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Impact of additional blue light in the production of small plants of *Abies laciocarpa* and *Picea abies* propagated by seeds and stem cuttings

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Norge Miljø –og Biovitenskapelige Universitet

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## Abbreviations

*A.laciocarpa* = *Abies laciocarpa*

DM = Dry matter

DW = Dry weight

FW = Fresh weight

HPS = High pressure sodium

HPS+BL = High pressure sodium + blue light

LEDs = Light emitting diodes

*P. abies* = *Picea abies*

PAR =Photosynthetic active radiation

RH = Relative humidity

VPD = Vapour pressure deficit

B = Blue light

R = Red light

G = Green light

FR = Far red light

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## Abstract

The goal of this study was to examine how additional blue light affected growth and development of *Picea abies* and *Abies laciocarpa* seedlings and stem cuttings. The experiment was conducted at the Center for climate regulated plant research, Norwegian University of Life Sciences, (Ås Norway) in closed growth chambers with high pressure sodium (HPS) as the main light source. The seedlings germinated in a greenhouse compartment (3 weeks) and moved to closed growth chambers with different light treatment. The light was provide continuously with no dark period and the temperature was set to 22° C, relative humidity 85% CO<sub>2</sub> level was ambient (400ppm). The light quality was by High Pressure Sodium (HPS) lamps (300µmol m<sup>-2</sup> s<sup>-1</sup>) or a combination with High Pressure Sodium + blue light emitting diodes (HPS+BL: 300 µmol m<sup>-2</sup> s<sup>-1</sup> + 75µmol m<sup>-2</sup> s<sup>-1</sup>). Thus, the photosynthetic active radiation was 300µmol m<sup>-2</sup> s<sup>-1</sup> in both treatments and the red to far-red ratio was 3.5.

After 2 months of growth, morphology, water loss and chlorophyll content of seedlings as well as dry matter accumulation in stems, needles, and roots were measured. The results showed that the responses of *P. abies* seedlings to light quality were more stronger than in *A. laciocarpa*. In *P. Abies*, thicker stem diameter, pronounced branches, higher significant differences in fresh weight and dry weight of roots and needles was found in produced with additional blue light compared to HPS alone. In addition, *P. abies* has a significant higher water loss when exposed to blue light compared to HPS. In chlorophyll content measurement the only significant difference was found in *A. laciocarpa* exposed to HPS+BL. In this treatment 50% higher content of chlorophyll *b* was found compared to HPS. More terminal buds were also observed in additional blue light in *A. laciocarpa* compared to HPS. However, *P. abies* did not produce any terminal buds during the experimental period. On the other hand, *A. laciocarpa* showed a different growth response to blue light than *P. abies* and a higher dry weight were found in stems and needles when they were exposed to HPS alone compared to additional blue light. The result from this thesis shows that the different species behave differently in response to additional blue light. Additional blue light seems to have a positive effect on the growth of *P. abies* but not for *A. laciocarpa*.

However, the potential after-effects of growth under different light quality and different environmental conditions after planting to forest site are still unknown.

Furthermore, both species can be propagated by stem cuttings but no significant effect of light quality on rooting was observed in this experiment due to huge variation between the cuttings.

**Key words:** Temperature, light quality, *Picea abies*, *Abies laciocarpa*, seedlings, stem cuttings

# 1. Introduction

## 1.1. Properties of light, the pigments and receptors involved in light sensing

Light is a form of radiant energy, narrow band of energy within the continuous electromagnetic spectrum of radiation emitted by the sun (HOPKINS WG y HÜNER, 2009). Light has characteristics of a particle and a wave. A wave characterized by a wavelength or frequency a distance in space between wave crests. The energy divided into units or particles called *photons* are what plant sense and the energy contained in a photon called a *quantum* (Hopkins, 1995). The light reaching plant is a flux, the amount of energy falls on flat sensor area per unit time expressed in watts per square meter ( $\text{W m}^{-2}$ ) called *irradiance* while *photon irradiance* is the number of incident quantum striking the leaf expressed in moles per square meter per second  $\text{mol m}^{-2}\text{s}^{-1}$  (Taiz and Zeiger, 2010). The radiation has different average energy and wavelength 100-400nm UV radiation ( $332\text{-}471 \text{ kJ mol}^{-1}$  photons), 400-740nm visible light ( $166\text{-}290 \text{ kJ mol}^{-1}$  photons), and longer 740nm is infrared ( $85 \text{ kJ mol}^{-1}$  photons) (Hopkins, 1995). Photosynthetic Active Radiation (PAR) ranges from 400-700nm are utilized in photosynthesis violet (400-425nm) blue (425-490nm), green (490-550nm), yellow (550-585nm), orange (585-640nm), red (640-700nm), far-red (700-740nm) (Hopkins, 1995). The light level increases to a saturation point photosynthesis increases. Respiration normally functions in light or darkness. There are three parameters in describing light quantity (irradiance/intensity), quality (spectral distribution) or duration (length) (HOPKINS WG y HÜNER, 2009).

Light absorbed by pigments and pigments that absorbed physiological light is photoreceptors and 85-90% is absorbed by leaf, the rest is either reflected, transmitted to the leaf (Taiz and Zeiger, 2002). Photoreceptors are proteins or pigments that has two groups the mass and sensory pigment such as carotenoids, phycobilin, flavonoids and chlorophyll. Chlorophyll is mass pigment that that absorbs very strongly in blue (400-450 nm) and red (600-650 nm) wavelength of light, and less in the green wavelength (550nm) (Taiz and Zeiger, 2002). UV-B receptors, cryptochrome, phototropin and phytochrome are sensory pigments. All pigments active in photosynthesis can be find in chloroplast. Red far red (R:FR) ratio spectrum determines the ratio active phytochromes (PFR) and inactive phytochrome

(Pr) (Taiz and Zeiger, 2002). Plants grown in shaded area try to elongate their stem and leaves in order to achieve better position in the canopy to catch more light. Chlorophylls *a* and *b* are green pigment that captured the energy of light. Other photosynthetic pigments are carotenoids, xanthophylls, phaeophytin are important in photosynthesis as they increase the range of wavelength use in photosynthesis (Taiz and Zeiger, 2002).

Action spectral differ in details for various plant systems known as phototropically sensitive in which blue is effective called cryptochrome (Salisbury and Ross, 1985). Blue light involved in wide range of plant processes such as phototropism, photomorphogenesis, stomatal opening and leaf photosynthetic function (Hogewoning et al. 2010). One pigment that absorbed red and far red are effective in causing photomorphogenesis known as phytochrome (Salisbury and Ross, 1985). The two types of photoreceptors in blue light are cryptochromes and phototropin (Lin, 2002). The chromophore for cryptochrome is flavin and most common are riboflavin, and its own nucleotide derivatives, flavin mononucleotide, and flavin denucleutides, this flavoprotein are important in cellular oxidation-reduction reactions (HOPKINS WG y HÜNER, 2009). *Cryptochromes* and phytochromes are important in photomorphogenetic responses such as cell elongation, stem elongation and inhibition and photoperiodic flowering whereas, *phototropin* (phot1 and phot 2) blue light photoreceptor for phototropism, senses the direction of light and important in blue light stomatal opening, chloroplast movement, leaf expansion (Takemiya et al., ; Lin, 2002). According to Lin (2002) combined absorption spectra of red/far red light receptors *phytochromes* and the blue light receptors (cryptochrome and phototropins) overlap with those photosynthetic pigments to control the development and energy production in plant i.e. the arabidopsis cryptochrome in mediating de-etiolation, gene expression, and photoperiodic flowering performed cryptochrome and phytochrome acts in response to blue/UV-A and red/far-red spectra of light (Fig 1) also etiolated seedlings of dicotyledonous plants develop hypocotyl elongation and small unopen cotyledons but exposure to light inhibit hypocotyl elongation, stimulation of cotyledon opening and expansion also establishment of photoautotrophic (Lin, 1998). Plants are able to sense changes in the spectrum, intensity and direction of light. Photoreceptors sense light signals for the plants to adjust its growth and development i.e. hypocotyls bend toward light to maximize photosynthesis in cotyledon on the other hand roots curve away from blue light in order stay in growing media for nutrient uptake (Lin, 2002). Whereas, chloroplasts move toward weak light for maximum photon capture but move away from

high irradiance to avoid photoinhibition (Lin, 2002). Stomata pores formed around guard cell and opens when lights on for gas exchange but closes when it is dark to minimize water loss. Moreover, blue light known to induce these movement responses.

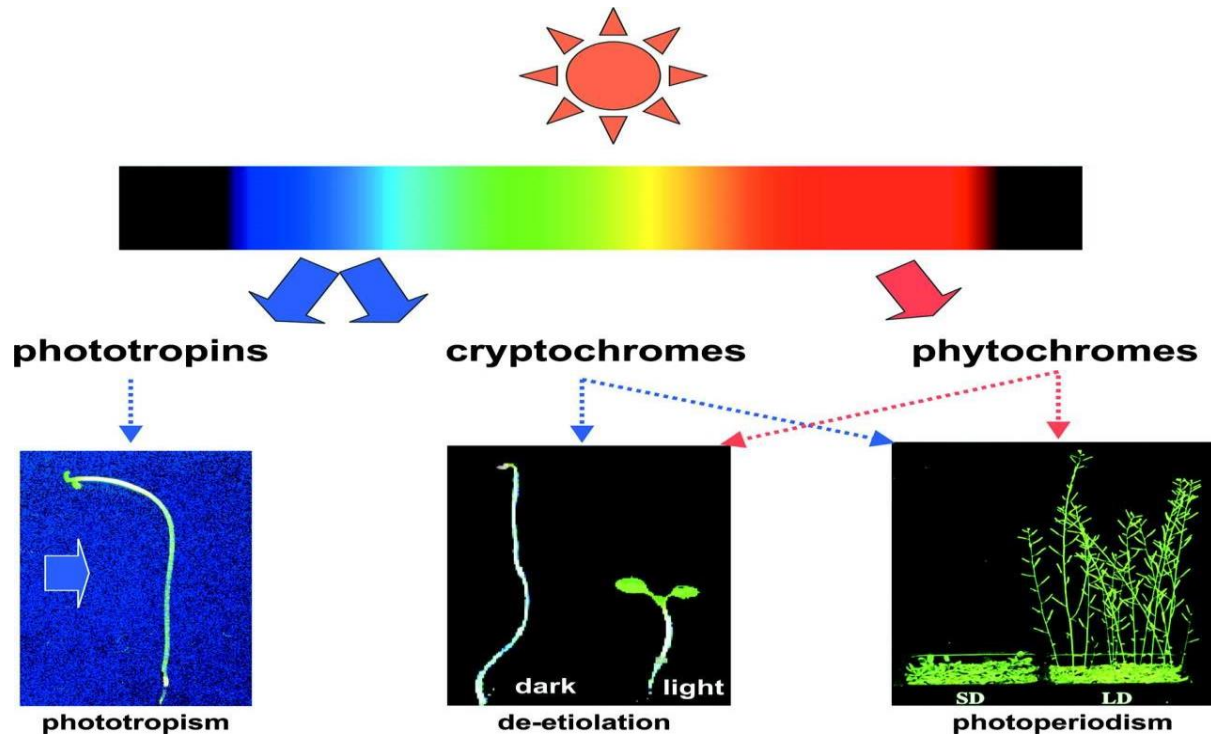


Figure 1. Functions of Blue light receptors in phototropism, photomorphogenesis and photoperiodic flowering. Solid arrows depict light, and dashed arrows depict signal transduction of photoreceptors. Image accessed from (Lin, 2002)

## 1.2. Morphological and physiological response to blue light

Plant development and physiology are strongly influence by the light spectrum for growth and development and involvement of photoreceptors was been demonstrated for a wide range of spectrum-dependent plant responses.

In phototropism, stems grow toward unilateral light sources by asymmetric growth on their shade side (Taiz and Zeiger, 2002). Blue light depolarized the membrane of hypocotyls cells before the inhibition of growth rate (Taiz and Zeiger, 2002). Blue region contains relevant information on the physiology and health status of a plant (Wang and Folta, 2013). The manipulation of blue light

proportion in the light source is one of the strategies to inhibit stem extension growth of roses and other ornamentals (Terfa et al., 2012).

Many studies found greater stem length in plants grown under supplementary HPS treatment than plants grown under supplemental LED. For instance, in the study of (Islam et al., 2012) poinsettia grown under LED 20% blue and 80% red showed 20%-34% reduction in plant height compared to plants grown under HPS with 5% blue. Also, in the study of (Terfa et al., 2013a). *Rosa x hybrid* plants grown under LED (20% blue light) showed a significant reduction in stem elongation compared to plants grown under HPS (5% blue light). In the study of (Bergstrand and Schüssler, 2013). The morphology of ornamental plants that grown under different light spectra of red/blue (8:1) LED and white LED a reduction in stem elongation grown during autumn period and a delayed in developmental was found in chrysanthemum, kalanchoe and euphorbia with a significant difference. Also, in other study of Hernandez et al., (2014) the cucumber transplants grown for 26 to 37 days under HPS had hypocotyl length of 36% to 50% greater than in blue and red treatment LED. According to (Johkan et al., 2010) seedling of lettuce treated with blue light improved seedling quality, promote growth after transplanting and compact plant, Therefore, compact morphology of seedlings and any crops that suitable for blue LED light is useful for transporting. Moreover, the effects of LED blue light known that inhibit stem elongations in most plant species.

Blue light stimulates ion and water uptake in the guard cell photoplasts, which in the intact guard cells provide mechanical force that drives increases in stomatal apertures (Taiz and Zeiger, 2002). Blue light modulates guard cell osmoregulation via its activation of proton pumping and via the stimulation of the synthesis organic solutes (Taiz and Zeiger, 2002). More blue light can improve the drought stress tolerance in roses that grown in high relative humidity (Terfa et al., 2012). Blue light have the potential to overcome negative effect of high relative humidity on stomata functionality and improve postharvest life (Terfa et al., 2012). Blue light known to have many effects on plant growth, development of stomata function, photosynthesis, carbohydrate status and rate of senescence (Terfa et al., 2012). According to Wang (2009) cited in Terfa et al., (2012) cucumber plant that grown in blue light quality treatment has higher total of sugars and sucrose content compared to white, red and green lights.

LEDs with 80% red and 20% blue showed higher chlorophyll and anthocyanin content and more thorns in *Rosa x hybrida* 'Toril' compared to HPS grown plant (Terfa et al., 2012). In addition, LEDs improves the post-harvest life in roses probably because of carbohydrate status of compared to (Terfa et al., 2012). In the study of (Terfa et al., 2013a) high proportion of blue light increased photosynthesis per unit leaf area, enhanced growth and morphological changes in roses but no significant effect on the total dry matter production and flowering time were found. In other species such as lettuce the anthocyanin and carotenoid concentrations were increased by 31% and 12% under blue light with 130  $\mu\text{mol m}^{-2} \text{s}^{-1}$  respectively compared to UV-A 18, G-130, R-130, and FR160  $\mu\text{mol m}^{-2} \text{s}^{-1}$  using LEDs spectra (Li et al., 2007). Therefore, the growth and development of the plants can be manipulate by altering light spectra but the responses are species-specific. In the study of (Hernández and Kubota, 2014) the effect of supplemental light on the physiological response of cucumber transplant were the shoot fresh weight were greater 28% to 32% and shoot dry mass was higher by 28% in HPS treatment compared to LED treatments.

Timing and formation of bud set is dependent on light factors and daylength, temperature and other climate factors. In the study of (Fløistad and Patil, 2002) *P. abies* grown under natural short day conditions with high far red prevent terminal bud formation but with low R:FR increased stem elongation and reduced shoot dry mass. In, previous experiment in *A. laciocarpa* seedlings exposed to different light climate easily make buds also in long day conditions (Jetmundsen, 2015). In their experiment, 100% of the plants produced buds in short day (SD) plants in short day after 41 days but 30% of the plants produced buds in long days under UV-B plants did not reveal a clear effect of bud development (Jetmundsen, 2015). Further, according to Aas (2015) bud formation in *A. laciocarpa* is highly sensitive to light quality as 100% of the plants developed buds in SD after 66 days of light treatment compared to 92% plants with buds under red light treatment, 75% plants with buds under blue light treatment and 35% plants with buds under far red light treatment. Thus, bud formation is influence by different light quality and quantity factors. But less published information exist on the impact of blue light on *A. laciocarpa* and *P.abies* growth and development by seedlings propagation Riikonen et al., (2015) by cutting propagation (Ragonezi et al., 2010). Therefore, more knowledge is needed on the responses of conifers to different light quality especially blue light to produce high quality and cost-efficiently forest regeneration.

### 1.3. Greenhouse Production in Northern Europe

Greenhouse production systems are important in growing different crops even for smaller trees. In, northern Europe normally they propagate and germinate seedling of small trees inside greenhouse due to fluctuating climatic factors. Environmental factors such as light, temperature, relative humidity, CO<sub>2</sub>, have significant impact on the growth and development of small plants as they interfere the different morphological and physiological processes and fluctuation of these environmental factors outside of their normal ranges may result to negative physiological consequences for the plants. Light is the single most important environmental factor in regulating plant development (Fosket, 1994). Since, temperate region such as Northern Europe light is limited, especially during the winter season. Thus, commercial greenhouses commonly used artificial lighting in this period to grow plants. Artificial lighting is mainly used to increase the light intensity and to increase yield, to decrease time for crop development and improve plant quality (Bergstrand and Schüssler, 2013; Runkle and Both, 2011). Many growers produce crops all year round. Therefore, the use of supplementary lighting is beneficial to maintain plant quality and crop schedule. The light environment inside greenhouse is dependent on the natural light, the time of the year and the time of the day, the direct or diffuse light, the covering materials and the types of lamps. The light intensity has different purposes in propagation, photosynthesis and for growth and development. The daily light integral (DLI) the cumulative amount of light that a plant receives in a 24 hour period and important in photoperiodic experiments to provide similar DLI to be able to separate the effect of irradiance and photoperiod also used to control flowering. Individual different growers have different specific concepts on how to produce their desired plants. DLI is important in greenhouse crop production because it usually correlates with plant biomass such as roots, stems, flowers and fruit production thus, the higher the DLI the greater the plant growth (Runkle and Both, 2011). Light transmission through glazing depends on the percentage transmission of the glazing materials and angle of incidence. In addition, shading is importance under high solar load conditions to prevent plant stress, it reduces direct solar beam radiation and increases diffuse radiation. In Norway, greenhouse can be a limiting growth factor during winter due to low light level and short days and high light and long days during summer, therefore it is important to manipulate different climatic factors to the optimum level in produced quality crops.



## 1.4. Supplemental artificial lighting

Studies with the use of more advanced light technology and smart use of light are of interests for growers as well as for plant physiologists. Most HPS and LED fixtures have equal efficiency, but the initial capital cost per photon from LED is 5 to 10 times higher than HPS (Nelson and Bugbee, 2014).

### 1.4.1. High Pressure Sodium (HPS)

Different lamp types can be used in greenhouse production such as HPS, LED, HPI, fluorescent tubes, incandescent lamps, and plasma lamps. Different lamp types have different spectral distribution of light, energy efficiency i.e. the photosynthetic photon flux per watt, the price, the lifetime and investment. In greenhouse production High Pressure Sodium (HPS) is still the most widely used as supplemental light source (Bergstrand and Schüssler, 2013; Runkle and Both, 2011). The light from this lamp consists mainly of yellow, orange and red light but very little with blue light (Figure 6). The main reason why this is commonly used is because it has a relative high energy efficiency of 40% and it also emits heat (infrared radiation) and many growers use HPS to heat their greenhouses and the heat radiation increases plant temperature which improves crop growth and development (Runkle and Both, 2011). HPS bulb also has a relative long lifetime 14000 hr. The main problem with HPS is the blue light portion (5-8%) and lack of blue light can stimulate shoot elongation and lead to lower plant quality (Wheeler et al., 1991). Supplementary light level improves the post-harvest life in roses because of carbohydrate status of the plant, but in terms of post-harvest longevity of roses were decreasing due to higher water loss and wilting (Terfa et al., 2012). Thus, HPS emit moderate efficient, long bulb life that emit increase orange light significant amount of heat that save heating fuel, the heat radiation increase plant temperature which improve crop growth and development.

### 1.4.2. Light Emitting Diodes (LEDs)

Light Emitting Diodes (LEDs) are of increasing interest to plant production and research due to high energy efficiency, adjustable light intensity and spectrum and low radiant heat load (Bergstrand and Schüssler, 2013). They emit spectrum that tailored for specific crop stages of

production or for desired growth characteristics (Wollaeger and Runkle, 2014). And LED can be used as interlighting and placed very close to the plants without damaging the leaves (Wollaeger and Runkle, 2014). LEDs have higher blue spectra than HPS (Fig. 6). According to Terfa et al (2012) roses grown in LED with 80% red and 20% blue light had shorter stem and shorter pedicle and more thorns in roses compared to HPS lamps. According to Valoya.com the LEDs that they manufactured is cost effective investment because the product is highly durable, increased yield, less maintenance and energy savings, less heat radiation leads to less water evaporation, which result to less plant stress that could be more effective in nutrient uptake. In addition, Valoya conducted an experiment to find the best possible light spectrum for tree seedling in expression of frost tolerant genes of Norway spruce that grown in artificial light intensity 100  $\mu\text{mol}$  with no sunlight condition and according to them AP67 showed the best response as it provide strong plants, full cold hardiness and very strong roots. In general, many studies shown that growth and development of plants in HPS are compared with different LED, and the responses seem to vary with species and cultivars.

### 1.5. Production of small trees by seeds and stem cuttings

Trees can be propagated by seedlings and cuttings and seed germination is sensitive to light quality and quantity such as *Abies*, *Picea* and *Pinus* (Kozłowski and Pallardy, 1997). Vegetative propagation by rooting of stem cuttings has been practiced for centuries. This method is useful to improved reforestation due provide the fast multiplication of selected superior trees. Conifers are one of the most economically important among gymnosperms covering approximately 60% of the forested areas worldwide and used for the production of soft lumber, pulp and paper (Ragonezi et al., 2010). Although conifers play a major role in reforestation but there is problem the research in stem cutting propagation method is not sufficiently developed due to poor rooting capacity that may affect the survival trend when planting on forest site (Ragonezi et al., 2010). Although the capacity of rooting is complex in physiological standpoint as usually depends on number of factors and rooting among species. There are trees that are relatively easy to induce roots such willows, non-aspen poplars, monterey pine, junipers whereas spruces, pines chestnuts are difficult to root according (Kozłowski and Pallardy, 1997). The extent and density of rooting systems are influenced by light intensity as it affect the availability of carbohydrates and hormonal growth

regulators in the roots and roots growth varies among species and different light quality (Kozłowski and Pallardy, 1997). Woody plants use large amount of carbohydrates in metabolism and growth but differs among species and genotype in accordance with their growth characteristics. In temperate region many deciduous trees which the amount of reserve carbohydrates of stem and branches decrease rapidly during early summer and increase in autumn and decline slowly in winter for such sugar maple, gray birch, apple and peach trees (Kozłowski and Pallardy, 1997). Light intensity affects crown size, influencing branching, bud formation, shoot expansion, leaf distribution and structures in woody plants and high rate of photosynthesis and increase in biomass usually increases linearly with amount of intercepted light (Kozłowski and Pallardy, 1997) Kramer at al., 1979. In temperate region plants are expose to low temperature during winter and low temperature is lethal for growing tissue as it developed buds to enter dormant state and dormancy of bud is cessation of observable growth (Wilkins, 1984). The primary factor for inducing dormancy bud is day length while nutrition, water status, temperature and irradiance are consider as other factors that can modify the time of onset of dormant (Wilkins, 1984). Stem elongation reduced by inhibitory effect of water deficits on both bud formation and bud elongation (Kramer at al., 1979).

### 1.5.1. *Picea abies*

Norway spruce is native to the European Alps, the Balkan Mountains and the Carpathians, and extended to Scandinavia and introduced by British Isle in early 1500 AD, planted widely in North America (Sullivan, 2012). Norway spruce is shade tolerant tree Riikonen et al., (2015) and planted to windbreaks and shelterbelts in Western prairies and grows in more humid environment, widely planted for Christmas trees and as an ornamental (Sullivan, 2012). Norway spruce is an evergreen tree, grows to 30-61m long, cones are 10-18cm long according to Collingwood et al 1964. In addition, the root system is shallow with several lateral roots and no taproot (Sullivan, 2012). The early growth stage of *P. abies* is slow but increase to maximal rates from 20-60 years Sullivan (1994). Senescence occurs at less than 200 years of age and *P. abies* can be propagated by cutting and micro propagation techniques according (OuYang et al., 2015; Kozłowski 1997; Ragonezi et al., 2010) In Norway, the major forest type is coniferous evergreen forest and the main species are *Picea abies* and *Pinus sylvestris* in which area covered by 5.5 million hectares. These

two conifers are economically important species for wood production in commercial forestry (FAO). In Norway *P. abies* is also used as a Christmas tree.

### 1.5.2. *Abies lasiocarpa*

Subalpine fir is a native coniferous, evergreen tree and indigenous to Western United States and distinguished by the long, narrow conical crown terminating in conspicuous spike like point widely distributed to North America (Uchytel, 1991). Subalpine fir flower are monoecious, the male flower are abundant and female flower are fewer (Uchytel, 1991). The seed lie dormant under snow and germinate in coming spring in normal environment (Alexander et al., 1990). Also, wind dispersal and when trees are 4-5 ft. tall and 20 years old the cones begin to produce but seed production is not significant until trees are older and taller (Alexander et al., 1990). Seedlings root growth are very low in initial year and usually outplanted 2-3 years (Alexander et al., 1990; Uchytel, 1991)). Also at high elevation, a one-year-old seedling has less than 2.5cm tall also subalpine fir trees the growth is not rapid, trees 25 to 51 cm in diameter in 150-200 years old under closed- forest conditions (Alexander et al., 1990). The tree has shallow root system limit the depth of root penetration and develop lateral root system by (Alexander et al., 1990). The insect that caused significant mortality to *Abies lasiocarpa* is balsam woolly adelgid *Adelges Piceae* (Uchytel, 1991). Subalpine fir in Rocky Mountains of Idaho and Montana assists in protecting water sheds and rehabilitating the landscape and provides habitat for animals, forage for livestock, recreational opportunities and scenery according to U.S Department of Agriculture Forest Service 1974 cited in (Uchytel, 1991). Also used as lumber in building constructions, boxes, crates, placing mill products, sashes, doors, frames and food containers (Alexander et al., 1990). In Norway *A. lasiocarpa* is used as Christmas tree.

### 1.6. Propagation of *P. Abies*, *A. lasiocarpa* and aim of the study

*P. abies* and *A. lasiocarpa* are normally propagated by seeds in greenhouses the first year of growing. The production usually starts in January/February and in this period they need artificial lighting. The second year they are placed outdoor and later they are planted out in tree plantation or into the forests. *A. lasiocarpa* easily makes buds and then the growth stops. The reason why

they so easily make buds is not known yet but it is believed to be due to low irradiance and short days (Wilkins, 1984). *P. abies* is more robust and continues to grow also under a lower irradiance. However, knowledge on their growth responses to blue light is scarce. In many plants, blue light is believed to be an irradiance sensor (Terfa et al., 2013). Thus, when the irradiance is low, but the content of blue light is high, the plant behaves as it grows in higher irradiance (Terfa et al., 2013). Hence, it is of interest to study if additional blue light can affect bud formation and growth of small tree seedlings.

The propagation of seedlings takes a long time and it has been questioned if they can be produced by cuttings. However, knowledge about rooting of woody species is scarce. In other studies, additional blue light has been shown to improve rooting of woody cuttings like *Hydrangea macrophylla* (S. Torre, personal communication). However, it is not known if blue light can improve the rooting capacity of *P. abies* and *A. laciocarpa*. Hence, the aim of the study was to investigate the following:

1. Effects of additional blue light on morphology and development of *Picea abies* and *Abies laciocarpa* during seedling production.
2. Effects of additional blue light on chlorophyll content and water loss analysis of *Picea abies* and *Abies laciocarpa* seedlings.
3. Effects of additional blue light on fresh and dry weight distribution of *Picea abies* and *Abies laciocarpa* seedlings.
4. Effects of additional blue light on the rooting and survival of *Picea abies* and *Abies laciocarpa* stem cuttings.

## 2. Materials and Methods

### 2.1. Experiment I Seed germination and Pre-cultivation of seedlings

#### 2.1.1. Stratification of *Abies lasiocarpa*

Seeds of Subalpine fir (*Abies lasiocarpa*) from the provenance CØN10 from 53.39°N latitude, 122.23°W longitude, 1000-1200 meters above sea level from 'George Mt' in British Columbia, Canada (seed number F13-005, The Norwegian forest seed center, Hamar Norway) were stratified on 13<sup>th</sup> of May 2015. In this process, the seed dormancy is broken in order to promote seed germination. Seeds placed on petri dish with lid on moist filter papers (Fig.2). The petri dishes covered with aluminum foil paper to induced darkness and they were stored in cold storage room at about 4<sup>o</sup> C for three (3) weeks before sowing in soil.



Figure 2. Seeds from *Abies lasiocarpa* (right) placed on wet filter paper in petri dishes (left) ready for sowing 3 weeks after placement in cold storage (4°C).



Figure 3. Peat and perlite (3:1) were mixed and used as growing media in the experiments (left). A small pots were filled with growing media (right) and two seeds were seeded in each pot.

### 2.1.2 Seedling of *A. laciocarpa* and *P. abies*

The 3<sup>rd</sup> of June 2015, the seeds of Norway spruce *Picea abies* from the provenance CØ1 from 59°N latitude, 0-149 meters above sea level Halden Østfold, Norway (seed lot 98063 The Norwegian forest seed center, Hamar Norway), and the stratified seeds *Abies laciocarpa* were sown in pots. Two seeds per species were sown in each individual black plastic pots size/mm (ø60x51) and placed in white tray system size/mm (596x396x53) (Art no. 780619 produced by VEFI A/S Drammen Norway). Pots were filled with peat and perlite mixture with the ratio of 3:1 (peat:perlite) by combining these two growing media (Fig.3). The sphagnum peat was from Go jord, Vesktjord, produced by Degernes Vesksttorv, Torvstøfabrikk Norway and the perlite was from RHP, Agra-Perlite. The seedlings were covered by white plastic to increase air humidity and water as needed. After 2 weeks, some seeds were germinated then short sticks were added to support the white plastic not to disturbed the apical meristem of the plants. During pre-cultivation the plants were kept in the greenhouse with glass roof (90% PAR transmission) and polycarbonate walls (83% PAR transmission) at the Center for climate regulated plant research, Norwegian

University of Life Sciences, Ås Norway (59°39'47''N 10°47'38''E). The average air temperature was 20 ( $\pm 2^{\circ}$  C), and the average daily relative humidity (RH) 70 ( $\pm 5$  %), corresponding to 0.5 kPa water vapour deficit (VPD) and ambient carbon dioxide level of 400ppm controlled using PRIVA system (The Netherland). The supplemental light given by High Pressure Sodium lamp (HPS Osram NAVT 400W, Munich Germany), the light level was ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) given 16 hours every day from (06:00-22:00) but the supplemental light was set turned off automatically when the sun is stronger than  $200\text{W/m}^2$ . The pre-cultivation ended after 19 days on 22 of June 2015. It was made sure that there was only one plant grown per pot, others were removed carefully with a small scissor. The plants were arranged and equally distributed to different system trays with respective name codes and transferred to the different light treatments in controlled growth chambers. There were 36 pots of *P. abies* and 18 pots of *A. laciocarpa* in each growth chambers. At the time of transfer to the growth chambers the plants height of *A. laciocarpa* were 0.4 -1.9 (cm) with 0-5 needles while in *P. abies* plant height were 0.4 – 2.0 (cm) with 4-10 needles. The plant height were measured from the rim of the pot up to apical meristem.



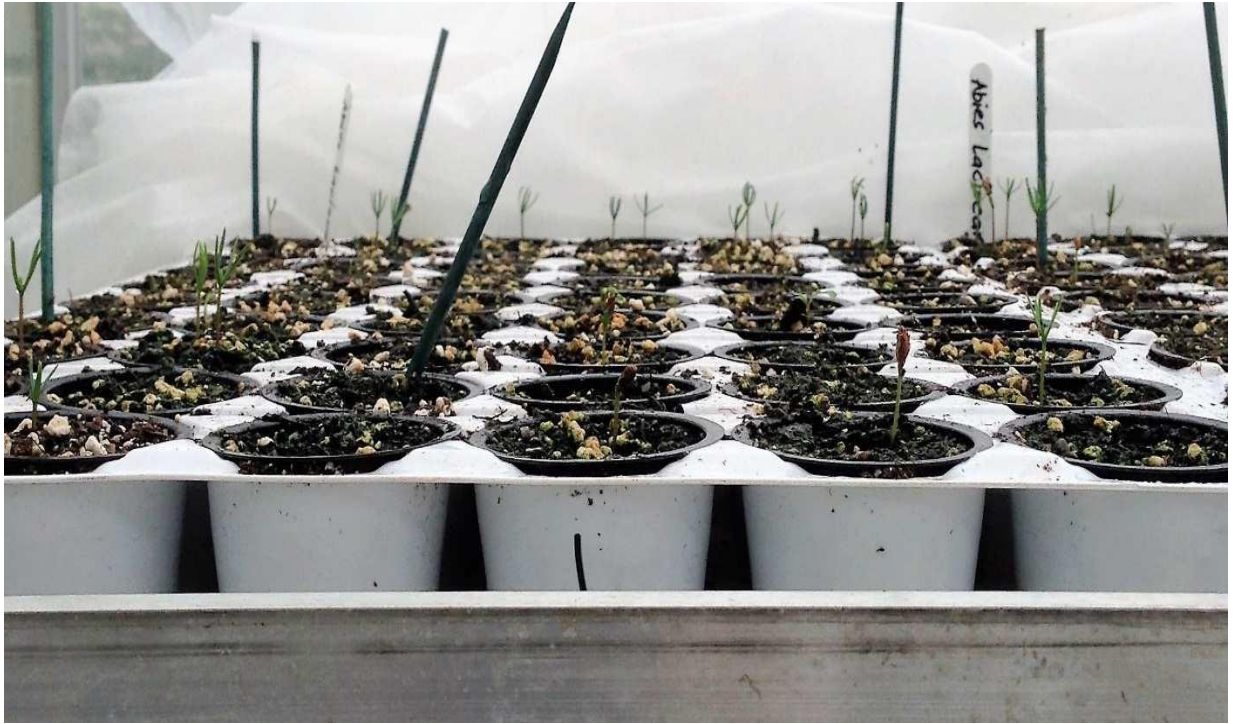


Figure 4. Plants were grown about 0.4 – 2.0 cm after 19 days from sowing *A. laciocarpa* (top), *P. abies* (bottom) ready for transfer in growth chambers.

## 2.2. Experiment II: Stem cutting propagation method

There were two bundles of *Picea abies* (Gran) certificate for reference number: KV14014, Proveniensi: L2, Sankested: L2, Plant type M60, Seeds batch number: 4184 and two bundle of *Abies laciocarpa* (Fjelledelgran) certificate for reference number: AL13606, Proveniensi: Grassie Mt, Plant type M60, Seeds batch number: F10-005 were taken out of cold storage at a commercial nursery (Skogplanter Midt-Norge As) and sent to Ås. All plants were at two years old (Fig.5).

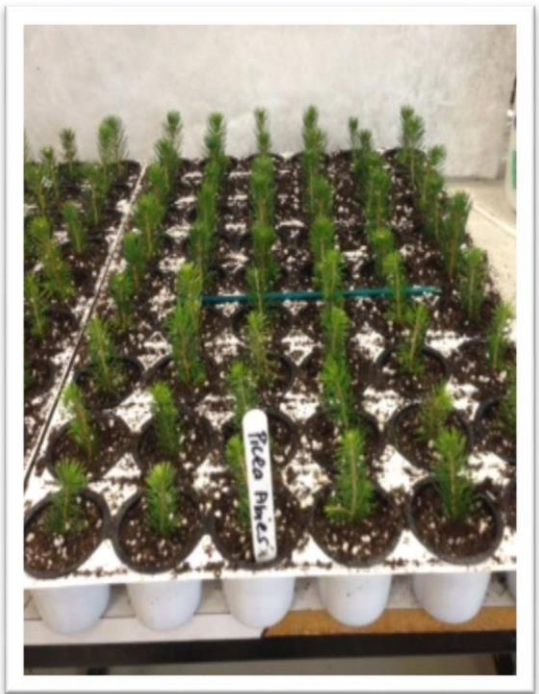


Figure 5. Two years old *P. Abies* (left) and *A.laciocarpa* (right) above from a commercial nursery (Skogplanter Midt-Norge As) and below were sticking in pots filled with peat and perlite.

Cuttings were taken from the plants by using a scissors. Each cutting were about 5-8 cm long. The needles on the lowest 1 cm of the cuttings removed and they were placed directly into the black pots filled with peat and perlite mixture (the growing media used in experiment II was the same in experiment I). In *A. laciocarpa* twenty four (24) cuttings were taken from main stems (top-cutting) and thirty (30) cuttings were taken from the side shoots. In *P. abies* thirty (30) cuttings were taken from the main stems and twenty (24) cuttings were taken from the side shoots. Water was been sprayed to their needles to add moistures during the cutting period and after planting. The plants were placed directly into different chambers with different light quality (see below) right after the sticking. The light treatment for cuttings ended 19<sup>th</sup> of August 2015 but since the roots were very small/no roots, the cuttings were transferred to a greenhouse compartment for further development one more month. The growing conditions in the greenhouse was as mentioned above (2.1)

### 2.3. Experiment growth conditions both experiment I and II

The experiments were performed in two growth chambers with different light quality from 22 June 2015 to 18 August 2015. These growth chambers were manufactured by Center for climate regulated plant research, Norwegian University of Life Sciences, Ås Norway. In one of the chambers the light was provided continuously 24 h daily by four High Pressure Sodium (HPS) lamps (400 Watt) from GAN-4-550 AL 230V Superagro system (Gavita Norway) enriched with 8 incandescent bulb, (50 Watt each from Osram, Munich Germany) the light in the second chamber was provided by the same lamp type as for the first chamber (HPS and incandescent bulbs but in addition 6 bars blue light (400-500nm, peak at 460 nm) from Philips green power Light Emitting Diode (LED) 100 Watt (Phillips, The Netherland). The irradiance of both growth chambers were the same  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  measured at the top of the plants by Li-Cor Quantum / Radiometer/ Photometer, Model LI-250 Light Meter, and Serial no. LMA-301 Made in United States of America. In the treatment with blue light (HPS+BL) the blue LEDs contribute to about  $75 \mu\text{mol m}^{-2} \text{s}^{-1}$  to the total irradiance (Table 1). In addition, the spectral distributions of different light quality of different growth chambers were measured using SpectraWiz Spectrometer Operating Software (c) 2003 StellarNet-Inc.com Home of EPP2000 Fiber Optic Spectrometers (Fig.6). In both chambers, the light was given continuously with 24 hours a day with no dark period.

The red:far red ratio was 3.5 measured with a Skye instruments 660/730 sensor (made in Wales). The temperature set point was 22<sup>0</sup> C and the average relative humidity (RH) 85% in day and night in both growth chambers during experimental period corresponding to 0.5 kPa water vapour deficits (VPD) and has normal ambient carbon dioxide level of 400ppm controlled using PRIVA system (The Netherland). The light levels were monitored every week. During the experiment unwanted weeds were removed, yellow sticky cards were placed in the chambers for unwanted insects and the plants were watered when needed. The plants were watered with fertilizers containing Superba 9-5-25+4.2 Mg the +S+Micro-nutrients and calcinit from Yara, Oslo Norway.

Table 1. Description of the light treatments given in the two chambers

Light treatment	Photosynthetic active radiation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		R/FR ratio
	HPS + IB	Blue LED	
HPS	300	-	3.5
HPS+BL	225	75	3.5

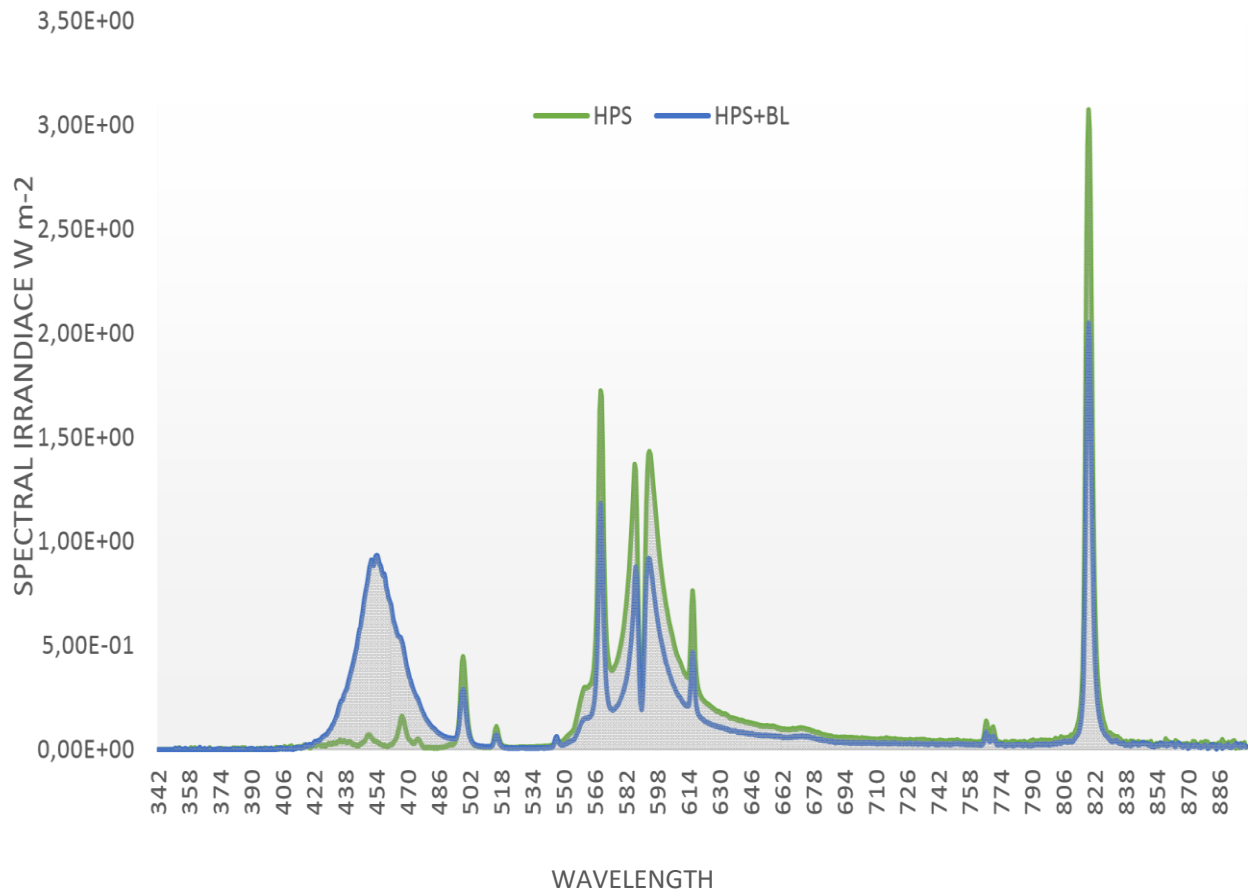


Figure 6. Spectral distribution of different light treatments High Pressure Sodium (HPS) and High Pressure Sodium + Blue light (HPS+BL) that used in the experiments using SpectraWiz Spectrometer Operating Software (c) 2003 StellarNet-Inc.com Home of EPP2000 Fiber Optic Spectrometers. Spectra were recorded at the top of the plant canopy.



Figure 7. Set up of experiment in growth chamber with High Pressure Sodium (HPS) light quality treatment.

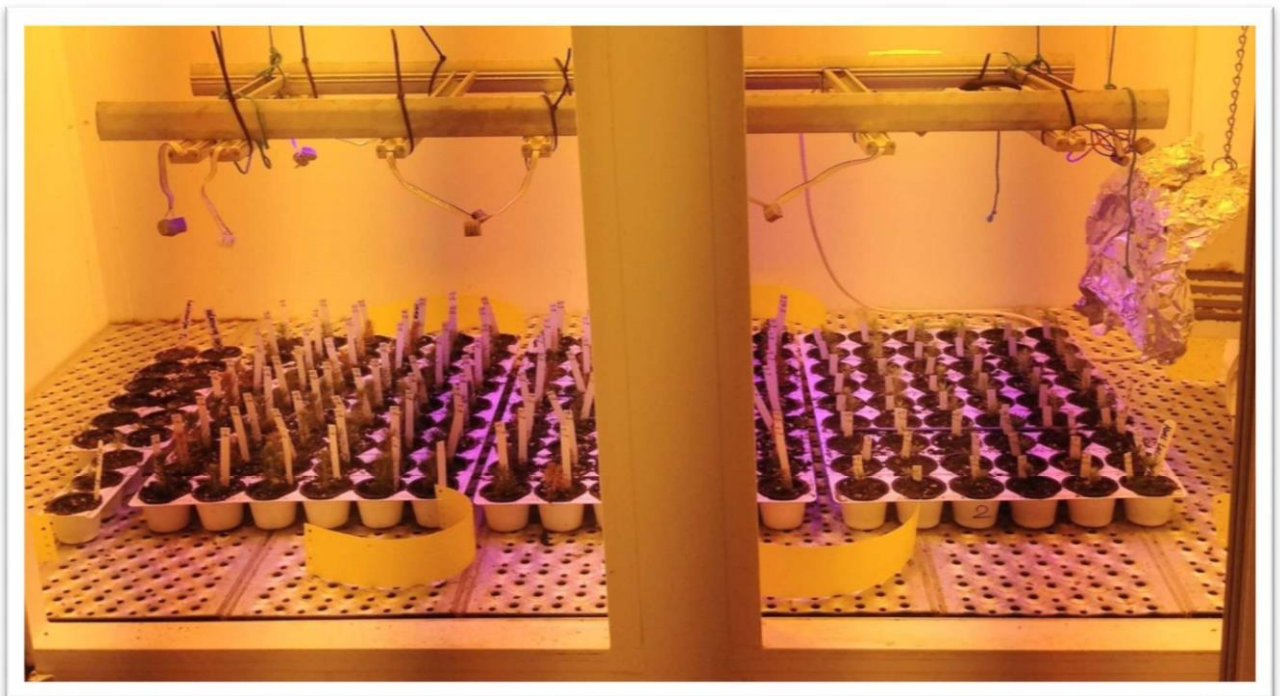


Figure 8. Set up of experiment in growth chamber with High Pressure Sodium +LED (Blue light) light quality treatment.

## 2.4. Growth Analysis, measurement during and at the end of the experiments

### 2.4.1. Measurement during whole experiment

Height measurements were done every week during the experimental period for eighteen seedlings from each species (*A. laciocarpa* and *P. abies*) except *A. laciocarpa* that grown under HPS+BL that has 17 plants. The height measurement was done from the rim of the pots up to the apical meristem. The buds formation of *A. laciocarpa* were monitored weekly in percentage. The bud formation was characterized by the formation of brown and green bud scales at the apices of the plant and the bud break was characterized by the first clear needle that was formed from the apical bud.



Figure 9. Example of green (left) and brown (right) terminal bud of *A. laciocarpa* (From Cazanji O. C. term paper, NMBU, 2013). The sampling was done once every week.



## 2.4.2. Seedlings

After two months of growth (19 August 2015) final measurement of the experiment I seedlings were executed. Then, eight plants of *A. laciocarpa* and 10 *P. abies* plants in each treatment were randomly selected for morphological measurements. The growth parameters were total plant height, plant diameter, and fresh weight of needles, stems and roots. In measuring plant height (cm) a transparent ruler in centimeter was used to measure from the rim of the pot to the apical meristem of the plants. The plant diameter using Mitutoyo digimatic vernier caliper. Counted the final buds of *A. laciocarpa*, and counted the branches of *P. abies* and measured the branch length in centimeter. For measurements of plant diameter (at the middle of the stem) a Mitutoyo digimatic vernier caliper was used from soil surface up to apical meristem of the plant. The number and length of the side branches were counted/measured and the buds were scored according to the grouping described above, to see the color and bud structure using binocular instrument. The stem of the plants were been cut above the soil surface and the needles were detached from the stem using fingers or forceps. The stems and the needles were then separated and put in different paper bags after weighing the fresh weight in gram (FW). Roots were carefully washed with water and the numbers of roots were counted and the longest root was measured in cm (from primary root to the end of the root. Fresh weights of roots were measured in grams. All fresh weight of samples (needles stems and roots) was kept in the dry storage 20° C for about 1 week. After a week, dry weight of the samples were been measured in grams.



Figure 10. Plants that grown in growth chambers for 2 months 3 rows from front were *A. laciocarpa* and from 4<sup>th</sup> until end rows were *P. abies* (top) treatment with HPS (below) treatment with HPS+BL. The height of plant were measured from the rim of the pot up to apical meristem based on the 18 plants of both species (shortest and highest) and no. of needles based on 5 plants used in chlorophyll measurement (few and many). In *A. laciocarpa* were 0.6-3.5 cm plant height and no. of needles 28-60 for HPS treatment while in HPS+BL treatment plant height 1.3- 3.2 cm and 34-74 no. of needles. For *P. abies* plant height 2.8-6.5 cm and 122-232 no. of needles in HPS treatment while in HPS+BL treatment plant height were 2.8-7.0 cm and 158-280 no. of needles.



Figure 11. Plants from seedlings *P. abies* (left) under HPS light quality treatment and *A. laciocarpa* (right) from HPS+BL light quality treatment

### 2.4.3 Cuttings

The final measurement of stem cutting were done 23.09.2015 on all plants that had survived during the experimental period. Stem cuttings of both species were counted in percentage (%) plants that survived weekly, at the end of light quality treatments and growing extendedly in greenhouse compartment. Different growth parameters such as stem diameter in millimeter by Mitutoyo digimatic vernier caliper measured 0.5 mm above callus, number of roots were counted and root length was, measured on the 3 longest root length (cm), fresh and dry weight of roots in grams were also measured (like described for seedlings).



Figure 12. Plants from stem cuttings *P.abies* (left) under HPS light quality treatment and *A.laciocarpa* (right) from HPS+BL light quality treatment.

#### 2.4.4 Water loss and chlorophyll content measurement

Water usage (mg water/needle/hr) was measured on 5 seedlings per species per treatment. The pots were covered with aluminum foil and whole plants were weighed two times (20 hr between the weighing). The water usage (mg/needles/hr) was calculated by dividing the water loss (in mg) on the number of needles and hours (20). The needles of each plant were detached and counted, and weighed the fresh weight of needles in grams (g) Fig. 13. Then the needles from each plant and species were placed in tubes containing 5ml of N, N-Dimethylformamide (SigmaAldrich Norway AS) for 3 days in a cold storage room (4° C) for chlorophyll extraction (Fig.13). After three (3) days the samples were been measured using the spectrophotometers (Unicam Helios Beta, Auxin Texas). The spectrophotometer was calibrated with N, N-Dimethylformamide (absorbance 0). The absorbance for the solutions was measured at two wavelength of 647nm and 664nm. These wavelength correspond to the maximum of absorbance of chlorophyll b and chlorophyll a respectively. The formula that used to determine the content of chlorophyll a and b with the absorbance measurement were: *chlorophyll a* =  $12.64 A_{664} - 2.99 A_{647}$  and *chlorophyll b* =  $-5.6664 + 23.26 A_{647}$ . The data was expressed on the basis of needle number.

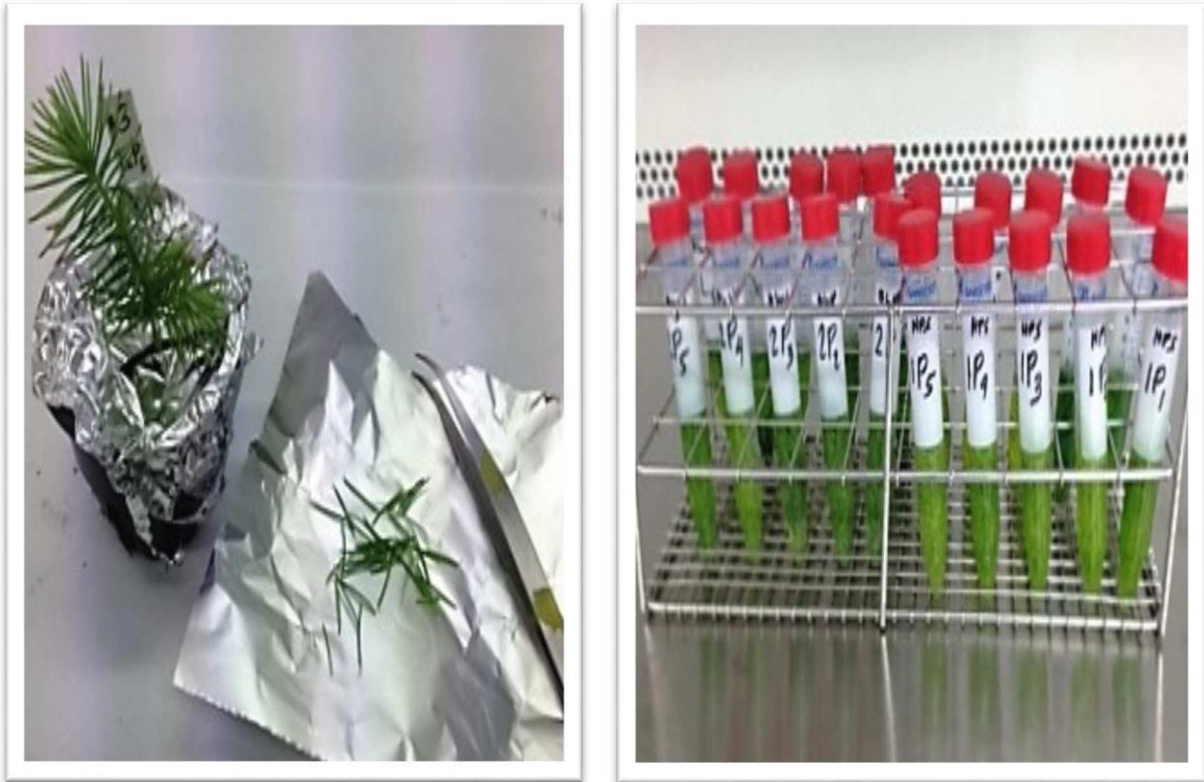


Figure 13. Needles were detached and counted, and weighed the fresh weight of needles in grams (g) left. Needles from each plant and species were placed in tubes containing 5ml of N, NDimethylformamide (right).

## 2.5 Data analysis and statistics

All data were calculated and plotted by using Microsoft excel worksheets. The statistics in all experiment were performed using Minitab 17 (Minitab, Inc. State College, PA, and United States of America). To study the effect of light quality in different species (*Abies laciocarpa* and *Picea abies*) were analyzed separately by a one-way ANOVA analysis. Tukey Pairwise was used to make a comparison among the treatment means and p values <0.05) was considered as significant difference.

## 3.0 Results and discussions

### 3.1 Experiment 1: Seedlings

#### 3.1.1 Cumulative growth pattern of seedlings

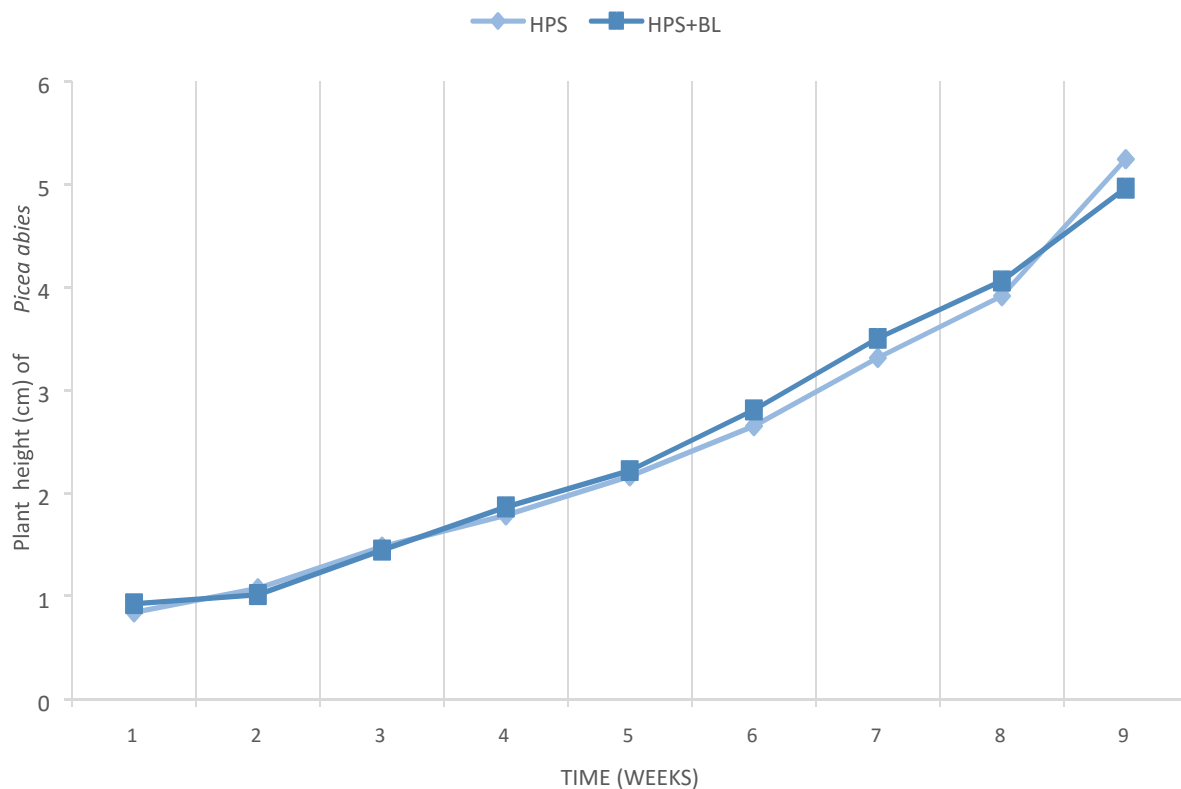


Figure 14: The average weekly cumulative growth of *P. abies* seedlings exposed to different light treatments: High Pressure Sodium (HPS) and High Pressure Sodium + Blue Light (HPS+BL). The height of the plants were measured from the rim of the pot to the apical meristem the same day every week, from the start of the light treatments until the end of experiment (23 June 2015 - 18 August 2015). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h) of 300 ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) light level but both have the same daily average relative humidity 85%, 22° C temperature and 400ppm ambient level of CO<sub>2</sub>. Results are mean of N=18 plants.

Figure 14 shows the cumulative growth pattern of *P. abies* seedlings grown in different light quality treatments. All plants showed an increase in growth from week one to week nine but no significant differences were found between the light quality treatments.

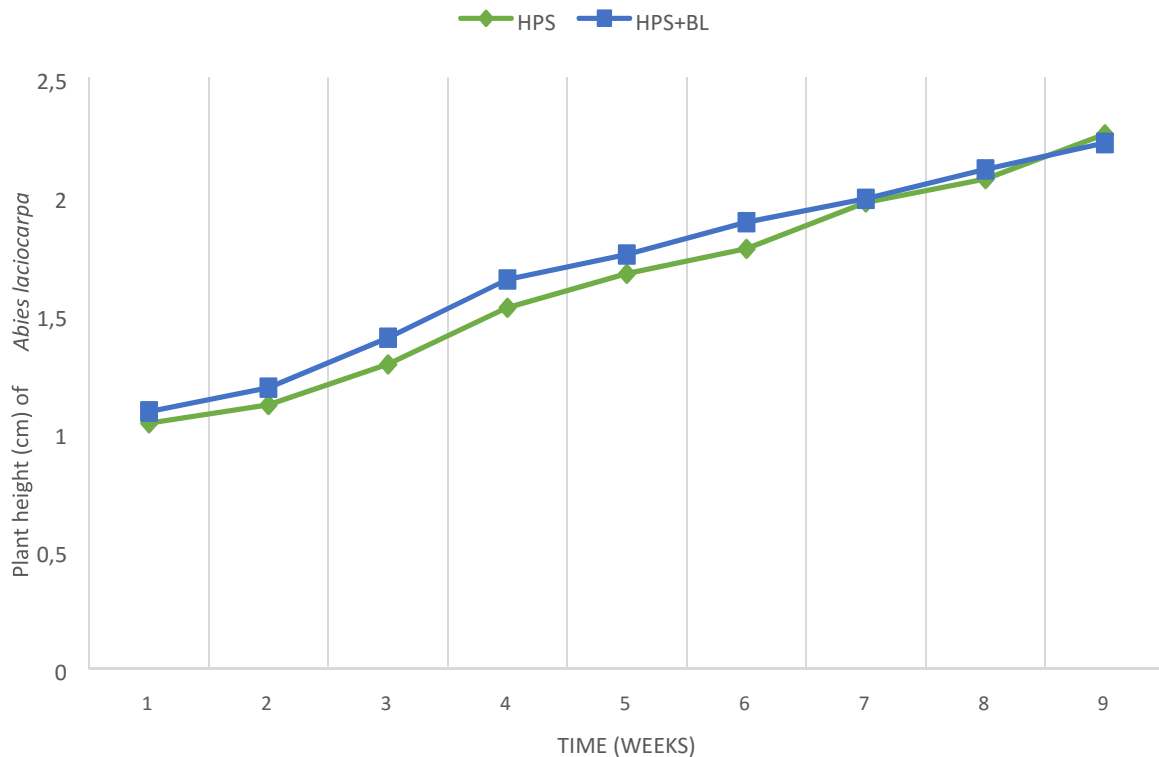


Figure 15: The average weekly cumulative growth of *A. laciocarpa* seedlings exposed to different light quality treatments: High Pressure Sodium (HPS) and High Pressure Sodium + Blue Light (HPS+BL). The height of the plants were measured from the rim of the pot to the apical meristem the same day every week, from the start of the light treatments until the end of experiment (23 June 2015 - 18 August 2015). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h) of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  light level but both have the same daily average relative humidity 85%,  $22^\circ \text{C}$ , and 400ppm ambient level of  $\text{CO}_2$ . Results are mean of 18 plants from HPS and 17 plants from HPS+BL



Figure 15 shows the cumulative growth pattern of *A. laciocarpa* seedlings grown in different light quality treatments. All plants show a slowly increase in growth from week one to week nine but no significant differences were found between light quality treatments. In many plant species additional blue light leads to shorter plants (Taiz et al 2002; Terfa, 2012), but in this experiment no such effect was seen.

Both species showed an increased growth from week 1 to week 9, but the growth and development of *P. abies* were faster compared to *A. laciocarpa*. The average number of the needles produced during the experimental period was also different and *P. abies* produced in average 200 needles per plant during the experimental period but *A. laciocarpa* produced only 50 needles. Maybe *A. laciocarpa* seedlings have a higher light requirement and the irradiance of 300 is too low to induce fast growth in this species. However, it is well known that *A. laciocarpa* has a slow growth rate in first year of growing stage (Alexander et al., 1990; Uchytíl, 1991). If plants were grown for at least two months in the greenhouse compartment before treated with different light quality or if the plants were grown for a longer time in the light quality treatment, maybe another response would appear.

### 3.1.2 Bud formation of *A. laciocarpa*

*P. abies* seedling did not make any buds during the experimental period. However, *A. laciocarpa* developed green and brown terminal buds during the experiment (Figure 9, Table 2).

Table 2. Bud formation of *A. laciocarpa* seedlings exposed to High Pressure Sodium (HPS) light or HPS + blue light (BL) treatment. There were 17-18 plants grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was 22° C., the level of CO<sub>2</sub> was ambient (400ppm).

Bud score	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
<b>HPS</b>						
Green bud (%)	-	-	-	-	6%	6%
Brown bud (%)	11%	11%	6%	11%	6%	6%
Total	11%	11%	6%	11%	12%	12%
<b>HPS+BL</b>						
Green bud (%)	-	-	12%	6%	6%	6%
Brown bud (%)	24%	24%	29%	18%	36%	29%
Total	24%	24%	41%	24%	42%	35%

Table 2 shows the bud formation in *A. laciocarpa* seedlings grown in different light quality. There were more buds formed in HPS+BL compared to HPS only. In week 4, it was observed 11% brown buds in HPS and 24% brown buds in HPS+BL. The same pattern was observed in week 5. In week 6, 6% of the plants grown in HPS showed green buds, while 29% brown and 11% green buds were observed under HPS+BL. In week 7, 11% brown were observed in HPS, while 18% brown and 6% green buds were observed in HPS+BL. In 8<sup>th</sup> week 12% brown and green buds in HPS (Table 2) and 36% of the plants in HPS+BL had brown buds. Calculation from the data in week 9 showed that plants from HPS with brown buds formed in week 2 turned to green and then tiny needles appeared or bud burst. Hence, this shows that *A. laciocarpa* is very unstable and alternate between vegetative growth and terminal bud formation. In addition, previous experiment have shown that *A. laciocarpa* seedlings exposed to different light climate easily make buds also in long day conditions (Jetmundsen, 2015). In their experiment, 100% of the plants produced buds in short day (SD) plants in short day after 41 days but 30% of the plants produced buds in long days under UV-B plants did not reveal a clear effect of bud development (Jetmundsen, 2015).

Further, according to Aas (2015) bud formation in *A. laciocarpa* is highly sensitive to light quality. In their experiment 100% of the plants developed buds in SD after 66 days of light treatment compared to 92% plants with buds under red light treatment, 75% plants with buds under blue light treatment and 35% plants with buds under far red light treatment. In this experiment *A. laciocarpa* formed buds that changed back to vegetative growth again. The buds are probably not fully dormant and therefore it can go back to vegetative growth. It is known that fully dormant buds required prolonged or severe promotive treatment to break dormancy and renew growth (Hopkin et al., 2009). More buds were formed in HPS+BL compared to HPS in *A. laciocarpa*. It can indicate that blue light is involved in dormancy induction of this species but it can also be stress related. A high proportion of blue light can induce stress to some species for instance in the cultivation of spinach blue irradiation is not suitable due to extreme decreased in shoot dry weight (Ohashi-Kaneko et al., 2007). In *P. abies* no buds were observed during the experimental period and indicate that this species is very robust. This has also been observed by Riikonen et al., (2015) that Norway spruce seedlings did not form terminal buds during their experimental period in any of the light treatment (1) 25% B + 70% R + 5% FR, (2) 25% B + 75% R, (3) 55% B + 45% R, (4) HPS: 6% B + 44% G + 41% R + 9% FR whereas, the scots pine seedlings were studied and formed buds after 10 to 11 weeks from sowing. Thus, different species behave differently and some are more robust than others.

### 3.1.3 Water loss measurement

Table 3. Effects of light quality treatments: (HPS and HPS + Blue light) on water loss measurement of *A. laciocarpa* and *P. abies* (means  $\pm$  SE,  $n = 5$  for both species and treatment). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was 22° C., the level of CO<sub>2</sub> was ambient (400ppm). The water loss measurements were done from 19-20 August 2015.

	HPS	HPS+BL	Significant Differences
<b><i>A. laciocarpa</i></b>			
Water loss/hour/ needle	0.0036 $\pm$ 0.0006	0.0027 $\pm$ 0.0004	*
Water loss/hour/ FW	0.7687 $\pm$ 0.2893	0.5721 $\pm$ 0.1339	NS
<b><i>P. abies</i></b>			
Water loss/needle/ hour	0.0006 $\pm$ 0.0001	0.0014 $\pm$ 0.0005	*
Water loss/ FW /hour	0.1574 $\pm$ 0.0318	0.4023 $\pm$ 0.1136	*

Significance levels based on the overall effects of light treatment interactions on specific plant species according to General Linear Model Analysis of variance and Tukey Pairwise Comparisons: Response = morphological parameters.

Significance levels: NS, not significant ( $p < 0.1$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

Table 3 shows the effects of light quality treatments (HPS and HPS+Blue light) on water loss measurement of *A. laciocarpa* and *P. abies* seedlings. Water usage (mg water/needle/hr) was measured on five seedlings per species per treatment. The water usage (mg/needles/hr) was calculated by dividing the water loss (in mg) on the number of needles and hours (20). *A. laciocarpa* grown under HPS had 0.009 mg/needle higher water loss per hour compared to needles grown under HPS+BL light treatment (Table 3). The same trend was found also when calculating the water loss based on fresh weight (Table 3). An opposite effect of light quality on water loss was found in *P. abies*. In this species, a significant higher water loss was found in plants exposed to HPS+BL compared to HPS (Table 3). The plants exposed to HPS+BL showed more than 50% higher water loss compared to HPS.

Normally blue light stimulate stomatal opening and it is common to see higher stomatal number in blue light (Terfa et al 2013; Taiz et al. 2002; Kendrick and Kronenberg, 1994). Stomatal opening is promoted by both red and blue light according to Salisbury and Ross (1985), but more sensitive to blue than red light for (Salisbury and Ross 1985; HOPKINS WG y HÜNER, 2009). Opening and closing of stomata is to balance water loss allow the intake of CO<sub>2</sub> to facilitate photosynthesis. In the case of *P. abies* and in the needles had a higher water loss in the treatment with additional blue light and could be due to more open stomata and maybe more stomata that allow higher water loss or transpiration. *A. laciocarpa* behaved opposite but only in water loss/needle. In this species, the data was also more variable (Table 3). However, the lower transpiration in additional blue light in this species can indicate that the plant were stressed as discussed above. Stomatal closure is an indication of stress and often will high abscisic acid (ABA) level be induced under stressful conditions. ABA is also involved in stomatal closure. It would have been interesting to measure ABA level in *A. laciocarpa*. A higher level has been found in *Rosa x hybrida* with increasing blue light proportions (Terfa, 2013).

### 3.1.4 Chlorophyll content measurement

Table 4. Effects of light quality treatments: (HPS and HPS + Blue light) on chlorophyll content measurement of *A. laciocarpa* and *P. abies* (means  $\pm$  SE,  $n = 5$  for both species and treatment) divided on number of needles or fresh weight (FW). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm).

	HPS	HPS+BL	Significant Differences
<b><i>A. laciocarpa</i></b>			
Chlorophyll A/ needles	$0.57 \pm 0.21$	$0.60 \pm 0.18$	NS
Chlorophyll B/ needles	$0.39 \pm 0.31$	$0.66 \pm 0.29$	NS
Chlorophyll A/FW	$113.47 \pm 33.03$	$125.78 \pm 44.69$	NS
Chlorophyll B/FW	$65.90 \pm 29.91$	$130.94 \pm 29.84$	*
Chlorophyll A/B needles	$2.04 \pm 1.06$	$1.07 \pm 0.61$	NS
Chlorophyll A/B fW	$2.04 \pm 1.06$	$1.07 \pm 0.61$	NS
<b><i>P. abies</i></b>			
Chlorophyll A/ needles	$0.14 \pm 0.03$	$0.12 \pm 0.03$	NS
Chlorophyll B/ needles	$0.10 \pm 0.06$	$0.09 \pm 0.04$	NS
Chlorophyll A/FW	$38.50 \pm 5.17$	$35.39 \pm 8.11$	NS
Chlorophyll B/FW	$24.35 \pm 9.44$	$30.03 \pm 5.91$	NS
Chlorophyll A/B needles	$1.84 \pm 0.84$	$1.67 \pm 1.48$	NS
Chlorophyll A/B FW	$17.95 \pm 13.52$	$33.37 \pm 30.07$	NS

Significance levels based on the overall effects of light treatment interactions on specific plant species according to General Linear Model Analysis of variance and Tukey Pairwise Comparisons: Response=morphological parameters.

Significance levels: NS, not significant ( $p < 0.1$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

The needles in plants exposed to HPS+BL looked more green but no significant differences were found in chlorophyll *a*, chlorophyll *b* and chlorophyll *a/b* ratio in the end of the experiment (Table 4). The only significant difference was found in *A. laciocarpa* exposed to HPS+BL based on FW measurements. In this treatment 50% higher content of chlorophyll *b* was found in HPS+BL compared to HPS (Table 4).

Normally plants produced in more blue light have higher chlorophyll content because chlorophyll is a light absorbing pigment that absorbed blue and red light wavelength (Taiz et al. 2002). In *Rosa x hybrida* 'Toril' grown under LED 80% red and 20% blue showed higher chlorophyll and anthocyanin content and more thorns compared to HPS grown plant (Terfa et al., 2012). Whereas, in the study of Islam et al., (2012) the leaves of poinsettia grown under LED 20% blue and 80% red had lower chlorophyll content and total dry mass accumulation compared to plant grown under HPS lamp with 5% blue. In other species such as lettuce *Lactuca sativa L.* the pigment concentrations of anthocyanin and carotenoid were increased by 31% and 12% under blue light with  $130 \text{ umol m}^{-2} \text{ s}^{-1}$  respectively compared to UV-A 18, G-130, R-130, and FR-160  $\text{umol m}^{-2} \text{ s}^{-1}$  using LEDs spectra (Li et al., 2007). Manipulation of different light quality is useful to achieved higher productivity or high nutritional quality of different crops but the effectiveness of light quality treatment is depending on plant species. Thus, growth and development responses and accumulation of pigments of plants is dependent on species-specific. The variable results in the presented experiment can be explained by the method. The method used in this experiment in extracting chlorophyll content was not the best because of huge variation between the samples. The seedling was used in the extraction but maybe it would be better to use similar number of needles in each sample, i.e. 10 needles in each plant instead of using all needles from each plant instead of using the whole plant because of big variation between plants.

### 3.1.5 Morphological measurement in the end of the experiment

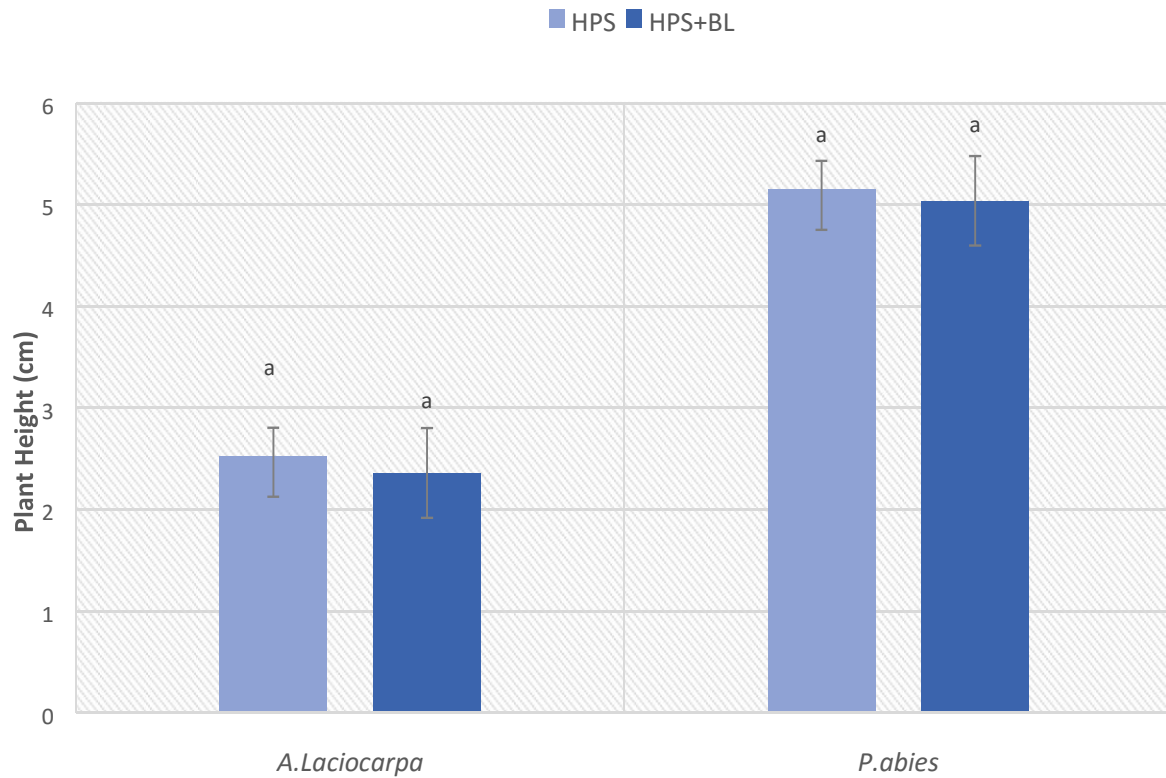


Figure 16: Effect of different light quality treatments: High Pressure Sodium (HPS) compared with HPS+ blue light (BL) on plant height (cm) of *A. laciocarpa* and *P. abies* measured after 9 weeks of growth. The length was measured from the rim of the pot to the plant apical meristems at end of the experiment 18 august 2015. The results are mean  $\pm$  SE of (n= 8 *A. laciocarpa*) (n= 10 *P. abies*) per treatments). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Different letters in different species indicate significant differences by Tukey Pairwise Comparisons ( $p < 0.05$ ).

Figure 16 shows the plant height of *A. laciocarpa* and *P. abies* seedlings grown in different light quality treatments. There was no significant difference in plant height between HPS and



HPS+BL in any of the species. *P. abies* plants were about 50% taller than *A. laciocarpa* plants at the end of the experiment.

The plant height of *A. laciocarpa* and *P. abies* seedlings exposed under HPS+BL were not significantly shorter compared to HPS. In the study of Riikonen et al., (2015) with Norway spruce seedlings taller plants were found under HPS than those grown under 25 B/R and 55 B/R light quality treatments. In this experiment and Riikonen et al., (2015) study that Norway spruce grown under the highest proportion of blue light did not show a significant height reduction. Blue light normally leads to shorter stems in fact, 400-500 nm blue region of action spectrum for inhibition of stem elongation is believed to be sensed by the cryptochrome and affect both cell elongation and cell number (Taiz et al., 2002; Lin, 2002). In the study of Islam et al., (2012) poinsettia grown under LED 20% blue and 80% red showed 20%-34% reduction in plant height compared to plants grown under HPS with 5% blue. Also, in the study of Terfa et al. (2013) *Rosa x hybrida* plants grown under LED (20% blue light) showed a significant reduction in stem elongation compared to plants grown under HPS (5% blue light). In the study of (Bergstrand and Schüssler, 2013) the morphology of ornamental plants that grown under different light spectra of red/blue (8:1) LED and white LED a reduction in stem elongation grown during autumn period and a delayed in developmental was found in chrysanthemum, kalanchoe and euphorbia compared to plants grown under HPS with significant difference. However, it seems that different plant species respond differently.

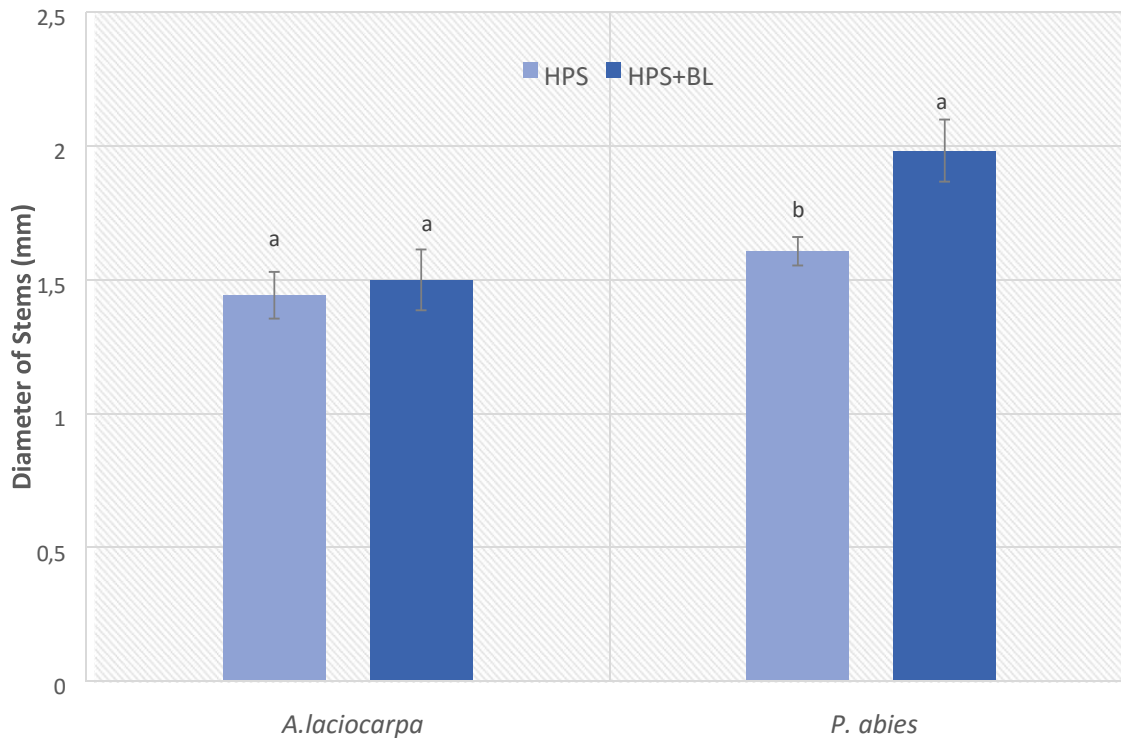


Fig. 17: Effect of different light quality treatments: High Pressure Sodium (HPS) compared with HPS+ blue light (BL) on the stem diameter (mm) of *A. laciocarpa* and *P. abies* measured after 9 weeks of growth and measured (at the middle of the stem) from the level of soil surface until apical meristem of the plant. Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Different letters in different species indicate significant differences by Tukey Pairwise Comparisons ( $p < 0.05$ ).

Both species exposed to HPS+BL had increased stem diameter compared to HPS. However, only *P. abies* showed a significant difference (Fig.17). In this species the plants exposed to HPS+BL had 48% thicker stems compared to plants exposed to HPS. No significant difference in plant stem diameter between HPS and HPS+BL was found in *A. laciocarpa*.

The same result was found in the study of Riikonen et al., (2015) that Norway spruce and scots pine seedlings grown under the highest proportion of blue light had increased stem diameter resulting in the highest shoot sturdiness. The increased stem diameter could be, at least partly, a

result of the higher water loss from this treatment. Plants that need to transport a high amount of water usually develop their vasculature tissue to do so and can result in thicker stems.

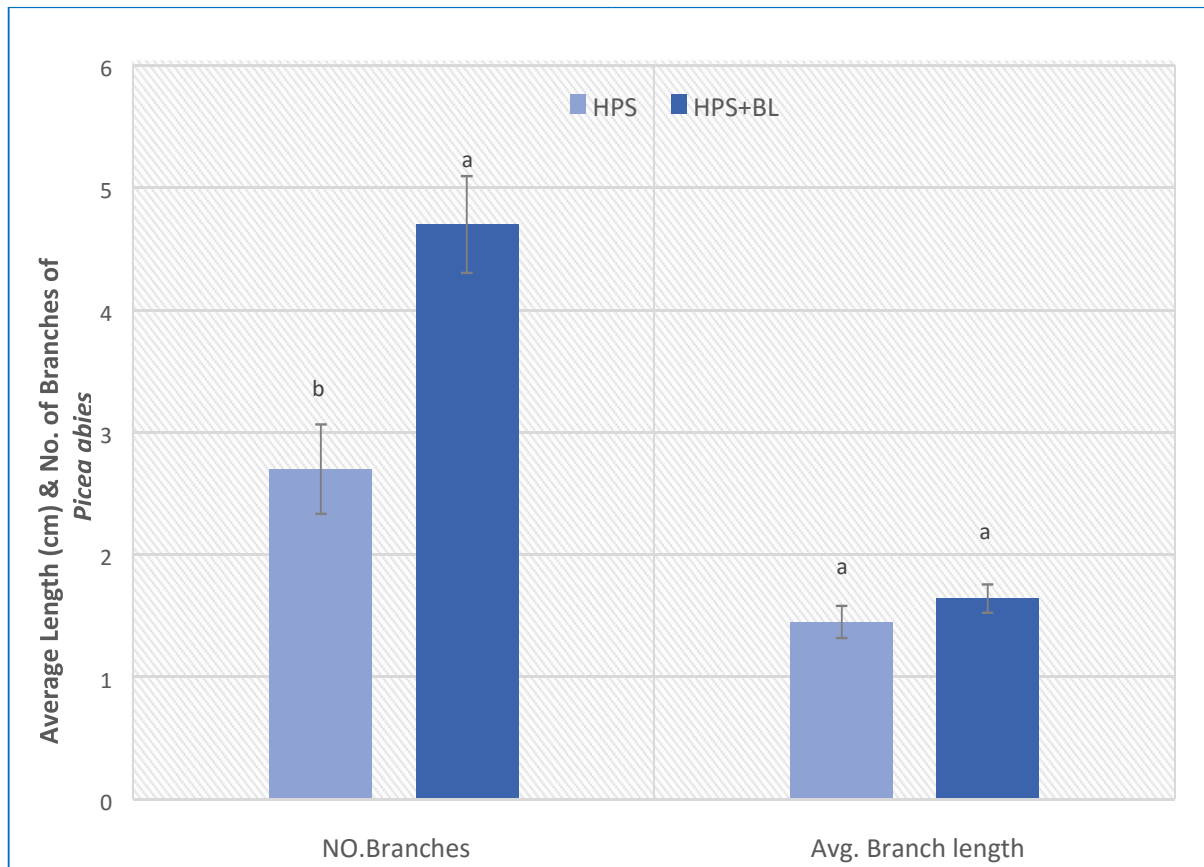


Figure 18: Effect of different light quality treatments: High Pressure Sodium (HPS) compared with HPS+ blue light (BL) in number of branches and average branch length (cm) in *P. abies* measured after 9 weeks of growth treatment 18<sup>th</sup> August 2018. The results are mean  $\pm$  SE of (n= 10 *P. abies*) per treatments. Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was 22° C., the level of CO<sub>2</sub> was ambient (400ppm). Different letters in different species indicate significant differences by Tukey Pairwise Comparisons (p<0.05).

Figure 18 shows number of branches and average branched length (cm) of *P. abies* seedlings grown in different light quality treatments. *P. abies* plant exposed to HPS+BL had higher number of branches (28%) compared to HPS and a significant differences was observed between two light quality treatments. However, the branch lengths were not significantly different between the two light qualities. *A. laciocarpa* did not make any branches.

The fact that *P. abies* plant exposed to HPS+BL had significantly higher number of branches is in accordance to the result of Riikonen et al., (2015). They found that scots pine seedlings grown under the highest proportion of blue light had increased branched density and more pronounced branched length.

### 3.1.6 Biomass accumulation in different plant parts

#### 3.1.6.1 Fresh weight of *A. laciocarpa* seedlings

Table 5. Effects of light treatment (HPS and HPS + Blue light) on morphological parameters of *A. laciocarpa* Subalpine fir (means  $\pm$  SE,  $n = 8$  for each treatment). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Measured on 19 August 2015. The numbers in brackets are percentage (%) of total Fresh Weight (g).

Parameters	Light Treatment		Statistical Significance
	HPS	HPS+BL	
Fresh Weight Needles(g)	$0.28 \pm 0.03$ (43%)	$0.24 \pm 0.03$ (38%)	NS
Fresh Weight Stems (g)	$0.07 \pm 0.01$ (12%)	$0.09 \pm 0.01$ (14%)	NS
Fresh Weight Roots (g)	$0.29 \pm 0.04$ (45%)	$0.30 \pm 0.04$ (49%)	NS
Avg. Longest Root (cm)	$11.32 \pm 1.48$	$8.75 \pm 0.79$	NS

Significance levels based on the overall effects of light treatment interactions on specific plant species according to General Linear Model Analysis of variance and Tukey Pairwise Comparisons: Response=morphological parameters.

Significance levels: NS, not significant ( $p < 0.1$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

Table 5 shows the effect of light quality on fresh weight (g) parameters of *A. laciocarpa* seedlings. No significant differences were found in fresh weight (g) of needles, stems or roots. Also no significant difference was found in the length of the longest root (Table 5). Although in terms of total fresh weight distribution a higher percentage was found in fresh weight of needles under HPS treatment compared to HPS+BL (Table 5).

### 3.1.6.2 Fresh weight of *P. abies* by seedlings

Table 6. Effects of light quality treatments: (HPS and HPS+Blue light) on the morphological parameters of ‘*Picea abies*’ Norway Spruce (means  $\pm$  SE (percentage),  $n = 10$  for each treatment). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Measured on 19 August 2015. The numbers in brackets are percentage (%) of total Fresh Weight (g).

Parameters	Light Treatment		Statistical Significance
	HPS	HPS+BL	
Fresh Weight Needles(g)	$0.51 \pm 0.04$ (50%)	$0.78 \pm 0.08$ (51%)	**
Fresh Weight Stems (g)	$0.08 \pm 0.01$ (8%)	$0.12 \pm 0.01$ (8%)	*
Fresh Weight Roots (g)	$0.41 \pm 0.06$ (41%)	$0.64 \pm 0.10$ (42%)	NS
Avg. Longest Root (cm)	$16.73 \pm 2.28$	$13.07 \pm 1.56$	NS

Significance levels based on the overall effects of light treatment interactions on specific plant species according to General Linear Model Analysis of variance and Tukey Pairwise Comparisons: Response=morphological parameters.

Significance levels: NS, not significant ( $p < 0.1$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

Table 6 shows the effects of light quality on fresh weight parameters of *P. abies* seedlings. HPS+BL light quality treatment induced a significant higher fresh weight of needles were 35% higher and the fresh weight of the stems were 33% higher in plant exposed to HPS+BL compared to HPS respectively. Although a higher root fresh weight (g) was observed in plants exposed to HPS+BL light compared to HPS only but the data was not significantly different (Table 6). HPS light treatment induced 12% longer roots (cm) compared to HPS+BL but the data was not

significantly different. The percentage distribution of the total fresh weight between the different light quality treatments was almost the same (Table 6).

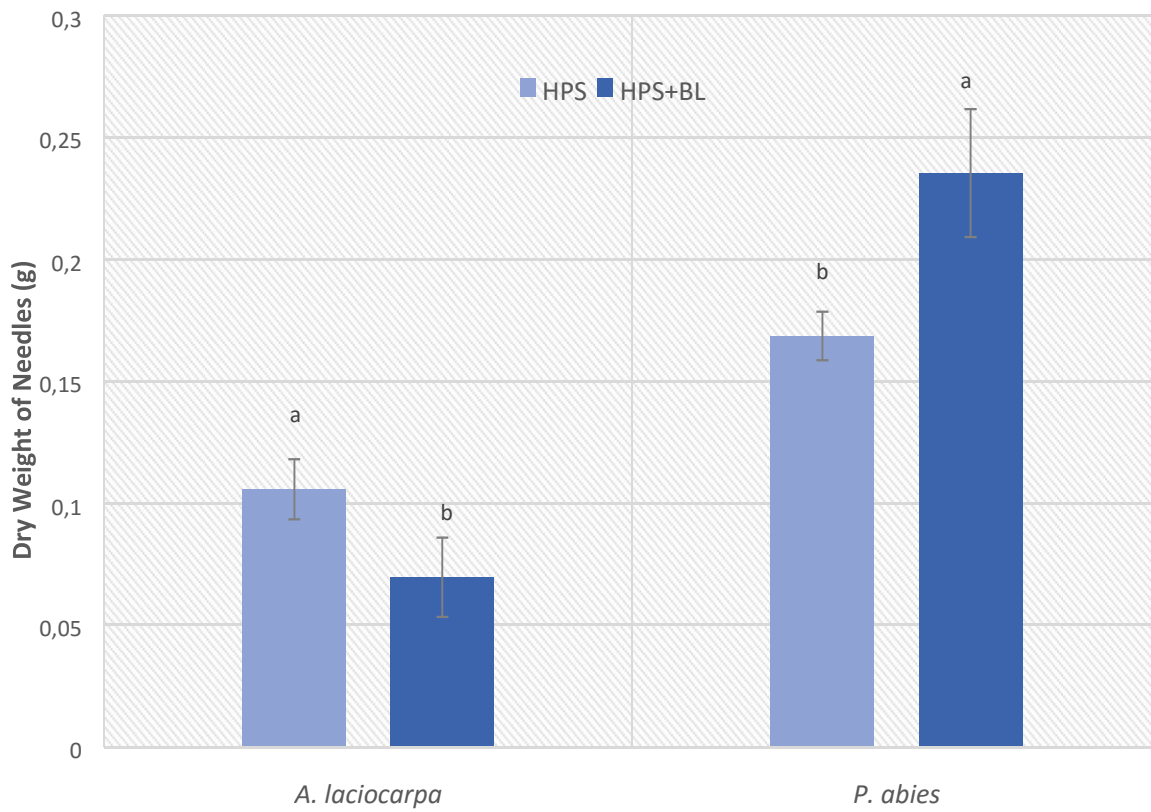


Fig.19: Effect of different lights treatments: High Pressure Sodium (HPS) compared with HPS+ blue light (BL) on plant dry weight (DW) needles (g) of *A. laciocarpa* and *P. abies*. The needles were removed from the stem using finger or forceps. Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Measured 1 week after being placed in dry chamber at  $20^\circ \text{C}$ elsius, 26 August 2015. Different letters in different species indicate significant differences by Tukey Pairwise Comparisons ( $p < 0.05$ ).

Figure 19 shows the dry weight needles of *A. laciocarpa* and *P. abies* seedlings grown in different light quality treatments. *P. abies* plants exposed to HPS+BL had a significantly higher of needles (g) compared to HPS only. The dry weight increased 58% in plants exposed to HPS+BL compared to HPS. On the other hand, the dry weight of needles (g) in *A. laciocarpa* were significantly higher in the HPS treatment compared to HPS+BL found higher in HPS treatment compared to HPS+BL (20%). The result from DW of needles show similar pattern as the water loss results in the two species. In *A. laciocarpa* a higher water loss and the highest DW was found in the HPS treatment compared to the HPS+BL. On the other hand, *P. abies* showed the highest water loss and the highest DW in HPS+BL compared to HPS. This could indicate that additional blue light induce stress or growth inhibition in *A. laciocarpa* but lack of blue light induce stress and growth inhibition in *P. abies*.

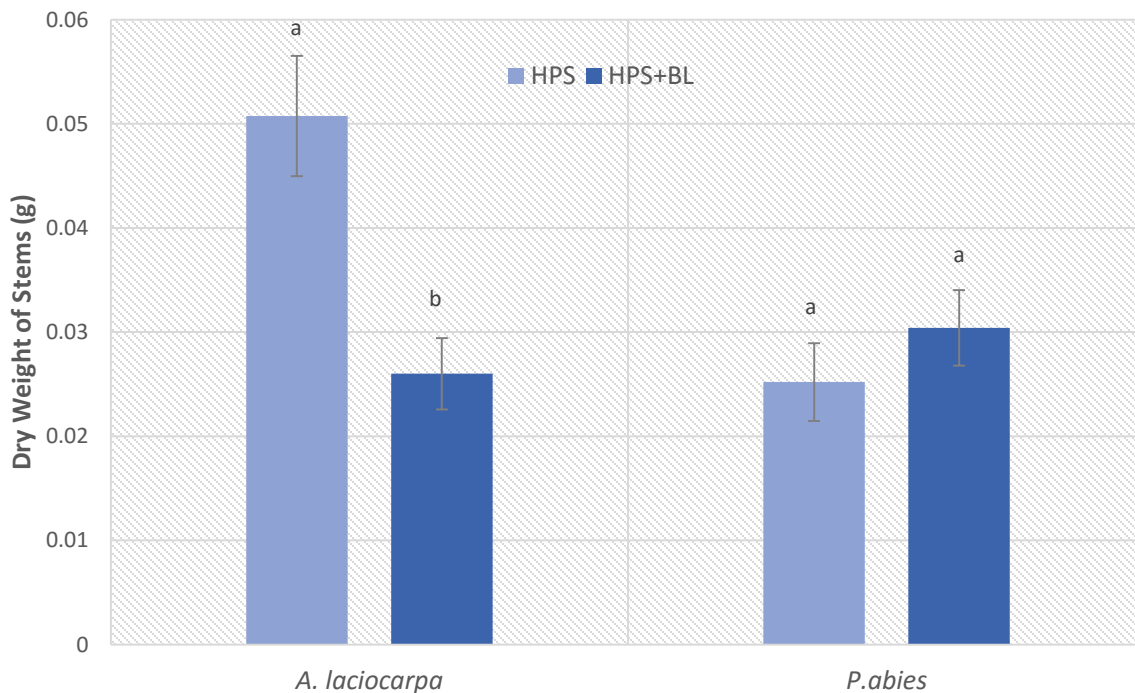


Figure 20: Effect of different lights treatments: High Pressure Sodium (HPS) compared with HPS+ blue light (BL) on plant dry weight stems (g) of *A. laciocarpa* and *P. abies*. The stems were cut from the level of soil surface and stems were separated by removing the needles using finger or forceps. Plants were

grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was 22° C., the level of CO<sub>2</sub> was ambient (400ppm). Measured 1 week after being placed in dry chamber at 20° Celsius, 26 August 2015. Different letters in different species indicate significant differences by Tukey Pairwise Comparisons (p<0.05).

Figure 20 shows dry weight of stems (g) in *A. laciocarpa* and *P. abies* seedlings grown in different light quality treatments. *A. laciocarpa* plants exposed to HPS had a significant higher dry weight of stems (g) 32% compared to HPS+BL. On the other hand, the dry weight of the stems (g) in *P. abies* were not significantly different in the two light treatments. The fact that 30% of the *A. laciocarpa* produced with additional blue light made buds during the experiment can explain the significant difference in stem DW in this species. In *P. abies* none of the plants formed buds.



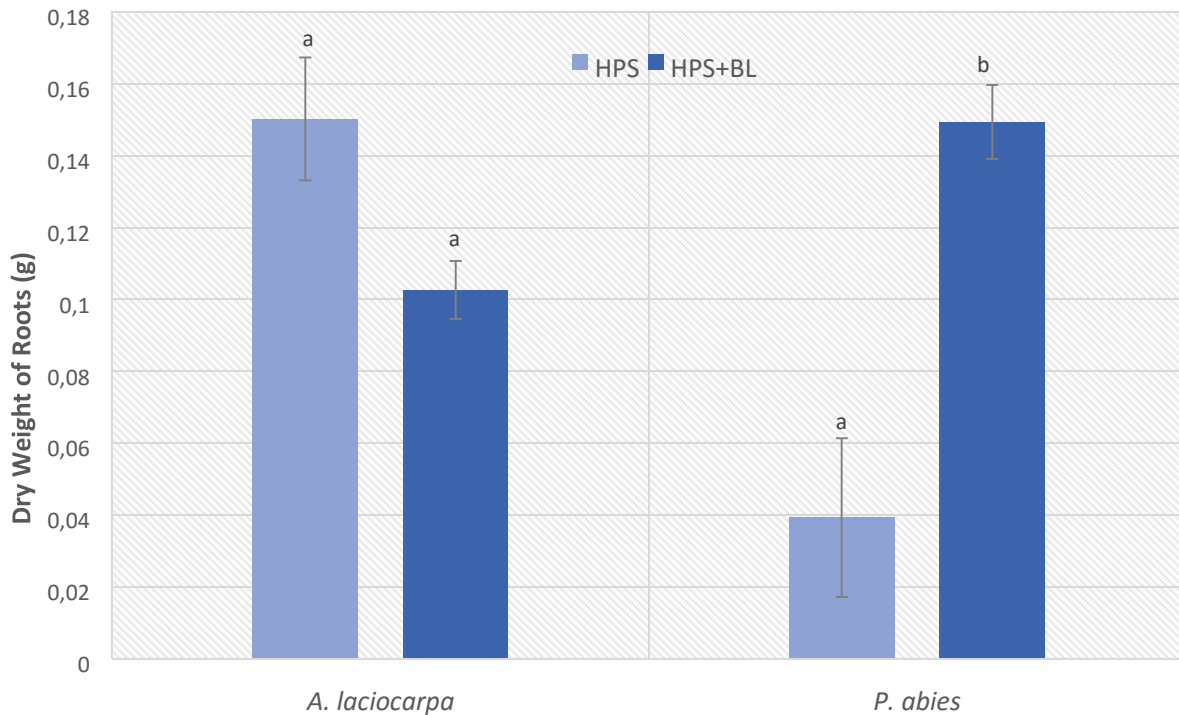


Figure 21: Effect of different lights treatments: High Pressure Sodium (HPS) compared with HPS+ blue light (BL) on plant Dry Weight Roots (g) of *A. laciocarpa* and *P. abies*. Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Measured 1 week after being placed in dry chamber at  $20^\circ \text{C}$ elsius, 26 August 2015. Different letters in different species indicate significant differences by Tukey Pairwise Comparisons ( $p < 0.05$ ).

Figure 21 show dry weight roots (g) of *A. laciocarpa* and *P. abies* seedlings grown in different light quality. In *P. abies* plants exposed to HPS+BL had a significant higher dry weight of roots (g) compared to HPS (58%) and it was found to be 4 times higher (Fig. 21). On the other hand, the dry weight roots of *A. laciocarpa* were higher in HPS treatment compared to HPS+BL but the data was not significantly different from each other.

The fresh weight biomass accumulation in different plant parts needles, stems and roots of *A. laciocarpa* and *P. abies* seedlings showed a higher weight accumulation when plant exposed to additional blue light except for *A. laciocarpa* species that accumulate higher in fresh weight of needles grown under HPS by 5% compared to HPS+BL. Also in figure 22-25 shows the root

system external structure of both species exposed to different light quality treatments in which *P. abies* with additional blue light the roots are thicker, many root hairs compared to roots under HPS (Figure 24 & 25). Also in *A. laciocarpa* the same trend was observed, plants exposed to HPS+BL have thicker roots and many root hairs compared to plants under HPS (Figure 22 & 23).

In the study of Riikonen et al. (2015) the growth under the light treatment of 25% B + 75% R result in the highest root-to-shoot ratios in *P. abies* and Scot pine seedlings but the highest root growth capacity and water use efficiency was found in Scots pine. Also blue light is known to increase dry matter production and carbohydrate accumulation in plants (Terfa et al., 2012). But, in the study of Bergstrand and Schüssler, (2013) in chrysanthemum and kalanchoe plants the fresh and dry mass (g) accumulation are significantly lower under red/blue LED (350W) compared to HPS (250 W). Thus, the alteration of different light quality treatments affect differently between species. Also, the growth and survival of seedlings planted in the different field are influence by seedling quality, nursery practices, and handling of nursery stock. According to (Kozłowski and Pallardy, 1997) loss of smaller absorbing roots or root-hair like during stock handling leads to dehydration of transplanted trees. Also, according to Pule, (2003) *P. Abies* can grow in different soil physical and chemical conditions as long as with sufficient soil aeration however, the root is very sensitive to any dislocation of its primary taproot. Thus, the important requirements for survival of transplanted trees are high root-shoot ratio, rapid growth of roots into a large volume of soil and to maintain high rates of absorption of water and mineral nutrients. Thus, plants with more developed roots are expected to have a higher survival rate in the fields despite the prevailing circumstances effect of different climatic factors.



Figure 22. Pictures of *A. laciocarpa* seedling the root system grown under HPS+BL



Figure 23. Pictures of *A. laciocarpa* seedling the root system grown under HPS



Figure 24. Pictures of *P. abies* seedling the root system grown under HPS+BL



Figure 25. Pictures of *P. abies* seedling the root system grown under HPS

### 3.1.7 Dry matter distribution of *A. laciocarpa* and *P. abies*

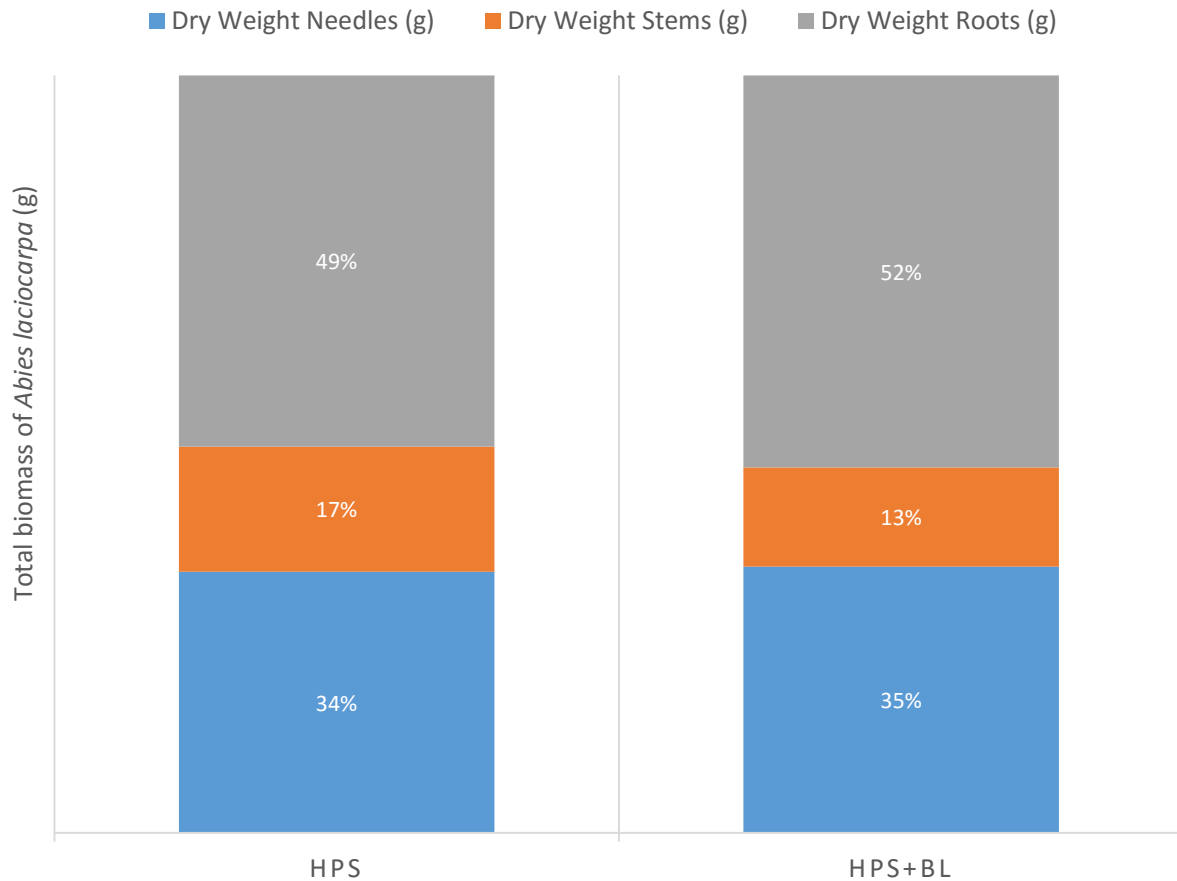


Figure 26: Total biomass (DW) and mean distribution of DW biomass between the needles, the stems and the roots of *A. laciocarpa* (n= 8 for each light treatments). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Measured 1 week after being placed in dry chamber at  $20^\circ \text{Celsius}$ .

Figure 26 shows the percentage distribution of dry weight biomass of *A. laciocarpa* seedlings grown in different light quality treatments. The distribution was almost the same in the different light quality and only small differences appeared.

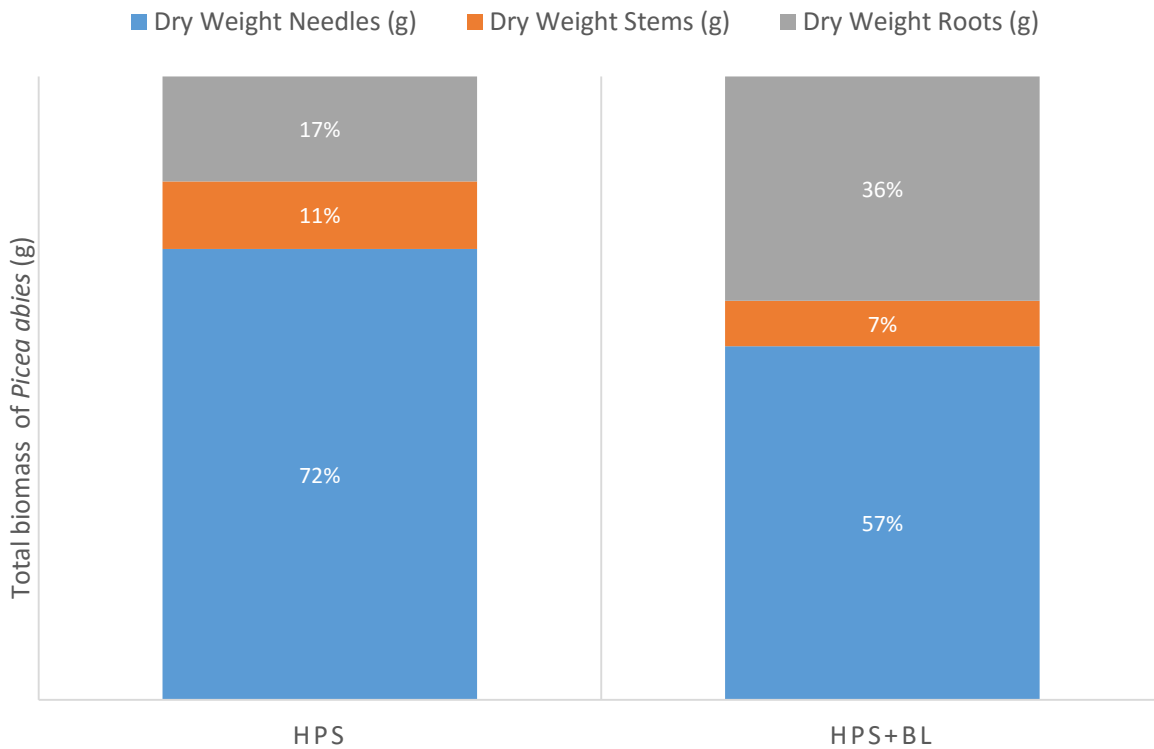


Figure 27: Total biomass (DW) and mean distribution of DW biomass between the needles, the stems and the roots of *P. abies* (n=10 for each light treatments). Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). Measured 1 week after being placed in dry chamber at  $20^\circ \text{C}$ elsius.

Figure 27 shows the DM distribution in *P. abies*. In this species a clear change appeared in the different light quality treatments. In HPS+BL more of the DM was allocated to the roots (20%) compared to the HPS. In the HPS treatment more of the DM was allocated to the needles (15%) compared to HPS+BL. The DM allocation to the stem was almost similar in the two light quality treatments (Fig. 27).

*P. abies* is more responsive to blue light and additional blue light caused changes in water loss and dry matter distribution. More dry mas allocated to the roots in blue light as they are sensitive and responsive to blue light. According to Terfa et al., (2012) blue light known to increase dry matter production and carbohydrate accumulation in plants. Nevertheless, in the study of Terfa



et al., (2013) higher blue light increased photosynthesis of rose leaf area and enhanced growth and morphological changes but does not affect total dry mass production and flowering time. *P. abies* grown under HPS+BL have higher water loss as they have bigger roots, they usually lose and up take more water. Maybe because they have bigger roots they were able to transpire more.

### 3.2 Experiment II: Stem Cuttings

#### 3.2.1. Plant survival of the main stems and side shoots

**Table 7.** Number of plant survival (% of total) propagated by the stem cuttings 9 and 4 weeks after sticking. The cuttings were treated with different light qualities (HPS and HPS+BL) for 9 weeks. Then the cuttings were transferred to greenhouse conditions for 4 weeks before evaluation.

Stem cuttings	No. of stem cutting propagated 23.06.15	Survival (% of total)			
		Growth Chambers 18.08.15		Greenhouse 22.09.15	
		HPS	HPS+BL	HPS	HPS+BL
<b><i>Abies laciocarpa</i></b>					
Main Shoots	24	63%	25%	58%	25%
Side Shoots	30	77%	67%	77%	67%
Total	54	70%	48%	70%	48%
<b><i>Picea abies</i></b>					
Main shoots	30	73%	60%	70%	53%
Side Shoots	24	67%	58%	58%	50%
Total	54	70%	59%	65%	52%

Table 7 shows the number of *A. laciocarpa* and *P. abies* cuttings that survived during the experiment 2 months in growth chambers in different light quality (HPS and HPS+BL) and after 1 month in the greenhouse (greenhouse conditions as described in materials and methods).

The number of *A. laciocarpa* cuttings that survived from different light treatments after two months in the chambers were the same that survived after 1 month in greenhouse compartment. Higher survival (%) as found in the side cuttings compared to the main shoots and more plants survived in HPS compared to HPS+BL. In addition, in *P. abies* more plants survived in HPS compared to HPS+BL but in this species only small difference between the main shoot and the side shoots were found and the survival was almost similar in the two cutting types.

According to Leakey 1983; Hannerz et al.1999; Vigl and Reward 2014 cited in (OuYang F. et al 2013) cuttings with a larger stem diameter and longer length provide better survival and growth under normal conditions. In this experiment, we found the same result the cuttings from the main shoots of *P. abies* had higher percentage of survival and growth by 6% under HPS and 2% under HPS+BL after the light quality treatment compared cutting from side shoots which had thinner stem diameter. In contrary with the above result, *A. laciocarpa* had higher percentage of survival and growth of cuttings were from side shoots with 14% under HPS and 42% under HPS+BL compared cuttings from main shoots.

### 3.2.2. Root morphology of cuttings

#### 3.2.2.1 *A. laciocarpa*

Table 8: Effects of light quality treatments: (HPS and HPS+Blue light) on Root systems of *A. laciocarpa* Subalpine fir from stem cuttings (means  $\pm$  SE,  $n = n = 38$  HPS treatment; 26 HPS+BL) grown for 9 weeks. Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h) 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was 22° C., the level of CO<sub>2</sub> was ambient (400ppm). Then, transferred to greenhouse compartment for 4 weeks until the end of experiment.

Parameters	Light Treatment		Statistical Significance
	HPS	HPS+BL	
No. of Roots	1.97 $\pm$ 0.61	2.38 $\pm$ 0.64	NS
Avg. (3) Longest Roots (cm)	2.31 $\pm$ 0.69	2.42 $\pm$ 0.54	NS
Diameter (0.5 cm above callus)	3.56 $\pm$ 0.25	3.31 $\pm$ 0.21	NS
Fresh Weight Roots (g)	0.08 $\pm$ 0.03	0.06 $\pm$ 0.02	NS
Dry Weight Roots (g)	0.02 $\pm$ 0.01	0.01 $\pm$ 0.00	NS

Significance levels based on the overall effects of light treatment interactions on specific plant species according to General Linear Model Analysis of variance and Tukey Pairwise Comparisons: Response=morphological parameters.

Significance levels: NS, not significant ( $p < 0.1$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

Table 8 shows the effect of light quality in different parameters *A. laciocarpa* cuttings. No significant difference was found between the two light quality treatments. However, parameters such as stem diameter (mm), fresh weight of roots (g), and dry weight of roots (g) were found higher in HPS treatment compared to HPS+BL treatment by 4%, 14% and 34% respectively. Whereas parameters such as no. of roots and average root length were found higher in HPS+BL compared to HPS.

### 3.2.2.2 *P. abies*

Table 9. Effects of light quality treatments: (HPS and HPS+Blue light) on root systems of *P. abies* from stem cuttings (means  $\pm$  SE,  $n = n = 35$  HPS treatment; 28 HPS+BL) grown for 9 weeks Plants were grown in different controlled growth chambers for 58 days under continuous lighting (24h)  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ . In both chambers the daily average relative humidity was 85%, the temperature was  $22^\circ \text{C}$ ., the level of  $\text{CO}_2$  was ambient (400ppm). But then, transferred to greenhouse compartment for 4 weeks until the end of experiment.

Parameters	Light Treatment		Significant Difference
	HPS	HPS+BL	
No. of Roots	$6.51 \pm 1.09$	$6.00 \pm 0.88$	NS
Avg. of (3) longest root (cm)	$7.57 \pm 1.12$	$8.81 \pm 0.85$	NS
Diameter (0.5 cm above callus)	$2.85 \pm 0.11$	$3.05 \pm 0.17$	NS
Fresh Weight Roots (g)	$0.33 \pm 0.04$	$0.45 \pm 0.06$	NS
Dry Weight Roots (g)	$0.06 \pm 0.01$	$0.07 \pm 0.01$	NS

Significance levels based on the overall effects of light treatment interactions on specific plant species according to General Linear Model: Analysis of variance and Tukey Pairwise Comparisons: Response = morphological parameters.

Significance levels: NS, not significant ( $p < 0.1$ ); \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

Table 9 shows the effect of light quality in different parameters *P. abies*' cuttings. No significant difference was found between the two light treatments.

In this experiment, the optimum rooting results were obtained from *P. abies* compared to *A. laciocarpa* with 80% higher number of roots (Table 8 & 9) and obtained from main shoots cuttings *P. abies* which had thicker stem diameter (Table 9) the effectiveness of rooting by larger cuttings can be explained by different factors according to OuYang et al., (2015) first, the level of endogenous auxins and other rooting inducing factors is lower in smaller cutting that leads to decreased rooting percentage or probably no roots in shoot cuttings, second factor larger cuttings stored more carbohydrates because root growth is dependent on the carbohydrates in leaf and stem, third factor the lower amount of macronutrients such as N, P, K will negatively affect the roots of stem cuttings. In this experiment, it was observed the root structure from the cuttings are different from the seedlings, in stem cuttings less tiny hair-like structure, more succulent and fragile rooting

systems. It is clear that we can propagate these two species through stem cuttings and seedlings but light quality seems to have a stronger effect on the seedlings than stem cuttings when it comes to root development. The potential after-effects of growth under different environmental conditions after transferred to outside conditions for 2-3 years and even after planting to forest site are still unknown.



Figure 28. Pictures of *A. laciocarpa* grown under HPS+BL cuttings from main shoots (top) cuttings from side shoots (down).



Figure 29. Pictures of *A. laciocarpa* grown under HPS cuttings from main shoots (top) cuttings from side shoots (down).

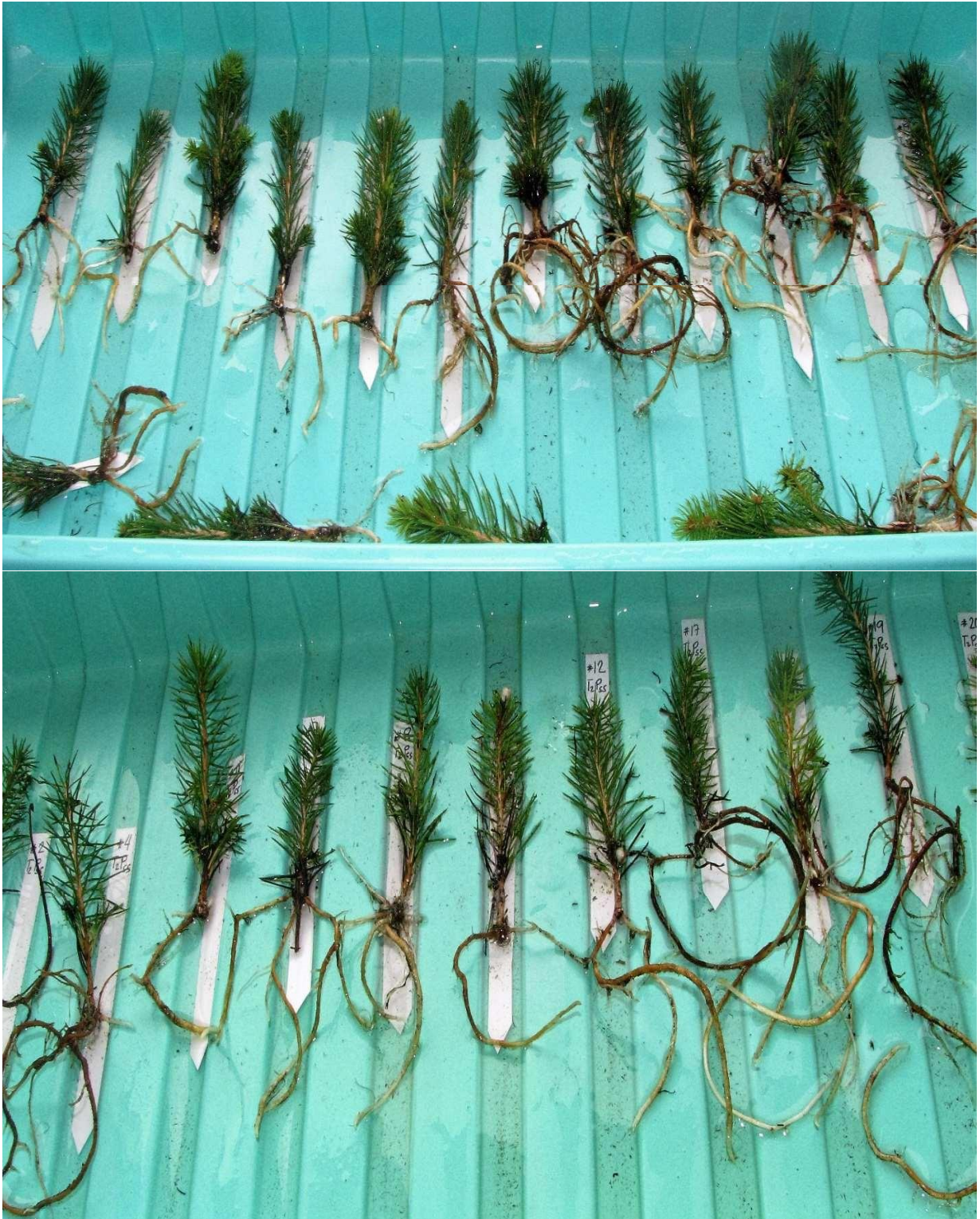


Figure 30. Pictures of *P. abies* grown under HPS+BL cuttings from main shoots (top) cuttings from side shoots (down).





Figure 31. Pictures of *P. abies* grown under HPS cuttings from main shoots (top) cuttings from side shoots (down).

## 4.0 Conclusions

Plant development and physiology are influenced by different light spectrum of the growth environment. In this experiment, the growth and morphology of the seedlings were modified by different light quality spectra.

- *P.abies* was more responsive to additional blue light compared to *A. laciocarpa* but no effect on plant height was found in any of the species.
- *P. abies* showed higher water loss, higher dry matter content, bigger roots and thicker stems in additional blue light compared to HPS.
- *A. laciocarpa* showed a weak response to additional blue light and in most cases an opposite response compared to *P. abies*.
- In *A. laciocarpa* the blue light had no effect on the DM distribution.
- The result from this thesis shows that the different species behave differently in response to additional blue light.
- Both species can be propagated by stem cuttings but no significant effect of light quality was observed in this experiment.
- The potential after-effects of the growth under different light quality are still unknown.

## 5.0 References

- Aas .O.T. 2015. Effect of light quality and temperature on elongation growth, dormancy and bud burst in Norway spruce (*Picea abies*) and Subalpine fir (*Abies laciocarpa*). Master Thesis, Department of Plant Science, Norwegian University of Life Sciences
- Alexander, R. R., Shearer, R. C. & Shepperd, W. D. 1990. *Abies lasiocarpa* (Hook.) Nutt. *Silvics of North America*, 1, 60-70.
- Bergstrand, K. & Schüssler, H. 2013. Growth, development and photosynthesis of some horticultural plants as affected by different supplementary lighting technologies. *Europ. J. Hort. Sci*, 78, 119-125.
- Cazanji, O. 2013. Effect of quality in day extension light on growth and winter bud formation in *Abies laciocarpa*. Term Paper. Department of Plant Science, Norwegian University of Life Sciences
- Fløistad, I. S. & Patil, G. G. 2002. Growth and terminal bud formation in *Picea abies* seedlings grown with alternating diurnal temperature and different light qualities. *Scandinavian journal of forest research*, 17, 15-27.
- Fosket, D. E. 1994. *Plant growth and development: a molecular approach*, Academic Press Inc.
- Hernández, R. & Kubota, C. 2014. Growth and morphological response of cucumber seedlings to supplemental red and blue photon flux ratios under varied solar daily light integrals. *Scientia Horticulturae*, 173, 92-99.
- Hopkins, W. G. & Hüner, N. P. 1995. *Introduction to plant physiology*, Wiley New York.
- Hopkins Wg Y Hüner, N. 2009. *Introduction of Plant Physiology*. John Wiley and Son, USA.
- Islam, M. A., Kuwar, G., Clarke, J. L., Blystad, D.-R., Gislerød, H. R., Olsen, J. E. & Torre, S. 2012. Artificial light from light emitting diodes (LEDs) with a high portion of blue light results in shorter poinsettias compared to high pressure sodium (HPS) lamps. *Scientia Horticulturae*, 147, 136-143.
- Jetmundsen M.R., 2015. The effect of UV-B and temperature on photoperiodic control of growth and bud set in Subalpine fir (*Abies laciocarpa*) and Norway Spruce (*Picea abies*). Master Thesis. Department of Plant Science, Norwegian University of Life Sciences

- Johkan, M., Shoji, K., Goto, F., Hashida, S.-N. & Yoshihara, T. 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience*, 45, 1809-1814.
- Kendrick, R. & Kronenberg, G. 1994. *Photomorphogenesis in Plants*, (Dordrecht, The Netherlands: Martinus Nijhoff/W. Junk Publishers). *Kendrick2Photomorphogenesis in Plants1994*.
- Kozłowski, T. T. & Pallardy, S. G. 1997. *Growth control in woody plants*, Elsevier.
- Li, X., Shiqi, L., Liandong, Q., Qingling, L. & Wenyan, Y. 2007. Effect of Light Quality on Leaf Lettuce Photosynthesis and Chlorophyll Fluorescence [J]. *Chinese Agricultural Science Bulletin*, 1, 022.
- Lin, C., Yang, H., Guo, H., Mockler, T., Chen, J. & Cashmore, A. R. 1998. Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. *Proceedings of the National Academy of Sciences*, 95, 2686-2690.
- Lin, C. 2002. Blue light receptors and signal transduction. *The Plant Cell*, 14, S207-S225.
- Nelson, J. A. & Bugbee, B. 2014. Economic analysis of greenhouse lighting: light emitting diodes vs. high intensity discharge fixtures.
- Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K. & Kurata, K. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environmental Control in Biology*, 45, 189-198.
- Ouyang, F., Wang, J. & Li, Y. 2015. Effects of cutting size and exogenous hormone treatment on rooting of shoot cuttings in Norway spruce [*Picea abies* (L.) Karst.]. *New Forests*, 46, 91105.
- Puhe, J. 2003. Growth and development of the root system of Norway spruce (*Picea abies*) in forest stands—a review. *Forest Ecology and Management*, 175, 253-273.
- Uchytel, R. J. 1991. *Abies lasiocarpa*. WC Fischer (compiler), *The Fire Effects Information System [Database]*, Missoula, MT: USDA Forest Service, Intermountain Research Station, Intermountain Fire Sciences Laboratory.
- Ragonezi, C., Klimaszewska, K., Castro, M. R., Lima, M., De Oliveira, P. & Zavattieri, M. A. 2010. Adventitious rooting of conifers: influence of physical and chemical factors. *Trees*, 24, 975-992.

- Runkle, E. & Both, A. 2011. Greenhouse energy conservation strategies. *Michigan State University-Extension Buletin*.
- Salisbury, F. & Ross, C. 1985. Stress physiology. *Plant Physiology*. 3d ed, Wadsworth Publishing Company, Belmont.
- Sullivan, J. 2012. Picea abies. *Fire effects information system, US Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer)*. <http://www.fs.fed.us/database/feis/>. Accessed, 8.
- Takemiya, A., Inoue, S.-I., Doi, M., Kinoshita, T. & Shimazaki, K.-I. 2005. Phototropins promote plant growth in response to blue light in low light environments. *The Plant Cell*, 17, 1120-1127.
- Taiz, L. & Zeiger, E. 2002. *Plant Physiology*. 3rd. Ed. Pub. Sinauer.
- Taiz, L. & Zeiger, E. 2010. *Plant Physiology Online*. Created by Sinauer Associates Inc.
- Terfa, M.T. 2013. Growth, stomatal responses and postharvest characteristics of *Rosa x hybrid*-*The influence of air humidity and light quality*. Philosophia Doctor (PhD) Thesis. Department of Plant and Environmental Sciences, Norwegian University of Life Sciences
- Terfa, M., Poudel, M., Roro, A., Gislerød, H., Olsen, J. & Torre, S. Light emitting diodes with a high proportion of blue light affects external and internal quality parameters of pot roses differently than the traditional high pressure sodium lamp. VII International Symposium on Light in Horticultural Systems 956, 2012. 635-642.
- Terfa, M. T., Solhaug, K. A., Gislerod, H. R., Olsen, J. E. & Torre, S. 2013. A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of *Rosa x hybrida* but does not affect time to flower opening. *Physiol Plant*, 148, 146-59.
- Valoya.com. Retrieved on 13 December 2015 on <http://www.valoya.com/>
- Wang, Y. & Folta, K. M. 2013. Contributions of green light to plant growth and development. *Am J Bot*, 100, 70-8.
- Wheeler, R., Mackowiak, C. & Sager, J. 1991. Soybean stem growth under high-pressure sodium with supplemental blue lighting. *Agronomy journal*, 83, 903-906.
- Wilkins, M. B. 1984. *Advanced plant physiology*.
- Wollaeger, H. M. & Runkle, E. S. 2014. Growth of impatiens, petunia, salvia, and tomato seedlings under blue, green, and red light-emitting diodes. *HortScience*, 49, 734-740.



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