

NORWEGIAN UNIVERSITY OF LIFE SCIENCES



**Responses of air humidity and light quality on growth and stomata
function of greenhouse grown *Rosa × hybrida***

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ABSTRACT

Single node stem segment with one mature leaf of *Rosa x hybrida*, cv. Toril were grown in chambers. Plants were exposed to $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 20 h day^{-1} followed by a 4 h dark period and supplementary light was provided by LED (20% blue light and 80% red light), HPS and HPS+UV-B Lamp. Each light source was provided by moderate (60%) and high (90) RH in different chambers. The UV-B light was provided 1 hour twice a day, in the middle of the dark period and in the middle of light period. The UV-B treatment was given in combination with HPS lamp as supplementary light. Thus, the main aim of the study was to investigate if light quality can improve the stomata function under high RH.

The experiment was divided into two parts, first HPS and LED and second the effect of UV-B. In the first experiment, the stems were significantly longer under HPS compared to LED, under moderate RH, LED suppressed pedicel length and induced shorter pedicels compared to HPS. The number of flowers and leaf area was not significantly affected by either RH or light quality. Comparatively, moderate RH had significantly more leaves per stem higher, dry weight of stem and higher dry weight of pedicel length and flower compared to high RH, but no significant effect of light quality was found on these parameters. At high RH, g_{sw} was still high during darkness for both HPS and LED and indicate lack of dark induced closure. However, LED reduced the conductance under high RH during light and darkness indicating reduced stomatal aperture and a better closure capacity when grown with LED. Moderate RH had a lower conductance under LED compared to HPS but both light qualities induced closure in darkness. Detached leaves from plants grown at high RH showed a rapid water loss compare to leaves grown at moderate RH. Stomatal length and pore aperture were significantly higher in plants grown at high RH compared to moderate RH. Plants developed at high RH with LED showed a desiccation tolerance than plant grown under high RH and HPS. In second experiment, both high and moderate humidity showed a similar pattern with the conductance decreasing through the time of one hour exposure of UV-B. The UV-B did not induce better closure rather the opposite was found and plants developed at high RH and exposed to UV-B had a higher water loss in a desiccation test. Comparison of flavonoids and anthocynins on high and moderate humidity of

UV-B treatment was found to be non-significant. Hence, the conclusion of this study is that light quality can be used a tool to improve the stomata function under high RH. By using LED with more blue light (20%) a better dark induced closure was found and a better desiccation tolerance was found compared to the traditional HPS lamps. The use of UV-B did not improve the stomata function under high RH but rather induced an even higher water loss. The interactive effect between light quality and air humidity needs further investigation but light quality can be one of the useful tools since LEDs are believed to be an important light source in greenhouse in the future.

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ABBREVIATIONS

%	Percent
⁰ C	Degree Celsius
ABA	Absciscic Acid
B	Blue
C3	Carbon fixation
CO ₂	Carbon dioxide
DIF	Difference between day and night temperatures
DT	Day temperature
g _{sw}	Stomatal conductance
HPS	High pressure sodium
LED	Light emitting diodes
Nm	Nanometer
NT	Night temperature
PAR	Photosynthetic active radiation
PPF	Photosynthetic photon flux
PPFD	Photosynthetic photon flux density
Pfr	Phytochrome in its far-red light absorbing
R	Red light (generally 600-700nm)
R: FR	Ratio of red light to far-red light
RH	Relative humidity
UV	Ultraviolet rays
Vpd	Vapor pressure deficit
(SKP)	Senter for Klimaregulert Forskning

1 INTRODUCTION

Flower opening as well as long keeping quality to a satisfaction level are important factors determining consumer satisfaction and choice (Evans et al. 1995). Currently, most quality manipulation strategies are focused on the postharvest phase but the shelf life of the product is also dependent on the environmental condition during growth and can be improved by changing the climate to the effect the stomatal functionality, regulating water loss and carbohydrate status (Fanourakis et al. 2013).

Light is regarded as the most important environmental factor acting as both as a sole source of energy as well as source of external information affecting growth and development. Red and blue light are efficiently absorbed by photosynthetic pigments than other spectral region (McCree 1971) . Maximum quantum yield occurs near 600nm, declining rapidly at wavelengths shorter than 400nm and greater than 680nm. To enable year round plant production, supplementary light is necessary to enhance photosynthesis with focusing on light intensity, duration and light quality. In the past, High Pressure Sodium (HPS) lamps were preferred as supplemental lighting. Nowadays, LEDs is the gaining popularity mainly because of their potentially higher energy efficiency and possibility to control light quality. We mainly concerned in our study about light quality because it influences plant morphology and developmental processes, mostly mediated by a set of blue, red and far-red photoreceptors (i.e. cryptochromes, phototropins and phytochromes). Light quality can prompt leaf deformations and epinasty, which can negatively influence on biomass production. Other important effects of light quality involve on the development of stomatal density and the control of stomatal aperture, which both attribute to stomatal conductance as well as leaf hydraulic resistance (van Ieperen 2012).

Relative humidity is defined as the ratio of the amount of water vapor in the air relative to the amount of water vapor that would be present at saturation and thus relative humidity is measured routinely in the greenhouse. Different air humidity (high 90% and moderate 60%) in

combination with light quality were studied in this research. Roses usually get unfunctional stomata when they are grown under high RH. They get higher rates of water loss, compared to roses grown at moderate RH, which show less responsive stomata to both water stress and darkness (Fanourakis et al. 2013). Roses with high water loss usually have a reduced potential to last during sale and in the consumer house. Thus, tools needed to be able to grow roses with functional stomata also in periods when the air humidity is high (autumn, winter).

The interaction between air humidity and light quality is not well studied. Hence, this project was designed to evaluate the interaction between light and humidity on modification of morphology and stomatal characteristics of *Rosa* × *hybrida*, to test if light quality can improve the stomata function under high RH without negative effects on morphology or flowering.

2 LITERATURE REVIEW

Roses are important perennial ornamental flower grown in greenhouse as well as in open field condition. It belongs to genus *Rosa* and family *Rosaceae* (Anonymous 2012). Rose shoot development follow a number of steps (Figure 1). After pinching, a new shoot appear at a leaf axil below the cut. This process is also known as bud break. Another visible events is unfolding of first leaf, shoot development can be followed by unfolding of subsequent leaves. Likewise, another visible event is emergence of flower bud. These flower buds grow and develop until the shoot is ready for harvest. Commercial cultivars are self-inductive that mean every shoot (except blind shoot) has capacity to form a flower (Halevy 1986).

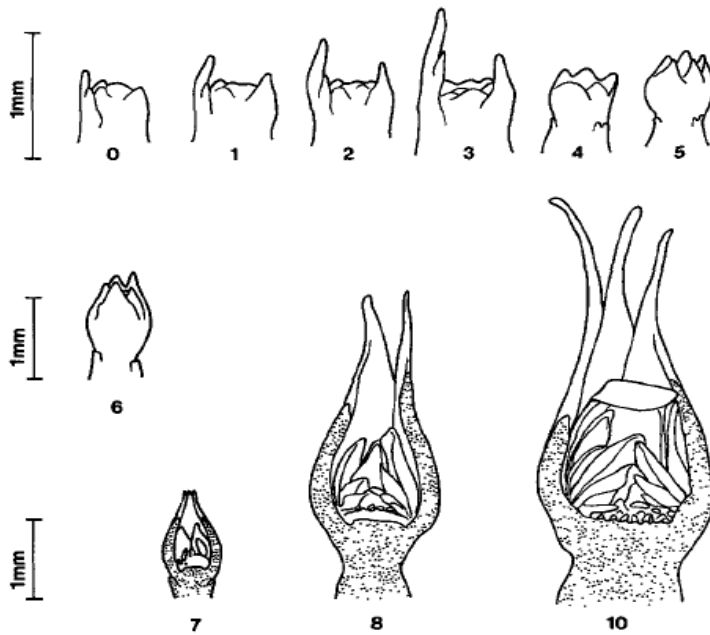


Figure 1. Different stages in the development of the reproductive apex of the rose flower from stage 0 to 10 (Horridge & Cockshull 1974).

Yield of the plant depends on environmental factors like light (intensity, quality and integral), carbon dioxide level, temperature, and relative air humidity. Four important processes in plants are controlled by light: photosynthesis, photoperiodism, phototropism and photomorphogenesis (Taiz and Zeiger, 2002). Photomorphogenesis is important part of our research in greenhouse production. Photomorphogenesis is defined as a change in plant shape (stem length, internode

length, leaf area) and is induced by a specific light quality and is not dependent on photosynthesis. Photomorphogenesis is controlled by photoreceptors that initiate changes. Two main photoreceptor are important in controlling morphogenesis and they are known as the phytochrome system and the cryptochrome (Moe & Heins 2000). The phytochrome system is sensing light mainly in the red and the far-red area and the cryptochrome in the blue area (Taiz and Zeiger, 2002). Usually, red light and blue light is causing short and compact plants. On the other hand, far-red and lack of blue light is causing more stem elongation and tall plants (Moe & Heins 2000).

2.1 Light

2.1.1 Use of Supplementary lighting in greenhouses

Norway is located far north from about 59° N to 71° N, and winter production of greenhouse plants therefore becomes rather limited without the application of supplemental lighting. Earlier, midwinter production of flowers was mostly forcing of bulbous and tuberous plants. The possibilities of supplementing natural daylight with artificial light has been an area with huge interest in Norway and makes it possible to produce plants the year around (Moe et al. 2005).

Light can affect several components of productivity and quality of roses, such as bud breaking, rate of flower abortion, formation of renewal shoots, time period between harvests, length, weight and diameter of stem and flower buds, leaf area and pigmentation of petals. Due to these reason supplementary lighting of relatively high levels of irradiance, especially during periods of low solar radiation have been common in pot roses and cut roses. Supplementary lighting results in increasing numbers of flowers. High irradiance improves flower yields; enhanced bottom breaks and help in stimulating auxillary shoot development and finally reduced the number of blind shoots (Zieslin & Mor 1990). The last year the number of growers producing cut roses has decreased but still several growers are producing pot roses in Norway the year around. If the lighting period is increased from 18 to 24 hours in a day there is an increment in the number of flowers by 34 % and the number of days until flowering is reduced by 12% (Pettersen et al.

2007). Moreover supplementary lighting give the best result during first 2 week of shoot growth by means of reducing the bud abortion (Maas & Bakx 1995).

2.1.2 Use of different lamps HPS and LED

High pressure sodium (HPS) lamps are the most common lamp type in greenhouse production. HPS are found to be better than high pressure mercury vapor lamp because of its higher luminous efficiency per unit of electrical energy and high radiant emission. However, HPS has only 5% blue light and the normal level in the natural light is about 18%. Now a day's using 100-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ is normal for a successful year round production. In year round production of cucumber the yield per m^2 is approximately 120-160 kg and cut roses 300-400 kg. When the most effective cultivation methods are applied and optimal light levels are used, the yield can increase upto 100 kg or higher for tomatoes (40-60 kg with only natural light), above 200 kg of cucumbers and 600-700 rose pieces (depending of cv.) per square meter per year (Moe et al., 2005).

HPS lighting increased tomato yield by 2.5 times and seedless cucumber yield by 25% in February and March when natural irradiance was low. The total spring season yield was increased by only 25% or 7%, respectively. Likewise, by using supplementary lighting for the rose plant in winter the yield was increased by 28.8% during winter (Blom & Ingratta 1983). The horticulture light industry is always trying to find better light sources to use in growth rooms and greenhouses. Nowadays light emitting diodes (LEDs) are becoming popular and they are believed to be future lighting systems in greenhouses (Figure 2). The LEDs are solid state lighting and the industry is rapidly developing and the cost for such lamps is decreasing. It is long-lasting and can provide narrow band spectral emission (Patil et al. 2001). Thus it is possible to design a light spectrum that is optimal for a specific process i.e. flowering control, morphology control or stomata opening or closing (Terfa et al., 2012b).



Figure 2. Different LED lamps (Pode 2010).

LED plastic cup traps increased the efficiency in catching the pests *Bemisia tabaci* by 100%. LED plastic cup are available and cheaper than yellow sticky traps for monitoring of whiteflies in greenhouse and is more relevant with whiteflies parasitoids release *Bemisia* nymph control (Chu et al. 2003). Moreover, by the exposure of red LED light during the dark interval is as effective as continuous illumination in suppressing powdery mildew in greenhouse rose (Suthaparan et al. 2010) .

2.1.3 UV Light

UV regions of the spectrums are divided into UV-A (320–400nm), UV-B (280–320nm) and UV-C (<280nm). Of these, UV-A and UV-B penetrate the stratospheric layer and have biological importance (Sakalauskaitė et al. 2013). During the growing period plant experiences different environmental fluctuation and, a sudden change in UV radiation can induce stress and the plant tissue starts to produce certain level of secondary metabolite like, flavonoids and anthocynins as a protection. As it was reported by different researchers on red maple (*Acer rubrum L.*) for

instance, cool temperatures, high sun radiation and drought conditions promoted anthocyanin production (Hoch et al. 2001). Similarly, Bilger et al. (2007) reported that biosynthesis of flavonoids which is one of the epidermal UV screening substance, was higher in *Vicia faba* leaf and stem when exposed to low temperature. Such climate change can also affect the growth and development of plant. In a commercial greenhouse production system, different chemical growth retardants are commonly used to control morphology. This is not currently recommended because it can be harmful for human and environment. Hence, application of low UV-B fluence rate has been found interesting as an environmental friendly tool to reduce the elongation of shoot and promote the biosynthesis of secondary metabolites (Treutter 2006).

Thus, UV radiation is a new emerging tool in greenhouses to control the plant height. UV-B applied on *Avena fatua* and *Setaria viridis* was found not to affect plant height of *Avena fatua* by different levels of UV-B radiation while the plants of *Setaria viridis* were found much more susceptible to the UV-B radiation, resulted in shorter plants at the levels of 8 and 12 kJ m⁻² d⁻¹ UV-B radiations than 0 and 4 kJ m⁻² d⁻¹ (Zuk-Golaszewska et al. 2003). It has also been found that the plant height was significantly decreased with increasing UV-B radiation in *Acorus calamus*, and resulted in compact plants. When plants were exposed to high level of UV-B, they were 47% shorter than no UV-B radiation (Kumari et al. 2009).

Moreover, 6 hours continuously exposure to UV-B from mid-morning to mid-afternoon each day (280 to 320nm) in soybean significantly decreased height, fresh and dry weights, leaf chlorophyll and carotenoid contents, and CO₂ uptake rates (Vu et al. 1981). Two cultivars of the rice in Japan with or without supplemental UV-B radiation named UV-resistant 'Sasanishiki' and UV-sensitive 'Norin 1', were grown from 1994 to 1997 with UV-B emitting fluorescent lamps, with a 0.1-mm-thick cellulose di-acetate film as a filter. In year 1994, 1995 and 1997 a significant decreases in tiller number was observed and decreases in grain were recorded in all seasons (Kumagai et al. 2001). Application of UV-B with irradiance of 0.1 to 1.2 W m⁻² by the exposure 2 minutes to 2 hours significantly suppressed powdery mildew (*Podosphaera pannosa*) in pot rose (*Rosa × hybrida* 'Toril'). Furthermore a reduction in spore germination, infection efficiency, disease severity, and sporulation of surviving colonies were found. Thus, 90% of the

disease severity was reduced with a daily exposure to UV-B than without exposure (Suthaparan et al. 2012).

2.1.4 Light receptors and light quality

Plants have different photochemical that help them to harvest light, produce characteristic colors, perceive the length of the day and they trigger many physiological and developmental responses. They are found in all flowering plant as well as cryptophytes.

2.1.5 Phytochromes and cryptochromes

Phytochromes are involved in sensing of red (R) and far red (FR) light (Figure 3). Cryptochromes and phototropins are sensing mainly in blue light sensing area (400-500nm). Phytochromes are a family of 12 soluble proteins consisting of a light absorbing chromophore pigment and a polypeptide chain i.e., apoprotein. Phytochrome plays mainly a role in chloroplast development, initiation of seed germination in response to light, control of flowering and inhibition of elongation growth through inhibition of cell elongation (Reed et al. 1994).

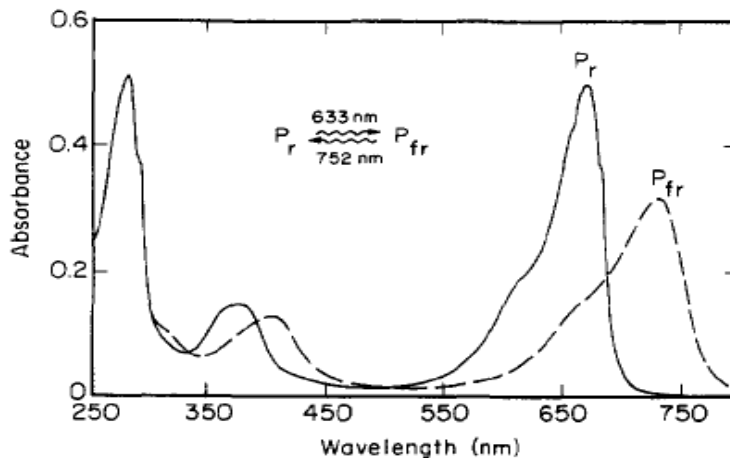


FIGURE 1: Absorption spectra of the P_r and P_{fr} forms of phytochrome

Figure 3. The two forms of phytochromes differ in their absorption spectra (Fodor et al. 1990).

2.1.6 Blue and red light

Photosynthetic active radiation (PAR) is between 400 and 700nm. Red and blue light are the most efficiently absorbed wavelength by photosynthetic pigments than any other spectral region. Evans (1987) reported that the maximum quantum yield occur near 600nm and decline rapidly at wave length shorter than 400nm and greater than 680nm with a high at 475nm. Moreover red light is an important tool for the development of the photosynthetic apparatus and driving photosynthesis (Sæbø et al. 1995). Moreover light quality affects photosynthesis by the combining effect on photosynthetic apparatus and by the accumulation of carbohydrate from chloroplasts. Sæbø et al. (1995) further reported that epidermal cells of *Betula pendula* were found to be largest by the application of blue light as compared to red light. Samuoliene et al. (2010) reported that red LED treatment shows smaller sized fruits but that combination of red and blue LED spectra is highly significant for development of strawberries.

Li and Kubota (2009) reported that fresh weight, dry weight, stem length and leaf width significantly increased by 28%, 15%, 14%, 44% and 15%, respectively, with supplemental Far-red light compare to white light. Blue light affects the formation of chlorophyll, stomata opening and photomorphogenesis (Heo et al. 2002; Senger 1982). Moreover Blue light enhances dry matter production in plant species like wheat (Zeiger et al. 2002). Blue light also affects the biochemical properties of photosynthesis in leaves such as chlorophyll (Chl) *alb* ratios, Chl *alb*-binding protein of photosystem II (LHCII), and photosynthetic electron-transport (Leong & Anderson 1984; Senger & Bauer 1987).

Blue light is known to stimulate ‘sun-type’ characteristics on the leaf level, even at a rather low irradiance ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$), and thus provides a leaf development normally associated with acclimation to high irradiance. Moreover blue light is qualitatively required for photosynthesis and quantitatively mediates leaf responses resembling those of irradiance intensity (Hogewoning et al. 2010). The effects vary among species to species. In the long day plant *Petunia x hybrida* an enhanced flowering was found in monochromatic Blue compared to R (Fukuda et al. 2009). In the facultative long day-plant *Arabidopsis thaliana*, Blue light over a range of irradiances was

found to be effective in promoting flowering under long (Guo et al., 1998; Imaizumi et al., 2003). In the day neutral plant *Cyclamen persicum* simultaneous exposure to irradiances of (80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) monochromatic B and R light enhanced flowering compared to fluorescent light tubes (Heo et al. 2003).

In contrast, B and R light given separately delayed flowering. In the short day-plant *Chenopodium rubrum* an irradiance-dependent Blue light promotion of flowering was observed but the effect was counteracted by R light in the wavelength area 500-700 nm (Sawhney 1977). Likewise, in *Xanthium pennsylvanicum* and *Lemna perpusilla* B light allowed flowering when given continuously or when it replaced inductive darkness (Hillman 1965).

Dougher and Bugbee (2004) conducted experiment on soyabean under long term light exposure by increasing the blue light fraction from less than 0.1% to 26% and found that the inter node length was decreased by reducing the cell division. Moreover, it is shown that an increase in blue light fraction from 6% to 26% reduced soybean leaf area by decreasing cell expansion. When the blue light fraction increased from (0% to 6%), it showed a 3.1-fold increase in cell expansion and a 1.6-fold increase in cell division.

2.2 Relative air humidity

Mortensen and Gislørød (2000) reported in cut roses that increasing the relative air humidity from 67 to 94% (corresponding to vapor deficits (vpd) of 750 and 139 Pa, respectively) results in increased shoot length, leaf size and shoot fresh weight (Table 1). Moreover, rose grown at high RH (>85%) had a much shorter vase life when they were tested at low air humidity (1170-1710 Pa vpd), whereas the difference was small when tested at high humidity (440 Pa vpd). Similarly, when roses grown at high RH (91% had a much higher rate of water loss from detached leaves compared to three lower RH levels (64, 74 and 83%).

Evans (1987) reported that when the air humidity was increased, the vase life was decreased in roses from 8-13 to 2-5 days and the weight loss of the leaves from shoots at 90% was much higher as compared to 65% RH. The reason why roses grown under high RH have such high water loss because of malfunctioning stomata (described in 2.4).

Table 1. A decrease in VPD from 660 to 155 Pa, delayed flowering time by 4.2 days, increased shoot length about 8.7 cm and increased the number of leaves by 2.6. Leaf area, leaf size and specific leaf area were unaffected. The keeping life increased with increasing VPD (Mortensen 2000).

The influence of air humidity (VPD) on growth, flowering and keeping life of chrysanthemum cv. Framint^a

VPD (Pa)	Days until flowering	Stem length (cm)	Shoot dry wt. (g)	Dry wt. (%)	No. of inter-nodes	No. of branches	Total leaf area (dm ²)	Leaf size (cm ²)	Keeping life (days)
660	51.2 ± 0.3	73.5 ± 0.6	24.5 ± 1.8	15.9 ± 0.3	31.7 ± 0.3	6.7 ± 0.2	24.3 ± 1.5	43 ± 2	17.3 ± 0.3
420	52.5 ± 0.4	76.9 ± 0.8	27.0 ± 2.3	16.1 ± 0.4	32.1 ± 0.3	6.6 ± 0.2	21.9 ± 2.0	46 ± 1	18.0 ± 0.4
155	55.4 ± 0.4	82.2 ± 0.8	29.9 ± 3.5	16.3 ± 0.4	34.3 ± 0.4	6.8 ± 0.2	20.3 ± 1.0	42 ± 2	19.6 ± 0.6
Significance level	****	****	n.s. ^b	n.s. ^b	***	n.s. ^b	n.s. ^b	n.s. ^b	***

^a Standard errors (±S.E.) are given and $n=14$ except for keeping life ($n=10$).

^b Not significant.

*** $p < 0.01$.

**** $p < 0.001$.

2.3 Temperature and Carbon dioxide enrichment

Temperature has a significant effect on plant growth and development. The difference between day and night temperature influence internode length, plant height, leaf orientation, shoot orientation, chlorophyll content, lateral branching and petiole and flower stalk elongation in plants. As the differences between day and night temperature (DIF) increases, internode length

and petiole length seems to be increased (Senger 1982). Lower DT than NT reduced plant height significantly in *Cucumis sativus* and *Fuchsia x hybrida* cv. 'Beacon' (Heo et al. 2002).

For the photosynthesis, carbon dioxide is the main component. CO₂ enrichment in greenhouses can increase dry weight, plant height, number of leaves and lateral branching. Optimal CO₂ required for roses in green house is 700 and 900 µl l⁻¹. A concentration higher than 900 µl l⁻¹ can cause reduction in growth and induce injuries of leaves (Zeiger et al. 2002).

2.4 Shelf life of roses

Blockage of xylum vessels due to bacteria growth or air emboli inhibit water transport to the flower and is the main cause for the short vase life for cut roses. Therefore treatment with chemicals containing bactericides or lowering the pH helps in increasing vase life of cut flowers (Senger & Bauer 1987). However, roses grown under high RH have very high water loss from the leaves due to malfunctioning stomata and are very sensitive to xylem blockage. Also pot roses grown under high RH can have a shorter shelf live. If they are well watered it is usually not a problem. However, drought stress during shipping and sale can reduce the postproduction shelf life and marketability. A problem with pot roses is that they dry out very easily during sale. The wholesalers, flower shops and supermarkets usually do not water the plants (Waterland et al. 2010).

2.5 Senescence

Senescence is defined as the post-maturation decline in survivorship and fecundity that relates with advancing age (Rose & Charlesworth 2002). Plants with malfunctioning stomata usually do not undergo a natural senescence process but will end their life because of wilting (Torre et al. 2003). To grow plants with functional stomata is possible if the RH is kept high or if the plants are sprayed daily with the plant hormone abscisic acid (Arve et al. 2013).

Abscisic acid is responsible for stomatal closure and will reduce the water loss from shoots bearing leaves. However, ABA has a role in enhancing aging of the flowers and some biochemical processes associated with RNAase activity and reduction in protein content (Halevy et al. 1974). Abscisic acid also promotes petal growth and respiration leading to aging and accelerates senescence (Heo et al. 2003).

A water deficit in leaves or petals induces premature senescence, bent neck and wilting. Membrane permeability and hydrolysis of cell component also affected (Guo et al. 1998). The lack of soluble carbohydrate creates petal discoloration and weakening of amino acids and protein (Imaizumi et al. 2003). Ethylene is the most important enzyme in the process of senescence. Thus, ethylene inhibitor STS (Silver ThioSulphate) is found to be effective in suppression of senescence (Altvorst & Bovy 1995). Calcium treatment reduced the ethylene production with age and flowers and petal senescence by the protection of membrane protein and phospholipids. Similar conclusion was made by (Torre et al. 1999). Furthermore, calcium increased ATPase activity in the aging petals and delayed electrolyte leakage from the cells. Thus, calcium has a significant role in protection of membrane and maintaining solute transport and tissue strength.

2.6 Stomata function is affected by air humidity and light

Stomata are defined as small pores on the surface of leaves and stems that is bounded by a pair of guard cells (Figure 4). Its function is to control water vapor and carbon dioxide interior of leaf and atmosphere. Mainly gas exchange is regulated by the aperture of stomatal pore and the number of stomata on epidermis.

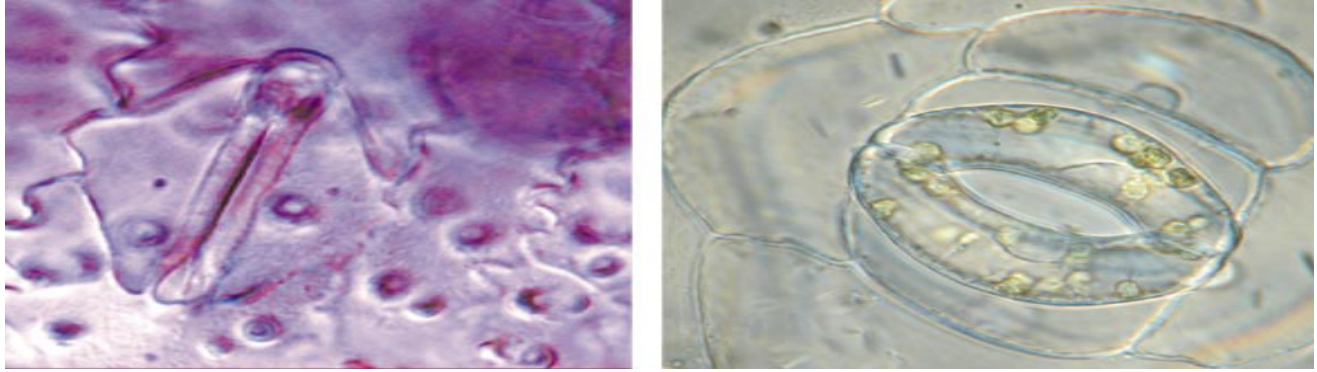


Figure 4. Dumb-bell-shaped stoma of rice typical of the grasses (left) and the kidney-shaped stoma typical of *Arabidopsis* and *Commelina* (right) (Hetherington & Woodward 2003).

The regulation of the stomata plays an important role for the water loss from leaves. Most of the C3 plant closes the stomata during night to increase the hydration. During morning, when the water potential is negative, stomata will open for the uptake of CO₂ and nutrient from the soil (via transpiration stream). The plant hormone ABA is responsible for decreasing turgor pressure and stomata closure (Hillman 1965). High relative humidity greater than 85% induced disorder in leaf anatomy, stomata morphology and function (Figure 5). However a daily application of ABA conveys the negative effect of high RH (Fanourakis et al. 2013).

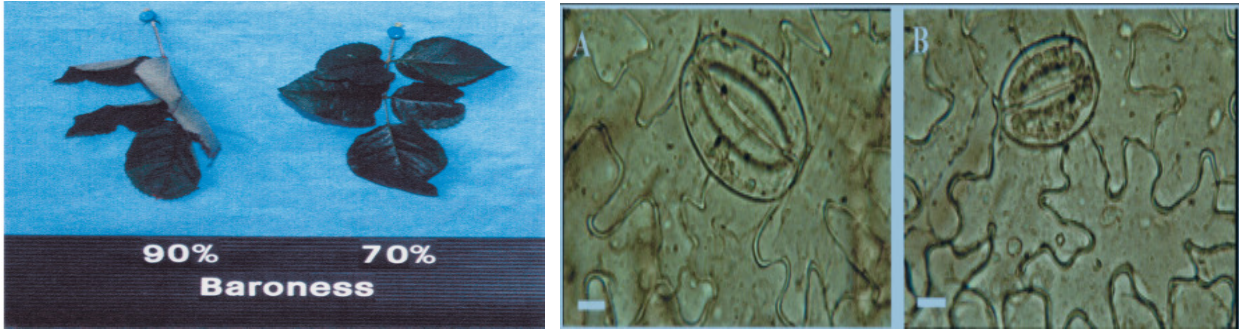


Figure 5. Detached rose leaves developed at 90% and 70% RH. After 30 minutes the leaves from 90% is already wilting (left). Size and morphology of stomata cells from 90 % and 70 % RH (right).

Arve et al. (2013) found that pot roses grown under high RH have a higher transpiration and there is no stomatal response to high RH leaves darkness. In moderate RH, the rose leaves

contained higher concentrations of ABA compared to high RH leaves in the light and the dark. Especially the amount of ABA increased during darkness in leaves from moderate RH compared to moderate RH. Under continuous light (24 hr) stomata developed inferior compared to 20 hour photoperiod. Thus, to get fully functional stomata, a dark period is important in roses (Arve et al. 2013; Mortensen 2000).

3 MATERIALS AND METHODS

3.1 Plant material

Rosa x hybrida, cv. Toril was grown from a single node stem segment with one mature leaf. The cuttings were taken from the middle and lower position of fully developed stems with open flowers. After 2-3 weeks, the cuttings were rooted and transferred to 12 cm pots containing a standard fertilized sphagnum peat media (Floralux, Nittedal, Norway). The pH and EC level were 5.7 and 1.5, respectively, in all experiments (Superba: NPK 9-5-25+Mg+S+Micro and calcinit from Yara, Oslo, Norway). During pre-cultivation the plants were kept in a greenhouse compartment (glass roof and polycarbonate walls) at Center for climate regulated plant research, Norwegian University of Life sciences, Ås, Norway (59°39'47"N10°47'38"E). The temperature was 21°C and average daily relative air humidity (RH) 70%, corresponding to a 0.5 kPa water vapour deficit (VPD). Supplementary light was provided by high-pressure-sodium-lamps (HPS, Osram NAV T-400W, Munich, Germany) given 20 h every day, followed by a 4 h dark period. At average the supplemented irradiance was 100 (± 10) $\mu\text{mol m}^{-2} \text{s}^{-1}$ (measured with a Li-Cor, Model L1-185, quantum sensor, LI-COR Inc, Lincoln, Nebraska, USA). The pre-cultivation ended when the plants had 1-1.5 cm long shoots. Thereafter, the plants were transferred to the different light treatments growth chambers.

3.2 Experimental growth conditions

The experiments were performed in growth chambers without any influence of natural light (describe the chambers). In these experiments the plants were exposed to 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 20 h day⁻¹ supplementary light provided either by LED lamps (round LED-light with 3 chains, delivered by Sola-co, China) containing 80% red (R; peak wavelength at 630nm) and 20% blue (B; peak wavelength at 465nm) or HPS (5% B) lamps (Figure 6). The phytochrome photostationary state (PSS) was calculated based on the method developed by (Sager et al. 1988) and was 0.85 and 0.89 for HPS and LED, respectively.

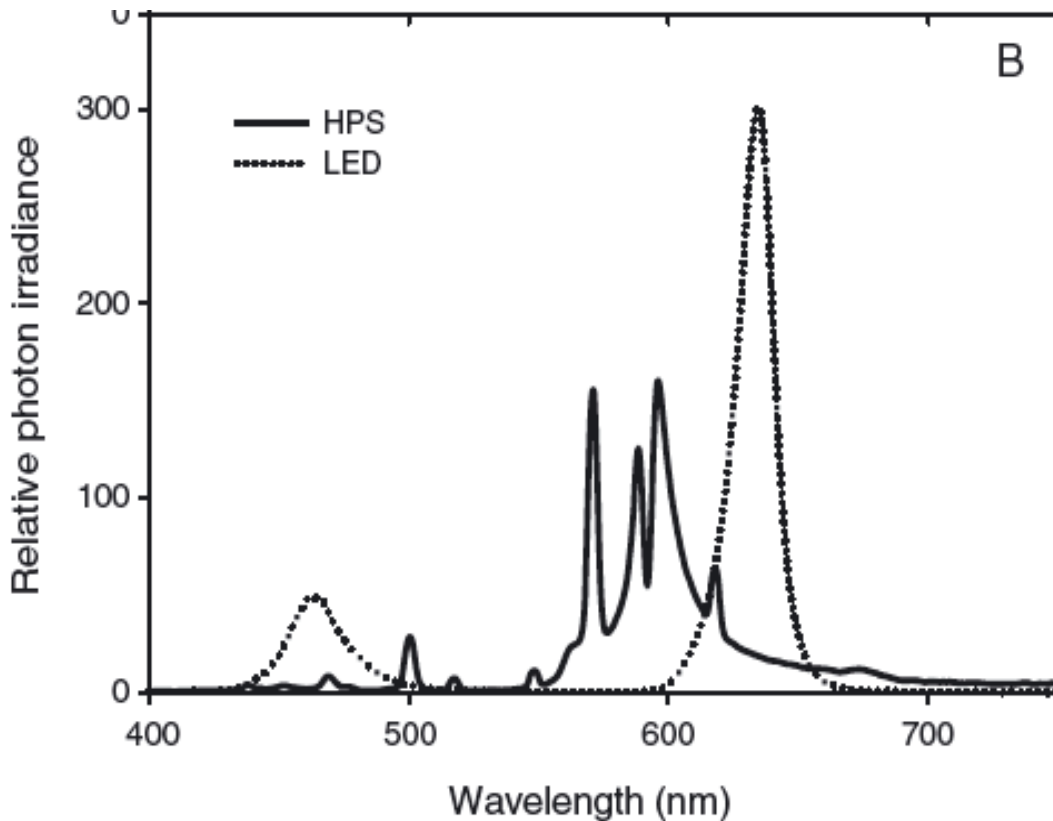


Figure 6. Irradiance spectra of the lamps used in the experiments; HPS Lamps Osram NAV T-400W (Solid lines) and LED lamps (Round LED-light 92W with 3 chain, SoLa-Co) (Dotted lines) (Terfa et al. 2013).

The temperature set point was 20°C in growth chambers ($\pm 0.5^\circ\text{C}$) during the experimental period. A PRIVA greenhouse computer was connected for recording, controlling and storing of the climate data both in greenhouse and growth chambers. The plants were grown until they reached the commercial stage of flower development. In order to get significant results with some degree of accuracy, all the experiments were repeated twice. First replication from mid of April 2011 til mid of June and second one from mid of June til mid of August 2011.

3.3 Experimental set up

For these experiments, six growth chambers with each chamber having 20 pots were arranged and in all chambers the plants were receiving $100\mu\text{mol m}^2/\text{s}$ photosynthetic photon flux density (PPFD) for 20 hours per day. Supplementary light was provided by LED, HPS as described above. Another supplementary light the UV-B fluorescent were provided with spectral (280–320nm) as shown (Figure 7).

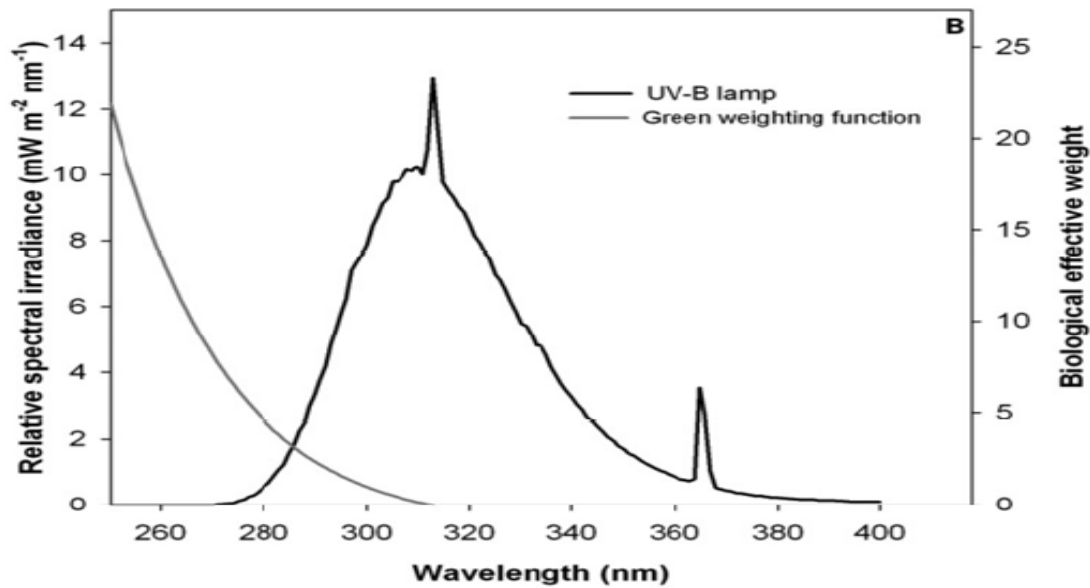


Figure 7. UV-B fluorescent tubes (Suthaparan et al. 2012)..

In two chambers (one with 60% RH and one with 90% RH) the plants were grown with HPS light but supplemented with UV-B tubes Model UVB-313 EL (Q-PANEL lab products, Cleveland, OH, USA) two times per day as described (Figure 8). Each light source was provided by moderate (60%) and high (90%) RH in different chambers.

Table 2. Climate and light conditions in experiment 1 and 2 inside the chambers

Experiment	Light sources	Intensity (μ mol/m ² /s)	RH (%)	Temperature	CO ₂ (ppm)
1	LED	100	90 and 60	20°C	400
1	HPS	100	90 and 60	20°C	400
2	UV-B	100	90 and 60	20°C	400

The dark period for UV-B, LED and HPS were 16:00 to 20:00 pm. The UV-B light was provided twice a day from 6 to 7 am (in the middle of the light period) and 17:30 to 18:30 pm (in the middle of the dark period).

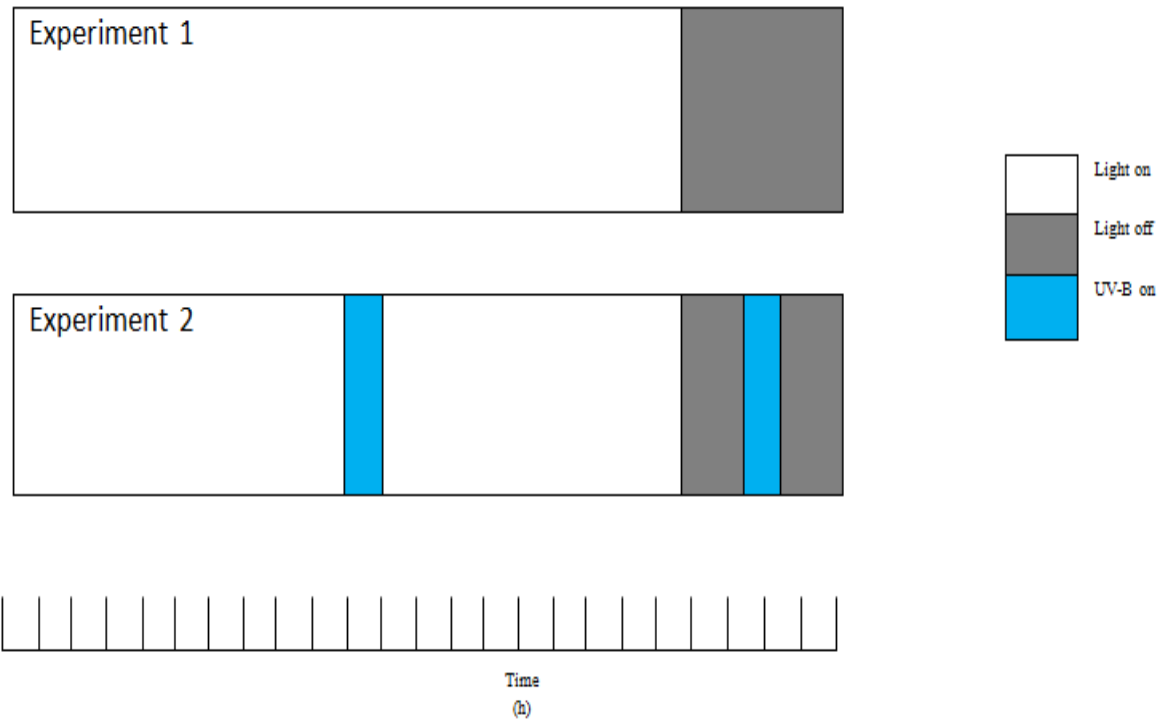


Figure 8. Experiment 1 effect of more blue light HPS and LED (20 hour light followed by 4 hr dark from 16 to 20:00) and Experiment 2 effect of UV-B (exposure of UV-B 1 hour twice a day).

3.4 Data collection (Measurement of different parameters)

Comparison of LED and HPS and two air humidity levels (high and moderate) was called experiment 1 and the UV-B exposure combined with high and moderate RH was called experiment 2. We studied the following parameters.

Experiment 1: HPS and LED

- i) Crop growth measurements
- ii) Gas exchange measurements
- iii) Water loss measurement
- iv) Sump analysis (stomata aperture)
- v) Stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$) measurements

Experiment 2: UV-B

- i) Gas exchange measurements
- ii) Stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$) measurements
- iii) Pigments Measurement

3.4.1 Crop growth measurements

Crop growth measurements were measured in order to analyze morphological and developmental characteristics of *Rosa × hybrida*. Eight plants per chamber from HPS and LED were measured for morphological analysis. The shoot length was measured as the total length from the base of the shoot until start of the pedicel. The pedicel length was measured from the end of the stem until the receptacle and the number of thorns was counted on the main stem. Number of leaves, number of flowers and leaves area were recorded. Dry weight of shoot, leaves pedicles and flowers were separated and measured after 72 hours in a dry oven at 70°C.

For the analysis of growth parameters, plant height (base of shoot until start of the pedicel) and internodes length were measured (using meter), leaf area (using LI-3100C Area Meter, resolution of 0.1 or 1mm², Figure 9) and dry matter (using Analytical sensitive balance, Model HR-60, USA, resolution 0.001gm).

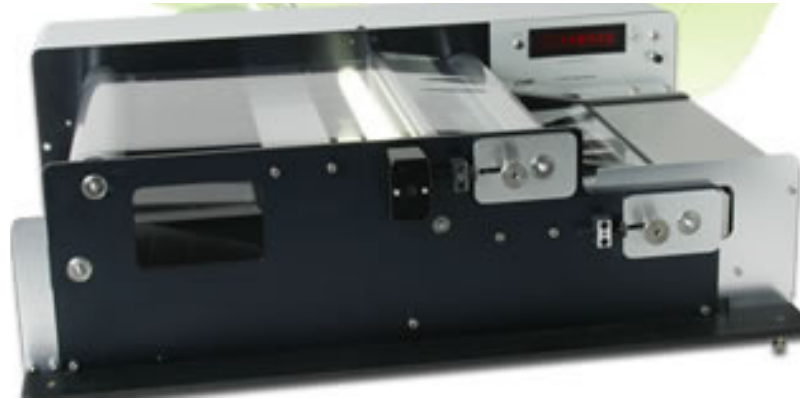


Figure 9. Leaf area meter

(Source: http://www.licor.com/env/products/leaf_area/LI-3100C dated:30.04.2013)

3.4.2 Gas exchange measurements

A CIRAS 2 portable photosynthesis system with PLC6 (U) Automatic Universal Leaf Cuvette (PP Systems, 2001, Hertfordshire, U.K.) was used to measure stomata conductance. In this instrument Silica gel and Carbondioxide cylinder were changed every day.

Stomatal conductance ($\text{molm}^{-2}\text{s}^{-1}$) was measured on the adaxial side of fully expanded leaflets with five leaflets of rose plants grown at moderate (60%) and high (90%) relative humidity. The cuvette was attached to the leaf for 24 hour and the readings were stored in a computer. Repetition was made for 3 plants per chamber. The CO₂ concentration was 400 μmol^{-1} air flow

was $250 \mu\text{mol s}^{-1}$ and the leaf chamber temperature 22°C . Measurements were taken every 15 minutes for 24 hours interval.



Figure 10. A portable ciras analyzer (left) and a CO₂ regulator (right)

(Source: <http://www.bandp.co.kr/center/pdf/ciras2.pdf> dated 30.04.2013)

3.4.3 Water content measurement

In order to study stomata response to dehydration, desiccation tests were done with first five leaflets from eight plants grown under high (90%) and moderate (60%) RH. The leaves were put on the table and the initial weight was measured immediately. Afterwards measurements were taken at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120 and 180 minutes interval. Finally, leaf area (using LI-3100C Area Meter, resolution of 0.1 or 1mm^2) and leaf dry weight (using Analytical sensitive balance, Model HR-60, USA, resolution 0.001gm) was measured after 72 hours.

3.4.4 Microscopy analysis of stomata

Epidermal imprints were made of fresh intact upper leaves by Suzuki's Universal Micro-printing (SUMP) method using SUMP liquid and SUMP plate B (SUMP laboratory Tokyo, Japan) as described by (Tanaka et al. 2005). Three leaves per chamber with repetition were done in SKP. Sump plates were cut into two halves. Afterwards layer was detached followed by polishing in it. Those polishing layer was attach below, five leaves lets from single plants. After three minutes it was detached and kept in slide. 3 samples during morning at 7:00 (light) per chamber and 3 samples at 18:00 middle of dark period (16 to 20:00) were taken. From each sample 10 photos were taken. Randomly 3 photos were selected. Hence on an average 6 stomata were taken from each photo. 3 mean values from replication 1 and 3 mean value from replication 2 were selected. Hence 6 mean values per chamber were analysed statistically.

The SUMP imprints were observed under a microscope with (Leitz, Labolux K, Type 0.2, Wetzler, Germany) and stomata images were obtained with a Leica camera (LEICA DC200, Heerbrugg, Switzerland). Stomatal morphology (length and aperture) were measured with the use of UTHSCSA Image tool for windows version 3.00 (University of Texas Health Science Center, San Antonio, TX, USA).

3.4.5 Stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$) measurements

Leaf conductance ($\text{mmol m}^{-2}\text{s}^{-1}$) was measured at moderate (60%) and high (90%) relative humidity to study stomatal responses to water stress. An AP4 Porometer (Delta-T Devices-cambridge UK) is the instrument that we measures stomata responses in relation to darkness and dry air. Measurements were taken from 5 plants from each experiment on abaxial side of the leaves during lighting period. Lighting period followed by dark period. Immediately afterwards plants were placed in a dark chamber and reading were taken after one hour and then followed by measuring the reading in dark period till third day.



Figure 11. AP4 Porometer for the measurement of Leaf conductance ($\text{mmol m}^{-2}\text{s}^{-1}$)

(Source: <http://www.delta-t.co.uk/product-display.asp?id=AP4%20Product> dated 30.04.2013)

3.4.6 Pigments Measurement

For the determination of plant pigments anthocyanin and flavonoid three leaves were taken from each chamber and sensory data was collected. During data collection, non-destructive method was applied using non-contact optical sensor (Multiplex R 3 FORCE-A 91893), a hand-held, multi-parametric fluorescence sensor used for quantification of leaf screening potential, flavonoids and anthocyanins. The measurement was started with a warm up of the instrument for 10 minutes. Afterwards, air value and blue value were taken before the main measurement to calibrate. The blue standard has fluorescent properties similar to that of a leaf without flavonols or anthocyanins present (Ghozlen et al. 2010). Multiplex reading was taken at 3rd, 4th and 5th

node having fully developed leaf of the whole plants. Afterwards upper and lower sides of detached leaves were record.



Figure 12. Multiplex for the determination of plant pigments

(Source: <ftp://ftp.dynamax.com/DynamaxPDF/Multiplex2.pdf> dated 30.04.2013)

3.5 Data analysis

The experiments were repeated twice in the growth chamber. Since the trends of the results in the experiment were similar, the data are presented as an average value for each treatment. Moreover significant differences between means were tested for normally distributed general linear models (GLM) and Tukey's test. Differences among treatment means were tested with $P < 0.05$. Data will be analyzing using Minitab statistical software version 16.1.1 (Minitab 16.1.1, windows version, State College, Penn state University, Pennsylvania, USA). Graphical presentations of water loss were performed by sigma plot.

4 Results

4.1 Experiment 1: HPS and LED

4.1.1 Growth analysis

Table 3. Morphological and developmental parameter of *Rosa × hybrida* grown under different light sources HPS and LED at 90 and 60% RH. Significant differences were calculated at *P*-values <0.05, n.s. indicates non-significant differences. The values are average ± SE (n=8).

RH during growth (%)	Light	Stem length (cm)	Pedicle length (cm)	No of flowers	No of leaves	Leaf area	Dry weight of stem	Dry weight of all pedicles and flowers
High 90%	LED	18.6	8.24	2.18	7.75	333.4	0.52	1.57
	HPS	21.67	8.7	2.62	7.87	383.4	0.79	2.03
Moderate 60%	LED	19.99	6.95	2.62	9.0	410.7	0.81	2.38
	HPS	22.13	7.92	2.93	8.25	390.4	0.80	2.6
Statistical significance								
RH		n.s.	P= 0.0001	n.s.	P=0.034	n.s.	P=0.031	P= 0.036
Light quality		P= 0.048	P= 0.0001	n.s.	n.s.	n.s.	n.s.	n.s.
RH × light quality		n.s.	P= 0.04	n.s.	n.s.	n.s.	n.s.	n.s.

The stems were significantly longer under HPS compared to LED but RH had no effect on stem length (Table 3). On the other hand, pedicel length was affected by both light quality and RH, and an interaction between RH and light quality was found. Under moderate RH, LED suppressed pedicel length and induced shorter pedicels compared to HPS. The longest pedicels were found under high RH irrespective of the light quality and LED had only a small suppressive effect on pedicel length (Table 3). Comparatively moderate RH had significantly more leaves per stem, higher dry weight of stem and higher dry weight of pedicel length and flower compared to high RH, but no significant effect of light quality was found on these parameters (Table 3). Moreover, the number of flowers and leaf area was not significantly affected by either RH or light quality and no interaction was found (Table 3).

4.1.2 Gas exchange measurements of plants grown with HPS and LED under moderate and high RH

The diurnal stomatal conductance (g_{sw}) of *Rosa × hybrida* growing at high and moderate RH was measured with gas exchange analyzers (CIRAS 2). At moderate RH there was only a small difference in conductance between HPS and LED plants. In general, at moderate RH conductance was 40-50% lower compared to high RH (Figure 13). During darkness the g_{sw} was less than $10 \text{ mmolm}^{-2}\text{s}^{-1}$ indicating stomatal closure. At high RH g_{sw} was still high during darkness (greater than $30 \text{ mmolm}^{-2}\text{s}^{-1}$) for both HPS and LED and indicate lack of dark induced closure. However, LED reduced the conductance under high RH during light and darkness indicating reduced stomatal aperture (Figure 13).

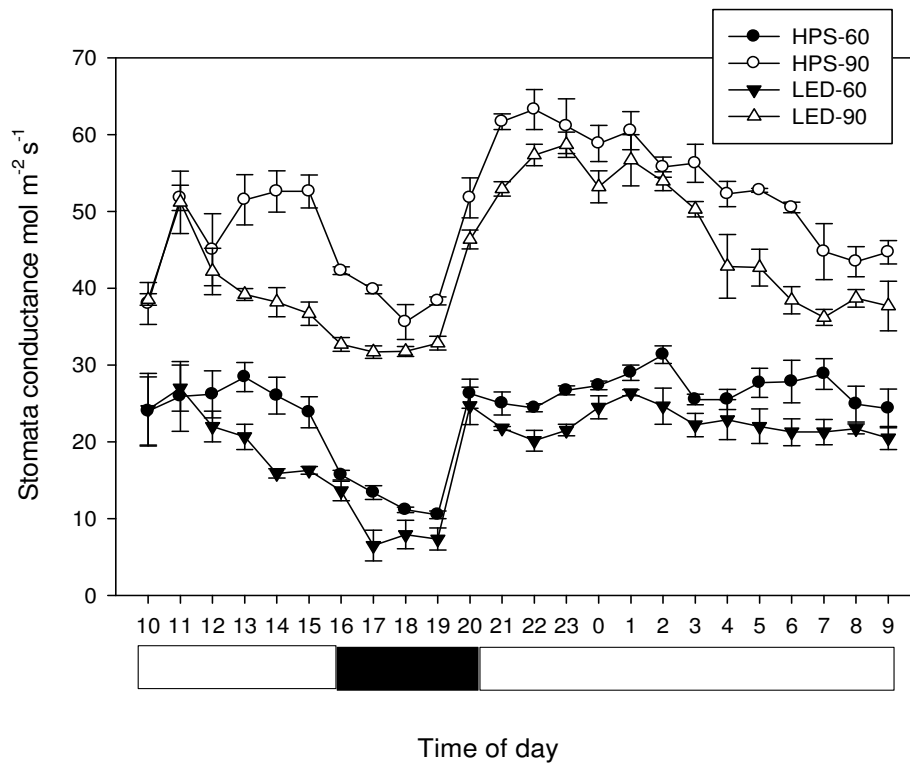


Figure 13. Stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$) measured with a CIRAS on the adaxial side of fully developed *Rosa*×*hybrida* leaves during growth at different RH (90 and 60%) in combination with HPS and LED as the light source. The measurements were done three times during a 24 hours' time interval of light/dark cycle. The dark was given from 16.00 to 20.00. Average \pm SE (n=3).

4.1.3 Water loss measurement of HPS and LED

Stomatal response to dehydration after detachment were analysed from upper leaves of plants grown under HPS and LED under high and moderate RH. Detached leaves from plants grown at high RH showed a rapid water loss compared to leaves grown at moderate RH (Figure 14). During the first hour after detachment, both HPS and LED from high RH lost in similar pattern but at end (180 minutes) lost leaves developed under HPS had lost around 53% whereas leaves developed under LED had lost 39%. Moreover, at moderate RH, leaves grown with HPS lost 23% water whereas LED lost 17%. There was a statistically significant difference between

plants developed under different RH after 180 minutes (RH, light quality and RH × light quality was found to be statistically significant at 5% probability level).

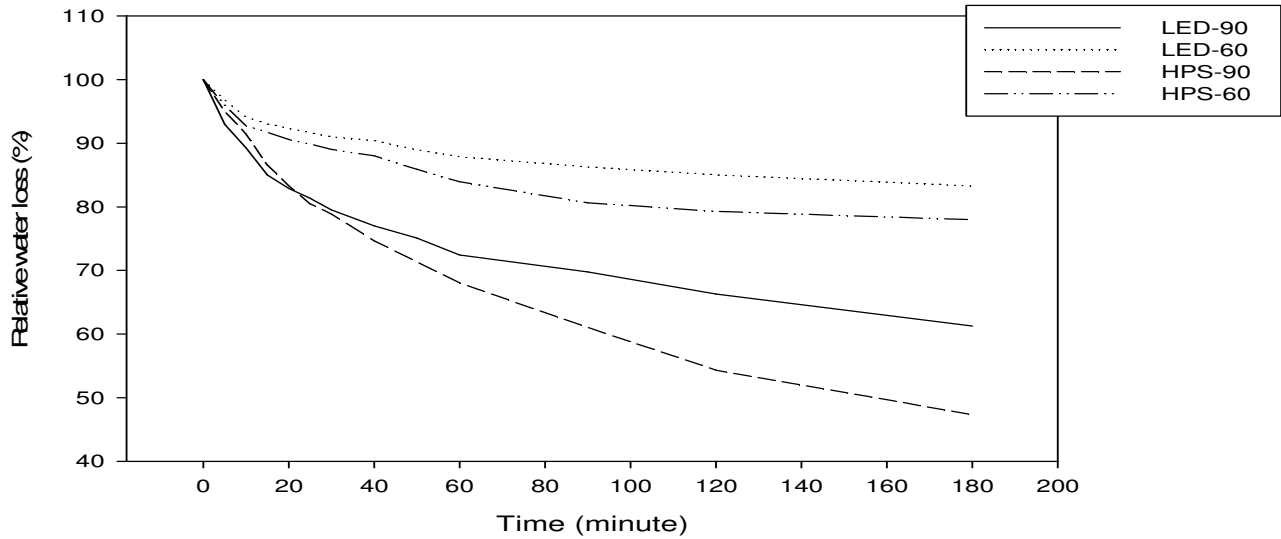


Figure 14. Relative water loss (%) of detached upper leaves of *Rosa x hybrida* grown under HPS and LED under high and moderate RH. The water loss was recorded during 0 to 180 minutes after detachment (n= 8).

4.1.4 Microscopy analysis of stomata (Sump analysis) of HPS and LED

To view the idea about the effect of high RH and light quality on the stomatal development and ability to close stomata, imprints were made on leaves during both light and dark and stomatal characteristics were measured. In general, stomatal length and pore aperture were significantly higher in plants grown at high RH compared to moderate RH (Table 4). Similarly the light quality had a significant effect of stomatal length and aperture.

The length of stomatal pore grown at high RH and HPS measured during light and dark was 1.08 and 1.13 times larger than those grown under moderate RH. The pore aperture during light and dark in plants grown under high RH for HPS was 1.24 and 1.55 times larger than those

grown under at 60% RH with HPS. Moreover the pore aperture during light and dark in plants grown under high RH and LED was 1.66 and 1.44 times larger than those grown under moderate RH.

Table 4. Stomatal characteristics of *Rosa* × *hybrida* comparing high and moderate RH in combination with HPS and LED during light and darkness. Group information using Tukeys method. Average ± SE (n=6). Significant differences were calculated at *P*-values <0.05, n.s. indicates non-significant differences.

Pore Character acteristics	Light	RH during growth				Statistical significance	
		RH (90%)		RH (60%)			
		light	dark	light	dark		
Length(μm)	LED	31.48	28.86	31.37	28.58	RH	P=0.019
	HPS	36.76	36.21	33.78	32.03	Light quality	P=0.0001
aperture(μm)	LED	9.29	6.96	5.59	4.83	RH × light quality	P= n.s.
	HPS	8.18	7.69	6.56	4.96	RH	P=0.0001
						Light quality	P=0.009
						RH × light quality	P= n.s.

4.1.5 Stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$) measured with a porometer after transfer to darkness and dry air of plants grown with LED and HPS under moderate and high RH.

Table 5. Porometer reading ($\text{mmolm}^{-2}\text{s}^{-1}$) of plants moved from the growth environment to a common environment (40% RH, 20°C) in darkness and measured on leaves in the light (start value), after 1 hour in darkness, two days in darkness and day 3 days in darkness. Average \pm SE (n=5). Significant differences were calculated at *P*-values <0.05.

RH during growth (%)	Light	Starting value light*	During dark			
			1 hour in darkness	Two days in darkness	Three days in darkness	Ratio between start value and day 3
High 90	LED	468.6	38.6	10.83	15.05	31.13
	HPS	349.2	186.8	92.5	43.30	8.06
Moderate 60	LED	125.4	4.63	2.68	3.168	39.58
	HPS	179.8	5.30	6.14	3.052	58.91
Statistical significance						
RH		P=0.0001	P=0.0001	P=0.005	P=0.0001	
Light quality		ns	P=0.002	P=0.01	P=0.008	
RH × light quality		ns	P=0.002	P=0.016	P=0.007	

*The first measurement was taken in the light before the light was turned off

The conductance in the light was significantly affected by RH but not by light quality (Table 5). Plants produced under high RH had about 3 times higher conductance compared to moderate RH (Table 5). The leaf conductance was highly reduced from the first reading in the light to the third day of dark storage (Table 5). A significant interaction was found between RH and light quality after 1 hr in dark and after 2 and 3 days in darkness (Table 5). Plants developed at high RH with LED showed a better dark induced closure than plants grown under high RH and HPS. Plants developed at moderate RH showed a strong dark induced closure irrespective of the light quality (Table 5). The ratio between the initial measurement in the light and the third day of dark induced closure for HPS 90, HPS 60, LED 90, LED 60 was 8.06, 57.92, 31.13, 39.58.

4.2 Experiment 2: UV-B

4.2.1 Gas exchange measurements of plant grown with UV-B

The diurnal stomatal conductance of *Rosa × hybrida* growing at high and moderate RH was measured with a gas exchange analyzers (CIRAS 2). In the UV-B treatment, the g_{sw} of *Rosa × hybrida* grown at both humidity showed a similar pattern with the conductance decreasing through the time of one hour exposure of UV-B treatment showing a higher value for high RH compared to moderate RH (Figure 15).

Stomatal conductance was found to be lower at moderate RH compared to high RH. Stomatal conductance during (one hour 17:30 to 18:30 exposure of UV-B and dark period from 16 to 20:00) was found least below $15 \text{ mmolm}^{-2}\text{s}^{-1}$ which was around 65% lower than high RH. However exposure of one hour UV-B during morning (6 to 7:00) nothing happen and the pattern was similar in both moderate and high RH (Figure 15).

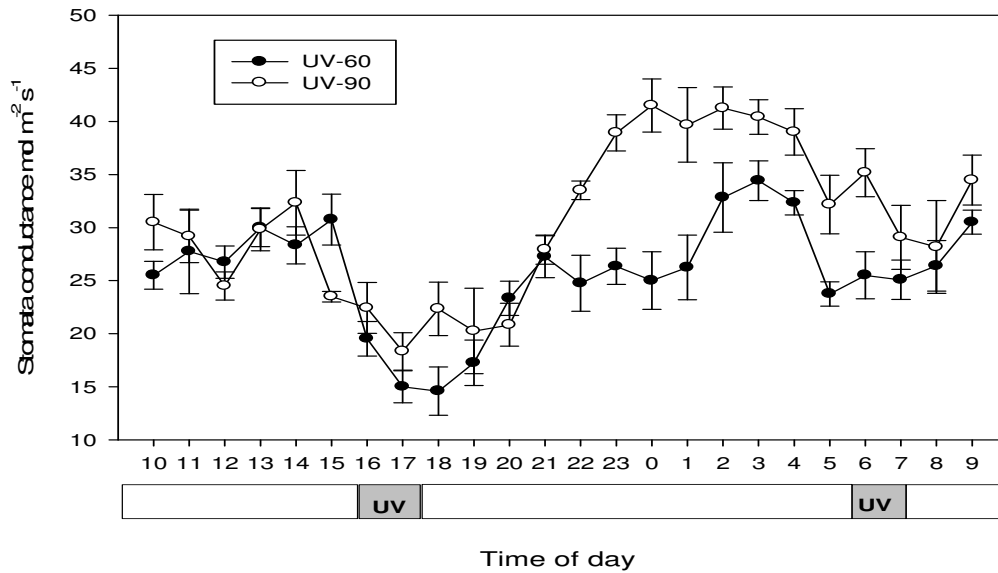


Figure 15. Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) responses for UV-B at different RH 90 and 60% measured on the adaxial side of the first expanded leaves with five leaflets of *Rosa × hybrida*. The measurements were done three times during 24 hr time interval of light/dark cycle. The dark was given from 16.00 -20.00 and two times a day UV-B was provided (one hour 17:30 to 18:30 and another hour 6 to 7:00). Average \pm SE (n=3).

4.2.2 Water loss measurement of UV-B

Stomatal responses to dehydration were analysed from a detached upper leaves from 8 plants grown under 90 and 60% relative humidity under HPS in combination with UV-B exposure. (Figure 16), detached leaves of *Rosa hybrida* grown at high relative humidity showed a rapid water loss compared to leaves grown at low RH. During first 1 hour of dehydration, both HPS 90 and HPS+UV-B 90 lost the same rate of water. More than 50% water were loss from HPS 90 and 60% water loss for HPS+UV-B 90 while this value was limited about 15 % loss in HPS 60 and UV-B 60. There was a statistically significant difference between plants developed under the

different RH after 180 minutes (RH as well as light quality was found to be statistically significant) and HPS+ UV-B was losing more water than HPS alone when growing at high RH.

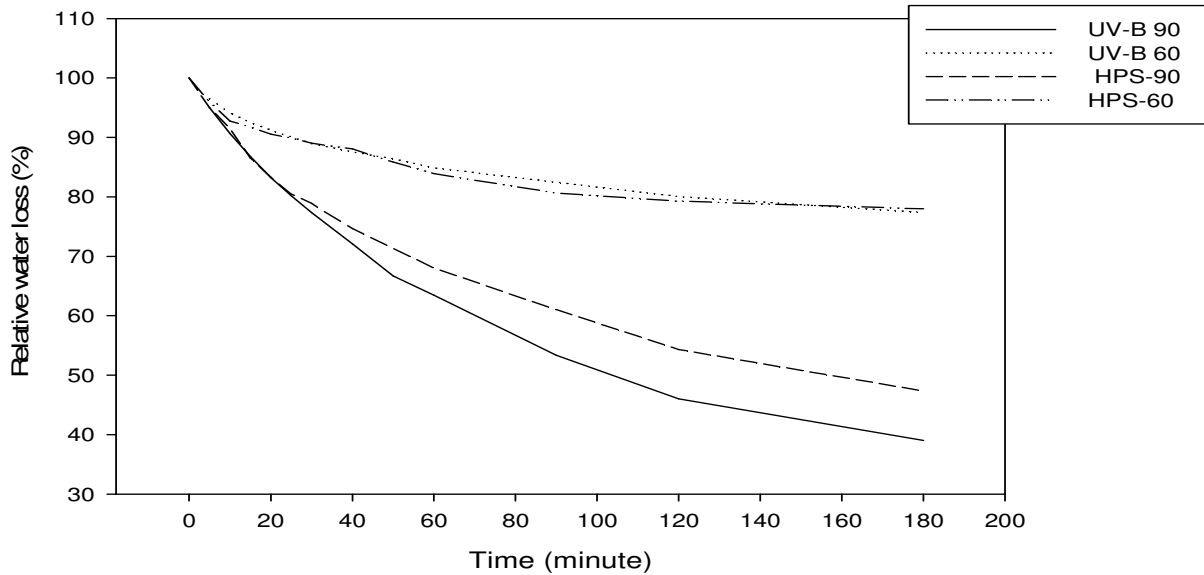


Figure 16. Relative water loss (%) from detached upper leaves of *Rosa ×hybrida* grown under HPS+ UV-B and HPS under high and moderate RH. The water loss was recorded during a period of 0 to 180 minutes after detachment (n=8).

4.2.4 Stomatal conductance ($\text{mmolm}^{-2}\text{s}^{-1}$) measurements of UV-B

Table 6. Value of Porometer reading at initial light level, after 1 hour in dark, day 2 in dark and day 3 during dark measurement. Significant differences were calculated at P -values <0.05 .

	Starting value light	1 hour in darkness	Two days in darkness	Three days in darkness
UV-B 90	466.3	184.8	92.4	80.3
UV-B 60	194.6	22.65	15.53	9.60
P-value	0.0001	0.003	0.007	0.001

Highest mean value was observed for UV-B 90 whereas least for UV 60 at starts lighting period, after 1 hour in darkness, two days in darkness and three days in darkness. Starting value at light for UV-B 90 was 5.8 times greater than third day in darkness whereas starting value at light for UV-B 60 was 20.27 times greater than third day in darkness. Thus UV-B 60 had greater reduction of porometer value compared to UV-B 90. Statistically significant were observed at *P*-values <0.05 (Table 6).

4.2.5 Pigments Measurement of UV-B

Table 7. Effect of UV-B and Relative humidity on accumulation of Flavonoids and Anthocyanin on different side of Rose leaf grown under growth chamber (n=3).

Treatments	Flavonoids		Anthocyanin	
	Upper leaf	Lower leaf	Upper leaf	Lower leaf
UV-B 90	0.28	0.25	0.05	0.033
UV-B 60	0.28	0.24	0.04	0.029
P-Value	0.98	0.65	0.19	0.60
	NS	NS	NS	NS

Measurement of flavonoids and anthocyanin with sample size of 3 of each upper and lower leaves showed statistically non-significant at *P*-values <0.05. Treatment of UV-B 90 and UV-B 60 RH had highest mean value of 0.28 in both cases of upper leaves at different treatment system of flavonoids. Similarly there is slightly difference in case of anthocyanin at upper leaves while the value was slightly higher for the treatment of UV-B 90 of lower leaves in both cases of flavonoids and anthocyanin, but the data is not significant.

5 Discussion

5.1 Experiment 1: HPS and LED

5.1.1 Morphology of roses

5.1.1.1 Stem and pedicel length

Our present study showed that the stem length was affected by light quality and shorter stems were found when plants were developed under LED (20% B and 80% R) compared to HPS (Table 3). Similar kind of result were observed by where rose plants grown under HPS had longer stems, compared to LED at both high and moderate RH (Terfa, M. T. et al. 2012). Moreover, the fact that stem length was found to be insignificant by RH is in accordance with similar studies where the rose ‘Baroness’ had similar stem length under high and moderate RH (Torre & Fjeld 2001).

Reduction in height by 20–34% was achieved when poinsettia plants were grown under the similar lamps as in the presented experiment. Thus, LED with a higher portion of blue light (20%) can suppress stem length more than the traditional HPS lamps (5% B). HPS also contain far red light but since the PPS was very similar in LED and HPS. It indicates that the blue light sensed by the cryptochrome is the main reason for shorter plants under LED and not in phytochrome. Blue light is also effective in the control of stem extension of poinsettia (Islam et al. 2012) and in a number of horticultural plants species including pepper (Brown et al. 1995). Also the pedicel length was suppressed under LED but only when the RH was moderate (Table 3). Under high RH the pedicel length was enhanced compared to moderate RH irrespective of the light quality. The pedicel has a more soft tissue and contains more water compared to the stem. The pedicel is probably more responsive to RH than the stem. Thus, high RH is probably overriding the effect of light quality and stimulates pedicel expansion.

5.1.1.2 Number of leaves, leaf area and flowering time

The leaf area was not significantly affected by either RH or light quality and no interaction between RH and light quality was found at 5% level of significance. However, the numbers of leaves were significantly affected by RH. On the other hand, the time to first open flower was not affected by either RH or light quality. Thus, in moderate RH the time to open flower was faster compare to high RH.

The number of flowers were found higher when plants were developed under HPS compared to LED and at moderate RH the number of flowers were greater compared to high RH but data was not significant (Table 3). Terfa et al. (2012) found the time to open flowers to be similar under HPS and LED light conditions. Similarly, in poinsettia no difference in time to open flowers was found between LED and HPS, indicating that poinsettia tolerates LED with a high proportion of Blue light in the spectrum without any negative effects on the time of the marketing stage (Islam et al. 2012) . Similarly, the flowering in roses also seems to be very robust when it comes to light quality effects.

Furthermore under continuous lighting humidity had no effect on the number of flowers and days to flowering (Pettersen et al. 2007). However when extending the lighting period from 18 to 24 hour per day a reduction in number of days until flowering and an increased number of flowers (34%) and a decreased number of days of flowering (12%) were observed (Pettersen et al. 2007). In the presented study the irradiance used under both light qualities was the same ($100 \mu\text{mol m}^{-2}\text{s}^{-1}$). Thus, it seems like the PAR light is more important for flowering time than light quality.

5.1.1.3 Dry weight

Dry weight is a measure for the amount of carbon fixed in the photosynthesis and the respiration. Our present study showed that dry matter of stem as well as dry matter of all pedicles with flowers was affected by RH and a higher dry weight were found when plants were developed

under moderate RH compared to high RH. The optimal RH for growth in most plants is between 70-85% RH. Photosynthesis was not measured in this experiment and it is not known why plants developed at high RH have a dry matter compared to moderate RH plants.

An increase in RH from 70 to 81% showed an increase in plant diameter and plant dry weight of *Begonia* whereas a further increase to 93% caused a decrease. Plant quality was found to be best at 70 % air humidity (Mortensen 2000). Light quality affects physiological process during growth and development of plant particularly during photosynthesis. Also, Light quality alters photosynthesis apparatus in leaves as well as calvin cycle enzymes. Wang et al. (2009) showed that the sucrose contents of cucumber plants grown under blue light were slightly higher than those grown under white, Red and green lights.

Few studies compare the effect of HPS light with LED. In our experiment we used LED, which was a mixture of 80% red and 20% blue, while in most studies monochromatic red or blue light was used. Kim et al. (2004) showed in a chrysanthemum a significantly higher dry weight in LED with a mixture of 50% red LED and 50% blue LED compared to 100% blue or 100% red LED.

Euphorbia pulcherrima were grown for 4 month providing artificial lighting for 16 hour day and 8 hour per day for flower induction. Observation was found that shoot elongation and fresh weight were highest for plants grown under HPS lamps than compared to LED as a supplementary lighting. Plants grown under HPS lamps had the highest photosynthesis compare to red/blue LED where combination was made 12.5% blue light 460nm and 87.5% red light 640 nm (Bergstrand & Schüssler 2012) . In roses the dry weight was not affected by light quality. Roses are very tolerant plants to light and high irradiance and are probably also robust to light quality.

5.1.2 Effect of light and humidity on Water loss measurement

Vase life of cut roses and keeping quality of pot roses is closely linked to the ability of leaves to control water loss. Roses grown under high humidity generally have a reduced keeping quality and symptoms of suffering from water stress like bent neck, wilting of leaves and improper opening and wilting of flower (Mortensen & Fjeld 1995; Torre & Fjeld 2001). Measurements of water loss from detached leaves have often proved to be a very good method to evaluate the potential keeping quality of roses. Our present result (Figure 14) showed that detached leaves of *Rosa x hybrida* grown at high relative humidity showed that rapid water loss compared to leaves grown at low RH.

Similar kinds of result were observed by others when leaves were exposed to high and moderate RH. (Mortensen & Fjeld 1998) reported that loss of water from leaves grown at high RH was much greater than that at 65% RH and increased with increasing lighting periods (16, 20 and 24 hr) using HPS lamp with a photosynthetic photon flux of $170 \mu\text{mol m}^{-2} \text{s}^{-1}$. In The presented study moderate RH grown leaves under HPS and LED lost in similar pattern and had a good desiccation tolerance irrespective of the light quality. However, leaves produced under high RH showed less water loss when grown under LED compared to HPS. The results indicate that LED is causing a better stomata function compared to HPS when growing at high RH.

5.1.3 Effect of light quality and air humidity on leaf conductance (CIRAS) and stomata morphology (Sump analysis)

When measuring the conductance (Figure 13) continuously during growth the main difference was found between plants at moderate and high RH. At moderate relative humidity a very low g_{sw} was found during day and night at both light qualities. In the night a conductance of less than $10 \text{ mmolm}^{-2}\text{s}^{-1}$ was observed indicating stomatal closure. This indicates that stomata developed under moderate RH close during darkness.

Similar kind of result was observed in the rose ‘Rebecca’ (Arve et al. 2013). The g_{sw} of ‘Rebecca’ growing in high relative humidity was high during darkness ($30 \text{ mmol m}^{-2}\text{s}^{-1}$) whereas very low g_{sw} ($3.5 \text{ mmol m}^{-2}\text{s}^{-1}$) in moderate relative humidity. In moderate humidity 60% results in stomatal closure (Arve et al. 2013). On the other hand, the g_{sw} of plants growing in high RH was still high for both HPS 90 and LED 90, but the LED was lower in darkness compared to HPS indicating a better dark induced closure when grown under LED compared to HPS.

The stomatal pore length were larger in plant grown under high RH measured during light and dark was 1.3 and 1.6 times larger than those grown under moderate RH. Likewise, stomatal pore aperture during light and dark under high RH was 1.9 and 3 times larger than moderate RH (Arve et al. 2013). Several studies have indicated similar result. Likewise, (Torre et al. 2003) found significantly higher number of stomata, greater length and greater aperture in plant grown under high RH compared to moderate RH.

Moreover in roses produced under LED (20% B and 80% R) the stomata frequency, index and number of stomata were higher than that of HPS lamp (Terfa et al. 2012). Large number of stomata developed under high relative humidity in *Populus × canescens* are found and believed to be because of low endogenous ABA levels. Large stomata are due to ABA insensitive plants (Arend et al. 2009).

The aperture measurements were done on stomata in light and dark. The interaction between light quality and air humidity was found to be non-significance in the presented study. This does not correspond to the water loss data or the conductance data. The comparative mean values between High RH and moderate RH during lighting as well as dark was not so larger in our experiment (Table 4). There may be several reasons for this. One reason may be that the stomata measured were not sufficient. There are lots of stomata of various size and shape and may be the numbers have to be increased to get clear results. Secondly, the image taken from the imprints were not clearly visible and it was difficult to measure the aperture. This can give a big error.

5.1.4 Effect of light and humidity on Leaf conductance after storage in darkness (Porometer measurement)

Our present study showed that the value of porometer reading of fully developed leaves were highly reduced from the initial reading during light to day third in darkness for both moderate and high RH plants (Table 5). Similar kind of result was also observed in ‘Baroness’ grown at moderate relative humidity and decreasing leaf conductance was found from day one to day third during both light and dark (Torre et al. 2003). In the study of Torre et al. (2003) a reduction in conductance was not found mainly in the plants grown at moderate RH. It is possible that different rose cultivars respond differently to both RH and darkness. The leaf conductance of pot roses grown at a constant high relative humidity under continuous lighting was higher during light as well as dark when placed under indoor climatic conditions than either plant under low humidity with 24 hours lighting Period.

5.2 Experiment 2: UV-B

5.2.1 UV-B and stomata function

In some species like Ericaceae and *Vicia faba* UV-B has been reported to induce stomatal opening or closing (Jansen & Van Den Noort 2000). The plant hormone ethylene is believed to be involved in UV-B responses. Ethylene is known to affect growth and development in many aspects and in some species ethylene is involved in stomata closure and opening. Ethylene induced stomatal opening is found to be inhibited by ABA (Wilkinson & Davies 2010). UV-B exposure has been found to induced hydrogen peroxide which is key component in *Vicia faba* stomatal closure. The effect was found to be mediated by ethylene (Desikan et al. 2006).

During the analysis of gas exchange measurement, our result showed in the UV-B treatment, the g_{sw} of *Rosa × hybrida* grown at both humidity had a similar pattern in conductance. However stomatal conductance was lower at moderate RH during one hour (17:30 to 18:30) exposure of UV-B and same time there was dark period from 16 to 20:00 (Figure 15). During the exposure of

one hour (6 to 7:00) exposure of UV-B without dark period nothing happens and the pattern was similar in both moderate and high RH. Likewise the porometer reading of leaves exposed to darkness was highly reduced from initial during light period to day third during dark for treatment UV-B (Table 6) but the leaves did not show a better closure than the leaves without UV-B (only HPS). Thus, it can be concluded that UV-B exposure will not improve the stomata function under high RH and will not lead to improve dark induced closure. Rather, the water loss (Figure 16) from leaves developed at HPS + UV-B was even higher than from HPS.

5.2.2 Pigments measurement

The UV radiation is now emerging as a new tool to use in greenhouse, mainly for bringing changes in plant morphology and in the control of plant diseases like powdery mildew. Supplemental UV radiation generally inhibits elongation growth of plants but increases secondary metabolite production like polyphenols such as anthocyanin and rosmarinic acid, organic sulfur compounds such as glucosinolate, and terpenoid compounds such as β -carotene, lutein. (Goto 2012). Moreover Supplemental UV-B had beneficial effects on assimilating, leaf area, fresh biomass and dry biomass on basil (*Ocimum basilicum* L) and other biochemical constituents (Sakalauskaite et al. 2013).

The anthocyanin and flavonoid of UV-B were measured in our experiment. Our present study showed no effect of UV-B exposure on accumulation of flavonoids and anthocyanin in the rose leaves (Table 7). However, anthocyanins concentration was increased in lettuce leaf (*Lactuca sativa* L) by 11 and 31% with supplemental UV-A (Li & Kubota 2009). Likewise UV-B induced anthocyanin synthesis in maize (*Zea mays* L.) by several fold compared to white light (Khare & Guruprasad 1993). Also, rose plants grown under LED with 20% B and 80% R light had a much higher anthocyanin levels compared to HPS. Likewise glucose, fructose and sucrose contents were 1.5, 1.6 and 1.4 times higher respectively, in leaves of LED grown plants than under HPS (Terfa, M. T. et al. 2012). Thus, blue light seems to be more important for inducing anthocyanin and flavonoids in rose leaves than UV-B. The UV-B was only given for 2 hours daily and this is

probably not enough to induce an increase. Maybe a longer exposure time with UV-B can induce anthocyanin and flavonols.

6 Conclusion

6.1 Experiment 1: HPS and LED

- The stems were significantly longer under HPS compared to LED but RH had no effect on stem length.
- Pedicel length was affected by both light quality and RH, and an interaction between RH and light quality was found. Under moderate RH, LED suppressed pedicel length and induced shorter pedicels compared to HPS.
- The number of flowers and the leaf area was not significantly affected by either RH or light quality and no interaction was found.
- Roses produced at moderate RH had significantly more leaves per stem, higher dry weight of stem and higher dry weight of pedicel length and flower compared to high RH, but no significant effect of light quality was found on these parameters.
- Moderate RH had a lower conductance than high RH in light and dark irrespective of the light quality.
- The conductance measurement showed a better dark induced closure under LED compared to HPS, especially when the HPS was high.
- Detached leaves from high RH lost more water compared to moderate RH. HPS 90 and LED 90 lost 53% and 39% of the initial water whereas HPS 60, LED 60, lost around 23%, 17%.
- Stomatal length and pore aperture were significantly higher in plants grown at high RH compared to moderate RH. The light quality had a significant effect of stomatal length and aperture but no interaction between RH and light quality was found.
- The leaf conductance ($\text{mmolcm}^{-2} \text{s}^{-1}$) was highly reduced from the first reading in the light to the third day of dark storage.
- Plants developed at high RH with LED showed a better dark induced closure than plants grown under high RH and HPS.
- Plants developed at moderate RH showed a strong dark induced closure irrespective of the light quality.

- A light source with more blue light will improve the stomata function under high air humidity conditions and can be a tool to improve shelf life of ornamentals like roses in periods when air humidity is high.

6.2 Experiment 2: UV-B

- Both high and moderate air humidity (HPS + UV-B) showed a similar pattern in conductance and water loss as plant not exposed to UV-B (only HPS).
- HPS + UV-B did not improve the stomata function under high RH, rather it is induced a higher water loss than high RH under HPS.
- The content of flavonoids and anthocyanin in rose leaves was not affected by RH or UV-B treatment.
- The use of UV-B as a supplementary light in the greenhouse is a new tool and needs further investigation, especially the effect on stomata function.

7 Reference

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