Effects of heat stress on *Euphorbia peplus* growth in hydroponics and subsequent ingenol production

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Abstract

Plant secondary metabolites (PSM) are phytochemicals with high relevance to the pharmaceutical and food industries. The accumulation of PSM occurs often under stress as a protective and/or adaptive mechanism. Environmental factors such as temperature, relative humidity, fertilization, light intensity, and atmospheric CO_2 concentration have all separately a significant impact on plant growth and PSM production. Therefore, the purpose of this study is to examine the effects of heat stress on *Euphorbia peplus* growth and the subsequent ingenol production. Then, three temperature levels (18, 24, 30°C) were applied. Plants were cultivated in controlled environment chambers using a hydroponic system. Harvesting was conducted over three distinct periods (30 days, 45 days and 60 days). Throughout the crop, agronomic measurements were made (plant height, dry weight percentage), as well as an UPLC analysis of ingenol plant content. In response to heat stress (30°C), the plant's height decreased between the 45th and 60th day of cultivation, the plant bends over and loses leaves. In opposite, the plant's height carried on to increase at 18 and 24°C for all the periods of observation. At 30°C, the plants accumulated more dry matter after 45 and 60 days than the plants grown at other temperatures with a higher plant content of ingenol.

Keywords: PSM, heat stress, *Euphorbia peplus*, ingenol, hydroponics

INTRODUCTION

Plant secondary metabolite (PSM) is a compound that is not fundamental to maintaining plant processes, but that is important for the plant to interact with its environment for adaptation and defense (Akula and Ravishankar, 2011). These molecules are often characteristic of a species stresses and play a major role in plant response to stress (Kennedy and Wightman, 2011). On the basis of their biosynthesis pathways, these compounds are divided into three major groups: terpenoids, alkaloids, and phenylpropanoids (Chen et al., 2013). In fact, plant metabolites are considered biosynthetic compounds that can accumulate in plants and serve as therapeutic medicinal for humans (Verma and Shukla, 2015). Plants produce very small amounts (less than 1% dry weight) of these compounds, which are strongly influenced by the phenological stages of the plant (Ramachandra Rao and Ravishankar, 2002).

PSMs are influenced by many factors, including genetic, morphogenetic, and environmental factors, which can be classified as biotic and abiotic (Verma and Shukla, 2015). There are several types of abiotic stress affecting plants, including temperature, drought, radiation (light), chemical stress (nutrient solution), and atmospheric composition (CO_2, humidity) etc. (Mahajan and Tuteja, 2005; Akula and Ravishankar, 2011; Rouphael and Kyriacou, 2018).

In the *Euphorbiaceae* family, *Euphorbia* is the largest genus, containing over 2000 species (Shi et al., 2008).

This latex has long been used as an alternative therapy for skin diseases, including cancerous lesions (Berman, 2012).

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In this study, we focused on *Euphorbia peplus*, a C3 plant (Batanouny et al., 1991). This plant is considered as a weed and produces a latex that contains active compounds. As a result of its discovery in the 1990s for cancer therapy, this plant has been the subject of numerous medical studies. Indeed, prior research has focused on specialized metabolites from the whole plant, and many bioactive diterpenoids with diverse structures, including jatrophan, ingenan, and tetracyclic diterpenoids, have been isolated and identified (Jakupovic et el., 1998; Hohmann et al., 1999, 2000; Song et al., 2010). Among these compounds is ingenol, a compound discovered for the first time at the end of the 20th century (Hohmann et al., 2000).

However, the chemical synthesis of this compound involves several relatively slow steps, with a low yield at the end (Jorgensen et al., 2013). Furthermore, the natural production of this molecule from plants is low (Zerbe et al., 2013), so it is important to intensify the production of this plant and focus on in vivo production.

This work aims to stimulate ingenol biosynthesis pathway and biomass production in *Euphorbia peplus* grown in shipping container equipped with a hydroponic system by applying heat stress.

MATERIALS AND METHODS

Plant material and growing conditions

Euphorbia peplus seeds were purchased from a horticulture shop in (Liège, Belgium). They were sown in rock wool and placed beneath a PPFD (photosynthetic photon flux density) of 200 µmol m^{-2} s^{-1}. At the stage of the second true leaf (15-20 days), plants were transplanted into rock wool substrates (7×7.5×7.5 cm) and placed in a hydroponic system.

The experiments took place in a container equipped with a controlled-environment vertical production unit provided by Urban Crop Solutions (Waregem, Belgium). The container was controlled through a remote monitoring application that allowed the user to program culture conditions and to get an overview of the environmental factors.

Temperature experiment

Three temperatures were tested (18, 24 and 30°C) under 200 µmol m^{-2} s^{-1}. The culture parameters were set as follows: CO_2 concentration = 950 µmol mol^{-1}, pH = 6.2, electro-conductivity = 2000 µS cm^{-1}, relative humidity = [60-70%], and a photoperiod of 18h day/6h night. Each temperature treatment was applied to four culture trays containing 12 plants to obtain 48 plants per treatment. Moreover, for each treatment three harvests (48 plants per harvest for each stage) were performed at 30 days (stage 1), 45 days (stage 2), and 60 days (stage 3).

Plant height measurements

Plant heights were measured from the base of their stem to their apex using a graduated slat (mm) (Figure 1).

Dry weight

The dry weight at the vegetative system was determined using a Mettler PM460 Delta Range scale (precision = 0.01 g) after drying in an oven with a ventilation system (Wötsch VTU 125/200) at 40°C until constant weight (Figure 2).

Ingenol content

Ingenol quantification method was internally developed by Celabor (Herve, Belgium) (Bafort et al., 2022) using a UPLC-DAD-MS/MS system from Waters (Milford,USA).

Briefly, the dried plants were ground on a sifter with a diameter of 250 µm. Then, 100 mg of the powder obtained was blended with 2 mL methanol and placed in an ultrasonic bath for three cycles of five minutes each, with vortexing in-between. Subsequently, the mixture was filtered into a UPLC vial using 0.22 µm PTFE from Millipore (Billerica, USA) and injected for analysis.

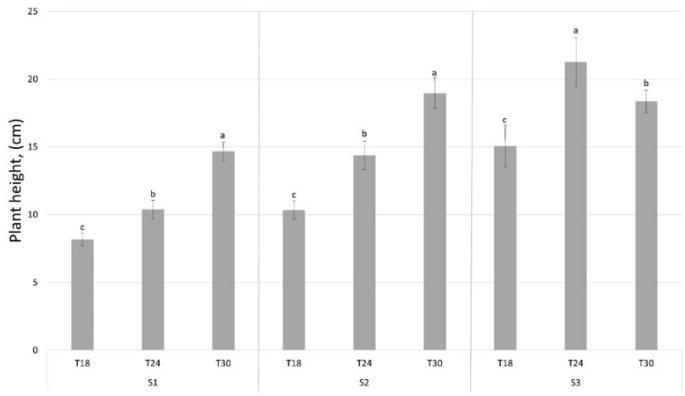

Figure 1. Plant height in response to thermal treatments. T18=18°C, T24=24°C, T30=30°C, S1 (stage 1: 30 days), S2 (stage 2: 45 days), and S3 (stage 3: 60 days) (Data represent the mean of 48 biological replicates followed by SD). Tukey mean comparisons were performed to study the significance of differences between treatments. Value showing different letters are significantly different a>b, P<0.05.

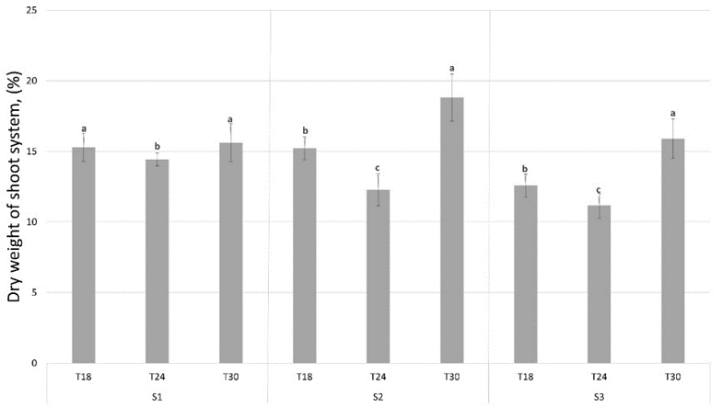

Figure 2. Effects of thermal treatments on the dry weight of shoot system. T18=18°C, T24=24°C, T30=30°C S1 (stage 1: 30 days), S2 (stage 2: 45 days), and S3 (stage 3: 60 days) (Data represent the mean of 48 biological replicates followed by SD). Tukey mean comparisons were performed to study the significance of differences between treatments. Value showing different letters are significantly different a>b, P<0.05.

The stock solutions of ingenol (1.0 mg mL^{-1}) from Bio-Connect (Huissen, The Netherlands) was prepared in methanol and was diluted with methanol to prepare a series of concentrations (8, 6, 4, 2,1, 0.5, 0.25 ppm) to establish the calibration curve (Figure 3).

RESULTS AND DISCUSSION

Plant height

In response to a temperature increase, the plant elongates more strongly at temperature T30 during the first 45 days, but the rate of growth slows after acute stress, resulting in a slight decrease in height in stage 3. As a result of stress, the plant bends over and loses leaves. These results are in line with those reported by (Alhaithloul et al., 2019), who demonstrated that the height of *Mentha piperita* and *Catharanthus roseus* decreased after a temperature of 35°C compared to 17 to 22°C.

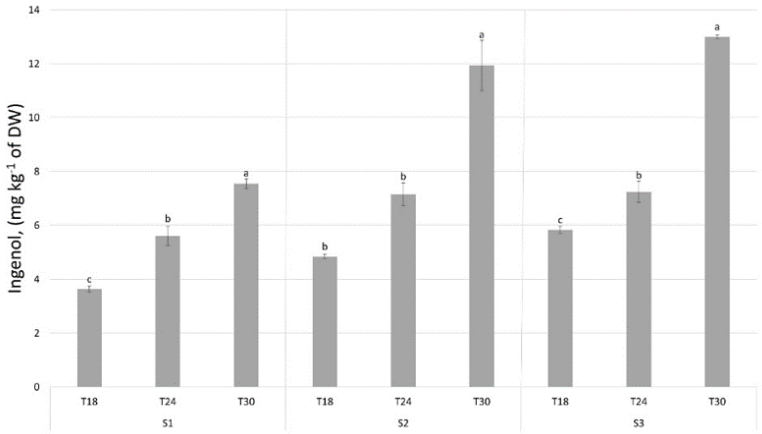

Figure 3. Effects of culture temperature on the amount of ingenol (mg kg^{-1} of DW) in *Euphorbia peplus*. T18=18°C, T24=24°C, T30=30°C S1 (stage 1: 30 days), S2 (stage 2: 45days), and S3 (stage 3: 60 days) (Data represent the mean of 48 biological replicates followed by SD). Tukey mean comparisons were performed to study the significance of differences between treatments Value showing different letters are significantly different a>b, $P<0.05$.

In contrast, we observe that at 18°C, the apical growth is slower compared to other treatments and that the height of the plants did not exceed 15 cm at stage 3. However, under 24°C the plants appeared to grow more rapidly, with a significant difference in height compared to those grown at 18°C.

Dry weight (DW)

Depending on the stage of growth, the temperature has a significant effect on the dry weight of the aerial part. For the first 30 days, there is no significant difference between T18 and T30. In 45 days, the percentage of dry matter has significantly increased with T30 treatment, but not with T18 or T24 treatment. In stage 3 (60 days), as a result of senescent leaves loss at the end of culture, we observe that this percentage has decreased with each treatment and the plants grown at 24°C accumulate less dry matter. Heat stress can reduce yield and dry matter production in many crops, including maize (Giaveno and Ferrero, 2003).

Ingenol content

Results revealed that the ingenol content in the plants increases significantly as the temperature rises. Plants grown at 30°C have almost twice as much ingenol as those grown at 24°C at stage 3 of harvest (60 days).

Secondary metabolites are key components of plant defense systems, which are stimulated by stress (Abbas et al., 2017; Yang et al., 2018). In accord with our results, at high temperatures, terpenoids accumulate in *Betula pendula* and *Populus tremula* (Ibrahim et al., 2010). This phenomenon is regulated by zeatin riboside and isopentenyl adenosine in *Pinus radiata* (Escandón et al., 2018).

CONCLUSIONS

Heat stress significantly decreased growth and biomass accumulation in *Euphorbia peplus*. An increased accumulation of ingenol at early and late stages of growth in response to stress confirmed that this plant has a tendency to produce more diterpenoids. The plants grown at 30°C are stressed, and they produce more ingenol, but they have low biomass compared to the plants grown at 24°C.

Among the treatment conditions, 24°C gives the best results for the quantity of ingenol produced per plant.

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