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ARTICLE

Carrot flowering initiation: light effect, photosynthetic pigments, carbohydrates

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ABSTRACT The present study was aimed at the investigation of the influence of illumination spectrum on physiological processes in carrots (*Daucus carota* L.) during their evocation, flower initiation and differentiation. The process of flower initiation and morphogenesis was studied in a phytotron facility under treatment with different illumination spectra. The dominating 640-nm component and supplementary components (455-nm, 660-nm and 735-nm). High-performance liquid chromatography with a refractive-index detector was used for the detection and separation of carbohydrates. Total quantification of photosynthetic pigments was performed by spectrophotometric method. A considerable influence of illumination spectrum on physiological processes in the carrot, especially on the morphogenesis rate, was observed. We conclude that flower initiation processes in carrots can be controlled by tailoring the illumination spectrum and photon flux density. This enables one to accelerate plant cultivation in phytotron conditions. Conclusions: (i) The elimination of both red and far-red or only blue light appeared to suppress floral initiation, under such conditions carrots grew vegetative. In contrary, the elimination of solely far-red light resulted in faster flowering differention. (ii) The elimination of solely red or blue light resulted in a low synthesis rate of photosynthetic pigments and conditioned high sucrose content in carrot root. Meanwhile, the elimination of solely far-red light resulted in the opposite effect. (iii) Dominating 640-nm light was found to considerably contribute to the excitation of the carotenoid antennal complex of the photosynthetic system.
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KEY WORDS

carrots,
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evocation

Light quality (spectral composition), quantity (photon flux density), and duration (photoperiod) have a profound influence on the morphogenesis of biennial plants. However, there is very little knowledge on how to control flower initiation processes of biennial plants using LEDs. Flowering induction and evocation consist of two periods. The first period of flowering induction is photoinduction. During photoinduction, metabolites of the photomorphogenetic system are transported to apical meristems where they can de-block the genes of the inflorescence axis formation. This moment is the starting point of the Ist evocation period, which is accomplished with the formation of the inflorescence axis. The second period of flowering induction of wintering plants is thermoinduction. The induction metabolites determine the formation of inflorescence axis structures (the evocation period II). These processes require flower initiation and differentiation (Duchovskis 2004). The carrot plant is known to flower only after vernalization, *i.e.* low temperatures are needed for flower initiation (Gláucia et al. 1994). The role of illumination spectrum in these processes is not known well enough. Therefore, the growth, development and flowering initiation of different species of plants grown under specific wavelengths and narrow bandwidth must be characterized and understood.

Light is the energy source for photosynthesis, and it controls many aspects of the plant development. In recent studies photomorphogenetic responses of plants to red and blue light from broad spectrum sources were examined (Brown et al. 1995; Yorio et al. 1995). Pepper, lettuce, radish, spinach, broccoli, cabbage, wheat, strawberry, etc. have been successfully grown under red and blue light applied typically within vegetative growth (Kim et al. 2006; Tarakanov 2006; Yanagi et al. 2006). However, little is known on the use of light-emitting diodes to support plants after flowering induction (after vernalization) through the further life cycle.

Photosynthetically active radiation (PAR) referred to as blue light (from 400 to 500 nm) and red light (600 – 700 nm) is involved in photosynthesis, photomorphogenesis, and chlorophyll synthesis. Far-red irradiance, the spectral band from 700 to 800 nm is not important for photosynthesis but considerably influences photomorphogenesis (Casal and Sanchez 1994). Both red light, via phytochrome, and blue light, via photoreceptor(s), is effective in inducing photomorphogenetic responses. Although red light has great potential to drive photosynthesis, plants are adapted to utilize a wide-spectrum of

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Table 1. Illumination conditions for 6 growth treatments.

Treatment	455 nm	640 nm	660 nm	735 nm
L1	7.6 (4.5%)	150 (89.7%)	7.1 (4.3%)	2.5 (1.5%)
L2	1.4 (0.8%)	159 (94.5%)	7.5 (4.5%)	0.0
L3	7.6 (4.7%)	150 (93.7%)	0.0	2.5 (1.6%)
L4	0.0	159 (93.8%)	7.5 (4.4%)	3.0 (1.8%)
L5	0.0	150 (100%)	0.0	0.0
L6	1.4 (4.5%)	159 (95.5%)	0.0	0.0

Table 2. Orgagenesis as a function of treatments.

Treatment	Organogenesis stages according to Kuperman (%)			
	II	III	IV	V
L1		65	25	
L2		27	35	38
L3		40	60	
L4	22	11	67	
L5	100			
L6	100			

light to control photomorphogenetic responses (Kendrich and Kronenberg 1994). Carbohydrate-dependend regulation of photosynthetic gene expression is believed to occur through hexokinase, which is a sugar sensor (Jang et al. 1997).

It is well-known that phytochrome is light sensing protein that plants require for developmental responses and that plays an important role as a photoreceptor in the photoperiodic reaction of floral initiation (Thomas and Vince-Prue 1997).

In this study we present results on manipulation of carrot flowering initiation by blue, red and far-red light, and the links with the variation in carbohydrates and photosynthetic pigment contents.

Materials and Methods

Carrots *Daucus carota* L. var. *Garduolė 2* were initially grown in vegetative tumbler, 54x34x15 cm in size, placed in a greenhouse (16 h photoperiod and 21/16°C day/night temperature). Peat (pH ≈ 6) was used as a substrate. Carrots with 9 leaves in rosette were removed from the greenhouse and kept in phytotron chambers under low temperature (4°C) for 120 days. Subsequently evocation, flower initiation and differentiation processes were investigated under illumination with the photoperiod of 16 h and 21/16 ± 2°C day/night temperatures maintained for one month. Illumination with different spectra was generated by an light-emitting diodes based illuminator (Table 1). The dominating 640-nm component delivered by AlGaInP LEDs and supplementary components from AlGaN (455-nm) and AlGaAs (660-nm and 735-nm) LEDs (Tamulaitis et al. 2005). After a month of growth under different regimes of illumination, all plants were grown under a high-pressure sodium lamp (Son-T Agro, *Philips*), and their further development was studied. The level of plant development was determined according to Kuperman (1982).

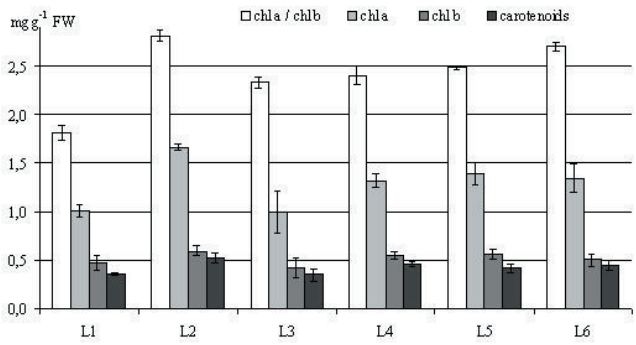


Figure 1. Effects of illumination spectrum on photosynthetic pigment contents.

Carbohydrates sample preparation: 1-2 g of fresh tissue per sample was grounded and diluted with 4 ml bidistilled water. The samples were pre-purified using 0.2 μm syringe filters.

Analysis of fructose (Fru), glucose (Glu), sucrose (Suc) and maltose (Mal) were performed on a Shimadzu 10A HPLC model equipped with a refractive index detector (RID 10A), column oven (CTO-10AS VP), degasser (DGU-14A), and pump (LC-10AT VP). Separations were performed on an Adsorbosil NH_2-column (150 mm x 4.6 mm). Mobile phase: 75% acetonitrile. Flow rate: 1 ml/min.

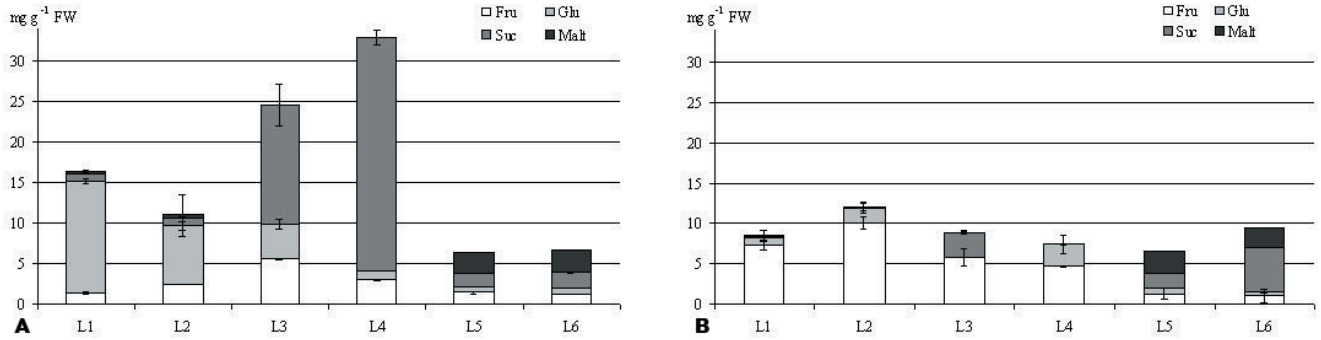
Spectrofotometric analysis (spectrophotometer Genesis 6, USA) and quantification of total chlorophylls a and b and carotenoids was performed at 440.5nm, 662 nm, and 644 nm wavelengths, respectively. Photosynthetic pigment samples preparation: 0.2 g of fresh weight (from roots and leaves) was grounded with $CaCO_3$ and extracted in 100% acetone, according to Vetsthtein (Gavrilenko 1975).

Results

Different effects of illumination spectrum on the carrots physiological process, especially on morphogenesis rate during evocation, flower initiation and differentiation, were observed, as illustrated in Table 2. All plants passed the flower initiation and evocation process in growth runs L1, L2 and L3. Under treatment without blue component (L4), 22% of plants remained in the vegetative state (organogenesis stage II). Carrots treated under solely red LEDs (640 nm, L5) and under combination of red and blue LEDs (640 nm and 455 nm, L6) generally showed no development and all 100% remained in the vegetative stage (Table 2).

The influence of different illumination spectrum on the content of the photosynthetic pigments indices was established (Fig. 1). The lowest contents of all pigments were

Figure 2A, B. Effects of treatments on sugar content.



found in growth runs L1 (with all four spectral components) and L3 (without 660 nm). The highest concentrations of chlorophylls a and b and carotenoids were detected in L2 runs, where plants were treated without far-red light. The lowest and the highest chlorophyll a/b ratios were observed in growth runs L1 and L2 respectively. No significant differences in photosynthetic pigment concentrations in growth runs L4 (without blue light), L5 (only with 640 nm component) and L6 (without 660 nm and 735 nm components) were found.

Figure 2 shows that though the variation between fructose and glucose and between sucrose and maltose in different growth treatments was different, but we found that when the total amount of monocarbohydrates is high, the levels of dicarbohydrates are low (L1, L2, L3 and L4). Contrarily where the concentrations of monocarbohydrates were low, the amount of dicarbohydrates were high (L5 and L6). Considering dicarbohydrates, the sucrose concentration significantly increased in carrot root in growth runs L3 and L4, although maltose was not detected here (Fig. 2A). In the carrot leaves (Fig. 2B) maltose also was not detected in growth treatments L3 and L4. Also sucrose was not present in leaves in treatment L4. Different behavior was observed for monocarbohydrates. Contrary to carrot leaves, roots exhibited higher glucose and lower fructose levels (L1 and L2). In growth treatments L5 and L6 the carbohydrate distribution between roots and leaves are the same.

Discussion

In agreement with other authors (Yanagi et al. 2006), our results show that the elimination of both red (660 nm) and far-red (735 nm) light that is utilized in the reversible transformation at phytochrome (growth runs L5 and L6) suppresses floral initiation. Although plants were treated by low positive temperatures required for floral induction, after treatment with above-mentioned illumination (L5 and L6) carrots remained in the vegetative stage (Table 2). Also, for these treatments the sugar concentration was low and distribution of mono and dicarbohydrates was the same in roots and in leaves. The elimination of only far-red (L2), red (L3) or blue (L4) light did not have such a dramatic effect on the suppression of plants flowering (Table 2). These results imply that at least far-red is required to invoke floral initiation, probably mediated by phytochrome response. The effect of the absence of blue light (L4) might be lower than that of the absence of far-red light (Table 2). Similarly to our results a study of the light wavelength range required for floral induction, because floral initiation by red and far-red lighting seems to be mild in strawberry plants (Yanagi et al. 2006). In higher plants there are general pathways in the transduction of photoperiodic/photomorphogenetic signals. Effects of different environmental stimuli (e.g., flowering occurs in response to long photoperiod as well as to low red : far-red ratio) often result in the same developmental or morphogenetic pattern depending on the plant life strategy. According to our results, when red: far-red ratio was equal to zero (L2 treatment) even 38% reached V^{th} organogenesis stage. In treatments L1, L3 and L4 red : far-red ratio was equal to 50, and development of carrots was lower (Table 2). In other words, photoperiodic and photomorphogenetic light signals trigger similar stress-avoidance response. In long-day plants with competitor strategy the same conditions influence rapid flowering and bolting response (Tarakanov 2006).

Photosynthetic system plays an important role in plant development. In photoperiodic plants, there is strong experimental evidence that leaves produce promoters and inhibitors of flowering when exposed to favorable and unfavorable conditions, respectively (Bernier and Prilleux 2005). These signals are transported from the leaves to the apical meristem with metabolites of photosynthesis process. Plants are adapted to utilize a wide-spectrum of light to control photomorphogenetic responses (Kendrick and Kronenberg 1994). Various parts of light spectrum serve as signals providing organism with important information from their environment. Appropriate combinations of red and blue light have great potential for use as a light source to drive photosynthesis due to the ability to tailor irradiance output near the peak absorption

regions of chlorophyll. There are close relations between plant photosynthesis and photoperiodic response based on source-sink relations (Tarakanov 2006). Chlorophyll b and inactive phytochrome form (P_r) have absorption spectrum at 660 nm, the elimination of this red light (L3) resulted in a low photosynthetic pigments synthesis rate (Fig. 2) and conditioned a high sucrose content in carrot root (Fig. 2A). In the contrary, the elimination of far-red light (L2) stimulated synthesis of chlorophylls and carotenoids (Fig. 1). The absorption spectrum of carotenoid and chlorophyll a is at blue light region. The elimination of this blue light did not influence the content of chlorophyll a but dramatically influenced sucrose distribution: a high level was found in carrot root (Fig. 2A) and no sucrose was found in leaves (Fig. 2B). The transported sucrose is effective as a florigen even if its main action is the energy supply (King 2006). Conversion of sucrose to glucose can control flowering. According to Bernier and Perilleux (2005), mutants that block photosynthetic carbon metabolism usually exhibit late flowering as could be expected for a plant that shows flowering due to a photosynthetic response.

During the reproductive phase of development a lot of new structures are formed, the photosynthetic apparatus is complete. Photosynthetic pigments can participate like structural material for carbohydrates biosynthesis. Both, light quantity and quality are known to affect the contents and the ratio of individual proteins and pigment-protein complexes of the photosynthetic apparatus. It is well known that blue light promotes stomatal opening and influences the biochemical properties of photosynthesis. Menard et al. (2005) showed that plants grown under blue fluorescent lamps had higher chlorophyll a/b ratios, smaller amounts of light-harvesting chlorophyll a/b-binding protein of photosynthetic system II per unit chlorophyll content, and higher ribulose-1.5-bisphosphate carboxygenase/oxygenase activities per unit leaf area compared to plants grown under red fluorescent lamps. According to our results the elimination of blue light (L4) had no such influence on chlorophyll a/b ratio (Fig. 2). The stability of photosynthetic pigment contents in growth runs without blue (L4), both red (660nm) and far-red (L6) and only with 640 nm lighting (carotenoid absorption maximum) shows very strong participation of photosynthetic system antennal complex (Fig. 1). Antennas permit organisms to increase greatly the absorption cross section for light without having to build an entire reaction center and associated electron transfer system for each pigment, which would be very costly in terms of cellular resources.

To summarize, the following conclusions can be drawn. (i) The elimination of both red (660 nm) and far-red (735 nm) or only blue (455 nm) light appeared to suppress floral initiation, under such conditions carrots grew vegetative. In contrary, the elimination of solely far-red light resulted in faster flowering differention. (ii) The elimination of solely red (660 nm) or blue (455 nm) light resulted in a low synthesis rate of photosynthetic pigments and conditioned a high sucrose content in carrot root. Meanwhile, the elimination of solely far-red light resulted in the opposite effect. (iii) Dominating 640-nm light was found to considerably contribute to the excitation of the carotenoid antennal complex of the photosynthetic system.

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References

Bernier G, Prilleux C (2005) A physiological overview of the genetics of flowering time control. Plant Biotechnol J 3:3-16.

Casal JJ, and Sanchez RA (1994) Impaired stem-growth responses to blue-light radiance in light-grown transgenic tobacco seedlings overexpressing Avena phytochrome A. Physiol Plant 91:268-272.

Duchovskis P (2004) Flowering initiation of wintering plants. Horticulture and vegetable growing 23(2):3-11.

Gavrilenko VY (1975) Big practice of growing physiology. Moscow. (in Russian).

Gláucia M, Dias-Tagliacozzo and Válio FM Ivany (1994) Effect of vernalization on fowering of *Daucus carota.* R Bras Fisiol Veg 6:71-73.

Jang JC, Leon P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. The Plant Cell 9:5-19.

Kendrick RE, Kronenberg GHM (1994) Photomorphogenesis in plants, 2nd edn. The Netherlands: Kluwer Academic Publishers.

Kim HH, Wheeler RM, Sager JC (2006) Evaluation of lettuce grown using supplemental green light with red and blue light-emitting diodes in a controlled environment –a review of reseach at Kennedy Space Center. Acta Horticulturae 711:111-119.

King R (2006) Light-regulated plant growth and flowering; from photoreceptors to genes, hormones and signals. Acta Hort 711:227-233.

Kuperman FM, Rzhanova EI, Murashev VV, L'vova IN, Cedova EA, Akhundrova VA, Shcherbina IP (1982) Biology of cultivated plants development. 'Vischaia shkola', Moscow (in Russian).

Menard C, Dorais M, Hovi T, Gosselin A (2006) Development and physiological responses of tomato and cucumber to aditional blue light. Acta Hort 711:291-296.

Tamulaitis G, Duchovskis P, Bliznikas Z, Breive K, Ulinskaite R, Brazaityte A, Novickovas A, Zukauskas A (2005) High-power light-emitting diode based facility for plant cultivation. J Phys D 38:3182-3187.

Tarakanov IG (2006) Light control of growth and development in vegetable plants with various life strategies. Acta Hort 711:315-321.

Thomas B, Vince-Prue D (1997) Photoperiodism in Plants, 2nd ed. Academic Press, San Diego.

Yanagi T, Yachi T, Okuda N, Okamoto K (2006) Lihgt quality of continuous illuminating at night to induce floral initiation of Fragaria chiloensis L. CHI-24-1. Sci Hortic 109:309-314.

Yorio NC, Mackowiak CL, Wheeler RM, Sager JC (1995) Vegetative growth of potato under high-pressure sodium, high-pressure sodium SON-T Agro, and metal halide lamps. HortScience 30:374-6.