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Exogenous putrescine changes redox regulations and essential oil constituents in field-grown *Thymus vulgaris* L. under well-watered and drought stress conditions

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ABSTRACT

Drought stress affects a vast range of morphological, physiological and biochemical characteristics in plants; however, exogenous application of osmotically active materials such as polyamines has been regarded as a good alternative to counter the adverse effects of various environmental stresses on plant functions. The present study investigates the response of *Thymus vulgaris* L. plants to different concentrations of putrescine (0, 20 and 40 mg L^{-1}) under well-watered and drought stress conditions in a factorial experiment based on randomized complete block design with three replications during 2-year field trial. Drought stress significantly impaired the plant growth and biomass accumulation, and altered leaf water status, photosynthetic pigment contents, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels, free proline and soluble protein contents, ROS scavenging enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX), essential oil content and chemical profiles of the essential oil compared to the respective control values. However, exogenously applied putrescine (particularly at 20 mg L^{-1}) improved leaf water content, accumulated dry matter, reduced cell injury indices and up-regulated antioxidant enzyme activities. Moreover, stressed plants treated with the highest concentration of putrescine increased their essential oil content by 23.07% compared to non-stressed plants without putrescine application. A total of 14 compounds predominantly consisted of monoterpenes such as Thymol, Carvacrol, γ-Terpinene and *p*-Cymene were identified in the essential oil of the plants in both years. Percentage of the major essential oil constituents substantially enhanced under drought stress, whereas exogenous application of putrescine further improved the content of these specific volatile compounds under well-watered and stressed plants compared to the respective control. A significant negative correlation was observed between essential oil percentage and H_2O_2 ($r_{0.01} = -0.928$) and MDA ($r_{0.01} = -0.947$) contents in plants under drought stress. The results of this study indicated that foliar application of putrescine in proper level may act as elicitor to trigger physiological processes and induce valuable metabolites biosynthesis, which may compensate the negative impacts of drought stress on plant biomass and essential oil quality and quantity.

1. Introduction

Thyme (*Thymus vulgaris* L.), belonging to the mint (Lamiaceae) family, is an aromatic and medicinal plant native to the western Mediterranean region of Europe, has a long history of use in culinary spices and ancient herbal medicine, and is a chemically variable species cultivated worldwide (Zarzuelo and Crespo, 2002). The volatile phenolic oil of thyme has been reported to be among the world's top 10 most popular essential oils (Letchamo et al., 1995), with antioxidative, antibacterial, antihelminthic antiseptic, antimycotic, anaesthetic, sedative, and food preservative properties (Fatma et al., 2014; Zarzuelo and Crespo, 2002). Chemically, thyme essential oil is a mixture of secondary metabolites commonly comprised of monoterpenes, and the major active constituents of this oil are the phenolic isoprenoids such as Thymol and Carvacrol (Amiri, 2012; Nickavar et al., 2005).

Although, the secondary metabolites are not directly involved in the well-defined primary metabolic processes such as growth and development, they are crucial for plant adaptation to a diverse range of stresses in the environment (Ghorbanpour et al., 2016; Ramakrishna and Ravishankar, 2011).

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Water, core of the sustainable development, has been considered as the single most important factor restricting plant productivity in agricultural systems particularly in semi-arid and arid regions of the world, where regular and prolonged drought exist (Fageria et al., 2006). Drought stress affects a vast range of plant morphological, physiological and biochemical characteristics, for example, it results in altered water relations, suppressed cellular activities (Hatami et al., 2017), reduced content of chlorophyll and carotenoids (Guo et al., 2016), induced generation of reactive oxygen species (ROS) that cause damage plasma membrane integrity and protein functioning (Farooq et al., 2009; Baiazidi-Aghdam et al., 2016), disturbed the redox state homeostasis, leading to metabolic dysfunction and severe yield losses in subjected plants (Gholami Zali and Ehsanzadeh, 2018). Plants counter drought stress by adopting various strategies including accumulation of compatible solutes (e.g. proline and glycine betaine), adjustment in photosynthetic parameters, synthesis of stress-related primary and secondary metabolites, activating antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX), changes in gene expression (Morshedloo et al., 2017; Farooq et al., 2009).

Antioxidant defense systems may not be adequate to mitigate the harmful effects of ROS under prolonged drought stress conditions. Therefore, the application of osmotically active substances (e.g. polyamines) in plants has been adopted as a potential candidate to cope with the adverse effects of various environmental stresses. Polyamines including spermidine, spermine, and putrescine, a calss of phytohormone-like aliphatic amine compounds, are widely involved in a range of developmental and physiological processes (Shi and Chan, 2014). They also play important modulating roles in plant cells against environmental stresses, such as maintenance and regulation of cellular homeostasis, integrity and permeability of plasma membrane, inhibition of chlorophyll degradation, stimulation of specific protein biosynthesis and nitrogen-containing alkaloids (Kusano et al., 2008; Tiburcio et al., 2014; Bouchereau et al., 1999).

Although, numerous research have been performed on the role of polyamines in crop plants, very little has been reported about the effects of exogenous application of putrescine on essential oil components in medicinal and aromatic plants under drought stress. Therefore, the present study was carried out to investigate the role of exogenous putrescine on various physio-biochemical attributes and essential oil constituents in field-grown *Thymus vulgaris* L. under normal irrigation and drought stress conditions.

2. Materials and methods

2.1. Site description

A two-year (2015 and 2016) field experiment was conducted at the research farm of Azarbaijan Shahid Madani University, located in Tabriz, Iran (latitude 37°49′N, longitude 45°56′E, and altitude 1303.8 m above mean sea level). The research location is classified as semi-arid climate with regular seasons according to Koppen climate classification system. The mean annual precipitation is approximately 280 mm, a great deal of which falls as snow over the winter months and rain in autumn and spring seasons. The trends and variations of minimum and maximum daily air temperatures and precipitation registered during the growing seasons in 2015 and 2016 are given in Fig. 1.

2.2. Plant materials

The seeds of the *Thymus vulgaris* L. were obtained from the Pakan Bazr Co. Esfahan, Iran. The viability of seeds was tested prior to the experiment and was 96% on average. Then, fifteen seeds were planted into each plastic pot (25 cm in length and 30 cm in diameter), totally 116 seeds were sowed in pots filled with sandy loam soil, pH: 7.4, and kept under light/dark cycle conditions of 16/8 h at 25 °C and 75% relative humidity placed in a greenhouse (date: 2015.02.20, and 2016.02.22). Thereafter, with growing seedlings until reaching to length of 10–12 cm, they were transplanted to field (date: 2015.04.20 and 2016.04.20). There were four rows in each plot with a length of 4 m and 50 cm (distance between rows) and 30 cm (distance of seedlings on the planting line). In the field experiment, totally 18 plots were considered and 52 uniform seedlings were planted per each plot. During entire study, no fertilizer or pesticide was used and weeds were controlled manually as needed. Physical and chemical characteristics of the field soil (0–30 cm depth) were described in Table 1.

2.3. Experimental design

The study was arranged as factorial experiment based on a randomized complete block design (RCBD) with three replications. Treatments were consisted of two factors. The first factor was the water availability (well-watered and drought stress) and the second factor was the concentration of putrescine (0, 20 and 40 mg L^{-1}). The well-watered and drought stress plots were irrigated regularly to avoid wilting until 50% of the plots reached the flowering stage. After this stage, drought stress plants were no longer irrigated, while well-watered plants were further irrigated until early stages of flowering (flowering beginning). Spraying Putrescine on *Thymus vulgaris* L. plants was performed before flowering initiation phase (*i.e.*, after 60 days of seedling transplanting for three continuous weeks). All shoot parts of each plant was exogenously sprayed/covered with employed concentrations of Putrescine in a volume of 50 mL in each time with hand atomizer. At the early stages of flowering (when 50% of the plants entered this stage), the plant was removed from the plots for each treatment (date: 2015.07.22, and 2016.07.17), and a part of the shoots was considered for measuring the morphological traits (dry weight of shoots) and essential oil extraction. The other part was quickly placed in liquid nitrogen and transferred to a −80 °C freezer at the laboratory for measuring physiological traits as follows:

2.4. Measurements

2.4.1. Growth parameters

At full flowering stage, plant height (from soil surface to the tip of the stem) was recorded. Moreover, aerial parts of the plants were cut from soil surface at full bloom stage in both trial years. After harvesting, dry weight (kg/ha) of harvested plants was immediately noted.

2.4.2. Relative water content (RWC)

RWC was determined by using fully developed young leaf (4th leaf). They were quickly sealed into clear plastic bags and fresh weights (FW) were recorded immediately after samples transferred to laboratory. Then, they were immersed in double distilled water inside the covered Petri dish for 6 h at room temperature and the turgid weight (TW) was recorded. The leaf samples were oven dried at 70 °C for 48 h and then dry weight (DW) was determined. Finally, RWC was estimated according to Levitt (1980) using the following formula:

$$\mathrm{RWC}(\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

2.4.3. Photosynthetic pigments

For chlorophyll (Chl) and carotenoids extraction, 0.5 g of fresh leaves was ground using mortar and pestle containing 10 mL of acetone (80% V/V). The light absorption of leaf extract solution was read at 645, 663 and 470 nm for Chl *a*, Chl *b* and carotenoids contents in a UV–vis spectrophotometer, respectively. Photosynthetic contents were estimated according to the following equations (Lichtenthaler, 1987) and expressed as mg g^{-1} fresh weight (fw^{-1}).

Chl *a* (mg g^{-1} fw^{-1}) = 12.25 × $A_{663.2}$–2.79 × $A_{646.8}$

Chl *b* (mg g^{-1} fw^{-1}) = 21.50 × $A_{646.8}$–5.10 × $A_{663.2}$