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Various strategies elicited and modulated by elevated UV-B radiation and protectant compounds in *Thymus* species: Differences in response over treatments, acclimation and interaction

Alireza Shayganfar[a], Majid Azizi[a,*], Mousa Rasouli[b]

[a] Department of Horticultural Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[b] Department of Landscape Engineering, Faculty of Agriculture, Malayer University, Malayer, Iran

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ABSTRACT

Nowadays, ultraviolet radiation, particularly from the ultraviolet-B (UV-B) range, is considered a regulating factor triggering a global response in the plants rather than a simple stressing factor. Predicted scenarios of climate change indicate the increasing trend of UV-B radiation in Mediterranean ecosystems as the center of origin of *Thymus*. The present study aimed to investigate the probable responses of three entirely different *Thymus* species in their natural habitats under elevated ultraviolet-B (UV-B), and to seek conditions that can induce desirable changes using UV-B radiation as an effective elicitor and protectant compounds as antioxidants. Altogether, under elevated UV-B radiation, the total amount of phenolic compounds was increased in all three species, while the total rate of alcoholic compound of linalool was decreased in *Thymus fedtschenkoi*. Principal components analysis suggested that the created scattering was caused by protectant compounds, especially under enhanced UV-B radiation. Despite the great differences in responses of each species to the applied treatments, there were similarities between the *Thymus daenensis* and *Thymus fedtschenkoi* species. However, *Thymus vulgaris* had the most distance. The most changes, both morphologically and phytochemically, were observed in *Thymus fedtschenkoi*. *Thymus daenensis* had the most accordance with the enhanced UV-B conditions, most likely because of growing on the southern slopes.

1. Introduction

Stratospheric ozone depletion increases the ultraviolet-B (UV-B) radiation (280–315 nm) in the biosphere (McKenzie et al., 2011). The ozone layer is not expected to recover until 2070 due to the decrease of the temperature in the stratosphere as an effect of climate change (Caldwell et al., 2007; WMO, 2010; Kataria et al., 2013). Predicted scenarios of climate change over the next decades include an increase in the levels of UV radiation reaching the Mediterranean ecosystems (Zerefos et al., 1997; Foyo-Moreno et al., 2003; Giorgi et al., 2004; IPCC, 2007; WMO, 2010; Verdaguer et al., 2012). Taking into account the climate model predictions, it is clear that further studies are needed to improve the understanding of the responses of plants to increasing UV radiation in their habitats.

Ultraviolet-B radiation is an important component of the environment acting as an ecophysiological factor with the potential to alter plant growth and photosynthesis (Ballaré et al., 2011). Ultraviolet-B radiation has considerable consequences on many levels, including anatomy, morphology, physiology, biochemistry, phenology, and yield, even though these responses varied markedly within and between species (Searles et al., 2001; Kakani et al., 2003; Kataria and Guruprasad, 2014). During the last decade, it has become increasingly clear that UV-B, as a growth modulator, is able to turn metabolic switches between primary and secondary metabolism (Zhang and Björn, 2009; Jansen and Bornman, 2012; Schreiner et al., 2012). Thus, treatment with UV light, particularly from the UV-B range, is an example for an effective elicitor application (Schreiner et al., 2014). On the other hand, perusal of relevant literature reveals that UV-B radiation inflicts damage to the photosynthetic apparatus of green plants at multiple sites (Chen et al., 2008; Kataria et al., 2014). Hence, an idea for study is to investigate the circumstances that in spite of reducing the damage to the photosynthetic apparatus can stimulate and manage the production of desirable secondary metabolites.

In recent years, melatonin (a biogenic indoleamine) has emerged as a research highlight in plant studies. The most frequently mentioned functions of melatonin are related to abiotic stresses. It has also been suggested that melatonin protection against reactive oxygen species covers not only chlorophyll, but also photosynthetic proteins in general

* Corresponding author.
E-mail address: azizi@um.ac.ir (M. Azizi).

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(Zhang et al., 2015; Lazar et al., 2013). Glutathione, another non-enzymatic antioxidant, is an essential metabolite with multiple functions in plants. It protects cell constituents against oxidation and eliminates the oxygen radicals formed during the photosynthesis process together with ascorbate (Mittler, 2002). An equal mixture of iron-zinc nano-fertilizer was the other used protectant treatment. Up to 80% of the cellular iron is found in the chloroplasts, which is consistent with its major function in photosynthesis (Hänsch and Mendel, 2009). Zinc, as a component of proteins, influences not only the proper development of the chloroplasts, but also the maintenance of the photosynthetic activity of the system (Aravind and Prasad, 2004).

Given that the habitat of thyme (*Thymus* spp.) is rangelands and rocky-gravelly grounds in high altitudes, and the Mediterranean region is the center of diversity of this genus (Jalas, 1971; Morales, 2002). It consists of the conditions with the highest potential to receive UV-B radiation (Zerefos et al., 1997; Foyo-Moreno et al., 2003; Giorgi et al., 2004; IPCC, 2007; WMO, 2010; Verdaguer et al., 2012). Three different *Thymus* species were chosen for consideration. The aromatic medicinal properties of the genus *Thymus* have made it one of the most popular plants from all over the world. *Thymus daenensis* Celak, which is well-characterized by its lanceolate leaves, is an endemic species in Iran (Rechinger, 1982; Rustaiee et al., 2013). It is a standing plant with up to 30 cm height, cushion-shaped, linear-to-lanceolate leaves, and pale-purple flowers. Essential oil of this plant is a rich source of phenolic compound thymol (78.1%) (Rustaiee et al., 2010). *Thymus fedtschenkoi* Ronniger is a semi endemic plant of Iran that also grows naturally in some parts of Turkey and the Caucasus. It is small, highly branching and woody at basal parts, with triangular-ovate- to-oblong-ovate leaves, short and compact head inflorescence, and white to pale-pink flowers, which grows naturally on some stony mountain slopes of Iran (Rechinger, 1982; Rustaiee et al., 2011a,b). Linalool as an acyclic monoterpene alcohol is the major component in the essential oil of this plant (83.1%) (Rustaiee et al., 2011a,b). *Thymus vulgaris* L., more commonly known as thyme, an evergreen herb and not native in Iran, was considered as a criterion for comparison. There are numerous studies about the essential oil of *T. vulgaris* (indicating the various chemotype). However, mostly thymol, *p*-Cymene, γ-terpinene, and carvacrol component were reported (Morgan, 1989; Torras et al., 2007; Rustaiee et al., 2013; Borugă et al., 2014; Satyal et al., 2016).

Essential oil composition of aromatic plants, as antioxidant substances (Adorjan and Buchbauer, 2010; Sanchez-Gonzalez et al., 2011), is greatly influenced by various factors such as environmental conditions, edaphic factors, and harvesting time (Figueiredo et al., 2008; Barra, 2009) and is highly integrated with the physiology of the whole plant. In this study the changes of essential oil compositions as well as other plant responses have been evaluated under elevated UV-B radiation and photosynthetic apparatus protectant compounds.

2. Materials and methods

2.1. Plant material and growth conditions

The field experiment was conducted in the research farm of the University of Malayer under more realistic field conditions, including visible light and background ambient UV-B level (Latitude: 34°15′N, Longitude: 48°51′E, Altitude: 1814 m, Malayer, Hamedan Province, Iran).The experiments were carried out from March to August 2016.

The plants of *T. daenensis* and *T. fedtschenkoi* were collected from their natural habitats in Malayer (1840–50 m above sea level, 34°15′N, 48°35′E, on the southern slope) and Soobashi Mountains (2430–40 m above sea level, 34°11′N, 48°15′E, on the northern slope) in late February 2016, respectively. *T. vulgaris* transplants were purchased from the Hamedan Botanical Garden.

A randomized complete block design with a split-plot arrangement was used to investigate the main and interaction effects between two UV radiation levels and four protectant treatments with three replicates. Hence, two blocks were considered based on two UV radiation conditions (solar ambient ultraviolet and elevated UV-B). Then, each block was divided into four plots, according to four protectant treatments (glutathione, melatonin, nano-fertilizer and control) In each plot three *Thymus* species were transplanted (*T. daenensis*, *T. fedtschenkoi* and *T. vulgaris*), and finally, from each species three bushes were chosen as replicate.

2.2. UV-B radiation

In order to achieve steady and fresh growth, the plants were trimmed in mid-May two and a half months after planting. In UV-B block, the elevated UV-B treatment begun two weeks after trimming in late May and continued until harvesting time at the full flowering stage in late August. Supplemental UV-B radiation was provided by Q panel UV-B 313 40 W, 120 cm fluorescent lamps (Q Panel Inc. Cleveland, OH, USA).

The lamps were wrapped with cellulose diacetate foil (Ultraphan URT, 0.1 mm, Digefra GmbH, Munich, Germany) to remove wavelengths < 280 nm (UV-C radiation). Cellulose diacetate filters were pre-solarized for eight hours and changed weekly to ensure uniformity of UV-B transmission. In order to prevent shading of metal frames and fluorescent lamps, the plots direction was considered in east-west orientation and then at the northern margin of each plot. The lamps were mounted on frameworks so that UV radiation was reflected on the plants. The direction of the lamps was perpendicular to the planted rows. The lamps were installed in a steel and shiny frame with the distance between lamps of about 25 cm at a distance of 50 cm above the center of each plot. The spectral irradiance was determined by an Optronic Model 742 spectroradiometer (Optronic Laboratories Inc., Orlando, FL, USA). The spectral irradiance was weighted according to the generalized plant action spectrum (Caldwell, 1971) and normalized at 300 nm to obtain biologically effective UV-B radiation (UV-B_{BE}). During the experiment, the lamp height was adjusted monthly to maintain a constant distance of 50 cm from the center of each plot. Therefore, providing supplemental irradiances of 5.98 kJ m^{-2} of UV-B_{BE} enhanced. Exposure resulting from 25% ozone depletion was simulated, corresponding to a 50% increase of ambient UVB_{BE} (McKenzie et al., 2007). Nevertheless, the plants in the UV-B block received ambient plus supplemental levels of UV-B and in another block plants received only ambient levels of solar UV radiation. Ultraviolet-B was provided to the plants during the solar noon period (11:00–14:00 h).

2.3. Protectant treatments

After five days of UV-B treatment, protectant treatments were applied every five days at sunset. In each block, plots randomically received one of the glutathione, melatonin (both from Sigma-Aldrich, Inc. Germany), nano-fertilizer (Khazra, Sodour Ahrar Shargh knowledge-based Co, Iran) and control treatment so that at every turn, each of the plants were completely sprayed with 200 ml of 100 mg/L melatonin solution, 550 mg/L glutathione solution, and 5000 mg/L of nano-fertilizer solution (equal mix of nano-iron and nano-zinc fertilizer). For more absorption, a slight amount of surfactant was added in each spraying.

2.4. Morphological and phenological measurements

In the morphological study, six important agro-morphology traits were evaluated, including weight loss (difference between fresh and dry weight), height, internode, leaf (and flowers or inflorescences) amount, stem amount, and leaf to stem ratio (based on dry weight) (Table 1).

Due to very slow initial growth, about a month after beginning the UV-B treatment and simultaneously with each irrigation, the phenological data were recorded every ten days in five note taking, from 53 to 93 days after trimming, as shown the stickers on the columns in Figs. 1