EFFECT OF LIGHT INTENSITY ON THE PHOTOSYNTHETIC PARAMETERS FOR PLANTS FROM THE *LAMIACEAE* FAMILY

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Abstract. *Alongside water and nutrients, solar radiation is of most importance for regulating photosynthesis, plant growth and adaptation. The lack or too much solar radiation can become a stress factor in a relatively short time (minutes, hours), while other stress factors, like water content in the soil needs a few days or weeks. Many studies have shown that plant biomass and photosynthetic parameters (net assimilation rate, stomatal conductance) decrease in low light conditions. By determining the photosynthetic parameters and photosynthesis irradiance curve, we aimed to see if several correlations could be made. High values for stomatal conductance and net assimilation were recorded in all four plants, but the highest were observed for Origanum vulgare and the lowest for Satureja montana. The higher assimilatory pigments quantity over the unit area (mg/m^2) were determined for Thymus vulgaris 718.32 chlorophyll b and 1464.76 chlorophyll a, maintaining the ratio between chlorophyll a and chlorophyll b to that of ~2, corresponding with the literature of values found in medicinal and aromatic plants.*

Keywords*: aromatic plants, photosynthesis, light intensity, assimilatory pigments, Lamiaceae.*

INTRODUCTION

In many plant habitats, solar radiation and therefore light intensity varies in time (seasonal and daily changes) allowing the plants to develop acclimation of the changing light regimes, also developing possible anatomical and physiological alterations.

Under both natural and agricultural conditions, among many other environmental factors, solar radiation is of most importance for regulating photosynthesis, plant growth and adaptation, therefore the lack or too much solar radiation can become a stress factor in a relatively short time (minutes, hours), while other stress factors, like water content in the soil needs a few days or weeks, and the minerals deficit in the soil can induce stress after a few months (REZAI ET AL., 2018; ZERVOUDAKIS ET AL., 2012). Many previous studies have shown that overall plant biomass as well as photosynthetic parameters (net assimilation rate, stomatal conductance) decrease in low light conditions, but in contrast, the average plant height increased (CASTRONUOVO ET AL., 2019; OLIVEIRA ET AL., 2016; ZERVOUDAKIS ET AL., 2012).

Lamiaceae is a family of medicinal and aromatic plants of great economic importance, which produces a wide variety of secondary compounds, being highly recognized for its essential oils secreted by glandular trichomes. Usually it comprises a large group of herbaceous, shrubs and subshrubs, perennial or annual plants, having antispasmodic, antioxidant, antitumor, excitatory, carminative, antiarthritic, tonic and many other health effects (AVRAM, 1974; LUPITU ET AL., 2018; MOISĂ ET AL., 2018; MOISA ET AL., 2018; MOISA ET AL., 2019; RHNID, 2012; V. HEYWOOD ET AL., 2007). This family includes 236 genera and around 7173 species grouped in families and subfamilies (HARLEY ET AL., 2004).

Medicinal plants usually refer to plants that could be used directly, without further isolation of compounds (foods with healing properties), but similarly, they could also be used as medical agents and starting materials for compound isolation (aromatic plants for essential oils, sources of phenolic compounds, alkaloids) (MÁTHÉ, 2015). In view of above facts, the medicinal plants belonging to *Lamiaceae* family cultivated in west side of Romania show great promise as a dietary and therapeutic source involved in human health. (TOMESCU ET AL.2015).

The aim of this study was to measure the physiological parameters and assimilatory pigments for *Thymus vulgaris* L. var. *Donne Valley*, *Satureja montana* L., *Satureja hortensis* L. and *Origanum vulgare* L. var. *aureum* cultures established at the Lovrin Agricultural Research and Development Station.

MATERIALS AND METHODS

Plant material and growing conditions

Three perennial plant species (*Thymus vulgaris* L. var. *Doone Valley*, *Origanum vulgare* L. var. *aureum*, *Satureja montana* L.) were brought as seedlings in the spring of 2016, namely on April 24th, 2016 at Lovrin Agricultural Research and Development Station 20º 47 'Longitude E' and 45º 57 'latitude N, Timiș county. They were transplanted directly into the field in a uniform land and with permanent sun exposure. Depending on the cultivated species, the following aspects were taken into account: the size of the parcels, the density of the plots and the optimum distance between the plants (0.7 m), in order to ensure the possibility of further care work.

The annual plant species (*Satureja hortensis* L.) was sown directly in the field at the beginning of May 2017, at a seeding depth of 1-1.5 cm and a distance of 20 - 25 cm between the rows. When the plants were fully sprung, they were thinned down leaving a distance of ~ 10 cm between the plants in rows.

During the vegetation periods, the phenological stages were followed as well as the general condition of the plants, avoiding the occurrence of specific diseases and pests.

Determination of photosynthetic processes

Measurements of the photosynthetic parameters were performed in the summer of 2017 using a portable gas exchange device GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) as in Copolovici et al. (Copolovici et al., 2017), The measurements conditions are: PARtop = 1000 mmol.m^2.s^{-1} light intensity, 25˚C leaf temperature, 70% chamber humidity and 400 ppm CO_2 concentration. Each measurement was performed by enclosing a 2-3 cm^2 plant offshoot within the analysis cuvette and maintained until plant stabilization). The assimilation rate (A), stomatal conductance (GH_2O) and intermolecular CO_2 were calculated.

Analysis of assimilation pigments

Leaves pigment extraction (chlorophyll and carotenoids) was realized according to Copaciu et al. (COPACIU et al., 2013) method, with specific modifications. Offshoots were cut off and scanned to get the leaves area and then immediately immersed in liquid nitrogen. The frozen leaves were grinded using a mortar and pestle over an ice bed, adding a constant flow of liquid nitrogen, and the extraction was performed using cold pure acetone and calcium carbonate (Sigma-Aldrich, Steinheim, Germany). The mixture was centrifuged at 0-4˚C and 5000 rpm for 3 minutes, with a Hettich centrifuge (Rotina 380 R, Hettich GmbH, Tuttlingen, Germany), using 2 mL centrifuge tubes. The supernatant was collected, and three extractions have been performed, until the supernatant became colorless. The combined extracts were then brought to a volume of 10 mL and filtered through a 0.22 µm PTFE disk membrane filter. The final extracts were analyzed by ultra-high-performance liquid chromatography (UHPLC–MS Nexera X2, Shimadzu 2010, Kyoto, Japan), equipped with a DAD (M30A, Shimadzu, Tokyo, Japan), and a reversed phase column Nucleosil 100-3-C18 (4.0 mm i.d. x 125 mm length, maximal particle size 3 µm, Macherey-Nagel GmbH, Duren, Germany). The column was maintained at 10˚C and the total flow rate has been 1.5 ml.min^{-1} (COPACIU ET AL., 2013).

For the pigments separation, we used the following solvents for the chromatographic elution: TFA acidified ultra-pure water (A) and UHPLC grade acetone (B) (Sigma-Aldrich, Steinheim, Germany). The elution started by mixing 25% A and 75% B for 7.5 min, proceeding with a linear gradient towards 100% B between 7.5 and 20 min, then changing back to 25% A and 75% B from 20 to 25 min. The calibration for the UHPLC was made using commercially available lutein, chlorophyll a, chlorophyll b and β-carotene standards (Sigma-Aldrich, Steinheim, Germany), at specific wavelengths.

All results represent the average of three distinct analysis performed on separate plants, with calculated standard deviations.

RESULTS AND DISCUSSIONS

Photosynthetic parameters

The photosynthetic parameters for *Origanum vulgare* presented the highest values in regard to stomatal conductance and a net assimilation rate. Lower values were recorded for *Satureja montana* (Table 1) a plant with very small bud.

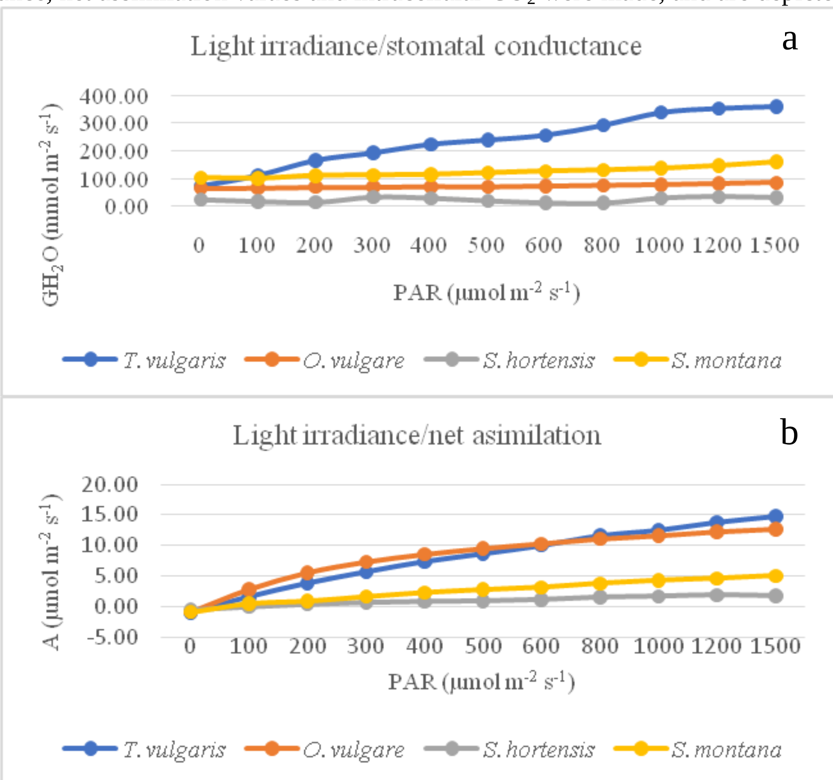
Table 1

Photosynthesis parameters for studied plants

Plant name	CO_2 (ppm)	PARtop µmol m^{-2} s^{-1})	GH_2O (mmol m^{-2} s^{-1})	A (µmol m^{-2} s^{-1})	Ci (ppm)
Thymus vulgaris L. var. *Donne Valley*	400	1000	126.19 ± 8.49	6.06 ± 0.64	306.00 ± 8.73
Origanum vulgare L. var. *aureum*	400	1000	196.78 ± 0.59	13.47 ± 0.01	273.32 ± 0.29
Satureja hortensis L.	400	1000	191.27 ± 0.65	9.89 ± 0.07	302.42 ± 1.10
Satureja montana L.	400	1000	64.55 ± 0.15	3.92 ± 0.08	294.74 ± 2.16

The influence of light to photosynthesis parameters

For each plant taken into this study, periodic measurements at different photosynthesis active radiance were performed (0, 100, 200, 300, 400, 500, 600, 800, 1000, 1200 and 1500 µmol m^{-2} s^{-1}). The obtained curves present a photosynthetic response of the plant to the light. Therefore, correlations between light intensity and stomatal conductance, net assimilation values and intracellular CO_2 were made, and are depicted in figure 1 a-c.



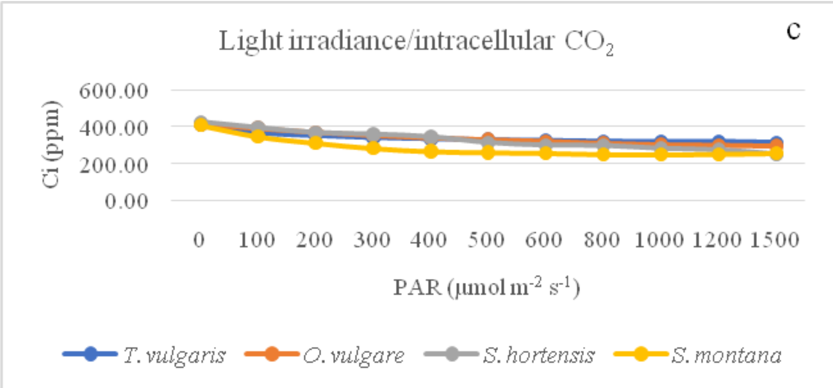


Fig. 1. Correlations between light intensity and photosynthetic parameters, a - stomatal conductance correlation with light intensity; b - net assimilation rate correlation with light intensity and c - intracellular CO_2 correlation with light intensity for the studied plants

In figure 1 a and b an increase of photosynthetic parameters is observed, and in the case of *T. vulgaris* with stomatal conductance varying from 71.91 to 359.12 mmol m^{-2} s^{-1}, and a net assimilation rate from -0.99 to 14.80 µmol m^{-2} s^{-1}. Intracellular CO_2 decreased from 414.47 to 315 ppm. Similar behavior was observed for all plant samples with minor differences.

Analysis of assimilation pigments

From the four studied plants samples, *Thymus vulgaris* has the highest content of assimilatory pigments, followed by *Origanum vulgare*, *Satureja hortensis* and *Satureja montana*. The chlorophyll a and chlorophyll b ratio was ~2, corresponding with the values determined for medicinal and aromatic plants encountered in literature (EL-QUDAH, 2014; LEE, 2005).

Table 2

Assimilatory pigments quantity determined in the studied plants

Assimilatory pigments (mg/m^2)	Lutein	Chlorophyll b	Chlorophyll a	β-Carotene	a/b ratio
Thymus vulgaris L. var. *Donne Valley*	96.68 ± 0.43	718.32 ± 2.31	1464.76 ± 2.50	27.75 ± 0.77	2.04
Origanum vulgare L. var. *aureum*	37.23 ± 0.37	250.13 ± 1.58	492.12 ± 6.12	8.26 ± 0.37	1.97
Satureja hortensis L.	17.40 ± 0.67	135.41 ± 0.45	256.64 ± 1.16	6.11 ± 1.13	1.90
Satureja montana L.	8.68 ± 2.1	63.30 ± 0.71	121.98 ± 4.50	26.67 ± 1.07	1.93

A sample chromatogram of assimilatory pigments analysis is depicted in figure 2, following the same footprint for all four plants, and separate UV spectra for each assimilatory pigment are presented in figure 3 a-d.

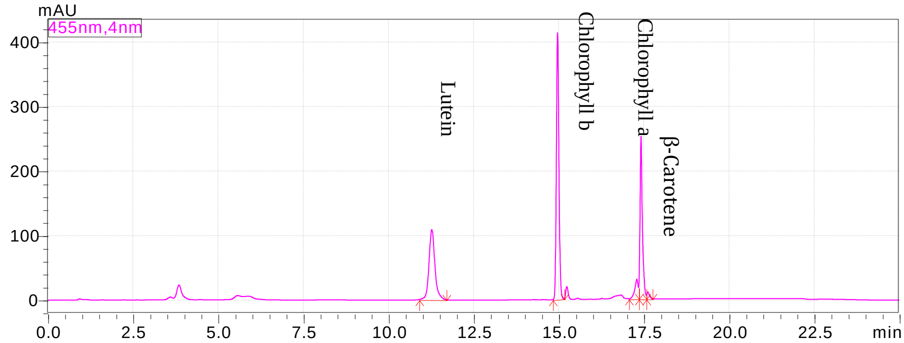


Fig. 2. Example of assimilation pigments chromatogram (*Thymus vulgaris* L. var. *Doone Valley*)

CONCLUSIONS

High values for stomatal conductance were recorded in all four plants, but the highest was observed for *Origanum vulgare*, GH_2O = 196.78 mmol m^{-2} s^{-1}, and the lowest for *Satureja montana*, GH_2O = 64.55 mmol m^{-2} s^{-1}. Net assimilation value followed the same pattern with the highest being at A = 13.47 µmol m^{-2} $s^{-}1$ and the lowest at A = 3.92 µmol m^{-2} $s^{-}1$.

The higher assimilatory pigments quantity over the unit area (mg/m^2) were determined for *Thymus vulgaris* 718.32 chlorophyll b and 1464.76 chlorophyll a, followed by *Origanum vulgare, Satureja hortensis* and *Satureja montana* with lowest values 63.30 and 121.98. Even though the differences are quite significant, the ratio between chlorophyll a and chlorophyll b was ~2, corresponding with the literature of values found in medicinal and aromatic plants.

From all four studied species, *Thymus vulgaris* has the smallest plant height, forcing it to be in a permanent competition for sunlight with all the surrounding vegetation. However, one of the acclimation strategies developed by plants growing in shade conditions is a three-fold increase in chlorophylls, adapting the plant to different environmental conditions (TAIZ AND ZEIGER, 2002). Leaves with higher pigmentation being more efficient in absorbing light per unit of leaf biomass, allowing the plant to balance carbon uptake under lower light conditions (ZERVOUDAKIS ET AL., 2012).

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