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[1]Department of Agronomy, Institute of Agronomy and Plant Breeding, Justus Liebig University Giessen, Giessen, Germany
[2]Institute for Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine, Vienna, Austria

Characterization of glandular trichomes in four *Origanum vulgare* L. accessions influenced by light reduction

Marzieh Shafiee-Hajiabad[1*], Johannes Novak[2], Bernd Honermeier[1]

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Summary

Effect of reduced light intensity (minus 26%) on trichomes characteristics and herb yield responses of three subspecies of *Origanum vulgare* (ssp. *vulgare*, ssp. *hirtum* (Link) letswaart and ssp. *viride* (Boiss.) Hayek) and a hybrid (ssp. *viride* (Boiss.) Hayek × *O. majorana* L.) was investigated. It was found that the investigated genotypes showed different morphological characteristics which were significantly influenced by light reduction. The applied treatment decreased the total indumentum of the leaves as well as the density and size of capitate glandular trichomes and size of peltate glandular trichomes. The theoretical volume of essential oil on one peltate glandular trichome as well as the total theoretical volume of essential oil on the adaxial side of the leaves was reduced under this condition. The indumentum of all samples included non-glandular trichomes, peltate glandular trichomes and capitate glandular trichomes type I. Capitate glandular trichomes type II with low density were observed only on *O. vulgare* ssp. *viride* and on the hybrid. Based on the findings of this study, it can be suggested that the applied light reduction was a limiting factor for the growth of *O. vulgare* L. plants. It shifted the carbon allocation to increase plant surfaces. As a result, the density of trichomes, which is proposed to be a fixed factor in *O. vulgare* L. plants, decreased.

Abbreviations: CGT: capitate glandular trichomes; GT: glandular trichomes; FLI: full light intensity; NGT: non-glandular trichomes; Ohi: *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart; Ovi: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek; Ovu: *Origanum vulgare* L. ssp. *vulgare*; Oxm: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L.; PGT: peltate glandular trichome; RLI: reduced light intensity, SEM: scanning electron microscopy

Introduction

Plant trichomes are epidermal appendixes, which protect the plants against external factors by reducing leaf temperature, preventing water loss of leaves, increasing light reflectance, and reducing leaf abrasion (Bosabalidis and Sawidis, 2014; Wagner et al., 2004). Glandular trichomes (GTs) are plants' active bio-factories for production and storage of the essential oil (Dai et al., 2009). The formation of GTs' is a species related parameter (Bosabalidis, 2004; Dai et al., 2009), however, plants can regulate their response to environmental stresses by changing the physical properties (size and density) of their GTs (Wagner et al., 2004). In different medicinal plants the effect of environmental stimuli such as temperature (Bosabalidis and Sawidis, 2014), altitude and season (Glas et al., 2012; Kofidis et al., 2003), drought stress (Gonzales et al., 2008; Olmos et al., 2007), light intensity (Nihoul, 1993; Yamaura et al., 1989), and toxicity of minerals (Huang et al., 2010; Panou-Filotheou et al., 2001) on GTs formation has been found.

* Corresponding author

Origanum vulgare L. (Lamiaceae), is widely used as a spice as well as a medicinal plant due to its antimicrobial, antiviral and antioxidative effects (Capecka et al., 2005; Kulisic et al., 2004; Siroli et al., 2014). Two types of GTs that form on the areal surface of *O. vulgare* L. plants are the peltate glandular trichomes (PGTs) and the capitate glandular trichomes (CGTs). Uniseriate non-glandular trichomes (NGTs) are also present on the surface of *O. vulgare* L. (Werker et al., 1985). PGTs consist of one basal cell, one stalk cell, and a secretory head composed of four central and eight (occasionally up to ten) peripheral cells. They secrete and accumulate the essential oil into a space between the walls of head cells and the cuticular layer, which enclose them (Bosabalidis, 2004; Werker et al., 1985). CGTs are smaller than PGTs and form two types in *O. vulgare* L. plants. Type I consists of one basal cell, one stalk cell, and one rounded to somewhat elongated head cell. Hairs of type II consist of a basal cell, one to three stalk cells and one narrow, sometimes pear-shaped head cell (Werker et al., 1985).

Furthermore, light intensity has an important role both in GTs formation and essential oil production (Fernandes et al., 2013). There are studies about the influence of light intensity on PGTs in Lamiaceae plants (Fernandes et al., 2013, Li et al., 1996). Conversely, there is no published data about size and density of CGTs under different light conditions in *Origanum vulgare* L. Accordingly, this experiment was aimed to investigate the physical properties of both capitate and peltate glandular trichomes as well as yield parameters of *O. vulgare* L. accessions under the influence of reduced light intensity (RLI) condition.

Materials and methods

Experimental design

A pot experiment (6 L per pot) was carried out in 2012 in the research station of Rauischholzhausen (University Giessen). Four accessions of *Origanum vulgare* L. include three subspecies and one hybrid from the German Genebank IPK Gatersleben (http://www.ipk-gatersleben.de/) were used as plant material: (1) *O. vulgare* L. ssp. *vulgare* (Ovu), (2) *O. vulgare* L. ssp. *hirtum* (Link) letswaart (Ohi), (3) *O. vulgare* L. ssp. *viride* (Boiss.) Hayek (Ovi), and (4) *O. vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L. (Oxm). The seeds were planted on April 17th and transferred to pots on April 24th 2012 with twenty replications per treatment.

The soil used was a loess soil from the research station taken from the layer of 0-20 cm. The sieved soil (<2 mm) was homogenized, air-dried, mixed with sand (loess soil: sandy soil = 1: 2 w/w), and fertilized with nitrogen (0.5 g per pot as NH_4NO_3), phosphor (0.3 g per pot as $Ca(H_2PO_4)2H_2O$), potassium (1.5 g per pot as K_2SO_4), magnesium (0.1 g per pot as $MgSO_4 \cdot 7H_20$), and calcium (3.0 g per pot as $CaCO_3$). The mixed soil was characterized by a pH value of 6.7 (in H_2O). The plants' water supply was maintained at 60% of water holding capacity of the soil.

The pots were lined up from the north-west to the south-east direction. The treatments included (1) RLI (reduced light intensity treat-

ment): pots were located under a wire-house with a woven metal net with 16.7 × 16.7 mm square mesh and 1.86 mm wire diameter and (2) FLI (full light condition in outside of the greenhouse). The light intensity was measured daily between 9 and 11 a.m. close to the pots by the use of a light meter (EBLX4, Hartman & Braun AG, Frankfurt Germany). The mean daily light intensity during the plants growth cycle (May to August) inside and outside of the wire-house was calculated (43,761 lux and 59,067 respectively). Therefore, the wire-house reduced the light intensity by an average of 26%.

Scanning electron microscopy (SEM) analyses

Leaf samples were taken at the beginning of anthesis (10% of the plants in bloom) before the harvest on July 4th 2012. In order to obtain comparable results the leaves from the third node of the apex were used for SEM analyses. Four pots (plants) were considered for replication; from each pot four representative leaves were investigated. The fresh leaves were immediately submerged in a glass bottle containing acidified 2,2-dimethoxypropane (DMP; 1 drop 0.2 M HCl added to 30 ml DMP) (HALBRITTER, 1997). After 24 hours, the samples were transferred to water-free acetone followed by a critical-point-drying (CPD 030, BAL-TEC GmbH, Germany) in CO_2 at 40 °C. The specimens were mounted on aluminum stubs using double-sided adhesive tape and sputter coated with a thin film of gold (Balzers SCD 004, Liechtenstein).

The leaves were analyzed by means of an LEO Gemini DSM 982 scanning electron microscope (SEM, LEO, Oberkochen, Germany) equipped with a field emission cathode. Acceleration voltage was set to 3 kV. Images were recorded using a secondary electron detector with the voltage of the collector grid biased to +300 V in order to improve the signal-to-noise-ratio and to reveal optimal topographical contrast. The settings of the SEM for tilt angle, spot size, scanning mode and magnifications used were kept constant throughout the study. The size and density of trichomes was measured directly on the SEM screen as described in detail elsewhere (SHAFIEE-HAJIABAD et al., 2014). All of measurements were done on the adaxial side of the leaves.

In order to measure the theoretical essential oil volume in PGT, based on its shape, the volume of the essential oil-filled subcuticular cavity was considered as a hemisphere ($2/3\ \pi r^3$) and the volume was calculated (PANOU-FILOTHEOU et al., 2001). The estimated volume of PGT was multiplied by their density to obtain the essential oil volume per square millimeter on adaxial side of the leaf. The final essential oil volume on adaxial side of one leaf was calculated by multiplying the essential oil volume per square millimeter and the leaf area.

Yield parameters analyses

The plantlets were transferred to pots and the plant length was measured continuously (five times) during the plant growth cycle. The plants were harvested, total fresh mass per pot was determined. Total dry mass, herb and stem per pot were determined after drying the plant material in an air-circulating dryer at 39 °C.

In order to measure leaf area, the third node leaves from the top were used (directly opposite leaves which were used for scanning electron microscopy analysis were collected). The leaf area was measured manually by the use of standard millimeter paper.

Statistical analyses

A completely randomized experimental design was conducted as a two-factorial pot experiment. The analysis of trichomes characteristics and leaf area was conducted with four replications and the analysis of yield parameters was carried out with 20 replications. The analysis of variances was carried out using the IBM SPSS program version 20 on the all parameters. A two-way between-groups analysis of variance was conducted to explore the impact of accessions and light intensity on the evaluated parameters. LSD values were calculated and used for the comparison of means of different treatments within one factor.

Results

Micro-morphological studies

The indumentum of investigated accessions includes warty uniseriate non-glandular trichomes, peltate and capitate glandular trichomes. In all accessions each PGT consists of 12 head cells, one stalk, and one basal cell. The head cells are covered with a cuticular layer (Fig. 1 A). CGT type I was observed on the leaves of all accessions (Fig. 1 B) and CGT type II with very low density was found only on Ovi and Oxm (Fig. 1 C). Another form of CGT with one big rounded head cell (30-50 μm), one stalk cell and one basal cell was identified on the leaves of Ohi and rarely on Ovi and Oxm (Fig. 1 D).

Effect of RLI: The applied treatment affected the size of PGTs and their estimated volume of essential oil. The size and density of CGTs was significantly influenced by light intensity. Accordingly, the RLI treatment reduced the density and size of capitate glandular trichomes, the size of peltate glandular trichomes, and their estimated volume of essential oil. The total number of glandular trichomes and theoretical volume of essential oil in one leaf was decreased in the RLI condition (Tab. 1) (Fig. 2).

Effect of accessions: The Ovu plants had the smallest PGT (Fig. 2 A and B) while Ohi had the largest size of PGT (Fig. 2 C and D). The size of PGT was not statistically different between Ovi and Oxm (Fig. 2 E, F, G and H). The estimated volume of essential oil in one PGT followed the same order. Furthermore, the size of CGT was higher in Ovi followed by Ovu and Oxm. Ohi had the smallest CGT (Tab. 2).

The density of trichomes was statistically different between investigated accessions. However, the highest density of all trichomes, PGT, CGT, and NGT were observed on the adaxial side of the Ohi leaves. Ovu and Ovi had higher density of PGT in comparison with Oxm. Conversly, the density of capitate glandular trichomes was higher in Ovu and Ohi compared to the other accessions. Moreover, NGT appeared to have very low density on the adaxial side of Ovu, Ovi and Oxm leaves (Tab. 2).

The number of all glandular trichomes on one leaf was superior in Ohi and Ovu when compared to Ovi and Oxi. However, the theoretical volume of essential oil (on the adaxial side of leaves) per leaf square millimeter and per leaf was greater on Ohi than on other accessions (Tab. 2).

Yield parameters

Effect of RLI: RLI modified plant fresh mass, herb to stems ratio, plant length, and leaf area. Plant fresh mass and leaf area was greater in the RLI treatment compared to FLI; although the ratio of herb to stems was superior in FLI condition (Tab. 1). RLI increased the plant length in all investigated accessions (Fig. 3).

Effect of accessions: Ovu had highest fresh mass, total dry mass, herb dry mass, and stem dry mass (Tab. 2). The vertical growth of Ovu was greater than Ohi, followed by both Ovi and Oxm which had comparable growth (Fig. 3).

The leaf area was larger in Ovu and Oxm when compared with the

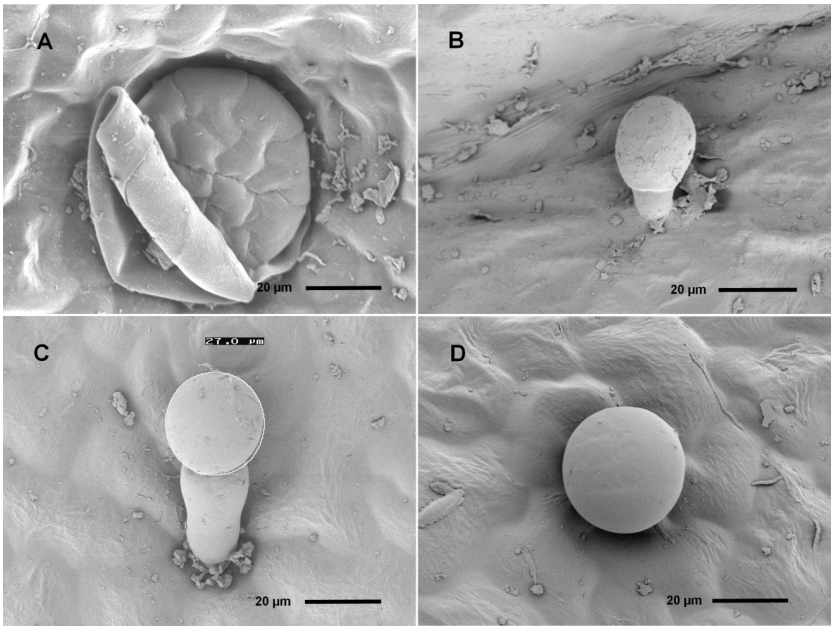

Fig. 1: Glandular trichomes of *Origanum vulgare* L. accessions.
A) Peltate glandular trichome, in the picture the secretory head cells and the cuticular layer are visible.
B) Capitate glandular trichome type I with one basal cell, one stalk cell and one head cell.
C) Capitate glandular trichome type II with one basal cell, two stalk cells and one head cell.
D) Capitate glandular trichome with a big head cell.

Tab. 1: Mean values (± standard error) of evaluated parameters in two different light intensities (RLI, FLI).

Parameters	Light intensity		p value
	RLI	FLI	
FM (g/pot)	44.08±1.86 a	36.69±1.94 b	0.01
HDM/SDM	4.93±0.78 b	7.87±0.80 a	<0.01
LA (mm^2)	191.21±9.63 a	178.23±9.63 b	0.04
CGT (No. per mm^2)	6.57±0.55 b	9.01±0.58 a	<0.01
D.PGT (µm)	72.73±0.61 b	75.73±0.64 a	<0.01
D.CGT (µm)	20.95±0.21 b	22.05±0.22 a	<0.01
GT (No. per mm^2)	15.65±1.08 b	18.69±1.13 a	0.05
No. of GT/leaf	2696.45±235.85 b	3492.13±245.48 a	0.03
V. EO/PGT (µl)	104.07±26.3 ($\times 10^{-6}$) b	118.9±27.38 ($\times 10^{-6}$) a	0.01
V. EO/leaf (µl)	0.072±0.02 b	0.096±0.02 a	0.03

RLI: reduced light intensity, FLI: full light intensity, FM: fresh mass, HDM/SDM: ratio of herb dry mass to stems dry mass, LA: leaf area, CGT: density of capitate glandular trichomes, D.PGT: diameter of peltate glandular trichome, D.CGT: diameter of capitate glandular trichome, GT: density of all glandular trichomes, No. of GT/ leaf: estimated number of all glandular trichomes in one leaf, V. EO/ PGT: theoretical volume of essential oil in one peltate glandular trichome, V. EO/ leaf: theoretical volume of essential oil of the adxial side of one leaf.

other two accessions. The total dry mass and herb dry mass of Ovi was lower than other accessions. The ratio of herb to stem was higher in Ovu and Oxm compared to Ohi and Oxm (Tab. 2).
Furthermore, the dry stem mass of *O. vulgare* L. accessions was significantly influenced by the interaction of light intensity and accessions (p-value: 0.01, not shown in a table). Greatest stem dry mass was observed in Ovu cultivated in the FLI (7.31% a). This parameter was reduced to 6.43% (b) in Ovu in the RLI condition. In contrast, Oxm had a dry stem mass of 1.89% (c) in RLI (1.89% c) compared to the FLI 0.46% (d). In Ovi and Ohi this parameter was similar between RLI (Ovi: 0.51% (d) and Ohi: 1.89% (c)) and FLI (Ovi: 0.42% (d) Ohi: 1.94% (c)).

Discussion

Micro-morphological parameters

Effect of RLI: When plants are exposed to variable light intensities, plant trichomes as the first defense mechanism of plants are expected to meet morphological changes as well as alterations in their secretion quantity and/or quality. Furthermore, the essential

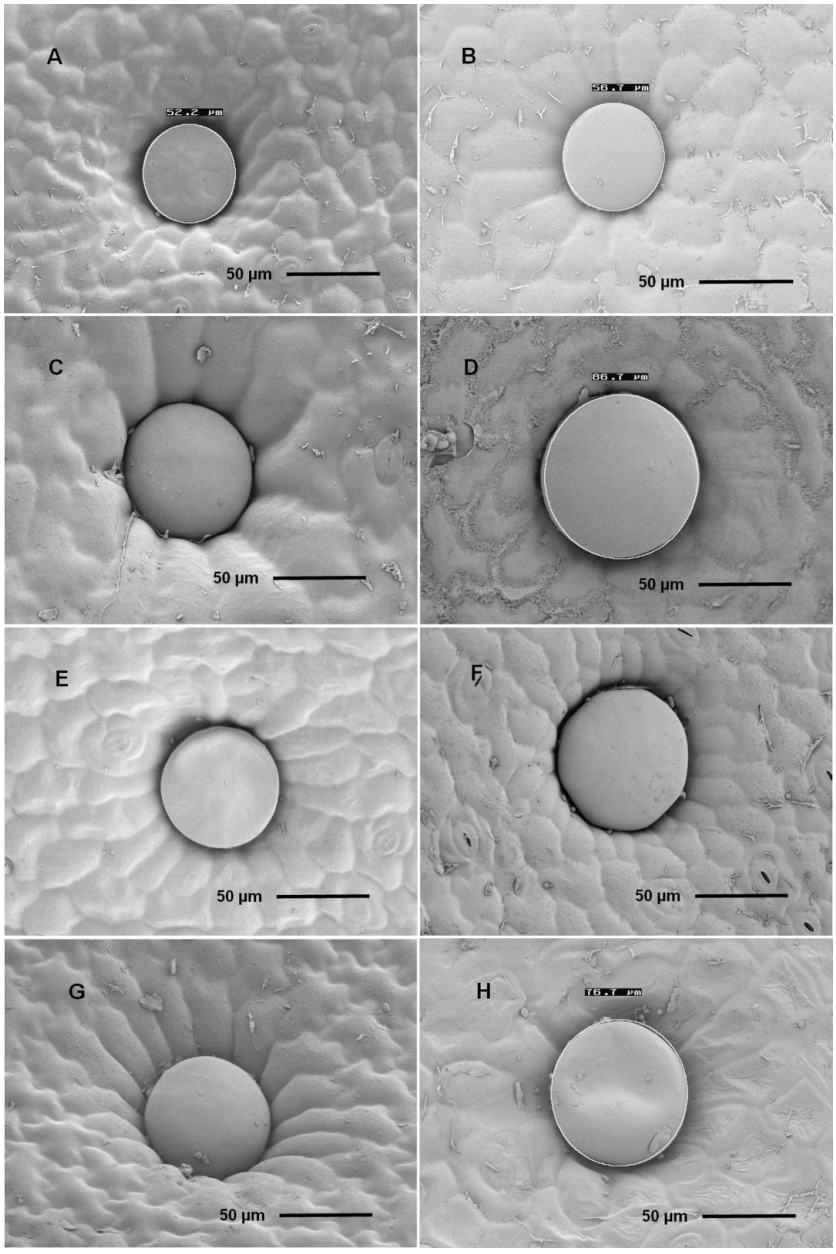

Fig. 2: Size of peltate glandular trichomes of four *Origanum vulgare* L. accessions under two light intensity conditions (RLI: reduced light intensity, FLI: full light intensity (SEM × 50).
A, C, E and G) RLI condition; B, D, F, H) FLI condition
A, B) *Origanum vulgare* L. ssp. *vulgare*
C, D) *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart
E, F) *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek
G, H) *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L.

oil production of plants depends on carbon which is a fixed process in photosynthesis (SANGWAN et al., 2001), thus changes in light intensity alter essential oil secretion via variations in leaf micro- and macro-morphology. Morovere, UV-B light is an essential factor for the formation of PGTs and CGTs (IOANNIDIS et al., 2002).

It may be suggested that larger size of PGTs and CGTs and higher theoretical volume of essential oil in PCTs under the FLI condition was caused by greater essential oil secretion of each gland. The essential oil biosynthesis is influenced by other factors such as photosynthetic radiation and plant-insect interaction. Higher intensity of photosynthetic radiation leads to more photosynthesis products which may transfer to secondary metabolites such as essential oil (CHANG et al., 2008). Moreover, under higher light intensity, plants are more vulnerable to insects. Therefore higher secretion of essential oil in each gland acts as a defense strategy (KOFIDIS et al., 2003). Accordingly, increasing the size of PCTs and CGTs as well as the density of CGTs and the density of GTs in the higher intensity of light could be an adaptive strategy of plants.

Two distinct parameters, leaf area and the number of initiated hairs per leaf, influence the density of trichomes on leaves. In most cases, leaf area is controlled by growth and environmental factors. Additionally, the number of hairs initiated per leaf is based in the plants genetics (ROY et al., 1999). Accordingly, it is proposed that the number of GTs per leaf is fixed in each accession. Moreover, studies

Tab. 2: Mean values (± standard error) of evaluated parameters of four *Origanum vulgare* accessions (Ovu: *Origanum vulgare* L. ssp. *vulgare*, Ohi: *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart, Ovi: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek, Oxm: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L.).

Parameters	Accessions				p value	LSD
	Ovu	Ohi	Ovi	Oxm		
FM (g/pot)	84.91± 2.76 a	35.64±2.59 b	15.48±2.59 d	25.52±2.8 c	<0.01	7.34**
TDM (g/pot)	21.14±0.8 a	7.80±0.79 b	3.06±0.75 c	6.20±0.81 b	<0.01	2.12**
HDM (g/pot)	14.49±0.61 a	6.11±0.58 b	2.82±0.58 c	4.85±0.62 b	<0.01	1.63**
SDM (g/pot)	6.87±0.28 a	1.94±0.26 b	0.33±0.26 c	1.14±0.29 bc	<0.01	1.01**
HDM/ SDM	9.88±1.07 a	4.12±1.07 b	9.42±1.17 a	2.17±1.14 b	<0.01	3.07**
LA (mm^2)	241.23±13.63 a	121.26±13.63 b	141.16±13.63 b	235.22±13.63 a	<0.01	39.78*
PGT (No. per mm^2)	7.81±1.05 b	20.28±1.13 a	5.12±1.05 bc	4.70±1.05 c	<0.01	3.19**
CGT (No. per mm^2)	10.20±0.78 a	10.98±0.84 a	5.66±0.78 b	4.34±0.78 b	<0.01	2.29**
NGT (No. per mm^2)	0.25±0.72 b	3.77±0.78 a	0.14±0.72 b	0.54±0.72 b	<0.01	2.18**
D.PGT (µm)	61.08±0.87 c	84.16±0.94 a	75.83±0.87 b	75.86±0.87 b	<0.01	2.69**
D.CGT (µm)	21.58±0.29 b	19.76±0.31 c	23.49±0.29 a	21.17±0.29 b	<0.01	0.88**
GT (No. per mm^2)	18.01±1.53 b	31.25±1.65 a	10.78±1.53 c	9.04±1.53 c	<0.01	5.15**
AT (No. per mm^2)	18.21±1.79 b	35.01±1.93 a	10.92±1.79 c	9.57±1.79 c	<0.01	4.00**
No. of GT/ leaf	4376.19±333.54 a	4212.78±360.29 a	1540.24±333.54 b	2247.396±333.54 b	<0.01	1010.04**
V. EO/ PGT (µl)	60.09 ±37.19 (× 10^{-6}) c	156.91±40.18 (× 10^{-6}) a	114.28±37.2 (× 10^{-6}) b	114.67±37.14 (× 10^{-6}) b	<0.01	15.28 × 10^{-6}**
V. EO per mm^2 (µl)	46.5±25.58 (× 10^{-5}) b	273.9±25.58 (× 10^{-5}) a	58.1±25.58 (× 10^{-5}) b	55.7±25.58 (× 10^{-5}) b	<0.01	101.2 × 10^{-5}**
V. EO / leaf (µl)	0.112±0.03 b	0.331±0.03 a	0.082±0.03 b	0.123±0.03 b	<0.01	0.13**

** at α <0.01

FM: fresh mass, TDM: total dry mass at 39°C, HDM: herb dry mass, SDM: stems dry mass, HDM/ SDM: ratio of herb dry mass to stems dry mass, LA: leaf area, PGT: density of peltate glandular trichomes, CGT: density of capitate glandular trichomes, NGT: density of non-glandular trichomes, D.PGT: diameter of peltate glandular trichomes, D.CGT: diameter of capitate glandular trichomes, GT: density of all glandular trichomes, AT: density of all trichomes (glandular and non-glandular trichomes), No. of GT/ leaf: number of glandular trichomes per leaf, V. EO/ PGT: theoretical volume of essential oil of one peltate glandular trichome, V. EO per mm^2: theoretical volume of essential oil per mm^2, V. EO/ leaf: theoretical volume of essential oil of the adxial side one leaf, LSD: least significant difference.

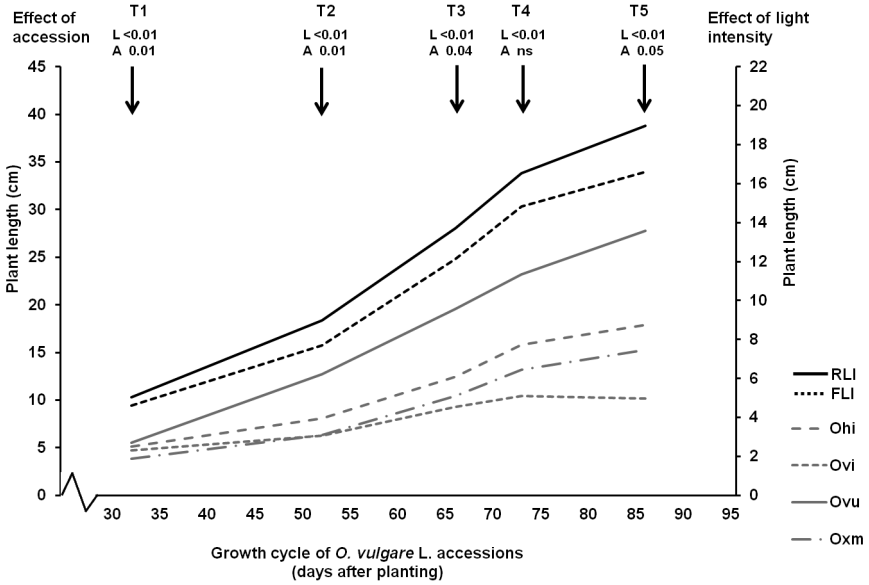

Fig. 3: Plant length of *Origanum vulgare* L. accessions under RLI and FLI conditions during the growing season.
Ovu: *Origanum vulgare* L. ssp. *vulgare*, Ohi: *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart, Ovi: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek, Oxm: *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayek × *O. majorana* L., T1 – T5: time of measurement (days), L: Light (p value), A: Accession (p value), RLI: reduced light intensity, FLI: full light intensity.

on CGTs have shown that they are active at very early stages of a leaf's development while PGTs are mostly active on mature areas of leaves (BOSABALIDIS, 2004; TURNER et al., 2000; WERKER, 1993). Therefore, it can be suggested that in the investigated accessions the differentiation of CGTs should be before cell division and/or cell expansion, and the differentiation of PGTs after it. It is suggested that in the current experiment, the light intensity conditions had no direct effect on GT's density. However, it modified the density of trichomes indirectly through alteration in leaf area. Consequently, the distance between CGTs was exceeded, leading to reduction of CGTs' density. As PGTs formed normally after cell division and/or cell expansion, their density was not affected by increasing leaf area. Correspondingly, in *Sinapis arvensis* it was found that the number of GTs per leaf was fixed. The density of trichomes was decreased by leaves' growing up over the time and increasing the leaf area (ROY et al., 1999).

The results from the current study support previously researched findings. For example in clove basil (*Ocimum gratissimum*) the density of GTs did not change by different light intensity levels. However, leaf area increased under shadow treatment (FERNANDES et al., 2013). Moreover, higher density of GT has been observed by increasing light intensity in sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*) (LI et al., 1996) as well as *wild mustard (Sinapis arvensis*) (ROY et al., 1999). Conversely, in *Mentha* × *piperita* L. it was observed that the number of PGTs was not fixed at the time of the leaf's emergence, rather with increasing the leaf length the density of PGTs decreased (MAFFEI et al., 1989).

Effect of accessions: Micro-morphological characteristics such as size and density of glandular trichomes are taxonomically significant to identify the oregano accessions (WERKER et al., 1985). New evidence from this study of GTs of Ovi and Oxm builds on previous work done on PGTs with 12 head cells and CGTs type I identified on the leaves of Ohi and Ovu (SHAFIEE-HAJIABAD et al., 2014). These conclusions are further supported by *Origanum dictamnus* where PGTs with 12 head cells were also reported (BOSABALIDIS and SAWIDIS, 2014).

The size variety of the PGTs has been previously observed in different genotypes of *O. vulgare* L. plants (BOSABALIDIS and KOKKINI, 1997; IETSWAART, 1980). BOSABALIDIS and KOKKINI (1997), investigated wild plants from different regions in Greece found that PGTs were larger in Ohi than in Ovu and Ovi. In agreement with their results the current study found Ohi had the largest PGT. Contrary to that, it was observed that the size of PGTs in Ovi and Oxm were larger than in Ovu.

The difference in the size of GTs may be explained by different numbers or different size head cells in one GT or in the case of PGTs by different volumes of accumulated essential oil (BOSABALIDIS et al., 1998; BOSABALIDIS, 2004). As shown in the investigated accessions, the number of head cells were equal (SHAFIEE-HAJIABAD et al., 2014), thus either the size of each head GT cell or the volume of accumulated essential oil caused the variance between accessions. Additionally, it may be proposed that the genetic relation between Ovi and Oxm is the reason for the similarity in size of PGTs in these accessions.

Ohi is known as an accession with high density of trichomes whereas Ovu and Ovi compared to Ohi are sparsely covered with GTs (GAVALAS et al., 2011; SHAFIEE-HAJIABAD et al., 2014). However, our results do not entirely agree with BOSABALIDIS and KOKKINI (1997) who found that the GT of Ovu was denser compared to Ovi.

With regard to NGTs, KOFIDIS et al. (2003) investigated Ovu (grown at high altitude: 1790 m), Ovi (grown at mid altitude: 945 m), and Ohi (grown at low altitude: 200 m) at their habitat of origin. They discovered the density of NGTs was greater in Ovu compared to Ovi and Ohi. Their conclusions showed lower temperature habitats of the Ovu plants induced an adaptive procedure protecting them against freezing. Under the experimental conditions of this study, without any cold stress, Ohi had the greatest density of NGTs.

Although Ovu is known as a subspecies with low essential oil content, the current experiment showed the total number of trichomes per leaf of Ovu and Ohi were equal. The higher density of CGTs in Ovu when compared to Ohi may be responsible for this incident. However, the volume of essential oils per square millimeter and per leaf was the same in Ovu, Ovi, and Oxm; although the estimated essential oil volume of Ovi and Oxm was larger compared to Ovu. This may be explained by the density of PGTs which was higher in Ovu than two other accessions. Therefore, the combination of the estimated essential oil volume (per mm^2 leaf area and per whole leaf) and the trichome density led to the same results in all accessions.

Yield parameters

Effect of RLI: plants growing under low-light conditions exhibit a variety of photosynthetic and biochemical adaptations to optimize their metabolism. These adaptations normally lead to alterations in plant growth with changes in the allocation of biomass to different organs (CHANG et al., 2008; MORELLI and RUBERTI, 2000). The first visible response of plants to low-light condition is plants' elongation (MORELLI and RUBERTI, 2000). The mechanism of plant's elongation is regulated through up-regulations of signal pathways where the phytohormone auxin and the phytochrome are interacting factors (WIT et al., 2014). Furthermore, plants under lower light intensity expand the cell's size of aerial organs in order to increase their photosynthetic surface (FERNANDES et al., 2013).

Reduction in irradiance leads to decrease the rate of photosynthesis and therefore the growth rate of the plant (CHANG et al., 2008). Moreover, the greater herb to stem dry mass ratio observed in FLI suggests a carbon allocation shift occurred to produce new reproductive organs. However, decreasing leaf thickness is an adaptive response of plants who live in a shaded habitat (TAIZ and ZEIGER, 2002); this can lead to a decrease in the stem dry mass ratio.

Congruent findings suggested, lower light intensity increased the leaf area in *Salvia officinalis* and *Thymus vulgaris* (LI et al., 1996), *Ocimum gratissimum* L. (FERNANDES et al., 2013) and *Origanum vulgare* L. ssp. *hirtum* (TIBALDI et al., 2011). However, *Ocimum basilicum* L. was observed under 75% heavy shading which resulted in plants that were shorter, lower weight, and had a smaller leaf area. The heavy shading also strongly decreased the rate of photosynthesis (CHANG et al., 2008). Additionally, the results showed the higher light intensities inhibited growth of *Ocimum gratissimum* L. (FERNANDES et al., 2013).

Effect of accessions: As shown in Tab. 2, Ovu had considerably greater fresh and dry biomass as well as higher plant length compared to other accessions (Fig. 3). In Ovi, low amounts of stem dry mass caused the herb, stem dry mass ratio to increase making it comparable to Ovu; although Ovi had the smallest biomass. Alternately, AZIZI et al. (2012) observed that the plant length of Ohi was superior when compared with Ovu, Ovi, and Oxm; the dry mass of Oxm was higher than Ohi, Ovi and Ovu respectively. Despite this, the Ov accessions which grow naturally in different altitudes in Greece, the plants of Ohi were taller than Ovu and Ovi; and leaf area was smaller in Ohi when compared to other accessions (KOFIDIS et al., 2003). In a comparable study done in Lithuania, the Ovu and Ohi plants had a similar plant fresh and dry matter yield (BARANAUSKIENĖ et al., 2013). Principally there is a relative low correlation between yield parameters and genetic matrices of Ov accessions (AZIZI et al., 2012) and these parameters are mostly influenced by growth and environmental condition.

Conclusion

Our results revealed that RLI decreased the size of PGT, as well as size and density of CGT. The lower production of herb to stem dry mass ratio obtained under applied treatment indicates that light intensity is a limiting factor for the growth of *O. vulgare* L. accessions. Moreover, it is proposed that the density of trichomes per leaf is a fixed factor; the plants in response to the low light condition and in order to increase the photosynthesis surfaces expand their length and leaf area. Therefore, the combination caused a reduction in GT density under RLI in this experiment. Additionally, as the size of glandular trichomes and the estimated volume of essential oil decreased in RLI, a conclusion may be draw suggesting light is a fundamental factor in essential oil production. Observing new types of CGTs, on analyzed accessions, is very important in understanding the impact of reduced light on plants. More research about the impact of light reduction on other plant varieties' structure, ontogeny, and secretion should be done.

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Address of the authors:
Marzieh Shafiee-Hajiabad, Bernd Honermeier, Department of Agronomy, Institute of Agronomy and Plant Breeding, Justus Liebig University Giessen, Schubertstraße 81, D-35392 Giessen, Germany
E-Mail: Marzieh.Shafiee-Hajiabad@agrar.uni-giessen.de
E-Mail: Bernd.Honermeier@agrar.uni-giessen.de
Johannes Novak, Institute for Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria
E-Mail: Johannes.Novak@vetmeduni.ac.at

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