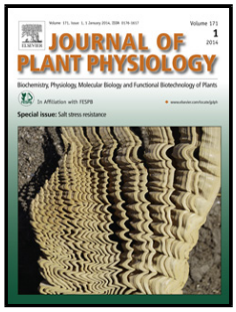

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Early growth of Scots pine seedlings is affected by seed origin and light quality

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Abstract

Plants have evolved a suite of photoreceptors to perceive information from the surrounding light conditions. The aim of this study was to examine photomorphogenic effects of light quality on the growth of Scots pine (Pinus sylvestris L.) seedlings representing southern (60°N) and northern (68°N) origins in Finland. We measured the growth characteristics and the expression of light-responsive genes from seedlings grown under two LED light spectra: (1) Retarder (blue and red wavelengths in ratio 0.7) inducing compact growth, and (2) Booster (moderate in blue, green and far-red wavelengths, and high intensity of red light) promoting shoot elongation.

The results show that root elongation, biomass, and branching were reduced under Retarder spectrum in the seedlings representing both origins, while inhibition in seed germination and shoot elongation was mainly detected in the seedlings of northern origin. The expression of ZTL and HY5 was related to Scots pine growth under both light spectra. Moreover, the expression of PHYN correlated with growth when exposed to Retarder, whereas CRY2 expression was associated with growth under Booster.

Our data indicates that blue light and the deficiency of far-red light limit the growth of Scots pine seedlings and that northern populations are more sensitive

to blue light than southern populations. Furthermore, the data analyses suggest that ZTL and HY5 broadly participate in the light-mediated growth regulation of Scots pine, whereas PHYN responses to direct sunlight and the role of CRY2 is in shade avoidance. Altogether, our study extends the knowledge of light quality and differential gene expression affecting the early growth of Scots pines representing different latitudinal origins.

Abbreviations

AK, adenosine kinase; B, blue light; CRY, cryptochrome; FR, far-red light; G, green light; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HY5, elongated hypocotyl 5; LED, light emitting diode; PHY, phytochrome; qPCR, quantitative real-time PCR; R, red light; sPLS, sparse partial least squares; TUBA, alfa-tubulin; UBQ, ubiquitin; ZTL, zeitlupe

Keywords: Gene expression; Photomorphogenesis; Photoreceptor: Pinus sylvestris; Seedling morphology; Spectral composition

1. Introduction

Light quality (i.e. spectral composition) is an important factor that regulates plant growth and development during several processes including germination, photomorphogenesis, and floral induction (Smith, 1982; de Wit et al., 2016). Far-red light (FR, 700–800 nm) dominates in the forest understorey (Smith,

1982) since red (R, 600–700 nm) and blue lights (B, 400–500 nm) are absorbed efficiently by the chlorophyll of leaf canopy. Plants adapt to varying light quality by expressing different phenotypes. For instance, plants growing beneath dense forest canopy acclimate to a low R:FR by producing an array of developmental responses including enhanced shoot elongation (Casal, 2012). B in turn has been shown to reduce shoot elongation (Taulavuori et al., 2005; Sarala et al., 2011; Hernandez and Kubota, 2016), and hence plants grown under high B levels and high R:FR remain short and have an increased leaf size for photosynthesis (Franklin et al., 2016).

Investigations on the effects of light quality have focused less on belowground growth although it is known that roots have photoreceptors as well (Henke et al., 2015; Mo et al., 2015). Light triggers many photomorphogenic responses in roots, including primary root growth, lateral root emergence, and tropic responses (reviewed by Lee et al., 2017). According to optimal partitioning theories, plants adapt to environmental changes by partitioning biomass among organs from below- and aboveground resources to optimize growth (e.g. Bloom et al., 1985; McConnaughay and Coleman, 1999). For example, in limited light conditions, plants tend to allocate more resources to shoots than roots, which is analogous with shade avoiding responses. However, the photomorphogenic responses do not necessarily follow a typical trade-offs pattern related in

resource allocation between roots and shoots. For instance, B may inhibit shoot elongation, but resources driven from elongation may be retained in other aboveground parts like leaf biomass (Huche-Thelier et al., 2016).

Plants have several types of light-perceiving photoreceptors, which gather information from the surrounding environment. In natural light conditions, plants are exposed to various wavelengths simultaneously, and thus crosstalk is initiated between multiple photoreceptors. Phytochromes are among the best-characterized photoreceptors. They are able to capture R and FR, and they regulate many developmental processes such as seed germination and hypocotyl development (reviewed by Casal, 2012). Other well-known receptors are cryptochromes and zeitlupe, which perceive B and ultraviolet radiation. Cryptochromes control for example seedling de-etiolation, elongation growth and entrainment of the circadian clock (reviewed by Ahmad, 2016), while zeitlupe controls circadian clock by regulating the stability of other clock proteins (Kim et al., 2007). Light-responsive genes are well characterized in Arabidopsis, and they include five phytochromes (PHYA to PHYE) and two cryptochromes (CRY1 and CRY2). In gymnosperms, PHYN is the orthologue of angiosperm PHYA and PHYO is the orthologue of PHYC (Mathews et al., 2010). The expression of light-responsive genes is regulated by a set of transcription factors. A bZIP transcription factor ELONGATED HYPOCOTYL

5 (HY5) promotes photomorphogenesis in various light conditions (Osterlund et al., 2000; Li et al., 2010) by binding directly to the promoter of several light-inducible genes (Hiltbrunner et al., 2006). For example, enhanced HY5 expression has been implicated in shade-avoidance responses (Ciolfi et al., 2013).

Light emitting diode (LED) lighting has opened new insights in optimizing plant growth (Dueck et al., 2016, and references therein). Plant responses to different light spectra can differ among different species (Taulavuori et al., 2016, 2018), but so far LED light investigations have been mostly concentrating on horticulturally important herbs grown under commercial production conditions. Thus, more studies are needed to clarify the specific responses of tree species.

Scots pine (Pinus sylvestris L.) is an important timber tree and the most widespread pine species in the world (Gardner, 2013). Survival of boreal forest trees depends on the precise synchronization of annual growth and dormancy cycles with the seasonal climatic changes: In southern populations, night length has been suggested to be the most important factor regulating the length of growth period, while in northern populations, the light quality seems to be a more significant factor (Olsen, 2010). In northern latitudes close to Arctic Circle, long growing season is characterized by midnight sun during which sun shines in very low position. Consequently, the FR content increases northwards,

while the diffuse B content also increases (Taulavuori et al., 2010). Previous studies have documented an increase in the requirement of FR with increasing latitude of origin for maintaining the growth in Norway spruce (Picea abies (L.) H. Karst.) and Scots pine (Clapham et al., 1998, 2002; Mølmann et al., 2006; Ranade and García-Gil, 2013; Razzak et al., 2017). The light-mediated responses can also vary between different conifer species since, for example, Scots pine is shade-intolerant while Norway spruce is shade-tolerant.

The aim of our study was to examine photomorphogenic effects of light quality on shoot and root growth of Scots pine, and to relate growth responses to the expression of certain light-responsive genes. We selected two commercially available light spectra, from which the other should retard and the other should boost shoot elongation. Scots pines of southern and northern origins were studied since their local light environments significantly differ from each other (e.g. Taulavuori et al., 2010), and moreover, it is proposed that northern populations may be more sensitive to B than southern populations (Sarala et al., 2011).

2. Materials and methods

2.1. Plant material

Scots pine seeds were obtained from the state-owned forestry company Siemen Forelia Ltd for the experiment. We used two pooled seed samples collected from natural populations from which one represented southern origin (Miehikkälä; 60°N, 27°E) and the other northern origin (Sodankylä; 68°N, 25°E). Pre-tested germination percentage of both seed origins was higher than 90%. The experiment was established in two identical computer-controlled (Fidelix Ltd, Vantaa, Finland) climate rooms (Arctest Ltd, Espoo, Finland) in which temperature was set to 16°C and relative humidity to 61%. The temperature of 16°C was used since it is the average temperature of the two origins in July over the period 1981-2010 (data from Finnish Meteorological institute), i.e. confirming optimal photosynthesis vs. respiration, growth and survival. Seed material i.e. both seed origins were evenly distributed in both climate rooms. One seed per pot (8 x 8 x 6 cm in size) were sown in Kekkilä Professional Substrate FPM 420 (NPK 17-4-16) in the depth of 1 cm and filled with sand.

2.2. Experimental setup

We employed two light spectra established by commercially available LED lights (Valoya Ltd, Helsinki, Finland) to establish two light treatments: (1) “Retarder” using AP9 spectrum, and (2) “Booster” using AP67 spectrum (see spectra in Supplemental Fig. S1). According to Kotiranta et al. (2015), the former induces compact growth and the latter induces shoot elongation. The

Retarder trait of AP9 is an obvious consequence of lacking FR, and consequent ability to stimulate elongation (e.g. Casal, 2012). The Booster trait of AP67, in turn, is a combination of three facts. First, relative proportion of B is much lower than in Retarder in agreement to improved elongation under removal of B (e.g. Sarala et al., 2010). Second, Booster consists of moderate proportion of green light, which is shown to increase growth (Johkan et al., 2012). Finally, Booster produces also moderate proportion of FR to improve the elongation. Therefore, we have two contrasting light treatments, which affect via multiple mechanisms, and provide insight into light adaptations to different light environments (See Supplemental Table S1 for proportions of B, G, R and FR). As both light treatments contained seeds from southern and northern sites, we had four final experimental units: southern and northern origins exposed to both Retarder and Booster lights. Light intensity of above seedlings was adjusted to 300 µmol $m^{-2}s^{-1}$ by the installation of a lamp system at an appropriate height (approximately 40 cm) above the growing table. Each experimental unit included 96 pots. Day length was set to 22 h by timers. Water was supplied at a few days' intervals and no additional fertilizers were given. The experiment was started on 1 July and continued until 27 August.

2.3. Growth measurements

Germination rate and shoot elongation were measured in six sessions during the experiment on the following days after sowing: (1)19, (2) 22, (3) 28, (4) 38, (5) 54 and (6) 57. In the last session, a set of eight plants from each experimental unit were randomly chosen, and shoot length, root length, root branching and root biomass (dry weight) were measured. Shoot and root length were measured in 1 mm accuracy. Tap root and the number of lateral roots were counted. Soil was carefully washed from roots, which were then dried for two days at 80°C followed by one day in desiccator before weighing.

2.4. RNA extraction and cDNA preparation

Total RNA was isolated from needles, which were gathered from the seedlings at the end of the experiment (27 August). Five samples (n=5) from each experimental unit were used for gene expression analyses. The procedure of Chang et al. (1993) was used for RNA isolation with some modifications. In brief, 100 mg of the needles were homogenized with TissueLyser (Qiagen, Hilden, Germany). Thereafter, 750 µl of preheated extraction buffer was added to each sample and the tubes were incubated for 10 min in 65°C. An equal volume of chloroform:IAA (24:1) was added to the supernatant and the tubes were centrifuged at 12 000 g at 4°C for 20 min. The chloroform:IAA (24:1) extraction was repeated. One-fourth volume of 10 M LiCl was added to the supernatant and RNA was precipitated overnight at 4°C. Next day, the tubes

were centrifuged at 10 000 g for 20 min at 4°C. The supernatant was removed, and the pellets were dissolved in 200 µl of RNase-free water. The samples were extracted with an equal volume of chloroform:IAA (24:1) and centrifuged at 12 000 g for 20 min at 4°C. Two volumes of absolute ethanol was added to the supernatant and the samples were precipitated at -70°C for 40 min. The tubes were centrifuged at 12 000 g for 20 min at 4°C and the pellet was dried in Savant Speed Vac (Thermo Fisher Scientific, Waltham, MA, USA) for 3 min. Finally, the pellet was resuspended in 20 µl of RNase-free water, and RNA was treated with DNase I (Fermentas, Waltham, MA, USA) to remove possible genomic DNA. RNA concentrations were analysed with Nanodrop ND-1000 (Thermo Fisher Scientific) and the integrity of total RNA was assessed on gel. The cDNA was synthesized from the total RNA using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) with anchored oligo(dT) primers according to the manufacturer's instructions.

2.5. Quantitative real-time PCR

Quantitative real-time PCR (qPCR) was used for the relative quantification of gene expression according to MIQE guidelines (Bustin et al., 2009). Primers were designed with Primer3 software (Koressaar and Remm, 2007; Untergasser et al., 2012) and ordered from TAG Copenhagen A/S (Frederiksberg, Denmark). The qPCR amplifications were done with LightCycler® 480 instrument (Roche,

Basel, Switzerland). The 20 µL reaction mixture consisted of 10 µL SYBR Green I Master mix (Roche), 0.5 µM gene-specific primers (see Supplemental Table S2 for reference genes and Supplemental Table S3 for light-responsive genes) and 2 µL cDNA (1/10 dilution). Primers showing at least 90% efficiencies were accepted. The qPCR amplification was initiated by incubation at 95°C for 10 min, followed by 45 cycles of 10 s at 95°C, 10 s at 60°C, and 20 s at 72°C. The melting curves of qPCR runs showed a single product. Two technical replicates were used in data analysis. GeNorm method (Vandesompele et al., 2002) in qbase+ software (Biogazelle, Zwijnaarde, Belgium) was applied to identify the set of reference genes that were used for normalization of the gene expression data: adenosine kinase (AK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alfa-tubulin (TUBA) and ubiquitin (UBQ). The relative expression of the target genes was calculated with the $2^{-\Delta\Delta Ct}$–method.

2.6. Statistical analyses

The statistical analyses for shoot elongation, root elongation, root biomass and root branching were performed on the results from the last measurement day (57 days after sowing). Two-way ANOVA was conducted to examine the effects of light quality and seed origin on the growth of Scots pine seedlings. Shapiro-Wilk's test was used to assess normality of each experimental unit and Levene's test to assess homogeneity of variances. In case of violations of the assumptions

of normality and homogeneity of variance, square root transformation was used for statistical assays. The transformation was applied to the results from CRY2 and PHYO expression surveys, and to the measurements of root length, biomass and branching. The HY5 expression results violated the assumption of homogeneity of variances, but as the group sample sizes were equal, the results were normally distributed, and the ratio of largest to smallest group variances was less than three, we were able to apply two-way ANOVA. Two-way ANOVA is quite robust to heterogeneity of variance in these circumstances (Jaccard, 1998). The simple main effects and main effects were tested if reasonable. Moreover, we conducted chi-square test of homogeneity followed by z-test of two proportions with a Bonferroni correction to study whether the germination rates differed between the experimental units (Supplemental Fig. S2A). Differences in median elongation rates between the units were analysed with Kruskal-Wallis H test followed by Dunn's post hoc procedure with a Bonferroni correction (Supplemental Fig. S2B). The statistical analyses were run with SPSS v24.0 (SPSS, Chicago, IL, USA) at a significance level of 0.05.

Sparse partial least squares (sPLS) regression has been proven as an effective method to assess relations between analyzed variables, being capable to predict the response of a set of variables based on a matrix of predictors (Lê Cao et al., 2008). We used sPLS-based method to integrate the gene expression results with

the mean values of the growth measurement data. MixOmics R package (Rohart et al., 2017) was used for building regression models. These models were tuned based on total Q2 (threshold for component consideration was established on 0.0975) and variables were selected if their individual Q2 was greater than 0.35. The cutoff value for establishing variable relations within networks was set to 0.14. The generated correlation networks were further edited in Cytoscape v.3.3.0 (Cline et al., 2007) to add legends and color edges based on the loadings each variable had over the first two components.

3. Results

3.1. Light quality and Scots pine origin affected the growth of seedlings

Germination percentage and shoot elongation were assessed six times during the experiment (Supplemental Fig. S2). Germination rate reached 70–80% in each experimental unit except in northern origin under Retarder, where it remained below 50% (Supplemental Fig. S2A). Shoots of southern origin elongated faster than shoots of northern origin, and shoots of both origins elongated faster under Booster than Retarder (Supplemental Fig. S2B).

At the end of the experiment, the randomly selected eight plants (eight-week old) from each experimental unit were used for further examination of shoot

length, root length, root biomass, and root branching (Fig. 1). Two-way ANOVA results of the growth measurement data are summarised in Table 1. There was a significant interaction effect between the seed origin and light quality ($P < 0.05$) on shoot length (Fig. 1A). The analysis of simple main effects revealed that shoots of northern origin were significantly longer ($P < 0.005$) under Booster than Retarder. Shoots of southern origin were also significantly longer ($P < 0.001$) than shoots of northern origin while exposed to Retarder. Moreover, the main effect of seed origin on shoot length was tested, which showed that shoots of southern origin were significantly longer ($P < 0.05$) than shoots of northern origin.

The interaction effect between the seed origin and light quality on root length (Fig. 1B) and root biomass (Fig. 1C) was not significant. Instead, light quality had a significant main effect on root length and root biomass. The seedlings grown under Booster were associated with significantly longer roots ($P < 0.005$) and larger biomass ($P < 0.001$) than the ones grown under Retarder. Moreover, seed origin had a significant main effect on root biomass since southern origin was associated with a significantly larger mean root biomass ($P < 0.005$) than northern origin.

There was a significant interaction effect between the seed origin and light quality ($P < 0.05$) on root branching (Fig. 1D). The analysis of simple main effects revealed that the roots of northern origin were significantly less branched ($P < 0.001$) under Retarder than Booster. Moreover, testing the main effect of light quality on root branching revealed that roots were significantly less branched ($P < 0.001$) under Retarder than Booster.

3.2. Light-responsive genes showed variation in expression levels

The expression analyses were performed to eight-week old needles, which were gathered at the end of the experiment. The light-responsive genes had a similar expression pattern across the four experimental units (Fig. 2); the relative expression levels of the genes were at the highest rate under Retarder in the northern origin. Two-way ANOVA results of gene expression data are presented in Table 2. A significant interaction effect between the light quality and seed origin ($P < 0.05$) was observed on the expression of transcription factor HY5. The analysis of simple main effects revealed that HY5 was expressed more intensively ($P < 0.001$) under Retarder than Booster in the seedlings of northern origin. HY5 was also upregulated ($P < 0.001$) in northern origin relative to southern origin under Retarder.

The expression of other studied light-responsive genes did not show a significant interaction effect between the seed origin and light quality. Consequently, the analysis of main effect was performed to the results. The light quality affected CRY2 and PHYN expression, as both genes were significantly more expressed ($P < 0.05$) under Retarder than Booster. Seed origin had also a significant effect on the expression of ZTL, since ZTL was expressed in higher rate ($P < 0.05$) in the seedlings representing northern than southern origin. Main effects were also assessed with PHYO, but the results were not significant.

3.3. Differential gene expression correlated with growth variables

The sPLS model, which integrates gene expression and growth measurement data, clustered the Scots pines of southern and northern origins into two clearly distinguishable corresponding groups while exposed to both Retarder (Fig. 3A) and Booster (Fig. 3B). The correlation circle plot of sPLS model grouped all growth variables together to the opposite sector to the genes in Retarder (Supplemental Fig. S3A) indicating that growth variables had similar correlation pattern with the genes. In Booster, correlation circle plot (Supplemental Fig. S3B) showed that growth variables were also grouped together except for germination, which appeared in another sector. Interaction networks (Fig. 3C and D) revealed that ZTL and HY5 correlated positively with all growth

variables when exposed to Retarder or Booster. Moreover, PHYN correlated negatively with growth variables under Retarder (Fig. 3C), whereas CRY2 showed positive correlation with growth variables under Booster (Fig. 3D). In Retarder, correlations between genes and growth factors were relatively strong ($r < -0.59$) while, in Booster, correlations were subtle ($r < -0.14$, $r > 0.14$). The observed correlations in Booster were considered reliable since another interaction network, in which the light quality and seed origin were used as variables in addition to the growth measurements, showed similar kind of interaction (Supplemental Fig. S4).

4. Discussion

Plant responses to light quality is species-specific (Taulavuori et al., 2016, 2018), and more studies are needed to clarify the specific morphological responses and the underlying signalling pathways in forest trees. We examined the photomorphogenic effects of two LED light spectra on Scots pine growth and studied the expression of light-responsive genes in the end of the experiment.

4.1. Blue light limits the germination of seeds representing northern origin

It has been shown in several plant species including conifers that R promotes (Nyman, 1963; Kvaalen and Appelgren, 1999) and FR inhibits seed germination (Durzan et al., 1979). Tanno (1983) observed that continuous FR and B inhibit the germination of Laportea bulbifera, but only FR inhibits germination after the removal of seed coats. Moreover, the germination of Brachypodium-species has been shown to be sensitive to both B and FR but, after ripening, they are only sensitive to B (Barrero et al., 2012). Fernbach and Mohr (1992) also showed that hypocotyl growth is sensitive to B. Indeed, light penetrates rarely below 4–5 mm in soil (Tester and Morris, 1987), and the short wavelengths do not penetrate soil as effectively as the longer ones (Ciani et al., 2005; Galen et al., 2007). Therefore, it is likely that the inhibiting effect of B on germination occurs at the late germination stage when the hypocotyl hook is reaching soil surface. In our study on Scots pine, Retarder spectrum inhibited germination only in the seeds of northern origin (Supplemental Fig. S2A), which suggests that the northern Scots pine populations are more sensitive to B.

4.2. The lack of FR inhibits shoot elongation in northern origin

Compact shoots and well-elongated shoots were produced as expected for Retarder and Booster spectra, respectively (Fig. 1A and Supplemental Fig. 2B). The effect of FR on the elongation of seedlings has previously been reported, for example, in dicot tomato (Solanum lycopersicum, Kotiranta et al., 2015) but

also in coniferous species including Scots pine (Ranade and García-Gil, 2013, 2016) and loblolly pine (Pinus taeda, Warrington et al., 1989). Furthermore, our previous studies have shown that the removal of B from light spectrum improves shoot elongation of Scots pine (Taulavuori et al., 2005; Sarala et al., 2007, 2011). According to Sarala et al. (2011), the removal of B was more beneficial for northern than southern population, indicating that northern populations are more sensitive to B. Consistently, our key finding here shows that shoot elongation decreased under supplemental B, and especially in the seedlings of northern origin.

The origin of seeds had a significant effect on shoot elongation. Recently, similar findings have been reported in the seedlings of deciduous trees, indicating that shoot elongation potential is inherently low in the northernmost tree populations (Reich and Oleksyn, 2008; Taulavuori et al., 2017). Accordingly, the hypocotyl growth of Arabidopsis has shown to decrease clinally towards the northern origin of population, indicating the role of natural selection (Stenøien et al., 2002). Thus, genetic material becomes narrower at the edges of species' distribution area (Mimura and Aitken, 2007; Savolainen et al., 2007).

Retarder significantly reduced shoot elongation in the seedlings of northern origin, which may also be related to the lack of FR. According to Smith (1982) the usual daylight R:FR is around 1.15, and the ratios in the present investigation were 58.0 for Retarder and 3.12 for Booster. Given that it is the low R:FR that drives for shoot elongation under shade avoidance reaction (e.g. Casal et al., 2012), it is understandable that the lack of FR reduces elongation growth. Indeed, previous reports have shown a clinal increase in requirement of FR in northern latitudes for elongation growth of Scots pine (Mølmann et al., 2006; Ranade and García-Gil, 2013). These reports are in accordance with the result of the present study: The lack of FR reduced shoot elongation only in northern origin, indicating a significant interaction between light quality and seed origin on shoot length (Table 1).

4.3. Root growth is inhibited in northern origin

Scots pine roots of northern origin had less biomass compared to southern origin under both light qualities (Fig. 1C). This is in accordance with Oleksyn et al. (1992), who indicated that root biomass of Scots pine was lower in populations at 60°N than 50°N. Concerning the optimal allocation theory (Bloom, 1985), resource allocation to roots might be expected to increase under FR-deficient light because of the reduced shoot elongation. Moreover, improved

root growth capacity was recently reported when Scots pine and Norway spruce were exposed to FR-deficient light (Riikonen et al., 2016). However, in the present study, FR-deficient light significantly reduced root length, biomass, and branching (Fig. 1B−D) in the seedlings of both origins. The results are also contradictory to previous findings on Scots pine, since additional FR (i.e. lower R:FR) has been shown to increase shoot elongation and biomass, while reducing allocation into roots and decreasing the number of short root tips (de la Rosa et al., 1998). In the present study, resource allocation did not exist between shoots and roots, and hence it is possible that resources were allocated to other aboveground organs such as needles (Huche-Thelier et al., 2016). Moreover, in the present study, we used different wavelength combinations compared to previous studies in Scots pine. The Booster consisted of 16% green light (G, 500–600 nm), while Retarder had only 1% of G. According to Johkan et al. (2012) G may affect photomorphogenesis by decreasing shoot to root ratio, but plant responses to G may also be species-specific (Hernandez and Kubota, 2016).

4.4. The expression of light-responsive genes is associated with the growth of seedlings

We observed similar expression patterns of light-responsive genes across the four experimental units (Fig. 2) and found the gene expression to be associated

with growth variables (Fig. 3A and B). A significant interaction effect between the light quality and seed origin was found in the expression of transcription factor HY5 (Table 2). Moreover, HY5 expression was linked to all growth variables under both light qualities (Fig. 3C and D). Recently, HY5 was shown to be translocated from shoot to root upon light activation and to coordinate the growth of these organs (Chen et al., 2016). Shoot-sensed R:FR influences the expression of HY5 i.e., HY5 may regulate root growth and development together with auxin and gibberellin during shade-avoidance responses (van Gelderen et al., 2018). Moreover, B has been shown to enhance the expression of HY5 (Sellaro et al., 2009). Our results suggest that supplemental B and lack of FR increased the expression of HY5 in northern origin. ZTL expression was also found to correlate with all growth variables under both light qualities (Fig. 3C and D), and it was expressed differently between northern and southern origins (Table 2). As a B photoreceptor, ZTL expression can be expected to differ between northern and southern origins, since northern populations of Scots pine might be more sensitive to B (Sarala et al., 2011). Altogether, our results suggest that HY5 and ZTL have important roles in the regulation of shoot and root growth in Scots pine while exposed to different light conditions.

The expression of phytochrome gene PHYN was affected by the light quality (Table 2), while PHYO expression did not differ between the experimental

units. Previously, the function of PHYN has been suggested to differ from PHYO under the distinct light conditions of Scots pine populations (Alakärppä et al., 2018). We found PHYN expression to associate with growth variables while exposed to high B and R:FR of Retarder (Fig. 3C), which is plausible since phytochromes detect R:FR. Moreover, the light quality affected the expression of CRY2 (Table 2), which has also been connected with Scots pine adaptation in our previous study (Alakärppä et al., 2018). In this study, the expression of B receptor CRY2 was related to growth variables under Booster (Fig. 3D), which is interesting since Booster included high intensity of R.

It has been proposed that the detection of B by cryptochromes or phytochromes has a central role in the growth regulation of northern Norway spruce populations (Mølmann et al., 2006). Cryptochromes have also been shown to promote growth in a shaded environment with diminished B in Arabidopsis (Pedmale et al., 2016). Our results give evidence of cryptochromes being able to function under low R:FR light conditions also in conifers. Moreover, the synergism between R and B has been shown to require the interaction of cryptochromes and phytochromes in the regulation of various photomorphogenic and photoperiodic responses (Ahmad et al., 1998; Casal and Mazzella, 1998; Más et al., 2000; Mølmann et al., 2006; Sellaro et al., 2009; Wang et al., 2018). Thus, every photoreceptor has a complex light signalling

network, which enables multiple responses to changing light quality (Kong and Okajima, 2016). Overall, our results suggest that several photoreceptors participate in light-mediated growth responses in Scots pine, and that the growth responses vary between populations across latitude of origin.

4.5. Conclusions

To conclude, the two light spectra and two seed origins had a significant effect on Scots pine growth. Shoots representing northern origin were significantly shorter when exposed to Retarder than Booster spectrum, which indicates that the northern Scots pine populations may be sensitive to B. Root elongation, biomass, and branching were reduced when the seedlings, representing both northern and southern origins, were exposed to low R:FR. We found consistent expression patterns with the studied light-responsive genes in the seedlings. Moreover, the expression of ZTL and transcription factor HY5 correlated with the growth data under both light qualities, which suggest that these genes have a pivotal role in the light-mediated growth responses of Scots pine. CRY2 and PHYN expression levels were connected to growth data under Booster and Retarder, respectively, while PHYO expression did not show any correlation with growth variables. The results indicate that CRY2 participates in shade avoidance responses and PHYN in responses to direct sunlight. Our results

allow a better understanding on how different spectral compositions and expression of light-responsive genes affect the early growth of Scots pine.

Author contributions

H.H. and K.T. designed the experiment. T.T. measured the growth characteristics. E.A., K.K., N.N. and S.J.-L. conducted the qPCR study. E.A., E.T., K.T. and L.V analysed the data. The paper was written by E.A., E.T., H.H. and K.T. with input from all authors.

Conflict of interest

None declared.

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Figure legends

Figure 1. The growth measurements of Scots pine seedlings of southern and northern origins exposed to Retarder and Booster LED lights: shoot length (A), root length (B), root biomass (dry weight) (C), and number of root branches (D). Vertical bars represent standard error of means (SE).

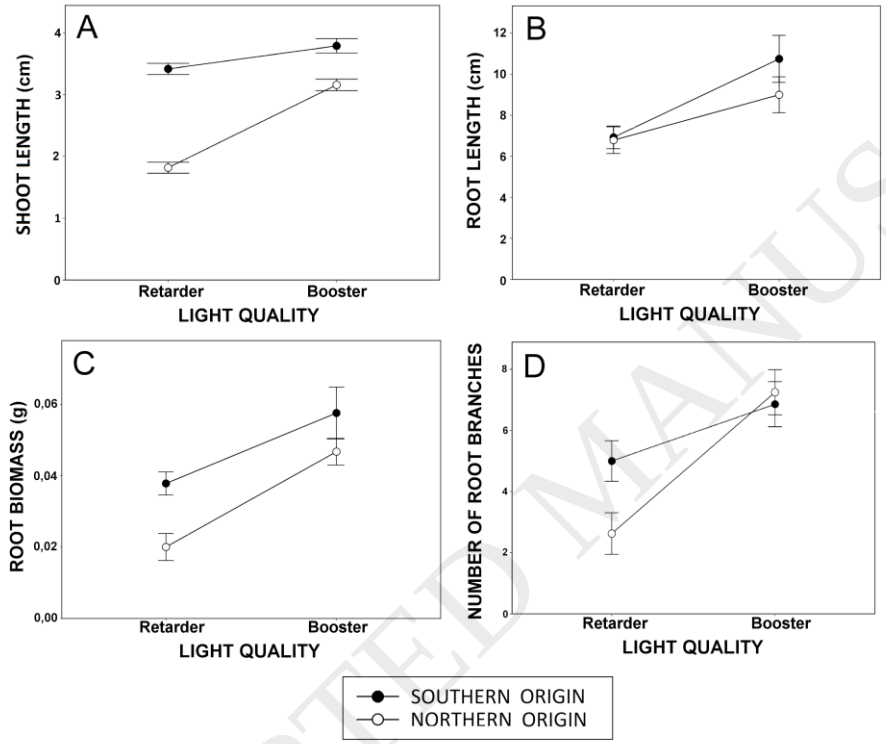

Figure 2. The expression levels of light-responsive genes in the eight-week old Scots pine seedlings representing southern and northern origins exposed to Retarder and Booster LED lights. Error bars indicate standard error (SE).

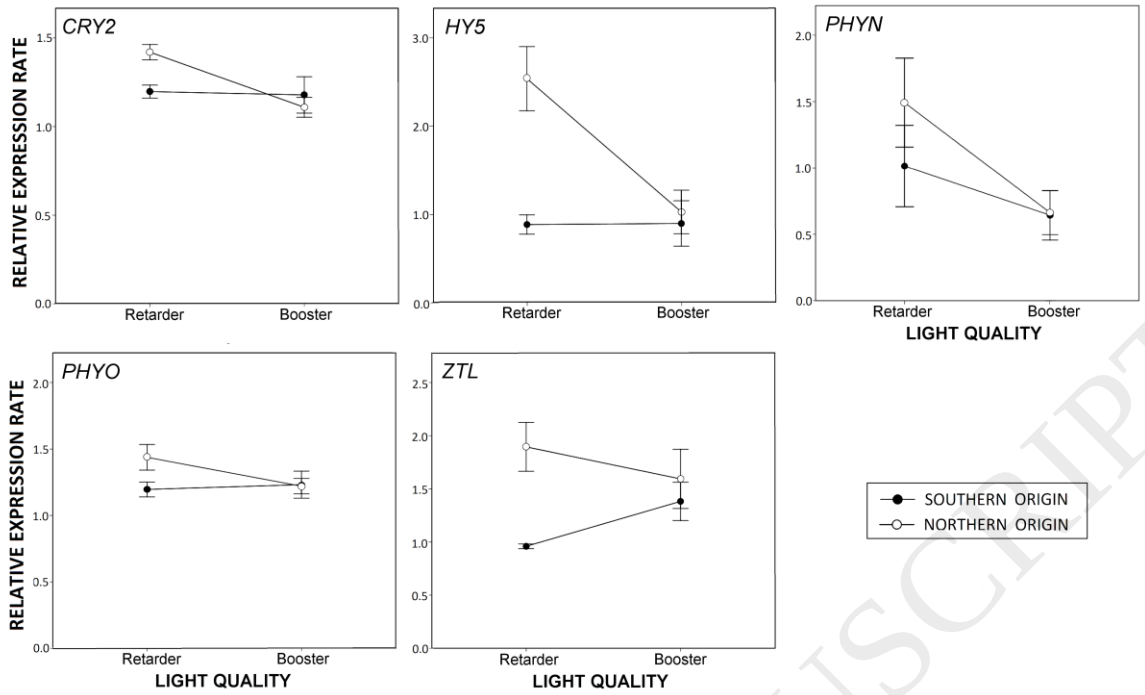

Figure 3. The sPLS-based classification of samples representing southern and northern seed origins of Scots pine considering the changes in gene expression and growth variables under Retarder (A) and Booster (B) LED lights. Components 1 and 2 corresponding to the XY space are represented. The ellipses represent a confidence interval of 0.9. The sPLS-network was constructed using relative gene expression data and growth variables in Retarder (C) and Booster (D). Color key represents the value of the correlation between gene expression and growth variables.

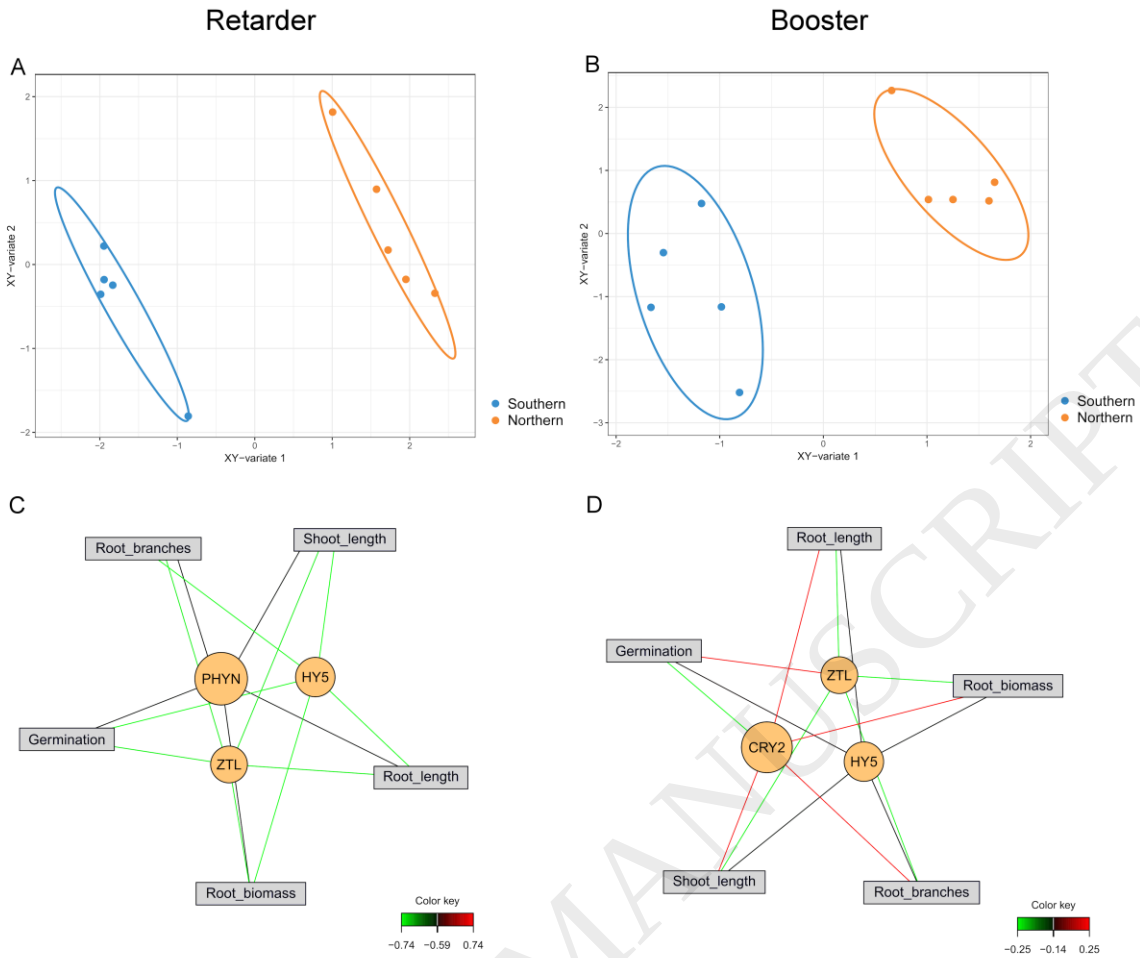

Table 1. Two-way ANOVA statistics for shoot length, root length, root biomass and root branching of Scots pines of southern and northern origins exposed to Retarder and Booster LED lights. O x L = interaction effect between light quality and seed origin, NS = not significant.

Growth measurement		**df**	**F**	**P <**
Shoot length	Origin	1	10.96	0.005
	Light	1	6.89	0.05
	O x L	1	4.94	0.05
Root length	Origin	1	1.07	NS
	Light	1	12.14	0.005
	O x L	1	0.68	NS
Root biomass	Origin	1	11.37	0.005
	Light	1	27.70	0.001
	O x L	1	2.15	NS
Root branching	Origin	1	3.45	NS
	Light	1	22.70	0.001
	O x L	1	5.06	0.05

Table 2. Two-way ANOVA statistics of the expression levels of light-responsive genes in Scots pines of southern and northern origins exposed to Retarder and Booster LED lights. O x L = interaction effect between light quality and seed origin, NS = not significant.

Gene		**df**	**F**	**P <**
CRY2	Origin	1	0.45	NS
	Light	1	7.61	0.05
	O x L	1	2.76	NS
HY5	Origin	1	10.50	0.01
	Light	1	7.41	0.05
	O x L	1	7.65	0.05
PHYN	Origin	1	0.92	NS
	Light	1	5.36	0.05
	O x L	1	0.78	NS
PHYO	Origin	1	0.67	NS
	Light	1	2.20	NS
	O x L	1	0.90	NS
ZTL	Origin	1	7.12	0.05
	Light	1	0.08	NS

	O x L	1	2.84	NS