leaves were pooled together from different plants and from different boxes (25 mg of fresh weight), frozen in liquid nitrogen. Plant material was extracted using 300 µL of 70% ethanol, sonicated for 15 minutes in ice and centrifuged for 10 minutes at 3000 rpm. The pellet was resuspended 3 times and the supernatants were combined to give a final volume of 900 µL. The extract was used for Folin - Ciocalteau, ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays following Csepregi et al. (2017) with some modifications. The extract was also used for the UV-absorbing pigments following Csepregi & Hideg (2018) with some modifications. Antioxidants and pigments were measured after the UV exposure stage as well as after the recovery stage on five independent experiments.

3.2.7.2 Folin–Ciocâlteu assay

An aliquot of 100 µL of ethanolic extract was mixed with 500 µL Folin- Ciocâlteu reagent (1:10 dilution in distilled water). After 5 minutes at room temperature, 500 µL of Na_2CO_3 6% (w/v) was added. After incubation for 1.5 hours in the darkness, the antioxidant capacity was determined by measuring the absorbance at 765 nm using a UV - VIS spectrophotometer (Shimadzu UV - 160 A, Kyoto Japan). A calibration curve was made using gallic acid as the standard and using different concentrations: 0.01, 0.05, 0.1, 0.25, 0.5 and 1 mg/mL. The results were reported in gallic acid equivalents.

3.2.7.3 Ferric-reducing antioxidant power (FRAP)

Ethanolic extract (50 µL) was mixed with 950 µL of FRAP solution and incubated for 30 minutes, mixing every 10 minutes, before measuring the absorbance with a UV - VIS spectrophotometer at 593 nm. The FRAP solution was obtained by mixing 12.5 mL of acetate buffer 300 mM (pH 3.6) with 1.25 mL of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution 10 mM in HCl 40 mM and 1.25 mL of $FeCl_3$ 20 mM in distilled water. A calibration curve was made using different concentrations of ascorbic acid as the standard: 0.01, 0.05, 0.1, 0.25, 0.5 and 1 mg/mL. The results were reported as ascorbic acid equivalents.

3.2.7.4 Trolox equivalent antioxidant capacity (TEAC)

2,2'Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical ($ABTS^{\bullet+}$) solution was prepared by mixing 9.7 mL of 50 mM phosphate buffer (pH 6), 100 µL of 10 mM ABTS in 70% ethanol, 100 µL of 100 mM H_2O_2 in distilled water and 100 µL of 1.25 µM horseradish peroxidase in the phosphate buffer. After 15 minutes, 950 µL of $ABTS^{\bullet+}$ solution was mixed with 50 µL of the ethanolic extract and the absorbance was measured immediately at 730 nm using a spectrophotometer. A calibration curve was made using different concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard: 0.00375, 0.0115, 0.0262, 0.0525, 0.105 mg/mL. The results were reported as Trolox equivalents.

3.2.7.5 Total UV-absorbing compounds

A volume of 300 µL of ethanolic extract was mixed with 1.5 mL of acidified ethanol (EtOH: H_2O: HCl, 70:29:1) in a quartz cuvette. The absorbance was recorded in the UV range (280 nm – 400 nm) with a spectrophotometer. The results were integrated

across the whole wavelength range and the total UV absorbing compound concentration was calculated. Alternatively, absorbance was separately integrated across the UV-A (315 nm – 400 nm) or UV-B (280 nm – 315 nm) wavelength range. A calibration curve was made using different concentrations of quercetin as standard: 19.85, 39.70, 59.56, 79.41 and 99.26 µM.

3.2.8 Chlorophyll *a* fluorescence analysis

Analysis of photosynthetic efficiency was performed using an Imaging-PAM Chlorophyll fluorometer with ImagingWin 2.47 software (Walz, Effeltrich, Germany). All samples were dark-adapted for 20 minutes, prior to the measurement of Fv/Fm. To measure the steady-state yield of photosystem II, and the quenching parameters, a background of 185 µmol m^{-2} s^{-1} of blue actinic light was applied for five minutes until a steady state was reached. Using a saturating pulse, Y(II) (Maxwell & Johnson, 2000), Y(NPQ), and Y(NO) were then calculated (Klughammer & Schreiber, 2008). Measurements were taken randomly on six healthy plants for each treatment from five independent experiments. For the plants that present necrotic areas, measurements were measured on the remaining, healthy section of the leaves. Chlorophyll *a* fluorescence measurements were performed on plants prior to UV exposure, at the end of eight days of UV exposure, and at the end of the recovery stage.

3.2.9 Morphological parameters

Morphological parameters, including total leaf area, specific leaf area (SLA), fresh and dry root weight, and the length of the primary and secondary roots were measured as described in Crestani et al. (2023). In essence, the total leaf area was quantified using ImageJ software (version 1.52a) (Wayne Rasband, National Institute of Health, United States) across the oldest six leaves. SLA was calculated as a ratio between leaf

area and leaf dry weight for the same leaves used to measure the leaf area. Root dry weight was measured after drying cleaned roots for five days at 60 °C. The length of the primary and secondary roots was calculated using SmartRoot, an extension of ImageJ. In addition, the root/leaf ratio was calculated as a ratio between root dry weight divided by the total leaf dry weight. All the morphological parameters were measured before UV exposure, at the end of the UV-exposure stage and at the end of the recovery stage. Total leaf area and root/leaf ratio data comprised the main stem leaves, although it was noted that small leaves had formed on branches of UV-exposed plants (see Crestani et al., 2023) at the end of the recovery stage. All the morphological parameters were obtained from six plants after each stage, independently replicated three times, with the exception of total leaf area and SLA which were obtained from five independent replicates.

3.2.10 Statistical analysis

Two tails t-tests, or the correspondent nonparametric Mann-Whitney test, were used to establish significant differences between non-UV treated and UV-treated plants All statistical analyses were performed using the software IBM SPSS Statistic v28 (Armonk, New York, US).

3.3 Results

M. spicata plants were grown in closed containers covered with either UV-blocking (Mylar) or UV-transmitting (CA) filters. After eight days of UV exposure, plants were transplanted to soil and monitored for a further seven days. Proline content, stomatal density and stomatal aperture, total antioxidant capacity, UV absorbing pigments, the efficiency of PSII and some morphological parameters were measured after the UV

exposure stage and again after the recovery stage while necrotic areas were measured only after the recovery stage.

3.3.1 Necrotic areas

Necrotic spots occurred only after the recovery period and only in non-UV exposed plants (Figure 3.2 A). No necrotic spots were noted on plants that received a UV treatment (Figure 3.2 B). In contrast, 52% of non-UV exposed plants presented one or more necrotic areas. In total some 75 necrotic areas were identified across 75 leaves.



Figure 3.2. *After the recovery stage, non-UV exposed plants show necroses on leaf surfaces (**A**). A comparison of non-UV exposed leaves (left) with UV exposed leaves, which do not present necrotic areas (**B**).*

These necrotic areas were found mainly on leaves five and six (L5 – L6; 54 necrotic spots), followed by leaves seven and eight (L7 – L8; 14 necrotic spots) and leaves three and four (L3 – L4; six necrotic spots) and leaves nine and ten (L9 – L10; two necrotic spots) (Figure 3.3 A). The average necrotic area was respectively 0.149 cm^2 on L3 – L4, 0.074 cm^2 on L5 – L6, 0.107 cm^2 on L7 – L8, and 0.039 cm^2 on L9 - L10 (Figure 3.3 B). The percentage of leaf surface damaged by necroses was calculated, and constituted 38.6% on L3 – L4, 19.6% on L5 – L6, 27.3% on L7 – L8 and 6.2% on L9 – L10 (Figure 3.3 C).

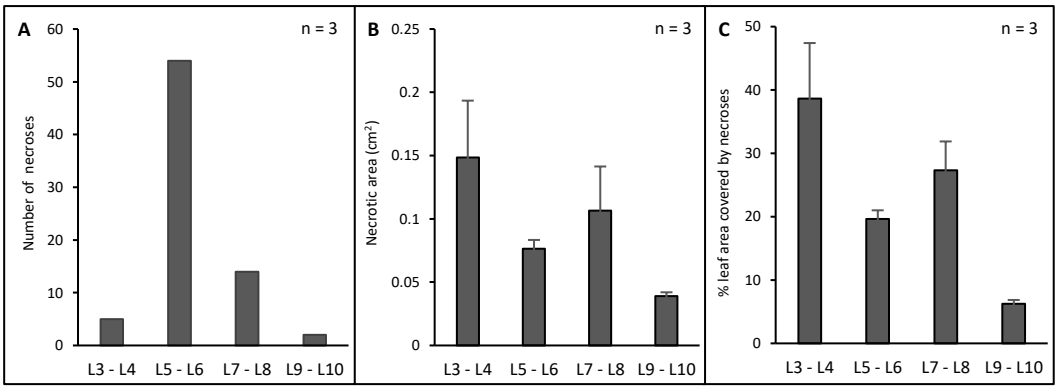


Figure 3.3. *At the end of the recovery stage, seven days after transplanting, necrotic areas were identified on non-UV exposed plants. Parameters measured were number of necroses across the leaves* ***(A)****, the dimension of the necrotic area (cm²)* ***(B)*** *and the % of the leaf area covered by necroses (****C****). Before UV exposure plants did not show any necroses****.*** *Also, UV-primed plants displayed no necroses.*

To assess if the formation of necrotic spots was related to a change in the humidity, plants were exposed to either 40% or 90% relative humidity (RH) during the recovery stage. A considerable number of damaged leaves were detected in plants exposed to 40% relative humidity. In comparison, the number of leaves with necroses was much higher on leaves from plants not exposed to UV at 40% humidity when compared to 90% humidity ($p = 0.029$). No necrotic spots were detected to plants pre-treated with UV (Figure 3.4).

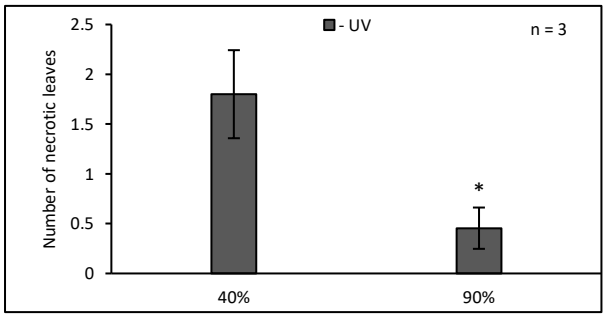


Figure 3.4. *During the recovery stage, plants that did not receive UV (grey bar) were exposed to low (40%) and high (90%) humidity. After seven days the mean number of necrotic leaves per plant was recorded. No necrotic spots were present in the UV pre-treated plants. Asterisks indicate a significant difference between the UV treatments with* $p < 0.05$ *(*).*

3.3.2 Proline content

The proline concentration was significantly higher in non-UV exposed plants than in UV-exposed plants after eight days of UV exposure (p = 0.008). A comparison after seven days recovery on soil showed that the proline concentration increased in previously UV-exposed plants, while it decreased in non-UV exposed plants (Figure 3.5).

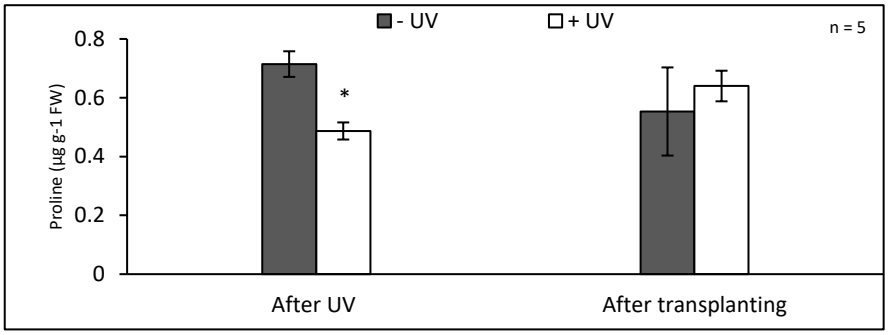


Figure 3.5. *Proline content (µg g^{-1} FW) was measured immediately after the eight days UV exposure phase, or seven days after transplanting. White bars represent UV-primed samples. Grey bars represent non-UV controls. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*). Fold change between after UV and after transplanting is reported in Supplementary Table 3.1.*

3.3.3 Stomata

The stomatal density on the abaxial surface is not affected by UV, as measured either immediately after UV exposure or after recovery (Figure 3.6 A, 3.7 A & 3.7 B). Immediately after UV exposure, the stomatal density on the adaxial leaf surface was slightly, but not significantly, increased in UV-exposed plants. After the recovery stage, the stomatal density on the adaxial leaf surface of plants that had received UV was significantly increased, compared to non-UV exposed plants ($p < 0.001$) (Figure 3.6 B, 3.7 C & 3.7 D).

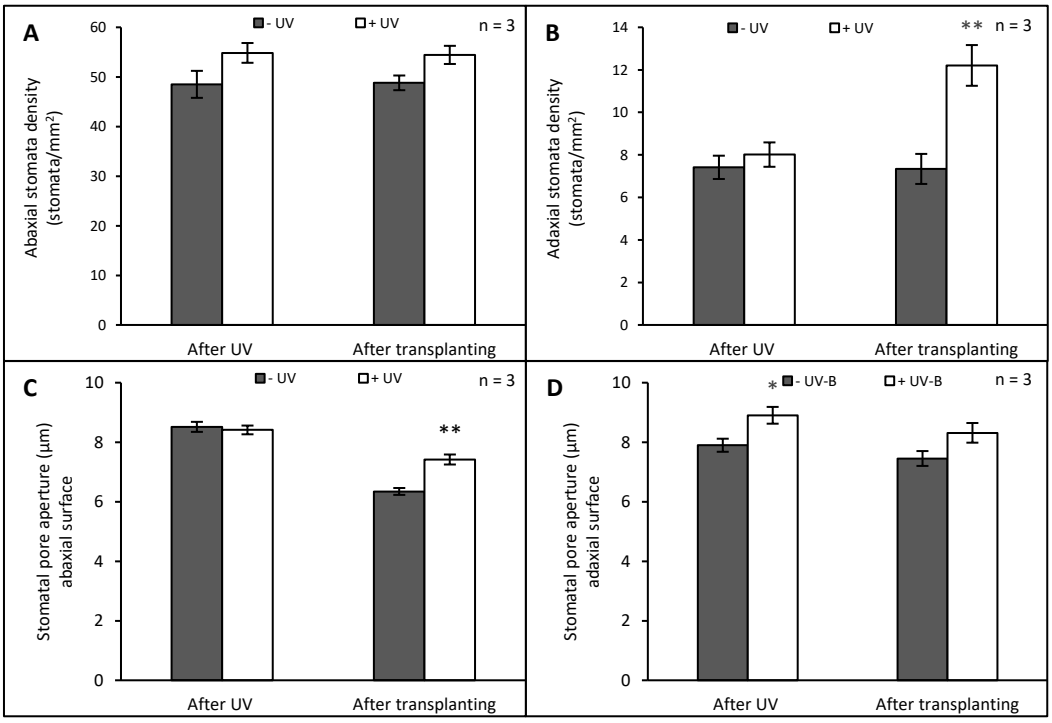


Figure 3.6. *The stomatal density (stomata/mm^2) on the abaxial (**A**) and adaxial surfaces (**B**), the stomatal pore aperture on the abaxial (**C**) and the adaxial surfaces (width) (**D**). Parameters were measured after the eight days of UV exposure phase, and seven days after transplanting. Before the UV exposure stage, the mean of the stomata density per mm^2 was 43.87 on the abaxial surface and 7.63 on the adaxial surface, while the mean of the stomata pore aperture was 3.02 µm (width) on the abaxial surface and 2.95 µm (width) on the adaxial surface. White bars represent UV-primed samples while grey bars represent non-UV controls. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 3.1.*

On the abaxial surface, the stomatal aperture was not immediately affected by UV treatment, but a significant difference was recorded after the recovery stage ($p < 0.001$). Stomata were more open in the UV-treated plants (Figure 3.6 C). On the adaxial surface, the stomatal pore aperture was greater in UV-exposed plants, but this difference was statistically significant only directly after the UV-exposure stage and not after the recovery stage ($p = 0.006$) (Figure 3. 6 D).

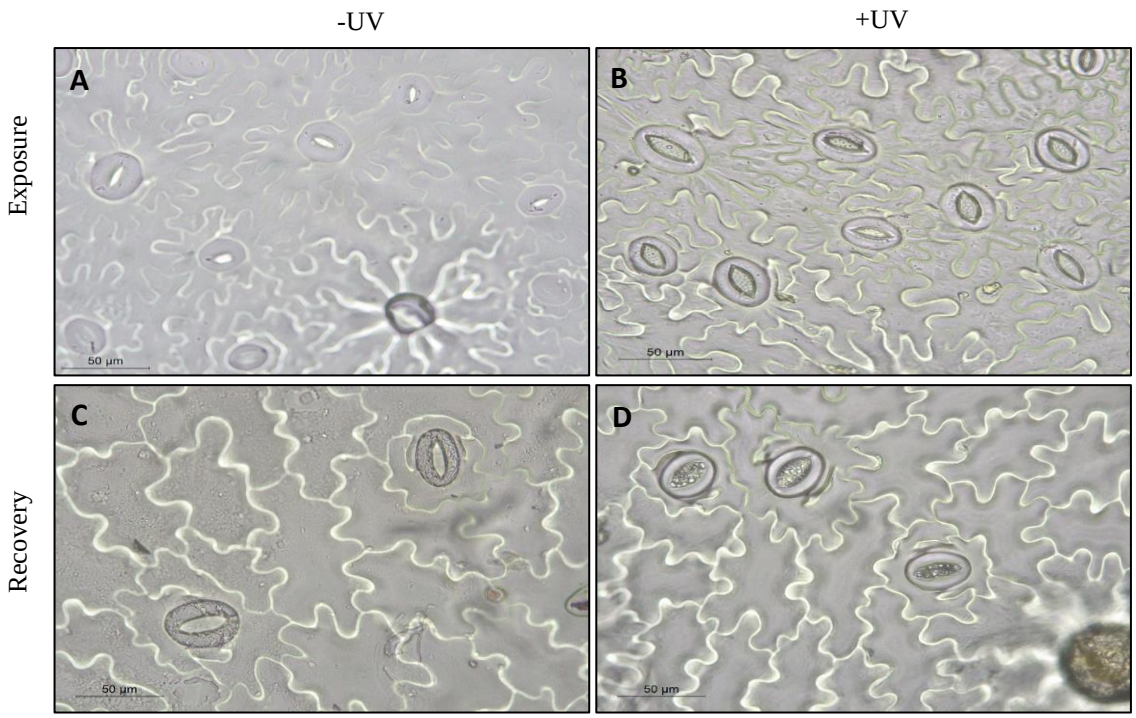


Figure 3.7. *Stomata impressions at a magnification of 400X were obtained from the abaxial surface of non-UV exposed control plants (**A**), the abaxial surface of UV-exposed plants (**B**), the adaxial surface of non-UV exposed plants (**C**), the adaxial surface of UV-exposed plants (**D**) analysed at the end of the recovery stage.*

3.3.4 Antioxidants

The concentration of total antioxidants measured with the Folin-Ciocâlteu assay increased in non-UV exposed plants over time. Immediately after UV exposure, UV has a positive effect on the total phenol concentration resulting in a significantly higher concentration in UV-treated plants compared with the non-UV exposed plants ($p < 0.001$). However, this effect was temporary. After one week recovery, the total phenol concentration in previously UV-exposed plants was significantly reduced while in non-UV exposed plants it significantly increased ($p < 0.001$) (Figure 3.8 A). A similar trend was also observed for TEAC. Immediately after the UV exposure stage, TEAC was significantly lower in non-UV exposed compared to UV-exposed plants ($p = 0.002$). During the recovery stage, TEAC increased in both UV treatments, but the

increases were far more marked in non-UV treated plants with a fold change of 1.35 times compared to 0.35 times of the +UV plants (Supplementary Table 3.1). As a result, TEAC was significantly higher in non-UV samples after the recovery stage ($p < 0.001$) (Figure 3.8 B). Similarly, immediately after UV exposure, ferric reducing antioxidant power is significantly higher in UV-exposed plants compared with the non-UV treated ones ($p < 0.001$). Following transfer to soil, FRAP values for non-UV exposed plants increased 1.75 times while +UV plants showed an increase of 0.12 times (Supplementary Table 3.1). Consequently, –UV plants have a significantly higher FRAP at the end of the recovery stage than UV-exposed plants ($p = 0.001$) (Figure 3.8 C).

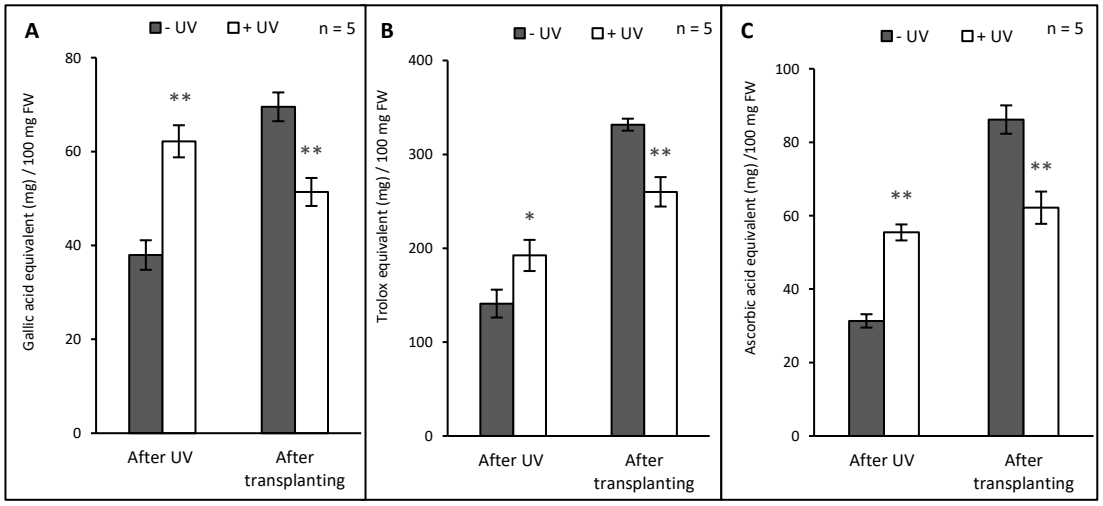


Figure 3.8*. Total antioxidant capacity was measured as total phenols (gallic acid equivalent (mg) / 100 mg FW)* ***(A)****, as TEAC (Trolox equivalent (mg) / 100 mg FW)* ***(B)*** *and as FRAP (ascorbic acid equivalent (mg) / 100 mg FW)* ***(C).*** *Measurements were made at the end of the UV-exposure phase, and again at the end of the recovery phase. White bars represent UV-primed samples. Grey bars represent non-UV controls. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 3.1.*

Analysis of total UV absorbing compounds revealed a significant increase in UV-exposed plants measured immediately after the UV exposure phase ($p = 0.019$). This

increase reflects the rise in UV-A and UV-B absorbing compounds but is significant only for UV-B screening pigments ($p < 0.001$). On the contrary, overall similar contents of total UV, UV-A, and UV-B absorbing pigments were recorded after the recovery stage (Figure 3.9).

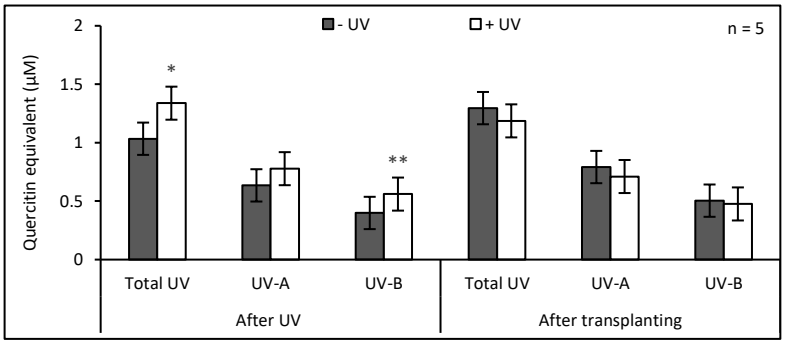


Figure 3.9. *Total UV, UV-A and UV-B absorbing compounds were measured immediately after the UV exposure phase, and seven days after transplanting. White bars represent UV-primed samples. Grey bars represent non-UV controls. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**). Fold change between after UV and after transplanting is reported in Supplementary Table 3.1.*

3.3.5 Chlorophyll *a* fluorescence

To compare the impacts of UV on photosynthetic efficiency, chlorophyll *a* fluorescence was analysed immediately after UV exposure and seven days after transplanting on soil. The maximum quantum yield of PSII, Fv/Fm, (Figure 3.10 A) was not affected by UV either, immediately after UV-exposure nor after the recovery stage. Also, the effective quantum of PSII, Y(II) showed no change between the two UV treatments at the end of the UV-exposure stage, but a significant reduction in Y(II) was noted in UV-exposed plants at the end of the recovery stage ($p = 0.01$) (Figure 3.10 B).

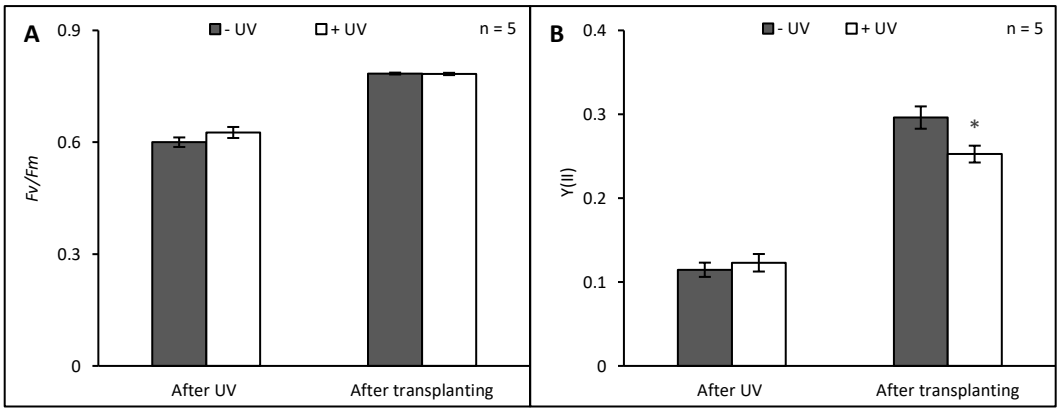


Figure 3.10. *The maximum quantum yield of PSII, Fv/Fm (**A**) and the effective quantum of PSII, Y(II) (**B**) were measured immediately after the UV exposure phase and seven days after transplanting. Before the UV exposure stage, the mean of Fv/Fm was 0.698 while the mean of Y(II) was 0.233. White bars represent UV-primed samples. Grey bars represent non-UV controls. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*). Fold change between after UV and after transplanting is reported in Supplementary Table 3.1.*

The energy dissipated through regulated, non-photochemical quenching, given by Y(NPQ) (Figure 3.11 A), showed no significant differences between UV treatments immediately after UV exposure. However, there was a significantly higher Y(NPQ) in UV-exposed plants after transplanting ($p < 0.001$). At the same time, the quantum yield of non-regulated, non-photochemical energy dissipation in PSII, Y(NO) (Figure 3.11 B), was not significantly affected by UV at either stage.

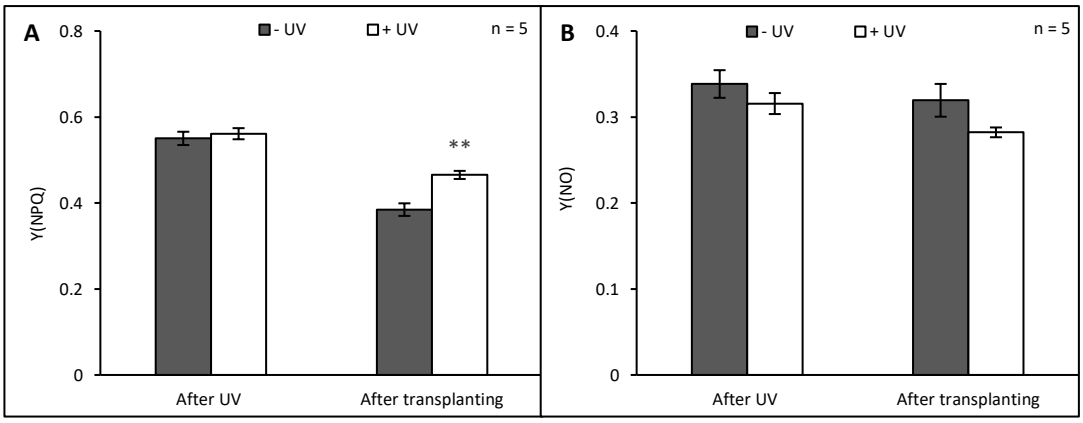


Figure 3.11. *The energy dissipated through regulated non-photochemical quenching, Y(NPQ) (**A**), the quantum yield of non-regulated, non-photochemical energy dissipation Y(NO) (**B**) were measured both immediately after the UV exposure phase and seven days after transplanting. Before the UV exposure stage, the mean value of Y(NPQ) was 0.478 while the mean value of Y(NO) was 0.287. White bars represent UV-primed samples. Grey bars represent non-UV controls. Asterisks indicate a significant difference between the UV treatments with* $p \leq 0.001$ *(**). Fold change between after UV and after transplanting is reported in Supplementary Table 3.1.*

3.3.6 Morphological parameters

UV-treated plants displayed a significant reduction in total leaf area, both immediately after UV-exposure ($p < 0.001$) and again after the recovery stage ($p < 0.001$), when compared with non-UV exposed plants. A substantial drop in SLA was recorded seven days after transplanting, with considerably lower SLA values in UV-exposed plants compared to non-UV ones ($p = 0.001$). Fresh root weight was lower for UV-exposed plants after both experimental stages ($p = 0.031$ & $p < 0.001$). A non-significant difference in root dry weight was noted immediately after UV treatment, and this difference in root dry weight became significant seven days after transplanting to soil ($p = 0.003$) when the root biomass recorded for UV-exposed plants was lower than that for non-UV exposed plants. Primary and secondary roots were both significantly shorter in UV exposed plants in comparison with the non-UV exposed ones, after the UV- exposure stage ($p = 0.011$ & $p = 0.006$). Yet, no significant difference in primary

and secondary root length was measured after the recovery stage. Finally, no difference between UV-treatments was found for the root/leaf ratio, both immediately after UV treatment and after the recovery stage (Table 3.2).

***Table 3.2**. Morphological parameters across different UV treatments, sampled immediately after the UV exposure phase or seven days after transplanting on to soil. Parameters include the total leaf area (cm^2), SLA (cm^2 mg^{-1}), root fresh weight (mg), root dry weight (mg), primary roots length (cm), secondary roots length (cm) and root/leaf ratio. Before UV exposure, the mean of leaf area was 0.261 cm^2, the mean of SLA was 0.448 cm^2 mg^{-1}, the mean root fresh weight was 8.00 mg, the mean root dry weight was 0.54 mg, the mean length of primary roots was 2.47 cm, the mean length of secondary roots was 0.80 cm, and the mean root/leaf ratio was 0.184. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.001$ (**) while non-significant results were identified as n.s.. Fold change indicates the difference between plants sampled after the UV and plants sampled after transplanting both in –UV and +UV treatments.*

Parameter	*–UV after exposure*	*+UV after exposure*	*Significance*	*–UV after transplanting*	*+UV after transplanting*	*Significance*	*Fold change –UV*	*Fold change +UV*
Total leaf area (cm^2)	0.316 ± 0.01	0.250 ± 0.01	**	0.447 ± 0.04	0.270 ± 0.03 [1]	**	0.41	0.23
SLA (cm^2 mg^{-1})	0.51 ± 0.04	0.72 ± 0.12	n.s.	0.37 ± 0.02	0.29 ± 0.02	**	-0.26	-0.60
Root fresh weight (mg)	16.9 ± 1.46	12.2 ± 1.4	*	26.7 ± 1.63	16.33 ± 1.25	**	0.58	0.34
Roots dry weight (mg)	0.99 ± 0.09	0.77 ± 0.05	n.s.	2.41 ± 0.24	1.50 ± 0.17	*	1.42	0.94
Primary roots length (cm)	2.91 ± 0.24	2.25 ± 0.33	*	2.99 ± 0.55	2.97 ± 0.27	n.s.	0.03	0.32
Secondary roots length (cm)	0.81 ± 0.06	0.69 ± 0.19	*	1.16 ± 0.37	0.70 ± 0.12	n.s.	0.42	0.04
Root/leaf ratio	0.27 ± 0.05	0.23 ± 0.03	n.s.	0.29 ± 0.03	0.28 ± 0.05 [1]	n.s.	0.07	0.22

[1] Total leaf was measured only on the main stem leaves and not on the small leaves on newly emerging branches.

3.4 Discussion

In this study, it is shown that priming plants with UV enhances stress resistance. The data show that UV exposure generates more compact plants that cope better with the changes in environmental conditions as evidenced by variation in proline content, antioxidant capacity, stomatal opening and morphological changes.

3.4.1 UV pre-treatment elicits stress resistance

Plants raised under *in vitro* conditions, in the absence of supplemental UV exposure, developed large numbers of necrotic spots once transplanted into soil. These necrotic spots are likely to be associated with the dramatic change in environmental conditions when a plant is transferred from a closed *in vitro* growth container to soil-based cultivation in a growth room. *In vitro* conditions include a high relative humidity, low levels of CO_2 and a lack of wind exposure (Debergh et al., 1992). It was hypothesised that the dramatic decrease in RH, caused by transferring plantlets to growth-room conditions, is responsible for the development of abiotic stress in the *in vitro* raised plantlets, and the subsequent development of necrotic areas. Indeed, drought stress is often associated with formation of necrotic spots (Ribeyre et al., 2022). Moreover, by raising the RH to 90% necrosis was avoided. It was considered most likely that drought stress, due to an imbalance between water uptake and transpirational water loss, was the cause of necrosis. Remarkably, plants that had been raised under *in vitro* conditions, in the presence of supplemental UV, did not develop necrotic spots, once transferred to growth room conditions, not even under a low RH. These data are in agreement with a recent meta-analysis revealing a degree of cross-resistance between UV and drought (Jansen et al., 2022). Thus, based on published literature and the data presented in this thesis, it was hypothesised that UV could induce drought resistance and this possibility would merit further research. Here, it is shown that UV-priming

induces an array of acclimatory responses including morphological alterations, changes in stomatal density and aperture, changed proline content, and an elevated content of antioxidants and UV-absorbing pigments which may potentially contribute to drought resistance.

3.4.1.1 UV-induced changes in plant morphology

This study reports UV-induced decreases in total leaf area, as well as root length and weight. The results support previous findings wherein total leaf area decreased across different plant species including *Arabidopsis* (Hectors et al., 2012), cucumber (Qian et al., 2021), and basil (Dou et al., 2019). It has been theorised that these changes in leaf area are part of a defence response to minimize UV exposure and protect plants from UV stress (Robson et al., 2015A), however, this has not been proven. Conversely, a decrease in leaf area is an effective response to limit transpirational water loss and increase drought resistance (Xu & Zhou, 2008). Thus, UV-induced changes in leaf area might be considered a key player in increasing the putative drought resistance reported in this study.

Interestingly, it was noted that UV negatively affects plant root systems with a decrease in both primary and secondary root length. This observation is consistent with earlier works. For example, UV inhibits root growth in UV-sensitive *Arabidopsis* mutants (*rus-1* and *rus-2*), (Tong et al., 2008; Leasure et al., 2009) a response that is mediated by the *RUS1* and *RUS2* genes (Yang et al., 2020). It is likely that this effect is linked to the measured auxin concentrations in UV-exposed plants (Chapter 2). Drought resistance is commonly associated with increased root development (Brunner et al., 2015) which in turn leads to an improved water uptake capacity. For example, Jansen et al. (2022) revealed a substantial increase in root: shoot ratio in plants exposed

to drought. Thus, the data reported in this study do not support a role for UV-induced root formation in the plant resistance against abiotic stress. This particular morphological response needs to be investigated in more detail, as it may relate to the growth of plants on agar, and therefore be the result of direct UV exposure to roots.

3.4.1.2 UV-induced changes in stomatal responses

Under ideal growth conditions, plants lose more than 99% of water through stomatal transpiration (Kane et al., 2020). Therefore, the question arises whether UV-induced changes in stomatal responses can explain the increase in putative drought resistance. Both the light spectrum and intensity are known to alter stomatal development and consequently their density as well as aperture (Gitz & Liu-Gitz, 2003). Previous studies have reported that plants exposed to UV show a reduction in stomatal density. For example, Gitz III et al. (2013) found a significant reduction of stomatal density in soybean, while Poulson et al. (2002) reported a decrease in stomatal density in Douglas fir after exposure to ambient UV-B radiation. Conversely, Golob et al. (2018) reported both UV-induced decreases as well as increases in stomatal density, depending on plant nutrition. In this study, both adaxial and abaxial stomatal density remained unchanged, despite UV exposure, and therefore it is concluded that this parameter does not explain any potential increase in drought resistance.

UV also modulates the stomatal aperture directly (Jansen & Van Den Noort, 2000). For example, Derebe et al. (2019) reported substantial decreases in stomatal conductance in taro cultivars grown at different altitudes in Ethiopia. Indeed, many studies report that UV-B causes stomatal closure (He et al., 2011; Tossi et al., 2014; Li et al., 2017). On the contrary, this study shows a slight UV-mediated increase in the stomatal aperture. Jansen & Van Den Noort, (2000) demonstrated that the UV

effect on stomatal opening in *Vicia faba* plants depends on the metabolic state, with UV strengthening the background stomatal response. Consistent with this finding, it was observed in this study that UV stimulates further stomatal opening under high humidity, and low CO_2 conditions as found in *in vitro* containers. The data on enhanced stomatal apertures do, however, not explain the observed increase in drought resistance in mint plants.

3.4.1.3 UV-induced changes in cellular defences

Environmental stressors, such as UV-B and drought, can initiate signalling cascades that can induce biosynthesis of various stress-defence metabolites, including proline (Liang et al., 2013), antioxidants (Sharma & Dubey, 2005; Csepregi et al., 2017) and UV-screening pigments (Feng et al., 2007). Proline is an osmolyte that plants accumulate when exposed to environmental stressors such as salinity, high temperatures, drought and UV (Hayat et al., 2012; Jansen et al., 2022). Saradhi et al. (1995) noted a time-dependent increase of proline across different plant species such as rice, mung bean and mustard during UV exposure. It was assumed that this increase indicated the involvement of proline in defence response against UV-generated radicals. Similarly, Salama et al. (2011) found that UV increases the proline content of different varieties of desert plants such as *Plantago major, Malva parviflora, Rumex vesicarius, Sismbrium erysimoids* compared to the control. Although proline accumulation is commonly associated with stress defence, our data demonstrate that UV causes a small decrease in proline content, thus implying it is not responsible for any stress resistance in mint.

The antioxidant capacity of a plant cell can help protect against oxidative damage caused by a wide range of stressors (Gill & Tuteja, 2010). Here, it is shown that UV

exposure results in substantial increases in antioxidant activity measured as gallic acid, Trolox and ascorbate equivalents. In parallel, a strong increase in UV-screening capacity is measured. Induction of phenolic and flavonoid biosynthesis in UV-exposed plants may be regulated through the UV-B photoreceptor UVR8 (Demkura & Ballaré, 2012). The two acclimation responses are likely to be closely related to phenolics and other flavonoids playing a key role in increasing cellular antioxidant activities (Hideg et al., 2013). There is strong evidence that flavonoids and antioxidants also play a central role in protection against drought, both by preventing oxidative damage and through regulatory redox signalling (Laxa et al., 2019). Thus, it is concluded that the upregulated antioxidant defences in UV-exposed mint potentially contribute to improved stress resistance of the plants.

3.4.2 The role of UV in stress -related parameters after a period of recovery

A strong acclimation response could be observed in both UV-primed and control plants during the recovery phase. Common acclimatory responses to drought are morpho-physiological re-adjustments including root expansion, stomatal closure, and accumulation of compatible solutes such as proline (Bandurska et al., 2013). The data show an increased stomatal closure in non-UV exposed plants. This is most likely associated with drought stress, as stomatal closure is typically the first response of plants that experience drought (Laxa et al., 2019). A previous study showed increases in ABA in control plants during the recovery phase (Crestani et al., 2023), and that is consistent with the observed stomatal closure (Lim et al., 2015).

Apart from minimising stomatal transpiration, both UV-primed and non-UV primed plants increase their water uptake capacity through an increase in root biomass during the recovery stage. Secondary roots expand to increase the water uptake, thus

minimising potential drought effects on plant growth (Shoaib et al., 2022). The data in this paper show that plants are actively acclimating to the new, drier growth conditions.

In parallel, both UV-primed and control plants appear to minimise water loss by decreasing SLA. A reduction in leaf area versus leaf biomass is typically associated with drought acclimation (Wellstein et al., 2017). Interestingly, although UV-primed plants did not show macroscopic sign of drought stress (i.e., necrosis) a particularly strong decrease in SLA is noticed. It appears that UV-primed plants are more effective in acclimating to drier conditions compared to non-UV primed plants, suggesting that the substantial necrotic stress in the latter plants impeded acclimation. A likely explanation for the decrease in SLA is an increase in leaf thickness (Milla et al., 2008). Leaf thickness tends to be positively associated with stomatal density (Beerling & Kelly, 1996), presumably to facilitate adequate CO_2 supply with the increased mesophyll thickness. Consistently, in this paper, it was noted that the lower SLA in UV-primed plants is associated with a higher stomatal density and aperture. The lack of increase in stomatal density in non-UV exposed plants is likely associated both with a higher SLA and in parallel with drought stress that occurs in these plants.

Overall, photosynthetic efficiency increased in all plants during the recovery phase. Acosta-Motos et al. (2019) showed a progressive decrease in Y(NPQ) in *Stevia rebaudiana* over 28 days after the plants were moved to *ex vitro* conditions. This effect is likely to relate to an increase in CO_2 availability, resulting in a lower protective Y(NPQ) but a higher Y(II) as was also observed in this study. Further, it could be speculated that UV-primed plants, not showing visible signs of UV stress and with higher stomatal gas exchange, will display a higher photosynthetic activity compared to plants not primed with UV. This is not the case. UV-primed plants display a lower

Y(II) and a higher Y(NPQ) value, implying energy dissipation through the xanthophyll cycle (Thomson et al., 2003). Photosynthetic efficiency was measured in the non-necrotic regions of the leaf. Due to substantial necrotic leaf damage in control plants, there is less photosynthesising leaf area relative to root biomass in UV-primed plants. The imbalance will have increased the sink effect in plants not primed with UV. Such a sink effect is associated with a decrease in NPQ and an increase in overall photosynthetic efficiency (Thomson et al., 2003). Thus, a peculiar outcome of this study is that drought-stressed plants have a higher photosynthetic efficiency locally compared to UV-primed plants that display stress resistance. However, this is likely to be a short-term affect that will not affect overall long-term plant photosynthetic productivity, and agro-technological applications.

The most common plant stress response is an increase in plant antioxidant defences (Kasote et al., 2015). Consistent with transplanting stress, a strong upregulation in antioxidant defence capacity is noted in all plants. This re-emphasises the conclusion that plants were actively acclimating. However, a stronger upregulation is noted in non-UV primed plants, consistent with the increased stress experienced by these plants. Interestingly, the higher antioxidant activity in non-UV primed plants is not associated with increased accumulation of total flavonoids and other UV-absorbing pigments, nor with proline accumulation. Rather, it is speculated that plants displaying drought stress will have accumulated other antioxidants, for example, ascorbate, glutathione, and tocopherol or have changed their flavonoid profile (Szarka et al., 2012).

3.4.3 UV-priming as a driving tool for sustainable crop production

In the context of a changing climate and consequential shifting plant production systems, there is a pressing need to explore novel ways to produce high-quality, cost-effective, hardened crops and ornamental plants. Drought stress is one of the most limiting factors in agricultural productivity, with estimated annual crop losses of 34% in the least developed and low to middle income countries (FAO, 2021). In the current study, it has been theorised that UV induces drought resistance which can be beneficial in a horticultural setting. For instance, modern agriculture involves the transport of agricultural products worldwide and often involves unfavourable conditions including desiccating environments (e.g., airplanes, ferries, and trucks). Here, it is proposed that UV radiation can be used to produce hardened crops that can tolerate such sub-optimal growth conditions. UV induced drought resistance is associated with an increase in antioxidant capacity, as well as a decrease in leaf area. These responses are common amongst many crop species, and therefore it is speculated that priming plants with UV-B can be used in the production of many commercial crops as a means of enhancing resistance to negative environmental factors.

3.5 Supplementary information

3.5.1 Change between after UV and after recovery

Supplementary Table 3.1. *Fold change of protection-related parameters measured after the UV and after transplanting both in –UV and +UV treatments.*

Parameter	–UV After UV	–UV After transplanting	Fold change	+UV After UV	+UV After transplanting	Fold change
Proline (µg g^{-1} FW)	0.71	0.55	**-0.23**	0.49	0.64	**0.31**
Abaxial stomata density (stomata cm^{-2})	48.50	48.81	**0.01**	54.85	54.44	**-0.01**
Adaxial stomata density (stomata cm^{-2})	7.41	7.34	**-0.01**	8.01	12.21	**0.52**
Stomata pore aperture (µm) Adaxial surface	2.93	3.40	**0.16**	2.61	2.91	**0.12**
Stomata pore aperture (µm) Abaxial surface	2.85	3.13	**0.10**	2.72	2.79	**0.03**
Folin- Ciocâlteu	37.95	69.53	**0.83**	62.19	51.39	**-0.17**
TEAC	141.01	331.60	**1.35**	192.30	260.08	**0.35**
FRAP	31.33	86.17	**1.75**	55.43	62.17	**0.12**
UV-A absorbing pigments	0.01	0.01	**0.25**	0.01	0.01	**-0.09**
UV-B absorbing pigments	0.01	0.01	**0.26**	0.02	0.01	**-0.15**
Total UV absorbing pigments	1.03	1.30	**0.25**	1.34	1.19	**-0.11**
Fv/Fm	0.60	0.78	**0.31**	0.63	0.78	**0.25**
Y(II)	0.11	0.30	**1.58**	0.12	0.25	**1.05**
Y(NPQ)	0.55	0.38	**-0.30**	0.56	0.47	**-0.17**
Y(NO)	0.34	0.32	**-0.06**	0.32	0.28	**-0.11**

3.6 Reference

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Chapter 4

Unlocking the potential of UV radiation: enhancing production of secondary metabolites in *Mentha spicata* L. for improved crop value

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Abstract

In plants, metabolite profiles change in response to environmental conditions. These changes in secondary metabolites may contribute to plant protection against biotic and abiotic factors, including ultraviolet (UV) radiation. The objective of this study was to determine the association between UV-exposure and contents of commercially valuable secondary metabolic products, with an aim to improve the horticultural quality of the crop. Specifically, UV mediated changes in the content of amino acids, phenolics, flavonoids, monoterpenes and sesquiterpenes, carotenoids, tocopherols and phytosterols were quantified. *Mentha spicata* plantlets were grown in tissue culture boxes for 30 days and then exposed to ~0.5886 W m^{-2} UV for eight days. Metabolite contents were quantified either immediately after the final UV exposure, or after seven days 'recovery' under visible light only. Immediately after UV exposure, it was found that UV promotes the production of flavonoids over phenolic acids. Furthermore, the majority of monoterpenes and sesquiterpenes, constituents of valuable mint essential oil, were highly induced by UV treatment. In contrast, contents of other secondary metabolites including carotenoids and tocopherols did not increase as a consequence of UV exposure. A comparison between the plants sampled immediately after UV exposure and after seven days recovery shows that there was an overall increase in the content of carotenoids, mono and sesquiterpenes, phenolics and amino acids following recovery, while contents of sterols and tocopherols decreased. The ability of UV to alter the secondary metabolite profile, provides an exciting opportunity to enhance crop value, facilitating the development of improved products which have higher levels of industrially relevant essential oils while having added benefits of enhanced flavour, colour, bioactive content and, ultimately, human health-giving properties.

4.1 Introduction

Secondary metabolites are specialized compounds that, amongst others, fulfil important roles in the interactions of plants with the surrounding environment (Erb & Kliebenstein, 2020). In plants, secondary metabolite profiles change in response to environmental conditions, and these changes may contribute to the protection of plants against biotic and abiotic factors (e.g., drought, salinity, cold and UV) (Akula & Ravishankar, 2011; Pagare et al., 2015; Yang et al., 2018). Furthermore, secondary metabolites act as pollinator attractants, antioxidants and signalling molecules (Schreiner et al., 2012), thus, contributing to the regulation of plant growth and development (Zaynab et al., 2018; Pang et al., 2021). Secondary metabolites synthesised by plants comprise a wide range of structurally different molecules including phenolics, flavonoids, terpenoids, steroids, and nitrogen or sulphur-containing compounds such as alkaloids some of which are commercially valuable (Pott et al., 2019). Perhaps not surprisingly, given the dynamic character of secondary metabolite profiles, biosynthesis and accumulation of these compounds is tightly regulated by numerous environmental factors, including light intensity and spectral composition (Schreiner et al., 2017).

Plants detect light through a variety of wavelength-specific photoreceptors (Hirose et al., 2013) including phytochromes for red and far-red but also blue light (Thoma et al., 2020), cryptochromes and phototropins for blue/ UV-A radiation (315nm – 400 nm) and UVR8 for UV-B wavelengths (280 nm – 315 nm) (Fraser et al., 2016; Paik & Huq, 2019). UVR8, in conjunction with COP1 and HY5 transcription factors, controls a variety of morpho-physiological responses to UV wavelengths up to 350 nm (Mewis et al., 2012; Fraser et al., 2016; Rai et al., 2020). UVR8 activity is directly linked to the expression of key genes in the flavonoid and terpenoid pathways (Jenkins, 2014;

Shamala et al., 2020). Apart from UVR8-associated changes in the secondary metabolite profile, plant responses to UV are also modulated through non-specific signalling pathways involving Reactive Oxygen Species (ROS) and/or defence-induced hormones such as ethylene, jasmonic acid and salicylic acid (Schreiner et al., 2017). A commonly reported plant UV response is an increase in UV-absorbing, photoprotective secondary plant metabolites, such as phenolic and/or flavonoid compounds (Schreiner et al., 2017; Sharma et al., 2019; Takshak & Agrawal, 2019; Thoma et al., 2020). UV up-regulates the expression of key genes in the phenylpropanoid biosynthetic pathway, increasing the content of phenolic acids and/or flavonoids (Neugart et al., 2012; Daryanavard et al., 2023; Singh et al., 2023). For example, Chen et al. (2019) reported that phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), and catechol-O-methyltransferase (*COMT*) expression levels were upregulated in UV-exposed *Triticum aestivum* plants. Similar results were found by Inostroza-Blancheteau et al. (2016) who reported enhanced gene expression of *PAL*, chalcone isomerase (*CHS*) and flavonoid 3-hydroxylase (*F3'H*) in UV-exposed *Vaccinium corymbosum*. However, the induction of phenylpropanoid biosynthesis genes can be variable and depends on UV wavelength, and the specific plant organs among other factors (Qian et al., 2019). Therefore, the accumulation of phenolics and flavonoids is a compound-specific process which varies depending on environmental and developmental parameters. For example, Hectors et al. (2014) showed a significant accumulation of kaempferol over quercetin in *Arabidopsis* leaves at different developmental stages. This has been related to quercetin having a higher antioxidant capacity and therefore a more important role in photoprotection against UV, compared to kaempferol (Csepregi & Hideg, 2018). Prolonged exposure to UV is not the only factor that leads to an accumulation of phenolics, their content can also

be affected by both diurnal and nocturnal cues the time of day (Barnes et al., 2008) as well as a wide variety of other environmental factors including cold, drought, salinity and light (Šamec et al., 2021). The biosynthesis of phenolics initiates from two aromatic amino acids, phenylalanine and tyrosine (Tarasevičienė et al., 2021) which are converted to cinnamic acid and *p*-coumarate respectively, thus initiate the phenylpropanoid pathway (Sharma et al., 2019) (Supplementary Figure 4.2 & 4.3). The aforementioned phenylalanine ammonia-lyase and the bifunctional phenylalanine/ tyrosine ammonia-lyase play a central role in the pathway not only because they are the first enzymes involved in the dedicated synthesis of phenolics from phenylalanine and tyrosine but also because their activity changes in response to a variety of environmental stressors (Barros & Dixon, 2020). The presence of UV enhances the production of most amino acids, particularly those derived from the shikimate pathway, facilitating their role as precursors of secondary metabolites involved in defence (Sun et al., 2022). The photoprotective role of phenolics is complemented by other secondary metabolites such as terpenoids (Takshak & Agrawal, 2019) and particularly carotenoids (Badmus, et al., 2022B). Both groups of metabolites also have substantial commercial value. Flavonoids and carotenoids are extensively used as food colourants, preservatives and antioxidants as well as in pharmaceutical and cosmetic products (Zakynthinos & Varzakas, 2016; Dias et al., 2021) and animal feed (Zakynthinos & Varzakas, 2016) underlining their importance as commercial commodities. Carotenoids have an important protective function not only as pigments but also as dissipators of excess light as heat (Sankari et al., 2017; Shen et al., 2017; Emiliani et al., 2018). Badmus et al. (2022B), showed that UV induces increases in antheraxanthin, neoxanthin, violaxanthin and lutein content in *Arabidopsis* leaves. The increase in violaxanthin is observed in many studies, as

revealed by a recent meta-analysis (Badmus et al., 2022A). It is thought that other terpenoids, such as mono- and sesquiterpenes, are also involved in photoprotection, mainly as antioxidants (Escobar-Bravo et al., 2017), as suggested by their accumulation in response to UV (Jansen et al., 2008). Shamala et al. (2020) found a time and UV-dependent increase in volatile terpenoid content in *Camelia sinensis* plants. The study highlighted prolonged up-regulation of most genes in the sesquiterpene biosynthetic pathway. In contrast, most genes in the monoterpene biosynthetic pathway seem to be up-regulated only for short periods during UV exposure (Shamala et al., 2020). However, the stimulatory effect of UV on monoterpenes and sesquiterpenes is not ubiquitous, with notable differences between different plant species (Thines et al., 2007). All terpenoids have a common biosynthetic intermediate, mevalonic acid (Rohdich et al., 2001). Interestingly, mevalonic acid is not only the precursor for the terpenoids but also the starting point for the synthesis of sterols (Rogowska & Szakiel, 2020), triggering the question whether their biosynthesis is similarly enhanced by UV exposure. Sterols form a structural component of the cell membrane, but also support the plant defence system in mitigating abiotic stressors, including UV (Du et al., 2022). This class of metabolites seems to play a protective role in conjunction with terpenoids in *Vitis vinifera* (Gil et al., 2012).

UV-mediated changes in plant metabolite profiles are not well documented except for the model plant *Arabidopsis thaliana*. This knowledge gap is particularly problematic in the case of commercially valuable plants such as mint (*Mentha spicata*), the economic value of which is to a large extent determined by its secondary metabolite profile and particularly essential oils. Mono- and sesquiterpenes are key components of essential oil, however, to fully understand their accumulation it is necessary to place

terpenoid biosynthesis in the context of the potentially competing biosynthesis pathways of phenolics and amino acids. The aim of this study was to determine the association between UV and commercially valuable secondary metabolites. As many harvested horticultural products may take several days to reach either a processing facility or the consumer, it is important to understand how long the effects of UV on the plant metabolite profile persist. Therefore, metabolite profiles were determined both immediately after UV exposure, as well as after seven days recovery under non-optimal conditions mimicking transport and storage conditions.

4.2 Material and Methods

4.2.1 Plant material and UV-exposure stage

Mentha spicata L. (Moles Seeds Ltd., Stanway, U.K.) seeds were sterilized and grown on agar in tissue culture boxes (RA40 plastic micro boxes, Sac 02, Deize, Belgium) for 16 days after germination as described in Crestani et al. (2023A). Plants germinated 14 days after sowing. Following the initial growth period, the boxes were covered with either a UV-B blocking filter, Mylar (MY) polyester film (125 µm thickness; Tocana Ltd., Ballymount, Ireland) or a UV-B transmitting filter, Cellulose Acetate (CA) (95 µm thickness; Kunststoff-Folien-Vertrieb GmbH, Hamburg, Germany). Cellulose acetate also blocks UV-C radiation emitted by the UV-B lamps (TL 40W/12 Philips, Germany). UV was applied for four hours daily between 12:00 and 16:00. The UV intensity was calculated as daily biologically effective dose (BE) as reported in Flint & Caldwell (2003). Under CA, the BE was 2.7937 kJ m^{-2} (0.2830 kJ m^{-2} for UV-A and 2.5258 kJ m^{-2} for UV-B) while under MY the BE was 0.3250 kJ m^{-2} (0.1797 kJ m^{-2} for UV-A and 0.1453 kJ m^{-2} for UV-B). A background of PAR was applied for 14 hours from 7:00 to 21:00 at the intensity of 180 µmol m^{-2} s^{-1}. The background PAR was enriched with far-red to obtain the red: far-red ratio of 1.6 calculated according to

Franklin & Whitelam (2005). Further details about the light conditions applied during the experiment are reported in Crestani et al. (2023A). Leaves were harvested immediately after eight days of UV exposure, ground in liquid nitrogen and stored at –80 °C until analysis. A total of six boxes were used for each treatment and each experimental replicate. All the compounds were analyzed from five independent replicates, each made up of at least five plants per box. For simplicity, plants under MY were labelled as "–UV" while plants under CA were labelled as "+UV". At least six plastic boxes per treatment (Mylar or CA) were used for individual replicates. Minimum of 20 plants were pooled together from different boxes and immediately frozen after the UV. Further 20 plants were transplanted on soil and frozen after the recovery period. Results were obtained as the average of five independent biological replicates for all the metabolites analysed.

4.2.2 Recovery period

Following the final UV-treatment, around 20 plants per treatment were transplanted on John Innes II compost (William Sinclair Horticulture Ltd., Lincoln, United Kingdom) and exposed to visible light as reported in Crestani et al. (2023A) for seven days. At the end of the recovery phase, plants were rapidly frozen in liquid nitrogen and stored at –80 °C until analysis. The analysis is the same as described for plants harvested immediately after the final UV exposure. All the compounds were analyzed on five independent replicates.

4.2.3 Amino acid extraction and determination

The extraction and quantification of amino acids were previously described in Crestani et al. (2023B). Briefly, 100 mg of plant material previously ground in liquid nitrogen was mixed with 1 mL of 6 M HCl and incubated for 24 h at 110 °C.

Evaporated samples were incubated with 1 mL of 1 M borate buffer and 2 µL diethyl ethoxymethylenemalonate (DEEM) for 50 minutes at 50 °C before transferring the samples to amber glass vials.

Separation of amino acids was carried out using an InfinityLab Poroshel column 120 EC – C18, 2.1 x 50 mm, 1.9 µm (Agilent Technologies, Santa Clara, CA, USA). Contents of individual amino acids were determined by an HPLC system (Agilent 1290 Infinity II LC HPLC Agilent Technologies, Santa Clara, CA, USA) connected with a diode-array detector (DAD) operating at 280 nm. To enable quantification, a standard amino acid mixture (Merck Life Science Limited, Arklow, Ireland) (Supplementary Figure 4.1 & Supplementary Table 4.1) was used at different dilutions. The content of amino acids is reported in µg g^{-1} of fresh weight (FW). For more details, see supplementary information.

4.2.4 Determination of phenolic acids and flavonoids

Phenolic compounds were determined following the procedure detailed in Klem et al. (2019). About 200 mg of fresh plant material ground in liquid nitrogen was lyophilized and then extracted using 4 mL of methanol: chloroform: H_2O (1:2:2, v/v/v) solution. The upper polar phase was separated and used for the determination of phenolic compounds whereas the lower phase was later used for the determination of sterols. The separation and quantification of phenolic compounds were carried out using an UltiMate 3000 HPLC combined with LTQ Orbitrap XL high-resolution mass spectrometer (HRMS) (Thermo Fisher Scientific, Waltham, MA, USA) equipped with electrospray ionization and using a Hypersil GOLD separation column (150 × 2.1 mm, 3 µm; Thermo Fisher Scientific). The content of phenolic acids and flavonoids is reported in peak area g^{-1} of dry weight (DW). For more details, see supplementary

information. The schematic shikimic acid pathway shown in supplementary figure 4.2 and supplementary figure 4.3 was generated using the software KingDraw (version 2.7) (KingDraw Business Corporation, Qingdao, China).

4.2.5 Determination of phytosterols

To analyse and quantify a spectrum of phytosterols, an aliquot of the lower phenolics extraction phase was evaporated under N_2 and derivatized at 60 °C for 30 min using 50 µL of pyridine, and 70 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% tetramethylsilane (TMS) solution. After that, samples were again evaporated under nitrogen stream, resuspended in 1 mL hexane and analysed using a gas chromatograph (GC; Trace GC Ultra) coupled with a triple quadrupole mass spectrometer (MS; TSQ Quantum XLS; Thermo Scientific, USA). A Rxi-5Sil MS column (length of 30 m, internal diameter of 0.25 mm, film thickness of 0.25 µm, Shimadzu, Kyoto Japan) was used for separation. Cholesterol was used as an internal standard. For more details, see supplementary information.

4.2.6 Determination of monoterpenes and sesquiterpenes

Monoterpenes and sesquiterpenes were extracted according to Večeřová et al. (2021) with some modifications. Fresh plant material (500 mg) was ground in liquid nitrogen and incubated for 24 h at 4 °C in 4 mL of cold heptane. The extract was filtered to remove residual plant tissues and evaporated using a stream of nitrogen. The pellet was resuspended in 970 µL of heptane, transferred to a 1.5 mL vial and mixed with 30 µL of bisabolol (Sigma Aldrich) with a concentration of 100 µg mL^{-1} as the internal standard. The standard was obtained by mixing linalool, α-pinene, (R)-(−)-α-phellandrene, (−)-β-pinene, myrcene, (R)-(+)-limonene, eucalyptol, ocimene, sabinene hydrate, (+)-terpinen-4-ol, (+)- pulegone, (−)-*trans*-caryophyllene, γ-

terpinene, *p*-cymene and menthol (Sigma Aldrich, Arklow, Ireland). The final concentration of the standard mix was 250 µL mL^{-1}. From this stock solution, different dilutions were made to construct a calibration curve. Volatile terpenoids were separated using a Rxi-5Sil MS separation column and determined by GC-MS (Thermo Scientific, USA). Terpenoid content is reported in µg g^{-1} FW. For more details, see supplementary information.

4.2.7 Carotenoid, chlorophyll and tocopherol extraction and quantification

Carotenoids, chlorophylls and tocopherols were quantified following Badmus et al. (2022B & C) with some modifications. Briefly, 50 mg of mint plant material, previously ground in liquid nitrogen (and stored at –80 °C), was extracted with 400 µL of 80% cold methanol (v/v) and sonicated in ice for 20 minutes. 800 µL of chloroform and 400 µL of NaCl 1 M were added to the extract. After centrifugation for five minutes at 3000 rpm at 4 °C, the organic phase was transferred to a new tube and the aqueous phase was re-extracted using 400 µL of chloroform. Samples were centrifuged once more at 3000 rpm for five minutes at 4 °C and the organic phases were combined and evaporated under vacuum till dryness (MiniVac evaporator, ScanVac). The pellet was resuspended in 200 µL of MeOH: MTBE (60:40 v/v) and filtered with 0.22 µm syringe filters (Millipore, Bedford, USA).

The content of carotenoids and chlorophylls was determined using an Agilent 1290 Infinity II LC HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a YMC carotenoids C-30 reverse phase column 250 mm x 4.6 mm, 3 µm resin diameter (YMC Europe GmbH, Dinslaken, Germany). The standard was made up by mixing pure standards of zeaxanthin, luteolin, neoxanthin, antheraxanthin, β-carotene, α-tocopherol, ɣ-tocopherol and δ-tocopherol (Sigma Aldrich, Arklow, Ireland).

Standards were mixed, evaporated, filtered, and resuspended in MeOH: MTBE (60:40 v/v) to obtain a solution with a final concentration of 10 ppm. Carotenoids, chlorophylls, and tocopherols content is reported in mg g^{-1} FW. For more details, see supplementary information.

4.2.8 Statistical analysis

Two-tailed t-tests, or the nonparametric Mann-Whitney test, were used to identify significant differences between the –UV and +UV plants. Data are reported as mean ± SE (Standard Error). The mean was calculated from five independent replicates. All statistical analyses were performed with IBM SPSS Statistic v28 (Armonk, New York, US).

4.3 Results

Mint plantlets were exposed to UV for eight days. The contents of amino acids, phenolic acids, flavonoids, mono and sesquiterpenes, carotenoids, tocopherols and sterols were quantified either immediately after the UV treatment, or after seven days of recovery on soil.

4.3.1 Amino Acids

4.3.1.1 Immediately after UV exposure

HPLC analyses revealed that across 17 amino acids analyzed, the content of 14 amino acids significantly increased in plants exposed to UV when measured immediately after the UV exposure ($p < 0.05$) (Figure 4.1 A). Proline and methionine were the only two amino acids where a decrease in content was recorded in +UV plants ($p = 0.002$ & $p = 0.016$). Contents of glutamic acid, aspartic acid and arginine were the highest

in UV-treated plants compared to non-treated plants (p = 0.009, p = 0.006 & p = 0.049). Only tyrosine, showed non-significant variation between the –UV and +UV treatments (Figure 4.1 A).

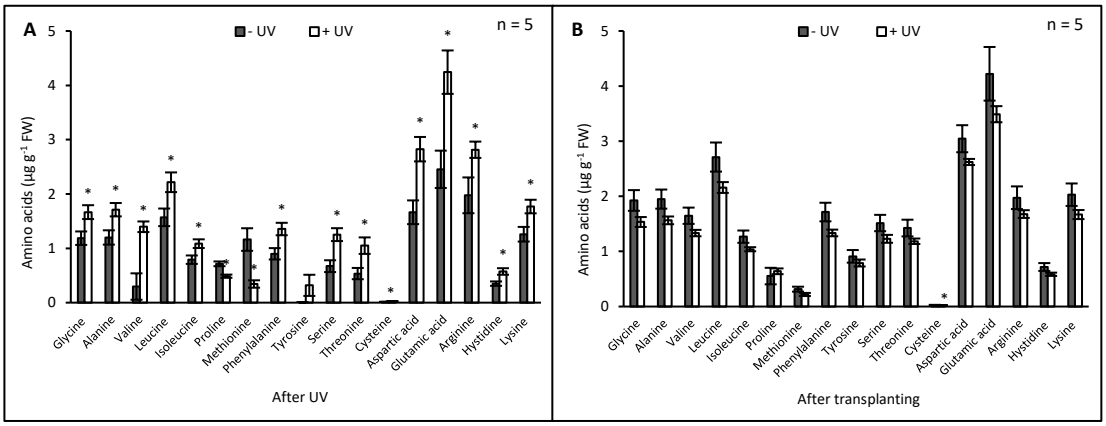


Figure 4.1. *Amino acids content (µg g^{-1} FW) was measured after eight days of UV exposure (**A**) or seven days later after transplanting on soil (**B**). Grey bars represent non-UV treated plants while white bars represent UV-treated plants. Asterisks indicate a significant difference between the UV treatments with p < 0.05 (*). Error bars represent the SE based on the means of five independent replicates. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

4.3.1.2 After seven days recovery

After recovery on soil, the amino acid content showed an opposite trend compared to what had been observed immediately after UV exposure, with amino acids being higher in –UV plants. In the recovery plants, no significant difference between the two UV treatments was found with the exception of cysteine which was significantly higher in –UV plants (p = 0.032) (Figure 4.1 B).

4.3.2 Phenolic acids and flavonoids

4.3.2.1 Immediately after UV exposure

The contents of phenolic acids in leaves, such as *3*-coumaric (p = 0.029) and syringic acid (p < 0.001), were significantly reduced in the UV exposed plants compared to

non-exposed plants. Also, the contents of chlorogenic acid, 3-hydroxybenzoic acid, vanillic acid and protocatechuic acid, were slightly reduced in +UV compared to –UV plants, however, these differences were not statistically significant ($p > 0.05$). Ferulic acid was the only phenolic acid that increased significantly in +UV plants ($p = 0.033$). Overall, the most abundant phenolic acid was caffeic acid which appeared not to be affected by the UV treatment and was present in almost the same content across both treatments. The total phenolic content also remained unchanged across –UV and +UV plants. Further, the flavonoid content increased consistently by 1.8-fold in +UV plants in comparison, with –UV plants.

The content of specific flavonoids, including apigenin and luteolin did not show any significant difference between the –UV and the +UV plants while contents of homoorientin ($p = 0.018$) and epigallocatechin ($p = 0.017$) significantly increased following UV treatment (Figure 4.2 A).

4.3.2.2 After seven days recovery

Variation in the content of phenolic acid and flavonoids was also noted seven days after transplanting on soil. The content of ferulic acid and protocatechuic acid was significantly lower in the +UV plants that had undergone recovery, compared to the –UV plants ($p = 0.008$ & $p = 0.006$). The content of other phenolic acids, including 3-coumaric acid, chlorogenic acid, 3-hydroxybenzoic acid, vanillic acid, syringic acid and caffeic acid was similar across the –UV and +UV treatment. The latter was also the most abundant phenolic acid present in mint plants. Apigenin was the only flavonoid for which no statistically significant difference was detected, but its content tended to be higher in the –UV plants after recovery. Luteolin content significantly decreased in the +UV plants ($p = 0.02$) while contents of both homoorientin and

epigallocatechin were higher in the +UV plants after transplanting (p = 0.016 & p = 0.008) (Figure 4.2 B).

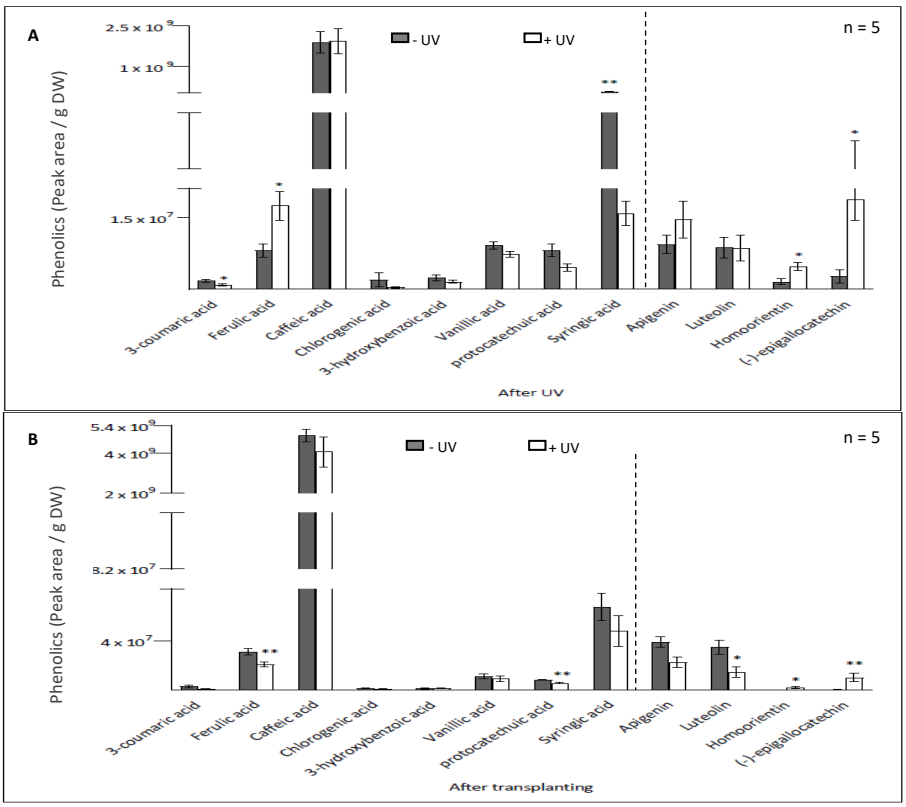


Figure 4.2. *Phenolics content (peak area/ g DW) was measured after eight days of UV exposure (**A**) and seven days after transplanting on soil (**B**). Grey bars represent non-UV treated plants while white bars UV-treated plants. Dashed line separated phenolic acids from flavonoids. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) and $p < 0.01$. Error bars represent the SE based on the means of five independent replicates. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

4.3.3 Sterols

4.3.3.1 Immediately after UV exposure

The analysis of sterols revealed no overall statistical difference in contents between the non-treated plants and the UV-treated plants in plants sampled immediately after the last UV exposure. Campesterol, stigmasterol and fucosterol were present in –UV and +UV plants in similar amounts, while β-sitosterol content was lower but not

significantly in +UV plants. Cycloartenol acetate is the only compound that was found to be significantly lower ($p = 0.016$) in UV-exposed plants when compared to the non-UV-exposed ones (Figure 4.3 A).

4.3.3.2 After seven days recovery

After the recovery phase, the content of sterols was reduced compared to samples taken immediately after UV exposure, across both –UV and +UV plants. Similar to the previous sampling stage, plants after recovery showed similar content of campesterol, stigmasterol and fucosterol irrespective of the UV treatment. The content of β-sitosterol was higher in +UV plants after seven days of recovery on soil, but the difference between –UV and +UV was not statistically significant.

Cycloartenol acetate content was below the detection limit in both –UV and +UV plants (Figure 4.3 B).

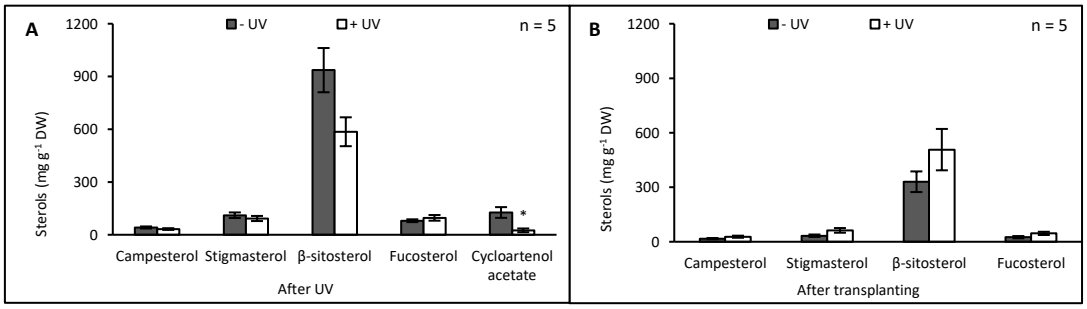


Figure 4.3. *Sterol content was measured after eight days of UV exposure (**A**) and seven days after recovery on soil (**B**). Grey bars represent non- UV treated plants while white bars UV-treated plants. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*). Error bars represent the SE based on the means of five independent replicates. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

4.3.4 Monoterpenes and sesquiterpenes

4.3.4.1 Immediately after UV exposure

Volatile terpenoids identified and quantified using GC-MS included 14 monoterpenes and eight sesquiterpenes. Contents of all volatile terpenoids tended to be higher in UV-exposed plants, with the exception of *p*-mentha-1,8-dien-3-one, the content of which was slightly, but non significantly lower in the UV-exposed plants. Some monoterpenes such as limonene oxide, p-menth-1-en-4-ol and α-terpineol were not detected in control plants and were only notable in UV-exposed plants. Within the group of monoterpenes, a significant increase in content due to UV treatment was recorded in limonene ($p = 0.008$), α-linalool ($p = 0.008$), limonene oxide ($p = 0.008$) and β-terpineol ($p = 0.028$). Although the content of sesquiterpenes was lower than that of monoterpenes, the content of the majority of sesquiterpenes was significantly enhanced in the plants exposed to UV. UV-treated plants accumulated significantly more sesquiterpenes such as cis-jasmone ($p = 0.008$), β-cubebene ($p = 0.016$), cadinene ($p = 0.008$), (+)-epi-bicyclosesquiphellandrene ($p = 0.016$) and germacrene D ($p = 0.003$). The contents of caryophyllene, α-farnesene and cubenol increased, but not significantly after the UV treatment. Across all the compounds analyzed, the most abundant was pulegone which was present in similar amounts in both –UV and +UV plants (Table 4.1).

4.3.4.2 After seven days recovery

After the recovery on soil, the content of monoterpenes was similar between the control and the +UV treatment with the exception of myrcene the content of which was higher but not significantly in –UV plants. Within the group of monoterpenes, the only compounds significantly different were β-pinene, α-terpineol and p-mentha-1,8-

dien-3-one. β-pinene was significantly higher in the plants previously exposed to UV ($p = 0.032$) while α-terpineol and p-mentha-1,8-dien-3-one were significantly higher in –UV plants ($p = 0.012$ & $p = 0.005$). Some monoterpenes that were not detected in the –UV treated plants immediately after UV exposure such as limonene oxide, α-terpineol and p-menth-1-en-4-ol were found in samples which recovered for seven days. The contents of limonene oxide and α-terpineol were slightly, but not significantly, higher in the –UV plants while p-menth-1-en-4-ol contents were similar in the different UV treatments. The majority of sesquiterpenes were present at similar concentrations in –UV and +UV plants. The contents of α-farnesene, caryophyllene and germacrene D increased in –UV plants seven days after recovery but not significantly. Similar to what was noted in plants analysed immediately after the last UV exposure, pulegone remained the most abundant compound with a similar content in –UV and +UV plants (Table 4.1).

Table 4.1. *Contents of volatile terpenoids (µg/g FW) were measured eight days after UV treatment and seven days after transplanting on soil in non-UV-exposed plants (–UV) and in UV-exposed plants (+UV). B.D. was used to indicate when the content was below the detection limit. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*) or $p \leq 0.01$ (**) while non-significant differences between treatments were identified as n.s. The mean from five independent (n = 5) was reported ± S.E. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

Terpenes	*Class*	*–UV (After UV)*	*+UV (After UV)*	*Sig.*	*–UV (After transplanting)*	*+UV (After transplanting)*	*Sig.*
α-pinene	Monoterpene	0.029 ± 0.019	0.068 ± 0.006	n.s.	0.124 ± 0.029	0.166 ± 0.1222	n.s.
Thujene	Monoterpene	0.025 ± 0.024	0.078 ± 0.010	n.s.	0.147 ± 0.030	0.220 ± 0.160	n.s.
β-pinene	Monoterpene	0.059 ± 0.052	0.182 ± 0.253	n.s.	0.402 ± 0.076	0.520 ± 0.392	*
Myrcene	Monoterpene	1.007 ± 0.926	3.557 ± 0.601	n.s.	7.440 ± 1.345	10.082 ± 7.457	n.s.
Limonene	Monoterpene	0.135 ± 0.042	1.753 ± 0.478	*	3.764 ± 0.638	4.063 ± 2.915	n.s.
Eucalyptol	Monoterpene	0.176 ± 0.077	0.198 ± 0.120	n.s.	0.79 ± 0.360	0.745 ± 0.460	n.s.
α-linalool	Monoterpene	0.009 ± 0.006	0.815 ± 0.437	*	0.327 ± 0.041	0.313 ± 0.140	n.s.
Limonene oxide	Monoterpene	B.D.	0.178 ± 0.033	*	0.392 ± 0.085	0.257 ± 0.155	n.s.
β-terpineol	Monoterpene	0.015 ± 0.007	0.074 ± 0.020	*	0.172 ± 0.040	0.135 ± 0.039	n.s.
p-menth-8-en-3-one	Monoterpene	2.157 ± 0.760	2.858 ± 0.712	n.s.	3.050 ± 0.623	1.991 ± 0.815	n.s.
p-menth-1-en-4-ol	Monoterpene	B.D.	0.032 ± 0.015	n.s.	0.055 ± 0.012	0.065 ± 0.021	n.s.
α-terpineol	Monoterpene	B.D.	0.855 ± 0.429	n.s.	6.220 ± 1.611	3.956 ± 1.011	*
Pulegone	Monoterpene	20.33 ± 6.973	21.68 ± 4.586	n.s.	23.389 ± 4.761	22.642 ± 4.564	n.s.
p-mentha-1,8-dien-3-one	Monoterpene	5.675 ± 4.510	3.828 ± 1.591	n.s.	15.882 ± 2.687	11.832 ± 2.368	**
Cis-jasmone	Sesquiterpene	0.327 ± 0.222	2.115 ± 0.379	*	3.916 ± 0.949	3.401 ± 0.458	n.s.
β-cubebene	Sesquiterpene	0.010 ± 0.010	0.087 ± 0.013	*	0.096 ± 0.027	0.113 ± 0.014	n.s.
Caryophyllene	Sesquiterpene	0.060 ± 0.044	0.256 ± 0.068	n.s.	0.430 ± 0.146	0.133 ± 0.03	n.s.
Cadinene	Sesquiterpene	0.013 ± 0.008	0.088 ± 0.011	*	0.085 ± 0.013	0.058 ± 0.007	n.s.
α-farnesene	Sesquiterpene	0.030 ± 0.030	0.115 ± 0.017	n.s.	0.306 ± 0.087	0.149 ± 0.013	n.s.
(+)-epi-bicyclosesquiphellandrene	Sesquiterpene	0.018 ± 0.018	0.117 ± 0.012	*	0.169 ± 0.036	0.131 ± 0.023	n.s.
Germacrene D	Sesquiterpene	0.198 ± 0.061	0.718 ± 0.106	*	1.205 ± 0.204	0.911 ± 0.098	n.s.
Cubenol	Sesquiterpene	0.060 ± 0.018	0.120 ± 0.013	n.s.	0.164 ± 0.042	0.182 ± 0.021	n.s.

4.3.5 Carotenoids, chlorophylls, and tocopherols

3.5.1 Immediately after UV exposure

Carotenoids measured in mint leaves include neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, β-carotene and VAZ (sum of violaxanthin, antheraxanthin and zeaxanthin).

Immediately after UV exposure, no significant difference was recorded between the plants from the –UV and the +UV treatments although a tendency to decrease the content of all carotenoids in +UV plants was noted. VAZ was reduced by ca. 33% in +UV plants, but the decrease was non-significant (Figure 4.4 A). After the UV exposure, the compounds with the highest content were neoxanthin and lutein with an average of 44.80 mg g^{-1} FW and 44.20 mg g^{-1} FW, respectively. Both –UV and +UV plants had similar content of chlorophyll *a* as well as a similar chlorophyll *a* to chlorophyll *b* ratio (Chl *a*/*b*) (Figure 4.5 C). A non-significant reduction in Chlorophyll *b* content, was recorded in plants exposed to UV (Figure 4.5 A).

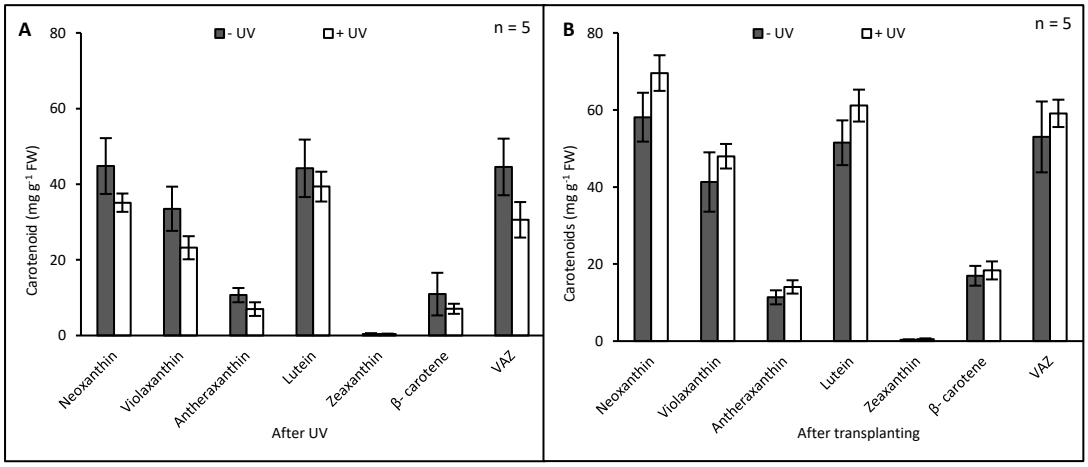


Figure 4.4. *Carotenoid content (mg g^{-1} FW) was measured after eight days of UV exposure (**A**) and seven days after recovery on soil (**B**). Grey bars represent non-UV treated plants while white bars represent UV-treated plants. No significant difference was found between the UV treatments. Error bars represent the SE based on the means of five independent replicates. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

In contrast to carotenoids, tocopherols tended to be induced by UV treatment. The content of α-tocopherol was higher but not significantly in +UV plants compared with –UV plants immediately after UV exposure. For δ-tocopherol, data did not show a significant difference between the –UV and the +UV treatment. In contrast, the content of ɣ-tocopherol was significantly stimulated by UV exposure ($p = 0.002$) reaching an average of 10.04 mg g^{-1} FW (Figure 4.6 A). The most abundant tocopherol recorded was α-tocopherol with an average content of 16.61 mg g^{-1} FW in UV-exposed plants.

4.3.5.2 After seven days recovery

During the recovery stage, the overall content of carotenoids increased compared to the previous stage. After the recovery on soil, a comparison between the –UV and +UV treatments showed a non-significant increase in the contents of neoxanthin, violaxanthin and lutein, as well as VAZ in +UV plants. After recovery, the most abundant compound was neoxanthin with an average of 69.57 mg g^{-1} FW in +UV plants (Figure 4.4 B).

As per carotenoids, the content of chlorophyll *a* and *b* increased in both –UV and +UV plants when compared with plants analysed immediately after the UV treatment. Similar contents of chlorophyll *a* as well as the chlorophyll *a* to chlorophyll *b* ratio (Chl *a*/ Chl *b*) were found across both UV treatments (Figure 4.5 C). Chlorophyll *b* content tended to be slightly higher in UV-treated plants, but the difference was not significantly different (Figure 4.5 B).

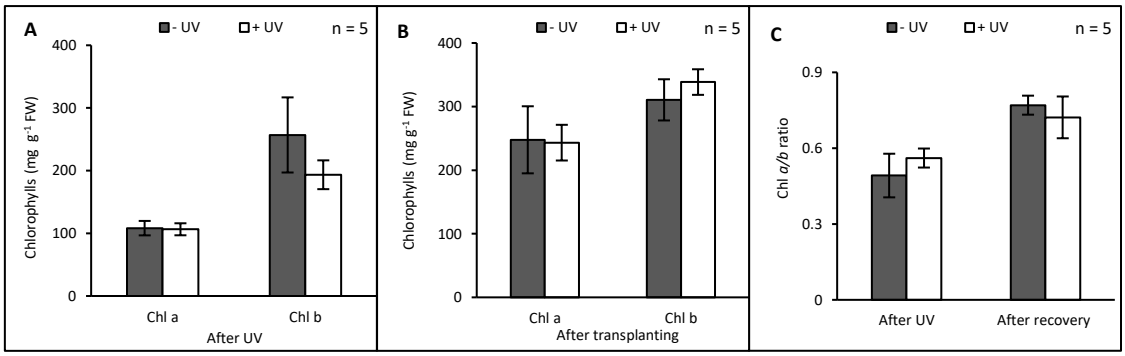


Figure 4.5. *Chlorophylls (mg g^{-1} FW) were measured after eight days of UV exposure (**A**) and seven days under UV-free condition (**B**). Chl a/b ratio was calculated after UV and after transplanting (**C**). Grey bars represent non-UV treated plants while white bars represent UV-treated plants. Error bars represent the SE based on the means of five independent replicates. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

In contrast to carotenoids and chlorophylls, tocopherol content was reduced compared to the previous sampling stage. The content of α-tocopherol and δ-tocopherol was similar between –UV and +UV plants while ɣ-tocopherol content was higher in –UV plants, but the difference was not significant. ɣ-tocopherol was again the most abundant tocopherol with an average content of 5.51 mg g^{-1} FW (Figure 4.6 B).

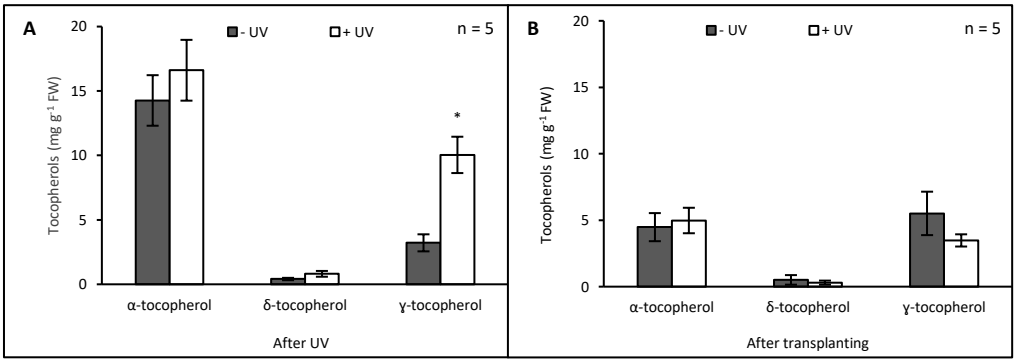


Figure 4.6. *Tocopherols (mg g^{-1} FW) were measured after eight days of UV exposure (**A**) and seven days after recovery on soil (**B**). Grey bars represent non-UV treated plants while white bars represent UV-treated plants. Asterisks indicate a significant difference between the UV treatments with $p < 0.05$ (*). Error bars represent the SE based on the means of five independent replicates. Fold change between after UV and after transplanting is reported in Supplementary Table 4.3.*

4.4 Discussion

Previous studies have shown that UV induced morphological and physiological changes in *Mentha spicata* grown under low intensities of UV radiation (Crestani et al., 2023A). These changes include a decrease in leaf area and increases in stem branching and numbers of leaves. Typically, morphological changes induced by low doses of UV are not accompanied by a decrease in overall plant biomass (Robson et al., 2015). Thus, any increase in metabolite concentration, comprises a real increase in metabolite yield. Here, distinct effects of UV radiation on the accumulation of different classes of metabolites are shown, with UV increasing the overall content of amino acids, flavonoids, monoterpenes and sesquiterpenes, while the contents of phenolics, carotenoids, chlorophylls, and sterols are mostly reduced. A different trend was recorded for plants that had been recovering for seven days under PAR only light, emphasising the transitory effect of UV on mint metabolite profiles.

4.4.1 Photoprotection: from primary to secondary metabolism

UV-B induces an extensive reprogramming of plant metabolic networks (Dotto & Casati, 2017). For example, in the presence of UV-B, plants have been reported to upregulate the production of several amino acids (Sun et al., 2022). Amino acids are key constituents of peptides, but their role in plant cells is much wider including functioning in a broad range of cellular processes associated with growth and development, and stress resistance (Hildebrandt et al., 2015). In general, the contents of plant amino acids vary dynamically in response to environmental conditions (Hildebrandt et al., 2015). For example, accumulation of amino acids has been observed in plants exposed to abiotic stress conditions highlighting their direct or indirect participation in stress response (Batista-Silva et al., 2019). Our results revealed an increase in most of the amino acids analysed, with the exception of proline

and methionine. Consistent with our results, a study conducted by Zhang et al. (2019), reported that methionine was the only amino acid whose content progressively decreased under four different UV exposure conditions. Since methionine is the key precursor of ethylene (Wawrzynska et al., 2015), this might indicate that there is a possible competition whereby the metabolite flux was directed to hormone biosynthesis and methionine is converted to ethylene. Indeed, it has been reported that ethylene content increases in UV-exposed plants (Azevedo et al., 2006; Vanhaelewyn et al., 2016). This ethylene-mediated response is mostly associated with UV-induced stress. However, in the current study, no macroscopic signs of stress were noted, and chlorophyll and carotenoid contents remained unchanged, suggesting a mostly regulatory UV effect. The increment in total amino acids found in this study displays the same trend found by Klem et al. (2022) who showed an overall increase in free amino acid biosynthesis of barley plants exposed to UV. The authors also found a positive correlation between nitrogen fixation and the accumulation of amino acids. Interestingly, aspartic acid and glutamic acid are present in the highest content in plants exposed to UV. These two amino acids not only are precursors of other amino acids such as threonine, leucine, isoleucine and methionine but also are involved in the tricarboxylic acid pathway (TCA) via their common precursor 2-oxoglutarate (Azevedo et al., 2006). The metabolite 2-oxoglutarate is an important regulator and precursor for the plant's primary and secondary metabolism including the phenolic biosynthesis pathway (Tohge et al., 2013). In parallel, we found an accumulation of aromatic amino acids, phenylalanine and tyrosine that are precursors of phenolic compounds via the phenylpropanoid pathway (Manela et al., 2015; Tzin & Galili, 2010). Thus, the accumulation of these amino acids is associated with a subsequent accumulation of phenolics known for their antioxidant and UV-absorbing properties.

4.4.2 Redistribution of the pool of phenolics and flavonoids is associated with changes in antioxidant activity

One of the most common UV-induced responses in plants is the synthesis of phenolic acids and flavonoids that are involved in cellular protection, shielding deeper tissues of the leaf from UV penetration and participating in the antioxidant response against ROS (Neugart & Schreiner, 2018; Rodríguez-Calzada et al., 2019). Our study showed that the overall content of phenolic acid decreases jointly with a parallel increase of flavonoids in plants exposed to UV. Therefore, the question arises whether the accumulation of flavonoids rather than phenolic acids is correlated to their different role in UV protection. In their study, Csepregi & Hideg (2018), tested the effective antioxidant capacity across numerous phenolics to correlate their structure with their photoprotective role. Summarizing their results, it was shown that flavonoids have a better antioxidant capacity than phenolic acids against H_2O_2 and $^1O_2$. Flavonoids' antioxidant capacity is strictly correlated to their molecular structure given particularly by the presence of a catechol group in ring B and a 3-hydroxy group and a 2,3-double bond in ring C. Consequently, plants tend to synthesise flavonoids rather than phenolic acids such as hydroxycinnamic acid due to the superior antioxidant capacity (Agati et al., 2020). For example, Turtola et al. (2005) found in hybrid willow plants that UV enhanced the content of flavonoids to a greater extent than that of phenolic acids. The authors speculated that this shift is caused by UV-B enhanced flavonoid accumulation while phenolic acids were induced in the presence of visible light only. In our study, the content of phenolic acids varies, with a tendency to be lower in plants exposed to UV except for ferulic acid. Ferulic acid has a modest UV absorption capacity and a low antioxidant capacity (Csepregi & Hideg, 2018) but it may contribute to UV-screening through binding to lignin or cutin (Jansen et al., 1998). The role and rapid induction of flavonoids in response to UV is well documented (Zoratti et al., 2014;

Barnes et al., 2016; Neugart et al., 2021). For example, Righini et al. (2019) and Wang et al. (2020) showed that *Arabidopsis* transgenic plants that are able to synthesise apigenin have higher protection against UV-B-induced damage. These findings, in line with our results, can be associated with the strong absorbance of apigenin in the UV range (Li et al., 1997). Flavonoids can be synthesized in different plant compartments including glandular and non-glandular trichomes (Agati & Tattini, 2010). Glandular trichomes are also the synthesis site of other secondary metabolites such as terpenes, waxes and lipids (Valkama et al., 2003). Even if flavonoid and terpenoid pathways are independent, they co-occur in plants to fulfil a variety of different functions in the plant-environment interaction (Kang et al., 2014). An intriguing question is whether terpenoid accumulation in plants such as mint, affects flavonoid accumulation.

4.4.3 UV enhances the synthesis of short-chain rather than long-chain terpenoids

The genus *Mentha* includes a wide number of species and hybrids with hundreds of subspecies and cultivars (Tafrihi et al., 2021). Due to the presence of different species and varieties, the composition of the essential oil (EO) is extremely variable (Singh & Pandey, 2018). Furthermore, the chemical diversity of essential oil is also subject to variation under different environmental conditions (Maffei, 1990). Essential oils consist of a mixture of different compounds with a volatile nature and include large amounts of monoterpenes and sesquiterpenes (Singh & Pandey, 2018). While the quality of EO cannot be assessed by terpene contents alone, our results showed that the application of UV-B results in changes in the composition of the terpene pool in essential oil, inducing an overall accumulation of mono and sesquiterpenes. A likely explanation for the accumulation of these compounds could be the direct effect of UV on the development of glandular trichomes, specialized structures on the leaf where

the EO are stored and synthesized (Turner et al., 2000). Indeed, the presence of trichomes can act as a physical barrier providing a protective role against UV, increasing the thickness of the epidermis, reducing water evaporation and helping to regulate the temperature (Tattini et al., 2000; Wang et al., 2021). Interestingly, Ioannidis et al. (2002) reported that the application of UV-B increases the oil content contained in the glandular trichomes in *Ocimum basilicum* and that this effect is more pronounced in mature leaves rather than developing leaves. Other studies also reported an effect of UV on glandular trichomes' size and density in *Artemisia annua* and *Betula pendula* respectively (Pandey & Pandey-Rai, 2014; Kostina et al., 2001). In contrast, Escobar-Bravo et al. (2019) reported a non-correlation between UV exposure and the increased number of glandular trichomes and variation in the EO content in tomato plants, suggesting a possible species-dependent response. Our data demonstrate that there is not only a quantitative variation in the mono and sesquiterpenes content but also that the composition of mono and sesquiterpenes varies under UV. The mechanism that leads to the synthesis of volatile terpenes needs to be clarified, but the stimulatory UV effect on mono- and sesquiterpenes is widely reported across different aromatic plant species (Jansen et al., 2008). In particular, a recent study conducted on basil found an increase in numerous monoterpenes including pinene, terpineol and linalool (Semenova et al., 2022). In contrast, Llusia et al., (2012) found more variable results whereby UV either induced or lowered terpene content depending on the plant species. Our data show that sesquiterpene content increased in spearmint under UV-B exposure. These data match the findings of Maffei & Scannerini (2000) and Dolzhenko et al. (2010) who noted an increase of germacrene D and β-caryophyllene in peppermint plants after UV exposure. The authors reported that the increase of sesquiterpenes is likely to be associated with an increase of farnesyl

pyrophosphate (*FPPS*) gene expression that is highly upregulated in the indoor UV-treated plants compared to the outdoor UV-treated plants (Dolzhenko et al., 2010).

It has been argued that as part of their stress response plants activate a broad range of antioxidant metabolites, including monoterpenes to minimise ROS accumulation (Jardine et al., 2017). Our previous study highlighted how UV exposure enhances the accumulation of antioxidants in mint plants under the same conditions reported in this study (Crestani et al., 2023B). Thus, UV-induced changes in mono and sesquiterpenes might be considered an important player in plant protection against reactive oxygen species.

Other secondary metabolites including carotenoids, tocopherols, chlorophylls and hormones such as gibberellins are synthesised via the same precursors as the monoterpenes (Nisar et al., 2015). Carotenoids are known to be strong antioxidants and are involved in plant photoprotection playing a central role in the dissipation of excessive light (Maoka, 2020). Many studies have found a stimulatory effect of UV on carotenoids, suggesting a conserved response (Badmus et al., 2022B; Martínez-Zamora et al., 2021; Sankari et al., 2017; Schreiner et al., 2012; Shen et al., 2017). However, the results of this study showed that the application of UV did not affect carotenogenesis suggesting that carotenoids are not involved in the acclimatory response against short-term UV exposure in mint. In line with our results, Nazari & Zarinkamar (2020) also reported a reduction in carotenoids in *Mentha aquatica* exposed to UV-B. The authors speculate that UV might degrade carotenoids and chlorophylls or alternatively affect their biosynthesis or the biosynthesis of their precursors. Since the content of their precursor (i.e., mevalonic acid) increases, it is more likely that UV controls the terpene pathway with a tendency to synthesise mono and sesquiterpenes rather than carotenoids and that this mechanism is species-specific.

Due to the overlap of the biosynthetic pathways, it is speculated that under UV, mint plants prioritize the synthesis of shorter terpenoids redirecting the resources to other metabolic pathways (Supplementary Table 4.2).

4.4.4 UV- modulates accumulation of tocopherols and phytosterols

In conjunction with carotenoids, tocopherols are reported to fulfil the role of antioxidants taking part in the protection of plants against environmental stressors (Hasanuzzaman et al., 2014). Tocopherols partially share their biosynthetic pathway with the terpenoids which provides for the isoprenoid chain and also with the shikimate pathway from which the aromatic ring is derived (Hasanuzzaman et al., 2014). Our results showed an overall increase in tocopherol content after UV exposure with the γ-tocopherol being statistically significant. However, Munné-Bosch & Alegre (2002) reported an increase in α-tocopherol. Such an increase can be associated with the plant response to UV-related damage to the chloroplast membranes and is often associated with stress tolerance. In line with our results, Emiliani et al. (2018) also found a significant increase in ɣ-tocopherols, and this was associated with an increase in carotenoids in *Arabidopsis* plants. The authors conclude that the increase in tocopherol can contribute to UV-B protection but does not play a central role in plant protection. Moreover, DeLong & Steffen (1998), conducted a study on spinach thylakoids showing that α-tocopherol is involved in the antioxidant response against lipid peroxidation. In the same way, Badmus et al. (2022C) reported a time-dependent accumulation of tocopherols in *Arabidopsis* plants exposed to UV. However, other studies reported that tocopherol levels tend to be lowered by UV treatment (DeLong & Steffen, 1998; Jain et al., 2003; Carletti et al., 2003). The variation in tocopherol content might depend primarily on the presence of other antioxidants produced by the

plant to counter ROS and avoid lipid peroxidation in the chloroplasts (Munné-Bosch & Alegre, 2002). Thus, it has been hypothesised that in mono- and sesquiterpene accumulating mint plants the role of tocopherols is not as pronounced as reported for *Arabidopsis* by Badmus et al. (2022C).

Phytosterols are also involved in stress protection. Indeed, most studies report an overall accumulation of sterols under UV (Rogowska & Szakiel, 2020). For example, a paper by Shahzad et al. (2021) found that an exogenous application of β-sitosterol offers protection against UV. Our results show, the levels of some sterols remain unaffected by UV, but others showed a marked decline such as β-sitosterol and cycloartanol acetate. Gil et al. (2012) found that changes in content of β-sitosterol in grapevine leaves are UV-dose dependent. In fact, low doses of UV enhance the synthesis of β-sitosterol while high doses of UV induce the synthesis of other protective metabolites that originate from the same metabolic pathway such as mono and diterpenes (Gil et al., 2012). Thus, it appears that results need to be interpreted in the context of UV-dose used. Cycloartenol is one of the precursors of other sterols including β-sitosterol, stigmasterol and campesterol (Cabianca et al., 2021). It has been reported by Du et al. (2022) that reduced activity of the cycloartenol synthase, a key enzyme in the synthesis of sterols, can cause phenotypic alterations in plants. Indeed, mint plants exposed to UV showed an altered phenotype with more compact plants and more branches (Crestani et al., 2023A). It is most likely that fluctuation in sterol composition can be associated with their primary role in regulating plant growth and development (Rogowska & Szakiel, 2020) rather than being involved in plant-stress protection.

4.4.5 Transitory effects of UV

The application of UV as an instrument to boost the content of metabolites is well established. However, the persistence of induced metabolites is still largely unknown. Nevertheless, this is an important aspect for applications. Depending on the specific crop, produce may take several days between harvest (and last UV exposure) and the consumer (Mohamed et al., 2024). Furthermore, produce would be exposed during that period to strikingly different environmental conditions, for example drought in an air-conditioned truck. Thus, for any UV-effect to be of benefit to consumers, induced metabolites need to be stable for several days. A limited number of studies have explored persistence of UV-induced changes after cessation of UV treatments in, what is often referred to as a "recovery period". For instance, Hectors et al. (2014) showed fluctuations in different classes of secondary metabolites in *Arabidopsis thaliana* plants after a 20-hour recovery period. Specifically, the content of both flavonoids and α-tocopherol increased while that of polyamines decreased after the recovery period. In contrast, Lidon & Ramalho (2011) explored dynamic effects of a two week recovery period on *Oryza sativa* L. On the final day of recovery, it was found that VAZ content was significantly increased in UV-B exposed plants compared to control plants, while α and β-carotene contents were similar to those in the control (Lidon & Ramalho 2011). In the current study, a comparison between the plants sampled immediately after UV exposure and after seven days recovery shows that there was an overall increase in the content of economically valuable carotenoids, mono and sesquiterpenes, phenolics and amino acids while contents of sterols and tocopherols decreased. Thus, effects of UV exposure remain for, at least, one week, with potential benefits for human consumers.

4.4.6 Production of enhanced quality in indoor farming

In nature, plants are exposed to a myriad of environmental conditions which can be unpredictable, beneficial, and/or damaging, thus plants have developed distinct responses to ensure survival (Loconsole & Santamaria, 2021). Moreover, these conditions directly affect the content of bioactive compounds, influencing the nutritional properties of fruits and vegetables (Toscano et al., 2019). This also gives growers and producers an opportunity to exploit the plants' innate response to stressors to develop plants with a more desirable metabolite profile. UV radiation can be effectively used on a large, commercial scale in indoor farming to promote the biosynthesis of secondary metabolites (Escobar-Bravo et al., 2017; Yoon et al., 2021). Indeed, the expansion of indoor and urban farming opens a window of opportunity to improve the production process in a cost-effective and targeted way by manipulating the light spectrum. This ability to alter the level and composition of secondary metabolites in fruit and vegetables can be used to improve flavour, colour, bioactive content and, ultimately, human health-giving properties (Schreiner et al., 2012; Seeburger et al., 2023) as well as increase its commercial value. Advances in LED technology have enabled us to use different wavelengths, and combinations of wavelengths, creating new opportunities for targeted manipulation of crops.

4.5 Conclusions

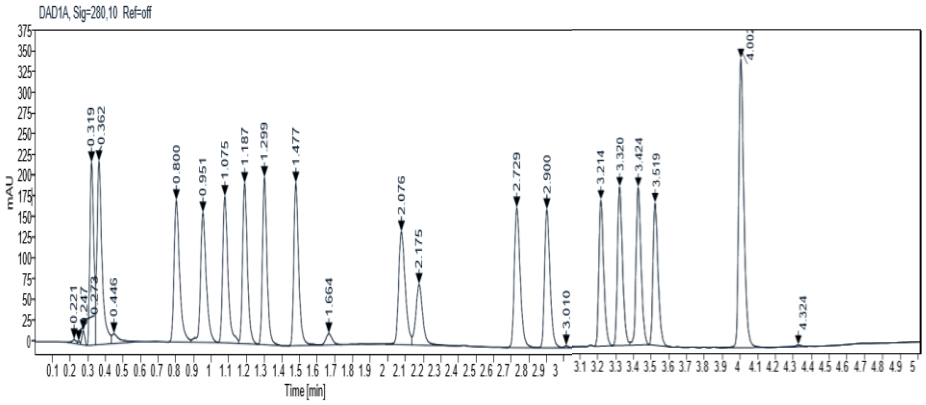
This study demonstrated that UV can be used as a powerful tool to boost the commercial value of *Mentha spicata*. UV induces key components of flavour and colour in mint including terpenoids, that are widely used in the pharmaceutical, cosmetic and food industries. It also demonstrates that the effects of UV persist after the end of the UV treatment, therefore the application can directly benefit consumers.

4.6 Supplementary information

4.6.1 Materials and Methods

4.6.1.1 Amino Acids

The composition of the mobile phases, named A and B, was acetonitrile and sodium acetate 25 mM, respectively. The program consisted of a linear gradient from 0 to 88% B from 0.00 to 0.30 minutes, followed by a decrease to 86% B from 0.30 to 1.00 minutes. Then, isocratic conditions were applied, and these consisted of 86% B from 1.00 to 1.3 minutes, followed by a linear decrease to 79% B from 1.3 to 2.2, then a further decrease to 69% B from 2.2 to 3.5 minutes and finally a linear gradient to 92% B from 3.5 to 5.1 minutes. The contents of individual amino acids were determined using a diode-array detector (DAD) at 280 nm, autosampler temperature 10 °C, column compartment temperature 18 °C and 0.613 mL min^{-1} flow rate.



Supplementary Figure 4.1*. Amino acid profile chromatogram detected with the HPLC detected from the highest concentration of the calibration standard.*

4.6.1.2 Phenolic acids and flavonoids

The column temperature was set at 30 °C and the flow rate was 0.3 mL min^{-1}. The eluents used for the mobile phase were acetonitrile (solvent A), and H_2O with 0.1% acetic acid (solvent B). Briefly, separation started in isocratic conditions with 10% A and 90% B for five minutes, followed by a 15 minute linear gradient to achieve 90 %

A, followed by maintaining these same conditions for five minutes. Then, in the final five minutes the gradient was reversed to achieve the initial condition of 10% A and 90% B. Compounds were analyzed in a positive and negative ion mode using HRMS in full scan mode. The mass range for the positive mode was set between 50-1000 m/z while for the negative mode between 65 and 1000 m/z.

Supplementary Table 4.1. *Retention time of the amino acids analysed from the calibration standard mix.*

Retention time (minute)	***Compound***
0.319	Aspartic acid
0.362	Glutamic acid
0.800	Serine
0.951	Histidine
1.075	Glycine
1.187	Threonine
1.299	Arginine
1.477	Alanine
1.664	Proline
2.175	Tyrosine
2.900	Valine
3.214	Methionine
3.320	Cysteine
3.424	Isoleucine
3.519	Leucine
4.002	Phenylalanine
4.324	Lysine

4.6.1.3 Phytosterols

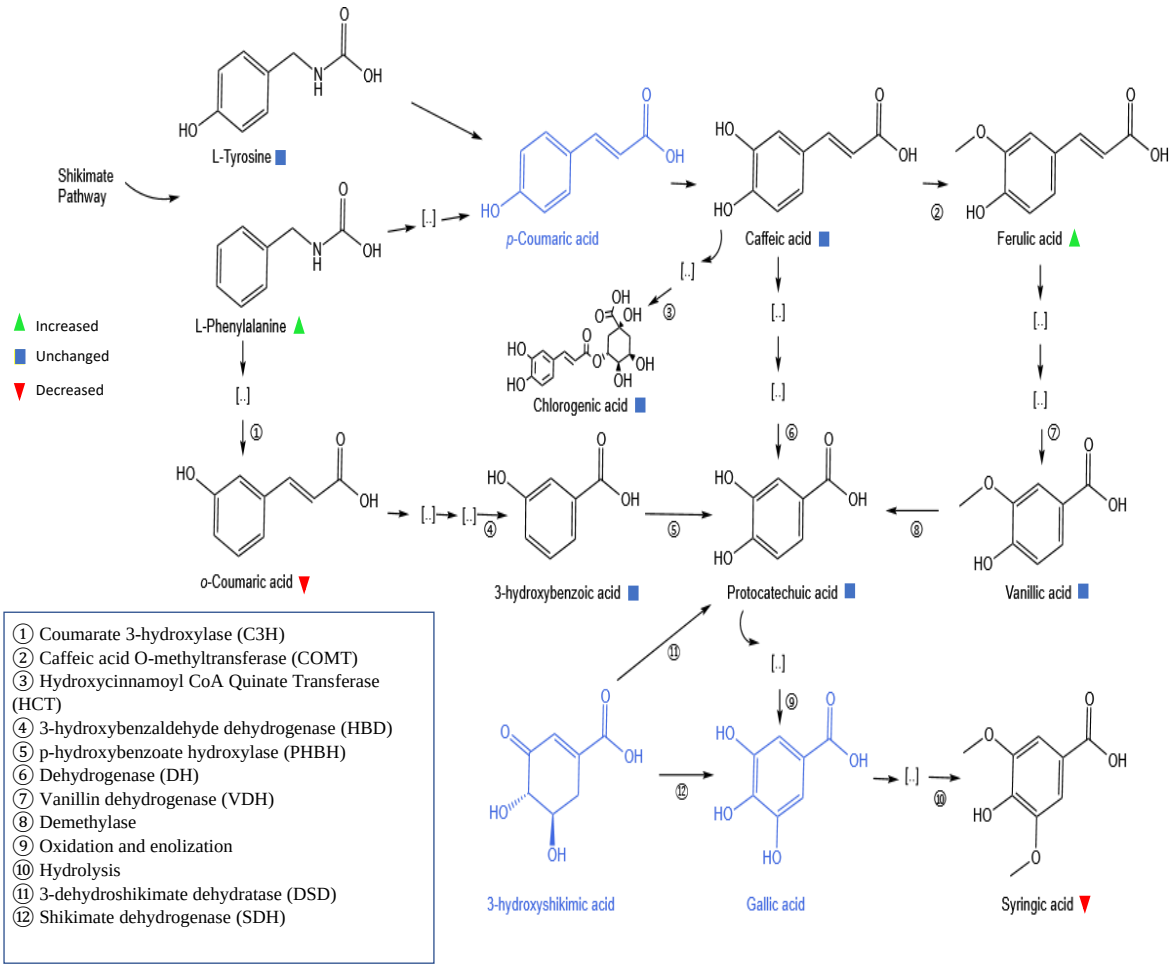
For phytosterols, a 15 min temperature programme was comprised of 1 min at 220 °C followed by a gradual increase at a rate of 6 °C min^{-1} to 290 °C (isothermal for 2.5 min). The injection temperature was 280 °C, the flow rate of the carrier gas (helium) was 1 mL min^{-1}, and the mode was spitless. The temperature of both the mass spectrometry transfer line and the ion source was set at 250 °C.

4.6.1.4 Monoterpenes and sesquiterpenes

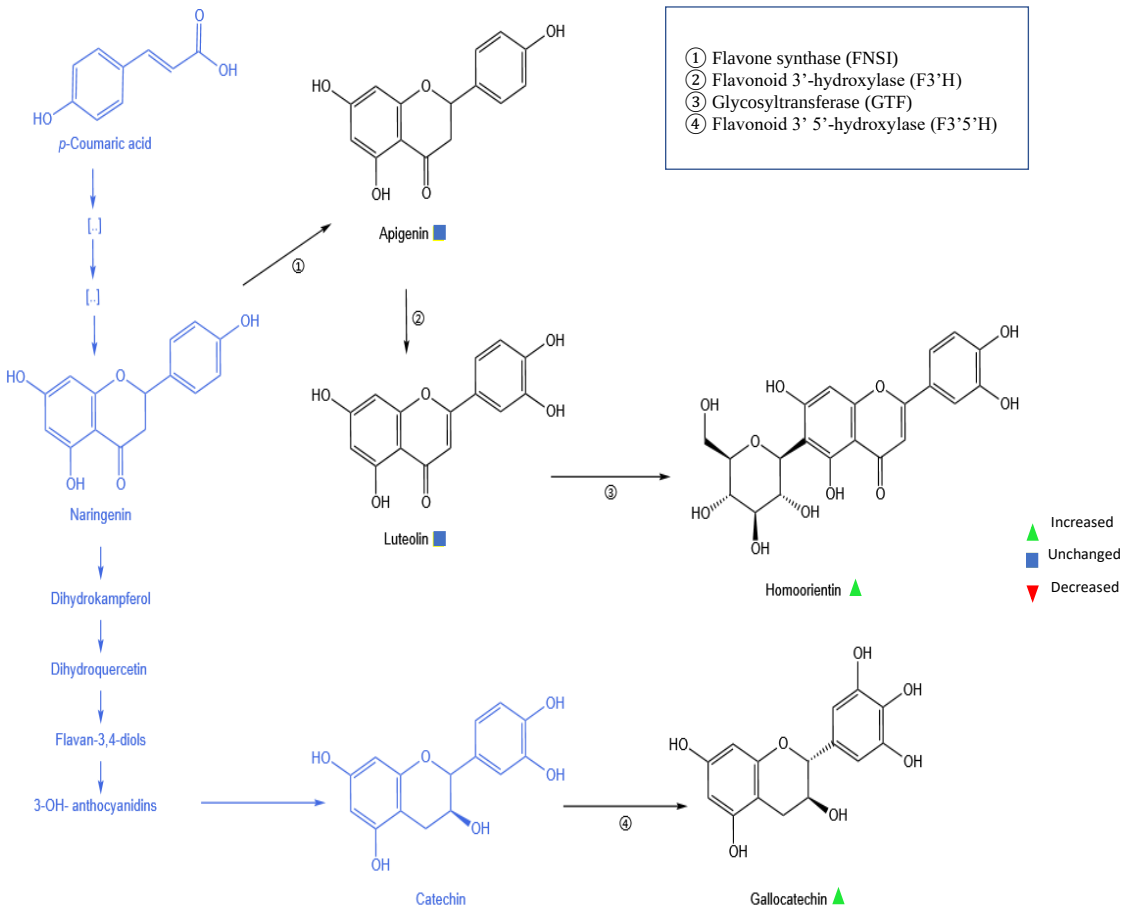
The GC was fitted with a 30 m column with 0.25 mm internal diameter and 0.25 µm film thickness (Rxi-5Sil MS). Extract (1 µL) was injected in spitless mode. The method was set up with an inlet temperature of 250 °C and helium carrier gas flow rate of 1 mL min^{-1} and 100 kPa pressure. The oven temperature was held at 60 °C for two minutes followed by a temperature gradient of 10 °C min^{-1} to 220 °C and finally an increase of 30 °C min^{-1} to 300 °C. The interface temperature was set at 250 °C. The mass spectrometer was used in scan mode with scans every 0.15 s and range between 40 and 200 m/z, and the ion source temperature was set at 200 °C.

4.6.1.5 Carotenoids and tocopherols

The eluents used for the mobile phase were methanol (solvent A), 80% methanol with 0.2% of ammonium acetate (v/v) (solvent B), and tert-methyl butyl ether (MTBE) (solvent C). Briefly, the gradient program of the mobile phase consisted of 95% A and 5% B in isocratic conditions for 12 minutes, followed by a linear gradient to 30% A and 65% C, from 12 to 30 minutes, and finally a linear gradient to 95% A and 0% C from 30 to 60 minutes. Solvent B was held in isocratic conditions in isocratic condition from 12 to 60 minutes. The maximum pressure was set at 1300 Bar. Carotenoids and chlorophylls were quantified with the DAD set at 280 nm, 450 nm and 650 nm while tocopherols were quantified with the FLD detector at 290 nm with emission at 330 nm. The autosampler temperature was set at 4 °C, column compartment temperature at 25 °C and a 1 mL min^{-1} flow rate.



Supplementary Figure 4.2. *Biosynthetic pathway of some phenolic acids via shikimic acid pathway. Molecules coloured in blue represent important intermediate, while the square brackets ([..]) represent the presence of intermediates the structure of which is not reported. Numbers identify the enzymes involved in the pathway while the triangles and the square symbols represent if the metabolites significantly increase, is unchanged or decrease in plants exposed to UV. The pathway is adapted from the KEGG pathway.*



Supplementary Figure 4.3. *Biosynthetic pathway of some flavonoids via shikimic acid pathway. Molecules coloured in blue represent important intermediate, while the square brackets ([..]) represent the presence of intermediates the structure of which is not reported. Numbers identify the enzymes involved in the pathway while the triangles and the square symbols represent if the metabolites significantly increase, is unchanged or decrease in plants exposed to UV. The pathway is adapted from the KEGG pathway.*

***Supplementary Table 4.2**. Heat map of secondary and primary metabolites measured after seven days of UV exposure. Class of compound include phenolic acid (peak area g^{-1} DW), flavonoid (peak area g^{-1} DW), monoterpene (µg g^{-1} FW), sesquiterpene (µg g^{-1} FW), carotenoid (µg mg^{-1} FW), chlorophyll (µg mg^{-1} FW), tocopherol (µg mg^{-1} FW) and amino acid (µg g^{-1} FW) Colours indicate the fold change between –UV and +UV plant. Values in red indicated a reduction in +UV treatment compared to –UV, value in blue compared a increment in +UV compared to –UV. Asterisks were reported instead of values when was not possible to calculate the fold change.*

Compound	Class	-UV	+UV	Fold change	Compound	Class	-UV	+UV	Fold change
3-coumaric acid	Phenolic acid	1842395	896051	-0.51	*(+)-epi-bicyclosesquiphellandrene*	Sesquiterpene	0.02	0.12	5.28
Ferulic acid	Phenolic acid	8103240	17389101	1.15	*Germacrene D*	Sesquiterpene	0.20	0.70	2.53
Caffeic acid	Phenolic acid	1892701213	1941838668	0.03	*Cubenol*	Sesquiterpene	0.06	0.12	0.94
Chlorogenic acid	Phenolic acid	2017041	376547	-0.81	*Neoxanthin*	Carotenoid	44.80	35.12	-0.22
3-hydroxybenzoic acid	Phenolic acid	2385564	1594233	-0.33	*Violaxanthin*	Carotenoid	33.50	23.20	-0.31
Vanillic acid	Phenolic acid	9140974	7316285	-0.20	*Antheraxanthin*	Carotenoid	10.68	6.98	-0.35
Protocatechuic acid	Phenolic acid	8106458	4610704	-0.43	*Lutein*	Carotenoid	44.20	39.36	-0.11
Syringic acid	Phenolic acid	72472022	15815060	-0.78	*Zeaxanthin*	Carotenoid	0.38	0.40	0.06
Apigenin	Flavonoid	9379657	14517467	0.55	*β-carotene*	Carotenoid	10.94	7.06	-0.35
Luteolin	Flavonoid	8693925	8617143	-0.01	*Chlorophyll a*	Chlorophyll	108.27	106.46	-0.02
Homoorientin	Flavonoid	1589815	4766532	2.00	*Chlorophyll b*	Chlorophyll	256.97	193.46	-0.25
(-)-epigallocatechin	Flavonoid	2694149	18700678	5.94	*α-tocopherol*	Tocopherol	14.27	16.61	0.16
α-pinene	Monoterpene	0.03	0.07	1.24	*δ-tocopherol*	Tocopherol	0.41	0.81	0.98
Thujene	Monoterpene	0.03	0.08	1.97	*γ-tocopherol*	Tocopherol	3.22	10.04	2.12
β pinene	Monoterpene	0.06	0.18	2.00	*Glycine*	Amino acid	1.19	1.67	0.40
Myrcene	Monoterpene	1.01	3.49	2.46	*Alanine*	Amino acid	1.20	1.71	0.43
Limonene	Monoterpene	0.14	1.71	11.62	*Valine*	Amino acid	0.30	1.40	3.71
Eucalyptol	Monoterpene	0.18	0.19	0.10	*Leucine*	Amino acid	1.57	2.22	0.41
α-linalool	Monoterpene	0.01	0.80	81.61	*Isoleucine*	Amino acid	0.79	1.09	0.37
Limonene oxide	Monoterpene	0.00	0.17	*	*Proline*	Amino acid	0.71	0.49	-0.32
β-terpineol	Monoterpene	0.02	0.07	3.63	*Methionine*	Amino acid	1.16	0.34	-0.70
p-menth-8-en-3-one, trans	Monoterpene	2.16	2.81	0.30	*Phenylalanine*	Amino acid	0.90	1.36	0.51
p-menth-1-en-4-ol	Monoterpene	0.00	0.03	*	*Tyrosine*	Amino acid	0.00	0.32	146.11
α-terpineol	Monoterpene	0.00	0.83	*	*Serine*	Amino acid	0.67	1.25	0.86
Pulegone	Monoterpene	20.33	21.35	0.05	*Threonine*	Amino acid	0.53	1.05	0.96
p-mentha-1,8-dien-3-one	Monoterpene	5.68	3.80	-0.33	*Cysteine*	Amino acid	0.01	0.02	0.45
cis-jasmone	Sesquiterpene	0.33	2.07	5.33	*Aspartic acid*	Amino acid	1.66	2.82	0.70
β-cubebene	Sesquiterpene	0.01	0.09	7.04	*Glutamic acid*	Amino acid	2.45	4.24	0.73
Caryophyllene	Sesquiterpene	0.06	0.25	3.17	*Arginine*	Amino acid	1.98	2.81	0.42
Cadinene	Sesquiterpene	0.01	0.09	5.28	*Histidine*	Amino acid	0.35	0.57	0.64
α-famesene	Sesquiterpene	0.03	0.11	2.68	*Lysine*	Amino acid	1.26	1.77	0.40

Supplementary Table 4.3. *Fold change of metabolites content measured after the UV and after transplanting both in –UV and + UV treatments.*

Compound	*–UV Fold change*	*+UV Fold change*	*Compound*	*–UV Fold change*	*+UV Fold change*
3-coumaric acid	0.59	0.17	***Neoxanthin***	0.30	0.65
Ferulic acid	2.82	0.20	***Violaxanthin***	0.23	1.07
Caffeic acid	1.59	1.10	***Antheraxanthin***	0.06	1.01
Chlorogenic acid	-0.26	0.73	***Lutein***	0.16	0.55
3-hydroxybenzoic acid	-0.52	-0.24	***Zeaxanthin***	-0.10	0.22
Vanillic acid	0.22	0.26	***β-carotene***	0.55	1.60
Protocatechuic acid	0.01	0.22	***Chlorophyll a***	1.29	1.29
Syringic acid	-0.07	2.03	***Chlorophyll b***	0.21	0.75
Apigenin	3.15	0.55	***α-tocopherol***	-0.69	-0.70
Luteolin	3.00	0.71	***δ-tocopherol***	0.24	-0.63
Homoorientin	-0.85	-0.59	***γ-tocopherol***	0.71	-0.65
(-)-epigallocatechin	-0.91	-0.46	***Glycine***	0.62	-0.08
α-pinene	3.16	1.48	***Alanine***	0.62	-0.09
Thujene	4.68	1.86	***Valine***	4.56	-0.05
β pinene	5.77	1.92	***Leucine***	0.73	-0.03
Myrcene	6.38	0.17	***Isoleucine***	0.60	-0.04
Limonene	26.76	1.37	***Proline***	-0.23	0.31
Eucalyptol	3.50	2.83	***Methionine***	-0.73	-0.36
α-linalool	32.68	-0.61	***Phenylalanine***	0.91	-0.01
Limonene oxide	100.00	0.48	***Tyrosine***	419.65	1.48
β-terpineol	9.87	0.85	***Serine***	1.26	-0.02
p-menth-8-en-3-one, trans	0.41	-0.29	***Threonine***	1.66	0.13
p-menth-1-en-4-ol	100.00	1.00	***Cysteine***	0.93	0.02
α-terpineol	100.00	3.75	***Aspartic acid***	0.83	-0.07
Pulegone	0.15	0.06	***Glutamic acid***	0.72	-0.18
p-mentha-1,8-dien-3-one	1.80	2.12	***Arginine***	0.00	-0.40
cis-jasmone	10.96	0.64	***Histidine***	1.05	0.02
β-cubebene	8.05	0.32	***Lysine***	0.61	-0.06
Caryophyllene	6.11	-0.47	***Campesterol***	-0.58	-0.15
Cadinene	5.14	-0.33	***Stigmasterol***	-0.70	-0.32
α-famesene	8.96	0.32	***β-sitosterol***	-0.65	-0.13
(+)-epi-bicyclosesquiphellandrene	8.21	0.14	***Fucosterol***	-0.68	-0.52

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Chapter 5

Impact of different UV wavelengths on the essential oil profile of spearmint

Abstract

The elucidation of photoreceptors signalling pathways, paired with recent advancements in the field of LED lighting technology, has created an exciting opportunity to use narrowband UV wavelengths to induce wavelength-specific responses in plants of interest. This allows both researchers and commercial growers to target specific plant metabolites by matching the most effective wavelengths that induce accumulation, whilst potentially avoiding other undesired UV responses. The aim of this study was to investigate the effect of different narrowband UV-A and UV-B wavelengths on the content of monoterpenes and sesquiterpenes as well as the expression of key genes encoding enzymes involved in the terpenoid biosynthesis pathway. Mint plantlets were treated with different UV-A and UV-B wavelengths emitted by single wavelength LEDs with peaks at 310 nm, 325 nm, 340 nm, and 365 nm. UV at an intensity of ~46 ± 2 μW cm^{-2} which was applied for 6 hours for eight days. It was found that the application of different UV wavelengths induced a differential synthesis of monoterpenes and sesquiterpenes with shorter wavelengths, particularly 310 nm and 325 nm, being more effective than others depending on the specific metabolite. It was also found that all the UV treatments altered the expression of important genes involved in the terpenoid biosynthesis pathway, relative to the control plants that received only a combination of red and blue light. The results of the current study show that by applying different wavelengths of UV, it is possible to differentially manipulate the composition of the mono and sesquiterpenes. This offers an important instrument to target the compounds of interest with precision, avoiding compounds that lower the commercial value of the final product.

5.1 Introduction

Light is a key factor that regulates plant growth and development. Plant responses to light are driven by different wavelength-specific photoreceptors that trigger molecular, morphological and metabolic changes (Trivellini et al., 2023). Phytochromes are involved in perceiving red, far-red and blue light, while phototropins and cryptochromes mediate responses to blue light as well as UV-A. Both UV-B and UV-A up to 350 nm, are sensed by a UV photoreceptor called UV RESISTANCE LOCUS 8 (UVR8) (Rai et al., 2020). UVR8 controls various aspects of plant growth (Jenkins, 2017). For example, UVR8 mediates photomorphological responses such as leaf area growth, stem elongation and stem branching (Jenkins, 2014; Tossi et al., 2019). Besides alterations in the morphology, UVR8 also controls genes involved in plant defence, including key genes in the flavonoid and terpenoid biosynthesis pathways (Shamala et al., 2020). Phototropins (PHOTs) and cryptochromes (CRYs) are also implicated in modulating the synthesis of different classes of secondary metabolites such as carotenoids and flavonoids (Lingwan et al., 2023). Furthermore, phototropins (PHOT1 and PHOT2) co-regulate leaf expansion, chloroplast movement, and stomatal opening and they are also involved in shade avoidance responses and phototropism (Takemiya et al., 2005; Christie, 2007). Cryptochromes, especially CRY1 and CRY2, also co-regulate plant growth and development processes (Wang & Lin, 2020). Similarly to PHOTs, CRYs are involved in controlling stomata opening, as well as regulating shade avoidance but they are also involved in hypocotyl elongation and in promoting flowering (Wang et al., 2014). Moreover, CRYs co-regulate the plant response to different biotic and abiotic stressors (Wang et al., 2014; D'Amico-Damião & Carvalho, 2018).

The elucidation of photoreceptors signalling pathways, paired with recent advancements in the field of LED lighting technology, has created an exciting opportunity to use narrowband UV wavelengths to induce wavelength-specific responses in plants of interest (Massa et al., 2008; Mitchell et al., 2015). LEDs offer numerous advantages over mercury-based and high-pressure sodium (HPS) lamps which are widely used for research purposes (e.g., growth rooms) as well as in commercial horticulture to supplement natural light (e.g., glasshouses) (Bantis et al., 2018). LEDs are more compact than traditional light sources, use less energy relative to their output, generate less heat, and have a long operating lifetime (Liang & Sun, 2022). LEDs are available with various emission spectra of horticultural relevance. Red (R) and blue (B) LEDs have been available for a long time, and are still the most commonly utilised LEDs in horticultural applications, often used in combination to generate optimised Photosynthetically Active Radiation (PAR) (Massa et al., 2008). While the use of red and blue LEDs is well established, the commercial use of UV-LEDs is still limited, and this relates to the pioneering character of these LEDs, their cost, the limited output of light, and the limited lifespan. However, technological advances in UV-LED technology have in recent years sparked interest in horticultural applications of UV LEDs (Wargent, 2016). A major advantage of UV-emitting LEDs is the narrow output spectrum which enables precision manipulation of the plant exposure spectrum (Weiland et al., 2023). This allows both researchers and commercial growers to target specific plant metabolites by matching the most effective wavelengths that induce accumulation, whilst potentially avoiding other UV responses. For example, growth, phenolic content and antioxidant capacity increased significantly in kale plants supplemented with UV-A (LEDs with peaks at either 370 nm or 385 nm) but the effect was more pronounced at the longer wavelength (Lee et

al., 2019). Different morphological and metabolic responses under different wavelengths of UV-A (LEDs with peaks at 366 nm, 390 nm, and 402 nm) were also reported by Brazaitytė et al. (2019) in mustard microgreens. The authors found that a higher β-carotene and lutein content could be detected in plants treated for 10 hours with 366 nm UV-A, while longer wavelengths required longer exposure times. In contrast, α-tocopherol and phenol content was strongly affected by 402 nm UV-A. Therefore, it is clear that even though these wavelengths are close to each other, the difference between them is enough to generate a distinct physiological response. This demonstrates the importance of wavelength as well as the dose applied.

Recently, the effect of UV-B LEDs on plants has also begun to be investigated, yet the number of published studies remains limited (McLay et al., 2020; Palma et al., 2021; Yoon et al., 2021; Choi et al., 2022; Tsurumoto et al., 2022; Weiland et al., 2023). Similarly to the aforementioned UV-A studies, different UV-B wavelengths have different effects on plants. For example, Tsurumoto et al. (2022) demonstrated that shorter UV-B wavelengths generate a more pronounced response in *Arabidopsis* plants. Narrow waveband, 280 nm, LEDs upregulate more effectively the genes involved in the phenylpropanoid pathway as well as the contents of lipids, polyamines and organic acids, than 310 nm wavebands. Other studies have shown that specific narrow waveband UV-B LEDs can boost the content of flavonoids. For example, McLay et al. (2020) applied narrow wavelength UV-B with an emission peak at 300 nm to control downy mildew infection in lettuce plants. This UV-B wavelength induced flavonoids, and appears to be associated with the protective role against the disease. A consistent increase of flavonoid glycosides in lettuce plants was also found by Weiland et al. (2023) in both indoor and greenhouse conditions where plants were exposed to narrow-band UV-B LEDs with an emission peak at 307 nm.

Hence, UV-emitting LEDs increase the possibility of selecting and inducing specific classes of metabolites, unlike traditional light sources that produce a broadband output (i.e., HPS lamps) (Paradiso & Proietti, 2022). The latter generates a more generic metabolite response due to a wider spectrum (Weiland et al., 2023) unless optical filters are used (Aphalo et al., 2012).

Potentially, narrow waveband UV-emitting LEDs can be used to boost the content of metabolites, increasing the quality of the product and at the same time the nutritional value (Taulavuori et al., 2017). One crop where this may apply is mint. Volatile terpenoids such as monoterpenes and sesquiterpenes are the main component of mint essential oil (Snoussi et al., 2015) that is widely used in numerous industrial applications (Gupta et al., 2023). The aim of this study was to investigate the effect of different UV-A and UV-B narrowband LEDs (with emission peaks at 310 nm, 325 nm, 340 nm and 365 nm) on the content of monoterpenes and sesquiterpenes as well as the expression of key genes encoding enzymes involved in the terpenoid biosynthesis pathway. We hypothesized that different UV wavelengths can specifically influence monoterpene and sesquiterpene metabolism, leading to higher nutritional value and sensory quality.

5.2 Materials and Methods

5.2.1 Plant material

Mentha spicata L. (Moles Seeds Ltd., Stanway, U.K.) seeds, previously surface sterilised with 0.5% Triton X and bleach, were sown on half-strength Murashige & Skoog media without sugar. Before being exposed to UV, plantlets were grown for 30 days under tissue culture conditions (RA40 plastic micro boxes, Sac 02, Deize, Belgium). Germination took place after 14 days, then seedlings were grown further 16 days until six leaves were formed. During this period, plants were exposed to ~50

μmol m^{-2} s^{-1} PAR for 14 h (07:00 -21:00) each day, provided by an LED lamp (AP673L, Valoya, Finland). Plants were kept at ~60% of relative humidity and 20 ± 2 °C. PAR was measured with a PAR meter (PAR special sensor, Skye Instrument Ltd, Powys, United Kingdom) while temperature and humidity were measured with a portable data logger (EL-USB-2, Lascar Electronics, Whiteparish, United Kingdom).

5.2.2 UV exposure

Plants were exposed to different UV-A and UV-B wavelengths emitted by single wavelength (WL) LEDs with peaks at 310 nm, 325 nm, 340 nm, and 365 nm (DUVXXX series,120° viewing angle, Roithner Electronic, Vienna, Austria). The spectral bandwidth at the half intensity was, between 303 and 317 nm (for 310 nm LEDs), between 318 and 330 nm (for 325 nm LEDs), between 332 and 343 nm (for 340 nm LEDs) and between 360 and 370 nm (for 365 nm LEDs). Individual UV WL were combined with red and blue LEDs (Roithner Electronic Vienna, Austria) to form separate devices. A device lacking UV LEDs was used as a control. The lamps were customised and built by the Electrical Engineering Department at University College Cork. In each unit, red, blue and UV wavelengths were dimmable and independently controlled. PAR was set up at ~33 ± 2 μmol m^{-2} s^{-1} with a ~0.75 R: B ratio. The UV intensity was set up at ~46 ± 2 μW cm^{-2}. The biologically effective dose calculated with the formula reported in Flint & Caldwell (2003) was 1.02 kJ m^{-2} under a 310 nm lamp, 0.151 kJ m^{-2} under a 325 nm lamp, 0.149 kJ m^{-2} under a 340 nm lamp and 0.107 kJ m^{-2} under a 365 nm lamp.

PAR was applied for 14 hours daily, between 07:00 and 21:00, while UV was applied for six hours daily between 10:00 and 16:00. In the current study, a low PAR background was used to avoid any interference of PAR in the UV-mediated production

of terpenoids. This enables a focus on the variations in UV-responses across different UV wavelengths.

UV was quantified using a spectroradiometer (Flame-S, Ocean Optics, Duiven, The Netherlands) combined with the software Oceanview version 1.6.7. A detailed heat map of the intensity for individual lamps and each WL is reported in supplementary table 5.1. The distance between the LEDs and the plants across the lamps was variable in order to achieve the same intensities of UV, red and blue. Plants were rotated daily for the duration of the experiment. After eight days of UV treatment plants were harvested, immediately frozen in liquid nitrogen, ground and stored at –80 °C until the analysis. Three tissue culture boxes per lamp were used for each replicate. A minimum of 20 plants were pooled together from different boxes and immediately frozen after the UV exposure. Results were obtained as the average of three independent replicates for all the metabolites analysed and the gene expression analysis.

5.2.3 Monoterpenes and sesquiterpenes analysis

Mono- and sesquiterpenes were characterized and quantified as detailed in Večeřová et al. (2021). 500 mg of fresh plant material, previously frozen in liquid nitrogen, was mixed with 4 mL of cold heptane and incubated for 24 hours at 4 °C. The extract, previously filtered, was evaporated to dryness with a stream of nitrogen. Then, 970 µL of heptane and 30 µL of bisabolol (100 µg mL^{-1}) as an internal standard (Sigma Aldrich, Arklow, Ireland) were added to each sample. The calibration curve was obtained by mixing different standards. The standard consists of a mix of linalool, α-pinene, (R)-(−)-α-phellandrene, (−)-β-pinene, myrcene, (R)-(+)-limonene, eucalyptol, ocimene, sabinene hydrate, (+)-terpinen-4-ol, (+)- pulegone, (−)-trans-caryophyllene, γ-terpinene, p-cymene and menthol (Sigma Aldrich, Arklow, Ireland) to reach the final

concentration of 250 µl mL^{-1}. Monoterpenes and sesquiterpenes were detected and quantified using gas chromatography equipped with a mass spectrometer (GC-MS). The method is summarised in table 5.1 following Večeřová et al. (2021).

Table 5.1. *GC-MS operating conditions used for the extraction of monoterpenes and sesquiterpenes.*

Parameter	***Condition***
Column type	Rxi-5Sil MS, 30 m x 0.25 mm ID x 0.25 µm
Pressure	100 kPa
Carrier gas	Helium
Injection volume	1 µL
Injection mode	Spitless
Flow rate	1 mL/ min
Initial oven temperature	60 °C for 2 min
Program temperature	10 °C min^{-1} to 220 °C 30 °C min^{-1} to 300 °C
Interface temperature	250 °C
Analysis mode	Scan, every 0.15 s, range 40 – 200
Ion source temperature	200 °C

5.2.4 RNA extraction and cDNA synthesis

RNA was extracted from frozen plant material, previously ground and stored at –80 °C, using Qiagen RNeasy plant mini kits (Qiagen, Manchester, UK) following the manufacturer protocol. An on-column DNase digestion was performed during the RNA extraction using RNase-Free DNase Set (Qiagen, Manchester, UK). RNA integrity was evaluated by subjecting the samples to agarose gel electrophoresis (1% gel in 0.5X TPE) (Figure 5.1 A). Briefly, 2 µL of the sample was mixed with 10 µL of formamide, 3 µL of formaldehyde, 2.5 µL of MEN buffer and a drop of ethidium bromide and heated for 10 minutes at 65 °C. RNA quality and concentration were determined with a Nanodrop ND -1000 (Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed using the AffinityScript qPCR cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA, USA) following the

manufacturing instructions. *Actin-41* was used as a reference gene based on a previous study on *Mentha* genus and tested on the cDNA of the samples as shown in figure 5.1 B. The primers of the genes of interest were either found in previous studies as reported in table 5.2 or designed with the help of Primer3. In the latter case, sequences of homologous genes were identified in GenBank by searching for species belonging to the same botanical family. Sequences were then aligned using ClustalW. Primers were purchased from IDT (Integrated DNA Technologies, Leuven, Belgium) and their sequences are reported in table 5.2. Before proceeding to the quantitative real time RT-PCR, primers were tested by agarose gel electrophoresis (1% gel in 0.5X TPE) with the addition of SYBR safe stain (1 µL / 10 mL gel).

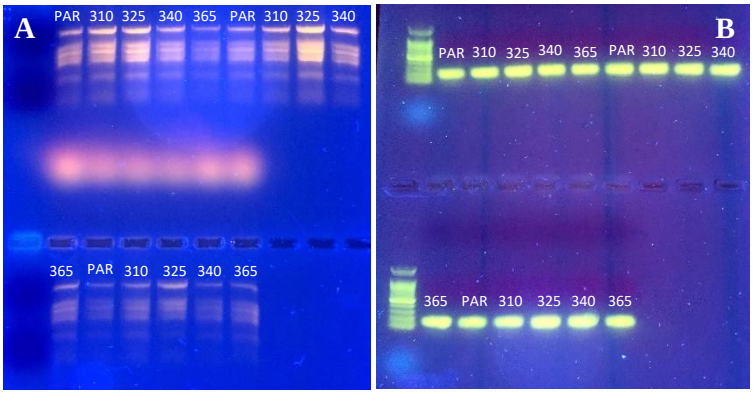


Figure 5.1. *RNA integrity of the samples was tested running the RNA extract on an agarose gel (**A**). cDNA synthesised from the samples was tested with the housekeeping gene Actin-41 (**B**). Numbers above lanes refer to exposure to PAR, or different UV wavelengths in nm.*

5.2.5 Quantitative real time RT-PCR analyses

Gene expression analysis was conducted using a Real-Time PCR System (7500, Applied Biosystem, Waltham, USA) by mixing the cDNA dilution (1:4) with primer pair and SYBR Green Extaq II (Takara Bio, Saint-Germain-en-Laye, France). ROX was used as a reference dye. The amplification program consists of 94 °C for 30

seconds followed by a cycling stage of 40 cycles at 94 °C for 10 seconds and 60 °C for 30 minutes. The melt curve stage consists of an initial phase at 94°C held for 15 seconds, followed by a decrease to 60 °C for 1 minute, a ramp to 95 °C for 15 seconds and a final decrease to 60 °C for 15 seconds. Prior to the formal analysis, serial dilutions of cDNA (1:4, 1:16 and 1:64) were performed for each primer set to assess their efficiency. Only primers with an efficiency above 80% were considered for the gene expression analysis.

Table 5.2. *Selected reference genes and genes involved in the monoterpenes and sesquiterpenes pathways present in Mentha spicata and primer sequences used for gene expression analysis using quantitative real-time PCR with efficiency above 80%.*

Gene Name	Abbreviation	Source species	Primer sequence 5'-3'	Reference
Actin-41	*ACT*	*Mentha piperita* L.	For: CTACGAAGGCTACGCACTCC rev: GCAATGTAGGCCAGCTTCTC	(Rahimi et al., 2017)
1-deoxy-d-xylulose-5-phosphate synthase	*DXS*	*Mentha piperita* L.	For: CCACCAGGCTTACCCACACAA rev: GCCACCGCCATCCCTAAAC	(Dolzhenko et al., 2010)
Geranyl pyrophosphate synthase	*GPPS*	Multi alignment	For: TCGGAGGGAATAATAAGCGG rev: CTTCTGAATCTCCTCCTCGG	Primer3
Limonene synthase	*LS*	*Mentha piperita* L.	For: CGGTGGTGGAGAAATACTGGGTTT rev: CCGTAATCAGAGCGTGACTTTGC	(Dolzhenko et al., 2010)
(−)-limonene-3-hydroxylase	*L3OH*	*Mentha piperita* L.	For: CCCCATCACCACCAACTCCA rev: CCCCATCACCACCAACTCCA	(Dolzhenko et al., 2010)
Isopiperitenol dehydrogenase	*IPD*	*Mentha canadensis* L.	For: GGGATTAGGGTTAACAGCGT rev: GGTAAACACTGCAATCCACC	(Yu et al., 2021)
Farnesyl Pyrophosphate synthase	*FPPS*	*Mentha piperita* L.	For: GGAGAACCATCCAACTGTAA rev: GCCTTTACAACCAGCCAAGA	(Dolzhenko et al., 2010)
Putative sesquiterpene synthase	*S-TPS*	*Mentha piperita* L.	For: GGAGAACCATCCAACTGTAA rev: GCCTTTACAACCAGCCAAGA	(Dolzhenko et al., 2010)

5.2.6 Statistical analysis

Statistical analyses were performed with SPSS IBM SPSS Statistic v28 (Armonk, New York, US). A two-tailed t-test was used to identify significant differences between the

control and the UV treatments. One-way ANOVA was used to compare the different terpenoid content while a t-test was used to compare each individual group with the control. Data are reported as mean ± SE (Standard Error).

5.3 Results

Mint plants were exposed to different UV treatments using UV-emitting LEDs with emission peaks at 310 nm, 325 nm, 340 nm and 365 nm. After eight days the content of monoterpenes and sesquiterpenes was measured. In parallel, the gene expression of the key genes of their biosynthetic pathway was analysed.

5.3.1 Monoterpene and sesquiterpene content

Monoterpenes and sesquiterpenes were quantified using GC-MS. The analysis revealed the presence of nine monoterpenes and seven sesquiterpenes. The majority of monoterpenes tended to be present at higher concentrations in plants exposed to 310 nm (Figure 5.2) while the majority of sesquiterpenes tended to be higher in both 310 nm and 325 nm exposed plants (Figure 5.3). Results of one-way ANOVA showed that significant differences across different wavelengths on individual metabolites were found only in thujene ($p = 0.020$) and β-pinene ($p = 0.045$). Post hoc test results revealed that the mean value of thujene and β-pinene was significantly different between PAR and 310 nm lamps ($p = 0.013$ & $p = 0.027$).

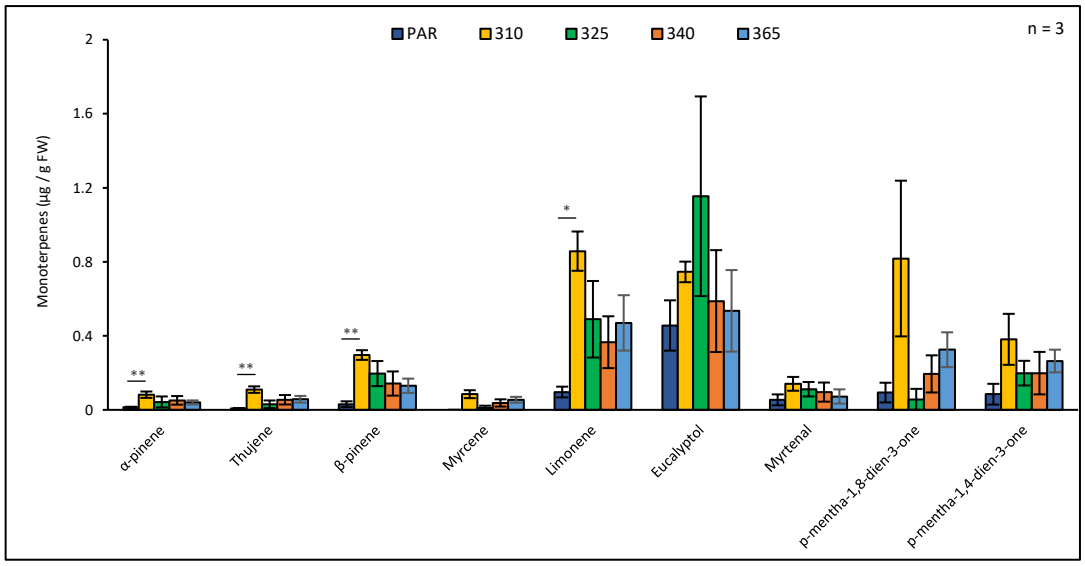


Figure 5.2. *Monoterpene content (µg/g FW) measured eight days after UV exposure. Blue bars represent plants not exposed to UV (PAR), yellow bars plants exposed to 310 nm, light green bars to 325 nm, orange bars to 340 and light blue bars to 365 nm. Asterisks indicate a significant difference between the UV treatment and the control of individual metabolites with $p < 0.05$ (*) or $p < 0.01$ (**). Error bars represent the SE based on the means of three independent replicates.*

When comparing the effects of exposure to individual UV wavelengths with the PAR control, only exposure to 310 nm caused significant effects. The 310 nm treatment significantly enhanced the content of some monoterpenes such as α-pinene (p = 0.001), thujene ($p < 0.001$), β-pinene (p = 0.007) and limonene (p = 0.023) while sesquiterpenes were not significantly affected by supplemental UV.

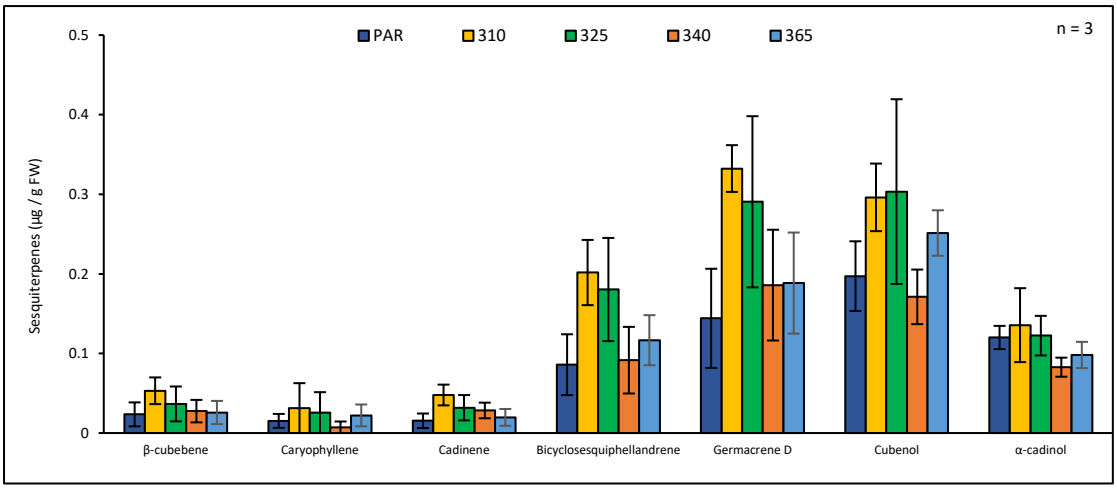


Figure 5.3. *Sesquiterpenes content (µg/g FW) measured eight days after UV exposure. Blue bars represent plants not exposed to UV (PAR), yellow bars plants exposed to 310 nm, light green bars to 325 nm, orange bars to 340 and light blue bars to 365 nm. Asterisks indicate a significant difference between the UV treatment and the control with $p < 0.05$ (*). Error bars represent the SE based on the means of three independent replicates.*

5.3.2 Gene expression analysis

Gene expression analysis was conducted on genes encoding enzymes that are involved in the monoterpene and sesquiterpene biosynthetic pathway. Expression of *DXS*, the first key gene involved in the Methylerythritol Phosphate (MEP*)* pathway leading to the formation of isoprenoids, was significantly upregulated in all the UV treatments when compared to the PAR control. Results showed an increase of 14.1-fold in *DXS* relative gene expression in plants exposed to 310 nm (p = 0.013) UV-B, followed by an increase of 10.2 times in plants exposed to 340 nm (p = 0.005), and an increase of 5.8 and 4.2 times in plants exposed to 325 nm and 365 nm respectively (p = 0.006 & p = 0.032). The expression of *LS*, a gene involved in the cyclization of *GGPS* to limonene, was also positively influenced by all the UV treatments, it was statistically different in plants exposed to 310 nm, 325 nm, 340 nm and 365 nm UV when compared with the PAR control. *LS* expression increased 20.1-fold in plants exposed to the 340 nm UV treatment (p = 0.001), while an increase of 9.6 and 6.1 times was

recorded in 310 nm (p = 0.001) and 325 nm (p = 0.003) treated plants respectively. *L3OH*, a gene that catalyses the enzymatic conversion of limonene to *trans*-isopiperitenol, was similarly significantly upregulated by 310 nm, 325 nm , 340 nm and 365 nm treatments. A wavelength of 310 nm induced an increase in gene expression of 20.3 times ($p < 0.001$), followed by 15.8 times in the 340 nm ($p < 0.001$) and 8.1 in the 325 nm treated plants (p = 0.002). The gene expression of *IPD*, a gene involved in the enzymatic catalysis to isopiperitenone, was also enhanced by all the UV treatments. *IPD* expression was upregulated 16 times following 310 nm UV treatment (p = 0.009), 12.4 times following 325 nm treatment (p = 0.004), 19.7 times following 340 nm treatment (p = 0.002) and 8.5 times following 365 nm treatment (p = 0.011).

FPPS was upregulated by all the UV treatments. FPPS catalyses the production of farnesyl pyrophosphate by condensing an isoprene unit to geranyl pyrophosphate. When compared to the PAR control, the expression of *FPPS* was higher in plants exposed to the 340 nm treatment with an increase of 37.85 times (p = 0.003) followed by 17.27 times in 325 nm UV plants (p = 0.001). Exposure to 365 nm and 310 nm UV radiation resulted in, respectively, an upregulation of *FPPS* expression of 13.90 and 9.75 times ((p = 0. 002 & p = 0.006).

Expression levels of *GPPS* and *S-TPS* genes in UV-treated plants were not significantly different in control and UV treatments. GGPS catalyses the condensation of two isoprene units to form geranyl pyrophosphate while S-TPS is involved in the enzymatic catalysis to germacrene D (Figure 5.4).

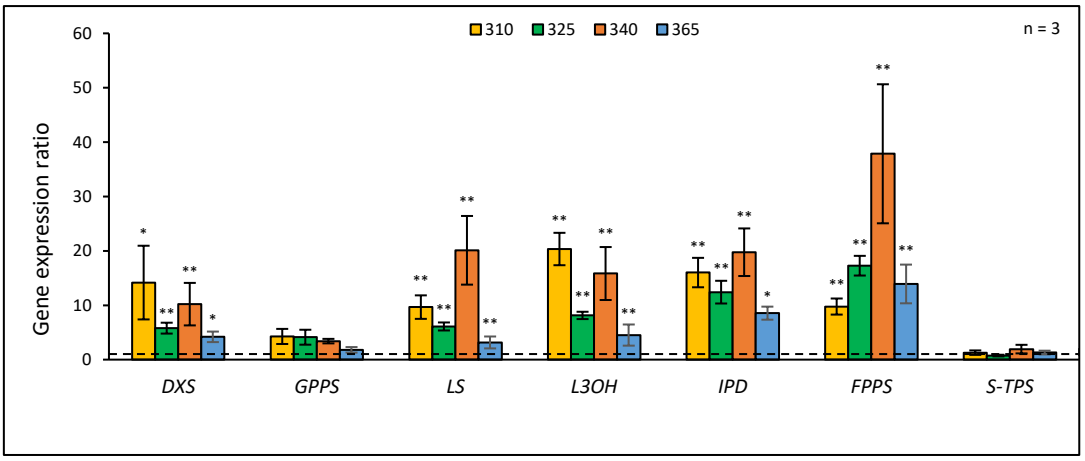


Figure 5.4. *Gene expression of genes involved in the monoterpenes and sesquiterpenes pathway. Yellow bars represent plants exposed to 310 nm, green bars to 325 nm, orange bars to 340 and light blue bars to 365 nm. Dashed line represents the control plants exposed to PAR. Asterisks indicate a significant difference between the UV treatment and the control with $p < 0.05$ (*) or $p < 0.01$ (**). Error bars represent the SE based on the means of three independent replicates.*

5.4. Discussion

The present study showed that the application of different UV wavelengths induced a differential synthesis of volatile monoterpenes and sesquiterpenes. However, some wavelengths were more effective than others depending on the specific metabolite.

5.4.1 UV treatment influenced the terpenoid biosynthesis

The production and accumulation of secondary metabolites, particularly essential oils, occurs in specialised structures present on the leaf surface, trichomes. These structures offer a protective role in mediating the interaction between the plant and the environment (Wang et al., 2021). Indeed, it has been reported that variation in trichome density might have a central role in the defence and tolerance against different biotic and abiotic stressors (Wang et al., 2021). As such, it is expected that UV exposure could induce the production of trichomes and alter the composition and quantity of their content.

A combination of broadband UV-B and UV-A has been found to induce an overall accumulation of mono and sesquiterpenes content (Crestani et al., 2024). Similarly, Maffei & Scannerini (2000) showed a significant increase of monoterpenes such as α-pinene, β-pinene, myrcene and limonene in *Mentha piperita* plants exposed to broadband UV-B. In another study, the same authors found UV-A regulated the content of essential oil constituents differently compared to UV-B (Maffei et al., 1999). UV-A enhanced the content of α-pinene but no differences were found in β-pinene and myrcene content and limonene content was reduced compared to the control (Maffei et al., 1999). However, applications of broadband UV can easily mask wavelength-specific effects. Therefore, the current study was done using narrow waveband, UV-emitting LEDs.

Our findings showed that the monoterpene content was mainly increased in plants exposed to narrowband UV-B, while UV-A seems to play a minor role. These findings are similar to those of Jadidi et al. (2023) who found an increase in essential oil content by exposing *Pelargonium graveolens* to broadband UV-B while plants treated with broadband UV-A were similar to the control. In contrast, our data demonstrated that monoterpenes were induced by all the UV wavelengths when compared to the visible light control. However, it is notable that some compounds including α-pinene, β-pinene, thujene and limonene are enhanced under 310 nm while eucalyptol responded better to 325 nm wavelengths.

Sesquiterpene content was lower compared to monoterpene content across all the UV treatments. Sesquiterpenes in our samples showed a more pronounced response at shorter UV wavelengths such as at 310 nm and 325 nm while longer UV-A wavelengths such as 340 nm and 365 nm showed a less pronounced effect. Nitz & Schnitzler (2004) also found that UV-B increased the content of germacrene D in

Ocimum basilicum leaves. On the contrary, UV-B did not have an effect on germacrene D content but β-caryophyllene significantly increased in *Ocimum sanctorum* (Kumari & Agrawal, 2011). β-caryophyllene was also enhanced by UV-B treatment in different turmeric species (Jaiswal & Agrawal, 2021). Often, no effects of UV-A wavelengths are reported in the literature, however mint plants supplemented with UV-A (peak 365 nm) showed no difference in germacrene D and β-caryophyllene contents compared with the control (Maffei et al., 1999). Thus, the induction of sesquiterpenes is clearly UV-wavelength dependent.

The data presented in this paper show that responses are influenced by the spectral composition of the UV applied. These findings complement literature data showing that responses are influenced by the intensity and the duration of UV exposure, and can be either UVR8-dependent or independent (Casati & Walbot, 2003; Jenkins, 2017; Meyer et al., 2021). The results of our paper suggest that supplementation with shorter UV wavelengths (310 nm) is more effective in the overall increase of the content of monoterpenes and sesquiterpenes. Therefore, the question arises whether the accumulation of specific volatile terpenoids is associated with the photoprotective role of these compounds in the plants, a protective role that is particularly critical at shorter, more bioactive, wavelengths. While the involvement of phenolics and carotenoids in photoprotection is well documented, not much is reported about the photoprotective role of volatile terpenoids which include both mono and sesquiterpenes. However, it has been hypothesised that volatile terpenoids are involved in antioxidant defence and may have a protective effect on lipid membranes (Gil et al., 2013). Indeed, it has been reported that some monoterpenes such as α-pinene and β-pinene have good antioxidant capacity against oxidative stress (Loreto et al., 2004). Thus, it is speculated here that the measured increase in monoterpenes could be associated with the protection of the

chloroplast membranes against UV-B. Indeed, it has been reported that chloroplasts are one of the main targets of UV-B radiation (Strid et al., 1994) and since monoterpenes are synthesized in the plastids (Li et al., 2023) they can possibly contribute to UV-B-induced stress tolerance. The fact that sesquiterpenes are synthesised in the cytosol (Li et al., 2023) can explain why their response was less pronounced than monoterpenes.

In the absence of stress, the induction of mono and sesquiterpenes could be hypothesised to be associated with the UVR8-dependent regulation of gene expression. However, to date, there is no evidence in the literature that these compounds are synthesised in a UVR8-dependent manner. Compounded with evidence found in this study, whereby initial data demonstrated a lack of clear evidence of UVR8 involvement in this regulation of associated transcription factor such as COP1 and PIF-1 and the content of metabolites, highlights the need to explore this signalling cascade in plants other than the model *A. thaliana*.

5.4.2 Assessing gene expression under different narrow band UV wavelengths

It has been shown that there are many molecular pathways involved in plant responses to UV-B radiation, involving different sets of genes (Jenkins, 2017). This study shows that all the UV treatments altered the expression of important genes involved in the terpenoid biosynthesis pathway, relative to the control plants that received only a combination of red and blue light. *DXS* is involved in an early step of the terpenoid pathway (see Figure 5.5). The overexpression of this gene can potentially explain the UV-induced overall increase in monoterpenes and sesquiterpenes content at a molecular level. In line with our results, a UV-mediated up-regulation of *DXS* was

also observed in other studies (Nazari & Zarinkamar, 2020; Tripathi et al., 2021). *GPPS* and *FPPS* are key enzymes that initiate monoterpene and sesquiterpene synthesis, respectively, their upregulation should reflect an increase in the content of monoterpenes and sesquiterpenes. However, while an increment in the content of monoterpenes was found in most of the UV treatments no difference in *GPPS* expression was found when comparing the UV treatments with the PAR control. Dolzhenko et al. (2010) showed that in mint plants grown indoors, there was a UV-B mediated upregulation in *FPPS* expression compared to *GPPS* while an opposite trend was recorded in plants grown in outdoor conditions. However, given the use of a broadband UV source and solar radiation, multiple UV wavelengths would have been present, so results do not necessarily contradict those in the current narrow waveband study. Moreover, the presence of other environmental stressors could have affected the gene expression differently. Indeed, it is reported that not only UV but also variations in light intensity, temperature, drought and possible pests and pathogens interactions can alter the plants' response and thus the gene expression (Wang et al., 2023).

Gene expression is not always linked to changes in the corresponding metabolic end products (Lange et al., 2011). For example, a mismatch was found in *FPPS* gene expression versus sesquiterpene accumulation. Although the content of total sesquiterpenes is more pronounced under 310 nm and 325 nm, the *FPPS* gene was strongly upregulated only under 340 nm even if a significant increase was also found for the other UV wavelengths. Similarly, a study conducted by Diemer et al., (2001) demonstrated that the overexpression of *LS* did not correspond to an increase in limonene content in transgenic mint plants. Notably, *LS* is involved in the synthesis of other monoterpenes such as α and β-pinene and myrcene (Colby et al., 1993), thus the

enzyme activity may reflect the synthesis of other molecules beyond limonene. However, this does not explain fully our results when the highest gene upregulation was found under 340 nm, but the accumulation of limonene peaked at 310 nm. One possible explanation of this observation is that gene-expression was measured at one specific time-point only, and this does not fully capture the potential circadian changes in gene expression over the course of the day. A further explanation is that the dynamic changes in gene expression might not match the metabolite content due to possible post-transcriptional (Apoorva et al., 2021) and post-translational enzyme regulation events (Mazzucotelli et al., 2008). Light regulates different transcription factors (i.e., HY5, PIF1) which are involved in the synthesis of secondary metabolites (Apoorva et al., 2021). However, the control of enzyme activity involves transcription factors that will ensure precise enzyme accumulation in response to light signals received by photoreceptors (Yadav et al., 2020). Thus, the mismatch between gene expression and metabolite content could derive from post-transcriptional and post-translational modifications that would require further investigation.

Moreover, limonene is one of the key intermediates of the monoterpenes pathway and precursor to isopiperitenone (Jin et al., 2014). Since isopiperitenone was present across all the treatments in two different forms *p*-mentha-1,4-dien-3-one and *p*-mentha-1,8-dien-3-one, the expression of *L3OH* and *IPD* genes was analysed. Both genes were highly enhanced by the UV treatments but only the two isopiperitenone isoforms were detected in the plant material. A possible explanation is that *L3OH* is a necessary intermediate in the biosynthetic pathway but the correspondent trans-isopiperitenol is rapidly converted by the activity of *IPD* to isopiperitenone (see Figure 5.5). A notable increase in *IPD* expression was also observed in peppermint plants exposed to other abiotic stressors such as drought (Rahimi et al., 2017) and salinity (Zhao et al., 2022).

A further explanation for the mismatch in the expression of key biosynthetic genes and metabolite accumulation relates to dynamic changes in gene expression during the treatment. For example, it has previously been found that the expression of *GPPS* in cumin plants exposed to drought increases rapidly and reaches a peak on day three to decrease immediately the following day till the last day of treatment (Ghasemi et al., 2023). Thus, it is possible that as for drought, UV rapidly increases the activity of *GGPS* as a rapid stress response but after these initial days, the synthesis of monoterpenes occurs from pre-transcribed *GPPS*. Finally, the discrepancy between the gene expression and the content of the correspondent metabolites can be attributed to the volatile nature of these compounds especially under strong UV-B light (Dolzhenko et al., 2010). UV-B radiation, in conjunction with increased temperature, enhances the release of volatiles (VOCs) in the environment (Malik et al., 2023). For example, it has been demonstrated that exposure of tea leaves to UV caused a subsequent release of volatile terpenoids to the detriment of the content of these compounds in the leaves (Jang et al., 2010). Thus, it is possible, that in our plants the combination of the UV and other experimental conditions (i.e., *in vitro*) could affect the endogenous content of monoterpenes and sesquiterpenes and consequently result in the inconsistent match of metabolite content with gene expression.

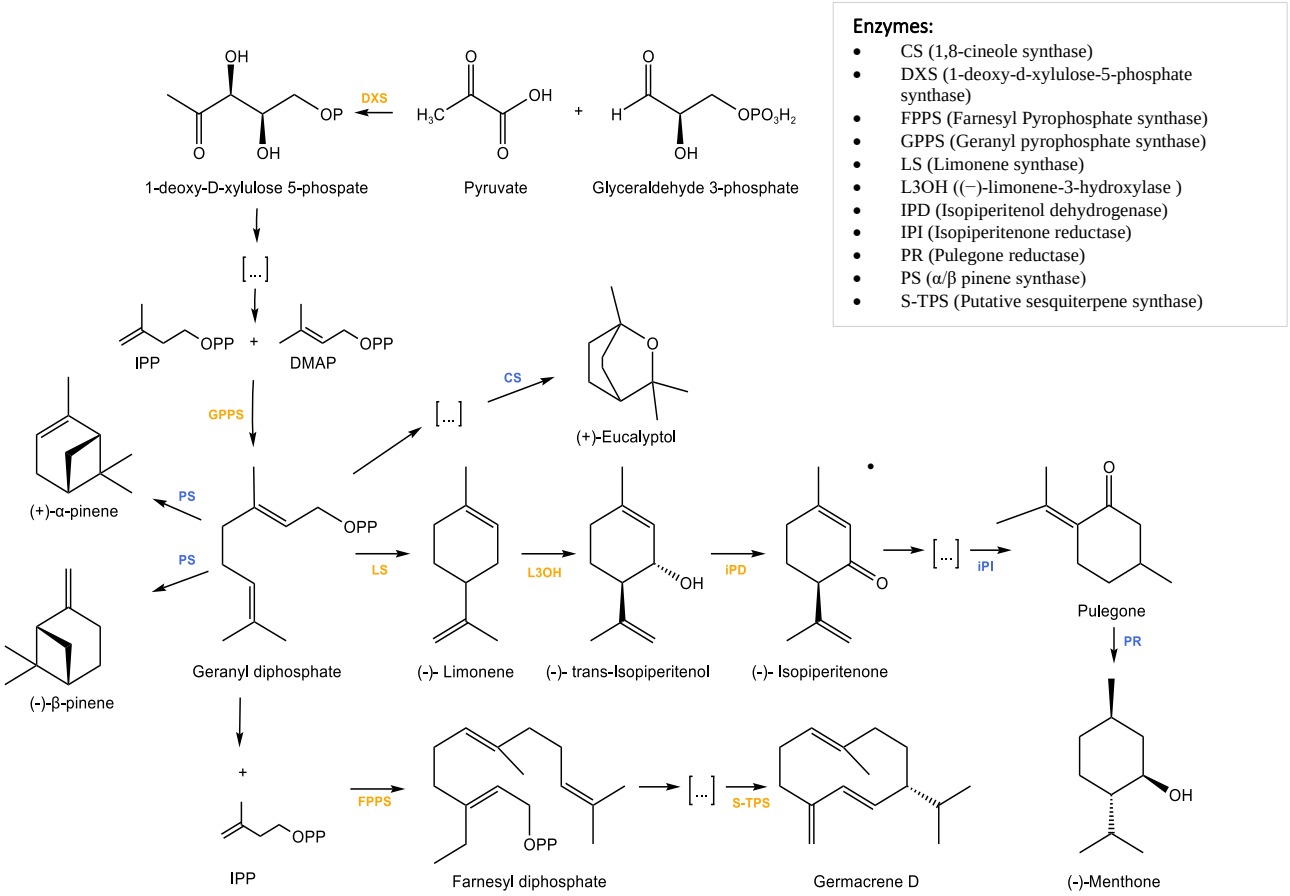


Figure 5.5. *Schematic biosynthetic pathway of some monoterpenes and sesquiterpenes. Square brackets ([..]) represent the presence of intermediates the structure of which is not reported. In orange are reported the key enzymes analysed during the gene expression analysis while in blue enzymes that were not analysed. The pathway is adopted from the KEGG pathway.*

5.4.3 Possible practical application of narrowband UV

A higher level of PAR can induce a photomorphological response similar to UV by increasing contents of defence and antioxidant secondary metabolites but also by triggering morphological changes such as increased leaf thickness and density of trichomes, and the related production of phenolics and terpenes (Escobar-Bravo et al., 2017). For example, in a study that used *Mentha arvensis* as a target plant, it was reported that different light intensities in the visible range of the spectrum affected the

production and composition of essential oil (Souza et al., 2016). The authors found a gradual increase in essential oil content with an increase in light intensity but simultaneously a reduction in essential oil quality. So, the application of high PAR could represent a possible alternative to UV to increase terpenoid content. However, a limiting factor remains the manipulation of the terpenoid composition that is fundamental for oil quality. The results of the current study show that by applying different wavelengths of UV, it is possible to differentially manipulate the composition of the mono and sesquiterpenes. This offers an important instrument to target the compounds of interest with precision, avoiding the compounds that lower the commercial value of the final product.

Understanding the dynamics between different wavelengths and the terpenoid pathway could help to customise the plant's metabolite profile. This is particularly important at more northern latitudes where days are short, and light intensities are low over the winter months (Moe et al., 2006). While increasing overall levels of PAR might not be economically viable, this study shows the potential to achieve similar results using low background intensities of UV radiation.

By using narrow waveband UV-emitting LEDs, it is potentially possible to avoid DNA damage and photosynthetic inactivation, mostly induced by short UV-B wavelengths, while inducing commercially valuable terpenoids.

5.5 Supplementary information

Supplementary Table 1. *Intensity map of red, blue, and different UV wavelengths with peaks at 310 nm, 325 nm, 340 nm, and 365 nm. Red and blue intensities are reported in µmol $m^{-2}s^{-1}$ while the UV in µWatt cm^{-2}. Each square represents a 5 x 5 cm area. Control lamp values are not reported but are similar to the other lamps.*

310 – Red

	1	2	3	4	5	6
1	14	14	14	14	14	12
2	15	15	15	15	15	13
3	15	15	16	16	15	13
4	15	15	16	16	15	13
5	14	15	15	15	14	12

310 – Blue

	1	2	3	4	5	6
1	18	19	20	19	19	17
2	19	21	21	21	20	18
3	20	21	21	21	21	19
4	20	21	21	21	20	18
5	18	20	20	20	19	17

310 - UV

	1	2	3	4	5	6
1	49	44	43	50	48	43
2	47	45	44	46	45	40
3	43	43	42	43	42	39
4	46	44	44	48	48	41
5	48	45	44	51	51	41

325 – Red

	1	2	3	4	5	6
1	14	15	15	15	15	14
2	15	16	16	15	16	15
3	14	15	15	15	15	14
4	14	15	15	15	15	14
5	14	16	16	15	16	15

325 – Blue

	1	2	3	4	5	6
1	18	20	19	19	20	19
2	19	21	20	20	21	20
3	18	20	20	20	20	19
4	19	20	20	20	20	18
5	19	21	20	20	21	20

325 - UV

	1	2	3	4	5	6
1	49	44	43	50	48	43
2	47	45	44	46	45	40
3	43	43	42	43	42	39
4	46	44	44	48	48	41
5	48	45	44	51	51	41

340 – Red

	1	2	3	4	5	6
1	14	16	16	16	16	15
2	14	17	17	16	17	15
3	14	16	16	16	16	15
4	14	16	16	16	16	15
5	15	17	16	16	17	16

340 – Blue

	1	2	3	4	5	6
1	16	20	20	19	20	17
2	18	22	21	21	22	19
3	17	20	20	20	20	18
4	18	20	20	20	21	18
5	19	21	20	19	21	18

340 – UV

	1	2	3	4	5	6
1	40	45	44	45	44	42
2	44	49	49	49	48	44
3	43	48	49	49	48	44
4	41	48	49	49	48	42
5	40	43	45	47	48	44

365 – Red

	1	2	3	4	5	6
1	15	15	14	14	15	16
2	15	15	15	15	15	16
3	14	15	14	14	15	15
4	14	14	14	14	14	14
5	14	15	14	14	15	15

365 – Blue

	1	2	3	4	5	6
1	20	20	19	19	20	20
2	20	20	19	19	20	20
3	19	19	19	18	19	19
4	18	19	18	18	19	19
5	19	20	19	19	19	20

365 – UV

	1	2	3	4	5	6
1	50	47	45	43	46	49
2	49	47	46	46	49	49
3	46	45	44	44	45	45
4	44	44	44	44	45	45
5	45	45	45	45	47	46

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Chapter 6

General Discussion and Conclusions

6.1 Overview

This thesis describes the effects of broadband and narrowband UV on *Mentha spicata* L. plants. Morphological responses were investigated and detailed in Chapter 2. The role of UV-priming, and resistance against other stressors was also investigated (Chapter 3). Secondary metabolites are induced in response to UV. Their accumulation is central in the plant acclimatory response, but these compounds are also important for their human health-giving properties. Variations in metabolic responses were investigated after UV exposure (Chapter 4). For the genus *Mentha*, the content and composition of essential oil are important for the commercial value of the crop. Therefore, monoterpene and sesquiterpene content were investigated under different narrowband UV wavelengths (Chapter 5). This thesis focused on the impacts of UV radiation on *Mentha spicata* L., a significant commercial crop. Results expand the existing knowledge that centres on the model plant *Arabidopsis thaliana*. In particular, this research showed new ways to increase plant quality and plant resistance by applying traditional broadband UV sources, as well as innovative new UV narrowband devices.

6.2 Key findings

- UV exposure induced morphological changes in mint plants grown *in vitro*. These effects persisted after transplanting to soil and are possibly associated with changes in plant hormones.
- Priming plants with UV induced better protection against soil transplanting stress. Plants pre-treated with UV did not develop necrotic areas and in parallel, showed an increase in UV-absorbing pigments and antioxidants.

- UV-induced complex changes in the secondary metabolite profile. On one side, UV promoted the production of flavonoids over phenolic acids. On the other side, UV enhanced the production of monoterpenes and sesquiterpenes. UV effects persisted during a period of recovery without UV.
- Different UV wavelengths induced increases in monoterpene and sesquiterpene contents and regulated the expression of terpenoid-related genes, in a wavelength-dependent manner.

6.3 UV-induced morphological changes without causing stress

Modifications of plant architecture represent a common acclimatory strategy in response to UV. This strategy is characterized by thicker leaves, shorter petioles and an increase in axillary branches (Robson et al., 2015; Qian et al., 2021). In this thesis, in line with the aforementioned findings, it is also documented that the exposure to UV induced an overall reduction in plant size and that these effects persist after a period of recovery in the absence of UV. Particularly, it was found that the reduction in leaf area and leaf thickening were accompanied by an increase in axillary branches. Changes in plant architecture can be regulated by the UV-B-specific photoreceptor, UVR8, and/or a non-specific signalling pathway which is associated with general stress (Robson et al., 2015; Jenkins, 2017). The former regulates genes involved in the accumulation of flavonoids, antioxidant defence, induction of morphological changes and DNA repair (Jenkins, 2014). The latter involves DNA damage, production of increased levels of ROS and activation of wound and defence genes (Jenkins, 2017). However, the increase in ROS does not always correspond to damage, indeed UV can act as a positive regulator (eustress) in the acclimation process (Hideg et al., 2013). Here, there was no evident stress in the UV-exposed plants as

demonstrated by the absence of any visible damage (Chapter 2), normal functioning of photosynthetic machinery and increased content of defence antioxidants (Chapter 3).

Both the UVR8-dependent and the independent pathways can work in parallel, coordinating the plant's UV response through hormone synthesis and translocation (Vanhaelewyn et al., 2016). Often, morphological alterations are associated with the regulatory effect of hormones, the content of which was found to be altered across different plant organs (Chapter 2). UV can induce changes in the level of hormones that regulate cell differentiation, elongation and ultimately plant growth (Vanhaelewyn et al., 2016). One of the most notable results in this thesis was the increase of axillary branching. This response is often associated with an increase in cytokinins, the activity of which is often antagonised by the activity of auxins (Kurepa & Smalle, 2022). Auxin metabolism is reported to be influenced by UV-B radiation (Hectors et al., 2012). A decrease in auxin content not only mediates the reduction in plant size but also affects flavonoid metabolism which may influence auxin transport across different plant organs (Hectors et al., 2012; Fraser et al., 2017). This explains the mutual interaction between UV-B and hormones, UV-screening flavonoids (Chapters 3 & 4) and ultimately morphology (Chapter 2).

The observed compact phenotype implies a reduction in plant biomass that can be an unfavourable outcome for some plant species. However, it has been shown in Chapter 3, that the UV-treated plants are more stress resilient than the untreated ones, reducing the loss caused by other environmental factors.

6.4 UV priming induces stress tolerance

In this thesis, it is documented that UV-induced stress resistance is associated with increased antioxidant capacity, as well as a decrease in leaf area, evidencing the beneficial role UV can play in the production of robust, stress-resistant crops. While the UV dose applied did not induce stress in UV-exposed plants, the transplanting on soil did. Indeed, plants primed with UV did not develop any necrotic spots following transplanting, demonstrating that the UV acclimatory response, which was characterised by altered phenotype, increased antioxidants and UV-absorbing pigments, contributed to the establishment of the plants following transplanting. Often plants are grown in protected environments for the entire production process. However, in some cases, plants are germinated and grown for the initial period in protected conditions before being transplanted to, for example, the field (Wargent, 2017). While propagation in a protected environment has the advantage of controlling plant growth and health, transplanting may constitute a stressful step due to the presence of suboptimal conditions. Previous studies reported the benefits of UV priming in protecting against transplanting stress of plantlets grown in nurseries (Wargent et al., 2011). UV applied in the early growth stages contributed to better acclimation in field conditions. Similarly, mint plants not treated with UV grown i*n vitro* experienced a combination of stressors, such as the change in humidity, that caused stress. The results in this thesis suggest that antioxidants played a central role in the acclimation process to new conditions. Antioxidant content increased in UV-exposed plants but then decreased during the recovery on soil. Also, the persistency of morphological alterations mediated by low doses of UV may have contributed to improving the resistance to other subsequent stressors. Moreover, stomatal closure in non-UV primed plants, and a parallel increase in ABA, may have prevented

transplanting stress caused by preventing excessive water loss (Cominelli et al., 2010) (Chapters 2 & 3). This aspect is important not only during the vegetative stage but also during the post-harvest stage and transportation. Maintaining the appropriate temperature, humidity, and ventilation during transportation is essential for preventing spoilage and preserving product quality. However, this is sometimes not possible, and as a result, crops and vegetables are subjected to post-harvest losses or damage.

Besides the potential of utilising low doses of UV to generate more resistant plants, the resulting smaller plants could represent a disadvantage if the final outcome is the generation of plant biomass. However, smaller plants do not necessarily correspond to a reduction in plant biomass because plants exposed to UV tend to have thicker leaves and axillary branches resulting in similar biomass to the non-treated ones. On the contrary, in some contexts more compact plants could represent an advantage because plants would need less space, and a smaller size could facilitate their transport and reduce their damage during transport (Qian et al., 2020).

It would be interesting to see if these same positive effects of UV demonstrated here, are applicable to a broader number of crops of horticultural interest. For example, by exposing plants to outdoor conditions during the transplantation stage or conditions similar to the current agricultural practice.

6.5 Harnessing UV-induced secondary metabolites: from defence mechanism to a quality product

UV-mediated changes occurred in the metabolite profile of the aromatic plant *Mentha spicata*. Synthesis of secondary metabolites is a defence response that consists of the accumulation of UV-screening pigments as well as enhanced production of terpenes and phenolics (Divekar et al., 2022).

UV has the potential to enhance the quality and content of valuable metabolic products in crops, offering new opportunities to optimise and manipulate plants' quality (Jansen et al., 2008; Schreiner et al., 2012; Hectors et al., 2014). These compounds, particularly phenolics, possess beneficial properties for human health acting like antioxidants and preventing chronic diseases (Schreiner et al., 2012). Other compounds, such as volatile terpenoids present in aromatic plant species, are important as flavouring and fragrances (Masyita et al., 2022). Thus, understanding the dynamics of relevant horticultural crops under UV is not only important in the plant-environment interaction but also for human nutrition and commercial applications.

From a practical point of view, it is important to consider if these compounds are subject to variation once UV treatment has ended. In Chapter 4, it was described that the content of phenolics persisted for at least seven days after the UV treatment had ended, providing evidence that products do not necessarily need to be used or consumed immediately after being UV exposed. This information is of relevance because it can help to program the timing of UV application while also presenting opportunities to improve agronomic practices. In addition to improved nutritional quality, UV can also be used to increase the shelf-life of products that deteriorate quickly (Mohd Idris et al., 2020). However, to fully ensure the efficacy of UV treatment and guarantee the improved nutritional properties of UV-treated crops and vegetables, understanding the effects of longer recovery periods is an understudied, yet necessary step.

6.6 New challenges in practical horticultural techniques

Unpredictable weather patterns and extreme weather events represent a threat to agriculture and food security. In light of the changing climate, the FAO has

emphasized the importance of improving food quality by increasing the nutritional value (FAO, 2015). Evidently, there is an urgent need to alter and renew current agricultural practices and one strategy involves the integration of traditional growing systems with the production of vegetables and crops in controlled environments (FAO, 2015). Indoor farming allows producers to increase the productivity of plant products using minimal space and reduced inputs (i.e., reduced use of soil, agrichemicals and water) (Avgoustaki & Xydis, 2020). Moreover, the possibility to control environmental conditions (i.e., light, temperature and humidity) avoids the losses that affect plant production under field conditions (Avgoustaki & Xydis, 2020). However, it must be noted that while there are many positives associated with indoor farming, that there are also drawbacks for instance, high energy consumption and costs associated with the required lighting and air control systems (Avgoustaki & Xydis, 2020).

Results of this thesis demonstrate the benefits of controlling plant environments when producing high-value crops. *In vitro* growth paired with UV exposure facilitated not only increased stress resistance of crops but also the generation of crops with higher levels of desired compounds. The application of UV in horticultural settings is known to control plant growth and quality (Loconsole & Santamaria, 2021) but additional benefits can be obtained through the application of narrowband UV, generated by LEDs, which allow more accurate manipulation of light (Wargent, 2016). LEDs enable the selection and application of specific UV wavelengths in order to optimise the quality of the final product as discussed in the previous section. Thus, depending on the desired outcome, the advantage of using narrowband UV can be seen in all the phases of production. During growth, UV enhances the production of secondary metabolites, increases the sturdiness of the plants, and protects against other stressors.

For instance, in Chapter 5 it was shown that different UV wavelengths induced the accumulation of different volatile terpenoids in mint plants, highlighting how it is possible to induce different responses with different UV wavelengths.

However, comparing the results of different studies is very complex not only because the knowledge about the effects of single wavelengths is limited but also because the application of different intensities, treatment duration and the use of different plant species make comparisons very difficult. Mapping a wider number of wavelengths across the UV spectrum would help to better target specific plant characteristics.

6.7 Is UV the future of horticulture?

Supplementing visible light with UV has numerous advantages for both growers and consumers as described in the previous sections.

The advancement of LED technology has drawn attention to UV-LEDs as an alternative source of UV radiation, as opposed to standard incandescent or fluorescent UV tubes. The potential benefits of UV LEDs are attractive for future horticultural applications. This includes not only their safety, which eliminates the environmental and health-related issues of mercury lamps, and their compactness, which makes them suitable for portable devices but also the possibility to precisely manipulate both light spectrum and intensity.

Yet, for horticultural applications, the most complex challenge is to expand the knowledge on their effect as research on UV-emitting LEDs is still limited to a few wavelengths and a few plant species. At present, even if UV-emitting LEDs offer long-term energy savings because of their better efficiency, the initial investment can be a barrier for growers and producers, especially in the absence of supporting studies on plants of horticultural interest. The presence of too many variables, including

questions surrounding what wavelength to apply, the intensity and the duration of treatments make applications complex despite the numerous advantages of UV-emitting LEDs. Additionally, there must be guidance, policies, and support in place to facilitate decision-making in the indoor farming industry. For instance, a full cost-benefit analysis would need to be carried out before implementing any new growing system. Moreover, there must be recognition of the associated costs and financial aid should be allocated to growers making the switch to supplemental UV-LED systems. It is anticipated that with increased use of UV-LEDs, these tools will become more affordable and accessible.

Thus, with continued research progress, paired with innovation and price reduction UV LEDs could play a significant role in shaping the future of agriculture for a more efficient, sustainable way to produce food.

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