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PHYTOCHEMICAL ACCUMULATION WITH PHOTOMORPHOGENESIS AND PHYSIOLOGY OF *Salvia officinalis* L.

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ABSTRACT

We have investigated the leaf development of sage including the stomata, trichome and clorophyll parameters, their response, and the interaction of the relationship between these parameters and quantity and content of the phytochemicals in the plant with different photoperiod applications. Sage plants were exposed to short-dat, middle-day and long-day conditions in a controlled environment for 3 months.
To confirm the morphological responses of stomata in response to photoperiod, stomatal density, stomatal sizes (Lg/Wg), stomatal area and relative stomatal area on both leaf surfaces were determined using SEM analyses. Phytochemicals parameters were determined using SPME and GS/MS analyses. Light period caused significant changes in morphoparameters on both surfaces of leaves. Significant changes in pythochemical quantity and content of sage were observed as well. In the light of the morphologic data such as plant growth, leaf surface area, stomatal and trichome parameters, chlorophyll and phytochemical content gathered from sage plants exposed to different photoperiod lengths, we hereby describe the circadian rhytm mechanism of the plant.

Key words: phytochemical, circadian clock, sage, stomata, trichomes

INTRODUCTION

Sage (*Salvia officinalis* L.), the largest genus of the *Lamiaceae* family, includes about 900 species widespread throughout the world. Sage has long been used for medicinal, pharmaceutical and culinary purposes because of its terpenoid, flavonoid and phenolic compounds. Essential oils obtained from sage shows antidiabetic, anticancer, anti-inflammatory, anti-mutagenic and antimicrobial properties and are used in treating many disases of the nervous system, heart and blood circulation, respiratory system, digestive system, metabolism and endocrine system [Wei et al. 2018]. On the other hand, sage oil is used as a food additive in the food industry, and due to its deodorant property it is also used in perfumery. It also shows insecticidal activity [Koutsaviti et al. 2018] and can play a role in reducing pesticide usage. Novadays, nanoparticles of essential oils are use in production and stabilization of nanostructures, therefore opening the path for their usage in many different areas [Gupta et al. 2017]. For these reasons, the species have been cultivated worldwide to improve essential oil production. Researches to imrove the essential oil content and quantity of sage have never been more important.

In sage, secondary metaboiltes are produced and stored mostly by glandular, peltate and capitate trichomes on leaf surface and rarely by the trichomes of the stem [Turner et al. 2000]. Capitate trichomes consist of a circular head containing four cells and a stalk with various morphologies, and are responsible for the production, accumulation and secretion of

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defensive proteins and compounds that are used in the defense mechanism against pests [Gao et al. 2017]. Peltate trichomes, formed by the separation of cuticula from the cell membranes of about eight disk shaped cells, are responsible for the production, accumulation and secretion of semi-volatile organic compounds that protect plants from biotic and abiotic stress [Martínez-Natarén et al. 2018].

Growth, organogenesis, formation of natural products, as well as biosynthesis and content of secondary metabolites of medicinal and aromatic plants, while being genetically controlled, are also strongly affected by environmental factors including light [Khan et al. 2018]. Light activates the defense mechanism of plants against biotic and abiotic stress factors and increases the production of protective molecules such as flavonoids [Zhang et al. 2017]. On the other hand, light affects chlorophyll concentration [Chen et al. 2017], causes photoinhibition and alters carbon assimilation rate [Tkalec et al. 2015]. Therefore, while being and energy source for photosynthesis, metabolism and growth of plants, light also serves as a signaling molecule. Optimal growth of plants depends on the mobilization of carbon and nitrogen that are accumulated in the light to promote metabolism and growth in the dark [Graf and Smith 2011]. This is maintained by the circadian clock, which regulates the growth, transformation of reserves and the metabolism by the coordination of light and dark in a one-day period [Greenham and McClung 2015]. It is also called a biological oscillator, a time keeper mechanism that ensures the adaptation of the plant to environmental changes. Plants adjust to light and temperature differences between light and day using this mechanism. Because, all physiological events such as carbon fixation, transpiration, the cell cycle, flowering time, stress responses and gene expression are regulated by circadian clock [McClung 2008]. Therefore, this mechanism also kicks in when plants are exposed to a stress factor and activates the biosynthesis, transportation and signal transmission mechanisms of some phytohormones to create a defense against the stress factor and maintain the growth and development of the plant [Seo and Mas 2015]. Physiological changes become apparent with the changes in morphological and anatomical structures. Furthermore, describing the relationship of the morphological and physiological parameters under changing environmental conditions (such as light and temperature) with the circadian clock mechanism improve our estimations of the product yield. For these reasons, changes in physiological, morphological and anatomical structures and the phytochemical content and quantity of sage exposed to varying light (L/D) conditions were investigated. For this purpose, the growth of *S. officinalis* under different light regimes, and the effects of morphological and physiological changes including photosynthetic pigments, leaf area, stomatal density, stomatal area, trichomes density and growth parameters on the production of phytochemicals were researched to determine the optimal circadian rhythm regime and to increase the medicinal value and economical benefits of sage.

MATERIALS AND METHODS

Salvia officinalis L. (sage) seedlings were commercial cultivars. Seedlings were transferred from the viols to 3 pots filled with peat + soil (10 each) and grown in normal environmental conditions for 15 days. After that pots were transferred to a growth chamber and the seedlings were left to grow in short-day (4/20 h; Light/Dark; L/D), middle-day (8/16 h: L/D) and long-day (16/8 h: L/D) under controlled temperature (23/21°C ±2°C L/D), humidity (75/80%), and light intensity (120 µmol quanta m^{-2} s^{-1}) for 3 months. Plants were irrigated periodically to prevent drought. Experiments were setup in a completely randomized design with 3 replicates. The leaves from the second nodule of the apex of each plant were used to compare the stomatal and trichome parameters.

Fresh weights of the plants from each treatment were measured. Then, plants were dried in an oven in 105°C for 48 h. The amount of obtained dry matter (%) was determined as the growth curve of the plants using following formula:

$$\text{Plant growth rate (\%)} = \left[\frac{\text{Weight of dry material}}{\text{Weight of fresh material}}\right] \times 100 \quad (1)$$

Approximately 30 leaf samples of each application were separated from the stem to determine leaf surface area. The leaves were laid on a graph paper, copied

and weighed. Also, 1 cm^2 of the same graph paper was cut and weighed. The leaf area was calculated according to Pandey and Singh [2011] using the following equations:

$$LA = \frac{x}{y} \quad (2)$$

where: x – graph paper weight of the leaf surface, y – similar graph paper weight of 1 cm^2 area.

The stomatal density (SD, number of stomata per mm^2) of the lower and upper epidermis of leaves was calculated in 50 areas as described by Rengifo et al. [2002].

$$SD = \left[\frac{s}{e+s}\right] \times 100 \quad (3)$$

where: s – stomata number, e – epidermal cells number.

Stomatal area (SA, µm^2) and Relative Stomatal Area (RSA, %) were determined according to the following equation [Orcen et al. 2013] in 50 areas. Using an ocular micrometer under light microscope at a magnification of ×40 on lower surfaces of the leaves stomata sizes (length and width) were determined (µ).

$$SA = \left[\pi \times \frac{Wg \times Lg}{4}\right] \quad (4)$$

where: Wg – stomata width (µm), Lg – stomata length (µm) were defined as the widest point perpendicular to that axis of the stomata, respectively.

$$RSA(\%) = [SA \times SD]/100 \quad (5)$$

Morphology and distribution of glandular trichomes (peltate and capitate) on both surfaces of the leaves from each application were classified as described by Turner et al. [2000]. To determine the density, trichomes in 50 microscopic fields (0.04 mm^2) were counted on both surfaces with independent measurements. Trichome sizes (µ) were determined using an ocular micrometer under stereo microscope on both sides of each leaf pair from ten plants at a magnification of X40.

SEM (Scanning Electron Microscopy) image analysis was used to examine the submicroscopic structures of trichomes and stomata. Fresh leaf samples were dried using a freeze-drier and immediately cut into pieces (2 mm^2). The lyophilized leaves were mounted on aluminum stubs using double-sided adhesive tape and sputter coated with a thin film of carbon. Trichome and stomata parameters of both surfaces of leaves were analyzed using LEO Stereoscan 360 SEM.

A PDMS coated fiber (100 µm) and a manual SPME holder (Supelco Inc.) were used for sample extraction. Firstly, the fiber was exposed to the GC inlet for 3 min for thermal desorption at 250°C before headspace sampling. Dry samples weighting 1 gr were placed in a 10 mL glass bottle, sealed with a rubber septum and stored in a drying cabinet at 25°C for 24 h. The SPME fiber (polydimethylsiloxane –PDMS) was exposed to each sample for 10 min by manually penetrating the septum at 25°C (0.25 cm depth). After the extraction, the SPME fiber was withdrawn from the bottle and inserted into the GC-MS injection port for 3 min for analysis. The GC-MS analysis was conducted using a gas chromatograph coupled to a mass spectrometer with electron impact ion source. A BP-5 Shimadzu fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 µm) 1.4 ml min^{-1}, was used as the carrier gas (23 psi). The detector and injection port temperatures were 260°C, split, 1 min (50 ml/min). The column initial temperature was 35°C. It was then raised to 140°C with a rate of 5°C min^{-1}, and finally raised to 250°C with a rate of 10°C min^{-1} and was held for 2 min. The active compounds from SPME sampling were identified on the basis of relative retention index (ESO 97, Database of Essential Oils, BACIS 1997) and by using mass spectrum database search (Varian NIST MS database 1992 and IMS Terpene Library 1992). The quantification of the components was performed on the basis of their total ion current (TIC) GC peak areas on the column.

Fifty leaves from each application were used to determine the total chlorophyll contents in a chlorophyll meter (Minolta SPAD-502 Chlorophyll Meter, Minolta Co. Ltd., Japan).

A completely randomized experimental design were used to identify significant differences among three different photoperiod treatments. All statistical analyses were performed using IBM SPSS Statistics 20 (SPSS Inc., IBM Company Headquarters, Chicago, IL, USA). Statistical significance was tested using one-way or

two-way analysis of variance (ANOVA). The means were compared using one-way and multivariate analysis of variance followed by Duncan's multiple range tests. The differences between individual means were deemed to be significant at $P \leq 0.05$, graphics were drawn in the software Excel for Windows 10. The analysis of morphological and physiological parameters was conducted with three replications, and results were presented as mean ± standard deviation (SD).

RESULT AND DISCUSSION

Results of these experiments describe the response of morphological and physiological parameters of the plants exposed to different photoperiod applications.

The sage plants (*Salvia officinalis* L.) grown under long-day period (16/8 h; L/D) were visually better than those grown in the other photoperiod treatments (Fig. 1). Stems and roots of sage plants grown under long-day period were longer, but these parameters were very thin. Stems of the plants grown under middle-day period (8 h) were shorter than those in other treatments, however they were well developed ($P \leq 0.05$). This shows that middle-day photoperiod supports stem development (Fig. 1). During dark periods plants transform the C reserves, such as starch that they store under light, to compounds that can be used as energy sources which is vital to plants' growth and development. Changes in the duration of the day upset starch degradation in the night, and starch and sucrose production in the day. Especially, the fact that starch production is inversely proportional to day length [Gibon et al. 2004] caused soybean plants' starch storage to increase from 60% under 14 h light period to 90% under 7 h light period [Smith and Stitt 2007]. While stem and root lengths increased from short-day to long-day periods, growth rate was the highest for middle-day period ($P \leq 0.05$). These results may indicate that starch synthesis and degradation is inversely proportional to day length. Because, when Arabidopsis plans were grown under abnormal day lengths (28 h or 17 h), their starch storages were depleted after 24 h which is the dawn according to the circadian clock and independent from the real dawn. Starch degradation was maintained by circadian control and plant development was reduced until the next dawn to stay alive. On the other hand, under short-day photoperiod, plants increase starch allocation to promote metabolism and growth during the night and therefore need more C reserves and this restricts growth [Mengin et al. 2017]. At the same time, organic acids that provide a substrate for respiratory metabolism at night can be accumulated. Even though very limited, growth is possible under these conditions with the regulation of growth by photoperiodic morphogenesis and the accumulation of various proteins by the hormone signal mechanism that acts in compliance with the circadian clock [Nomoto et al. 2012]. Photosynthesis rate of a plant is proportional to the chlorophyll content of its chloroplasts, which is rapidly synthesized when exposed to light [Coutinho et al. 2018]. Because, the amount of light to absorb that can be observed heavily depends on the amount of chlorophyll present. In this case, changes in chlorophyll content under photoperiod effect supports photoperiod which is effective in plant growth and development. For example, while the increase in the chlorophyll content were 85% under middle-day period, it was 38% and 29% under short-day and long-day periods, respectively. The reshaping of pigment-protein complexes under light indicates that chlorophyll a/b is under circadian control [Pan et al. 2015]. In a study where flaxinus plants were exposed to 0, 50% and 100% light, while total chlorophyll content was the highest at 100% light, highest chl a/chl b rate was at 50%. This rate is in parallel with photosynthesis [Tran 2018]. Plants that accurately regulate circadian clock period (light-dark cycle) gain a photosynthetic advantage. They have more chlorophyll, fix more carbon and grow faster. In fact, they are better survivors than other plants. The difference in plant height, leaf size, stem length and radial growth rate of two genotypes of populus plants grown under 10 : 10, 16 : 4 and 18 : 6 (light : dark) photoperiods for 125 days was explained by the close relationship between circadian clock and cell cycle [Edwards et al. 2018].

Plants undergo morphologic changes, such as changes in leaf area, stomata and trichome parameters, in response to environmental conditions including light [Souza et al. 2016]. These types of changes occur especially during the optimization of metabolism to ensure photosynthetic and biochemical adaptations to changing environmental conditions. In our study, photoperiod length modified morphological and physiological parameters ($P \leq 0.05$). Long-day peri-

od increased leaf surface area and leaf width (Fig. 1). Changes in the growing process due to photoperiod is in parallel with the participation rate of C reserves, which are produced during light period, to the biosynthesis mechanisms that has to occur during dark period to maintain growth [Ishihara et al. 2015]. While it can be expected that a plant should grow at the rate of illumination, there is a non-linear relationship between leaf area and plant biomass. Furthermore, changes in leaf area, total chlorophyll content and net photosynthesis due to changing environmental factors are regulated by circadian rhythm and reductions in product yield and biomass accumulation ensures adaptation to changing conditions [García-Plazaola et al. 2017]. Because, adaptations to external conditions such as photoperiod are provided by circadian clock mechanism.

SEM analyzes were used to determine the effect of photoperiod on the behavior of stomata on both sides of leaves. Stomata are the sole gates of the plants to the outside. In the presence of a stress factor they change in number and size to adapt to the new environment. Circadian rhythm regulates the balance between carbon uptake and water loss, and puts metabolism in order [Muir 2018]. On the other hand, circadian system causes changes in growth by regulating the metabolism, and in the development and differentiation process of stomatal apertures by organizing cell division [Edwards et al. 2018]. To confirm the morphological responses of stomata in response to photoperiod, stomatal density, stomatal sizes (Lg/Wg), stomatal area and relative stomatal area in both leaf surfaces were determined (Tab. 1). In the current study, significant

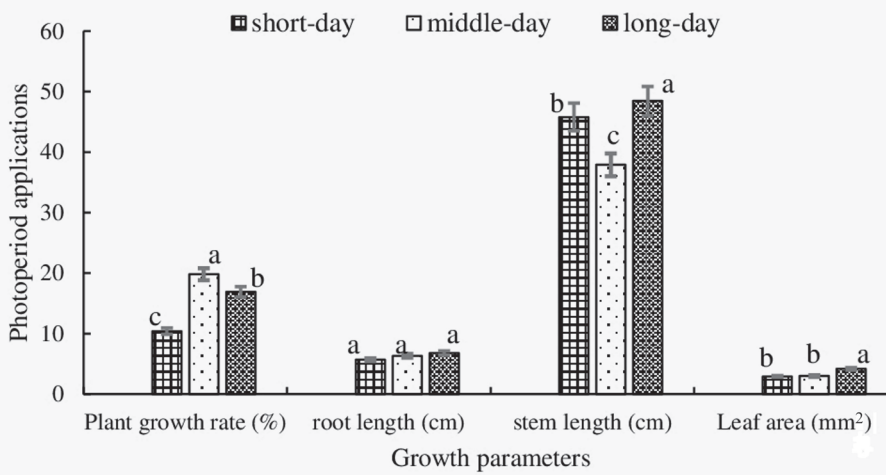

Fig. 1. Effects of photoperiod applications on the growth parameters of sage. Values that are followed by the same letter do not differ statistically at a significance level of 0.05

Table 1. Leaf morphoparameters of sage

Application (period/day)		Stomatal denstiy (mm^{-2})	Lg (µm)	Wg (µm)	SA ($µm^2$)	RSA (%)
Lower surfaces	short	1.79 ±0.5^{e}*	10.74 ±1.2^{c}	7.75 ±1.5^{c}	65.33 ±2.3^{c}	1.16 ±0.5^{de}
	middle	3.67 ±1.1^{ab}	15.34 ±2.2^{a}	12.04 ±1.8^{a}	144.98 ±3.8^{a}	5.32 ±1.9^{a}
	longer	3.08 ±0.5^{d}	11.95 ±1.1^{bc}	8.62 ±1.3^{bc}	80.86 ±3.7^{bc}	2.49 ±1.1^{c}
Upper surfaces	short	3.2 ±0.2^{c}	10.44 ±0.8^{c}	7.16 ±1.2^{c}	58.67 ±1.8^{cd}	1.87 ±0.5^{d}
	middle	3.96 ±1.6^{a}	12.68 ±1.3^{b}	9.29 ±1.1^{b}	92.47 ±2.5^{b}	3.66 ±0.8^{b}
	longer	3.57 ±0.9^{b}	9.95 ±1.1^{d}	5.96 ±1.3^{d}	46.55 ±3.1^{d}	1.66 ±0.6^{e}

* The values that are followed by the same letter do not differ statistically at a significance level $P \leq 0.05$

differences were found in density and size of stomata under different photoperiod applications ($P \leq 0.05$). Stomata were denser in upper epidermis than lower epidermis. Stomatal density in upper epidermis was the highest in middle-day period (3.96 mm^{-2}) and the lowest in short-day period (1.79 mm^{-2}). Values of stomatal area were similar except for the lower surfaces of middle-day period. Denser stomata mean more water loss. On the other hand, gas and humidity exchange rate increases with the stomatal area [Chen et al. 2018]. In the lower surfaces of the leaves, under middle-day photoperiod stomatal density was the second highest and stomatal area was the largest (144.98 μm^2). Stomatal width and length (12.04 μm and 15.34 μm) in addition to stomatal density is responsible for this value. Relative stomatal area was the largest in middle-day period and this value was higher on upper surface (5.32%), than lower surface (3.66%). Gas exchange and transpiration rates of stomatal parameters are directly related to guard cell size. Higher stomatal density on the leaf surface increases C uptake as well as transpiration. This is an unwanted situation. Circadian rhythm of plants regulates the balance between carbon uptake and water loss via stomatal parameters [Robertson et al. 2009]. Starch is a photosynthesis product and is accumulated in the presence of light and then transformed to maltose and glucose during the night in mesophyll chloroplasts [Smith et al. 2005]. Formation and usage of these different compounds in light/dark is regulated by plant's circadian clock [Pokhilko et al. 2014]. "C" is the initial element of C uptake mechanism which is necessary for photosynthesis and the synthesis of photosynthetic products with C obtained via stomata is only possible if the plants gets enough light. Therefore, stomatal movements due to changes in photoperiod has a regulating effect on CO_2 mechanism, especially in short-day conditions it is a limiting factor for CO_2 mechanism [Azoulay-Shemer et al. 2018]. On the other hand, light is a fundamental element that affects guard cells which is responsible for the regulation of stomata apertures. Continuous light will cause stomata pores to stay open, therefore the duration of light and loss in biomass has a linear relationship.

Trichomes (non-glandular and glandular) are derived from epidermis and have various functions in plants. Non-glandular trichomes are simple "hairs" that have different shapes and functions depending on their location on plants. Glandular trichomes are composed of a single basal cell located in epidermis, one or three stalk cells, and a head. Capitate glandular trichomes has one or two cells in the head and peltate trichomes has for or more broad cells [Turner et al. 2000]. These are secretory structures where plants produce and store phytochemicals. Their location and density, while having a genetical basis, can change depending on the plant's age and the stress factors it has been exposed to. These changes also affect the content and the amount of synthesized material [Escobar-Bravo et al. 2018]. For example, the increase in the amount of glandular trichomes that are responsible for artemis accumulation in *A. annua* plants increased artemis accumulation by 2.5% in flowers and 1.4% in leaves [Kjær et al. 2012]. Furthermore, it is also known that secretions of glandular trichomes protect the plant against various stress factors, especially biotic factors, and regulate its growth and development. In the present study, glandular trichomes were found on both surfaces of leaves (Fig. 2).

Number of glandular trichomes were higher on the upper surfaces of leaves than the lower surfaces in all applications (Fig. 3). Size and amount of glandular trichomes were different between applications ($P \leq 0.05$). Capitate hair density increased from 4.88 mm^{-2} in short-day period to 12.04 mm^{-2} in middle-day period on the lower surface of the leaves. Capitate hair density of the same surface was 9.04 mm^{-2} in long-day period. The difference in capitate hair density had similar effects on lower surfaces of the leaves and the densest value (12.98 mm^{-2}) was observed in middle-day period. The other values were 11.66 mm^{-2} and 10.5 mm^{-2} for long-day and short-day periods, respectively. Different applications didn't a significant difference in capitate and peltate hair size on both surfaces ($P \geq 0.05$). Density and size of glandular trichomes reveal important information about pythochemical content and quality. Especially for peltate trichomes, their location as well provides informa-

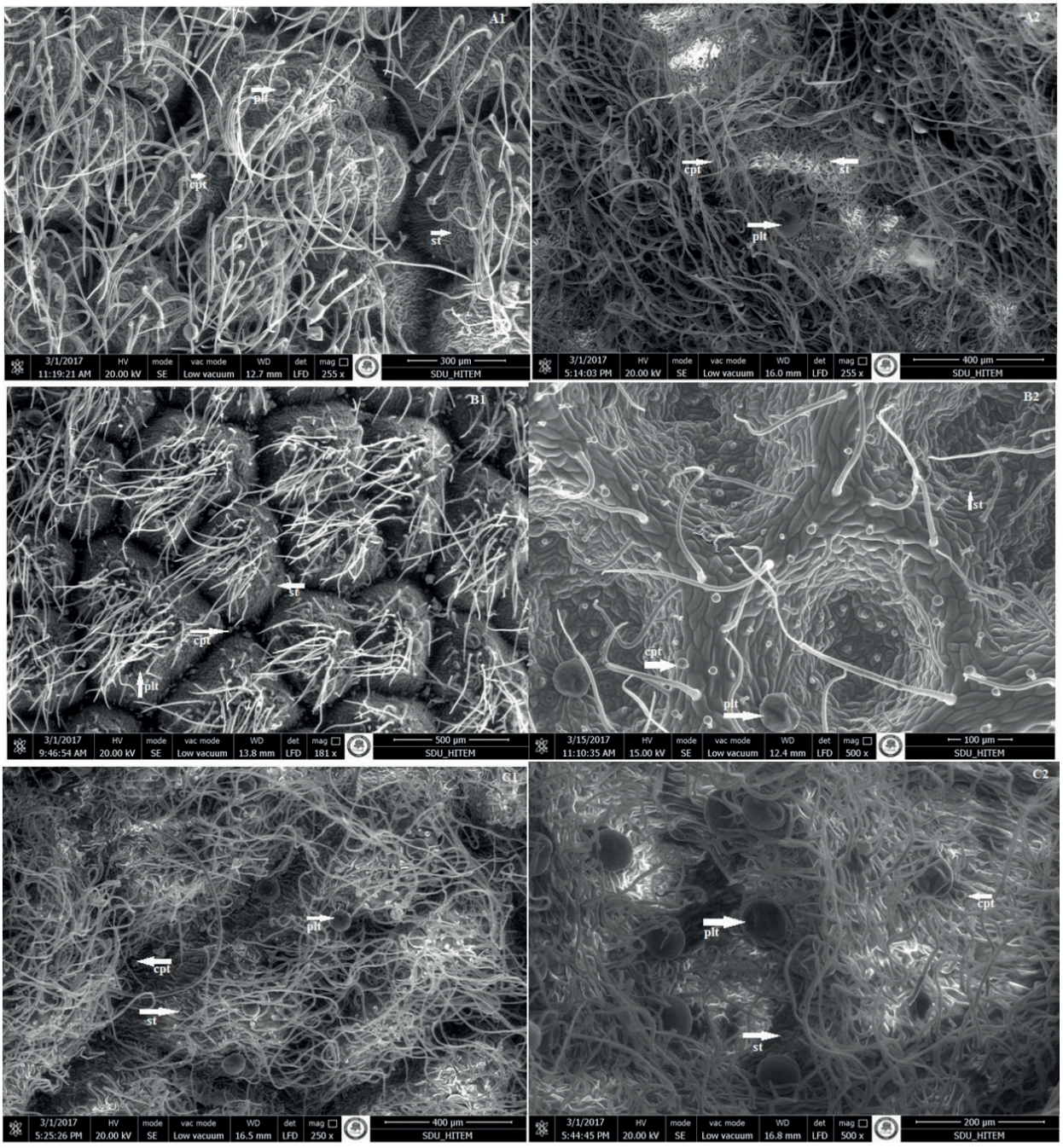

Fig. 2. SEM image of sage (stomata and trichomes); upper surfaces of leaves (A1, B1, C1), lower surfaces of leaves (A2, B2, C2) short-day; middle-day; long-day period, respectively; cpt – capitate, plt – peltate, st – stomata

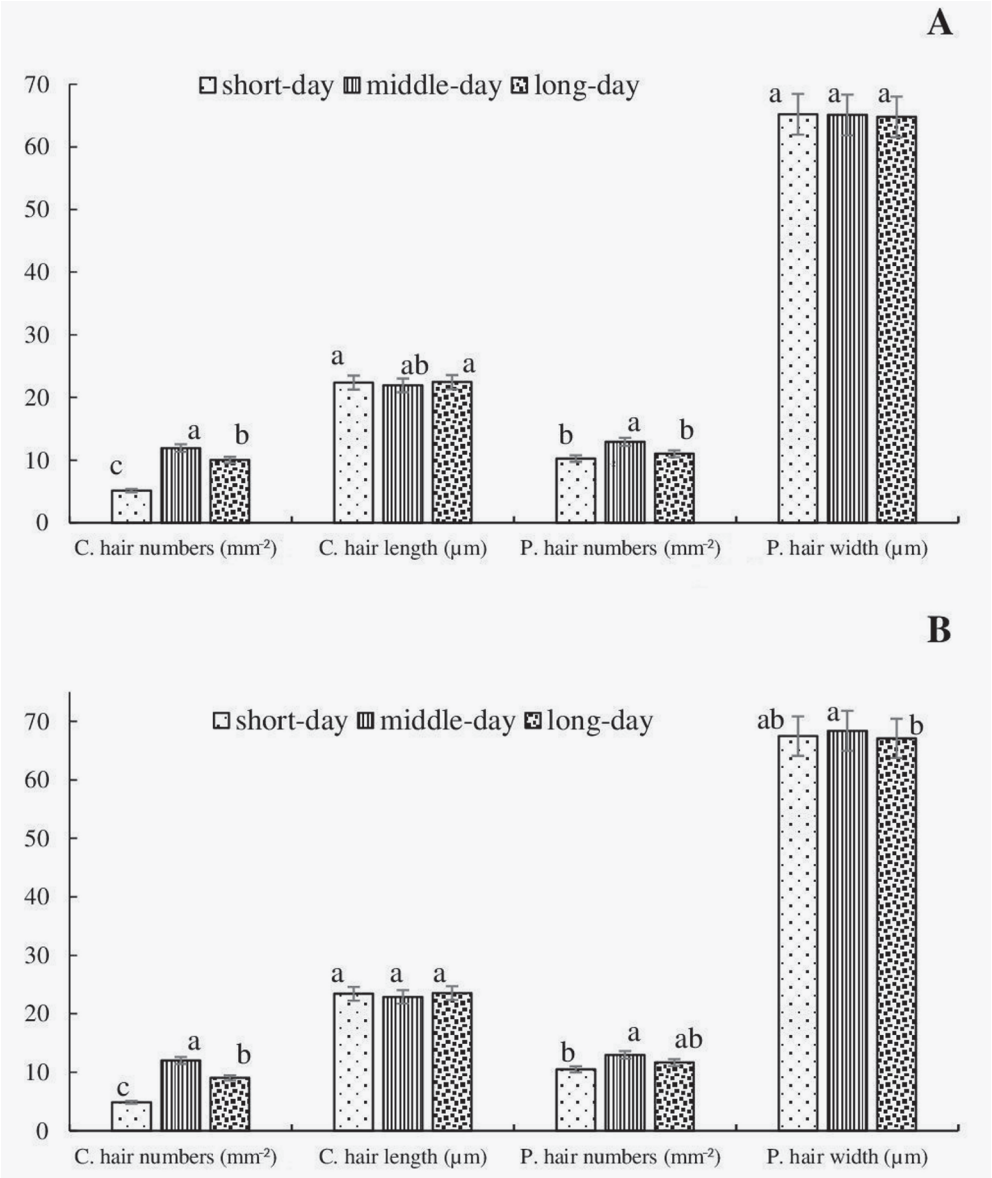

Fig. 3. Trichome parameters of sage; upper surfaces of leaves (A) and lower surfaces of leaves (B): (C. – capitate, P. – peltate). Values that are followed by the same letter do not differ statistically at a significance level of 0.05

tion. The increase in phytochemical content of *Thymus* due to photoperiod was supported by the increase in peltate trichomes [Sheidai et al. 2018].

GC/MS essential oil analysis of aerial parts of the sage plants used in this study determined 3 major (more than 10%), 12 minor (1 and 10%) and 33 (less than 1%) trace compounds. This compounds were, monoterpene hydrocarbons α-pinene and camphene; oxygenated monoterpenes α-thujone and camphor; sesquiterpene hydrocarbons α-humulene and β-caryophyllene; oxygenated sesquiterpene viridiflorol (Tab. 2). Proportion of these compounds were different

Table 2. Phytochemical composition and specific compound contents in sage

Compound (%)	Period		
	short-day	middle-day	long-day
cis-salvene	0.48	0.28	0.15
Hexylidenecyclopropane	0.07	0.04	0.03
Tricyclene	0.03	0.15	0.13
Thujene <α->	0.27	0.38	0.36
α-pinene	**1.15**	**3.96**	**3.45**
Camphene	**1.88**	**5.01**	**5.04**
Sabinene	0.22	0.31	0.30
β-pinene	**2.60**	**4.58**	**5.65**
β-myrcene	1.08	1.63	1.89
Phellandrene <α->	0.02	0.05	0.07
Terpinene <α->	0.19	0.26	0.26
Cymol	0.56	0.64	0.90
Limonene	**1.21**	**1.90**	**2.25**
1,8-cineole	**11.53**	**13.50**	**13.70**
γ-terpinene	0.37	0.48	0.49
Trans sabinene hydrate	0.31	0.38	0.33
α-terpinelone	0.11	0.25	0.22
α-dimethylstyrene	0.00	0.05	0.06
Sabinene hydrate <cis->	0.00	0.54	0.38
β-thujone	**20.84**	**40.18**	**17.92**
α-thujone	**5.27**	**7.49**	**8.74**
Thujyl alcohol	0.15	0.33	0.36
Camphor	**12.28**	**23.59**	**22.17**
Pinocamphone	0.14	0.00	0.00
Borneol	**1.32**	**1.49**	**1.58**
4-terpineol	0.33	0.40	0.40
Cymen-8-ol <para->	0.03	0.06	0.02
Terpineol <α->	0.15	0.13	0.09
Bornyl acetate	**0.08**	**0.22**	**0.48**
Sabinyl acetate	0.06	0.00	0.00
α-cubebene	0.00	0.06	0.06
Copaene <α->	0.00	0.13	0.17
Caryophyllene	**0.52**	**2.72**	**3.27**
β-cubebene	0.00	0.07	0.09
Aromadendrene	0.00	0.08	0.10
α-humulene	**4.84**	**1.47**	**1.47**
α-amorphene	0.00	0.29	0.40
γ-gurjunene	0.00	0.06	0.07
Muurolene <α->	0.00	0.06	0.08
Cadinene <γ->	0.00	0.11	0.16
Cadinene <delta->	0.00	0.33	0.48
Caryophyllene oxide	0.19	0.98	1.19
Viridiflorol	**5.83**	**2.75**	**2.75**
Bicyclogermacrene	0.26	0.16	0.19
Humulene oxide	1.89	0.61	0.64
Ledene	0.00	0.12	0.20
Epimanoo	4.25	1.04	1.26
Grouped components (%)			
Monoterpene hydrocarbons	8.13	17.06	18.01
Oxygenated monoterpenes	67.89	74.51	66.75
Sesquiterpene hydrocarbons	5.61	4.38	4.99
Oxygenated Sesquiterpenes	6.29	3.77	3.99

The most varying phytochemicals between applications are shown in bold.

for applications. Especially proportions of the compounds which determine the essential oil quality varied. For example, ratio of α-pinene was 1.15, 3.96, and 3.45 in short-day, middle-day, and long-day periods, respectively. Similarly, ratio of camphene was 1.88, 5.01, and 5.0.

Duration and amount of light affects the accumulation of some compounds and has significant effects on plant photochemistry [Khayyat and Roselin 2018]. While monoterpene hydrocarbon quantity had a linear relationship with duration of light, it increased by 109% from short-day to middle-day, but only increased by 5% from middle-day to long-day. Monoterpene hydrocarbones stand out in determining the essential oil quality of sage [Rguez et al. 2019], therefore their responses to the length of day were important. The amount of oxygenated monoterpenes was the highest in middle-day period. They include some essential compounds that determine the quality of sage oil, and this could mean that sage oil quality increased in middle-day condition.

Quality and quantity of phytochemicals of plants that produce secondary metabolites are different between growth, development and differentiation stages and biotic and abiotic factors that plants were exposed to during these stages also change them. Genes that are responsible for the biosynthesis of some phytochemicals are activated by circadian clock mechanism [Kerwin et al. 2011] and this shows phytochemical biosynthesis can be regulated by circadian clock [Goodspeed et al. 2013]. Biosynthesis mechanism of phytochemicals was delayed in *Artemisia annua* plants exposed to continuous light or dark and returned back to normal once photoperiod was normal again. Therefore, quality and quantity of light affects phytochemical accumulation metabolism. Light signals are perceived by photoreceptors and they regulate the accumulation of various phytochemicals depending on some stress factor and light [Oh et al. 2014]. As the duration of the day increases (longer period: 16 h), quantity of secondary metabolites that protect the plant from the negative effects of excessive, including flavonoids and phenolic acids, increases [Carvalho et al. 2010]. However, while 100% light density increases the essential oil quantity, the amount of pulegone, which is toxic for humans, also increases and therefore this is an unwanted situation [Souza et al. 2016].

Photoperiod also affects the metabolite quality of sage. Some compounds there weren't present in short-day were determined in longer light periods (Tab. 2). For example, dimethylstyrene, sabinene hydrate <cis->, α-cubebene, copaene <α->, β-cubebene, aromadendrene, α-amorphene, γ-gurjunene, muurolene <α->, cadinene <γ->, cadinene <delta-> and ledane weren't present in short-day. But for middle-day their proportions were 0.05%, 0.06%, 0.54%, 0.38%, 0.06%, 0.06%, 0.13%, 0.17%, 0.07%, 0.09%, 0.08% and 0.10%, and for long-day 0.29%, 0.40%, 0.06%, 0.07%, 0.06%, 0.08%, 0.11%, 0.16%, 0.33%, 0.48%, 0.12% and 0.20%, respectively. Only a handful of compounds were present in short-day but not in other periods, such as pinocamphone (0.14%), sabinyl acetate (0.06%), viridiflorol (0.12%).

Excessive light or dark has negative effects on monoterpene biosynthesis. In this situation normal photoperiod (12 h light/ 12 h dark) application activates circadian regulation and biosynthesis mechanism reverts back to normal [Lu et al. 2002]. On he other hand, there is a direct relation between daily monoterpene emission and photoperiod [Hendel-Rahmanim et al. 2007]. Therefore, the decrease in monotoerpene emission of plants there exposed to continuous light or dark shows that monoterpene emission is regulated by light and circadian clock [Chuang et al. 2017].

CONCLUSIONS

While light-dark cycle was different between applications all of the plants survived. Circadian clock regulation minimized the effects of changing environmental conditions and the plants continued their vital activities. Plants were exposed to 11, 12, and 15 hours of light, but due to circadian clock regulation plants altered their metabolic activities and had similar growth parameters as if they were not exposed to abnormal conditions. Therefore, circadian clock provides adaptation to changes between light/dark, regulates several metabolic activities, and enables plants to grow and develop by means of physiologic adaptation. However, adaptation is possible up to certain values of day length, its extreme values caused changes in phytochemical content as a result of its morphologic, anatomic and physiological processes.

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