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Effects of illumination on the growth and histogeny of garden cress seedlings under altered gravity

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The study is focused on the effects of blue (450 nm), red (660 nm) and far red (735 nm) spectral components of light on the growth of garden cress (*Lepidium sativum* L.) seedlings when the pathway of the induction of gravitropic responses is isolated by a 50 rpm horizontal clinostat. Custom-built light modules with one blue, four red and one far red light emitting diodes were fitted for the first time in the centrifuge–clinostat complex. Hypocotyls, roots and leaves of seedlings grown under altered gravity applying blue, red or far red light separately and a combination of all were compared with those grown under normal gravity conditions in the light and in the dark, respectively. The obtained data showed that the impact of light wavelengths of 450, 660 and 735 nm applied at a comparatively low density of the photon flux (5, 13, 0.8–1 μmol $m^{-2}s^{-1}$, respectively) had a stronger inhibiting effect on the elongation of hypocotyls and leafstalks when gravitropic stimulation was eliminated by clinorotation as compared with normal gravity. In blue light, the elongation of 1-*g* and clinorotated hypocotyls was reduced by 16% and 24%, in red by 29% and 43%, in far red by 57% and 64%, respectively. Irradiation of six light diodes available in the used modules at a fluence rate of 50 μmol $m^{-2}s^{-1}$ stimulated a more effective differentiation of palisade cells of clinorotated but not of 1-*g* leaves.

Key words: light, horizontal clinostat, seedlings, garden cress, growth, leaf tissues

INTRODUCTION

The relationship between photo- and gravi-responses has so far been insufficiently investigated because the constant presence of gravity on the Earth makes it difficult to determine whether plant growth is influenced by gravity or by light, or by a combination of the two stimuli. Synergistical and antagonistical impacts of light and gravity on plant growth responses were demonstrated over the past decade through the use of gravitropic or phototropic [1–4] mutants or using innovative techniques that reduce the effects of one stimulus on the other [2, 5, 6]. Studies of interactions between gravitropism and phototropism have shown that gravity and light can either enhance or reduce the effectiveness of impacts on plant growth and development [1, 7, 8]. Impacts of certain wavelengths [1], photon flux density [4] and direction [2, 9] of light were studied as factors modulating gravitropic response. The effects of the quality and quantity of light on plant morphology in hypogravity (or simulated weightlessness) have not been detailed or sufficiently studied. With reference to earlier data [1, 8], we can assume that the photophysiological effectiveness of certain components of the light spectrum might be more obvious when the net of gravi-responses is eliminated. Stimulation or slight effects on the growth of gravitropically negative hypocotyls were found in garden cress [10], lattuce [11] and *Arabidopsis* [12] seedlings in microgravity (MG) in experiments performed in the dark or in white light of a high fluence rate. The adjustment of light emitting diodes (LEDs) of certain wavelengths and a relatively low photon flux density of light for irradiation of clinorotated plants was one of the modes of determining the effects of separate components of the light spectrum and fluence rate on plant growth and tissue formation. Thus, the aim of the present research was to determine and compare the effects of 450 nm blue (B), 660 nm red (R) and 735 nm far red (FR) components of the light spectrum under gravity altered by a 50-rpm horizontal clinostat (HC) on the growth of seedling organs as well as an integrated action of the three wavelengths in different fluence rates on the differentiation of anatomical-morphological structures in leaves.

MATERIALS AND METHODS

Experiments were performed with garden cress seedlings (*Lepidium sativum* L.) cultivated in experimental containers fitted to a stationary vertical control (1 *g*) device and to 50-rpm HC. Garden cress seeds were planted on a transparent medium with ½ MS salts [13] and 0.2% (w/v) gelrite (Sigma) and were grown for 5 days on the HC or vertically in 1 *g* conditions (control), both with and without illumination. Custom-built light modules with LEDs were fitted in the centrifuge–clinostat complex. Four biocontainers (cartridges with a removable bottom and a light module which ensured irradiation of the plant from above) were adjusted to HC, whereas the other four – to the stationary

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control device. The light module of each biocontainer had six LEDs of three wavelengths. Illumination with B (peak wavelength 450 nm, photon flux density 5 μmol $m^{-2} s^{-1}$), R (660 nm, 13 μmol $m^{-2} s^{-1}$) and FR (735 nm, 0.8–1 μmol $m^{-2} s^{-1}$) LEDs were applied for irradiation on the 50-rpm HC and in the vertical control device. Each module of illumination had B and FR LEDs and four R LEDs (B + 4R + FR, which were capable of changing the fluence rate from 30 to a maximum of 90 μmol $m^{-2} s^{-1}$). The photoperiod was 12 h/d light, at 22 ± 2 °C. The influence of B, R and FR light and the combined effect (B + R + FR) on the length of roots, hypocotyls, leafstalks and laminas of leaves as well as fresh biomass of leaves per plant were evaluated.

At the morphological and cytological levels, leaves were analysed after illumination with B + 4R + FR applied in two fluence rates – 50 and 90 μmol $m^{-2} s^{-1}$. At the end of experiments, seedlings had two leaves of different lengths. Each leaf consisted of three laminas – one larger and two smaller. Cytomorphological analysis of the central part of the larger lamina formed at 300 μm from the central fibre of the first leaf was performed on transverse sections with a light microscope. Small (approximately 3 mm wide) sections of a mid-lamina tissue were excised from leaves fixed in a mixture of 70% ethanol, formalin and acetic acid (18 : 1 : 1 v / v / v /). The mid-leaf sections were dehydrated through a series of ethanol and embedded in paraffin [14]. The resulting blocks were sectioned (10–12 μm thick) and stained with periodic acid-Schiff´s and saffranin. The samples were examined on a light microscope photometer (SMP 03, Opton) equipped with a Moticam2000 and PENTAX **ist*.D digital cameras. The thickness of leaf tissues, the parameters of adaxial epidermis, palisade, spongy mesophyll and abaxial epidermis were determined employing the image analysis SigmaScan Pro 5 software (Jandel Scientific Software). The analysis data of garden cress on the HC were evaluated and compared with those at 1 *g*. Statistical analysis was performed using Excel (version 7.0). The data presented in figures and tables are given as a mean ± standard error (SE). Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of light on the growth of axial organs of 1-g and clinorotated seedlings

Measurements of the axial organs of garden cress seedlings showed that the length of hypocotyls and roots grown in the dark under 50 rpm clinorotation did not significantly differ from the control under normal gravity conditions (Fig. 1).

B light inhibited the elongation of 1-*g* and clinorotated hypocotyls by 16% and 24%, R by 29% and 43%, FR by 57% and 64%, respectively. Thus, the applied light inhibited the elongation more significantly of clinorotated than of 1-*g* hypocotyls. In FR light, hypocotyls were much shorter. Earlier it had been shown that the photon-sensing system of oat seedlings can be activated by 880 nm wavelength spectral component and interact with the gravity-sensing system [15], and it was assumed that even a small amount of FR radiation (700–800 nm) may cause growth suppression. Our data on garden cress seedlings support a significant growth inhibition caused by FR, which was more obvious on clinorotated roots. Differences between the length of 1-*g* and clinorotated roots were negligible in the dark and in B, R or B + R + FR illumination. However, roots showed a negative phototropism in B and a positive phototropism in R light more frequently. Similarly to R, the FR light considerably inhibited the elongation of roots, whereas the length of control roots was greater. Thus, the data obtained demonstrated that the impact of light wavelengths of 450, 660 and 735 nm applied in a comparatively low density of the photon flux (5, 13, 0.8–1 μmol $m^{-2} s^{-1}$, respectively) had a stronger inhibiting effect on the elongation of garden cress hypocotyls when gravitropic stimulation was eliminated by clinorotation as compared with normal gravity. A simultaneous effect of three spectral components of light suppressed the growth of hypocotyls (approximately by 57%) but not that of roots. A much higher photon flux density was probably the main factor that caused this reaction, because illumination with three LEDs enhanced the fluence rate and, as will be shown later, promoted the expansion of leaf lamina, thus shading roots from the above light.

Effects of light spectrum on leaf growth under simulated weightlessness and normal gravity conditions

Data in Fig. 2 show growth responses of leaves to darkness and light under altered and normal gravity conditions. Simulated weightlessness enhanced the elongation of leaves in the dark by 20.2% as compared with 1 *g*. Measurements of the leafstalk and leaf lamina showed that apical-basal axes of leaves clinorotated in the dark were longer as compared with the control due to an intensive elongation of leafstalks (Fig. 2A). So, these data confirm the data obtained in microgravity when the course of elongation of the overground part was promoted [11, 12]. The length of leafstalks clinorotated in the light was greater than in the dark, but, contrary to 1-*g*, did not significantly depend on the light wavelength applied. The applied B, R and FR illumination enhanced the total length of both 1-*g* and clinorotated leaves; however, a more intensive elongation of 1-*g* leafstalks was found. In B light, the apical-basal axes of leaves increased by 42.7% at 1 *g* and by 14.9% on the HC, as compared with the dark, while the total leaf length did not differ in both gravitational environments. It should be noted that the R and especially the FR light increased the length of 1-*g* leafstalks most significantly (approximately 2 and 2.5 times, respectively). The total area of garden cress leaves largely depended on the parameters of the lamina whose length only

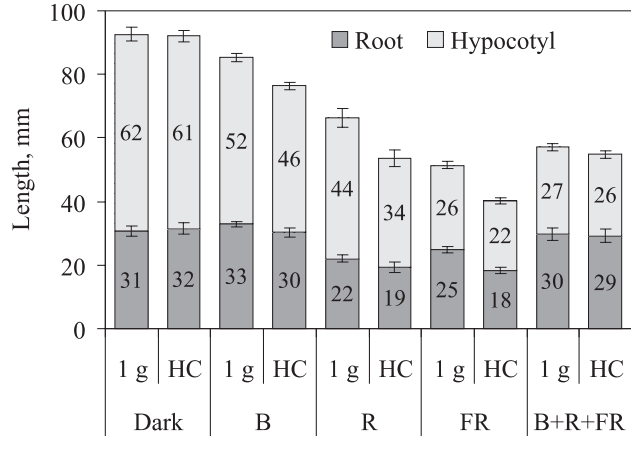

Fig. 1. Length of hypocotyls and roots of 1-*g* (control) and clinorotated (HC) seedlings of garden cress grown for five days in the dark or exposed to B, R or FR light or a combination of all (B + R + FR)

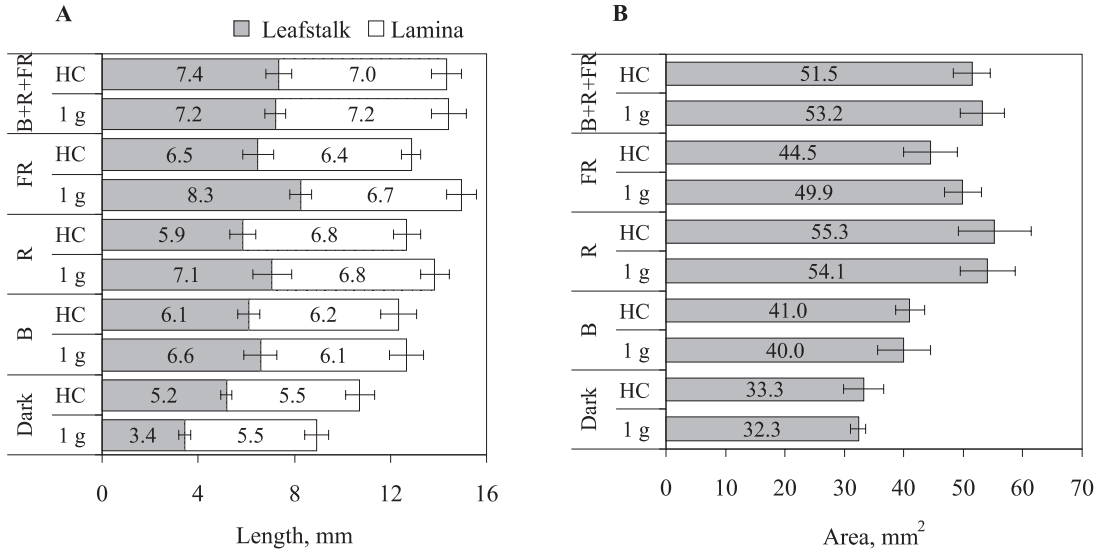

Fig. 2. Mean length (A) and area (B) of the leaves in the dark and after illumination in normal (1 *g*) and altered gravity (HC) conditions

slightly increased in the light, particularly under the influence of the R and FR light. However, no gravity-dependent differences in the areas of leaf laminas were determined (Fig. 2B). Promotion of leaf expansion by light was common to both 1-*g* and clinorotated plants, but it was strongly dependent on the components of the light spectrum. In fact, the area of garden cress leaves was illumination-dependent, and only differences between clinorotated and control leafstalks were significant. Analysis of data on the separate and integrated influence of the three components of the light spectrum under conditions of simulated weightlessness and normal gravity showed that the light suppressed the course of elongation more obviously on a 50 rpm HC.

Clinorotation in the dark slightly decreased the fresh biomass of leaves per plant as compared with the control (12.44 ± 1.31 and 9.83 ± 1.20 mg, respectively). The applied B and FR light did not significantly change leaf biomass. However, R and the B + R + FR combination of light increased the biomass by 19% and 25% at 1 *g* and by 67% and 44% on the HC, respectively. Thus, clinorotation stimulated the increment of fresh biomass of leaves in the R and B + R + FR light.

Inflence of two intensities of light fluence rates on histogenesis in 1-g and clinorotated leaves

At the morphological and cytological levels, clinorotated and 1-*g* leaves were analysed after cultivation in illumination with all six LEDs (B + 4R + FR) applied in 50 and 90 μmol m^{-2} s^{-1} fluence rates and compared with those in the dark. Increment of the light fluence rate from 50 to 90 μmol m^{-2} s^{-1} caused the augmentation of leaf area approximately by 32% on the HC and by 37% at 1 *g* as compared with the dark. Analysis of cell parameters in leaf sections showed the cause of the change in leaf thickness in the exposure to light (Table 1). The larger lamina consisted of 10–11 layers of cells in all studied conditions. The total thickness of 1-*g* and clinorotated leaves did not differ in the dark. However, gravity alteration caused differences in adaxial (upper) epidermis which was approximatelly by 10% less than at 1 *g*. Palisade cells did not yet form in these leaves which had a thick layer of tightly packed adaxial mesophyll cells. Illumination promoted differentiation of palisade cells in both 1-*g* and clinorotated leaves. The total thickness of a 1-*g* leaf was approximately by 10% larger at 90 μmol m^{-2} s^{-1} than at 50 μmol m^{-2} s^{-1} fluence rate of light. In contrast, the thickness of leaves clinorotated at 50 and 90 μmol m^{-2} s^{-1} light did not differ. The main differences in leaf cells size related to altered gravity were observed under a lower fluence rate of light (Table 2). Clinorotation in 50 μmol m^{-2} s^{-1} light stimulated the increment of the area of palisade and mesophyll cells. The effect of altered gravity on the growth of cells less exposed to light, i. e. the lower (abaxial) epidermis and lower mesophyll, was not considerable. The influence of 90 μmol m^{-2} s^{-1} photon flux density was stronger and stimulated the formation of a thicker layer of palisade parenchyma in these leaves. The palisade layer formed in 63% of clinorotated and 25% of 1-*g* leaves. It has been reported that leaf size is determined early in the development [16], but the histological differentiation of cells

Table 1. **Changes in the total leaf thickness and thickness of various tissue layers under exposure of garden cress to 50 and 90 μmol m^{-2} s^{-1} fluence rates of light**

Leaf tissues	Dark		Tissue thickness, μm			
			Photon flux density 50 μmol m^{-2} s^{-1}		Photon flux density 90 μmol·m^{-2} s^{-1}	
	1 *g*	HC	1 *g*	HC	1 *g*	HC
Upper epidermis	20.0 ± 0.6	18.1 ± 0.3	23.4 ± 1.4	17.0 ± 1.0*	20.4 ± 1.1	19.6 ± 1.7
Palisade layer	–	–	35.2 ± 5.2	44.8 ± 6.0	47.9 ± 4.1	49.4 ± 4.3
Spongy mesophyll	221.6 ± 3.0	224.1 ± 1.3	154.4 ± 5.7	185.9 ± 9.7*	178.5 ± 5.5	185.4 ± 5.6
Lower epidermis	16.6 ± 0.8	15.4 ± 1.4	21.2 ± 1.4	17.9 ± 1.1	19.3 ± 0.9	17.7 ± 1.2
Total	258.3 ± 2.8	257.7 ± 1.5	234.8 ± 7.5	265.8 ± 6.9*	266.2 ± 4.3	264.4 ± 5.7

* The difference between 1 g and HC is statistically significant at $p < 0.05$.

Table 2. **Mean area of cells of different leaf tissues grown in light of 50 and 90 µmol $m^{-2} s^{-1}$ fluence rates at 1 g and on the HC**

Leaf tissues	Cell area, µm²			
	Photon flux density 50 µmol $m^{-2} s^{-1}$		Photon flux density 90 µmol $m^{-2} s^{-1}$	
	1 *g*	HC	1 *g*	HC
Upper epidermis	529.7 ± 44.1	548.4 ± 40.2	589.6 ± 44.0	534.1 ± 11.6
Palisade layer	620.0 ± 29.3	714.2 ± 32.2*	699.9 ± 4.0	737.0 ± 31.5
Upper mesophyll	547.7 ± 22.0	735.4 ± 28.3*	673.1 ± 45.6	717.6 ± 44.5
Lower mesophyll	478.3 ± 22.3	641.9 ± 16.2*	626.7 ± 27.7	618.8 ± 21.0
Lower epidermis	474.7 ± 17.9	470.9 ± 35.1	470.3 ± 25.0	486.0 ± 45.0

* The difference between 1 *g* and HC is statistically significant at $p < 0.05$.

is definitely related to light spectral quality [17] and gravity conditions as shown in MG experiments [18].

On the basis of our experiments, we can assert that illumination promoted a more effective differentiation of leaf tissues during seedling cultivation under conditions when the induction of gravitropic reaction was eliminated by clinorotation. Thus, the role of gravity in the regulation of histodifferentiation processes can be assumed. In sum, our data confirm that photo- and gravi-physiological interactions can be modulated by precise parameters of gravity and light. To get a deeper insight into the role of light in plant gravisensing and gravitropism, experiments on a two-dimensional clinostat enabling a gradual variation of gravity and illumination parameters are under way.

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ŠVIESOS POVEIKIS SĖJAMOSIOS PIPIRNĖS DAIGŲ AUGIMUI IR HISTOGENEZEI PAKEISTO SVARUMO SĄLYGOMIS

S a n t r a u k a

Straipsnyje pateikiami bandymų, atliktų horizontaliame klinostate (50 aps./min) su mėlynos (450 nm), raudonos (660 nm) ir tolimos raudonos (735 nm) šviesos diodais, rezultatai. Pakeisto svarumo ir modeliuojamo apšvietimo sąlygomis buvo tirta sėjamosios pipirnės (*Lepidium sativum* L.) daigų augimas ir lapų histogenezė. Naujos konstrukcijos konteineriai su šviesos diodais (vienas mėlynos, keturi raudonos ir vienas tolimos raudonos šviesos) buvo įdiegti stacionarios vertikalios kontrolės įrenginyje ir horizontalaus klinostato-centrifugos komplekse. Daigai buvo auginami tamsoje, atskirai šviečiant mėlynai, raudonai ar tolimai raudonai šviesai arba visoms kartu. Nustatyta, kad 450, 660 ir 735 nm šviesos spektro komponentės (fotonų srauto tankis 5, 13, 0,8–1 µmol $m^{-2} s^{-1}$ atitinkamai) labiau slopino hipokotilių ir lapkočių tįstamąjį augimą pakeisto svarumo sąlygomis. Mėlyna šviesa sumažino hipokotilių tįsimą normalaus ir pakeisto svarumo sąlygomis 16% ir 24%, raudona – 29% ir 43%, tolima raudona – 57% ir 64%. Klinostate šviečiant visiems šešiems modulių diodams (50 µmol $m^{-2} s^{-1}$) lapų palisadinio audinio diferenciacija vyko sparčiau negu kontrolės sąlygomis.

Raktažodžiai: šviesa, horizontalus klinostatas, daigai, sėjamoji pipirnė, augimas, lapo audiniai