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EFFECT OF DIFFERENT SPECTRAL COMPOSITION LED LIGHTING ON SILVER BIRCH *IN VITRO* PLANTLET MORPHOLOGY

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Abstract
We tested effects of different spectral compositions on morphological parameters of ten silver birch (*Betula pendula* Roth.) clone *in vitro* cultures. Three different spectral compositions – red & blue (RB), red, green & blue (RGB) and red, green, blue, yellow & orange (RGBYO) LED luminaires and fluorescent light (FL) as control were used. Photon flux density of 110 µmol m^{-2} s^{-1} and 16/8h light/dark photoperiod was maintained for all luminaires. Stem and leaf morphological characteristics were affected by clone specific reaction to light treatment, while fresh and dry weight were affected only by clone. Partially opposite effect of red light amount in combination with yellow and orange light (RGBYO and FL luminaires) was observed on total plant length and main shoot elongation for two of ten birch clones. Length of third internode (four of ten clones) and leaf total and average area (three of ten clones) were stimulated by red light in combination with yellow and orange light. In general, our study shows that silver birch *in vitro* cultures exhibit clone specific, light-dependent morphological plasticity. We conclude that fluorescent lamps can be replaced with RGBYO, due to optimal spectral composition emitted by this luminaire and potential to adjust proportion of red light, thus providing individual approach for propagation of high-quality genotypes.
Key words: micro cuttings, clonal propagation, fluorescent lamps.

Introduction

The demand for wood and wood-based products is expected to increase globally in coming decades due to human population growth, economic growth, increasing prosperity and other reasons (Taylor, Lyons, & Jeffries, 2012). In European context Nordic and Baltic countries have the potential to increase its wood production, since they have favourable conditions for forest growth and already have extensive areas covered with forests. This is also important for mitigating climate change, since Nordic and Baltic countries have set a goal to reach carbon neutrality by 2050 (IEA, 2013; Nordiska Ministerrådet, 2009), with woody biomass supplying 50-97% of renewable energy (Rytter *et al*., 2016).

One of the strategies to increase wood supply is to enhance the productivity of planted forests by establishing superior genotypes of native species (Silva, Freer-Smith, & Madsen, 2018). Silver birch (*Betula pendula* Roth) has a high potential for cultivation in the Northern Europe and Baltic states, it is one of the most common tree species in the region (Brus *et al*., 2012; Forest Europe, 2011), it is highly productive and has a higher adaptation capacity to various environmental conditions compared to other native species (Dubois, Verkasalo, & Claessens, 2020). Birch timber is used for various products, like plywood, sawn-wood, wood-based panel, pulpwood, as well as for bioenergy production (Dubois, Verkasalo, & Claessens, 2020).

The use of LED luminaires is becoming more popular not only for indoor farming systems, but also as light source for plant propagation in controlled environment by tissue cultures (Fu *et al*., 2013; Morrow, 2008; Yang *et al*., 2018). Main advantages of LEDs over traditionally used fluorescent lamps include energy-efficiency, lower heat emission, compact size of LED modules, longer life-span compared to other lamp types, as well as the ability to adjust spectral composition and light intensity to specific requirements (Chen *et al*., 2020; Morrow, 2008). Knowledge on light-specific responses of species allows to adjust spectral composition of light so that spectral regions of emitted light correspond to absorption of photoreceptors, thus providing better growth by increasing photosynthetic, photomorphogenic, seed germination rates and accumulation of biomass (Bourget, 2008; Li-li *et al*., 2020; Massa *et al*., 2008; Morrow, 2008). Responses to different spectral regions of light are species specific – in general, it is observed that red (R) and blue (B) spectral regions affect morphogenesis and photosynthesis the most (Wang *et al*., 2015). However, plants have adapted to grow in broad spectral composition of sunlight, where development is determined by interaction of different spectral regions (Chen *et al*., 2020; Li *et al*., 2017; Liu *et al*., 2018). When cultivating *in vitro* cultures, where photosynthetic rate is limited, different spectral regions and their suitability for selected taxa can greatly affect photomorphogenesis (George & Davies 2008; Grout & Price, 1987; Kozai *et al*., 1991). Several researches on *in vitro* propagation of silver birch clones have been conducted; however, effects of lighting conditions on morphological characteristics of woody species have been rarely studied (Gailis *et al*., 2021; Meier-Dinkel, 1992). The aim of this study was to investigate the effect of different spectral composition LED treatment on *in vitro* microshoot morphology of silver birch (*Betula pendula* Roth).

Materials and Methods

This study was conducted in Laboratory of Plant Physiology of LSFRI 'Silava' during spring season of the year 2021. To evaluate the responses of silver birch clone *in vitro* cultures to different light spectrum, four spectral compositions of light were tested under controlled conditions. Tests were performed in a climatic chamber, where temperature was maintained at 25 °C and relative humidity at 30-40%. Four multi-store shelving systems with shelf size 120×100 cm and shelf height of 35 cm were placed in climatic chamber. Each shelf was equipped with luminaries placed 30 cm above the shelf surface. Non-transparent screens between shelf systems to prevent light contamination from other treatments were installed. The tested light treatments were combination of 1) red and blue LED light (RB), 2) red, green (G), and blue LED light (RGB), 3) red, green, blue, yellow (Y) and orange (O) LED light (RGBYO) (Table 1). Far-red (FR) diodes were incorporated in the RB and RGB luminaires to provide spectral region necessary according to phytochrome absorbance (Distefano *et al*., 2013; Liu *et al*., 2018). In RGBYO treatment, FR spectrum was provided by yellow diode, which emitted a broader spectrum light. Philips Master TL-D 36W (Koninklijke Philips N.V., Amsterdam, The Netherlands) warm white fluorescent lamps were used as the control. All LED luminaire treatments had red:blue (R:B) ratio of 3.2:1 and red: far-red (R:FR) ratio ranging from 28–36:1. The fluorescent light (FL) had R:B ratio of approximately 0.64:1 and R:FR 3:1 (Table 1), thus distinguishing it from others. The photon flux density of 110±10 µmol m^{-2} s^{-1} (range 400 to 750 nm) and 16/8h (light/dark) photoperiod was maintained for all light treatments. To ensure the uniformity and intensity of illumination, each shelf was divided into 10×10 cm sections and light spectrum and intensity was measured on each section using AvaSpec ULS2048 spectrometer (Avantes, Apeldoorn, The Netherlands).

Silver birch was represented by ten clones of open-pollinated progenies of plus-trees from Latvia (55°40′–58°05′N, 20°58′–28°14′E), which were obtained from a progeny trial in the central part of Latvia (56°44' N, 24°49' E) (Gailis *et al*., 2020). The studied clones were selected according to their field performance. In the clone collection, birch plantlets were cultivated on woody plant medium (WPM) (Lloyd & McCown, 1980), supplemented with WPM micronutrients, WPM vitamins, 0.1 mg L^{-1} zeatin, 20 g L^{-1} of sucrose, and 6 g L^{-1} agar (Sigma-Aldrich, St. Louis, MO). The pH of the medium was adjusted to 5.8 before autoclaving for 15 min (110 kPa, 121 °C).

Approximately 1.5 cm long silver birch plantlet apices were excised and planted in test tubes containing 5 mL of growth medium. Plant material was divided into three parts and randomly distributed on the different shelves for each light treatment. At least 30 plants in test tubes were used for each clone on each light treatment (at least 120 plants for each clone in total). To evaluate the effect of the light treatments on development of plantlets, microshoot cultures were cultivated under the experimental light treatments for 30 days.

Morphological parameters of regenerated cultures were determined: 1) main shoot elongation was determined for the longest and healthiest of shoots, independently of its origin; 2) total plantlet length was determined as a sum of lengths of main shoot, axillary and adventitious shoot(s); 3) length of third internode starting from apex was measured.

Leaf area parameters were measured by detaching and subsequently spreading newly formed leaves on a tablet and scanning them with Canon LC4800P

Table 1

Spectral composition % of total photon flux (from 400 to 750 nm) emitted by luminaires

Spectral regions and ratios	Red & Blue (RB)	Red & Green & Blue (RGB)	Red & Green & Blue & Yellow & Orange (RGBYO)	Fluorescent tubes (FL)
Blue 400 – 500 nm	23	18	17	17
Green 500 – 570 nm	0	22	17	25
Yellow 570 – 590 nm	0	0	3	7
Orange 590 – 625 nm	2	1	5	36
Red 625 – 700 nm	73	57	56	11
Far-red 700 – 750 nm	2	2	2	4
Red: Blue (R:B)	3.17	3.17	3.29	0.65
Red: Far-red (R:FR)	36.5	28.5	28	2.75
Blue: Green (B:G)	n/a	0.82	1.00	0.68

n/a – not applicable.

Table 2

Effect of different light spectral compositions on stem and leaf morphological properties

Morphological variables	Light treatment	Clone	Light by clone
Main shoot elongation, cm	ns	***	***
Total plantlet length, cm	ns	***	***
Length of third internode, mm	**	***	***
Leaf fresh weight, g	ns	***	ns
Stem fresh weight, g	ns	***	ns
Leaf dry weight, g	ns	***	ns
Leaf dry weight, g	ns	***	ns
Total leaf area, cm^2	***	***	***
Average leaf area, cm^2	***	***	***

ns – non significant ($p>0.05$); ** – significant at $p<0.01$; *** – significant at $p<0.001$.

scanner (Canon Inc., Tokyo, Japan). Obtained Tag Image File Format (.TIFF) images were analyzed with Winfolia Pro 2019 (Regent instruments Inc., Quebec, Canada) – average single leaf area was determined for each light treatment. Additionally, total leaf area was determined as the leaf area sum of one plant.

For determination of plant fresh and dry weight 1.5 cm long shoot segments with at least two leaves were planted in 300 mL glass jars containing 30 mL of the same medium as mentioned above. Eight shoots per jar were cultivated. For plant fresh and dry weight five plants from each jar were randomly chosen. Leaves were detached from stems and both were weighted separately. Dry weight was determined after drying plant material for 16 h at 60 °C. In total, five jars (replications) were used for each clone and each lighting treatment).

The effects of light treatments and clone on the morphology of plantlets were assessed using linear mixed-effect models or generalized linear mixed-effect models applying Poisson residual distribution according to data type analyzed. The statistical models in the general form were as follows:

$$Yijklm = \mu \pm LEDi \pm Cj \pm LEDi \times Cj \pm lk \pm \pm ikl \pm \varepsilon ijklm \quad (1)$$

where Yijklm is the response variable, μ is the overall mean; LEDi, Cj, and LEDi×Cj are the fixed effects of light treatment, clone, and the light treatment by clone interaction, respectively. The lk and ikl are the random effects, and εijklm is the random error.

The models were fit using the restricted maximum likelihood approach. The estimated marginal means for the levels of significant effects were compared using Tukey's HSD multiple comparison test. The data analysis was performed in R v. 4.1.2. (R Core Team, 2018) using packages 'lme4' (Bates *et al*., 2015) and 'emmeans' (Lenth, 2021).

Results and Discussion

Specific adaptation mechanisms to illumination are expressed through morphogenic characteristics by affecting size and form of a plant, thus determining *in vitro* propagation process in long term. Main objective of *in vitro* plant propagation is to obtain maximum number of high-quality explants in shortest period of time possible; therefore, conditions such as propagation medium content, spectral composition and intensity of light and length of propagation cycle should be adjusted according to requirements of selected species (Fu *et al*., 2013). Morphological characteristics of birch *in vitro* cultures were significantly affected by light treatments used in this study (Table 2). However, the response to light was clone-specific, indicating differences in exposure to light, highlighting the need for an individual approach. Significant effect of light and interaction of light and clone was observed on length of 3rd internode as well as average and total leaf area. Other stem morphological characteristics (main shoot elongation, total plant length) were significantly affected by interaction of light and clone, while stem and leaf fresh and dry weight was determined by clone (Table 2).

The development of the stem and leaves is affected by the processes of photomorphogenesis under the influence of different light signals (Cioć & Pawłowska, 2020; Wang *et al*., 2015), which show the reactions of clones to different light spectral composition in this experiment. Clone 54-229 formed significantly higher main shoot and total plantlet length, as well as larger total leaf area under FL compared to RGBYO treatment, while no significant differences with other LED treatments (Table 3). Results suggest that clone 54-229 specifically reacts to lowered red light amount in combination with yellow and orange light emitted by FL treatment (Table 1). On contrary, clone Med36 had significantly higher total plant length under RGBYO compared to FL, while main shoot length and length of third internode did not show significant differences

Table 3

Main shoot, total plantlet and length of third internode, average leaf area, total leaf area of silver birch *in vitro* plantlets under fluorescent (FL) light and different composition LED light (RGBYO, RB, RGB)

Clone	Light treatment	Main plantlet length, cm	Total plantlet length, cm	Length of third internode, mm	Average leaf area, cm^2	Total leaf area, cm^2
54-229	FL	1.65±0.28^{a}	3.59±0.50^{a}	3.90±0.79	0.42±0.07	2.25±0.40^{a}
	RB	1.16±0.27^{ab}	2.95±0.49^{ab}	2.94±0.78	0.27±0.07	1.27±0.38^{ab}
	RGB	1.42±0.27^{ab}	2.72±0.49^{ab}	3.66±0.79	0.41±0.07	2.01±0.39^{ab}
	RGBYO	1.01±0.27^{b}	2.50±0.48^{b}	2.76±0.87	0.39±0.08	1.50±0.39^{b}
54-286	FL	0.39±0.27	1.46±0.48	5.01±0.40^{a}	0.51±0.09	0.89±0.39
	RB	0.30±0.28	1.34±0.50	1.51±0.40^{b}	0.60±0.11	0.75±0.42
	RGB	0.22±0.30	1.33±0.53	1.72±0.50^{b}	0.54±0.10	0.71±0.40
	RGBYO	0.37±0.27	1.77±0.48	2.51±0.60^{b}	0.57±0.12	1.05±0.52
54-299	FL	0.51±0.26	1.69±0.46	5.33±2.29^{a}	0.73±0.10^{a}	1.26±0.39
	RB	0.54±0.25	2.22±0.46	1.38±0.39^{b}	0.68±0.12^{ab}	1.39±0.46
	RGB	0.33±0.27	1.58±0.48	2.00±1.16^{a}	0.79±0.09^{a}	1.60±0.42
	RGBYO	0.51±0.26	2.15±0.46	1.67±0.92^{b}	0.48±0.10^{b}	1.13±0.46
589-353	FL	1.82±0.27	3.07±0.48	2.60±0.72	0.31±0.07^{b}	1.70±0.38^{b}
	RB	1.94±0.28	3.53±0.5	3.00±0.75	0.29±0.08^{b}	2.06±0.41^{b}
	RGB	2.38±0.27	3.75±0.49	2.55±0.72	0.33±0.07^{b}	2.23±0.39^{b}
	RGBYO	2.09±0.27	3.83±0.48	3.38±0.69	0.54±0.07^{a}	3.67±0.39^{a}
589-805	FL	1.61±0.25	2.93±0.45	2.78±0.68	0.57±0.07^{a}	4.10±0.37^{a}
	RB	1.37±0.27	2.63±0.48	2.69±0.78	0.28±0.08^{b}	1.27±0.38^{b}
	RGB	1.54±0.26	2.84±0.47	2.40±0.73	0.55±0.07^{a}	3.88±0.40^{a}
	RGBYO	1.54±0.26	3.14±0.46	2.76±0.72	0.49±0.07^{a}	3.42±0.38^{a}
Ma29	FL	1.11±0.27	1.62±0.48	3.15±0.8^{b}	0.35±0.07	1.40±0.39
	RB	0.99±0.27	1.77±0.48	3.84±0.75^{ab}	0.24±0.07	1.01±0.37
	RGB	1.24±0.27	1.67±0.49	4.07±0.73^{ab}	0.35±0.07	1.56±0.38
	RGBYO	1.17±0.27	1.95±0.49	5.14±0.79^{a}	0.31±0.08	1.06±0.37
Med36	FL	1.89±0.27^{ab}	3.19±0.49^{b}	6.29±0.77^{a}	0.56±0.08	2.48±0.38
	RB	1.53±0.27^{b}	3.47±0.48^{ab}	3.6±0.72^{b}	0.47±0.07	2.23±0.37
	RGB	1.49±0.29^{b}	3.17±0.52^{b}	5.31±0.93^{a}	0.51±0.08	2.80±0.43
	RGBYO	2.33±0.28^{a}	4.29±0.50^{a}	6.78±0.76^{a}	0.51±0.07	2.53±0.40

^{a,b} – different letters in superscript represent significant differences ($p<0.05$) between light treatments for a concrete morphological variable of a concrete clone according to Tukey's HSD test results. Numbers after plus-minus signs represent 95% confidence intervals.

compared to FL. Clones 54-229 and Med36 showed response to red light in combination with yellow and orange, only this response was opposite. In general, both clones show response to combination of red light (therefore R:B and R:FR ratios as well) with yellow and orange. Similarly, other studies show that addition of yellow and orange light to red and blue light has positive effect on plant growth (Dougher & Bugbee, 2001; Li *et al*., 2017). No significant differences of total plantlet and main shoot length between light treatments for other clones were detected.

Effect of light and clone interaction on length of third internode was observed on four clones (Table 3): Clone 54-286 had significantly longer third internode under FL in comparison to LED treatments. Opposite effect was observed for clone Ma29, where length of third internode was significantly higher under RGBYO and RB as compared to FL. Both 54-286 and Ma29 indicate sensitivity to red light; however, responses of both clones are opposite. Clone 54-299 formed significantly longer third internode when grown under FL and RGB treatments as compared to RB and RGBYO, and could be explained by clone-specific reaction to green light amount, that is related to 'shade avoidance' response and is determined by B:G ratio of illumination (Smith, McAusland, &

Table 4

Total leaf area, average leaf area and length 3rd internode of silver birch *in vitro* plantlets under fluorescent (FL) light and different composition LED light (RGBYO, RB, RGB)

Light treatment	Total leaf area, cm^2	Average leaf area, cm^2	Length of third internode, mm
FL	2.2±0.12[a]	0.585±0.01[a]	4.15±0.7[a]
RB	1.69±0.13[b]	0.553±0.01[b]	2.71±0.64[b]
RGB	2.25±0.13[a]	0.589±0.01[a]	3.4±0.68[ab]
RGBYO	2.25±0.13[a]	0.606±0.01[a]	3.57±0.42[ab]

[a,b] – different letters in superscript represent significant differences ($p<0.05$) between light treatments for a concrete morphological variable of a concrete clone according to Tukey's HSD test results. Numbers after plus-minus signs represent 95% confidence intervals.

Murchie, 2017). Clone Med36 exhibited positive response to luminaires containing yellow and orange light – longer third internodes were observed under RGBYO and FL luminaires in comparison to RB. Results of Med36 agree with direct effect of light, where plants grown under RB luminaire formed significantly shorter third internode (Table 4).

Total leaf area and average leaf area was significantly smaller for plants grown under RB in comparison to other light treatments (Table 4). Clone 589-805 showed response similar to direct effect of light indicating that green, yellow and orange light affects leaf development (Table 1). Opposite clone-specific responses on total leaf area was observed on two clones – clone 589-353 had significantly higher total and average leaf area when grown under RGBYO, while clone 54-229 had the highest total leaf area when grown under FL (Table 3), indicating that, in addition to yellow and orange light effect, these clones have a specific response to red light as well. Clone 589-353 not only had significantly higher average leaf area, but total leaf area as well, when grown under RGBYO in comparison to other luminaires. This could be explained by clone-specific response to broad spectral composition emitted by RGBYO and related to light signals that plants are receiving when growing in full sunlight conditions. Similar results were observed in other studies where most effective leaf development occurred under broad spectral compositions (Baroli *et al*., 2008; Lake *et al*., 2001; Thomas, Woodwart, & Quick, 2003). Regarding average leaf area, clone 54-299 exhibited specific response to green light amount in luminaires – higher average leaf area was detected under FL and RGB treatments compared to other luminaires, indicating that leaf development of this clone was affected by B:G ratio, that signals about shade conditions (Jiang *et al*., 2011; Smith, McAusland, & Murchie, 2017). B:G ratio in RGBYO was 1, while in FL and RGB treatment it was 0.69 and 0.82, respectively.

In general, it can be concluded that the light spectrum containing blue, green, yellow, orange, red and far red spectra (RGBYO, FL) is important for birch growth *in vitro*. The specific reactions of birch clones (clone-light interaction effects) are related to the amount of red light, which means that an individual approach to determine the sensitivity of clones to red light is needed to optimize the propagation of each specific clone.

Conclusions

1. For silver birch clones in vitro, the main shoot length, total plantlet length, length of third internode, as well as average and total leaf area showed a clone-specific light-clone interaction response to the light spectrum.
2. The main shoot and total plant length of the birch clone responds specifically (two out of ten) to the amount of red light. Clones 54-229 and Med36 show the opposite reaction to the amount of red light, indicating the need for an individual approach to propagate these clones.
3. Silver birch clone in vitro cultures exhibited individual interaction effect of light and clone on length of third internode, average and total leaf area, where RB treatment underperformed. Clone-specific reactions, determined by interaction of light and clone, pointed out sensitivity to red light amount in combination with yellow and orange light.
4. LED luminaires with RGBYO spectral composition are an optimal solution to replace fluorescent lamps (FL) as primary illumination for silver birch propagation in vitro – spectral composition corresponds to silver birch sensitivity to red spectral region in combination with yellow and orange light. Knowledge of clone-specific responses to the spectral composition of light and the ability of LED luminaires to regulate the light spectrum can improve the propagation of birch clones.

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