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JIP-test as a tool for early diagnostics of plant growth and flowering upon selected light recipe

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Abstract

Light is a major factor controlling plant growth and development. To assess the impact of the applied light conditions, we aimed to sort out a tool for early diagnostics of the plant physiological state. We investigated the effect of a blue:red:far-red LED light formula recommended for improved plant performance (flowering). High (BR) and low (BRS) light intensity variants were compared to normal (W) and 'shadowed' (WS) white fluorescent controls. The efficiency of the JIP-test to determine changes during early growth of pea plants was compared to additional physiological characteristics (growth parameters, thermal stability of the thylakoid membranes, chlorophyll content, CO_2 assimilation, transpiration). Our data showed the onset of growth inhibition under BR light, while BRS light stimulated plants to reach the flowering stage similarly as the W control. We concluded that the JIP-test is appropriate for early, reliable, and nondestructive analysis of light recipes for plant growth and flowering.

Additional key words: biomass; chlorophyll fluorescence; differential scanning calorimetry; electron transport; net photosynthetic rate; *Pisum sativum*; water content.

Introduction

Photosynthesis consists of two main parts: the photochemical processes running at the level of thylakoid membranes producing NADPH and ATP, as well as CO_2 reduction pathways (mainly Calvin cycle) using ATP and NADPH for CO_2 assimilation (Rochaix 2011). The regulation of photosynthetic electron transport in the thylakoid membrane of chloroplasts is fundamental for the maximum photosynthetic yield and plant growth (Yamori *et al*. 2015). In leaves, the two photosystems PSII and PSI have maximum absorbance in the same spectral regions but the global quantum yield of PSII is generally higher than the PSI yield (Laisk *et al*. 2014). In natural environments, both the intensity and the quality, or spectral composition, of natural, ambient light fluctuate with time. Therefore, light not only drives photosynthesis but also acts as an environmental cue that informs plants about their environment (van Gelderen *et al*. 2018a). Light quality and duration drive major developmental changes, such as photomorphogenesis, photoperiodic induction of flowering, phototropism, shade avoidance, as well as defense (Ballaré 2014, Darko *et al*. 2014, van Gelderen *et al*. 2018a). To effectively utilize the amount of available light, plants adapt to light intensity during growth by regulating leaf morphology, the composition, structure, and function of thylakoid membranes and the overall rates of photosynthesis, which assures maximal photosynthetic yields (Leong and Anderson 1984, Kapchina-Toteva *et al*. 2014). Plants have very detailed light signaling mechanisms

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Abbreviations: ABS/RC – light absorption per active reaction center (RC); BR – blue and red light; BRS – blue and red light with shadow; *E* – transpiration rate; LED – light-emitting diode; PC – principal component; PI_{ABS} – performance index of PSII; PI_{total} – performance index of PSII and PSI; P_N – net photosynthetic rate; RC – active reaction center; RC/CS_0 – active RCs per excited cross section; W – white light; WS – white light with shadow; φ_{E0} – quantum yield for electron transport to the intersystem (between PSII and PSI) electron acceptors; φ_{P0} – quantum yield for the photochemical reaction in PSII; φ_{R0} – quantum yield for electron transport to PSI end acceptors.

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with photoreceptors dedicated to different wavelengths in the light spectrum and interactions between these photoreceptors themselves and their downstream signal transduction pathways (van Gelderen *et al*. 2018a).

Artificial lighting can be used to influence plant growth and development including the photosynthetic rate, plant form (photomorphogenesis), growth direction (phototropism), and flowering times (photonasty) (Darko *et al*. 2014). Light emitting diodes (LEDs) are particularly suited for horticultural lighting due to their narrow peak bandwidths, which allow for the creation of specialized lighting recipes, which are optimized for various applications, growing conditions, and plant species. LEDs have become a favorable choice as an artificial light source in various types of controlled plant cultivation systems because of their energy efficiency, long lifetime, and low heat emission (Ilieva *et al*. 2010, Phansurin *et al*. 2017). Because blue-red light is more effectively absorbed by photosynthetic pigments than green light, various LED systems producing only blue-red light have been designed and widely used (Massa *et al*. 2008, Yamori 2016). Although, studies have found that white light is either comparable or more effective than blue-red LED light in promoting whole-plant growth (Massa *et al*. 2008), the understanding of the benefits of LED light sources over fluorescent lighting is improving (Rehman *et al*. 2017, Monostori *et al*. 2018). Reports regarding the effect of blue-red and white lights on crop growth have shown conflicting results that could be explained by the differences in plant species, the spectrum, and the proportion of blue-red lights used in each experiment. Light quality affects flowering in various aspects. In *Cyclamen persicum*, plants grown under blue-red LED light (10 h per day) or white fluorescent light (12 h per day) for 63 d had a similar number of flower buds, while those grown under blue-red LED light had a much longer blooming period (Heo *et al*. 2003). Far-red light stimulates flowering in *Arabidopsis* and petunia (Lee and Amasino 1995, Haliapas *et al*. 2008). Moreover, a high ratio of red:far-red light delays flower initiation and inhibits flower development in some plants (Runkle and Heins 2001). This is in accordance with a recent report showing that a reduction in the red:far-red light ratio promotes flowering of petunia 'Madness Rose' plants (Park *et al*. 2016). The LED strategy offers new opportunities to modify plant growth and development. The available options are increasing and before starting more detailed analyses, a set of reliable assays could accelerate the choice of optimal light conditions.

Chlorophyll (Chl) *a* fluorescence is extensively utilized for detecting the plant health status and the influences of environmental stress effects on photosynthetic performance (Kalaji *et al*. 2011, 2014; Brestič *et al*. 2012, 2014; Yang *et al*. 2017). The JIP-test, a method extracting information from the Chl fluorescence transients, appeared to be fast, noninvasive, sensitive, and powerful approach for assessment of photosynthetic electron transport and related photosynthetic processes (Strasser and Strasser 1995, Strasser *et al*. 2004, Kalaji *et al*. 2012). The practical use of the model has clearly demonstrated that it can explain and predict the performance of photosynthetic samples under different conditions, especially when it is used in parallel with other techniques (Stirbet and Govindjee 2011, Kalaji *et al*. 2012).

In the present study, we investigated the effect of a blue:red:far-red LED light formula for improved plant performance (flowering) recommended by *OSRAM Opto Semiconductors*. To provide information regarding the effect of the artificial light sources on the relationship between photosynthesis, growth, and flowering, *Pisum sativum* L. seedlings on the 6th day after sowing were grown for additional 7 and 25 d under white and blue-red light recipe with different intensity. For assessment of the physiological state during early growth of pea plants subjected to different light treatments, we utilized the JIP-test for evaluation of PSII parameters related to light absorption and electron transport, as well as productivities of PSII and both, PSII and PSI. In order to have a more detailed picture on plant performance, additional analyses were made: shoot and root morphometric parameters (length, biomass, water content), thermal stability of the thylakoid membranes, and photosynthetic parameters in the second fully developed leaf (chlorophyll content, CO_2 assimilation, transpiration).

Materials and methods

Plant material and culture conditions: *Pisum sativum* L. cv. RAN 1 (garden pea) seeds were incubated on a filter paper for 4 d in the darkness, at 25 ± 1°C. The germinated seedlings were transferred for 2 d in tap-water containing hydroponic vessels under white light [PAR of 80 μmol(photon) m^{-2} s^{-1}, cool daylight fluorescent *TL-D 36W/54-765 1SL/25 Philips*, 6200 K color temperature, 16-h light/8-h dark photoperiod], relative humidity of 60%, at 25 ± 1°C. On day 6, when leaf development was in the initial stage, the plants were transferred under the same conditions to different light recipes for 7 or 25 more d: white (W) [80 μmol(photon) $m^{-2}s^{-1}$; fluorescent]; white shadowed (WS) [1 μmol(photon) m^{-2} s^{-1}; fluorescent]; blue:red:far-red [15:75:10% corresponding to the combination of diodes: 3 blue light (460 nm), 15 hyper red (660 nm), 2 far-red (730 nm), respectively] recommended by *OSRAM Opto Semiconductors* – with high intensity BR [240 μmol(photon) m^{-2} s^{-1}; LED] and with low intensity BRS [40 μmol(photon) m^{-2} s^{-1}; LED]. The BR and BRS light intensities corresponded to the maximal and minimal illumination, respectively, of the plant growth chambers manufactured by *Metagal OOD* (Sofia, Bulgaria) (Fig. 1S, *supplement*). The chambers with LED light were made by *KömaTex*, free-foam PVC sheet with good reflecting ability (https://www.komasheets.com/en/products/advertising-sector/koematex-advertisement/), and with four-lamps panel (each lamp was 16 W and contained the combination of diodes mentioned above). The measurement of PAR was conducted by spectroradiometer *Specbos1201* (*JETI Technische Instrumente GmbH*, Jena, Germany) (Velinova 2015). The spectral distribution and quantitative estimation of PAR of the LED source and the luminescence tubes are presented in Fig. 2S*A* (*supplement*). On the base of the absorption spectra of the photosynthetic pigments (Nishio 2000, Laisk *et al*. 2014), a rough quantitative estimation was made of the energetic input for the leaf photosynthetic machinery (*i.e.*, photon density absorbed by the leaf) at different spectral regions (Fig. 2S*B*): the photon density (in spectral regions 400–500, 500–600, 600–700, and 700–800 nm) was multiplied by a respective higher plant leaf absorptance coefficient (taken as approximation from figure 7 in Nishio 2000). The temperature was regulated by air-conditioner in the room where the chambers were positioned.

Pea growth parameters: Uniform plants were used to determine the effect of the light recipes on plant growth and development. The ability for flowering under the light conditions outlined above was evaluated on day 25 by counting the average flower number per plant (in stages: initial flower bud, flower bud, and developed flowers). Shoot and root growth parameters were estimated at both days 7 and 25: length; dry mass after 3-week air-drying; relative water content = [(fresh mass – dry mass)/fresh mass)] × 100. Parameters related to the second fully developed leaf were measured on day 7: fresh mass and leaf area by using the *ImageJ* software.

Thylakoid membrane preparation and differential scanning calorimetry measurements: For thylakoid membrane preparation, leaves were collected from plants after 7 d of growth under the light conditions outlined above. Intact thylakoid membranes were isolated as described in Petrova *et al*. (2018) and resuspended in a buffer containing 20 mM Tricine, 250 mM sorbitol, 5 mM $MgCl_2$, pH 7.6; before freezing and storing at –20°C until further use, the samples were supplemented with 30% glycerol. For differential scanning calorimetry measurements, thylakoids were washed in the same buffer up to a concentration of 600 μg(Chl) ml^{-1} and scanned in the 25–95°C temperature range with a scanning rate of 0.5°C min^{-1} by means of *DASM 4* calorimeter (*Biopribor*, *Privalov*, Pushchino, Russian Federation). The original thermograms were corrected for buffer and instrumental effects as in Petrova *et al*. (2018).

Chl concentration: The second fully developed leaf was analysed after 7 d of growth under the light conditions outlined above. Leaf Chl concentrations (mg cm^{-2}) were measured with a portable chlorophyll meter *atLEAF+* (*FT Green LLC*, Wilmington, DE, USA) using two readings (on the left and right part) per leaf. The *atLEAF+* measures leaf absorbance difference between 660 nm and 940 nm and its measurements strongly correlate with total Chl content (Zhu *et al.* 2012, Novichonok *et al.* 2016).

Chl fluorescence measurements: The second fully developed leaf was chosen for analyses after 7 d under the light conditions outlined above. Induction curves of Chl fluorescence were recorded *in vivo* using *M-PEA* fluorimeter (*Multi-Function Plant Efficiency Analyser*, *Hansatech Instruments*, King's Lynn, UK) for 1 s with PAR of 4,000 μmol(photon) m^{-2} s^{-1} and performing two readings (on the left and right part) per leaf. The whole plants were adapted to darkness for 1 h prior measurements, and following analyses were made in the morning between 10:00 and 12:00 h. The primary data processing was done in *M-PEA Data Analyser 5.4* program developed by Petko Chernev at the Department of Biophysics and Radiobiology, Faculty of Biology, Sofia University. The secondary processing including calculation of fluorescence parameters according to JIP-test (Strasser *et al*. 2004, Tsimilli-Michael and Strasser 2013) and plotting values was done in *Microsoft Excel*. Seven JIP-test parameters were examined in the experiment: (*1*) RC/CS_0 – density of active reaction centers. RC – Q_A-reducing (active) PSII reaction center(s), CS_0 – the illuminated cross section of the leaf; (*2*) ABS/RC – light absorption flux (of antenna chlorophyll molecules) per RC (also a measure of PSII apparent antenna size); (*3*) φ_{P0} – maximum quantum yield for PSII primary photochemistry (*i.e.*, photooxidation of RC chlorophyll P_{680} and reduction of primary quinone acceptor Q_A); (*4*) φ_{E0} – quantum yield for electron transport from RC to the acceptors between the PSII and PSI (mainly PQ); (*5*) φ_{R0} – quantum yield for electron transport from PQ to end electron acceptors at the PSI acceptor side; (*6*) PI_{ABS} – performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors; (*7*) PI_{total} – performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end acceptors.

Gas exchange: The second fully developed leaf was chosen for analyses after 7 d under the light conditions outlined above. Net photosynthetic rate (P_N) and transpiration (*E*) were measured using infrared gas analyser *Li6400* (*LI-COR Biosciences Inc.*, Lincoln, NE, USA) equipped with a red LED light sourced chamber (*LI6400-02*). Before measurements, the instrument was calibrated according to standard procedures (LiCor 2012). Measurements were taken in the morning between 10:00 and 12:00 h under controlled conditions within the chamber: actinic PAR of 100 μmol(photon) m^{-2} s^{-1}; ambient for experimental variants leaf temperature of 25°C; relative air humidity at 40 to 45% resulting in a leaf-to-air vapor pressure deficit ranging from 1.17 to 1.37 kPa; flow rate of 500 μmol s^{-1}; CO_2 concentration about 500 μmol(CO_2) mol^{-1}.

Statistical analysis: All of the measurements were performed in several biological and technical repetitions. The data are presented as mean ± SE. One-way analysis of variance (*ANOVA*) followed by *Holm-Sidak*'s statistical test with significance level 0.05 was performed with *Sigma Plot 11.0* software to estimate the difference between all the variants. In order to understand how well the seven chosen JIP-test parameters could differentiate the light variants, principal component analysis was performed in *R 3.3.3* using *prcomp* function after logarithmic normalization of the data set. The principal component analysis plot was done with *ggbiplot R package*. In *Sigma Plot*, the *Pearson*'s correlation coefficient (*R*) was calculated using average parameter values for the variants ($n = 4$) with a level of significance 0.05.

Results

Light recipe for flowering: *P. sativum* seedlings 6 d after sowing were grown for additional 7 (Fig. 1*A*) and 25 (Fig. 1*B*) d under W, WS, BR, and BRS conditions, respectively. The hydroponic culture did not contain any supplements in order to avoid potential growth effects. By day 7, the plants had fresh appearance due to nutrients uptake from the seed. On day 25, flower development was observed for all the plant variants except for WS (Fig. 1*C*,*D*). In BR plants, an arrest at initial flower buds stage occurred, while the BRS plants had equal number of flowers as the W control. From all the variants, the BRS plants kept the best status with fresh green leaves, while the BR plants appeared completely dry.

Growth changes in pea plant variants: The different light treatments affected significantly *P. sativum* shoot length, biomass, and water content (Fig. 2). The shoot length increased upon low light intensity in the BRS variant, as seen on both days 7 and 25 (Fig. 2*A*). In accordance, the low light intensity WS control had the most elongated shoot but after day 7 this process slowed down – by day 25 the WS shoot length increased 1.2 times, and in the case of BRS a 2.2-fold rise was measured. Similarly to BRS, the shorter W control had 2.4-fold enhanced shoot growth from day 7 to 25. In the BR variant, the shoot elongation on day 7 was equal to the W control, and by day 25 a slower growth, 1.5-fold, was observed. The root length slightly increased only in the BRS plants on day 25 (Fig. 2*B*).

The BR light promoted significant shoot and root biomass accumulation on day 7, and by day 25, the shoot dry mass remained at control levels, while the root dry mass continued increasing more than that of the other variants (Fig. 2*C*,*D*). The effect of BRS light on shoot biomass was comparable with the W control, and the root dry mass slightly decreased on day 25. The WS control expressed significant reduction in shoot and root dry mass on day 25.

The BRS shoot and root water content was equal to the W control. The BR water content on day 7 was comparable to the W and BRS variants, however, by day 25, the lowest water content was detected in BR shoots (nearly 10% reduction on day 25) and roots (2% reduction on day 25) (Fig. 2*E*,*F*). On the contrary, the WS low light intensity control had an average of 4% increase in shoot water content on days 7 and 25.

Thermal stability of thylakoid membranes: In leaves of pea seedlings after 7 d of growth under the light variants, the calorimetric profiles of W thylakoid membranes consisted of six transitions located approximately at 56, 60, 64, 67, 74, and 89°C (Fig. 3). Thus, the differential scanning calorimetry curves were similar to previous reports on the same pea cultivar grown hydroponically at different light conditions, where, however, two transitions located at 63 and 65°C instead of one at 64°C were observed, along with an additional peak at 81°C (Petrova *et al*. 2018). On the basis of several studies, the first heat-induced event (appearing as a shoulder) at 56°C can be assigned to the dissociation of the complex three-dimensional thylakoid architecture in vertical and horizontal planes, whereas the 74°C transition is regarded to be due to the denaturation of the LHCII (Dobrikova *et al*. 2003, Petrova *et al*. 2018). The identification of the rest of the transitions is dubious due to the significant overlap of the denaturation temperatures of the various photosynthetic complexes and therefore is not further considered in this study. The growth of plants in WS conditions resulted in a dramatic change in their thylakoid differential scanning calorimetry profile as compared to the W control – the enthalpy of the thermograms (*i.e.*, the integrated area under the thermal

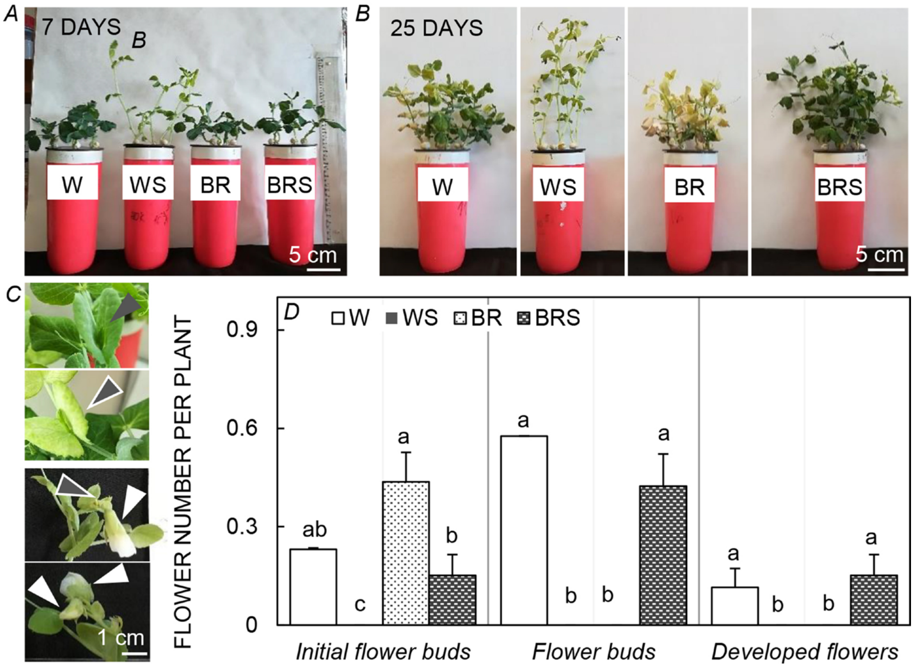

Fig. 1. *Pisum sativum* seedlings that from the 6th day were grown for additional 7 (*A*) and 25 (*B*) d under white and blue and red light with different intensity at 25°C, flowers on day 25 in different stages (*C*) are shown by arrows (*grey* – initial flower bud; *grey with white border* – flower bud; *white* – developed flower) and the average flower number per plant is evaluated (*D*). Data represent the mean ± SE ($n \geq 30$). One-way *ANOVA* (*Holm-Sidak*) statistical test is applied to estimate the difference between all the variants. *Different letters* denote statistically significant differences. Plant variants: W – white light; WS – white light with shadow; BR – blue and red light; BRS – blue and red light with shadow.

scan representing the internal energy of the system) increased nearly 3-fold, the 64°C transition became highly enhanced, the transitions at 74 and 89°C were not clearly resolved, and instead a broad transition at 83°C was visible. The switch to BR light resulted in a differential scanning calorimetry profile that resembled well the one of W thylakoids but had an additional high amplitude peak at 63°C. The BRS thylakoids did not differ from W ones regarding their calorimetric features.

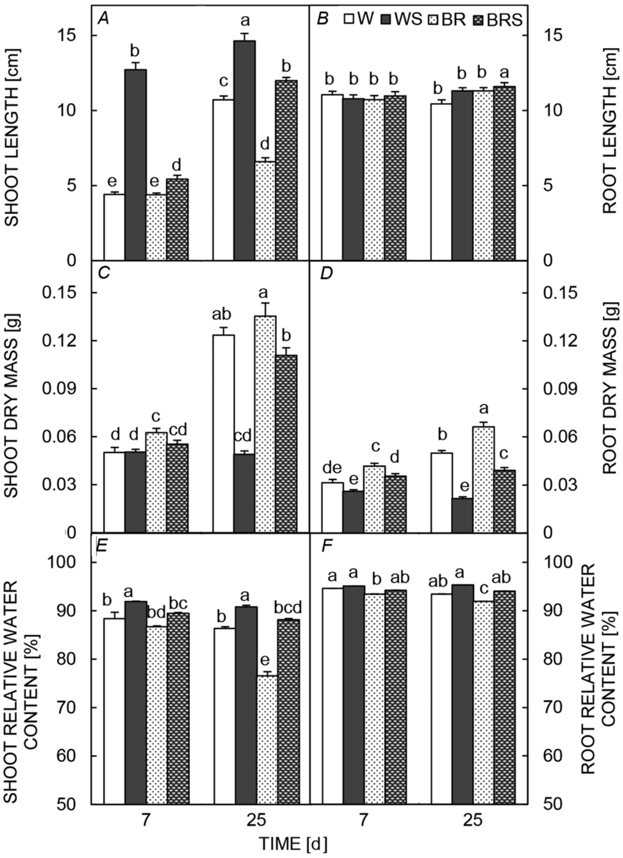

Fig. 2. Changes in shoot and root length (*A*,*B*), dry mass (*C*,*D*), and relative water content (*E*,*F*) of *Pisum sativum* seedlings that from the 6th day were grown for additional 7 and 25 d under white and blue and red light with different intensity. Data represent the mean ± SE ($n \geq 30$). One-way *ANOVA* (*Holm-Sidak*) statistical test is applied to estimate the difference between all the variants. *Different letters* denote statistically significant differences. Plant variants: W – white light; WS – white light with shadow; BR – blue and red light; BRS – blue and red light with shadow.

Growth changes in the second leaf of pea variants: To gain more focused insight in the physiological changes under the different light regimes, the second fully differentiated leaf of *P. sativum* after 7-d growth under the light variants was chosen for analysis. In the applied experimental conditions, the leaf area did not differ significantly between the BR, BRS, and W variants, and a 2.8-fold reduction was noted for the WS control (Fig. 4*A*). With respect to the leaf fresh mass, a tendency for a smaller biomass was observed under low light intensity for the WS and BRS variants with a significant 2-fold drop for the WS leaves (Fig. 4*B*). The data showed that the BR and BRS second leaves had the same leaf area and the BR leaves were heavier, which can suggest that the BR leaves were slightly thicker than the BRS ones, however, the data did not show strong statistical significance and further microscopic analyses could confirm this scenario. The Chl content per unit leaf area did not differ between BR, BRS, and W variants but it decreased two times in the WS variant (Fig. 4*C*).

JIP parameters and gas exchange: Further photosynthesis-related measurements were performed on the second leaf of all the plant variants (Figs. 5,6). To analyse the photochemical activity of PSII supercomplexes in the studied plant variants, we applied JIP-test (Strasser *et al.* 2004) (Fig. 5*A*). The analysis of JIP-test parameters revealed that in the BR and WS variants the absorption of light energy per active (Q_A-reducing) reaction center (ABS/RC) increased compared to the W control, which reflects a bigger apparent antenna size. In accordance, the number of active RCs per excited cross section (RC/CS_0) in BR and WS decreased. In these variants, the quantum yields of the photochemical reaction in PSII (φ_{P0}) and of PSII electron transport (φ_{E0}) were lower which led to respective reduction in the productivity of PSII (PI_{ABS}). In the BRS variant, the apparent antenna size was smaller (without change in RC/CS_0), and the electron transport in PSII was like the W control, which resulted in enhanced PI_{ABS}. In 'shadowed' conditions (WS and BRS light conditions), the quantum yield of electron transport to the

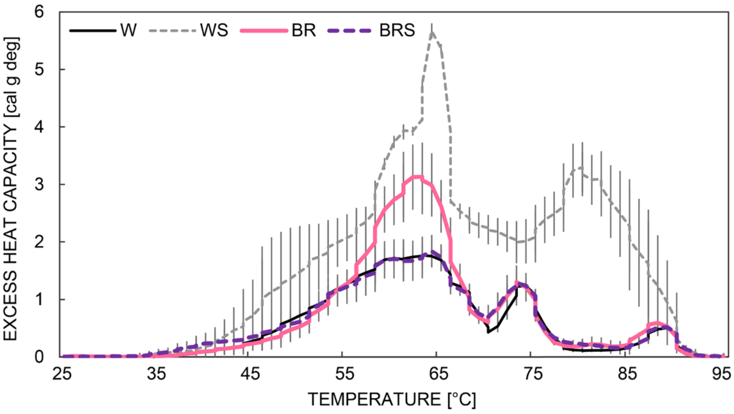

Fig. 3. DSC profiles of thylakoid membranes isolated from leaves of *Pisum sativum* seedlings that from the 6th day were grown for additional 7 d under white and blue and red light with different intensity. Data represent the mean ± SE ($n = 3$ with thylakoid membranes isolated from 25 plants per repetition of each variant). Plant variants: W – white light; WS – white light with shadow; BR – blue and red light; BRS – blue and red light with shadow.

end acceptors of PSI (φ_{R0}) was significantly reduced. Due to these results, all the light variants caused decrease of the total PSII and PSI productivity (PI_{total}) compared to the W control.

The total contribution of the two principal components (PC) in the variation of the data set is 92.8%: 63.4% for PC1 and 29.4% for PC2 (Fig. 5*B*). The formation of PC1 was caused mainly by differences in parameters characterizing the state of PSII: ABS/RC and φ_{P0}, φ_{E0}, PI_{ABS} in opposite direction. The PC2 formation was due to φ_{R0}, a PSI parameter. PI_{total} and RC/CS_0 contributed almost equally to both PCs. The experimental points (grouped by 68% probability to belong to the same variant) were not distributed homogeneously on the PC1/PC2 plane. Interestingly, each group occupied predominantly one of the four quadrants: BR – I, W – II, BRS – III, and WS – IV. PC1 separated BR from W conditions just slightly, but BRS from WS very clearly. PC2 separated moderately BRS from W and BR from WS. Both PC1 and PC2 separate W from WS and BR form BRS. In summary, significant overlapping between the groups was present except for W/WS and BRS/WS pairs and it was minimal between BR/BRS light variants. In addition, correlation analysis between the JIP parameters *vs*. the number of developed flowers showed the best correlation for the PI_{ABS} parameter ($R = 0.997$, $P=0.00319$).

The CO_2 assimilation declined under WS, BR, and BRS lights (Fig. 6*A*). The transpiration rate did not differ between the variants (Fig. 6*B*).

Discussion

In the recent years, the LED lighting enabled scientists to explore easily the effect of the light spectrum on plant growth and make impact on improving plant productivity (Darko *et al*. 2014, Rehman *et al*. 2017, Monostori *et al*. 2018). The light quality and the quantity, including light intensity and photoperiod, need to be adjusted specifically for the plant species of interest. In our study, we tested the effect of a blue:red:far-red LED light combination with high (BR) and low (BRS) intensity on pea plant growth and flowering. Approximate estimation of the energetic input for the leaf photosynthetic machinery at different spectral regions showed that the W fluorescent light input is mostly in the blue region similar to BR and in the BRS light is less, while both LED sources have increased red light input (Fig. 2S*B*; Nishio 2000, Laisk *et al*. 2014). The far-red light was maximal in the BR variant.

Most of the phenotypic analyses are time-consuming and require destruction of the plant material. In our investigation of the efficiency of the selected blue-red LED light recipe, we aimed to sort out a tool for early diagnostics of the plant physiological state, which would assist faster assessment of the impact of the applied light conditions. To evaluate the changes under the investigated LED light recipe with differing intensities, we first compared the general plant growth at early and late developmental stages. Despite the similar appearance of the plant variants at the early development, the low intensity BRS light increased shoot elongation, while the high intensity BR light stimulated root biomass accumulation and caused reduction in water content. At the later flowering stage, the BRS light promoted flower development similarly to the W control, while under BR light an arrest at the initial flower formation occurred accompanied by a significant shoot drying. Based on recent literature data, we summarised our results in a model according to which the light intensity controls allocation of sugars between the shoot and the root (Fig. 3S*A*, *supplement*; Sakuraba *et al*. 2018, van Gelderen *et al*. 2018a,b; Zhou *et al*. 2019). Under BRS light, a redirection of assimilates occurs towards the shoot and for flowering. The preferential distribution of photosynthetic products to shoots is explained as a strategy to increase the leaf interception of light and decrease the ratios of root to shoot biomass when plants are grown under low light intensity conditions (Gommers *et al*. 2013, Gundel *et al*. 2014). In the case of BR irradiance, we hypothesize that the intensive light caused increased uptake of nutrients from the seed and the shoot, which led to a much faster nutrient depletion than in the other light conditions (Fig. 3S*A*). Different studies demonstrated that high light intensity and red light can indeed promote nutrient assimilation and induce specific signaling cascades from the shoot to the root which promote root development, as well as exudation of organic acids in the rhizosphere (Cheng *et al*. 2014, Sakuraba *et al*. 2018, van Gelderen *et al*. 2018a,b; Zhou *et al*. 2019). These discoveries revealed that light is not only involved in the synthesis and transportation of sugars

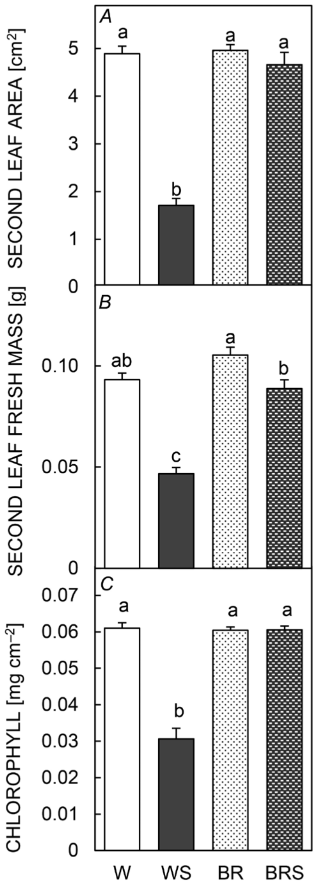

Fig. 4. Leaf area of the second leaf (*A*), fresh mass (*B*), and Chl content (*C*) of *Pisum sativum* seedlings that from the 6th day were grown for additional 7 d under white and blue and red light with different intensity. Data represent the mean ± SE ($n \geq 10$). One-way *ANOVA* (*Holm-Sidak*) statistical test is applied to estimate the difference between all the variants. *Different letters* denote statistically significant differences. Plant variants: W – white light; WS – white light with shadow; BR – blue and red light; BRS – blue and red light with shadow.

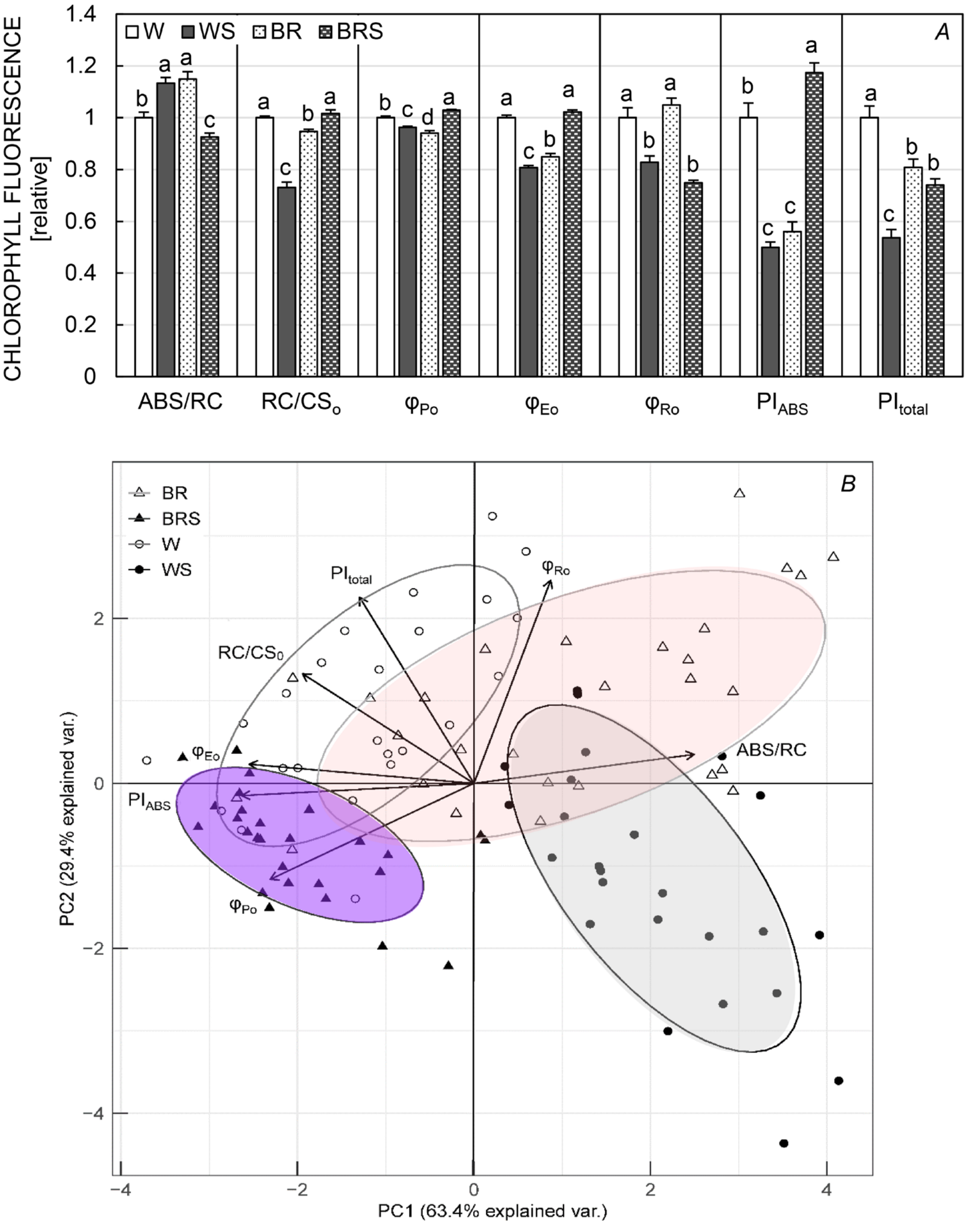

Fig. 5. Effect of light on Chl fluorescence defined by the JIP parameters (*A*): ABS/RC (light absorption per active reaction center), RC/CS$_0$ (active reaction centers per excited cross section), φ_{P0} (maximum quantum yield of the primary photochemical reaction in PSII), φ_{E0} (quantum yield of the intersystem (PSII to PSI) electron transport), φ_{R0} (quantum yield of electron transport to PSI end electron acceptors), PI$_{ABS}$ (performance index of PSII), PI$_{total}$ (performance index of PSII and PSI) in the second leaf of *Pisum sativum* seedlings that from the 6th day were grown for additional 7 d under white and blue and red light with different intensity. For each parameter the ratios to the W control are presented. Data represent the mean ± SE ($n \geq 24$). One-way *ANOVA* (*Holm-Sidak*) statistical test is applied to estimate the difference between all the variants. *Different letters* denote statistically significant differences. Principal component analysis (*B*) of the JIP-test parameters shown in (*A*). Plant variants: W – white light; WS – white light with shadow; BR – blue and red light; BRS – blue and red light with shadow.

but acts as a signal regulating root morphology. It has been suggested that the stimulatory effect of red light signaling on phosphorus uptake is apparent upon phosphorus deficit (Sakuraba *et al*. 2018). In the lower intensity BRS light variant, the blue light input is decreased compared to the W control, which could lead to slower nutrients assimilation. Therefore, in these conditions no severe nutrient deficiency symptoms and shoot dryness were observed.

We focused our further research on the detection of early changes in the photosynthetic performance in leaves compared to the W control. The BR and BRS lights did not alter significantly the thermal stability of thylakoid membranes in opposite to the obvious change in the WS variant. Notably, during thylakoid isolation and sample preparation procedures, the BR thylakoid suspensions contained excessive amount of starch, which has been

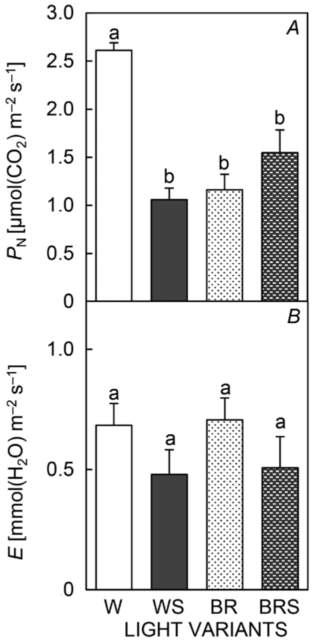

Fig. 6. Effect of light on CO_2 assimilation (P_N) (*A*) and transpiration (*E*) (*B*) in second leaf of *Pisum sativum* seedlings that from the 6th day were grown for additional 7 d under white and blue and red light with different intensity. Data represent the mean ± SE ($n \geq 10$). One-way *ANOVA* (*Holm-Sidak*) statistical test is applied to estimate the difference between all the variants. *Different letters* denote statistically significant differences. Plant variants: W – white light; WS – white light with shadow; BR – blue and red light; BRS – blue and red light with shadow.

reported to occur at irradiance that is above the optimum, as well as during phosphorus and nitrogen starvation (Chow and Anderson 1987a,b; Cetner *et al.* 2017). Further, the growth and the Chl content of the second pea leaf were similar between the BR and BRS variants and reduced in WS. However, the analysis of the Chl fluorescence revealed that the PSII efficiency is lower under BR light similarly to WS but contrasting to BRS, which was demonstrated at each step of light utilization. The apparent antenna size (ABS/RC) increased under BR light unlike generally reported smaller photosystems antenna size under high light (Wientjes *et al.* 2013). Since the Chl content remained at control level, the bigger ABS/RC ratio could reflect the decreased RC number per unit area induced by photooxidation (Takahashi and Murata 2008). It has been also hypothesized that under strong overexcitation of PSI with far-red light, the LHCII antenna moves to PSII to get quenched or to increase the PSII antenna size (Wientjes *et al.* 2013). On the other hand, bigger ABS/RC in 'shadowed' (WS) plants was due to significantly lower RC/CS_0 which is rather caused by disturbed RC formation in parallel to lower Chl synthesis and destabilization of the macroorganization and/or stacking of the membrane (as shown by the down-shift of the thermal transition at *ca.* 56°C to lower temperatures). Interestingly, greater ABS/RC was observed in nutrient deficient plants, too (Kalaji *et al.* 2014). In opposite, the low intensity BRS light decreased the PSII apparent antenna size without changing the RC number, and we speculate that the limited blue light absorption could cause such specific response. Next, the quantum yields of the photochemical reaction in PSII (φ_{P0}) and of the electron transport to the intersystem acceptors (φ_{E0}) were again lower under BR and WS lights. In BR plants, the increased energy dissipation (low φ_{P0}) has been suggested as a way for stressed plants to protect their leaves from excess absorbed light converting it into heat (Kalaji *et al.* 2014). High UV and blue light, and red light to a lesser extent, could cause PSII photodamage occurring at the oxygen-evolving complex (Takahashi and Murata 2008). In WS plants, the very low light intensity could not generate photoinhibition thus leaving structural impairment of RC complexes as a possible reason for the lower quantum yields. The higher φ_{P0} under BRS light regime suggests lack of photoinhibition and no need for heat dissipation. Other reports also support more effective functioning of PSII under moderately low irradiance (Ilieva *et al.* 2010).

With respect to the efficiency of electron transport to PSI end acceptors (φ_{R0}), under BR light φ_{R0} reached normal rate like the W control. It is possible that this effect results from the dynamic regulation of the excitation energy distribution between the photosystems, which is an important mechanism to keep the proper functionality of light utilization (Laisk *et al.* 2014). Different research groups showed that the response to high light conditions leads to significant enlargement of the unstacked membrane domains *in vivo*, which facilitates protein intermixing and substantial energy spillover towards PSI and lower PSII photochemical activity, and future detailed investigations could provide more information (Khatoon *et al.* 2009, Herbstová *et al.* 2012, Yamamoto *et al.* 2013). Under BRS and WS lights, φ_{R0} severely decreased which could be due to the lower light intensity. In accordance, it has been previously demonstrated that the absolute absorbance of pigments bound with PSI is lower than the absorbance of pigments bound with PSII (Laisk *et al.* 2014). Interestingly, in the BRS-grown plants, the red light intensity seems not to be limiting, which suggests a specific effect of the reduced blue light intensity on the electron transport to PSI. As an overall, the BR and BRS lights reduced the total performance of PSII and PSI (Fig. 3S*B*).

Principal component analysis of the obtained JIP-test data showed that despite the overlap between the analysed groups, differences between the effects of light recipes on photosynthetic machinery could be readily observed. PC1 separated the light variants according to the PSII activity in the respective plants – with lower values indicating higher performance (low light absorption, high photochemical and electron transport efficiency). The distribution according to the PC2 corresponded to PSI activity – with higher values indicating higher efficiency. Plants grown under W light expressed the highest overall performance of both PSI and PSII contrasting with WS plants having the lowest one. BR plants showed impaired PSII but intact PSI activity while BRS indicated the opposite – low PSI and intact PSII performance. Thus, BR and BRS variants have moderately low overall photosynthetic activity – between W and WS.

The principal component analysis may reveal correlation between photosynthesis and flowering. Specifically, the W and BRS variants, which have high PSII performance (negative PC1) on day 7, developed the same number of flowers later in their development, while WS and BR plants having inhibited PSII activity (positive PC1) did not exhibit flowers on day 25. Analysing the contribution of each parameter forming the PSII performance index

PI_{ABS} (ABS/RC, φ_{P0}, φ_{E0}) in the PC1 by the length and position of the parameter vectors in the graph, it could be assumed that flowering may be predicted most specifically by low apparent antenna size and high electron transport efficiency. We consider as the most accurate indicator the overall PSII parameter – PI_{ABS}, which correlated best with the number of developed flowers.

In accordance with the low productivity of the two photosystems (PI_{total}) under BR and BRS lights, the following biochemical reaction of CO_2 assimilation was also inhibited. Since the transpiration rate was not significantly changed between the variants, the gas exchange seems not to be disturbed. The high light intensity increases the accumulation of reactive oxygen species, which can result in both PSII photoinhibition and interruption of CO_2 fixation. It has been shown that the photosynthetic fixation of CO_2 can regulate the generation of reactive oxygen species (Takahashi and Murata 2008, Wang *et al.* 2018). The oxidative stress could shift the use of accumulated NADPH towards biosynthesis of secondary metabolites for quenching free radicals rather than for CO_2 fixation (Fig. 3S*B*; Selmar and Kleinwächter 2013, Kapchina-Toteva *et al.* 2014). At low light intensity, impairment of the cyclic electron transport around PSI caused a concomitant reduction in CO_2 assimilation rate, plant biomass and importantly, grain production in rice (Yamori *et al.* 2015). In addition, the CO_2 assimilation declines because the Rubisco content and respective activity is light-dependent (Fig. 3S*B*; Leong and Anderson 1984).

Conclusion: Early photosynthesis diagnostics could facilitate the adjustment of an optimal light recipe for growth and flowering of a plant species of interest. The clustering in the principal component analysis of JIP parameters outlined the major differences in the functionality of electron transport chain just after 7 d under the investigated light regimes and predicted different macro-effects in the later stages of plant development. We conclude that the JIP-test could be included in a set of analyses for early, fast, nondestructive, and reliable plant phenotyping to foresee developmental changes including ability for flowering.

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