

Impact of the varying intensity light on some morpho-anatomical characteristics and physiological parameters in young plants of *Pisum sativum* L.

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Abstract

In order to determine the extent to which reduced light intensity affects some morpho-anatomical characteristics and physiological parameters in young plants of *Pisumsativum* L. we compared certain plant parameters grown at full illumination (3200 lux) with plants grown at lower intensity illumination (1700, 1000 and 650 lux) in this research. The ultimate goal is a better understanding of the adaptations of the studied species (variety) to different light treatments. Low light intensity had a negative effect on the anatomical structure of the pea leaves and led to the development of thinner leaves compared to the plants grown at full illumination. The results obtained indicate that the thickness of the epidermis of the face and the back, the leaf thickness, the thickness of palisade tissue and the diameter of the conducting bundles decreased with decreasing illumination, while the thickness of the sponge tissue increased with decreasing illumination. The number of stomata both on the face and on the back of the leaves was lower at lower illumination, with the stomata cells being larger in size. The intensity of transpiration decreased with decreasing illumination, which was correlated with the decrease in the number of stomatal cells. The results show that lower light treatment had an inhibitory effect on the photosynthetic pigment content, which indicates the sensitivity of the studied species and raises the question of the level of adaptation and possible diminished yield of the species studied, if grown under poor light conditions.

Key words: light, peas, stomata, transpiration, photosynthetic pigments

Introduction

Legumes (*Legimonseae*) are a large group of plants that, because of their high nutritional value, represent an important source of food for humans and animals. As the world population continuously grows, the need and demand for these plants is increasing, and their production is growing in this respect as well. Pea, a very important agricultural crop, is widely used in human nutrition. During cultivation, it can be exposed to reduced light levels due to cultivation in the consociation of agricultural crops, which can significantly reduce and limit the level of production (Boardman, 1977; Akhter et al., 2009).

The growth of all autotrophic plants is directly influenced by the intensity of light, which is also the driving force behind the process of photosynthesis (Boardman, 1977; Allard et al., 1991). In nature, plants are often exposed to reduced light intensity and altered light quality resulting from the shading by the canopy or adjacent plants (Terashima et al., 2005; Yang et al., 2007; Zervoudakis et

al., 2012). The intensity of light varies greatly during the day; and season and depending on how the plants are grown this can result in reduced yields. Previous researches (Hart, 1990; Singh, 1994; Allard et al., 1991; Akhter et al., 2009; Yang et al., 2007; Zervoudakis et al., 2012) showed that growing plants under lower light conditions causes a change in the physiology and anatomy of the leaves. Light conditions can affect the morphology of the plant, decrease the specific mass of the leaf and increase the surface area. In this regard, the concentration and ratio of photosynthetic pigments changes directly interfering with the photosynthetic efficiency of the plant. The lack of light affects the ratio of energy absorbed and utilized in electronic transport and thus the overall functionality of the photosynthetic apparatus (Brouwer, 2012; Zhang et al., 2016). The leaves in the shade have a less developed palisade layer, while the mesophyll is primarily composed only of sponge cells with much more intercellular air space. As a consequence, the leaves in the shade are thinner than the leaves grown in full daylight. With the cultivation of plants in the shade, the intensity of photosynthesis, transpiration and the redistribution of biomass from vegetative parts to storage organs decreases (Nygren and Killomaki, 1993; Zervoudakis et al., 2012).

These researches are of particular interest, not only from a theoretical point of view but also from a practical point of view. Studying morpho-anatomical and physiological parameters can help selectionists create new varieties and hybrids.

Our research is directed at the influence of different light intensity on the morpho-anatomical characteristics and some physiological parameters of young pea plants. The aim was to determine the sensitivity, that is, tolerance to lower light intensity, by comparing the examined pea parameters at different light levels.

Material and Methods

In this paper, we monitored the influence of different illumination (3200, 1700, 1000, and 650 lux) on some morpho-anatomical and physiological parameters (number, stomata size, transpiration intensity, and photosynthetic pigment content) in young pea plants (*Pisum sativum* L.), of Petit Provencal variety. The plants were grown in complete nutrient solution according to Reid and York (1958). To monitor the growth of plants (root length and aboveground part) and biomass, a total of ten plants were taken from each treatment. Determination of the content of photosynthetic pigments was done in 3 repetitions, taking 0.5 g of leaves of five individuals from each treatment. Since the test species has amphistomatic leaves, their prints were taken from the abaxial and adaxial sides of the leaf, with three parts of the leaf (top, middle and base). For morpho-anatomical analysis, 15 sections were done for each group of plants. Reduced light levels are created by coating the frames with certain layers of gauze, namely with two, four and eight layers (designated I, II, and III). Plants grown at 1700 lux are designated as group I, 1000 lux as the second group and 650 lux as the third. Plants grown under full

light were taken as control (3200 lux). After 21 days from the set-up of the experiment, analyzes were performed and preparations were made.

Stomata prints were taken from three parts of the leaf; from the top, middle and base according to the standard Collodion method (Maksimović and Pajević, 2002). At the same time, the number of stomata per mm² of leaf area, length and width of stomatal cells (closure cell) were monitored. The preparations were processed on a Leica DM 500 microscope with 10x40 magnification, photographed with a Leica DFC 295 camera, and the results were analyzed in the accompanying software package. The content of photosynthetic pigments (Chla, Chlb and carotenoids) was determined spectrophotometrically in acetone extract by the standard method (Lichtenthaler, 1987) and readings were performed using a UV-VIS Shimadzu UV-160 spectrophotometer, Kyoto, Japan.

Anatomical analysis of the leaves was performed on permanent preparations that were obtained by the standard histological method for light microscopy. To prepare the anatomical preparations, the leaves were first fixed in 60% alcohol, dehydrated through a series of alcohols of different concentrations, molded in paraffin and cut on a sliding microtome according to the standard procedure (Blaženčić, 1994). For each group of plants (control, group I, II and III), 15 sections were processed. The cross-sectional preparations of the leaf thus prepared were photographed using a Leica DFC 295 camera, on Leica DM 50 microscope. The following features were analyzed on leaf preparations: thickness of the epidermis of the face and back of the leaf, thickness of the sponge and palisade tissue, total thickness of the leaf and diameter of the conducting bundles.

Results

Plant growth, transpiration intensity, number and size of stomata

Studies were conducted on plants that grew for 21 days under semi-controlled conditions under different light conditions (3200, 1700, 1000 and 650 lux). Plants grown at lower light intensities (1000 and 650 lux) were lower than controls, while at 1700 lux they were slightly higher than controls (Table 1). Plants grown at low illumination had significantly longer roots on average (14.83 cm) compared to the control (Table 1).

Table 1. Mean values of the length of the aboveground and underground parts, transpiration intensity, number and size of stomata (control-3600 lux, I group-1700 lux, II group-1000 lux, III group-650 lux)

Groups of plants	Length of aboveground part (cm)	Root length (cm)	Transpiration intensity (g·dm ⁻² ·h ⁻¹)	Number of stomata(mm ²)		Size of stomata (µm)			
				The face of the leaf	Reverse of the leaf	The face of the leaf		The reverse of the leaf	
						length	width	length	width
control	12.63	13.83	3.00	128.62	96.18	21.08	12	23.39	13.75
I	13.18	13.98	2.08	122.64	79.52	23.76	12.03	24.66	14.37
II	12.25	14.44	1.69	118.16	77.96	23.78	12.13	25.06	14.39
III	11.69	14.83	1.60	90.95	53.54	24.33	12.20	26.31	14.45

The intensity of transpiration is correlated with the intensity of illumination. With decreasing illumination, the intensity of transpiration decreased with respect to control plants in this order: I> II> III relative to the intensity of illumination. It is also noted that the intensity of transpiration was two times lower at illumination of 650 lux compared to the control.

The decrease in illumination led to a decrease in the number of stomata in young pea plants (Table 1). Changes were noted both on the face and on the back of the leaf. The number of stomata varied depending on the light intensity. Thus, the highest number was determined in the control group (96.18 mm² on the back and 128.62 mm² on the face of the leaf) and then gradually decreased with decreasing light intensity in this order: I> II> III group of plants. The different illumination of the pea plants also led to a change in the shape of the stomata apparatus. With decreasing of illumination, the width and length of the stomata apparatus increased, both on the face and on the back of the leaf (Table 1). The stomata from the initial kidney-like shape in the control group, with the lowest illumination took on the elliptic shape.

Leaf anatomy

The decrease of illumination in young pea plants caused slight changes in leaf structure (Table 2). The thickness of the facial epidermis in control plants was higher (12.605) compared to plants in Group I (11.980), Group II (11.632) and Group III (12.106). Reduced light intensity led to a decrease in the size of the epidermis cells of the back relative to the leaf face. Palisade tissue thickness also decreased with decreasing light intensity between control plants (62.522) compared to plants of group I (59.979), group II (55.846) and group III (46.117). The thickness of the sponge tissue was the smallest in control plants (96.394) and then increased slightly in plants in Group I (101.385), Group II (102.718) and Group III (102.055) with this layer up to 40% thicker in relation to the palisade. The thickness of the leaf also decreased with the decrease in light available. No differences were found between control (182.771) and group I (180.619) and II (177.257) plants, while larger differences were recorded between control and group III (161.356) plants. The diameter of the conductive bundles decreased as the light intensity decreased with no significant differences between control plants (93.251) and plants of I (92.829), II (86.751) and III group (83.100) recorded (Table 2).

Table 2. Mean values of leaf anatomy parameters (expressed in μm)
(control-3600 lux, I group-1700 lux, II group-1000 lux, III group-650 lux)

Groups of plants	Facial epidermis thickness	Palisade mesophyll thickness	Spongy mesophyll thickness	Back epidermis	Total thickness of the leaf	Diameter of the conducting bundles
control	12.650	62.522	96.394	7.801	182.771	93.251
I	11.978	59.979	101.385	7.275	180.619	92.829
II	11.632	55.846	102.718	7.061	177.257	86.751
III	12.106	46.117	102.005	7.078	161.356	83.100

Content of photosynthetic pigments

Treatment with different illumination intensity led to different effects on the photosynthetic pigment concentration in young pea plants. The values of total chlorophyll concentration (Table 3) ranged from 0.946 to 1.247 mg/g, with reduced light intensity causing a decrease in the content of photosynthetic pigments. The chlorophyll *a* concentration varied from 0.805 mg/g in the control to 0.559 mg/g at the lowest light intensity and was thus 31% lower than the control. Concentration of chlorophyll *b* decreased in group I and II in comparison to control, except in the third group where the concentration of this pigment was increased (0.453 mg/g). The values of the chlorophyll *a* and *b* ratios (Table 3) ranged from 1.234 (group III) to 1.959 (group II), with reduced light intensity significantly affecting the *a/b* ratio. The content of carotenoids varied from 0.204 to 0.307 mg/g, decreasing with decreasing light intensity (III). The lowest carotenoid value was observed in plants that were exposed to the lowest illumination intensity (0.204 mg/g), which was 34% lower than the control.

Table 3. Mean value of photosynthetic pigments (expressed in mg/g)
(control-3600 lux, I group-1700 lux, II group-1000 lux, III group-650 lux)

Groups of plants	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Chlorophyll <i>a+b</i>	<i>a/b</i>	carotenoids	Chlorophyll <i>a+b</i> / carotenoids
control	0.805	0.442	1.247	1.823	0.307	4.08
I	0.697	0.356	1.052	1.959	0.242	4.348
II	0.585	0.361	0.946	1.620	0.238	3.975
III	0.559	0.453	1.012	1.234	0.204	4.961

Discussion

Peas are a heliophyte species (Akhter et al., 2009), it is expected that grown at low light intensity it will grow less and reduced photosynthetic pigment content. It is known that low light intensity and shading often causes changes in plant development and such plants show faster elongation of stems and leaves (Yang et al., 2007; Franklin, 2008; Zervoudakis et al., 2012). In our study, plants grown at lower light intensity were slightly lower in growth than those grown at full light, with the roots of these plants being significantly longer. Nevertheless, the plants grown at full light (control) gave a more lush appearance, while the stems of the plants grown at lower intensity were thinner and feebler, corresponding to the expected phenotype of the plants growing at low light intensity (Table 1). Akhter et al. (2009) found that *Pisum sativum* L. grown at low light levels had a markedly different physiology and morphology than those grown under normal light, which may be related to the results obtained in this paper. On the other hand, Yang et al. (2007) Zervoudakis et al. (2012) found in their studies that plant height increases, as light intensity decreases and plants have an etiolated appearance. The process of leaf formation is conditioned by genes control and environmental conditions (Parkhurst and Loucks, 1972). Low light intensity showed a negative effect on the leaf anatomical structure. At an early stage of development, the leaves adapt to the conditions of the habitat, which is

reflected in the corresponding changes in metabolism (Marchetti et al., 1995), morphological structure (Gravano et al., 1999) and structure (Kull et al., 1999). Barna(2004) states that plants exposed to more light form leaves of smaller surface area, with more layers of mesophylls, thicker epidermis and cuticle, unlike plants that grow in shade and whose leaves are characterized by a larger surface area and thinner mesophyll, which corresponds with this research where the palisade layer was thicker in plants exposed to direct light during its development, while sponge layer was thicker in plants that grew in shade. According to Larcher (2003) the thickness of palisade tissue clearly indicates the light conditions in which a unit or leaf develops. Leaves that develop in the shade can be up to two times thinner than the leaves from the illuminated part of the canopy, and their sponge layer is usually thicker than the palisade. Thicker facial epidermis of leaves exposed to high light intensity is a form of leaf adaptation to protect photosynthetic tissue (Oguchi et al., 2005) which was also the case in our study. Accordingly, both sides of leaves grown at a lower intensity possessed a thinner epidermis than those of the group grown at full illumination (Table 1). The decrease in illumination led to a decrease in the number of stomata in young pea plants. Interestingly, the decrease in illumination did not have the same effect on the number of stomata on the face and back of the leaf. Namely, while on the face the number of stomata decreased with a decrease in the intensity of illumination up to 20% compared to the control, so far this decrease in the back of the leaf was up to 40%. In their research, Schulze and Hall (1982) have shown that a larger number of stomata smaller in size per unit of leaf area, provide better regulation of the water regime than a smaller number of larger stomata. However, in the conditions of optimal provision of plants with water, as was the case in our experiment, a larger number by the dimension of the smaller stomata openings transpires a larger amount of water from the same surface of the stomata openings but composed of a smaller number of larger stomata. The number of stomata per unit of leaf area depends largely on the environmental factors that govern the development of the leaf (Benjamin et al., 2006). Namely, it has been observed that the increase in the number of stomata in the conditions of increased illumination is not the result of light effect but the thermal effect of light. In their studies, Rahim and Fordham (1990) found that at full illumination, the number of stomata was twice that of plants grown at a lower intensity but with slight changes in stomatal cell length.

The transpiration intensity depends primarily on the anatomical structure of the leaf, on the morphological characteristics and on a number of environmental factors: temperature, humidity, airflow, light intensity, etc. (Nygren and Killomaki, 1993; Assmann and Schwartz, 1991; Casson and Gray, 2008). In this paper, we limited ourselves to the study of the illumination effect on the transpiration intensity, on the number and size of the stomata, starting from the fact that openness and the number of stomata can influence the intensity of water discharge. The results obtained in this paper showed that the intensity of transpiration was the highest at full illumination, which was partly caused

by a greater degree of opening of the stomata at higher illumination. Plants grown under lower illumination intensity had lower transpiration intensity, which was in agreement with the studies of Zervoudakis et al. (2012). However, some other plants may exhibit maximum intensity under moderate shade (Zhang et al., 2003) indicating better adaptability to different light conditions.

Different light treatments have led to different effects on the photosynthetic pigment distribution in young pea plants. In numerous studies (Assmann and Schwartz, 1991; Brouwner et al., 2012; Zervoudakis et al., 2012; Croft and Chen, 2017) that have dealt with the effect of light on plants it has been shown that in low illumination conditions the process which is most disturbed was photosynthesis. Lack of light leads to a decrease in photosynthesis intensity due to stomata closure and reduced CO₂ uptake, as well as metabolic damage (Croft and Chen, 2017). In this research, the chlorophyll content, a/b ratio, and carotenoid content were lower in leaves exposed to less light compared to leaves grown at full illumination. Namely, the plants that received the highest amount of light (control) had a higher concentration of chlorophyll *a*, whereas with the same treatment, the concentration of chlorophyll *b* decreased (Table 3). In their research, Croft and Chen (2017) indicate that chlorophyll *a* is more sensitive to reduced light intensity than chlorophyll *b*, which was recorded in our studies. Brouwner et al. (2012) in their work on *Arabidopsis thaliana* point to a strong correlation between chlorophyll content and illumination intensity, finding that the chlorophyll concentration doubles with increasing illumination intensity relative to the leaves in the shade. Zervoudakis et al. (2012) in their studies have shown that the concentration of photosynthetic pigments in plants grown in shade even doubles compared to plants grown in full illumination, which was contrary to the results obtained by us. The value of the chlorophyll a/b ratio is often used as an indicator of the response of plants to shade conditions (Hendry and Price, 1993). Given that the pea is a heliophytic plant, a lower a/b ratio in Group II and III indicates a better adaptation to the reduced light intensity.

Carotenoids play a significant role in light absorption and protection of chlorophylls from photo-oxidative damage. Any decrease in carotenoid concentration may adversely affect chlorophyll concentration (Brouwner et al., 2012). Furthermore, with decreasing light intensity, the carotenoid content also decreased. Reduced carotenoid content may also be a result of inhibition of their synthesis, or increased degradation of pigments or their precursors (Brouwner et al., 2012; Croft and Chen 2017).

Conclusion

Growing pea plants under different levels of reduced illumination resulted in reduced plant growth, less stomata and lower transpiration intensity, changes in leaf anatomy, and decrease in pigment content. The results obtained in this research showed that the studied species is sensitive to changes in

the light regime, which can greatly disturb the cultivation, yield and quality of cultivated plants in conditions of insufficient illumination.

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Утицај светлости различитог интензитета на неке морфо-анатомске карактеристике и физиолошке параметре у младим биљкама *Pisum sativum* L.

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Sažetak

Da bismo utvrdili u kojoj mjeri smanjen intenzitet svjetlosti utiče na neke morfo-anatomske karakteristike i fiziološke parametre kod mladih biljaka *Pisum sativum* L. u ovom radu upoređivali smo određene parametre biljakagajenih pri punoj osvjetljenosti (3200 lux) sa biljkama koje su gajene pri nižem intenzitetu osvjetljenosti (1700, 1000 i 650 lux). Krajnji cilj je bolje razumijevanje adaptacija istraživane vrste (sorte) na različit svjetlosni tretman. Nizak intenzitet svjetlosti pokazao je negativan uticaj na anatomsku građu listova graška te doveo do razvoja tanjih listova u odnosu na biljke koje su gajene pri punoj osvjetljenosti. Dobijeni rezultati ukazuju da sudebljina epidermisa lica i naličja, debljina liske, debljina palisadnog tkiva i prečnik provodnih snopića se smanjivali sa smanjenjem osvjetljenosti, dok se debljina sunderastog tkiva povećavala. Broj stoma ina licu i na naličju listovaje bio manji pri nižoj osvjetljenosti s tim što su stomine ćelije bile većih dimenzija. Intenzitet transpiracije se smanjivao sa smanjenjem osvjetljenosti što je u korelaciji sa smanjenjem broja stominih ćelija. Rezultati pokazuju da je niži svjetlosni tretman inhibitorno djelovao na sadržaj fotosintetičkih pigmenata, što ukazuje na osjetljivost istraživane vrste i ostavlja pitanje nivoa prilagođenosti i eventualnog smanjenog prinosa istraživane vrste ukoliko se gaji u uslovima nedovoljne osvjetljenosti.

Ključne riječi: svjetlost, grašak, stome, transpiracija, fotosintetički pigmenti

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