Effects of UV-B on the activity and gene expression of several antioxidant enzymes in peppermint (*Mentha x piperita* L.)

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Abstract

Peppermint infusion is a popular herbal drink with a number of benefits on cardiovascular, digestive, respiratory systems. This study aimed to analyze the effects of UV-B radiation on the essential oils and primary and secondary metabolism, including expression of genes involved in terpenoids biosynthesis pathway and scavenging of reactive oxygen species (ROS) in peppermint. *Mentha x piperita* L. plants grown in field were irradiated for one hour with UV-B light (310 nm, 3.8 Wm^{-2}), in the middle of the day, for two days. The five upper leaves were collected after 4, 20, 24, 28, 44, and 48 h following the first treatment. Plants of *M. x piperita* cultivated under the same circumstances without the treatment were utilized as the control at each time point. Results showed that the biosynthesis of peppermint essential oil is modulated by UV-B irradiation, but the plants had also their mechanisms of protection from UV by increasing the ROS-scavenging enzymes activity. Also, expression of some genes involved in pathway were modified in the presence of UV and showed a significant regulating effect in essential oil biosynthesis. Another confirmation of the protection from UV-B light was the stable concentration of photosynthetic pigments. Analysis of ROS-scavenging enzymes suggested that plants had an oxidative stress. In conclusion, this work is a step to understand the regulation of terpenoid biosynthesis and the effects of UV-B radiation on it. Complex analysis of whole plant' response will be helpful to get more insight on this.

Keywords: bioactive compounds, gene expression, peppermint, *Mentha x piperita* L., UV-B

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Introduction

Peppermint (*Mentha x piperita* L.) is the herbal species belonging to the family of Lamiaceae (Kashfi et al., 2020), which is cultivated in temperate climates in the United States, Asia, and Europe (Mahendran and Rahman, 2020). Peppermint species are typically used for flavoring agents (condiments and spices), herbal tea, and industrial purposes. Along with food and commercial applications, this plant is well-known for its anti-fungal, anti-viral, cold, and fever properties and traditional applications to treat various types of diseases e.g., throat inflammation and oral mucosa. Scientific studies confirm peppermint's biological benefits, including

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antioxidant, genotoxicity, anti-viral, anti-microbial, anti-inflammatory, larvicidal, anticancer, biopesticidal, and anti-diabetic properties. It was reported that 75% of the compounds in peppermint leaves, including hesperidin rosmarinic acid, luteolin 7-O-rutinoside, and eriocitrin can be extracted in the infusion (Cappellari et al., 2020). In this regard, peppermint aerial parts infusion and decoction can be used to create tonic, antispasmodic, digestive, carminative, and anti-inflammatory effects (Chiappero et al., 2019). Studies show that these health benefits of peppermint can be increased using stressful conditions in the pre-harvesting stage (Gholamipourfard et al., 2021; Kashfi et al., 2020). However, these stressful conditions make some changes in molecular processes like variation in metabolite profile and can decrease crop performance. Peppermint oil contains more than 200 chemical compounds, the most abundant being menthol (47-57%), menthone (6-24%), menthofuran (1-8%), and menthyl acetate (3-29%). Also, olefins, limonene and terpinolene, are present in low amount, 1-6% and 0.1-0.2%, respectively. The con centration of the compounds in essential oil is very important quality characteristic of medicinal herbs. For example, the minimum quantity of menthol should be 45% of total oil (Luciano et al., 2006).

The quality of peppermint essential oil can be influenced by environmental and seasonal factors. The blue light, UV-A and UV-B radiation, prompts a significant effect on photomorphogenesis, production, and chemical composition of *Mentha piperita* essential oil (Maffei et al., 1999; Maffei and Scannerini, 1999). UV light is typically found as part of the radiation received by the Earth from the Sun and has a wavelength range from 10 nm (extreme UV) to 400 nm. Though it is a totally different form of vision from what is seen with human and animal eyes, plants have the ability to see it because of their sensitivity to light. But light is not always safe for plants: some studies have shown that the misbalance of irradiations in different wavelengths can also be very harmful (Caldwell et al., 1994). On the other hand, balanced light flow can amend negative effects of harmful irradiations such as UV-B. For example, according to Adamse and Britz (1992), ambient photosynthetically active radiation can decrease UV-B radiation (PAR, 400–700 nm). Additional research revealed that whereas plants cultivated under UV-B-enhanced greenhouses condition showed a strong inhibition of growth and dry matter formation, field-grown plants showed no such impact (Dai et al., 1995). This is because there is more UV-A radiation in the field than there is in a greenhouse, which results in a higher UV-A/UV-B ratio. Day (2001) noted that there is significant uncertainty between indoor plant responses under high ratios of UV-B/UV-A and UV-B/PAR and the plants under outdoor spectral regimes.

In order to determine if plants are susceptible to the ambient as well as the higher levels of UV-B levels projected with prolonged ozone depletion, research needs to address more realistic UV and PAR circumstances. In field UV-B investigations, the two most popular techniques are either adding special lights to the ambient UV-B or reducing it with filters. Furthermore, different plants may respond differently to the same lighting circumstances. Moreover, latitude, the physiological and developmental stage of the plants, and the altitude at which the plants are cultivated can all influence the reaction (Ibañez et al., 2008). Also, different species vary widely in their response. For example, when it comes to changes in biomass decrease, native species are more resilient to increased UV-B exposure than agricultural plants (Krizek et al., 1997). A farmer's revenue is often lost as a result of significant fluctuations in an oil's commercial value resulting from variations in the chemical composition of the oil.

At the moment little is known about the role of terpenoids in UV-B-stress protection for plants. For this reason, a study was conducted with *Mentha x piperita*, a plant producing terpenoids. Light has a special effect on the composition of peppermint oil. Therefore, the study aimed to analyze the effects of UV-B radiation on several aspects of primary and secondary metabolism, including genes involved in terpenoids biosynthesis pathway and scavenging of reactive oxygen species (ROS) in peppermint. *Mentha x piperita* is an important commercial plant species, and the findings are beneficial from economical perspectives.

Materials and Methods

Plant material and growth conditions

Mentha x piperita L. plants grown in field were irradiated for one hour with UV-B light (310 nm, 3.8 Wm^{-2}), in the middle of the day, for two days. The five upper leaves were collected at 8, 16, 32, 40 and 48 h after the first treatment. Plants of *M. x piperita* cultivated under the identical circumstances without the treatment were utilized as the control at each time point. Whole leaves were sampled and preserved at -80 ℃ in liquid nitrogen for subsequent analyses.

Enzyme extraction

A slightly altered version of Zhang and Kirkham's (1996) extraction process was applied to the leaf samples for the extraction of ROS scavenger enzymes. The operating temperature for every procedure was 4 ℃. The plant material was pulverized in liquid nitrogen with a pestle and mortar, then resuspended in Na-phosphate buffer (50 mM, pH 7.5) containing: 1.0 mM EDTA, 0.5 mM PMSF, 10 mM KCl, 250 mM sucrose, 0.1 mM DTT, 1 mM MgCl2, and w / V PVPP (1%) in a 6: 1 (w / V) ratio. Following a four-layer gauze filtering step, the homogenate was centrifuged at 25,000 g, 4 ℃, and for 20 minutes. By addition of solid ammonium sulphate, the supernatant was raised to 80 percent saturation and stirred for at least 1 h. Following a 45-min centrifugation at 28,000 g for 4 ℃, the majority of the enzyme-containing pellets were resuspended in a small amount of 50 mM Na-phosphate buffer pH 7.5 and used right away for enzymatic tests (Zhang and Kirkham, 1996).

Enzyme assays

Superoxide dismutase (SOD, EC 1.15.1.1)

By measuring SOD's capacity to stop tetrazolium nitro blue (NBT) from being reduced by the photochemically generated superoxide anion, a method devised by Krishnan et al. (2002), the activity of SOD was ascertained. The enzymatic extract was generated in a final volume of 1 ml and the solution used for the experiment contained 50 mM Na-phosphate buffer (pH 7.8), 0.1 mM EDTA, 2 mM riboflavin, 75 mM NBT, and 13 mM methionine. The samples were exposed to a light source (4000 lux) for 15 min at a distance of 30 cm after riboflavin was added. As a response control, a non-irradiated sample that did not acquire any color was employed. The spectrophotometric assay was carried out at 560 nm absorbance (Krishnan et al., 2002).

Catalase (CAT, EC 1.11.1.6)

The activity of CAT was measured spectrophotometrically by measuring the change in absorbance at 240 nm caused by H_2O_2 consumption (ε= 39.4 mM^{-1} cm^{-1}) (Zhang and Kirkham, 1996). The solution comprised 50 mM Na-phosphate buffer pH 7.0, 15 mM H_2O_2, and the enzymatic extract in one ml. The reaction was triggered by the addition of H_2O_2.

Peroxidase (POX, EC 1.11.1.7)

POX activity was measured using the oxidation of guaiacol (ε = 26.6 mM^{-1} cm^{-1}) (Zhang and Kirkham, 1996). The reaction mixture contained 50 mM sodium phosphate, pH 7.0, 0.33 mM guaiacol, 0.27 mM H_2O_2, and enzyme extract. The reaction mixture's final volume was 1 ml. The reaction was started with the addition of guaiacol and observed spectrophotometrically at 470 nm.

Total protein determination

Proteins were measured using the Bradford technique (1976) using a standard of bovine serum albumin.

Total RNA extraction

Using A Tissue Lyser (Qiagen, Hilden, Germany) around 50 mg of frozen leaves were crushed in two 30-second bursts. After adding one milliliter of warm extraction buffer (2% CTAB (Cetyl trimethylammonium bromide) (w/v), 2% PVP (w/v), 100 mM Tris-HCl (pH 8.0), 25 mM EDTA 2.0 M NaCl, and 2% (v/v) β-mercaptoethanol), samples were immediately incubated at 65 ℃ for five minutes in a water bath by shaking. Centrifugation was used to separate the two phases for 10 minutes at 5000 g at room temperature following a double extraction using

the same ratio of chloroform to isoamyl alcohol (24:1). Next, the top phase was moved into a fresh tube. Total RNA was precipitated with LiCl (10 M solution, 0.25 vol) over night at 4℃. Pellet was split at 4 °C for 20 minutes at 10,000 g. After that, it was dissolved in 0.5 ml of SSTE-buffer, which included 1 mM EDTA (pH 8.0), 1.0 M NaCl, 0.5% w/v SDS, and 10 mM Tris-HCl (pH 8.0). Then, the pellet was purified twice more using an equivalent volume of acid phenol (pH 4.5–5), namely a 1:1 ratio of chloroform to isoamyl alcohol. Every time the phenol was added, the mixture was centrifuged for five to ten minutes at 4 ℃. After adding two liters of 100% ethanol, the RNA was precipitated before incubation for two hours at -20 ℃. After centrifuging the mixture for 20 minutes at 10,000 g, the pellet was separated and underwent two 80% ethanol washes. Next, DNase treatment (Ambion TURBO DNA-free) was carried out, and the quantity of RNA was further validated using a NanoDrop Nd-1000 (Thermo) spectrophotometer.

Reverse-transcription, PCR amplification, and semi-quantitative analyses

The PCR methodology used was as follows: 1.5 minutes of initial denaturation at 94 ℃; 35-45 cycles of 30 seconds at 94 ℃, 30 seconds at 52-60 ℃, and 30 seconds at 72 ℃. The last extension took place for 3.5 min at 72 ℃. One unit of Taq DNA polymerase (Fermentase), 0.2 µM of each primer, 0.2 mM of dNTPs, 0.5 µl of cDNA template, 2.5 µl of 10X PCR reaction buffer (Fermentas), 2.5 µl of $MgCl_2$, and sterile water to full volume were all included in the 25 µl PCR mixture. The primers are shown in Table 1.

Real-time PCR

Fluorescent reporter molecule identification and quantification serve as the foundation for the Real-Time PCR technology. The amount of PCR product in the process or the double-strain DNA determines how much this signal grows. It is feasible to track DNA amplification during the early exponential phase, when the accuracy of the connection between the fluorescent signal and the amplicon is highest, by logging the fluorescence emission at each cycle. Using the Mx3000P Real-Time PCR System, a quantitative real-time PCR was conducted. In order to run the reaction, 25 µl of a combination of 12.5 µl of Stratagene's 2x Brilliant SYBR Green QPCR Master Mix, 100 nM primers, 0.5 µl of cDNA, and 30 mM ROX was used. The primers used for PCR amplification were the same. The first polymerase activation was carried out at 95 °C for 10 minutes. Thereafter, 30 seconds at 52-60 ℃, 40 cycles of 60 seconds at 95 ℃, and 60 seconds at 72 ℃ were applied. With reference to the level of 18S ribosomal mRNA, relative RNA levels were calibrated and standardized. The RT product's dilution series threshold values were compared, and each primer pair's non-template control was then used to establish the PCR conditions.

Table 1
The primers used for the PCR in the study

Gene	Accession Number	Primers	Fragment Length
18S	NR_022795	F 5′-ATGATAACTCGACGGATCGC-3′ 5′-CTTGGATGTGGTAGCCGTTT -3′ R	166
CAT	AW255374 (Lange et al., 2000)	F 5′-AAACCCAACCCAAAATCTCAC-3′ 5′-GGACACCCAAATCATCAAAGA-3′ R	114
POX	EU439708	F 5′-GTCTCCACTTCCACGATTGC-3′ 5′-GAGTATGTCGGCGCAAGAAAC-3′ R	191
SOD	AW255115 (Lange et al., 2000)	F 5′-ACAGCAGCGAAGGTGTTAGTG-3′ 5′-TCCAACCGTGATGTTTCCAAG-3′ R	242
MECPS	AW255189 (Lange et al., 2000)	F 5′-TTGGAGTAGAGCCTCAGTCGG-3′ 5′-CAACTTAGGATTGGTGTCGGG-3′ R	255
DXPS	AF019383 (Lange et al., 1998)	F 5′-CCACCAGGCTTACCCACACAA-3′ 5′-GCCACCGCCATCCCTAAAC-3′ R	171
IPPI	AW255524 (Lange et al., 2000)	F 5′-CTCTTGGGGTGAGAAATGCT-3′ 5′-CATCTGAGGGGGCTTTGTA-3′ R	124

The Pfaffl method was used to determine the relative expression levels of the genes according to the following formula (Pfaffl, 2001):

$$\text{Relative Expression Ratio} = \frac{(E_{target})^{(Ct\ control - Ct\ sample)}}{(E_{standard})^{(Ct\ control - Ct\ sample}}$$

where E = Efficiency = $10^{(-1/Slope)}$.

Results

ROS scavenging enzymes activity and gene expression analyses

Considering that ROS are involved in UV-B-induced signaling, and at the same time ROS-production in response to UV-B can lead to oxidative stress, it was decided to measure enzymatic activities of three main ROS-scavenging enzymes: SOD, POX, and CAT. Also, the transcriptional levels of genes associated with the enzymatic functions were analyzed. In order to calculate enzymatic activity, total protein contents were measured, and the sharp drop in protein quantity was observed after both UV-B treatments (Fig. I).

The changes in activities and expression of genes for all the enzymes had a cyclic character. As for SOD, enzyme activity increased 8 h after each irradiation. These data were not correlated with the gene expression levels (Fig. II). On the other hand, POX showed a strong correlation with the gene expression level and enzyme activity - both of them were activated after the second UV-B irradiation (Fig. III).

Contrary to POX, CAT showed higher gene expression and enzymatic activity in irradiated plants after the first UV-B treatment, although the activation levels were not high as those observed for the other two enzymes (Fig. IV).

Expression of genes involved in essential oil biosynthesis

Starting from the essential oil analyses, the regulation of key-genes involved in the essential oil biosynthesis was studied. First of all, the level of expression of genes coding for enzymes of the mevalonate-independent pathway – DXPS, MECPS along with the IPP isomerase (IPPI), which are important for biosynthesis of the first precursor of

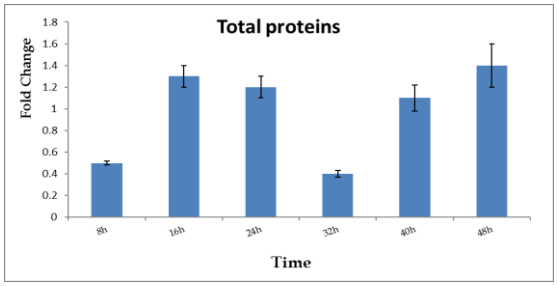

Fig. I. Changes in total protein content; Each data is presented as ratio (treatment/control). Data represent the mean ± SE.

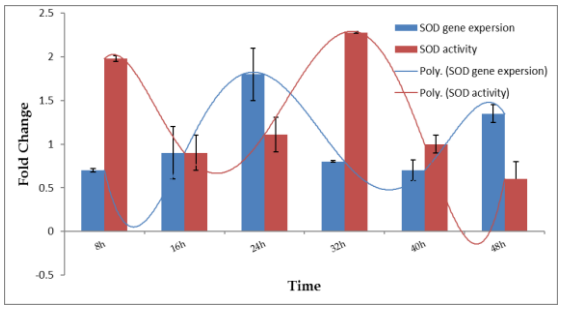

Fig. II. Changes in gene expression and enzymatic activity of SOD; each data is presented as ratio (treatment/control). Data represent the mean ± SE.

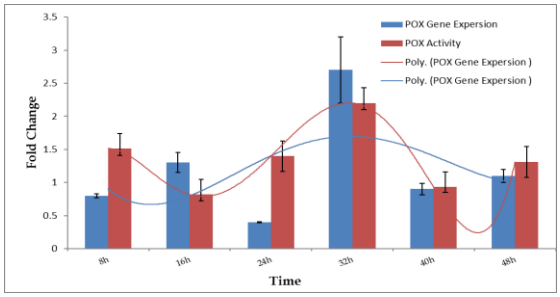

Fig. III. Changes in gene expression and enzymatic activity of POX; each data is presented as ratio (treatment/control). Data represent mean ± SE.

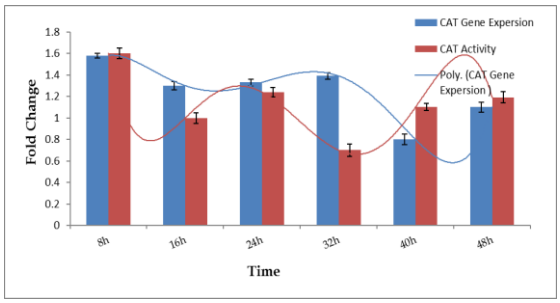

Fig. IV. Changes in gene expression and enzymatic activity of CAT; each data is presented as ratio (treatment/control). Data represent the mean ±SE.

monoterpene metabolism were checked. *MECPS* expression level was increased almost at all the time-points, except 40 h; the highest expression level - 3.5 times higher - was registered after 8 h

following the second UV-B treatment (32 h after the first UV-B treatment) (Fig. V). Also, *IPPI* expression increased, albeit in response to the first UV-B irradiation, at 8 h, 16 h, and 24 h time-points; in general, the expressions level at these points were twice higher in UV-B treated plants. In contrast, *DXPS* showed higher expression only at 16 h and 32 h after the first treatment, suggesting that mRNA accumulation was not immediately stimulated by UV-B light and required time after the irradiation (Fig. V).

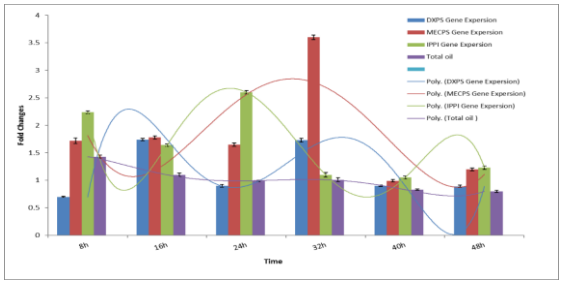

Fig. V. Changes in expression of genes coding enzymes of mevalonate-independent pathway; each data is presented as ratio (treatment/control). Data represent the mean ± SE.

Discussion

Much research has lately concentrated on the biochemical and physiological effects of UV radiation on plants, given the loss of stratospheric ozone and the resulting increase in UV-B irradiation that reaches the Earth's surface.

All cellular compartments create reactive oxygen species (ROS), which are typical byproducts of metabolism produced by a range of activities. Prior research has demonstrated that exposure to UV-B increases ROS that cause oxidative damage (Dai et al., 1997). UV stress can be reduced by scavenging active oxygen and other radical species using an enzymatic method. It has been shown that UV-B increases superoxide dismutase activity (Rao et al., 1996; Jansen et al., 1998); nevertheless the linked gene's expression either did not change at all or even decreased (Strid et al., 1994; Willekens et al., 1994).

Findings verified that SOD is activated at the post-transcriptional stage. It was shown that after exposure to UV light, Arabidopsis's peroxidase activity increased significantly (Rao et al., 1996). The induction of certain isoforms of the enzyme facilitated a well-regulated process of increase. The later increases observed in the *SOD* expression suggest that UV-B exposure may have a less strong role in induction of SOD compared to POX in peppermint.

POX seems to have the main role between these three scavenging enzymes. It is not clear why UV-B irradiation enhanced POX preferentially over the other two enzymes. Peroxidases have a lot of different roles in plants (Gaspar, 1991); one of the possible reasons may be the synthesis of secondary metabolites such as lignin, which can play a role in UV-B protection. Given that conjugates of lignin aid in UV-B screening, lignification may be advantageous for plants exposed to UV-B light (Jansen et al., 1996).

The other scavenger enzyme, catalase, was activated either on transcriptional, or on post-transcriptional levels after the first UV-B treatment, but the increasing was quite low. This may be due to the fact that there is more than one isoform of CAT, each of them can be up- and down- regulated simultaneously. Willekens et al. (1994) demonstrated different regulation of three CAT isomers in response to UV-B in tobacco plants. While gene expression of *CAT* enhanced after 8 h and 32 h, the enzymatic activity even dropped down after 28 h. This data suggests the activation of *CAT* by UV-B light but not an important role of CAT-related scavenging system in response to UV-B stress in peppermint plants.

The total soluble proteins extracted from the peppermint leaves exposed with UV-B were found to have decreased by around 40%, with a subsequent recovery occurring after 32 h, according to the quantitative assessment of soluble protein content of fresh weight. The possible explanation might be light absorption by proteins (Hollósy, 2002). These results can confirm the theory about decreasing the biomass accumulation provoked by UV-B (Kadur et al., 2007).

The studies of the effects of UV-B in field on photosynthesis demonstrate that photosynthesis of higher plants is generally unaffected, although biomass is often reduced. Moreover, plants grown

at high latitudes seem to be less sensible than plants at lower latitudes. As a species growing at elevated latitude, peppermint exhibited a sort of photosynthetic adaptation to UV-B stress. The mechanisms of this adaptation are unknown. This could be due to higher levels of antioxidants observed in high altitude plants (Polle and Rennenberg, 1992). Being a first "sunscreen" for plants, aromatic compounds and their biosynthesis pathways are affected by UV-B light. Induction of UV-B absorbing phenolic compounds like lignin, tannins, flavonoids or cumarins by enhanced UV-B radiation are commonly reported (Caldwell et al., 2007), but little is known about the effects of UV-B radiation on terpenes production.

It has been noticed that variations in light quality in peppermint and other species belonging to the same family affect the oil's chemical composition in different ways. Maffei and Scannerini (2000) demonstrated that UV-B particularly enhanced the contents of the different oxygenated monoterpenes such as menthofuran, menthone, and menthyl acetate in peppermints grown in controlled conditions. The present study's findings do not support these earlier findings, but they can be explained by adaptation of plants from field to higher irradiation levels. Our experiment showed that induction of mevalonate-independent pathway by UV-B: *DXPS*, *MECPS*, and *IPPI* transcripts resulted in up-regulation after both treatments. Also, biosynthesis and conversion of the first detectable precursors of menthol-limonene showed increases by UV-B (data not shown). On the other hand, the metabolism of pulegone has more complicated character and can lead to two different products - menthone and menthofuran. Comparison of gene expression analysis by qRT-PCR method and analysis of peppermint essential oil contents revealed some changes on genes' transcript levels, but no significant changes in the composition of essential oil (data not shown) and quantity of total oil were found. This may be due to regulation mechanisms at post-transcriptional level.

Lijima et al. (2004) in their study on different cultivars of basil found a loose correlation between transcript levels and enzyme activity levels for the terpene's biosynthesis. Quantitative RT-PCR analysis demonstrated no effect of enhanced UV-B level on the expression of three genes coding enzymes of the MEP pathway - MECPS and DXPS (Iijima et al., 2004). Instead, the biosynthesis of menthol seemed to be more sensible to increased ultraviolet irradiation at least on transcriptional level. Thus, *GPPS*, *PR*, *MFS*, and *MR* transcript levels increased after treatment. The biosynthesis of the sesquiterpene germacrene D also regulated at transcriptional level by additional UV-B irradiation (data not shown). Moreover, emission of volatiles can also contribute to the fact that there were no changes in essential oil composition. For example, it is known that biogenic hydrocarbons emitted to the atmosphere - especially terpenes - by plants are significant sources of hydrocarbons contributing to smog, which demonstrate the levels of VOC (volatile organic compounds) emission (Manahan, 2022). Other possible reason for the weak correlation between gene expression and produced terpenoids might be also their further partial modification by the activity of different enzyme classes such as the dehydrogenases, cytochrome P450 hydroxylases, and methyl-transferases and glycosyl (Aharoni et al., 2003).

Conclusion

Mentha x piperita is one of the most important plants used worldwide for valuable characteristics of its essential oil. Considering the actual increasing of UV-B in the sun radiation, it is important to estimate its economic consequences for the farmers growing this valuable herb. Containing mostly terpenes, peppermint essential oil composition might be affected by UV-B light. In this study it was found that biosynthesis of peppermint essential oil was modulated by UV-B irradiation, but the plants had also their mechanisms of protection from UV. Thus, expression of genes regulating essential oil biosynthesis was significantly higher while the concentration of certain compounds did not change so much. Another confirmation of the protection from UV-B light was the stable concentration of photosynthetic pigments. Analysis of ROS-scavenging enzymes suggested that the plant under study experienced an oxidative stress. In conclusion, this work is a step

to understand the regulation of terpenoid biosynthesis and the effects of UV-B radiation on it. A comprehensive analysis of the whole plant response is suggested for further studies.

References

Adams, R.P. 2017. Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing.

Adamse, P., and **S.J. Britz.** 1992. Amelioration of UV-B damage under high irradiance. I: Role of photosynthesis. *Photochemistry and Photobiology*. 56: 645-650.

Aharoni, A., A.P. Giri, S. Deuerlein, F. Griepink, W. J. de Kogel, F.W. Verstappen, H.A. Verhoeven, M.A. Jongsma, W. Schwab, and **H.J. Bouwmeester.** 2003. Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. *The Plant Cell*. 15: 2866-2884.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.

Caldwell, M., S. Flint, and P. Searles. 1994. Spectral balance and UV-B sensitivity of soybean: a field experiment. *Plant, Cell and Environment.* 17:267-276.

Caldwell, M.M., J. Bornman, C. Ballaré, S.D. Flint, and **G. Kulandaivelu.** 2007. Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. *Photochemical and Photobiological Sciences.* 6:252-266.

Cappellari, L.D.R., M.V. Santoro, A. Schmidt, J. Gershenzon, and **E. Banchio.** 2020. Improving phenolic total content and monoterpene in *Mentha x piperita* by using salicylic acid or methyl jasmonate combined with Rhizobacteria inoculation. *International Journal of Molecular Sciences* 21: 50.

Chiappero, J., L. del Rosario Cappellari, L.G.S. Alderete, T.B. Palermo, and E. Banchio. 2019. Plant growth promoting rhizobacteria improve the antioxidant status in *Mentha piperita* grown under drought stress leading to an enhancement of plant growth and total phenolic content. *Industrial Crops and Products.* 139:111553.

Dai, Q., S. Peng, A. Chavez, B. Vergara, S. Peng, K. Ingram, V. Neue, and **L. Ziska.** 1995. Effect of enhanced ultraviolet-B radiation on growth and production of rice under greenhouse and field conditions. In Climate Change and Rice. Peng, S, K.T, Ingram, H.U. Neue, and L.H. Ziska (Eds.). Springer-Verlag, Berlin, pp:189-198.

Dai, Q., B. Yan, S. Huang, X. Liu, S. Peng, M.L.L. Miranda, A.Q. Chavez, B.S. Vergara, and **D.M. Olszyk.** 1997. Response of oxidative stress defense systems in rice (*Oryza sativa*) leaves with supplemental UV-B radiation. *Physiologia Plantarum.* 101:301-308.

Day, T.A. 2001. Ultraviolet radiation and plant ecosystems. In Ecosystems, Evolution, and Ultraviolet Radiation. Cockell, C. S., and A. R. Blaustein (Eds.). Springer, pp: 80-117.

Gaspar, T., C. Penel, D. Hagege, and **Greppin, H.** 1991. Peroxidases in plant growth, differentiation, and development processes. In Biochemical, Molecular and Physiological Aspects of Plant Peroxidases. Lobarzewski, J., H. Greppin, C. Penel, and T.H. Gaspar (Eds.). Universite de Geneve Press Geneva, pp: 249-280.

Gholamipourfard, K., M. Salehi, and **E. Banchio.** 2021. Mentha piperita phytochemicals in agriculture, food industry and medicine: Features and applications. *South African Journal of Botany*. 141:183-195.

Hollósy, F. 2002. Effects of ultraviolet radiation on plant cells. *Micron.* 33: 179-197.

Ibañez, S., M. Rosa, M. Hilal, J.A. González, and **F.E. Prado.** 2008. Leaves of Citrus aurantifolia exhibit a different sensibility to solar UV-B radiation according to development stage in relation to photosynthetic pigments and UV-B absorbing compounds production. *Journal of Photochemistry and Photobiology B: Biology.* 90:163-169.

Iijima, Y., R. Davidovich-Rikanati, E. Fridman, D.R. Gang, E. Bar, E. Lewinsohn, and **E. Pichersky.** 2004. The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate

glands of three cultivars of basil. *Plant Physiology.* 136:3724-3736.

Jansen, M.A., T.S. Babu, D. Heller, V. Gaba, A.K. Mattoo, and **M. Edelman.** 1996. Ultraviolet-B effects on *Spirodela oligorrhiza*: induction of different protection mechanisms. *Plant Science.* 115: 217-223.

Jansen, M.A., V. Gaba, and **B.M. Greenberg** 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. *Trends in Plant Science* 3:131-135.

Kadur, G., B. Swapan, K. Sunita, Y. Sanjeev, T. Arjun, B. Sanjay, R. Abhinav, and **P. Mohanty.** 2007. Growth enhancement of soybean (*Glycine max*) upon exclusion of UV-B and UV-B/A components of solar radiation: characterization of photosynthetic parameters in leaves. *Photosynthesis Research.* 94:299-306.

Kashfi, A.S., Y. Ramezan, and **M.R. Khani.** 2020. Simultaneous study of the antioxidant activity, microbial decontamination and color of dried peppermint (*Mentha piperita* L.) using low pressure cold plasma. *LWT*. 123:109121.

Krishnan, N., S. Chattopadhyay, J. Kundu, and **A. Chaudhuri.** 2002. Superoxide dismutase activity in haemocytes and haemolymph of Bombyx mori following bacterial infection. *Current Science*. 321-325.

Krizek, D.T., S.J. Britz, and **R.M. Mirecki.** 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth of cv. New Red Fire lettuce. *Physiologia Plantarum* 103: 1-7.

Lange, B.M., M.R. Wildung, E.J. Stauber, C. Sanchez, D. Pouchnik, and **R. Croteau.** 2000. Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *Proceedings of the National Academy of Sciences.* 97: 2934-2939.

Luciano, P., M. Maffei, and **D. Castaldo.** 2006. Indagini preliminari sull'effetto della radiazione UV-B sull'espressione di alcuni geni coinvolti nella biosintesi di monoterpeni in *Mentha x piperita* L. *Essenze e derivati agrumari* 76: 5-9.

Maffei, M., D. Canova, C. Bertea, and **S. Scannerini.** 1999. UV-A effects on photomorphogenesis and essential-oil composition in *Mentha piperita*. *Journal of Photochemistry and Photobiology B: Biology.* 52: 105-110.

Maffei, M., and **S. Scannerini.** 1999. Photomorphogenic and chemical responses to blue light in *Mentha piperita*. *Journal of Essential Oil Research.* 11:730-738.

Mahendran, G., and **L.U. Rahman.** 2020. Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (*Mentha× piperita* L.)—A review. *Phytotherapy Research.* 34:2088-2139.

Manahan, S.E. 2022. *Environmental Chemistry*. CRC press.

Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research.* 29: e45-e45.

Polle, A., and H. Rennenberg. 1992. Field studies on Norway spruce trees at high altitudes: II. Defence systems against oxidative stress in needles. *New Phytologist.* 121:635-642.

Rao, M.V., G. Paliyath, and **D.P. Ormrod.** 1996. Ultraviolet-B-and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology.* 110:125-136.

Willekens, H., W. Van Camp, M. Van Montagu, D. Inze, C. Langebartels, and **H. Sandermann Jr.** 1994. Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbagini folia* L. *Plant Physiology.* 106:1007-1014.

Zhang, J., and **M. Kirkham.** 1996. Antioxidant responses to drought in sunflower and sorghum seedlings. *New Phytologist.* 132:361-373.