Growth, stomatal responses and postharvest characteristics of *Rosa x hybrida*

-The influence of air humidity and light quality

Vekst, spalteåpningsresponser og karaktertrekk etter produksjon hos Rosa x hybrida

- Betydningen av luftfuktighet og lyskvalitet

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ABSTRACT

Greenhouse production systems are important in the production of vegetables, herbs and ornamentals. Roses are among the most valuable crops in the greenhouse floriculture industry and are produced as pot plants or as cut flowers. Climatic factors like light and relative air humidity (RH) have an enormous influence on growth morphology and postharvest life of greenhouse grown roses. Light is one of the most important environmental factors, acting on plants not only as the sole source of energy for photosynthesis, but also as a source of external information, affecting growth and development. All its components: quality, quantity (irradiance) and periodicity can modulate plant growth and development either through an effect on photosynthesis or photomorphogenetic responses. In Northern latitudes supplementary lighting is necessary to keep up the production in periods when the natural irradiance is low. The RH in such greenhouses can also rise up to 90% in certain periods depending on the season and the production system. Plant production in continuous high RH (>85 %) may result in less functional stomata that are large and unable to close in environments which normally induce closure such as darkness, high VPD or abscisic acid (ABA) treatment. This results in poor postharvest quality due to uncontrolled water loss. Although several studies have been performed to find ways to avoid the development of malfunctioning stomata under high RH conditions, knowledge on this is still limited. The aim of the present study was to improve the knowledge on the effect of light quality on photosynthesis, morphology and development of roses. Further, the aim was to investigate interactions between RH and light climate in order to get a better understanding of the stomata control in light and darkness, as well as under different light qualities. Such knowledge is essential to be able to produce roses with a good water balance and a long postharvest life.

The effect of different light qualities provided by high pressure sodium (HPS; 5% blue light (B)) and light emitting diodes (LED; 20% B) on photosynthesis capacity, growth, morphology, flowering and postharvest characteristics of *Rosa x hybrida* 'Toril' plants were investigated. The results showed that the increased B light proportion highly affected the growth and morphology. Plants grown under 20% B light from LEDs had reduced plant height and leaf area and showed 20% higher photosynthetic capacity compared to plants produced under the traditional HPS lamps. Although floral initiation occurred at a higher leaf number in 20% B light, the time to open flowers was not affected and there was no difference in dry matter accumulation between the treatments. The plants produced with the LEDs also displayed a more sun-type leaf anatomy with more and longer palisade cells and a higher stomata frequency compared to HPS. This indicates that in roses plant morphology is more sensitive to B light than flowering. It was also observed that increased level of B light increased the level of carbohydrates, delayed senescence, and improved storability at 4°C.

The study of stomata of *Rosa x hybrida* plants developed under continuous high (90%) and moderate (60%) RH showed that compared to moderate RH, high RH reduced the

ability of the stomata to close when subjected to closing stimuli. The results also showed that plants grown under high and moderate RH regulate their ABA contents differently. ABA-glucose ester (GE) is an important storage form of ABA, which can be released through *β*-glucosidase activity when needed. Compared with high RH, *Rosa x hybrida* plants developed in moderate RH and 20 h photoperiod contained higher levels of ABA and *β*-glucosidase activity. The increase in ABA level during darkness in moderate RH was accompanied with a decrease in ABA-GE levels. However, the increase in ABA during darkness was absent and the *β*-glucosidase activity was low in plants developed under high RH with 20 h photoperiod. Continuous lighting (24 h) resulted in low levels of *β* -glucosidase activity irrespective of RH, indicating that a dark period is essential to enhance *β* -glucosidase activity. Furthermore, it was investigated if increasing the B light proportion during growth, could overcome the negative effect of high RH. The result showed that, increased B light proportion improved stomata function and dark-induced stomata closure under high RH conditions. The improved stomata function correlated with increased ABA content in general and a dynamic ABA peak during darkness. The increase in ABA was associated with the presence of high *β*-glucosidase activity and indicates that B light is important as a signal to enhance the activity of *β*-glucosidase enzyme.

Finally, the effect of natural levels of UV radiation at different altitudes in Ethiopia (high altitude; (2794 ma.s.l) and lower altitude (1700 ma.s.l.) on growth responses like morphology and flowering, postharvest water usage and life of three pot rose cultivars in Ethiopia were studied. The results showed that UV radiation significantly reduced stem length and leaf area at both altitudes; however the effect was more prominent at lower altitude. Besides, higher level of solar UV radiation delayed flowering by 7-10 days. Postharvest life and water usage were not significantly affected by UV radiation but rather by the altitude and plants produced at high altitude had a better control of water loss and a longer postharvest life compared to lower altitude-grown plants.

In conclusion, increasing the proportion of B light in assimilation lighting in greenhouse production of pot roses can be used to increase photosynthesis, reduce stem elongation, reduce the postharvest water usage of plants grown at high RH and to improve postharvest life without affecting production time. In high RH conditions, increasing B light can thus be applicable as a strategy to overcome the negative effects of high RH. In roses the enzyme *β* -glucosidase has a central role. It is a key enzyme in regulating the ABA pool and its activity was shown to be controlled by RH, photoperiod, and B light. However, the study shows that UV radiation is not important for stomata function and postharvest water relation of roses.

Key words: Abscisic acid (ABA), Blue light, Darkness, Relative air humidity (RH), Stomata, Ultraviolet radiation (UV), *β*-glucosidase

SAMMENDRAG

Veksthus som produksjonssystem er viktig i produksjon av grønnsaker, urter og prydvekster. Roser er en av verdens mest viktige prydvekster og dyrkes som snittblomst eller potteplante. Klimafaktorer som lys og relativ luftfuktighet (RF) har stor betydning for vekst, morfologi og egenskaper etter produksjon hos veksthusproduserte roser. Lys er en av de viktigste klimafaktorene, og drivkraften i fotosyntesen, men bidrar også til å gi planten viktig informasjon for vekst og utvikling. Både lysmengde, fotoperiode og lyskvalitet kan modulere plantevekst og utvikling enten gjennom endret fotosyntese eller via fotomorfologiske responser. På nordlige breddegrader er kunstig tilleggslys nødvendig for å opprettholde produksjonen i perioder med lite naturlig lys. Luftfuktigheten i veksthuset er ofte høy i denne perioden og kan, avhengig av produksjonssystem, være opptil 90%. Planter utviklet under kontinuerlig høy RF (>85%) danner spaltåpninger som ikke lukker under forhold som normalt fører til lukking, slik som mørke, tørr luft eller behandling med plantehormonet abscisinsyre (ABA). Ufunksjonelle spaltåpninger gir ofte dårlig holdbarhet hos roser på grunn av ukontrollert vanntap fra bladene når plantene flyttes ut av veksthuset.

Målet med denne studien var å utvikle kunnskap om hvordan lyskvalitet fra ulike kunstlyskilder påvirker fotosyntese, morfologi, utvikling og spaltåpningsfunksjon hos roser. Videre var målet å studere samspillet mellom lyskvalitet og RF for å undersøke hvordan spalteåpningsresponsen påvirkes av lyskvalitet under ulike RF-regimer (moderat og høy RF).

Resultatene viser at både vekst og morfologi hos *Rosa x hybrida* 'Toril' er følsomme for lyskvalitet, og spesielt er andelen av blått lys viktig. Lys-emitterende dioder (LED) med 20% blått lys og 80% rødt lys (20B/80R) ga redusert plantehøyde og bladareal, og 20% høyere fotosyntetisk kapasitet sammenlignet med planter dyrket under tradisjonelle høytrykksnatriumlamper (SON-T) med bare 5% blått lys. Roser dyrket under LED (20B/80R) hadde en lengre vegetativ vekstperiode men tid til første åpne blomst var den samme for LED og SON-T. Det var heller ingen forskjell i tørrvekt mellom de to lyskvalitetene. Planter produsert under LED (20B/80R) hadde imidlertid en mer sol-tilpasset bladanatomi med flere og lengre palisadeceller, høyere klorofyll-og antocyanin-innhold, og en høyere spaltåpningsfrekvens sammenlignet med SON-T. Dette viser at et lysspektrum med en høyere andel av blått har stor påvirkning på roser og at morfologien er mer følsom enn blomstringen. Det ble også observert at planter dyrket under LED (20B/80R) hadde et høyere innhold av løselige karbohydrater, og en forsinket aldring under lagring ved 4°C sammenlignet med SON-T.

Roser dyrket ved høy RF (90%) og moderat RF (60%) viste ulik spaltåpningsrespons og kontinuerlig høy RF reduserte spalteåpningenes evne til å lukke i mørke og i tørr luft (<50% RF) sammenlignet med moderat RF. Roser dyrket under moderat RF og 20 timer lys inneholdt mer ABA, spesielt i mørkeperioden, og det ble målt en høyere aktivitet av β-glucosidase i disse bladene. Roser dyrket ved høy RF derimot, vise ingen økning i ABA i mørke og β-glucosidase-aktiviteten var lav. ABA kan konjugere med glukose ester (GE) og danne ABA-glukose ester (ABA-GE) som er en viktig lagringsform. ABA kan frigis fra ABA-GE ved hjelp av enzymet β-glucosidase når det er nødvendig. Dyrking i kontinuerlig lys (24 timer) resulterte i lav β-glucosidase-aktivitet uansett RF-nivå og tyder på at en mørkeperiode er nødvendig for å aktivere dette viktige enzymet hos roser.

For å undersøke videre betydningen av blått lys på spaltåpningsfunksjon under høy RF ble det gjennomført en studie hvor andelen av blått lys og rødt lys ble manipulert og hvor de ulike LED behandlingene inneholdt 5%B/95%R, 20%B/80%R og 100%B. Resultatene viste at ved å øke andelen av blått lys fra 5% til 20% i vekstlyset var det mulig å forbedre spaltåpningsresponsen under høy RF. Roser dyrket under 20%B eller 100%B viste forbedret mørkelukking og mindre vannforbruk etter høsting. Bedring i spaltåpningsresponsen korrelerte med økt ABA-innhold og økt aktivitet av enzymet β-glucosidase.

Et forsøk med tre ulike potterosersorter ble utført på ulike høyder over havet i Etiopia: karakterisert som lavland (1700 m.o.h.) og høyland (2794 m.o.h.). I denne undersøkelsen var målet å teste betydningen av UV-stråling på produksjonspotensialet og holdbarheten hos roser. Selektiv plastfilm ble benyttet for å manipulere UV-strålingen og rosene ble dyrket under plastfilm som slipper gjennom UV (+UV) og sammenlignet med roser dyrket under plastfilm som ikke slipper gjennom UV (-UV). Resultatene vise at morfologien hos roser er svært følsom for UV-stråling. Strekningsveksten og bladarealet ble redusert hos planter eksponert for UV uansett høyde over havet men effekten var sterkere i lavlandet. UV-stråling forsinket også blomstringstiden med 7-10 dager. Holdbarheten og vannforbruket etter høsting var ikke påvirket av UV-stråling under dyrking men av høyde over havet. Plantene dyrket i høylandet hadde lavere vannforbruk etter produksjonen, viste mindre kronbladtørke og bladtørke og hadde dermed bedre holdbarhet sammenlignet med planter fra lavlandet.

Resultatene fra denne oppgaven viser at lyskvaliteten kan optimaliseres ved dyrking av roser i veksthus. Ved å øke andelen blått lys til 20% kan morfologien kontrolleres og strekningsveksten reduseres uten å forsinke blomstring. I perioder med høy RF kan blått lys bidra til å forbedre spaltåpningsfunksjonen og dermed bedre vannbalansen og øke den potensielle holdbarheten. Denne kunnskapen kan benyttes i praktisk produksjon til å produsere roser av god kvalitet i perioder med høy RF. Enzymet β –glucosidase har en sentral rolle i ABA-regulering og tilgjengelighet hos roser. Både RF, mørke og blått lys er viktige signaler for aktiviteten til dette enzymet.

Nøkkelord: Abscisinsyre (ABA), Blått lys, Mørke, Relativ luftfuktighet (RF), Spalteåpninger, Ultrafiolett stråling (UV), β-glucosidase

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Meseret Tesema Terfa

LIST OF PAPERS

I. Meseret Tesema Terfa, Knut Asbjørn Solhaug, Hans Ragnar Gislerød, Jorunn Elisabeth Olsen and Sissel Torre (2013). A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of *Rosa* × *hybrida* but does not affect time to flower opening. Physiologia Plantarum **148**, 146–159.

II. Meseret Tesema Terfa, Madhu S. Poudel, Amsalu G. Roro, Hans Ragnar Gislerød, Jorunn Elisabeth Olsen and Sissel Torre (2012). 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. Acta Horticulturea **956,** 635-642.

III. Louise Elisabeth Arve, Meseret Tesema Terfa, Hans Ragnar Gislerød, Jorunn Elisabeth Olsen and Sissel Torre (2013). High relative air humidity and continuous light reduce stomata functionality by affecting the ABA regulation in rose leaves. Plant, Cell and Environment **36**, 382–392.

IV. Meseret Tesema Terfa, Madhu S. Poudel, Hans Ragnar Gislerød, Jorunn Elisabeth Olsen, Sissel Torre (2013). Blue light improves stomata function and dark-induced stomata closure of rose leaves (*Rosa* x *hybrida* cv. Toril) grown at high air humidity. (Manuscript).

V. Meseret Tesema Terfa, Amsalu Gobena Roro, Jorunn Elisabeth Olsen and Sissel Torre (2013). Effects of UV radiation on growth and postharvest characteristics of three pot rose cultivars grown at different altitudes. (Manuscript).

ABBREVIATIONS

ABA= Abscisic acid

ABA-GE= ABA- glucose ester

AL= Artificial lighting

B = Blue

DM= Dry matter

DPA= Dihydrophaseic acid

FR= Far red

gs = Stomata conductance

HPS= High pressure sodium

LA= Leaf area

LEDs= Light emitting diodes

PA= Phaseic acid

PAR = Photosynthetically active radiation

PPS= Phytochrome photostationary state

R= Red

RH= Relative air humidity

SUMP= Suzuki's Universal Micro-Printing

UV = Ultraviolet

VPD= Vapour pressure deficit

1. INTRODUCTION

1.1 Greenhouse as a production system

Greenhouses are important in the production of vegetables, herbs and ornamentals. The greenhouse production system is based on control of the environment in such a way that it provides the conditions that are most favorable for optimal photosynthesis, maximum yield and quality. The productivity and quality of greenhouse products are influenced by various climatic factors during growth (Gruda, 2005). These conditions are also known to affect the plant morphology and its robustness and ability to tolerate stress after harvest (Gruda, 2005; Kang et al., 2002). The critical environmental parameters affecting plant growth and eventually the postharvest life in such growth systems are temperature, light, relative air humidity (RH), carbon dioxide, nutrition, availability of water, and the growing media. Climatic factors like light and RH have an enormous influence on the growth, morphology and postharvest life of greenhouse grown plants.

1.1.1 Greenhouse production at Northern latitudes

An important focus in the greenhouse industry in Northern latitudes is to have a high productivity and a good quality with a minimum of energy supply during production. Due to low natural radiation during winter at Northern latitudes (e.g. Norway), year round production is dependent on the use of supplementary artificial lighting (AL). Artificial lighting was supplied through different lamp types such as incandescent lamps or neon tubes. Later, mercury lamps and fluorescent tubes became predominant for assimilation light, while incandescent lamps remained widely used for flowering control due to richness in far-red (FR)light in the spectrum (Bergstrand and Schüssler, 2012). However, after the revelation of high pressure sodium (HPS) lamps in the 1970's, HPS has remained the most common technology used ever since (Bergstrand and Schüssler, 2012). Thus, the supplementary light during winter in the Northern hemisphere is usually applied by HPS, which have a high radiant emission, and a high electrical efficiency, a high R(red)/FR ratio (3.6) compared to natural light (1.2) but only 5% blue (B) light which is low as compared to the natural sunlight (15-18%).The majority of light emitted from HPS lamps is in the range of 565 to700 nm, primarily yellow (565 to 590 nm) and orange (590 to 625 nm), with a peak at 589 nm (Currey and Lopez, 2013). However, during the last decade the progress in solid-state lighting, based on light-emitting diodes (LEDs) has facilitated the research on light quality responses of

plants in general, and attracted much interest as a light source for assimilation lighting in greenhouses. There are several features of LEDs that make them attractive alternatives to HPS lamps. The most unique aspect of LEDs is the availability of narrow-spectrum light at wavebands of primary interest for plant growth and development, including B (450 nm), R (660 nm), and FR (730 nm) (Currey and Lopez, 2013). Advantages such as long lifetime, high efficiency, high controllability with respect to light intensity and quality and freedom of choice regarding design and placement of fittings are the most attractive features of the LED technology (Morrow, 2008; Pinho, 2008; van Ieperen and Trouwborst, 2008). Due to increasing interest in energy efficiency and minimizing the cost of energy in greenhouse production in Scandinavia in recent years, more efficient technologies are utmost needed. Besides, better utilization of the light by plants has attracted considerable interest. Optimization of light quality and greenhouse climate in relation to light supply is also of great relevance for maximizing production in relation to the amount of energy used (Aaslyng et al., 2006).

RH is economically the most difficult climate factor to control in a greenhouse and the most common methods until now have been to regulate the RH by opening and closing the vents and to warm up the humid air. This strategy will lead to high energy consumption. Thus, attention has been paid on either closed or semi-closed greenhouses systems where ventilation is avoided or reduced. This system with a minimum of ventilation will obviously lead to a higher RH if there is no system for de-humidification. The RH is one of the most important factors that influence the water status of plants and consequently affecting all processes that are associated with the transpiration such as water balance, transpirational cooling, and ion translocation (Mortensen and Fjeld, 1998; Torre et al., 1999). Harmful effects of extreme high RH to plants includes heat damage, which is likely to occur because of the reduction in transpirational cooling, reduced translocation of some ions (i.e calcium) from roots to the shoots, and reduced stomata function (Torre et al., 1999). The main postharvest problem of these plants is uncontrolled water loss from the leaves when they are transferred to a lower RH or postharvest rooms (Torre et al., 2001, 2003; Fanourakis et al., 2012).

1.1.2 Greenhouse production and floriculture in Ethiopia

In Ethiopia, greenhouse-growing of crops has become an important industry. Hence, the use of greenhouses or plastic tunnels with no heating for production of high valued crops

has intensively increased recently. Among all crops produced in Ethiopian greenhouses, cut flowers are the fastest growing in export business. The volume of export is still growing and shows great promises. Roses account for two thirds of Ethiopian flower export and it is estimated to expand even more. Today, the rose production in Ethiopia covers about 650-700 ha of production land. Even if the flower industry in Ethiopia is still young, it has already out-competed other African countries when it comes to size of production area (Haug et al., 2008). The country is now the second largest exporter of cut flowers in Africa (Gebreeyesus and Iizuka, 2012). This makes floriculture one of the most important branches of Ethiopian agriculture and indeed of Ethiopian economy as a whole. The two main locations where the commercial rose productions are intensively under way in Ethiopia are highlands (2,400-2,600 ma.s.l) around the capital, Addis Ababa, where the climate is characterized by high day temperatures and cool nights, and Ziway (mainly characterized as lowland; 1,100-1,800 ma.s.l) where the daily mean temperatures are higher (25°C in average) (Joosten, 2007). These production sites have obviously huge difference in different climatic factors such as daily mean temperature, day and night temperatures, UV radiation, and RH/water vapour pressure deficit (VPD). Hence, the expected differences in climate may play a big role in growth and postharvest behavior either directly or indirectly. To maintain a good quality and a long postharvest life, there is a need for more knowledge on effects of the seasonal variation during the year, and how the environmental factors during growth at these altitudes influence the keeping quality of the Ethiopian roses.

1.2 Environmental conditions affecting growth and postharvest life of roses

Environmental factors during growth are important in controlling plant growth, morphology and flowering in plants. In general, the external and internal qualities of roses are affected by environmental conditions during growth. Factors such as temperature, irradiance, light quality, photoperiod, nutrition and CO_2 concentration highly affect plant growth and morphology (Biran et al., 1973). Pre-harvest environmental conditions also have an enormous effect on the postharvest characteristics and water loss of cut roses (Halevy and Mayak, 1979a, b). Factors during growth such as irradiance, light quality and photoperiod (Fjeld et al., 1994; Mortensen and Gislerød, 1999; Terfa et al., 2012 (paper II)), day and night temperatures (Moe, 1975; Hamrick, 2003), carbon dioxide (Dole and Wilkins, 2005), relative air humidity (Torre et al., 2001; Pettersen et al., 2007; Fanourakis et al., 2012) are all shown to affect the postharvest shelf life. Previous studies on irradiance and roses have shown that

with increasing irradiance, plant growth and flowering also increased (Gislerød and Mortensen, 1997). This was due to increased photosynthesis and increased assimilate partitioning to young shoots, which in turn stimulate growth and flower development (Mor and Halevy, 1984). Increasing natural or supplementary light level has been also demonstrated to improve vase life of roses, and is thought to be mediated through the carbohydrate status of the plant (Fjeld et al., 1994). In this review only light quality and RH will be discussed in detail.

1.2.1 Light receptors and responses in plants

Light is one of the most important environmental factor acting on plants not only as the sole source of energy, but also as a source of external information, affecting growth and development. All its components: quality, quantity (irradiance) and periodicity can modulate plant growth and development either through an effect on photosynthesis or through a photomorphogenetic role. Spectral changes of illumination evoke different photosynthetic and morphogenetic responses, which can vary among different plant species. R and B light are more efficiently absorbed by photosynthetic pigments than other spectral regions (McCree, 1972; Inada, 1976). Maximum quantum yield occurs near 600 nm, and declines rapidly at wave lengths shorter than 400 nm and greater than 680 nm (Evans, 1987). Further, R light is important for the development of the photosynthetic apparatus (Saebø et al., 1995). B light affects the formation of chlorophyll (Chl), stomata opening and photomorphogenesis (Schuerger et al., 1997; Dougher and Bugbee, 1998; Heo et al., 2002). Several studies have also shown that B light influences the biochemical properties of photosynthesis in leaves such as Chl a/b ratios, Chl a/b-binding protein of photosystem II (LHCII), and photosynthetic electron transport (Leong and Anderson, 1984; Senger and Bauer, 1987).

In addition to the importance of B and R light in photosynthesis, these light qualities are also important in the regulation of plant growth by light receptors in photomorphogenesis. The R: FR ratio during the light period or at the end of the day (photoperiod) as well as the proportion of B light is known to affect stem elongation and morphology but the effect vary among species (Blom et al., 1995; Folta et al., 2003; Dougher and Bugbee, 2004; Islam et al., 2012; Terfa et al., 2013 (Paper I)). In this respect, the photoreceptors B/UV-A light absorbing cryptochromes and phototropins and the R and FR light absorbing phytochromes are of particular importance (Fig. 1) (Kami et al., 2010; Liu et al., 2011). Besides, it has been elucidated recently that UVR8 (UV resistant locus 8) is an UV-B receptor, which is involved

in UV-B perceiving and promoting plants UV acclimation (DNA damage repair, antioxidants, sunscreen pigments and inhibition of elongation) and survival in sunlight (Fig. 1) (Rizzini et al., 2011; Heijde and Ulm 2012; Tilbrook et al., 2013).

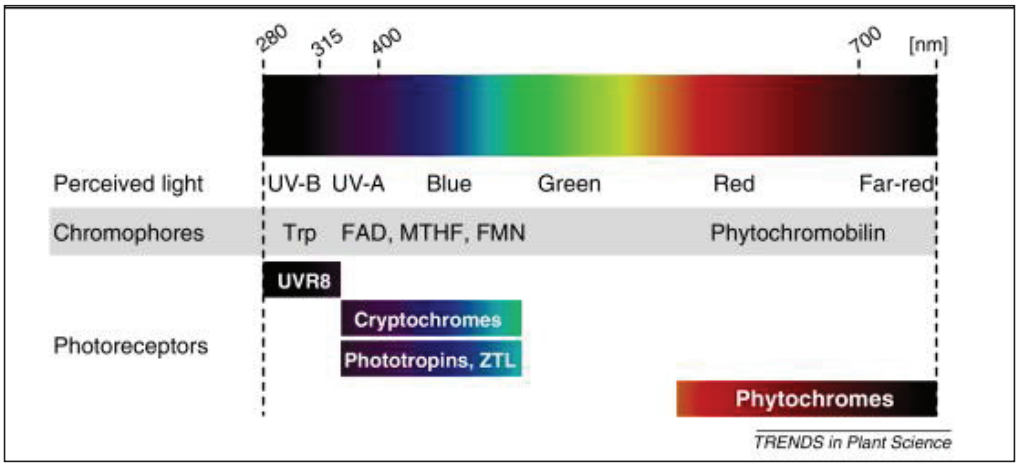


Figure 1. Photoreceptor-mediated light perception in higher plants. Plant photoreceptors perceive information from a large part of the light spectrum. UVR8 (UV resistant locus 8) is the only UV-B photoreceptor identified to date and uses specific intrinsic tryptophans (Trp) as an UV-B-activated chromophore. To absorb light in the UV-A/blue part of the spectrum, cryptochromes use flavin adenine dinucleotide (FAD) and methenyltetrahydrofolate (MTHF), and phototropins and the Zeitlupe family (ZTL) proteins use flavin mononucleotide (FMN) as their chromophores. Phytochromes are red/far red photoreceptors that use a plant-specific linear tetrapyrrol (phytochromobilin) for light capture Heijde and Ulm (2012).

Phytochromes as light receptors exist in two photoconvertible forms, the FR absorbing form (Pfr) which is considered as the active form, and the R light absorbing inactive form (Pr) (Smith, 2000). Phytochromes are capable of regulating almost all phases of plant development, but the control is conditional or facultative, rather than obligatory (Smith, 2000). Most common phytochrome responses are germination, de-etiolation, leaf expansion, Chl development, elongation, flowering and dormancy (Smith, 2000). The absorption spectra of the phytochromes show peaks at about 665 nm and 730 nm (Smith, 2000) for Pr and Pfr respectively. The relative proportion of active form (Pfr) to the total (Ptot) is denoted as the phytochrome photostationary state (PSS). It is this relative proportion of Pfr to Ptot that regulates a given photomorphogenic response. Although important in selecting a lamp source, in practice, the most relevant factor in photobiology is the fraction of phytochrome

present in the active (Pfr) form with respect to the total phytochrome (Ptot = Pfr + Pr) at photoequilibrium (Stutte, 2009).

B light, which acts via B/UVA photoreceptor(s), is effective in inducing different photomorphogenic responses and floral transition, but the effects vary among species (Guo et al., 1998; Imaizumi et al., 2003; Fukuda et al., 2011). In the facultative long-day plant (LD) *Arabidopsis thaliana* and *Petunia x hybrida*, B light is effective in promoting flowering under LD (Guo et al., 1998; Imaizumi et al., 2003; Fukuda et al., 2011). However, this response of B light was not notable in the LD plants *Scabiosa atropurpurea* and *Spinacea oleracea* (Withrow and Withrow 1940) and has no effect in day neutral roses (*Rosa x hybrida*) and short day (SD) plant poinsettia *Euphorbia pulcherrima* (Islam et al., 2012; Terfa et al., 2013 (Paper I)). B light is also involved in inhibition of growth of internodes and cell expansion or division (Appelgren, 2003; Folta et al., 2003; Dougher and Bugbee 2004). Ahmad et al. (2002) showed that hypocotyl elongation in *A. thaliana* is inhibited by B light via a cryptochrome-mediated response. However, these photomorphogenic responses are species dependent. Dougher and Bugbee (2001) defined long-term B light dose-response curves for leaf area (LA) and stem length in soybean and lettuce. They showed that stem length and LA in soybean decreased with increment of the B light proportions while LA in lettuce increased with an increasing B light fraction. Furthermore, in *Petunia x hybrida* monochromatic B light enhanced stem elongation strongly compared to R light (Fukuda et al., 2011). In roses and poinsettia, a high B light proportion supplemented by LED lamps inhibited elongation and produced more compact plants as compared to plants grown under HPS (5% B) lamps (Islam et al., 2012; Terfa et al., 2013 (paper I)).

UV radiation (especially UV-B) also has various effects on morphology, biochemical composition and molecular responses of different species. The responses depend on species, cultivar, experimental conditions, levels of UV-B and the interaction with other climatic factors like temperature and photosynthetically active radiation (PAR) (Frohnmeyer and Staiger, 2003; Reddy et al., 2004; Brown et al., 2005; Berli et al., 2012). Some of these UV-B induced ranges of plant responses are desirable from a horticultural perspective. Although UV-B was earlier mainly considered a plant stressor and a potential source for damage, currently an ambient or ecological dose of UV-B is believed to be an important signal for plants rather than a stressor (Jenkins, 2009; Jansen et al., 2012). Novel technologies to manipulate UV levels are emerging. For example by using different selective plastic films, either UV-blocking or UV-transparent, specific parts of the UV spectrum can be

manipulated. This provides new opportunities in protected crop production to use UV-B radiation for various purposes such as: controlling morphology in pot plants, color development and disease control, etc (Jansen et al., 2012).

1.2.2 The role of light quality in growth, flowering and postharvest life of roses

In roses generally, it has been reported that R light weakens the apical dominance while FR light strengthens it (Cline, 1991). R light also promotes the sink activity of the flower bud more than B or FR light, thus improving the growth and flower development (Mor et al., 1980). FR end-of-day light, compared with R light, markedly reduced flowering of the rose cultivar 'Mercedes' (Maas and Bakx, 1995). This response indicates the involvement of the photoreceptor phytochrome. However, increasing the B light proportion had no effect on flowering of rose 'Toril' (Terfa et al., 2013 (paper I)). Additonally, Maas and Bakx (1995) showed that decreased proportion of B light in the photosynthetic photon flux (PPF) increased the shoot length of 'Mercedes' roses. Low R:FR ratio commonly increases the levels of gibberellins (GA) as well as plants' sensitivity to GAs, thus enhancing internode length and inhibiting axillary bud growth (Hutchings and De Kroon, 1994; Smith, 2000; Olsen and Junttila, 2002; Islam, 2013). In greenhouse rose production HPS lamps are the most widely used lamps for supplementary lighting. In HPS lamps the R:FR ratio is approximately 3.6, while in natural light it is approx. 1.2 (Bredmose, 1993). HPS lamps also have a low B light proportion which is approximately 5%, compared to 15-18% in natural light. Hence, this decrease in B light proportion in addition to higher R:FR will promote shoot elongation rather than axillary shoot formation. Furthermore, in paper I we also showed that higher B light proportion supplemented by LED suppressed stem elongation and decreased LA in *Rosa x hybrida* 'Toril' as compared HPS (Terfa et al., 2013(paper I))

Light quality is important pre-harvest factor which can also have an impact on the postharvest behavior of roses either by affecting the hormonal content, carbohydrate status or stomata functionality (Rajapakse and Kelly, 1994; Blom-Zandstra et al., 1995; Garello et al., 1995; Mortensen and Fjeld, 1998; Terfa et al., 2012 (paper II)). Terfa et al. (2012 (paper II)) showed that LED with 20 % B light proportion improved storability of roses at 4 °C compared to HPS. This was mediated through increased level of carbohydrate and suppressed senescence, which are essential in postharvest. Light quality has also significant effect on the level of the abscisic acid (ABA) in rose petals which correlates with postharvest life of the flowers (Garello et al., 1995). The ABA content in petals at harvest was lower in plants

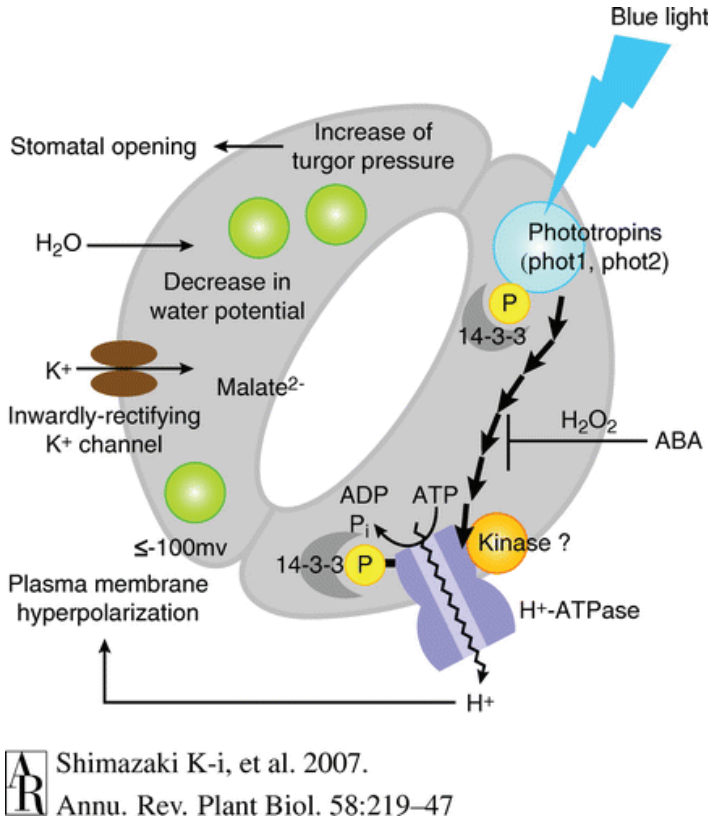
grown under HPS- lamps than in those grown under metal halide (MH) lamps. In vase, the ABA content in petals increased faster and the vase life was shorter for flowers from MH-grown plants, compared to those cut from HPS-grown plants. In contrast, van Doorn and Vojinovic (1997) reported that, neither low irradiance nor the difference in light quality (mainly R: FR ratio) had effect on inhibition of rose petal abscission rather it was dependent on water stress. Light quality can also affect postharvest water balance of roses by affecting stomata functionality. Blom-Zandstra et al. (1995) showed that orange light increases stomata conductance as compared to B or white light. They further showed that the night time transpiration of some rose cultivars was higher in orange light as compared to B or white light during growth. Studies have shown that the stomata behavior in response to conditions of the cultivation environment will also persist after harvest (Mortensen and Gislerød, 1999; Torre and Fjeld, 2001; Fanourakis et al., 2012). Thus this will affect the postharvest water relation and determine the potential postharvest life, especially for cut flowers, but also for some pot and bedding plants (Torre and Fjeld, 2001; van Doorn, 1997; Waterland et al., 2010a, 2010b). In contrast, Mortensen and Fjeld (1998) showed that different light qualities given by HPS and fluorescent light had no effect on either the water relation or postharvest life of cut roses.

1.2.3 Light regulation of stomata

Stomatal pores surrounded by a pair of guard cells in the plant epidermis regulate gas exchange between leaves and the atmosphere. Opening of the stomata allows both CO_2 entry for photosynthesis and the transpiration stream in higher plants (Assmann, 1993; Schroeder et al., 2001). The opening of stomata is mediated by an accumulation of K^+ in guard cells, and the accumulation is driven by an inside-negative electrical potential across the plasma membrane (Assmann and Shimazaki, 1999; Schroeder et al., 2001). Stomata closure occurs as a result of removal of osmotica such as K^+ from guard cells under drought, darkness, elevated CO_2, ABA or low RH (Shimazaki et al., 2007).

Stomatal opening is induced by light; including B and R light. Blue light acts as a signal and R light acts as both a signal and an energy source through photosynthesis (Zeiger, 1983). The stomata response to light is regulated by two major photoreceptor systems, photosynthesis in the guard cell chloroplast and a specific B light responses (Schwartz and Zeiger, 1984; Assmann, 1993). Blue light induced stomata opening involves the activation of the plasma membrane H^+-ATPase (Fig. 2; Kinoshita and Shimazaki, 1999; Briggs and

Christie, 2002), hyperpolarizing the membrane potential with simultaneous apoplast acidification, and drives K+ uptake through voltage-gated K+ channels (Shimazaki et al., 2007). The B light-absorbing cryptochromes, zeaxanthin and phototropins are receptors for B light stomata responses (Zeiger and Zhu, 1998; Kinoshita et al., 2001; Briggs and Christie, 2002; Mao et al., 2005). Zeaxanthin, a component of the xanthophyll cycle, is a candidate chromophore for the B light receptor of guard cells on the basis of action spectra for stomatal opening in intact leaves and malate synthesis in epidermal peel (Zeiger and Zhu, 1998; Shimazaki et al., 2007). Further, phototropins (phot1 and phot2) have also been identified as B light receptors mediating the H^+-ATPase activation in the plasma membrane (Kinoshita et al., 2001;Briggs and Christie, 2002; Kinoshita et al*.,* 2003; Shimazaki et al., 2007). The cryptochromes, independently of phototropins, also participate in B light induced stomata opening in *A. thaliana* (Mao et al., 2005). However, ABA signaling is thought to predominate over B light signaling in guard cells, since it is important for plants to prevent water loss under drought stress (Shimazaki et al., 2007; Kim et al., 2010). Under drought stress, ABA promotes stomatal closure to prevent water loss (Schroeder et al., 2001; Shimazaki et al., 2007). The ABA induced stomatal closure is driven by the effluxes of Cl^-, $malate^{2-}$, and K^+ from guard cells through Ca^{2+} and voltage-dependent anion channels and outward rectifying K^+ channels in the plasma membranes. Activation of these channels requires membrane depolarization, and the depolarization can be achieved at least partly by the inhibition of the plasma membrane H^+ATPase (Assmann and Shimazaki, 1999; Schroeder et al., 2001; Hetherington, 2001). ABA inhibits B light-dependent phosphorylation of the H+-ATPase, and the inhibition may be mediated by H_2O_2 in guard cells. This results in increase level of nitric oxide (NO) and Ca^{2+} promoting stomata closure (Shimazaki et al., 2007).



Shimazaki K-i, et al. 2007.
Annu. Rev. Plant Biol. 58:219–47

Figure 2. The process of stomata opening in response to blue light and abscisic acid (ABA) signaling in response to blue light (Shimazaki et al., 2007)

1.2.4 Air humidity and water relation

Relative air humidity affects the stomatal conductance, which controls transpiration and photosynthesis. Plant growth is usually normal in water VPD of 1.0-0.2 kPa, corresponding to RH of 55-90% at 20 ºC (Grange and Hand, 1987). Besides, numerous studies have shown that RH hardly affects stem length, but only the pedicel length, probably because of the softer tissue type (Mortensen and Fjeld, 1998; Torre and Fjeld, 2001). However, plant production in continuous high RH (>85%) may result in poor plant quality and nutrient deficiency due to reduced transpiration (Mortensen and Fjeld, 1998; Torre et al., 2001). Furthermore, the postharvest life of ornamentals has been found to be low when grown at high RH. The negative effect of high RH on water loss and shelf life has been

observed in various species such as *Rosa x hybrida* (Mortensen and Gislerød, 1999; Torre and Fjeld, 2001), *Begonia x cheimantha*, *Chrysanthemum morifolium,* poinsettias and *Kalanchoe blossfeldiana* (Mortensen, 2000). The problem of these plants is uncontrolled water loss from the leaves when they are transferred to a lower RH. Also, they usually show low degree of adjustability or tolerance to specific stresses after harvest. This generally affects the water balance of the plant which is dependent on the water uptake and transpirational water loss. A negative water balance occurs when the water uptake is lower than the water loss. This results in poorer quality of cut flowers, pot plants and reduced shelf life. In cut roses, high RH results in decreased vase life (VL) and reduced flower diameter (Mortensen and Gislerød, 2005; Fanourakis et al., 2012). Cut roses grown under high RH had higher rates of water loss, compared to roses grown at moderate RH, as a result of less responsive stomata to both water stress and darkness (Fanourakis et al., 2012; Arve et al., 2013 (paper III)).

Leaf transpiration occurs through stomata and the cuticle. However, cuticular transpiration is of minor importance since it is very small compared to stomata transpiration (Fanourakis et al., 2013). The increased water loss from plants developed in high RH must therefore be largely caused by increased stomata transpiration (Fanourakis et al., 2013). Several studies show that the increased water loss in plants developed in high RH is mainly due to reduced ability to close their stomata in response to closing stimuli such as darkness, dessication and ABA (Torre at al., 2003; Nejad and van Meeteren, 2005; Arve et al., 2013; Fanourakis et al., 2012). Besides, the stomata morphology of these plants were also different from moderate RH-grown plants, where rose leaves grown at high RH were found to have more and larger stomata than rose leaves developed at moderate RH (Torre et al., 2003; Arve at al., 2013 (paper III)). Several hypotheses have been proposed to explain the less responsive stomata at high RH. Some of the proposed explanations for this phenomenon are reduced Ca^{2+} due to reduced transpirational water uptake, changes in the guard cell anatomy, low ABA levels, reduced sensitivity to ABA, or absence of diurnal variation in ABA pool (Torre et al., 2001; Nejad and van Meeteren, 2007; Arve et al., 2013 (paper III); Fanourakis et al., 2013; Aliniaeifard and van Meeteren, 2013; paper IV). However, it is still not fully understood why stomata of leaves developed at high RH are less functional.

1.2.5 The involvement of plant hormone abscisic acid (ABA)

Abscisic acid is involved in several plant physiological processes such as stomatal closure, embryo morphogenesis and development of seeds, dormancy, and senescence (Garello et al., 1995; Seo and Koshiba, 2002; Wilkinson and Davies, 2002; Nilson and Assmann, 2007; Parent et al., 2009). The endogenous level of ABA in plant tissues is dynamically regulated by the balance between the biosynthesis and inactivation of the hormone (Zeevaart, 1980; Cutler and Krochko, 1999) (Fig. 3). The biosynthesis of ABA involves series of complex steps and enzymes from early steps of the carotenoid precursor synthesis in plastids to later stages in cytosol where xanthoxin is converted to ABA (Fig 3; Cutler and Krochko, 1999; Seo and Koshiba, 2002). The inactivation of free ABA involves either hydroxylation of ABA to the ABA catabolites phaseic acid (PA) and dihydrophaseic acid (DPA) or conjugation of ABA with glucose, creating ABA-glucose ester (ABA-GE) (Fig. 3; Lim et al., 2005; Priest et al., 2006).

Abscisic acid plays an important role in many environmental stress responses in plants. However, the endogenous level of ABA itself is regulated by many environmental factors like drought, salt, RH, light and suboptimal temperatures (Luan, 2002; Zhu, 2002; Nejad and Van Meeteren, 2007; Okamoto et al., 2009; Reynolds-Henne et al., 2010). Various studies in different species have shown that plants developed under high RH have lower ABA levels compared to moderate RH (Zeevaart, 1974; Nejad and Van Meeteren, 2007; Okamoto et al., 2009; Arve et al., 2012). This phenomenon was also related to less functional stomata in plants developed in high RH. This was demonstrated by ABA application on daily basis, which could overcome the negative effect of high RH and restore functional stomata in young leaves of rose (Fanourakis et al., 2011). Similar results have been shown in various species where continuous ABA application reduced transpiration rate and increased the shelf life (Pompodakis et al., 2004; Waterland et al., 2010a, 2010b; Kim and van Iesel, 2011). In another study, the transfer of plants from moderate to high RH decreased the ABA level and it increased again when these plants transferred back to moderate RH, but the stomata could not return to the fully functioning state after the high RH treatment (Nejad and van Meeteren, 2008). Arve et al. (2013 (paper III)) also recently reported that the dramatic increase in ABA level during dark in plants grown in moderate RH, was absent in rose leaves developed at high RH, indicating a difference in the regulation of the diurnal ABA-pool under different RH regimes. This was also correlated with stomata functionality. Over all, these studies

showed the presence of an interconnection between ABA and RH and their effects on stomata function.

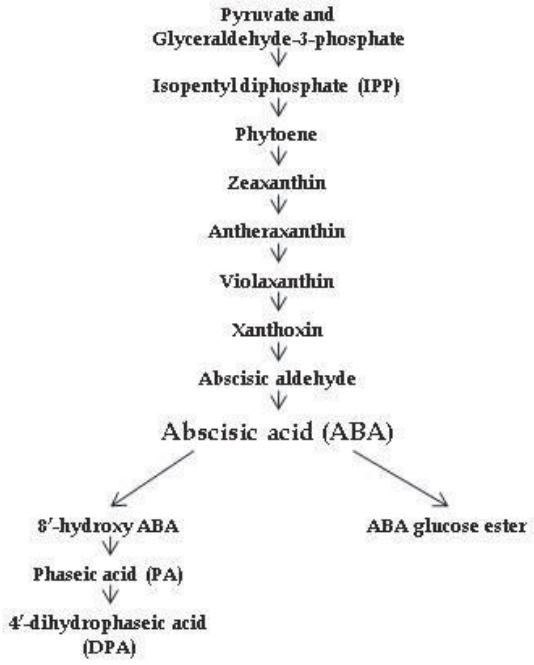


Figure 3. Biosynthesis of abscisic acid from pyruvate and glyceraldehyde-3-phosphate and ABA metabolism by oxidation to PA and DPA and conjugation to ABA glucose ester (Arve et al., 2011)

Furthermore, light regulates ABA levels through biosynthesis and degradation directly or indirectly (Xiong and Zhu, 2003; Tallman, 2004; Novakova et al., 2005; Arve et al., 2013 (paper III)). Specific light qualities like B light are also reported to regulate endogenous ABA levels during different developmental processes (Fellner and Sawhney, 2002). The diurnal pattern of stomata movements is affected by the diurnal alterations in metabolism of endogenous ABA, which partly associated with the effect of light on ABA precursors (Tallman, 2004). The ABA biosynthesis in guard cells can be affected by the removal of the ABA precursor through light-driven xanthophyll cycling, which converts violaxanthin to zeaxanthin (Eskling et al., 1997).As discussed above, zeaxanthin is proposed to be the B light-specific photoreceptor of guard cells (Zeiger and Zhu, 1998; Frechilla et al., 1999; Talbott et al., 2003) and conversion of violaxanthin to zeaxanthin is part of a

mechanism regulating endogenous guard cell ABA turnover. During the dark period ABA biosynthesis in guard cells is favored, maintaining stomata in closed position. Zeaxanthin accumulated in guard cells during the day will then start to be converted to violaxanthin, and this favors ABA biosynthesis, indicating a cross talk between ABA and light (Tallman, 2004). In our recent study, B light was shown to be involved in diurnal ABA homeostasis, which was in turn related to the diurnal stomata response (paper IV).

1.3 Strategies to minimize postharvest water loss (pre-harvest perspective)

To avoid water stress in postharvest life of roses the best strategy is to grow plants with functional stomata. Until now, different cultivation strategies have been used to avoid malfunctioning of rose stomata. For example, a short (4 h) temperature increase (21°C→27°C) during the day or variation in VPD can trigger the stomata closure (Pettersen et al., 2007; Mortensen et al., 2007) and improve the stress tolerance of the plants. In addition, giving the period of darkness has been shown to improve the water balance of roses. This is because growing roses in continuous lighting (24 h) have shown to induce a poor regulation of water loss, enhanced wilting and negative water balance (Blom-Zandstra et al., 1995; Mortensen and Fjeld, 1998; Arve et al., 2012). Elevated CO_2 concentrations have found to partly close the stomata (Morison, 1998). However, for roses 'Amadeus' grown at high RH increasing the CO_2 level had little effect on stomata function (Mortensen and Gislerød, 2011). An alternative efficient strategy for improving stomata responsiveness recently suggested by Mortensen and Gislerød (2011) was increasing air temperature (T_{air}) by 10 °C during a period of the day, instead of decreasing RH. However, some of these strategies are not favored by horticulturists because of increased energy consumption and high cost of production.

Additionally, the use of light quality on stomata opening and closure has been studied in many commercially important plant species. In roses it has been shown that, different rose cultivars respond differently to light quality and daily duration of lighting (Blom-Zandstra et al., 1995). The authors showed that, orange light increases stomatal conductance as compared to B or white light. In our recent experiment (paper IV) increasing level of B light improved stomata function and dark-induced stomata closure by reducing stomata conductance and transpiration. Hence, increasing the B light proportion in the assimilation lighting has a potential to improve production and quality. Thus, it is of great interest to study effects of light quality, alone and in combination with VPD fluctuations to

increase our understanding on how the climate during growth can control stomata function and general stress tolerance of roses. Documenting the effects of these growth factors will also give us knowledge to produce high quality greenhouse products with a low energy input.

2. AIMS OF THE STUDY

The purpose of greenhouse production is to produce high quality products with as little energy input as possible. This means providing a climate optimal for photosynthesis, fast growth and development, and a good product quality. A trend in the international rose market is the increased emphasis on quality. As competition in the world market increases, not only the external quality, but also the internal quality becomes more and more important. Relative air humidity and light are among numerous environmental factors influencing the stomata character and response. However, the sensing mechanisms involved in stomata RH response are still not well understood. This thesis, therefore, focused on how pre-harvest factors like light quality and RH influence on growth and quality parameters of roses.

The main aim of this study was to investigate effects of RH and light quality on growth, development and postharvest behavior of roses. Further, the aim was also to study the interactions between RH and light climate in order to get a better understanding of the stomata control and to be able to produce high quality roses with good water balance and longer postharvest life. The specific aims were to study the effect of light climate on the growth performance and quality of roses (Paper I, II and V). Further, the stomata response to different RH in light and darkness (photoperiod) and different light qualities were studied (Paper III and IV). Additionally, the role of the plant hormone ABA, with respect to air humidity and light climate interaction was also investigated (Paper III and IV).

3. MATERIAL AND METHODS

The studies were carried out at the Norwegian University of Life Sciences (UMB, Norway) and partly at Hawassa University (HU, Ethiopia). Pot roses (*Rosa x hybrida*) were used as a model plant since: (1) they are among the most important ornamentals commonly grown in greenhouses in the floriculture industry and, (2) roses respond to RH and there are several studies and accumulated knowledge on high RH-response of roses. Five different cultivars of pot roses ('Rebecca', 'Toril', 'Cygein', 'Tom-Tom', 'Snow white') were used to carry out the experiments. Reasons why pot roses are used rather than cut roses were (1) it is easier and faster to grow pot roses (takes 7-8 weeks from rooting to flowering), and (2) leaf responses to high RH is similar in pot roses as in cut roses as long as the cultivar used is sensitive to RH. The postharvest responses might be different since pot roses have intact roots. However, to achieve a long postharvest life, tolerance to drought and postharvest robustness is important for pot roses as well as for cut flowers.

In paper I and II, the effect of an increased proportion of B light (20%) provided by LED (round LED-light with three chains, delivered by Sola-co, Guangdong, China) on growth, morphology, flowering and postharvest quality was investigated as compared to traditional HPS lamps with 5% B light. The two lamps had almost similar phytochrome photostationary state (PPS). The experiments were performed both in greenhouse compartments as well as growth chambers. The leaf temperature was on average 1.5°C higher under HPS compared to LED due to the infrared radiation from HPS lamps.

To study the stomata sensitivity to RH in light and darkness (photoperiod), plants were developed at different RH (high (90%) and moderate (60%) RH) in 20 h photoperiod or 24 h photoperiod. Then, to determine the stomata sensitivity to RH in different light qualities and to investigate the interaction between RH and light climate, plants were developed under different light qualities (different proportions of B light provided by LED and HPS) and RH regimes (high (90%) and moderate (60%) RH) for 20 h photoperiod (Paper III and IV). In these studies stomata morphology and stomata responses were analyzed, diurnal conductance was recorded as well as ABA and β-glucosidase quantification was done. Even though, the ABA quantification methods used in both papers were different, the quantified ABA levels were in the same range in both papers (see materials and methods in paper III and IV).

Finally, the experiments in the last paper (paper V) were carried out in Ethiopia. In this experiment we investigated the effects of UV radiation on growth and postharvest

characteristics of three pot rose cultivars grown at different altitudes. The plants were grown at high altitude (2794 ma.s.l.) and lower altitude (1700 ma.s.l) under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (-UV). Growth parameters, flowering and postharvest water usage and characteristics were studied.

4. MAIN RESULTS AND DISCUSSION

4.1 Light quality effects on growth, morphology and postharvest characteristics of *Rosa x hybrida*

The change in light spectrum is known to have a strong influence on plant morphogenesis and growth (Whitelam and Halliday, 2007) but the effects are species dependent. The work in this thesis has focused particularly on the use of B light as a practical option to control morphology and improve postharvest characteristics of roses. Further, the effect of UV radiation in this regard has also been studied.

4.1.1 *Increased blue light changes morphology, increases photosynthesis but has no effect on flowering of roses*

In this study (Paper I), the responses in morphology, photosynthesis and flowering of *Rosa* × *hybrida* to different B light proportions provided by LEDs (20% B) and HPS (5% B) lamps were analyzed. There was a strong morphological and growth effect of the light sources but no significant difference in total dry matter (DM) production and flowering. Plants grown under LED with 20% B light had shorter internodes and reduced leaf expansion and exhibited more sun-type characteristics, with higher photosynthesis capacity and higher leaf mass per unit leaf area (LMA) than those grown under HPS lamps with 5% B. Furthermore, in LED light flower initiation occurred at a higher leaf number than under HPS, but no difference in time to open flowers was observed. Shorter stem length in LED lighting compared to HPS might be due to a strong sink competition from leaves, resulting in more assimilate partitioning toward leaves or B light inhibition of stem elongation. There are similar studies showing that B light is involved in inhibition of internode growth and cell expansion in a number of species (Appelgren, 2003; Folta et al., 2003; Dougher and Bugbee, 2004; Islam et al., 2012). Further, the much lower specific leaf area (SLA) and lower average leaf area (LA) under LED than HPS may be associated with a B light-mediated inhibition of cell expansion or division (Dougher and Bugbee, 2004). This was correlated with a change in

leaf anatomy and morphology of the plants. Plants grown under 5% B light (HPS) had thinner palisade and spongy mesophyll layers compared to those grown under 20% B light (LED). In contrast, plants from 20% B light (LED) had longer and higher number of palisade and epidermal cells than 5% B light (HPS) grown plants. These results are in line with previous works showing that leaf thickness, particularly the palisade mesophyll tissues of several plant species, decreased when plants were grown under either low light levels (Barreiro et al., 1992; Sims and Pearcy, 1992) or low levels of B light (Saebø et al., 1995). In spite of lower LA, LED lighting with 20 % B light resulted in a higher partitioning of assimilates towards leaves than stems compared to HPS grown plants (5 % B). Nonetheless, total DM accumulation in the whole plant did not show any significant difference among the two light sources. This phenomenon has also been observed in a number of species (Britz and Sager, 1990; Hogewoning et al., 2010).

Furthermore, plants grown under LED lighting showed higher photosynthesis than those grown under HPS lamps despite the fact that they had a lower average leaf area. This indicates that LED plants compensated for reduced leaf area by increasing photosynthesis per unit leaf area, so that the final result is about equal biomass. This was correlated with an increase in leaf mass per unit leaf area, higher stomata conductance and CO_2 exchange, total Chl content per area and higher Chl a/b ratio. In addition, LED-grown plants had higher electron transport rate (ETR) which is mainly a result of higher ΦPSII efficiency since the fraction of light absorbed from the different light sources by leaves was only slightly higher for LED than HPS. However, this increased photosynthesis did not cause early flowering in LED-grown plants. Even though roses have an autonomous flower induction, flower initiation is promoted by increasing temperature and irradiance (Zieslin and Halevy, 1975, Mortensen et al., 1992). The effect of irradiance on flower initiation is generally attributed to the effect of light on photosynthesis and the availability of assimilates for flower bud development. In the LED-grown plants, leaf anatomy and photosynthetic characteristics resembled high irradiance characteristic (sun-type) and higher contents of soluble carbohydrates (sucrose, fructose and glucose) were measured. Although high carbohydrate levels are known to facilitate floral initiation and development (Corbesier et al., 2002; van Doorn and van Meeteren, 2003) either the increased carbohydrate contents or photosynthesis in LED did not contribute to earlier initiation of flowering in roses. This might partly be due to (1) enhanced vegetative growth (more leaves and internodes) in LED-grown plants, (2) more assimilate partitioning to leaves than generative growth or (3) partitioning of assimilates

to other processes such as secondary metabolite production (e.g. LED-grown plants had more anthocyanin accumulation).

In general, the two lamp types used in this experiment have no (LED) or very little (HPS) FR. Accordingly, the relative amount of active phytochrome expressed as the PSS was 0.89 and 0.85 for LED and HPS, respectively, which is very similar. Therefore, the higher B light fraction of LED than HPS lamps apparently explains the lower SLA, greater DW partitioning to the leaf than stem and shorter stem in LED than HPS-grown plants. Besides, B light plays a role in modulating the leaf cell structures toward a more 'suntype' leaf character. The irradiance of 100 μmolm^{-2} s^{-1} in the experiment is relatively low for roses, which in horticulture normally are grown under high irradiances (Mortensen et al., 1992). However, even at this relatively low irradiance, B light stimulated 'suntype' characteristics on morphology of the plants.

4.1.2 Increased proportion of blue light improves postharvest life of roses

Postharvest senescence and stress tolerance are limiting factors in the marketing of ornamentals. The environmental conditions during growth are known to strongly influence the post-harvest behavior (Halevy and Mayak, 1979a; 1979b). In roses (*Rosa* × *hybrida*), it has been demonstrated that increasing natural or supplementary light level improves postharvest life. This effect is thought to be mediated through the carbohydrate status of the plant (Fjeld et al., 1994). On the other hand, postharvest longevity of roses has been found to decrease with increasing photoperiod mainly because of higher water loss and early wilting (Pettersen et al., 2007). Light quality is another important preharvest factor which can have an impact on the postharvest behavior of roses either by affecting the hormonal content, carbohydrate status or stomata functionality (Blom-Zandstra et al., 1995; Garello et al., 1995; Mortensen and Fjeld, 1998; Rajapakse and Kelly, 1994). Hence, in this study the effect of increased B light proportion on postharvest characteristics of roses was analyzed. The study clearly showed that, a better storability and longevity of flowers was observed for plants grown under LED compared to HPS when stored at 4°C in darkness. Typical symptoms of senescence, like bluing and wilting of petals were more pronounced in the flowers produced with HPS compared to LED after 4 weeks of storage. This observation was consistent with the measurement of petal cell-sap properties (pH and osmolarity of cell sap). A higher pH and osmolarity was measured in petals from HPS compared to LED, indicating accelerated senescence. Increased leakages of solutes from the cells, facilitating the movement of water

out of the cells are commonly observed during senescence. The aging of plant cells is usually associated with an extended decline in amino acids and proteins, as well as changes in the composition and structures of membranes which leads to change in cell pH and osmotic capacity (Borochov and Woodson, 1989).

The importance of carbohydrates for vase life and development of rose flowers has been well documented (Ho and Nichols, 1977; Halevy and Mayak, 1981; Marissen and La Brijin, 1995; van Doorn and van Meeteren, 2003). Besides, B light is known to increase DM production and carbohydrate accumulation in plants. Wang et al. (2009) showed that the total sugars and sucrose contents of cucumber plants grown under B light were slightly higher than those grown under white, R and green light. Also, in another experiment with roses we observed that increasing B light portion in the supplementary light source increased the levels of soluble carbohydrates in young leaves (Terfa et al., 2013 (paper I)). Hence, more available energy in form of carbohydrate might have suppressed the senescence and improved the storability of the pot roses. The faster degradation in cell structure of plants from HPS might also be due to poorer mechanical strength of the tissue. As mentioned above, B light is important in production and accumulation of carbohydrates. It is also known that the polysaccharides act as structural components and have a stabilizing effect on cell membranes and thus suppress action of ethylene and delay senescence (Ichimura and Hiraya, 1999).

4.1.3 Ultraviolet radiation has effect on growth and morphology but did not affect postharvest characteristics of roses

Numerous studies have shown that UV-B has various effects on morphology, biochemical composition and induces molecular responses of different species. Under natural conditions plants are exposed to different levels of UV radiation, especially UV-B, depending on geographic location, cloud cover, and solar altitude (Estupiñán et al., 1996; Rozema et al., 1997; Diffey, 2002). Even at the same geographical location and season the amount of UV-B reaching the ground varies with time of the day and also depends on the interaction between UV-B and other climatic factors. In the present study (paper V) the effect of UV radiation at different altitudes on growth, development and postharvest characteristics of three pot rose cultivars was investigated. The UV-blocking film used in the experiment blocked all UV up to 350 nm (all UV-B and the short UV-A; -UV) while the +UV film transmitted the full UV range. Thus, the main difference between the two films is in the UV-B region (280-320 nm) and the short wavelengths of UV-A. Clearly, from the study it was possible to observe the

typical UV-B responses such as, reduction in shoot length, leaf number and LA which resulted in reduced vegetative growth for plants grown under UV-transmitting film compared with the treatment where UV was blocked at both altitudes. All the rose cultivars tested responded similarly to UV radiation. This was similar to earlier findings in different species (Krizek et al., 1998; Jansen, 2002; Wargent et al., 2009; Berli et al., 2012).

Further, UV radiation also had effect on flowering time of the cultivars tested. +UV radiation delayed flowering by 7-10 days at both altitudes as compared to -UV radiation. However, the flower induction might have occurred earlier in +UV radiation since they had fewer number of leaves at flower opening. The delayed flowering is partly due to reduced LA in +UV radiation which caused low light interception and DM accumulation. It has been shown that carbohydrates are essential to facilitate floral initiation and development (Corbesier et al., 2002) and an important energy source facilitating flower opening (Ho and Nichols, 1977; Marissen and LaBrijn, 1995). The delay in flowering was more pronounced at high altitude where it took 2 -3 weeks to flower for all the cultivars regardless of the UV radiation. This might be due to a temperature effect, since at high altitude the mean daily temperature and night temperatures were low as compared to lower altitude. Flower initiation and development in roses is promoted by increasing temperature and irradiance (Zieslin and Halevy, 1975; Mortensen et al., 1992). Increased temperature is well known to facilitate flowering in many plant species (van Doorn and van Meeteren, 2003). It has been shown in roses that increasing temperature by 15°C facilitates flower opening by reducing the time from bud break to flowering by 40 days (Shin et al., 2001). This suggests that the temperature after visible bud formation significantly affects the rate of flower development and opening.

In general, the effect of UV radiation on growth and morphology was more prominent at lower altitude (with higher temperature) than high altitude (with lower temperature), despite the presence of high levels of solar UV-B radiation at high altitude. This might be due to the interaction of UV-B with other climatic factor such as temperature. The interactive effect of temperature and UV-B has been shown to affect plant growth in many species (Mark and Tevini, 1996; Kakani et al., 2003; Reddy et al., 2004). There exists cross-tolerance between different stressors such as UV-B, low temperature and drought (Manetas et al., 1997; Chalker-Scott and Scott, 2004; Poulson et al., 2006), and plants showed increased tolerance against UV-B, low temperature or drought because of increased acclimation to the other stressor (Chalker-Scott and Scott, 2004; Poulson et al., 2006). At higher altitude and latitude,

UV-B and cold temperature are naturally simultaneous or subsequent stresses. Hence, plants from these ecosystems are less sensitive to enhanced UV-B than those from low UV-B location (Chalker-Scott and Scott, 2004). This is partly due to increased tolerance towards UV-B as a result of low temperature.

UV radiation did not affect stomata conductance (gs), postharvest water usage and characteristics of the three pot rose cultivars. These factors were rather affected by altitude differences than UV radiation. Plants grown at high altitude had higher gs as compared to lower altitude. The higher transpiration at high altitude and vice versa in lower altitude might be due to effect of other climatic factors such as VPD and temperature since the gs was measured in the middle of the day when the temperature and irradiance reach their highest levels. The VPD and temperature recorded at high altitude were lower than low altitude. The lower gs measured at lower altitude might therefore be due to the higher VPD and higher air and leaf temperature that increase the transpirational flux, forcing the plants to close their stomata in order to conserve water. It is well known that plants grown under higher VPD characteristically have lower gs during growth (Arve et al., 2013 (paper III). Besides, the rate of water loss from plant leaves is basically dependent on density of stomata per leaf area and functional stomata. However, it has also been reported that gs is related to altitude or difference in air pressure in addition to VPD (Smith and Geller, 1979; Leuschner, 2000; Gale, 2004; Körner, 2007).

Further, the postharvest water usage and postharvest life was significantly affected by altitude but not UV radiation. The postharvest water usage was correlated to the postharvest life and characteristics measured. Leaf wilting and leaf drying are typical postharvest characteristics for water stressed plants (Torre and Fjeld, 2001). Hence, plants grown at the lower altitude showed higher percentage of leaf drying and wilting which might be due to water stress because of high transpiration rate. This led to shorter postharvest life for lower altitude plants as compared to high altitude. Postharvest transpiration was higher for plants from lower altitude than high altitude. They had twice as high water usage than high altitude plants when moved to postharvest room. Stomata behavior and water relations are one of the main factors determining the potential postharvest life of cut flowers as well as for some bedding and pot plants (Waterland et al., 2010a; 2010b). The postharvest water loss can be dependent on the stomata behavior during growth (Torre et al., 2001; Fanourakis et al., 2012). Even though plants grown at high altitude had experienced lower VPD (0.4 kPa) during growth, they probably responded by closing their stomata to avoid water loss when

moved to an environment where the VPD was very high (VPD in postharvest room: 1.2 kPa). However, for plants developed at the lower altitude, there was no significant change in VPD during growth (VPD: 1.12 kPa) and postharvest (VPD: 1.2 kPa). Since these plants did not sense any stimuli to close their stomata after transfer to the postharvest test room they continued to transpire as usual. Under natural conditions, plants are adapted to sudden environmental changes by physiologically adjusting themselves. One example is by dynamically controlling stomatal conductance, plants can effectively regulate long-distance water flow and water potential over a short term, which ultimately regulates stomata function (Hacke and Sauter, 1995; Laur and Hacke, 2013). This dynamics is influenced by the modulation of aquaporin abundance and regulation of aquaporin activity (Aroca et al., 2012; Laur and Hacke, 2013). Aquaporins are water channel proteins and are present in a wide range of animal, microbial, and plant membranes (Henzler et al., 1999; Baiges et al., 2002). Thus, plants have the ability to adjust their water uptake capacity to changing environmental conditions by regulating aquaporins in the plasma membrane of root cells (Laur and Hacke, 2013). Hence, in this experiment the ability of plants grown at high altitude to easily sense the changing environment and dynamically adapt to it by keeping their water balance and avoiding unnecessary water loss was a key factor for a better postharvest life.

4.2 Effect of relative humidity and light quality on stomata function

4.2.1 The role of relative humidity in stomata function

The role of RH in stomata responses has been studied in many species (Mortensen and Gislerød 1999; Fordham et al., 2001; Torre et al., 2003; Nejad and Meeteren, 2005). All the studies have clearly showed poor stomata functionality in response to elevated RH. In the present study stomata responsiveness to desiccation was tested to further analyze the degree to which the detached leaves close their stomata and retain water during a 3 h dehydration test. Similar to previous studies, stomata of high RH-grown rose plants failed to close and lost a high percentage of their weight during the test compared to moderate RH-plants. It has been shown that higher water loss of high RH- grown roses was mainly due to malfunctioning of the stomata and to a lesser extent to an increased cuticular transpiration rate (Fanourakis et al., 2013). Hence, the higher water loss in high RH-grown plants during the desiccation test was therefore due to reduced stomata closure. This is partly due to change in stomata morphology such as aperture, length or density. In general, rose plants grown under high RH had larger stomata length and aperture, which in turn resulted in larger

stomata area as compared to moderate RH .This is similar to previous studies in a number of species (Fordham et al., 2001; Torre et al., 2003; Karbulkova et al., 2008). Besides, some studies showed a negative relationship between stomata size and stomata functioning in different species (Aasamaa et al., 2001; Franks and Farquhar, 2007; Fanourakis et al., 2013). Hence, these changes in stomata characteristic exerted by high RH might influence the gs *per se* and have a direct role on the enhanced water loss (Fanourakis et al., 2013). Further, the higher diurnal gs measured in high RH-grown plants compared to moderate RH indicates the presence of higher transpiration in high RH-grown plants.

Darkness is thought to be one of the strong signals in stomata closure but the degree to which stomata close differs among species (Caird et al., 2007). In present study, stomata of plants developed under high RH showed no closure in response to darkness. This was exhibited in the stomata size measured in high RH-grown plants. Percentage in stomata aperture reduction between light and dark for high RH-grown plants was very small as compared to moderate RH-grown plants. Further, the diurnal gs tended to decrease during late day and night in plants from both high and moderate RH, while it remained higher for high RH-grown plants throughout the day and the night. The relative change in conductance was much larger in moderate RH (85% change) compared with high RH (28.5% change).This indicates that stomata developed under moderate RH close better during darkness than those developed at high RH. This is similar to previous studies on diurnal stomata conductance of *Rosa x hybrida* where plants developed under moderate RH showed a larger relative change in gs compared to high RH-grown plants (Fanourakis et al., 2012). Fanourakis et al. (2013), further showed that in four rose cultivars tested, plants grown at high RH failed to close their stomata in response to darkness. These authors further elucidated the rate of stomata closure was more impaired than the degree of stomata closure. Some of the possible reasons for less stomata responsiveness to darkness might be (a) lower foliar ABA content during leaf development at high RH as compared to moderate RH (Nejad and van Meeteren, 2007; Fanourakis et al., 2011; 2012) and , (b) impaired sensitivity to ABA. The latter was elucidated through foliar ABA feeding which resulted in short term increase in ABA content but failed to induce long term stomata closure (Fanourakis et al., 2013). Another possibility is the variation in the diurnal ABA concentration might be important to develop functional stomata.

4.2.2 Blue light improves stomata function and dark- induced stomata closure

Opening and closure of stomata is strongly controlled by light. The stomata response to light is regulated by two major photoreceptor systems, photosynthesis in the guard cell chloroplast and a specific B light responses (Schwartz and Zeiger, 1984, Tallman, 1992, Assmann, 1993). As mentioned above, the B/UVA light-absorbing cryptochromes, zeaxanthin and phototropins are suggested receptors for the B light stomata responses (Zeiger and Zhu, 1998; Briggs and Christie 2002, Kinoshita et al., 2003; Mao et al., 2005; Shimazaki et al., 2007). The hypothesis of this study was therefore, since B light plays a role in stomata opening and closure, B light would help stomata of high RH-grown plants to overcome the negative effect of high RH by improving functionality (i.e. stimulating the normal stomata opening and closure during development). The study clearly shows that the stomata characteristics and transpiration rates were significantly improved if plants were grown under 20% B (LED) as compared to under 5% B (HPS).As a response to stomata-closing stimuli, stomata of plants grown at high RH and 20% B (LED) responded strongly to the desiccation test and darkness by closing their stomata and eventually reducing transpiration as compared to 5% B (HPS). Thus, the transpiration and the stomata function under high RH were dependent on light quality. To further verify if the B light has a role and to separate the effects of the other wave lengths provided by HPS lamps, like orange and yellow, an experiment was carried out with distinct wavelengths of only B or R light at high RH, where the different B proportion were given (5% B, 20% B and 100% B). This also showed similar B light-induced improvement in stomata function and reduced water usage and transpiration during dark. The transpiration rate was highest during the light period for all the treatments but significantly decreased during dark for plants grown under 20% B and 100% B as compared to those grown under 5% B. Accordingly, the water usage recorded during the dark, when stomata are expected to close, was lower for plants grown at 20% B and 100% B. This situation indicates that B light improved stomata responses to darkness. This is similar to a previous study by Blom-Zandstra et al. (1995); showing that plants grown under B light had lower gs and water usage as compared to those grown under orange light. This improvement was partly due to improved stomata morphology since plants developed at high RH under 20% B and 100% B had smaller stomata aperture and area as compared to 5% B light. Water loss is not only determined by the size of the stomata pore but also the number stomata per area. Plants grown at higher B light proportion (20% B and 100% B) had higher

number of stomata as compared to 5% B. This correlated with our previous work with roses where a higher B light proportion increased the number of stomata per area (Terfa et al., 2013 (paper I)). Interestingly, although plants grown under higher B light proportion had higher number of stomata which correlated with higher day time transpiration; they were able to close their stomata better during the dark period. The ability of these plants to close their stomata when subjected to darkness or desiccation shows the improved stomata ability to quickly adapt and respond to the prevailing environmental condition. Besides, the significant reduction in night transpiration and water usage which complied with reduced stomata size entails improvement in stomata dark closure. This might party be due to the fact that B light itself plays a major role in stomata opening and closing. Hence, the stomata that are functional and normally adapted to regular opening and closing during development hence would be able to close properly when they are subjected to signals in stomata closure such as darkness, drought or ABA. Further, our other study on roses demonstrated that B light increases the production and accumulation of soluble carbohydrates (sucrose and fructose) in rose leaves (Terfa et al., 2013 (paper I)). Sugars have been shown to act as principal osmotica for stomatal opening and closure (Talbott and Zeiger, 1996; Kelly et al., 2013). This stomata closure was mediated through ABA (Kelly et al., 2013). Therefore, it can be assumed that the higher sugar accumulation in the leaf and possibly in apoplast and guard cells might also contribute to stomata closure through an osmotic effect or ABA (Kelly et al., 2013).

4.3 Effect of relative air humidity and light quality on abscisic acid

4.3.1 The role of relative air humidity on abscisic acid level

As discussed above, ABA plays an important role in stomata closure. However the endogenous level of ABA in plants could be affected by environmental factors such as RH. The amount of ABA and its metabolites PA, DPA, ABA-GE measured in this study was significantly lower in plants developed under high RH as compared to moderate RH. If only the amount of ABA is considered, the levels were still significantly lower at high RH compared to moderate RH. In moderate RH, the amount of ABA was significantly higher during dark compared to the light period while there was no significant change between light and dark in high RH, indicating the absence in diurnal ABA variation. This increase in ABA level during dark is believed to act as a signal for stomata closure during darkness (Tallman, 2004; Novakova et al., 2005; Aliniaeifard and van Meeteren, 2013).Various studies in

different species have similarly shown that plants developed under high RH have lower ABA levels compared to moderate RH (Zeevaart, 1974; Nejad and Van Meeteren, 2007; 2008; Okamoto et al., 2009). The low ABA level in plants developed in high RH or moved from low to high RH is partly due to inactivation of ABA by ABA 8'-hydroxylase but not altered biosynthesis (Tallman, 2004; Okamoto et al., 2009; Aliniaeifard and van Meeteren, 2013).

ABA is inactivated by oxidation to PA and further to DPA or by conjugation to ABA-GE (Nambara and Marion-Poll, 2005). In this study, the level of PA was lower at high RH, where the amount of ABA was also lower as compared to moderate RH. However, there was no difference in the ABA\PA ratio between high and moderate RH or between light and dark. Thus, inactivation of ABA to PA was similar in light and dark, and the relative amount of ABA being inactivated to PA was the same in both moderate and high RH. Further, the amount of ABA-GE was similar in both RH treatments during light. However, as the level of ABA was increased during dark the level of ABA-GE was decreased simultaneously in moderate RH, while it remained unchanged in high RH plants. This was consistent with the quantified β-glucosidase activity. The activity of β-glucosidase was about fivefold higher in plants grown in moderate RH compared with high RH. This indicated that more ABA-GE was converted to ABA in leaves developed under moderate RH. Hence, compared to the oxidation products, ABA-GE is more important metabolite in *Rosa x hybrida* to regulate diurnal ABA level, at least in moderate RH.

Further, to better understand the effect of day length and the importance of the dark period in stomata development the effects of RH under continuous lighting (24h lighting) was studied. The levels of total ABA and its metabolites as well as the level of PA in continuous light were significantly lower in plants grown at high RH compared to moderate RH. However, there was no difference in the levels of ABA, ABA-GE or β-glucosidase between 20h or 24 h lighting under high RH. In general, the levels of ABA and its metabolites in continuous light were more similar to the levels from high RH in 20 h photoperiod and lower than the levels from moderate RH in 20 h photoperiod. There were also no interactional effects between photoperiod and RH, except in PA, indicating that continuous lighting has the same effect irrespective of the RH. The lower amount of ABA in moderate RH and 24 h lighting indicates the importance of the dark period to increase ABA during night.

The diurnal pattern of stomata movements are affected by the altering endogenous ABA metabolism during the day (Tallman, 2004). In many previous and present studies, the absence of a significant change in ABA level during the light and dark periods in high RH

grown plants correlated with absence of stomata closure during dark. Hence, this absence of a dynamic ABA peak at the beginning of the dark period in high RH-grown plants might make the guard cells less sensitive to closing stimuli, and vice versa for moderate RH-grown plants. Besides, in most studies and particularly in this study, the differences in ABA levels between the treatments are rather small and they all contain physiologically sufficient levels of ABA to have a normal stomata function. This points out that the distribution of ABA in the leaf cells and the amount available in guard cells seem to be very important in governing closure. Hence, where the ABA is localized in the leaf might be more important than the total amount of ABA in the leaf.

4.3.2 Blue light affects diurnal level of abscisic acid

Since there is a cross talk between B light and ABA in guard cell signaling (Shimazaki et al., 2007; Kim et al., 2010; Hayashi and Kinoshita, 2011) the hypothesis of this study was that an increased B light proportion would affect the diurnal ABA pool in high RH-grown plants so that it affects the stomata response. In guard cells, ABA signaling is thought to predominate over B light signaling, since it is important for plants to prevent water loss under drought stress (Shimazaki et al., 2007; Kim et al., 2010). This study clearly showed that the highest level of total ABA was present in plants grown under high B light proportion (20% B and more) regardless of the RH regimes. However, the highest concentration was measured during the dark period. The increased ABA concentration during dark in moderate RH is believed to act as a signal for stomatal closure during darkness (Tallman, 2004; Novakova et al., 2005). In our previous study, in moderate RH, an increase in ABA level during dark was found while this was absent in high RH plants (paper III). However, in this experiment we observed a significant increase in the level of ABA during dark in high RH if plants were grown in a higher B light proportion (20% B and more). This showed that the light quality during the day is influencing the ABA levels at night. This increase in ABA during dark might arise from either increased biosynthesis of ABA or release from stored ABA-conjugate. This ABA might also have been transported from the root as a long distance chemical signal (Wilkinson and Davies, 2002) or from leaf cells (as a short distance signal) (Cutler & Krochko, 1999).

Further, light has been shown to regulate ABA biosynthesis and degradation directly or indirectly (Xiong and Zhu, 2003; Tallman, 2004; Novakova et al., 2005). There is a crosstalk between ABA and B light signaling in guard cell that would affect the ABA homeostasis. Light is one of environmental factors affecting sources of ABA during the day (Tallman, 2004; Aliniaeifard and van Meeteren, 2013). The observed lower ABA level during light and the sharp increase during dark in the leaves of rose plants grown under 20% B or more indicate a dynamic turnover of the ABA pool either by degradation, biosynthesis or conjugation. During light guard cell ABA biosynthesis would be restricted by the removal of the ABA precursor violaxanthin through light-driven xanthophyll cycling which converts violaxanthin to zeaxanthin (Tallman, 2004; Aliniaeifard and van Meeteren, 2013). Besides, as discussed above if Zeaxanthin is B light photoreceptor in guard cells (Frechilla et al., 1999; Talbott et al., 2003). Hence, the conversion of violaxanthin to zeaxanthin, as a part of a mechanism to regulate endogenous guard cell ABA turnover partly explains the lower ABA level during light in plants grown under 20% B or more. Furthermore, during the dark period endogenous guard cell ABA biosynthesis is favored to maintain stomata in closed position. Hence, the accumulated zeaxanthin during day as a result of light (B light photoreception) might start to convert to violaxanthin, which would favor endogenous ABA biosynthesis by guard cells (Tallman, 2004). On the other hand, regarding high RH, it has been shown that it is mainly the ABA degradation which is affected rather than the biosynthesis (Okamoto et al., 2009; Paper III). In addition, in our previous work it was suggested that the ABA metabolite ABA-GE is the predominant factor in changing the ABA pool during light and dark at least in moderate RH (Paper III). Hence, based on this knowledge, the β-glucosidase-activity was higher in moderate RH compared to high RH, similarly to what was found in paper III. Interestingly, even though plants grown at high RH generally had lower β-glucosidase activity, those grown under 20% B and more had a higher activity of this enzyme as compared to those grown under 5% B. A clear correlation between the enzyme activity and increased ABA during dark was also observed. This points out that the increase in levels of ABA during dark might arise from ABA-GE conversion. In addition, the dramatic decrease in transpiration and stomata size during dark for plants grown under high RH and 20% B was correlated with increased ABA level which improved stomata closure during dark. This increase in ABA might arise from the root. However, it is also logical to assume that the stored ABA (in the form of ABA-GE) is a rapid and easy way to regulate available ABA in guard cells to quickly respond to stomata-closing stimuli, whereas the bulk of transported

ABA from the root might help to keep the stomata in closed position throughout the dark period or until stomata receives a signal inducing opening. Hence, increase in the ABA content in general and a dynamic ABA peak during dark in plants grown under 20% B and more, was essential for stomata to sense dark as a stimulus.

5. CONCLUSIONS AND FUTURE PERSPECTIVE

5.1 Conclusions

- LED with a high B light proportion (20% B; 80% R) is efficient in increasing photosynthesis performance per unit leaf area, enhancing growth and morphological changes in *Rosa x hybrida* but does not affect the total DM production or flowering time as compared to the traditional HPS with low blue portion.

- LED-grown plants (20% B; 80% R) also had higher carbohydrate content, chlorophyll content and anthocyanin accumulation in the leaves. The leaves displayed a more sun-type leaf anatomy with more and longer palisade cells and a higher stomata frequency than HPS-grown plants.

- LED-grown plants (20% B; 80% R) had delayed senescence and improved postharvest storability at 4°C in darkness.

- *Rosa x hybrida* plants developed under high RH had reduced or no stomata response to closing stimuli, such as darkness or desiccation stress. This situation was improved when high RH plants were grown under increased proportion of B light (20% B or more). Increased B light proportion improved stomata functionality by improving stomata response to closing stimuli such as darkness and desiccations stress, and reducing transpiration.

- *Rosa x hybrida* plants developed under high RH, continuous light (24 h), and a low B light proportion in the light source have a lower ABA content in the leaves.

- The ABA level increased during dark in leaves developed under moderate RH as compared to high RH plants. The increased ABA level in moderate RH was accompanied by a simultaneous decreased in ABA-GE and a higher β-glucosidase activity indicating the increase in ABA was arise from conversion of ABA-GE.

- Roses grown under continuous lighting had low ABA content and a low β-glucosidase activity irrespective of the RH

- Increasing B light proportion (20% B or more) increased the ABA level in general and during dark specifically in high RH grown plants. This increase in ABA level was correlated with increased activity of β-glucosidase.

- This indicates β-glucosidase has a central role and is a key enzyme in regulating the ABA pool in *Rosa x hybrida* plants and its activity is regulated by RH, photoperiod, and B light

- UV radiation reduced shoot length and LA producing very compact plants. However, it delayed flowering and did not improve postharvest water usage or characteristics of *Rosa x hybrid*.

5.2 Concluding remarks and Further perspective

5.2.1. Practical application for greenhouse production of roses

Assimilation lighting in greenhouses production of pot roses at Northern latitudes can be made more productive by using a light source with more B light than the traditional HPS lamps without affecting production time. This could be an alternative strategy to replace chemical growth retardants to control plant elongation and morphology in production of ornamental plants such as pot roses. The use of B light to improve stomata function is an important finding that can be useful for growers in periods when the air humidity is high. Since the LED lamps are expensive and the efficiency ($\mu molm^{-2}s^{-1}$/Watt) of LEDs is not as high as desirable, pure LED as growth light is probably not the best solution at the moment. However, the widely used HPS can be combined with LED, i.e. HPS enriched with blue LEDs to increase the proportion of B light in periods when the RH is high, but this needs further testing. The development of LEDs as growth light in greenhouses is fast and the next generation of LED will maybe be cheaper and more efficient.

UV radiation also had a strong morphological effect on roses. In the production of pot roses either cladding material transmitting UV radiation or an artificial UV source could

be used as alternatives in Northern latitudes to grow more compact plants. The former would not have any effect in the winter because of very low level of solar UV radiation (UV-B) at Northern latitudes in this period. However, the use of artificial UV as a method to control plant morphology will need further investigations. On the other hand, cut rose in Ethiopia should be produced without UV radiation. UV transmitting film reduced the yield without improving postharvest quality. Altitude is rather important in this regard since it has a huge impact in determining growth and postharvest water usage and characteristics.

5.2.2. The physiology behind the lack of stomata function in roses and the role of RH and B light

To fully understand the reason behind reduced stomata function in high RH and the role of light quality (B light) further studies are necessary. It would be interesting to investigate the mechanism behind the role of B light in stomata function of roses produced in high RH. Measurements of turgor pressure in guard cells as well as testing if B light has an effect on ABA biosynthesis and ABA sensitivity of the guard cells would be of particular interest. Since B light is correlated with sucrose production, it would also be important to see if B light is affecting ABA through sucrose accumulation (Kelly et al., 2013). Further study is also needed to understand the role of ABA under high RH and different light qualities and how it interacts with other hormones known to affect stomatal movements like auxin and ethylene. However, it is difficult to harvest epidermal peels from rose leaves, and no mutants are available in roses. Since only limited numbers of genes are sequenced in roses, it is a difficult plant species to study hormonal biosynthesis and its regulation as well as stomata functionality at a molecular level. Hence studying this phenomenon in other RH responsive plant species would be easier and help to understand the underlying processes. One such option is the tomato plant, *solanum lycopersicum*, since the genome was recently sequenced (Tomato Genome, 2012) and tomato leaves are RH responsive, it can help to understand the hormonal biosynthesis and its regulation as well as stomata functionality at a molecular level. *Vica faba* could be an alternative to study signaling at cellular level in the guard cell since it is easy to harvest epidermal peels from leaves.

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PAPER I

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A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of *Rosa* × *hybrida* but does not affect time to flower opening

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Alterations in light quality affect plant morphogenesis and photosynthetic responses but the effects vary significantly between species. Roses exhibit an irradiance-dependent flowering control but knowledge on light quality responses is scarce. In this study we analyzed, the responses in morphology, photosynthesis and flowering of *Rosa* × *hybrida* to different blue (B) light proportions provided by light-emitting diodes (LED, high B 20%) and high pressure sodium (HPS, low B 5%) lamps. There was a strong morphological and growth effect of the light sources but no significant difference in total dry matter production and flowering. HPS-grown plants had significantly higher leaf area and plant height, yet a higher dry weight proportion was allocated to leaves than stems under LED. LED plants showed 20% higher photosynthetic capacity (A_{max}) and higher levels of soluble carbohydrates. The increase in A_{max} correlated with an increase in leaf mass per unit leaf area, higher stomata conductance and CO_2 exchange, total chlorophyll (Chl) content per area and Chl *a/b* ratio. LED-grown leaves also displayed a more sun-type leaf anatomy with more and longer palisade cells and a higher stomata frequency. Although floral initiation occurred at a higher leaf number in LED, the time to open flowers was the same under both light conditions. Thereby the study shows that a higher portion of B light is efficient in increasing photosynthesis performance per unit leaf area, enhancing growth and morphological changes in roses but does not affect the total Dry Matter (DM) production or time to open flower.

Introduction

Light is one of the most important environmental factors, acting on plants not only as the sole source of energy, but also as a source of external information, affecting growth and development. Spectral changes of illumination evoke different photosynthetic and morphogenetic responses, which can vary among different plant species. Red (R) and blue (B) light are more efficiently absorbed by photosynthetic pigments than other spectral regions (McCree 1972, Inada 1976). Maximum quantum yield occurs near 600 nm, and declines rapidly at wave lengths shorter than 400 nm and greater than 680 nm (Evans 1987). In addition, R light is important for the development of the photosynthetic apparatus (Saebø et al. 1995). B light

Abbreviations – B, blue; Chl, chlorophyll; DW, dry weight; ETR, electron-transport rate; FR, far red; HPS, high pressure sodium; LED, light-emitting diode; LM, light microscopy; LMA, leaf mass per unit leaf area; PAR, photosynthetic active radiation; PSI, photosystem I; PSII, photosystem II; PSS, phytochrome photostationary state; R, red; RH, relative air humidity; SLA, specific leaf area; SUMP, Suzuki's Universal Micro-Printing; TEM, transmission electron microscopy.

affects the formation of chlorophyll, stomata opening and photomorphogenesis (Senger 1982, Schuerger et al. 1997, Dougher and Bugbee 1998, Heo et al. 2002).

B light generally promotes stomatal opening more than other wavelengths and this has been shown to contribute to an increase in photosynthetic rate along with the enhancement of dry matter production in many plant species (Sharkey and Raschke 1981, Goins et al. 1997, Zeiger et al. 2002). Also, several studies have shown that B light influences the biochemical properties of photosynthesis in leaves such as chlorophyll (Chl) *a*/*b* ratios, Chl *a*/*b*-binding protein of photosystem II (LHCII), and photosynthetic electron transport (Leong and Anderson 1984, Senger and Bauer 1987). In addition, B light is shown to stimulate 'sun-type' characteristics on the leaf level, even at a rather low irradiance (100 $\mu mol\ m^{-2}\ s^{-1}$), and thus provides a leaf development signal normally associated with acclimation to high irradiance (Buschmann et al. 1978, Hogewoning et al. 2010b).

In several plant species B light, which acts via B/UVA photoreceptor(s), is effective in inducing different photomorphogenic responses and floral transition, but the effects vary among species (Cosgrove 1981, Mohr 1987, Barnes and Bugbee 1991, Guo et al. 1998, Imaizumi et al. 2003). In the facultative long-day plant *Arabidopsis thaliana*, B light over a range of irradiances is effective in promoting flowering under long days (Brown and Klein 1971, Eskins 1992, Guo et al. 1998, Imaizumi et al. 2003). Also, the long-day plant *Petunia* × *hybrida* showed enhanced flowering in monochromatic B compared to R (Fukuda et al. 2011). However, this response of B light was not notable in the long-day plants *Scabiosa atropurpurea* and *Spinacea oleracea* (Withrow and Withrow 1940). In the day neutral plant *Cyclamen persicum* simultaneous exposure to moderate irradiances (80–100 $\mu mol\ m^{-2}\ s^{-1}$) of 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 B light promotion of flowering was observed but the effect was counteracted by R light in the wavelength area 500–700 nm (Sawhney 1977). Similarly, in the other short-day plants *Xantthium pennsylvanicum* and *Lemna perpusilla* B light allowed flowering when given continuously or when it replaced inductive darkness (Withrow and Withrow 1940, Hillman, 1965). Thus, B light affects flowering differently in different species. However, since light sources, filters, irradiances, photoperiod and recorded flowering stages varied in earlier studies, generalization is often difficult.

B light is involved in inhibition of growth of internodes and cell expansion or division (Appelgren 2003, Folta et al. 2003, Dougher and Bugbee 2004). For instance, Meijer (1968) and Cosgrove (1981) reported that B irradiation rapidly inhibits elongation of cucumber and sunflower seedlings. Furthermore, Ahmad et al. (2002) showed that hypocotyl elongation in *Arabidopsis* is inhibited by B light via a cryptochrome-mediated response. However, these photomorphogenic responses are species dependent. Dougher and Bugbee (2001) defined long-term B light dose-response curves for leaf area and stem length in soybean and lettuce. They showed that both parameters in soybean decreased with increment of the B light proportion while leaf area in lettuce increased with an increasing B light fraction. Furthermore, in *Petunia* × *hybrida* monochromatic B light enhanced stem elongation strongly compared to R (Fukuda et al. 2011). Hence, the differences in response to B light point out that light quality responses of a species cannot necessarily be predicted on basis of results from others.

In advanced greenhouse production systems artificial lighting is necessary during winter in the northern hemisphere. The supplementary light source is usually applied by a gas-discharge lamp-type high pressure sodium (HPS), which have a high radiant emission, high photosynthetic active radiation (PAR) emission and a high electrical efficiency but only 5% B light. However, during the last decade the progress in solid-state lighting, based on light-emitting diodes (LEDs) has facilitated the research on light quality responses in plants in general, and attracted much interest as a light source for assimilation lighting in greenhouses.

On the other hand, artificial lighting with a too narrow spectrum is not spectrally optimal for normal plant growth (Brazaityte et al. 2006). In monochromatic R LED light, growth in several plant species, including lettuce, wheat, radish, spinach and pepper, was less vigorous than that of plants grown under white fluorescent lamps or metal halide lamps at an equal photosynthetic photon flux density (Bula et al. 1991, Hoenecke et al. 1992, Brown et al. 1995, Goins et al. 1997, Yorio et al. 2001). However, the additional B to R light from LEDs enhanced plant growth and photosynthetic capacity compared to the use of R LEDs alone. Taken together, it is clear that plants exhibit a high degree of physiological, morphological and anatomical plasticity to changes in spectral quality. Thus, optimization of lighting spectrum for photosynthesis, growth and flowering and better productivity is an important goal in advanced plant production systems.

As the responses to light quality differs greatly between species and affects overall plant growth and

development, investigating such effects in economically important plants is highly interesting in order to optimize production. Roses (*Rosa* × *hybrida*) are among the most economically valuable ornamental plants worldwide and are produced either as pot plants or cut roses in greenhouses. The flower induction in roses is known to be autonomous and is controlled mainly by growth temperature and irradiance and not by photoperiod (Zieslin and Halevy 1975, Zieslin and Mor 1990, Mortensen et al. 1992). As the B light receptors work as irradiance sensors (Eskins et al. 1989, López-Juez et al. 2007) as well as light quality detectors, as discussed above, they are important in determining the production potential of plants. As yet, very little data are available on the impact of B light on growth and flowering of the irradiance response of *Rosa* × *hybrida*.

The primary objective of this study was to evaluate if LED lamps with a high proportion of B could increase the production potential and reduce the time to flowering of *Rosa* × *hybrida* compared to HPS lamps which are commonly used in greenhouses. Particularly, the aim was to investigate the effect on photosynthetic characteristics, morphology and time to flowering in rose plants using the traditional HPS and LED light, which provide 5 and 20% B light proportions, respectively. The two growth light spectra used (Fig. 1) are different in many respects. However, the major spectral difference between the two light sources was blue light and this was subject to extensive study. To assess also the impact of the supplementary light sources under natural light conditions, experiments were performed both in greenhouse compartments and controlled conditions in growth chambers. Here, we report for the first time that B light increases the photosynthesis capacity and levels of soluble carbohydrates in roses but has no effect on the time to open flowers although flower initiation occurs at a higher leaf number.

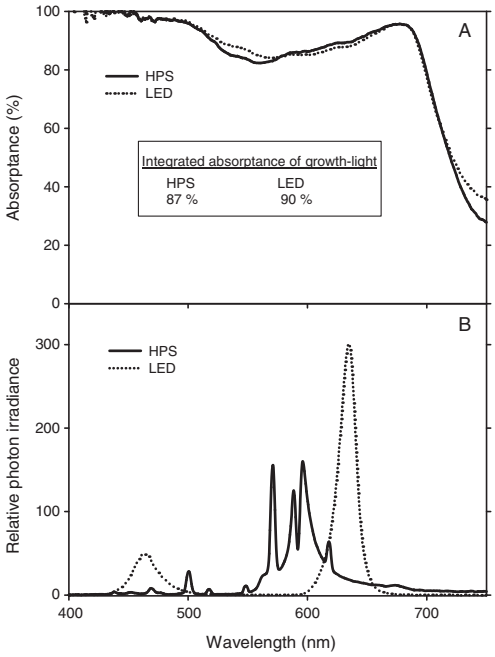

Fig. 1. Absorptance spectra (A) of rose leaves grown under HPS and LED lamps; irradiance spectra of the lamps used in the experiments (B): HPS lamps Osram NAV T-400W (solid lines) and LED lamps (round LED-light 92W with three chain, SoLa-Co) (dotted lines).

Materials and methods

Plant material and pre-cultivation

Rosa × *hybrida*, cv. Toril was grown from a single-node stem segment with one mature leaf in 12 cm pots containing a standard fertilized sphagnum peat media (Floralux, Nittedal, Norway). During pre-cultivation the plants were kept in a greenhouse compartment at the center for plant research in controlled climate (SKP), at Norwegian University of Life sciences, Ås, Norway (59°39′47″N 10°47′38″E). The temperature was 21°C, and average daily relative air humidity (RH) 70% corresponding to a 0.5 kPa water vapor deficit. Supplementary light by HPS lamps (Osram NAV T-400W, Munich, Germany) was given 20 h per day, followed by a 4-h dark period. At average the supplemented irradiance was 100 (±10) μmol m^{-2} s^{-1} (measured with a Li-Cor, Model L1-185, quantum sensor; LI-COR Inc, Lincoln, NE). The pre-cultivation ended when the plants had 1–1.5 cm long shoots.

Experimental growth conditions

Two growth experiments were performed in greenhouse compartments and two experiments were done in controlled growth chambers without any influence of natural sun light. During the experimental period the plants were exposed to 100 μmol m^{-2} s^{-1} for 20 h day^{-1} either by LED lamps (round LED-light with three chains, delivered by Sola-co, Guangdong, China) containing 80% red (R; peak wavelength at 630 nm) and 20% blue (B; peak wavelength at 465 nm) or HPS lamps. The irradiance was measured at plant level. The spectra for both lamps were measured with an OceanOptics SD2000 spectrometer (Fig. 1) (model SD2000; OceanOptics, Eerbeek, the Netherlands).

The spectrometer was calibrated against a National Institute of Standards Technology (NIST)-traceable calibration lamp (model LS-1-CAL; OceanOptics). 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. The temperature set point was 20°C in the greenhouse compartments (±2°C) and growth chambers (±0.5°C) during the experimental period. The RH was set to 70% in both greenhouse and growth chambers. The CO_2 concentration was 800 ppm (enriched with pure CO_2) in the greenhouse and ambient in the growth chambers. Leaf temperature during growth was measured by a thermocouple thermometer (model HD 9016; Delta OHM SRL, Caselle Di Selvazzano, Italy) and was measured to be in average 1.5°C higher under HPS compared to LED.

The first greenhouse experiment was carried out during December, 2009 to January, 2010 and the second experiment in February to March, 2010. The average natural solar radiation doses in these two periods were 4.3 and 24.9 mol m^{-2} day^{-1}, respectively, outside the greenhouse (Metrological data from Ås). Three separate shoots per pot were grown in the first greenhouse experiment and one shoot per pot in the second repeat. In the growth chamber experiments one shoot per pot was selected to grow up. The plants were grown until they reached the commercial stage of flower development, which are fully developed stems with open flowers.

Crop growth measurements

The total length was measured from the base of the shoot until start of the pedicel. The pedicel length was measured from the base of the pedicle to the base of the flower. The number of leaves and date of flowering (open flowers) were recorded. Once flowering is initiated no more leaves are formed. Fresh and dry weights (DW) of the leaves, flowers and stems (including stem and pedicle) were recorded at harvest. DW was determined after drying for 5 days at 70°C. Leaf area was also measured (Li-3100; Li-Cor Inc) at the end of the experiment for each treatment, and specific leaf area (SLA) was calculated from the area and DW of a leaf.

Anthocyanin, chlorophyll and carbohydrate analysis

Anthocyanins were estimated with the fluorescence excitation ratio method with a Multiplex 3 instrument (FORCE A, Université Paris-Sud ORSAY, Orsay Cedex, France) as described in Cerovic et al. (2008) and based on the principle from Bilger et al. (2001). The measurements were taken from the upper side of leaves. The measurements were calibrated against isolated pea chloroplasts as a reference with no anthocyanins. For chlorophyll analysis, leaf discs of 15 mm diameter from 20 individual plants were cut randomly and two discs per plant were extracted in10 ml *N*,*N*-dimethylformamide in the dark at 4°C for 6 days. Absorbance of the extracts was measured with a spectrophotometer (Shimadzu UV-2101PC, Vernon Hills, IL) and chlorophyll concentrations were calculated according to Porra et al. (1989).

Leaf samples for carbohydrate analysis were taken from 10 individual plants from four independent experiments at middle of a day for each sampling time. Samples were immediately frozen in liquid nitrogen and stored at −80°C prior to analysis. The carbohydrate concentrations were determined first by extracting the soluble carbohydrates (fructose and glucose) and then enzymatically hydrolyzing sucrose and starch. The analyses of sucrose, D-fructose, D-glucose and starch were done by using the Megazyme, K-SURFRG 12/05 kit (Megazyme International Ireland Ltd, Wicklow, Ireland).

Microscopy analysis of stomata and leaf morphology

Fully developed upper leaves from plants grown in the chamber experiments were collected and samples were taken interveinally close to the midribs from 10 plants from each light treatment for light microscopy (LM) and transmission electron microscopy (TEM; JEOL 1200 EX TEM; Jeol Ltd, Tokyo, Japan). Sample sections were fixed in 2% paraformaldehyde and 1.25% glutaraldehyde in 50 m*M* L-piperazine-*N*-*N*′-bis(2-ethane sulfonic) acid buffer (pH 7.2) for 24 h at room temperature. Fixed tissue was rinsed with the same buffer, dehydrated in an ethanol series (70 – 80 – 90 – 96 – 4× 100%). Samples for LM and EM were infiltrated with L.R. White acrylic resin (TAAB Laboratories, Aldermaston, Berkshire, UK), and polymerized at 60°C for 24 h. For LM study, semithin (1–2 μm) sections were cut with a glass knife. The sections were dried on slanted slides and stained with Stevenel's blue (del Cerro et al. 1980), mounted with immersion oil, and imaged and analyzed in a Leitz Aristoplan light microscope (Ernst Leitz Wetzlar, Wetzlar, Germany) with use of video microscopy and digital image processing. For TEM, ultra-thin (70 nm) cross-sections were cut with a diamond knife on an ultramicrotome, transferred to formvar-coated slot grids, poststrained with uranyl acetate and lead citrate. Cross-sections of rose leaves were measured for widths of whole-leaf, widths and number of palisade mesophyll, as well as epidermal tissues.

Epidermal impressions were made on 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 previously (Tanaka et al. 2005). All samples were taken interveinally close to the midrib on the abaxial side. The copied SUMP images were observed under LM and the number of stomata and epidermal cells were counted with UTHSCSA IMAGETOOL for windows version 3.00 (The University of Texas Health Science Center, San Antonio, TX). The stomata frequency and index were calculated from the number of epidermal and stomata cells per unit area of leaf surface according to Wilmer and Fricker (1996).

Gas exchange and chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured on fully expanded rose leaves grown under LED and HPS with a portable Pulse Amplitude Modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany). Prior to chlorophyll fluorescence measurements, the leaves were dark-adapted for 30 min to obtain maximum oxidized state of PSII-PSI (photosystem I) electron transport carriers (Horton and Hague 1988). PAM photosynthetic parameters were determined according to Rohacek (2002): the constant fluorescence F_o and the maximal fluorescence yield F_m induced by a saturating light pulse of 10 000 μmol m^{-2} s^{-1} for dark-adapted plant were used to evaluate the variable fluorescence $F_v/F_m = (F_m - F_o)/F_m$, which describes the maximum quantum efficiency of PSII. At steady state of variable fluorescence induced by actinic light, PSII in functional open state was evaluated as $\Phi_{PSII} = (F_m' - F_t)/F_m'$ (Genty et al. 1989). F_m' represents the maximum fluorescence yield induced by a saturating flash when leaves were exposed to continuous actinic light and F_t is the steady-state value of fluorescence immediately prior to the flash (Maxwell and Johnson 2000). The electron-transport rate (ETR) was calculated from the Φ_{PSII} as: $ETR = \Phi_{PSII} \times PAR \times (0.5) \times$ absorption fraction; where PAR is the PAR, 400 μmol m^{-2} s^{-1} and 0.5 is a factor that accounts for the partitioning of energy between PSII and PSI (Genty et al. 1989). The absorption fraction is the fraction of incident light absorbed by leaves, which was 0.90 and 0.87 for LED- and HPS-grown rose leaves, respectively. Leaf absorptance was calculated from transmittance and reflectance measured on 10 leaves per light treatment. The reflectance and transmittance measurement was done based on the method described in Solhaug et al. (2010). The absorptance was calculated every 1 nm of wavelength between 400 and 700 nm. The integrated absorptance of the growth lights was calculated by multiplying the relative leaf absorptance by normalized spectrum of growth light. Then the product values between 400 and 700 nm were summed up to get absorption fraction of the growth lights (Fig. 1).

Leaf gas exchange was measured on fully expanded leaves using a CIRAS-2 Portable Photosynthesis System with PLC6 (U) Automatic Universal Leaf Cuvette (PP Systems, 2001, Hertfordshire, UK). During all measurements, CO_2 concentration in the leaf chamber was 400 μmol mol^{-1}, the airflow was 250 ml min^{-1}, and the leaf chamber temperature 22°C, the humidity was approximately 70% (similar to the humidity during growth). The irradiance-response curves for gas exchange were measured by exposing leaves to the same LED and HPS light sources which were used for growth of the plants. For each lamp, the irradiance was increased stepwise in the following steps: 0, 50, 100, 150, 250, 400 and 600 μmol photons m^{-2} s^{-1}. The average values were calculated for each combination of irradiance and light source.

Calculations and statistics

The measured photosynthetic irradiance-response data were fitted with a quadratic equation (Prioul and Chartier 1977) using the PHOTOSYN ASSISTANT 1.1 software (Dundee Scientific, Dundee, Scotland, UK) to determine the light-saturated maximum photosynthetic rate (A_{max}). The experiments were repeated twice both in the greenhouse and growth chamber, and each experiment consisted of 10 individual plants. All growth and morphology data presented on Tables 1 and 2 were collected both from greenhouse and growth chamber experiments. Since the trends of the results in the experiments were similar the data are

Table 1. DW of leaves and stems of *Rosa* × *hybrida* plants grown under HPS (5% blue) and LED (20% blue) lamps. Different letters (within columns) indicate significant differences at *P*-values < 0.05. The values are averages ±SE (n = 20).

Growth environment	Light treatments	Leaves (g)	Stems (g)	Total DW (g)	Leaves (% DW)	Stems (% DW)
Greenhouse	LED	8.3 ± 0.2^{a}	7.0 ± 0.3^{b}	15.3 ± 0.6^{b}	53.9^{a}	46.0^{c}
	HPS	5.8 ± 0.1^{b}	9.9 ± 0.3^{a}	15.7 ± 0.3^{b}	37.1^{b}	62.9^{ab}
Growth chamber	LED	8.3 ± 0.1^{a}	7.1 ± 0.1^{b}	15.3 ± 0.2^{b}	54.4^{a}	46.1^{c}
	HPS	5.1 ± 0.1^{b}	10.8 ± 0.2^{a}	15.9 ± 0.2^{ab}	32.2^{bc}	67.6^{a}

Table 2. Morphological and developmental parameters of *Rosa* × *hybrida* plants grown under HPS (5% blue) and LED (20% blue) lamps. Different letters (within columns) indicate significant differences at *P*-values < 0.05. The values are averages ±SE (n = 20). SLA: specific leaf area indicating a leaf area per gram DW of a leaf. [a]Number of weeks to open flower with at least two sepals open at 180°.

Growth environment	Light treatments	Average leaf area (cm^2 per leaf)	SLA ($cm^2\ g^{-1}$)	Number of leaves per plant at flowering	Number of weeks to flowering[a]	Stem length (cm)
Greenhouse	LED	43.4 ± 0.6^c	9.5 ± 0.3^c	15.1 ± 0.2^a	6 ± 0.2^a	25 ± 0.2^b
	HPS	72.9 ± 1.2^a	19.4 ± 0.8^a	10.5 ± 0.2^c	6 ± 0.3^a	28.2 ± 0.4^a
Growth chamber	LED	37.5 ± 0.7^d	6.2 ± 0.2^d	11.8 ± 0.2^b	6 ± 0.1^a	21.7 ± 0.6^c
	HPS	56.3 ± 0.9^b	12.3 ± 0.5^b	9.8 ± 0.1^d	6 ± 0.2^a	28.6 ± 0.3^a

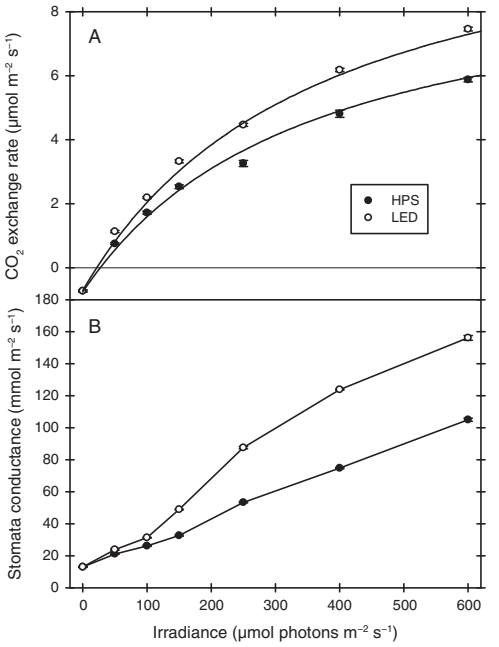

Fig. 2. Irradiance-response curves for CO_2 exchange (A) and stomata conductance (B) for leaves of *Rosa* × *hybrida* grown at 100 µmol m^{-2} s^{-1} under high pressure sodium lamps (HPS; 5% blue) or light emitting diodes (LED; 20% blue). Lines through the data points in (A) represent the best fit to a non-rectangular hyperbola calculated with the PHOTOSYN ASSISTANT 1.1 software (Dundee Scientific). The values are averages ± SE (n = 15).

presented as average values for each growth conditions, greenhouse (n = 20) and chamber (n = 20). However, the photosynthesis-related data (Fig. 2, Table 3) and leaf cellular analysis (Fig 3, Table 5) were collected only from growth chamber experiments. Tukey's HSD was used to make a multiple comparison among treatment means from significant Generalized Linear Model (GLM) ANOVA tests ($P < 0.05$) using MINITAB (16.1.1 windows version; Penn State University, Dubois, PA).

Table 3. Photosynthetic parameters of fully grown leaves of *Rosa* × *hybrida* grown under 100 µmol photons m^{-2} s^{-1} from HPS (5% blue) or LED (20% blue) lamps in growth chambers. A_{max} is estimated with the PHOTOSYN ASSISTANT 1.1 software on irradiance-response curves (Fig. 2). ETR and Φ_{PSII} were measured at 400 µmol photons m^{-2} s^{-1}. Different letters (within columns) indicate significant differences at *P*-values < 0.05. The values are averages ±SE (n = 15 for gas exchange parameter and n = 16 for chlorophyll fluorescence parameters).

Light treatments	A_{max} (µmol m^{-2} s^{-1})	F_v/F_m	Φ_{PSII}	ETR (µmol e^- m^{-2} s^{-1})
LED	12.6 ± 0.1^a	0.83 ± 0.01^a	0.49 ± 0.01^a	88.5 ± 1.7^a
HPS	10.2 ± 0.4^b	0.81 ± 0.02^b	0.32 ± 0.2^b	54.8 ± 2.4^b

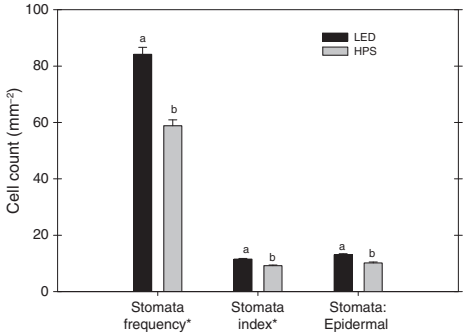

Fig. 3. Stomata characteristics of the abaxial side of leaves from *Rosa* × *hybrida* plants grown in growth chambers under different light qualities provided by light emitting diodes (LED; 20% blue) and high pressure sodium (HPS; 5% blue). Different letters indicate significant differences at *P*-values < 0.05. Error bars indicate + SE (n = 30). *Stomata frequency: number of stomata per a given area of leaf. *Stomata index: number of stomata per a given numbers of epidermal plus stomata cell (according to Wilmer and Fricker, 1996).

Results

Plant growth and development

The light sources with different B light proportions caused significantly different ($P < 0.05$) effects on development and morphology as well as dry matter

distribution between leaves and stem (Tables 1 and 2). The responses were slightly more prominent in the growth chambers compared to the greenhouse, although there was no difference in dry matter production and partitioning of the shoots (Table 1). Plants grown under LED with 20% B showed on average about 18% shorter shoot length and had 37 and 50% reduced leaf area and SLA, respectively (Table 2). Whereas 20% higher DW was allocated to leaves than stems in LED-grown plants, the case was the reverse in HPS plants, which showed higher DW in stems than leaves (Table 1). This is correlated with lower SLA values in LED. Number of days to flowering (open flowers) was similar for both light sources; all plants flowered after 6 weeks. However, LED exposed plants had on average 24% more leaves at flowering compared to plants grown under HPS lamps both in greenhouse and growth chambers (Table 2).

Leaf photosynthesis and gas exchange

The two light sources significantly affected the photosynthetic properties of leaves differently. The light saturated maximum CO_2 uptake (A_{max}) was 20% higher, and photosynthetic ETR at 400 µmol m^{-2} s^{-1} was 48% higher for leaves grown under LED lighting than HPS (Table 3). The dark-adapted F_v/F_m was slightly higher ($P < 0.05$) for plants grown under LED light (0.83) than under HPS lamps (0.81) (Table 3). The Φ_{PSII} (at 400 µmol m^{-2} s^{-1}) was 1.5 times higher for LED plants than for HPS plants (Table 3).

A considerably higher (1.5 times) stomata conductance (g_{sw}) was measured in plants from LED compared to under HPS (Fig. 2B). The g_{sw} of plants grown under the two light treatments responded similarly to the increase in irradiance during measurement. However, leaves from LED showed a higher increase in g_{sw} with increasing irradiance beyond 100 µmol m^{-2} s^{-1}, than leaves from HPS ($P < 0.05$; Fig. 2B). This result is consistent with the increase in photosynthesis rate (CO_2 exchange) with increasing irradiance (Fig. 2A). In line with the results on g_{sw}, the stomata frequency and index were notably higher in LED-grown leaves than HPS plants ($P < 0.05$; Fig. 3). Also, in LED plants there was a markedly larger number of stomata (29.8%) per a given unit area of epidermal cells as compared to HPS-grown plants (Fig. 3).

Significantly higher total chlorophyll (Chl) $a+b$ (7.3%), Chl a (12.4%) and Chl a/b (21.6%) were observed in LED lighting compared with HPS-grown plants ($P < 0.05$; Table 4). Nonetheless, the Chl b levels did not show any significant difference among the two light treatments ($P < 0.05$; Table 4). Leaves of plants grown under LED light had much higher anthocyanin levels than HPS plants (Table 4). The soluble carbohydrate contents per unit DW of leaf tissue also differed significantly between the treatments (Table 4). The 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 (Table 4). Nevertheless, starch content was not significantly different among the light treatments.

Table 4. The effect of different blue light proportions provided by LED (20% blue light) and HPS (5% blue light) lamps on the chlorophyll, anthocyanin (estimated as epidermal green light absorbance) and carbohydrate content of young leaves of *Rosa* × *hybrida* grown in growth chambers. Different letters (in rows) indicate significant differences at *P*-values < 0.05. The values are averages ±SE (n = 20).

	Light treatments	
Parameters	LED	HPS
Chl $a+b$ (µmol m^{-2})	1006.8 ± 13.8^a	933 ± 18.1^b
Chl a/b	3.7 ± 0.4^a	2.9 ± 0.2^b
Chl b (µmol m^{-2})	225.4 ± 16.4^a	248.5 ± 17.5^a
Anthocyanin (absorbance)	0.16 ± 0.01^a	0.12 ± 0.01^b
Glucose (g^{-1} DW)	0.31 ± 0.01^a	0.23 ± 0.03^b
Fructose (g^{-1} DW)	0.29 ± 0.02^a	0.18 ± 0.02^b
Sucrose (g^{-1} DW)	0.40 ± 0.03^a	0.29 ± 0.04^b
Starch (g^{-1} DW)	0.66 ± 0.03^a	0.67 ± 0.03^a

Cellular leaf morphology

The two light treatments affected rose leaf anatomy significantly ($P < 0.05$) different (Fig. 4, Table 5). Leaf thickness and palisade parenchyma cells were larger for plants grown under LED than HPS (Table 5). Also, plants grown under LED have a much higher number of palisade and epidermal cells as compared to HPS grown, which had fewer cells and more intercellular space (Fig. 4, Table 5).

Discussion

Plant growth and morphology

Plants grown under LED with 20% B light had shorter internodes and reduced leaf expansion exhibiting more sun-type characteristics, with higher photosynthesis capacity and higher LMA (leaf mass per unit leaf area) than those grown under HPS lamps with 5% B. Furthermore, in LED light flower initiation occurred at a higher leaf number than under HPS, but no difference in time to open flowers was observed.

Roses have an autonomous flower induction and flower initiation is promoted by increasing temperature and irradiance (Zieslin and Halevy 1975, Mortensen et al. 1992). A higher leaf temperature (1.5°C) was measured on plants under HPS compared to LED. The higher

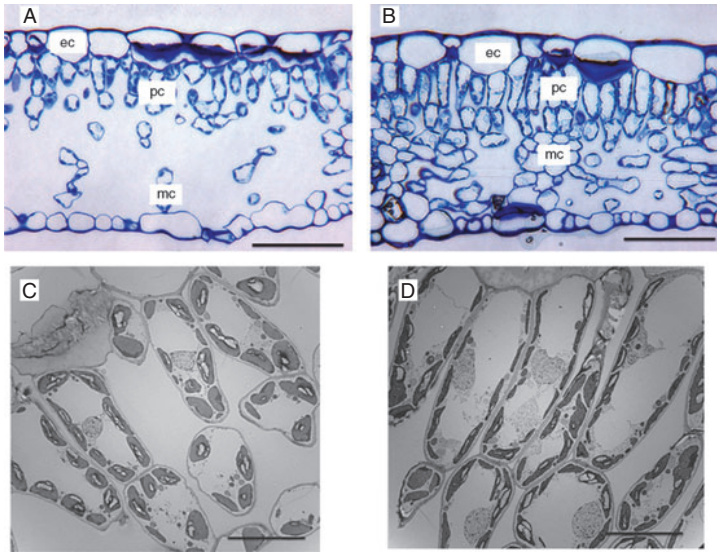

Fig. 4. Light photomicrographic cross sections of *Rosa* × *hybrida* leaves grown at 100 µmol m^{-2} s^{-1} in growth chambers under different proportions of blue light provided by high pressure sodium (HBS; 5% B) (A) and light emitting diodes (LED; 20% B) (B); ec, epidermal cell; mc, mesophyll cells; pc, palisade cells; bars, 100 µm (A and B). Transmission electron micrographs of palisade parenchyma cells of *Rosa* × *hybrida* leaves grown in growth chambers under different proportions of blue light provided by HPS (5% B) (C) and LED (20% B) (D); bars: 10 µm (C and D).

Table 5. Anatomical characteristics of *Rosa* × *hybrida* leaves grown in growth chambers under HPS (5% blue) and LED (20%, blue) lamps at a photosynthetic active irradiation of 100 µmol m^{-2} s^{-1}. Different letters (in rows) indicate significant differences at *P*-values < 0.05. The values are averages ±SE (n = 20).

	Light treatments	
Parameters	LED	HPS
No. of epidermis cell (µm^{-2}; adaxial)	11.6 ± 0.3^a	9.3 ± 0.5^b
No. of epidermis cell (µm^{-2}; adaxial)	14.5 ± 0.7^a	10.9 ± 0.5^b
No. of palisade cell (µm^{-2})	29.1 ± 0.6^a	23.6 ± 0.5^b
Leaf thickness (µm)	65.9 ± 0.7^a	60.3 ± 4.2^b
Length of palisade parenchyma (µm)	12.1 ± 0.4^a	8.9 ± 0.2^b

leaf temperature could have contributed to an earlier flower induction in HPS. However, no difference in time to open flower was found between the treatments. The effect of irradiance on flower initiation is generally attributed to the effect of light on photosynthesis and the availability of assimilates for flower bud development. In the LED-grown plants, leaf anatomy and photosynthetic characteristics resembled high irradiance characteristic (sun-type) and higher contents of soluble carbohydrates (sucrose, fructose and glucose) were measured. Although high carbohydrate levels are known to facilitate floral induction (Lejeune et al. 1993, Corbesier et al. 2002) the increased carbohydrate contents in LED did not contribute to earlier initiation of flowering in roses, rather the opposite was observed. LED-grown plants showed enhanced vegetative growth (more leaves and internodes per plant) compared to those under HPS and the effect was even stronger in greenhouse experiments with natural light from the outside compared to closed chamber systems (Table 2). LED-grown plants partitioned more energy toward other process than flower induction, for instance, distribution of dry matter to leaves rather than generative growth and production of secondary metabolites like anthocyanin. Previously it has been shown that anthocyanin formation is greatly affected by carbohydrates accumulation and sucrose supplementation is known to enhance the anthocyanin biosynthesis (Park et al. 1998, Kim et al. 2006, Solfanelli et al. 2006).

In roses, light in general has an effect on flower initiation not only by promoting photosynthesis, but also by altering the pattern of photosynthate distribution (Zieslin and Halevy 1975, Mor and Halevy 1980). Previous findings also indicate that B light is less effective in increasing the transport of assimilates to rose shoot tips compared to other wavelengths like R light. Further, application of far red (FR) light increased the R-promoting effect in roses but no such effect was

seen when combining FR and B (Mor et al. 1980). Thus, a light source containing low B (like HPS) is probably more optimal in promoting a rapid floral transition in roses.

Floral initiation under LED occurred at a higher number of leaves which indicates a more rapid leaf formation rate under LED compared to HPS. However, the leaf expansion was greatly reduced and the leaf area was 30 % smaller under LED compared to HPS (Table 2). On the other hand, it also shows that adding more B light has a potential to increase the production capacity but it needs to be combined with factors known to promote leaf expansion (e.g. FR light) (Newton et al. 1996).

The change in light spectrum is also known to have a strong influence on plant morphogenesis and growth (Whitelam and Halliday 2007). The two lamp types used in this experiment have no (LED) or very little FR (HPS). Accordingly, the relative amount of active phytochrome expressed as the PSS was almost similar under LED and HPS. The PSS was 0.89 [89% of the phytochrome was in active (P_{fr}) form] and 0.85 [85% of the phytochrome was in active (P_{fr})form] for LED and HPS, respectively. The difference in the state of the phytochrome system between the light treatments might not be considered as a plausible difference to cause the morphological alteration, since in both cases a high percentage of the phytochrome existed in an active form. Therefore, the higher B light fraction of LED than HPS lamps apparently explains the lower SLA, greater DW partitioning to the leaf than stem and shorter stem in LED- than HPS-grown plants.

The 18% shorter stem length in LED lighting compared to HPS at the end of the experiment (Table 2) might be due to a strong sink competition from leaves, resulting in more assimilate partitioning toward leaves (Table 1). This is in line with the observed B light inhibition of growth of internodes and cell expansion in a number of species (Appelgren 2003, Folta et al. 2003, Dougher and Bugbee 2004). Compared to 26% B light, soybean plants exposed to virtually no B light had a 4.7-fold increase in stem length, which was associated with a 4.5-fold increase in cell number (Dougher and Bugbee 2004). This suggests that the reduction in stem length in a high proportion of B light is due to reduced cell division. Maas and Bakx (1995) also showed that a decreased proportion of B light increased the shoot length of 'Mercedes' roses. Generally, this indicates that manipulating the B light proportion could be an alternative strategy to replace chemical growth retardants to control plant elongation and morphology in production of ornamental plants such as pot roses.

Further, the much lower SLA and lower average leaf area under LED than HPS (Table 2) may be associated with a B light-mediated inhibition of cell expansion or division (Dale and Milthorpe 1983, Dougher and Bugbee 2004). Hogewoning et al. (2010a) also reported that cucumber plants grown under HPS (5% B) had twice the area of plants grown under fluorescent tubes with 23% B proportion. In their experiment with soybean, Dougher and Bugbee (2004) showed a 23% decrease in leaf area when the B light fraction increased from 6 to 26%. This was associated with a 15% decrease in cell area (expansion) and 11% decrease in cell number.

Light spectrum has a strong influence on leaf anatomy and morphology (Saebø et al. 1995, Weston et al. 2000, Macedo et al. 2011). The difference in thickness of the leaves observed in this study (Fig. 4, Table 5) was mainly due to thinner palisade and spongy mesophyll layers in HPS-grown plants than those under LED. In contrast, plants from LED had longer and a higher number of palisade and epidermal cells than HPS plants. These results are in line with previous works showing that leaf thickness, particularly the palisade mesophyll tissues of several plant species, decreased when plants were grown under either low light levels (Barreiro et al. 1992, Sims and Pearcy 1992), high R:FR ratios (Barreiro et al. 1992), or low levels of B light (Saebø et al. 1995). Accordingly, B light plays a role in modulating the leaf cell structures toward a more 'sun-type' leaf character. The irradiance of 100 $\mu mol\, m^{-2}\, s^{-1}$ in the present experiment is relatively low for roses, which in horticulture normally are grown under high irradiances (Mortensen et al. 1992). However, even at this relatively low irradiance, B light stimulated 'sun-type' characteristics on morphology of the plants.

Plant DW and partitioning

In spite of lower leaf area, LED lighting resulted in a higher partitioning of assimilates toward leaves than stems compared to in HPS-grown plants (Table 1). Nonetheless, total dry matter accumulation in the whole plant did not show any significant difference among the two light sources. These results are similar to those found in cucumber where an increasing proportion of B light proportionally increased LMA and nitrogen content per unit area (Hogewoning et al. 2010b). B light deficiency has also been reported to be related with a lower LMA in soybean (Britz and Sager 1990). Thus, the difference in biomass partitioning in LED and HPS, which is reflected also in the higher levels of soluble carbohydrates in young leaves under LED (Table 4), is apparently attributed to the different proportions of B light. Wang et al. (2009) also showed that the total sugars and sucrose contents of cucumber plants grown under B light were slightly higher or comparable than those grown under white, R and green lights.

Leaf photosynthesis and gas exchange

A higher B light fraction, or a higher absolute amount of B light, is generally associated with the development of 'sun-type' leaves, which are characterized by leaves with a high LMA and a high photosynthetic capacity (Buschmann et al. 1978, Lichtenthaler et al. 1980, Matsuda et al. 2004, Matsuda et al. 2008). Here, we also found that rose plants grown under LED lighting showed higher photosynthesis (Fig. 2A, Table 3) than those grown under HPS lamps despite the fact that they had a lower average leaf area. This indicates LED plants compensated for reduced leaf area by increasing photosynthesis per unit leaf area, so the final result is about equal biomass. In cucumber, Hogewoning et al. (2010a) likewise found that A_{max} per unit leaf area is lower under lights with a lower proportion of B light. In their study, cucumber leaves grown under HPS (5% B) had lower A_{max} per unit leaf area compared with leaves grown under fluorescent tubes (23% B) and an artificial solar spectrum (provided by sulfur plasma lamps) (18% B). Also, in the presence of R LEDs supplemented with B light, wheat plants had higher leaf net photosynthesis rates than when grown under R LEDs alone (Goins et al. 1997). This was related to increased stomata conductance under more B light. Light-saturated maximum photosynthesis and stomatal conductance (g_s) are closely related in many species (Romero et al. 2004, Rosati et al. 2006). In a preliminary experiment with roses we also observed that photosynthetic CO_2 uptake was 5.4, 3.7 and 2.9 $\mu mol\, m^{-2}\, s^{-1}$ for HPS-grown plants, whereas LED-grown plants had higher photosynthesis with 7.0, 4.9 and 4.5 $\mu mol\ CO_2\ m^{-2}\, s^{-1}$ when measured with red, green and blue light, respectively, at 100 $\mu mol\, m^{-2}\, s^{-1}$ (Terfa et al., unpublished results). However the change in CO_2 fixation can also be affected by the change in leaf biochemistry apart from the stomata conductance (Rosati et al. 2006).

LED-grown plants exhibited significantly higher ETR than HPS-grown ones (Table 3), which is mainly a result of higher Φ_{PSII} efficiency since the fraction of light absorbed from the different light sources by leaves was only slightly higher for LED than HPS (Fig. 1). A higher level of total chlorophyll was found in LED-grown leaves compared to HPS (Table 4). However, this is not enough to explain the higher photosynthesis in LED plants since photosynthesis on a chlorophyll basis will be high in LED-grown plants. The work of Leong and Anderson (1984) also showed that plants grown under additional B light supplementation exhibited higher photosynthetic electron-transport activities per unit chlorophyll than R and white lights. Hence, the variation in photosynthesis measured as Φ_{PSII} efficiency, ETR and in CO_2 exchange of LED leaves was probably a result of thicker leaves with more photosynthetic apparatus per unit leaf area in LED leaves with a SLA that was about half of the SLA in HPS-grown plants (Table 2).

A higher Chl *a*/*b* ratio was found in LED-grown leaves compared to HPS (Table 4). The higher Chl *a*/*b* ratio was probably due to the effect of B light on the biosynthesis of chlorophyll and Chl *a*/*b* ratios. Senger and Bauer (1987) showed that plants grown under supplementary B fluorescent lamps had the highest Chl *a*/*b* ratios and more sun-like type chloroplast than plants exposed to less B light. A higher Chl *a*/*b* ratio indicate a high light-adapted photosynthetic apparatus with less Chl b containing light-harvesting antennae, and thereby a higher capacity for electron transport and more Calvin cycle enzymes on a chlorophyll basis (Evans 1988). High light-grown plants as shown for cucumber by Evans (1989) therefore have a higher photosynthesis on a chlorophyll basis.

The stomata conductance (g_{sw}) was significantly higher for LED leaves than HPS (Fig. 2B). This was correlated with a higher CO_2 exchange. Nevertheless, the g_{sw} under the two treatments responded similarly to increased irradiance. The higher g_{sw} in LED-grown plants might at least partly be due to a higher number and frequency of stomata per area of epidermal cells, as shown by the stomata index (Fig. 3). Hogewoning et al. (2010b) found that the g_{sw} in cucumber proportionally increased with increasing proportion of B light, and that was related both to higher number and aperture of stomata. A work of Wang et al. (2009) also showed that cucumber plants grown under B monochromatic light had increased g_{sw} compared to white, R, green and yellow monochromatic lights provided. In general, B light promotes stomata opening more than other light wavelengths (Sharkey and Raschke 1981, Zeiger et al. 2002), and this stimulation of stomata opening may contribute to the increase in stomata conductance and eventually increased gas exchange.

A significantly higher concentration of anthocyanins was measured in leaf tissue of LED-grown plants than under HPS (Table 4). Anthocyanin biosynthesis genes are regulated tightly by both the quantity and quality of light (Mancinelli et al. 1975, Solfanelli et al. 2006, Cominelli et al. 2008). Also, B light is effective in anthocyanin production in most plant species (Sponga et al. 1986). In an experiment with strawberry cells, B light played a major role in anthocyanin synthesis and accumulation (Kurata et al. 2000). Stutte (2009) also showed that lettuce leaves grown under more B light had enhanced anthocyanin biosynthesis and accumulation compared to other light qualities. These particular cases suggest that the B light receptors cryptochromes are involved in anthocyanin biosynthesis (Mancinelli et al. 1991, Briggs

and Huala 1999, Stutte 2009). Hence, the results shown in this study indicate that B light plays an important role in anthocyanin biosynthesis as an adaptation to change in spectral quality also in roses.

Conclusion

We show here for the first time that LED with a high B light proportion increased the overall photosynthesis capacity per unit leaf area, levels of soluble carbohydrates and enhanced growth in roses compared to the traditional HPS with low blue portion, while it reduced internode length and leaf expansion. Further, the higher B proportion in LED triggered qualitative and quantitative responses of leaves normally associated with acclimation to high irradiances. However, this did not affect yield (DW) or flowering time to a stage with open flowers, although flower initiation occurred at a higher leaf number. This indicated that plant morphology is more sensitive to B light than flowering in roses. On the other hand, under LED leaf formation rate was higher and assimilates were apparently translocated toward other processes such as leaves than regenerative processes. However, the increased photosynthesis capacity and soluble carbohydrate level of the LED-grown plants in comparison to HPS plants suggests that assimilation lighting in greenhouses could be made more productive by additional blue light.

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PAPER II

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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

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Keywords: light quality, morphology, postharvest physiology, roses, senescence, water loss

Abstract

Alterations in light quality affect plant quality but the effects vary significantly between species. In this study, we analyzed internal and external quality parameters of pot roses (*Rosa* × *hybrida* 'Toril') grown under different light qualities provided by light emitting diodes (LED, 80% red and 20% blue) and the traditional high pressure sodium (HPS) lamps. The experiments were conducted in closed growth chambers and in greenhouse during winter with supplemental lighting (100 µmol m^{-2} s^{-1}). LED-grown plants showed higher chlorophyll and anthocyanin content and more thorns than HPS-grown plants. The stem and pedicle length were significantly shorter in LED-grown plant compared to HPS although the total production period was not affected. There was no significant difference in the storability of dark stored plants at high temperature (24°C) between the two light qualities. However, at 4°C a better storability was found in LED-grown plants and the flowers were more vital and a lower pH and osmolarity was found in petals 4 weeks after storage indicating delayed senescence compared to HPS-grown plants. Further, desiccation tests were performed on detached leaves to study the drought stress tolerance. When growing the pot roses under high relative air humidity (90%), LED-grown leaves had significantly higher water content (WC) after 3 h of desiccation compared to leaves from HPS.

INTRODUCTION

Postharvest senescence and stress tolerance are limiting factors in the marketing of ornamentals. The environmental conditions during growth are known to strongly influence the post-harvest behavior (reviewed by Halevy and Mayak, 1979). In roses (*Rosa* × *hybrida*), it has been demonstrated that increasing natural or supplementary light level improves postharvest life. This effect is thought to be mediated through the carbohydrate status of the plant (Fjeld et al., 1994). On the other hand, postharvest longevity of roses has been found to decrease with increasing photoperiod mainly because of higher water loss and early wilting (Pettersen et al., 2007). Similar symptoms appear when growing roses under high (>85%) relative air humidity (RH) because the stomata fail to close (Torre and Fjeld, 2001).

Light quality is another important preharvest factor which can have an impact on the postharvest behavior of roses either by affecting the hormonal content, carbohydrate status or stomata functionality (Blom-Zandstra et al., 1995; Garello et al., 1995; Mortensen and Fjeld, 1998; Rajapakse and Kelly, 1994). The most common lamp type in commercial greenhouses is high pressure sodium (HPS) lamps. However, the use of light emitting diodes (LEDs) as a lighting system in greenhouse production is under development (Morrow, 2008). The LED technology opens up possibilities to select specific parts of the light spectrum to study and control different processes. Blue (B) light is known to have numerous effects on plant growth and development including stomata function, photosynthesis, carbohydrate status and rate of senescence (Senger and Bauer, 1987; Causin et al., 2006; Wang et al., 2009). However, limited work has been done on the

effects of high portions of B light on postharvest physiology responses of ornamentals. Hence, in this study pot roses were grown under LED and HPS providing 20 and 5% B light proportions respectively but with similar phytochrome photostationary state (PPS) (calculated according to Sager et al., 1988). The objective was to test differences in postharvest behavior of pot roses grown under the different light qualities. Further, the aim was to study the interaction between RH and light quality to see if the responses were different under high and moderate RH.

MATERIAL AND METHODS

Plant Material

Rosa × *hybrida* '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 1.75, 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 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 21(±2°C), and average daily relative air humidity (RH) 70(±5%), corresponding to a 0.5 kPa water vapour deficit (VPD). Supplementary light by high-pressure-sodium-lamps (HPS, Osram NAV T-400W, Munich, Germany) was given 20 h every day, followed by a 4 h dark period. At average the photosynthetic photon flux (PPF) was 100(±10) µmol m^{-2} 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 either in greenhouse compartments or growth chambers.

Experimental Growth Conditions

Two different experiments were performed and described as experiment I and II. In experiment I growth experiments were performed both in greenhouse compartments and controlled growth chambers while in experiment II only growth chamber experiment was performed. The greenhouse experiment was carried out during December 2009 to January 2010 and February to March 2010. The average natural solar radiations in these two periods were 4.3 and 24.9 mol m^{-2} day^{-1}, respectively (Metrological data from Ås). In all experiments the plants were exposed to a PPF of 100 µmol m^{-2} s^{-1} for 20 h day^{-1} with 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 630 nm) and 20% blue (B; peak wavelength at 465 nm) or HPS (5% B) lamps (Fig. 1). The phytochrome photostationary state (PSS) was calculated based on the method developed by Sager et al. (1988) and was 0.89 and 0.85 for LED and HPS respectively. The irradiance was measured at plant level. The temperature set point was 20°C in day and night both in greenhouse compartments (±2°C) and growth chambers (±0.5°C) during the experimental period. The relative air humidity (RH) during experiment I was 70% both in growth chambers (70±3) and greenhouse (70±6) while in experiment II the RH was either 60%±3 (moderate humidity) or 90%±2 (high humidity). The CO_2 concentration was 800 ppm (enriched with pure CO_2) in the greenhouse and ambient (400 ppm) in the growth chambers. 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.

Postharvest Handling and Measurements

In experiment I ten plants from each lighting treatments both from greenhouse and chamber were dark stored either in cold (4°C) or high temperature (24°C). The plants

were watered when needed. The longevity of the flowers was determined by recording wilting, blueing and discoloration of petals, dry spots and crispy areas on leaves. Besides, pH and osmolarity of the petals were analyzed after four weeks of storage as senescence indicators. Petals were collected from both treatments and stored at -80°C before the extraction of cell sap. The sap was extracted in water and expressed through cheese-cloth before the pH of the sap was measured. The osmolarity of cell sap was measured with a cryoscopy osmometer (Osmomat 030, Gonotec). Additionally, the relative chlorophyll values were measured every week with a chlorophyll content meter (Hansatech instruments Ltd., King's Lynn, England) to study the chlorophyll degradation rate.

Growth Analysis and Leaf Water Content

In experiment II 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. Anthocyanin contents were estimated on fully developed plants at three different positions (lower, middle and upper part) with the fluorescence excitation ratio method using a Multiplex 3 instrument (FORCE A, Université Paris-Sud ORSAY, Bâtiment 503, Orsay Cedex, France) as described in Cerovic et al. (2008) and based on the principle from Bilger et al. (2001). The measurements were calibrated against isolated pea chloroplasts with no anthocyanins as a reference.

Detached upper leaves from ten plants grown under LED (20% blue) and HPS (5% B) with high (90%) and moderate (60%) relative humidity (experiment II) were weighed right after detachment and after three hours of desiccation to determine the water content. The water content was calculated as the percentage of the difference in weight of the leaf at the start and end of the desiccation time. The desiccation tests were done at 20°C and 40% RH in the light (10 µmol m^{-2} s^{-1} provided by fluorescent lamps).

Statistics

The statistics in experiment I and II were performed using Minitab (Minitab 16.1.1, windows version, Penn State University, Pennsylvania, USA). To study interaction effect of humidity and light quality in experiment II a two-way ANOVA was used for analysis. Tukey's HSD was used to make a multiple comparison among treatment means from significant GLM analysis of variance (ANOVA) tests ($P<0.05$).

RESULTS AND DISCUSSION

A better storability and longevity of flowers was observed for plants grown under LED compared to HPS when stored at 4°C in darkness (Table 1). However, no difference was found between the light treatments when the plants were stored at 24°C (Table 1). After one week of dark storage the plants placed at 24°C had already lost their ornamental value and it was not possible to detect any differences between the light qualities. However, at long term storage (4°C for 4 weeks), which is a common storage temperature for roses, a significant difference in longevity was observed. Typical symptoms of senescence, like blue-ing and wilting of petals were more pronounced in the flowers produced with HPS compared to LED after 4 weeks of storage (Table 1). This observation was consistent with the measurement of petal cell-sap properties (pH and osmolarity of cell sap). The aging of plant cells is usually associated with an extended decline in amino acids and proteins, as well as changes in the composition and structures of membranes which leads to change in cell pH and osmotic capacity (Borochov and Woodson, 1989). A higher pH and osmolarity were observed in petals from HPS compared to LED, indicating an accelerated senescence (Table 2).

The phytochrome photostationary states (PPS) were 0.85 and 0.89 for the HPS and LEDs, respectively (calculated according to Sager et al., 1988), indicating that mainly the blue light, and not the PPS, is decisive for the observed responses. B light is known to increase dry matter production and carbohydrate accumulation in plants. Wang et al. (2009) showed that the total sugars and sucrose contents of cucumber plants grown under B light were slightly higher than those grown under white, R and green lights. Also, in a

previous experiment with roses we observed that increasing B light portion in the supplementary light source increased the levels of soluble carbohydrates in young leaves (Terfa et al., unpublished data). The importance of carbohydrates for vase life and development of rose flowers has been well documented (Ho and Nichols, 1977; Marissen and La Brijin, 1995).

The relative chlorophyll content in leaves was higher in LED grown plants compared to HPS at the start of harvest which is in accordance with previous experiments done with increased B light (Tripathy and Brown, 1995; Senger and Bauer, 1987). However, no significant difference in the rate of degradation of chlorophyll was found between the two light treatments during storage (Fig. 2). After 4 weeks of storage the chlorophyll content had decreased about10-12% in both light qualities and only 10% leaf drying was found at both light qualities (Table 1).

No significant difference was found in production period (data not shown) but plants grown under HPS had longer stems and pedicles and fewer thorns (Table 3) compared to LED at both high and low RH (Table 4). Maas and Bakx (1995) also showed that a decreased proportion of B light increased the shoot length of 'Mercedes'. Increased B-light is commonly known to inhibit extension growth (Laskowski and Briggs, 1988). This generally indicates that manipulating the B light proportion in the light source can be an alternative strategy to inhibit stem extension growth of roses. Further, the calculated anthocyanin values were higher in plants grown under LED compared to HPS, which is common under high B-light (Sponga et al., 1986), but the content of anthocyanin was not affected by RH (Table 3).

However, the pedicels were significantly longer under high RH compared to low RH in the presented experiment and an interaction between RH and light quality was found (Table 3). At moderate RH the LED had a stronger inhibiting effect on pedicel length than at high RH (Table 3). It was previously shown that a high RH enhances the pedicel length probably because of the softer tissue type (Torre and Fjeld, 2001).

The water loss from detached rose leaves was highly affected by the RH and the light quality and a significant interaction was found (Table 3). Leaves grown under high RH (90%) had lost in average 40% more water 3 h after detachment compared to moderate RH. This is consistent with previous findings of roses (Mortensen and Fjeld, 1998; Torre and Fjeld, 2001). Further, under moderate RH only small differences between LED and HPS was measured, but at high RH significantly lower water loss was found in leaves developed under LED compared to HPS (Table 3). The stomata functionality was improved and a better closing ability in darkness and during drought stress was detected in leaves developed at high RH when grown under LED compared to HPS (Terfa et al., unpublished results). A good water balance and drought stress tolerance is important for the postharvest life and storability of pot roses. The present study clearly shows that more B light can improve the drought stress tolerance of roses grown at high RH. A light source with high B light can have the potential to overcome, at least partly, the negative effect of high RH on stomata functionality and thus improve the postharvest life.

CONCLUSION

In conclusion, this study shows that there is a potential to reduce the stem length and improve the storability of pot roses by growing under LED compared to HPS. Plants grown under LED (20% B) and stored at 4°C had a better flower longevity and delayed petal senescence compared to roses grown under HPS (5%). Besides, reduced water loss was observed from leaves grown under high RH when LED was used as the light source, indicating an improvement in stomata functioning compared to HPS.

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Tables

Table 1. Number of plants with symptoms (% of total) of leaf and petal wilting, discoloration of petals and dry spots and brittle leaves during dark storage of *Rosa* × *hybrida* 'Toril' grown under LED and HPS until flowering and stored at 24°C and 4°C for 1 week and 4 weeks respectively. The values are presented as average values of greenhouse and chamber experiment since no significant differences was found between repeats and/or experimental site. The error bar indicates, Mean ± SE (n=40).

Postharvest symptoms	Light quality during growth	Storage temperature	
		24°C (1 week)	4°C (4 weeks)
Leaf and petal wilting	HPS	100	10
	LED	100	0
Discoloration of petals	HPS	100	35
	LED	100	10
Dry spots and brittle leaves	HPS	100	15
	LED	100	15

Table 2. Effect of blue (B) light by LED (20% B) and HPS (5% B) during growth on pH and osmotic values of rose petal cell sap after four weeks of storage at 4°C. Values indicate average values ± SE of four experiments in greenhouses and chambers. Different letters (in rows) indicate significant differences at *P*-values <0.05 (n=40).

Senescence indicators	Light quality during growth	
	LED	HPS
pH	4.18[b]	4.92[a]
Osmotic value (osmol/kg)	0.28[b]	0.36[a]

Table 3. Interactional influence of relative air humidity (RH; high, 90% and moderate, 60% RH) and light quality provided by LED and HPS on quality parameters and water content in detached leaves (measured 3 h after detachment) of pot rose 'Toril'. The anthocyanin content was measured on fully developed leaves at three different positions on the plant (lower, middle and upper part). The number of thorns was counted on the main stem. Significant differences are calculated at *P*-values <0.05, n.s. indicates non-significant differences. Values are averages ± SE (n=20). nd means not determined.

RH during growth (%)	Light	Stem length (cm)	Pedicle length (cm)	No. of thorns	Relative anthocyanin content	Water content (%)
High	LED	18.6	8.3	nd	0.40	65
(90)	HPS	21.7	8.7	nd	0.34	43
Moderate	LED	19.9	6.9	25.1	0.48	88
(60)	HPS	22.1	7.8	19.3	0.32	85
Statistical significance						
RH		n.s.	P=0.023	nd	n.s.	P=0.001
Light quality		P=0.019	P=0.001	P=0.01	P=0.025	P=0.045
RH × light quality		n.s.	P=0.034	nd	n.s.	P=0.01

Figures

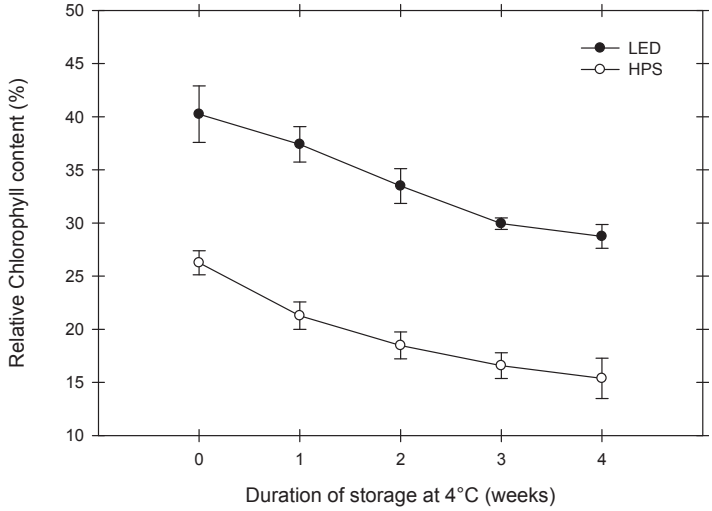

Fig 1. Irradiance spectra of the lamps used in the experiments; HPS Lamps Osram NAV T-400W and LED lamps (Round LED-light 92W with 3 chain, SoLa-Co).

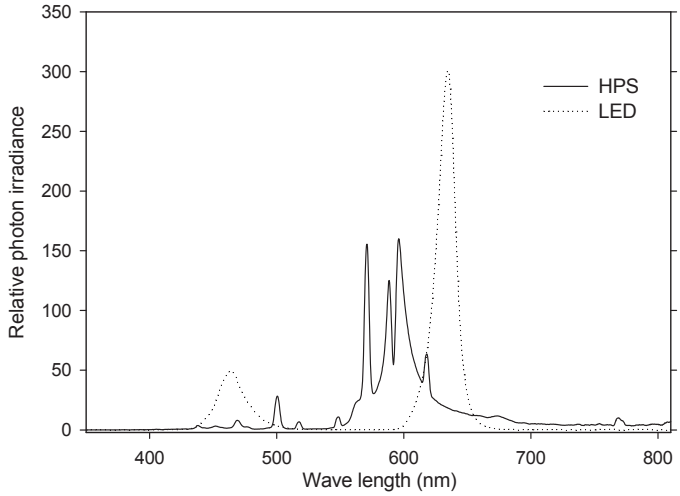

Fig 2. Change (%) in chlorophyll content in leaves of roses 'Toril' grown under HPS and LED during 4 weeks of storage at 4°C. The experiment was repeated twice in a greenhouse compartment and twice in a growth chamber. The values are presented as average of greenhouse and chamber experiment since no significant differences was found between repeats and/or experimental site. The error bar indicates, Mean ± SE (n=20).

PAPER III

Louise Elisabeth Arve, Meseret Tesema Terfa, Hans Ragnar Gislerød, Jorunn Elisabeth Olsen and Sissel Torre (2013). **High relative air humidity and continuous light reduce stomata functionality by affecting the ABA regulation in rose leaves.** Plant, Cell and Environment 36, 382–392.

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High relative air humidity and continuous light reduce stomata functionality by affecting the ABA regulation in rose leaves

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ABSTRACT

Plants developed under high (90%) relative air humidity (RH) have previously been shown to have large, malfunctioning stomata, which results in high water loss during desiccation and reduced dark induced closure. Stomatal movement is to a large extent regulated by abscisic acid (ABA). It has therefore been proposed that low ABA levels contribute to the development of malfunctioning stomata. In this study, we investigated the regulation of ABA content in rose leaves, through hormone analysis and β-glucosidase quantification. Compared with high RH, rose plants developed in moderate RH (60%) and 20 h photoperiod contained higher levels of ABA and β-glucosidase activity. Also, the amount of ABA increased during darkness simultaneously as the ABA-glucose ester (GE) levels decreased. In contrast, plants developed under high RH with 20 h photoperiod showed no increase in ABA levels during darkness, and had low β-glucosidase activity converting ABA-GE to ABA. Continuous lighting (24 h) resulted in low levels of β-glucosidase activity irrespective of RH, indicating that a dark period is essential to activate β-glucosidase. Our results provide new insight into the regulation of ABA under different humidities and photoperiods, and clearly show that β-glucosidase is a key enzyme regulating the ABA pool in rose plants.

Key-words: abscisic acid; ABA-glucose ester; β-glucosidase.

INTRODUCTION

Stomatal opening plays a critical role in regulating gas exchange required for photosynthesis and transpirational water loss needed for nutrient uptake and cooling. Throughout the day, there is a constant regulation of the stomata as the water loss through stomata is balanced against the need for CO_2 uptake (Tallman 2004). During the night, most C3 plants close the stomata to maximize the hydration when there is no need for CO_2 uptake for photosynthesis. In the early morning, when the plant water potential is the least negative, stomata open so that transpirational nutrient uptake can occur. Later in the day, the stomatal opening is closely regulated to make sure the plants retain enough water to maintain turgor. The control of stomatal opening and closing is regulated by the plant hormone abscisic acid (ABA), which in turn is regulated by the O_2:CO_2 ratio, air humidity, drought, temperature and light (Tallman 2004; Reynolds-Henne *et al*. 2010). Increased levels of ABA activate Ca^{2+} inwards channels and K^+ and Cl^- outwards channels, which decrease the turgor pressure, resulting in stomatal closure.

The regulation of endogenous ABA levels in plant tissues is mediated by the balance between biosynthesis and inactivation (Zeevaart 1980). ABA is synthesized through a series of steps from isopentenyl diphosphate (IPP), with zeaxanthin and then violaxanthin as two of the intermediate precursors (Seo & Koshiba 2002). Zeaxanthin and violaxanthin are also parts of the light-driven xanthophyll cycle, which regulates the dispatching of excess energy through non-photochemical quenching (Jahns, Latowski & Strzalka 2009). During light, violaxanthin is converted to zeaxanthin, reducing the amount of violaxanthin available for ABA biosynthesis (Tallman 2004). In *Nicotiana tabaccum*, the concentration of ABA in unstressed plants was at its maximum 3 h into the dark period, before decreasing and remaining low the rest of the dark period (Novakova *et al*. 2005). During the light period, the ABA concentration in unstressed plants was regulated by the need to conserve water, but never reached the level of the maximum peak during darkness. The increase in ABA levels in the beginning of the dark period is believed to ensure stomatal closure in order to preserve water and rehydrate when no CO_2 uptake is needed (Tallman 2004; Novakova *et al*. 2005).

ABA is inactivated by two main pathways: either oxidation or conjugation of free ABA (Nambara & Marion-Poll 2005). The oxidation pathway involves the hydroxylation of ABA to phaseic acid (PA), which is further reduced to dihydrophaseic acid (DPA) (Cutler & Krochko 1999; Nambara & Marion-Poll 2005). The second pathway is conjugation with monosaccharides, most commonly with glucose creating ABA-glucose ester (ABA-GE) (Lim *et al*. 2005; Priest *et al*. 2006). ABA-GE is hypothesized to be a storage form of ABA, which can be stored in the vacuoles and released when ABA is needed (Dietz *et al*. 2000). In a number of plant species like *Arabidopsis thaliana*, *Hordeum vulgare* (barley) and *Triticum spp.* (wheat),

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ABA-GE has been found to be hydrolyzed in response to water stress by β-glucosidase, leading to an increase in the active ABA pool (Dietz *et al*. 2000; Sauter, Dietz & Hartung 2002; Lee *et al*. 2006). β-glucosidases are also known to hydrolyze other conjugated plant hormones such as cytokinins, auxin, gibberellins and salicylic acid, as well as to degrade cellulose and anthocyanin (Minic 2008; Morant *et al*. 2008; Oren-Shamir 2009; Verma *et al*. 2010).

ABA plays an important role in environmental stress responses and the levels increase when plants experience adverse environmental conditions like drought, salt and suboptimal temperatures (Luan 2002; Zhu 2002; Reynolds-Henne *et al*. 2010). However, environmental factors such as air humidity are also known to affect the endogenous ABA levels of plants. For instance, in *Spinacia oleracea* (spinach), *Tradescantia virginiana* (Virginia spiderwort) and *A. thaliana*, ABA levels were lower in leaves developed under high compared with moderate humidity (Zeevaart 1974; Nejad & Van Meeteren 2007; Okamoto *et al*. 2009). *Arabidopsis* also showed lower ABA content due to increased inactivation of ABA to PA and DPA when moved from low to high relative air humidity (RH) (Okamoto *et al*. 2009).

Previous studies have shown that continuous high RH (>85%) during growth also results in the development of malfunctioning stomata, which are unable to close (Torre & Fjeld 2001; Torre *et al*. 2003). Similar observations have been obtained in experiments with leafy cuttings rooted at high RH (Fordham *et al*. 2001) and in micro propagated plants (Santamaria, Davies & Atkinson 1993). It has also been shown that when transferred to low RH conditions, *T. virginiana* plants developed under high RH (90%) had a higher leaf transpiration rate and stomatal conductance as well as larger stomatal apertures than plants developed under moderate RH (55%) (Nejad & Van Meeteren 2005). The development of malfunctioning stomata leads to significant water loss, low post-harvest stress tolerance, and limited survival of cuttings and *in vitro* grown plantlets upon transplanting (Torre *et al*. 2003). However, in roses, daily ABA applications could overcome the negative effect of high RH and produce functional stomata (Fanourakis *et al*. 2011).

Much of the work done on RH responses on stomata function and stress resistance has been performed on roses (*Rosa x hybrida*). Roses are one of the world's most economically important commercial ornamentals produced in greenhouses either as cut flowers or as pot plants. To save energy, ventilation of humid air is avoided and as a consequence the RH can increase to above 90% in some greenhouse systems and in certain periods of the year (Max *et al*. 2009). The critical threshold is found to be 85%, and an RH above this level affects the behaviour and anatomical features of stomata, stomatal function and post-harvest behaviour (Mortensen & Gislerød 1997; Pettersen, Moe & Gislerød 2007).

Further, to increase the productivity in greenhouse systems, extension of the natural photoperiod with the use of artificial lighting is common in periods when the natural irradiance is low. In some plant species, continuous lighting (24 h) induces severe injuries like leaf chlorosis and necrosis, and results in lower photosynthesis and accelerated leaf senescence (Velez-Ramirez *et al*. 2011). However, roses are tolerant to continuous lighting, and high growth rate and productivity are commonly observed (Mortensen & Gislerød 1999). The effect of high RH is clearly aggravated under continuous lighting and results in extremely high water loss and limited drought tolerance (Mortensen & Gislerød 1999; Mortensen, Pettersen & Gislerød 2007).

As daily application of ABA to roses results in functional stomata under high RH (Fanourakis *et al.* 2011) and the fact that ABA is a key regulator of the signal transduction pathway in stomatal closure in response to water deficit, it can be hypothesized that ABA is involved in RH responses. However, despite the progress in research on ABA metabolism, the mechanisms by which the ABA content is regulated by air humidity are less understood. The aim of this study was to study the effect of high air humidity on the ABA regulation and responses in rose leaves. To improve the understanding of plant responses to interactive effects of air humidity and photoperiod, the effects of high humidity under continuous lighting on ABA regulation were also investigated.

MATERIALS AND METHODS

Pre-cultivation and experimental growth conditions

Rosa x hybrida, cv. Rebecca plants were grown from a single node stem segment with one mature leaf. The cuttings were taken from the middle and lower positions 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 1.75, respectively, in all experiments (Superba: NPK 9-5-25 + Mg + S + Mikro and calcinit, Yara, Norway). During pre-cultivation, the plants were kept in a greenhouse compartment (glass roof and polycarbonate walls) at a temperature of 21 °C, and average daily RH of 70% [corresponding to a water vapour deficit (vpd) of 0.74 kPa], at the Center for Plant Research in Controlled Climate at the Norwegian University of Life Sciences, Ås, Norway (N 59°40.120′, E 10°46.232′). Supplementary light by high-pressure-sodium-lamps (HPS, Osram NAVT- 400W, Munich, Germany) was given daily for 20 h at a photon flux density of 100 ($\pm$10) μmol m^{-2} s^{-1} at 400–700 nm (measured with a Li-Cor, Model L1-185, quantum sensor, Lincoln, NE, USA). The pre-cultivation ended when the plants had 1–1.5 cm long shoots. The plants were then transferred to the different humidity treatments in growth chambers.

During the experimental period, the plants were exposed to 100 μmol m^{-2} s^{-1} for 20 h day^{-1} (4 h darkness) or continuous lighting (24 h) by Mercury lamps (Osram NAV T-400W). The temperature was 20 $\pm$ 0.5 °C; the RH in the

growth chambers was either 60 ± 3% (moderate humidity, vpd: 0.7 kPa) or 90 ± 2% (high humidity, vpd: 0.23 kPa); and the CO_2 concentration was 0.4 mg g^{-1}. A PRIVA (Priva, Ontario, Canada) greenhouse computer was connected for recording, controlling and storing the climate data in growth chambers and the greenhouse. Three different repeats of the experiments were carried out.

Leaf samples for analysis of ABA, its metabolites and β-glucosidase activity as well as stomata imprints, were taken from the first fully expanded leaves with five leaflets. The sampling was done after 6 weeks of treatment when the plants had flowered, in the middle of the light and dark periods in both humidity treatments with 20 h photoperiod. In continuous lighting, the samples were taken between 1700 and 1800 h. Samples were immediately frozen in liquid nitrogen and stored at –80 °C prior to extraction for ABA and β-glucosidase quantification.

Measurement of stomatal conductance

Stomatal conductance of the intact leaves was measured for 24 h during a diurnal cycle using a CIRAS-2 Portable Photosynthesis System with PLC6 (U) Automatic Universal Leaf Cuvette (PP Systems, 2001, Amesbury, MA, USA). During all measurements, the humidity and light in the leaf cuvette was the same as in the growth chamber, the CO_2 concentration was 400 μmol mol^{-1}, the airflow was 250 μmol s^{-1} and the temperature was 22 °C. Measurements were taken every 15 min for 24 h.

Stomata response to desiccation

To study the stomata response to dehydration, desiccation tests were done with detached upper leaves from 10 plants grown under high (90%) and moderate (60%) RH. The tests were performed in a test room with 50% RH, 15 μmol m^{-2} s^{-1} light intensity and 22 °C. The leaves were weighed 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120 and 180 min after detachment. After the desiccation test, the leaf area was determined with a leaf area meter (Li-Cor, LI-3100). The rate of water loss (transpiration rate) per leaf area was calculated using the following equation:

$$Transpiration\ rate = \frac{Change\ in\ leaf\ weight\ during\ the\ desiccation\ time}{leaf\ area}$$

Microscopy analysis of stomata

Epidermal impressions 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 previously (Tanaka *et al*. 2005). All samples were taken interveinally close to the mid-rib on the abaxial side of the leaf from 12 leaves from each air humidity treatment during both light and dark. The SUMP imprints were observed under a light microscope (Leitz, Labolux K, Type 0.2, Wetzlar, Germany) and stomata images were obtained with a Leica camera (Leica DC200, Heerbrugg, Switzerland). Stomatal morphology (length, width and area) were measured with the use of UTHSCSA ImageTool for windows version 3.00 (The University of Texas Health Science center, San Antonio, TX, USA).

ABA quantification

Chemicals and calibration curves

ABA-catabolites 4′-dihydrophaseic acid (DPA), ABA-β-D-glucosyl ester (ABA-GE), PA, 7′-hydroxy-ABA (7′OH-ABA), neophaseic acid (*neo*PA) and *trans*-ABA were synthesized and prepared at the Plant Biotechnology Institute of the National Research Council of Canada (PBI-NRC Saskatoon, SK, Canada), while *cis*-ABA was purchased from Sigma-Aldrich (Sigma Chemicals, St Louis, MO, USA). Deuterated forms of the hormones, which were used as internal standards, that is, *d*3-DPA, *d*5-ABA-GE, *d*3-PA, *d*4-7′-OH-ABA, *d*3-*neo*PA, *d*4-ABA, *d*4-*trans*-ABA, were synthesized and prepared at PBI-NRC (Abrams, Nelson & Ambrose 2003; Zaharia *et al*. 2005). The deuterated forms of selected compounds used as recovery standards, d6-ABA and d2-ABA-GE, were also prepared and synthesized at PBI-NRC. Calibration curves were created for all compounds of interest. Quality control samples (QCs) were run along with the tissue samples.

Extraction and purification

The samples were freeze dried and homogenized before analysis. A 100 μL aliquot containing the deuterated internal standards, each at a concentration of 0.2 pg μL^{-1}, was added to approximately 50 mg of homogenized plant tissue; 3 mL of isopropanol:water:glacial acetic acid (80:19:1, v/v) was then added, and the samples were agitated in the dark for 24 h at 4 °C. Samples were then centrifuged and the supernatant was isolated and dried on a Büchi Syncore Polyvap (Büchi, Flawil, Switzerland). Samples were reconstituted in 100 μL acidified methanol, adjusted to 1 mL with acidified water, and then partitioned against 2 mL hexane. After 30 min, the aqueous layer was isolated and dried as above. Dry samples were reconstituted in 100 μL acidified methanol and adjusted to 1 mL with acidified water. The reconstituted samples were loaded onto equilibrated Oasis HLB cartridges (Waters, Mississauga, ON, Canada), washed with acidified water and eluted with acetonitrile:water:glacial acetic acid (30:69:1). The eluate was then dried on a LABCONCO centrivap concentrator (Labconco Corporation, Kansas City, MT, USA). An internal standard blank was prepared with 100 μL of the deuterated internal standard mixture. QC standards were prepared by adding 100 and 30 μL (separately) of a mixture containing the analytes of interest, each at a concentration of 0.2 pg μL^{-1}, to 100 μL of the internal standard mix. Finally, samples, blanks and

QCs were reconstituted in a solution of 40% methanol (v/v), containing 0.5% acetic acid and 0.1 pg μL^{-1} of each of the recovery standards.

Hormone quantification by UPLC-ESI-MS/MS

The samples were subjected to UPLC-ES-MS/MS analysis and quantification (Ross *et al*. 2004). Samples were injected onto an ACQUITY UPLC® HSS C18 SB column (2.1 × 100 mm, 1.8 μm, Waters, Milford, MA, USA) with an in-line filter and separated by a gradient elution of water containing 0.02% formic acid against an increasing percentage of a mixture of acetonitrile and methanol (volume ratio: 50:50). Calibration curves were generated from the MRM signals obtained from standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding internal standard, as described by Ross *et al*. (2004). The QC samples, internal standard blanks and solvent blanks were also prepared and analysed along each batch of tissue samples. MassLynx™ and QuanLynx™ (Micromass, Manchester, UK) were used for data acquisition and data analysis.

β-glucosidase quantification

β-glucosidase was measured as described by Dietz *et al*. (2000), with some modifications. The leaf samples were taken from the freezer (–80 °C) and immediately homogenized in liquid nitrogen using a mortar and pestle. Samples (1.00 g) were extracted for 1.5 h at 4 °C in 10 mL 100 mM citrate buffer, containing 5% (w/v) poly (vinylpolypyrrolidone) (PVPP), 1 mM ethylenediaminetetraacetic acid (EDTA), 14 mM mercaptoethanol and 10% (w/v) glycerol. The samples were then centrifuged at 201 *g* for 2 min (Eppendorf 5810 centrifuge, Hamburg, Germany). The supernatant (100 µL) was mixed with 1 mL 100 mM citrate buffer containing 4 mM pNPG (p-nitrophenol-*β*-D-glucopyranoside) and incubated at 37 °C for 60 min (Termaks B 8054 Incubator, Bergen, Norway). The reaction was then terminated with 2 mL 1 M Na_2CO_3 and the amount of liberated p-nitrophenol was measured spectrophotometrically at 405 nm (Helios Alpha Spectrophotometer, Thermo Scientific, Surrey, UK). The concentration was calculated using the Beer-Lambert law, Absorbance = ε × length × concentration, and the molar extinction coefficient for p-nitrophenol ε = 18300 (Dietz *et al*. 2000). One unit of enzyme is then defined as the amount of enzyme needed to yield 1 nmol of p-nitrophenol per hour at 37 °C.

Statistical analyses

Significant differences between means were tested for normally distributed general linear models (GLM) and Tukey's test. Differences with $P < 0.05$ were considered significantly different. All statistical tests were performed in Minitab 16.1.1 (Minitab 16.1.1, windows version, State College, PA, USA).

RESULTS

Increased diurnal stomatal conductance and decreased response to desiccation in leaves developed at high RH under a 20 h photoperiod

We analysed the diurnal stomatal conductance of plants growing at high (90%) and moderate (60%) RH with a gas exchange analyser (CIRAS-2). A considerably higher conductance (g_{sw}) was measured throughout both the day and night in plants growing in high RH, compared with plants in moderate RH ($P = 0.041$, Fig. 1). However, the g_{sw} of plants grown under both humidity treatments showed a similar pattern with the conductance decreasing throughout the day and the lowest values during the dark, before increasing again when the light was turned on. The g_{sw} of plants growing in high RH was still high during darkness (30 mmol $m^{-2} s^{-1}$), while the plants grown under moderate RH showed very low g_{sw} (3.5 mmol $m^{-2} s^{-1}$), indicating stomatal closure. The difference in stomata conductance between light and dark was similar in both treatments (≈30 mmol $m^{-2} s^{-1}$). To estimate the relative change in the stomatal aperture between day and night, the day:night ratio was calculated. The relative change in conductance was much larger in moderate RH (85% change) compared with high RH (28.5% change, $P = 0.032$). This indicates that stomata developed under moderate RH close better during darkness

To further test the ability of the plants to close the stomata and retain water, a desiccation test was performed. After a 3 h desiccation, treatment plants grown at high RH had lost significantly more of their original weight (≈50%), while plants grown under moderate humidity had only lost 10–15% due to stomatal closure ($P < 0.001$, Fig. 2a). The transpiration rate was highest in the beginning, before the stomata had closed, and decreased throughout the

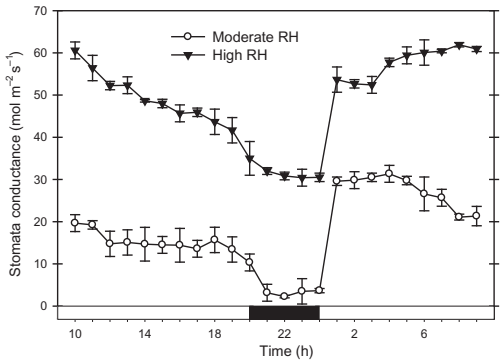

Figure 1. Diurnal stomata conductance (mol $m^{-2} s^{-1}$) measured on the adaxial side of the first fully expanded leaves with five leaflets of rose plants grown at moderate (60%) or high (90%) relative air humidity (RH). The measurements were done on fully grown plants with open flowers during a light/dark cycle of 24 h. Mean ± SE. $n = 9$.

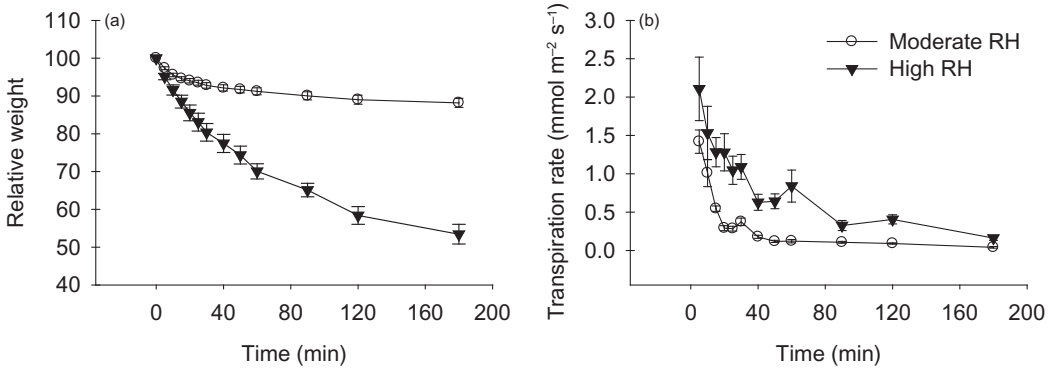

Figure 2. Relative weight (a) and transpiration rate (b) of leaves of rose plants grown under moderate (60%, circles) or high (90%, triangles) relative air humidity (RH) during 3 h of desiccation. Mean ± SE. $n = 10$.

desiccation test in plants developed in both moderate and high relative humidity (Fig. 2b). During the first 10 min, there was no statistically significant difference in the transpiration rate between the plants developed under the different RH conditions. However, after the first 10 min and throughout the rest of the test, a significantly higher transpiration rate was measured in plants developed under high RH ($P \leq 0.004$). This shows that the stomata of plants developed under moderate RH have better stomatal functioning and are able to retain more water.

To better understand the effect of high RH on the stomatal development and the ability to close the stomata, imprints were made of the leaves during both light and dark, and stomatal characteristics were measured. In general, the stomatal pore aperture and length were larger in plants grown at high RH than in plants grown at moderate RH (Table 1, Fig. 3). The length of the stomatal pore of plants grown under high RH, measured during light and dark, was 1.3 and 1.6 times larger than those grown under moderate RH ($P < 0.001$). Likewise, the stomatal pore aperture was also larger in plants grown under high RH ($P < 0.001$). The pore aperture during light and dark in plants grown under high RH was 1.9 and 3 times larger than in plants grown under moderate RH, respectively. As a consequence of the larger stomatal pore length and aperture, the pore area was also larger in plants grown under high RH ($P < 0.001$). During light, the pore area was 2.2 times larger and during dark 2.6 times larger in high RH than in moderate RH. Plants grown under moderate RH had larger pore aperture during light than during darkness, showing that the stomata developed in moderate RH close during the dark ($P < 0.001$, Table 1). In contrast, the pore aperture of plants developed under high RH did not change between light and dark, but remained open.

Reduced ABA content and no difference between light and dark under high RH in 20 h photoperiod

As ABA is an important signal for stomatal closure, the amount of ABA and its metabolites (DPA, ABA-GE, PA, 7′OH-ABA, *neo*PA, *trans*-ABA and *cis*-ABA) in the leaves was quantified. However, the levels of DPA, 7′OH-ABA, *neo*PA and *trans*-ABA were in all treatments mostly too low to be quantified (data not shown), and have therefore only been included in the value for total ABA metabolite content (i.e. in those cases where they could be quantified). The combined amount of ABA and its metabolites was significantly higher (in average 69%) in plants from moderate compared with high RH ($P < 0.001$). However, there was no significant difference in the combined amount of ABA and its metabolites between light and dark within

Table 1. Stomatal pore characteristics of pot rose cv. Rebecca grown at moderate (60%) or high (90%) RH

	Moderate RH (60%)		High RH (90%)	
	Light	Dark	Light	Dark
Pore length (μm)	31.2 ± 0.1^{b}	25.7 ± 0.2^{c}	41.7 ± 0.2^{a}	41.0 ± 0.5^{a}
Pore aperture (μm)	11.5 ± 0.1^{b}	7.0 ± 0.2^{c}	21.8 ± 0.2^{a}	20.7 ± 0.7^{a}
Pore area (μm^2)	174.3 ± 0.9^{b}	144.4 ± 0.9^{c}	378.4 ± 4.6^{a}	376.0 ± 3.5^{a}

$n = 28$–42.
Different superscript letters indicate significant differences.
Mean ± SE.
RH, relative air humidity.

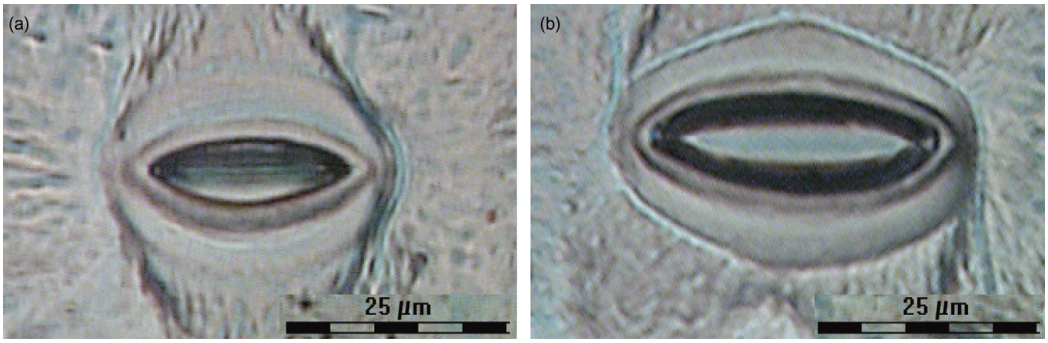

Figure 3. Light microscope images of stomata in 60% relative air humidity (RH) (a) and 90% RH (b). The images are of imprints of the abaxial side of the leaves during the light period.

either treatment (Fig. 4d). If only the amount of ABA is considered, the levels were still significantly higher in moderate RH both during light and dark ($P < 0.05$, Fig. 4a). During dark, the amount of ABA was significantly increased in moderate RH (approximately doubled, $P = 0.006$). However, there was no significant change between light and dark in high RH, indicating that the diurnal ABA level was constant and that unlike in moderate RH there was no signal to induce closure in the dark.

ABA-GE has been hypothesized to be a storage form of ABA, which can be converted to ABA (Dietz *et al.* 2000). The amount of ABA-GE was similar in both humidity

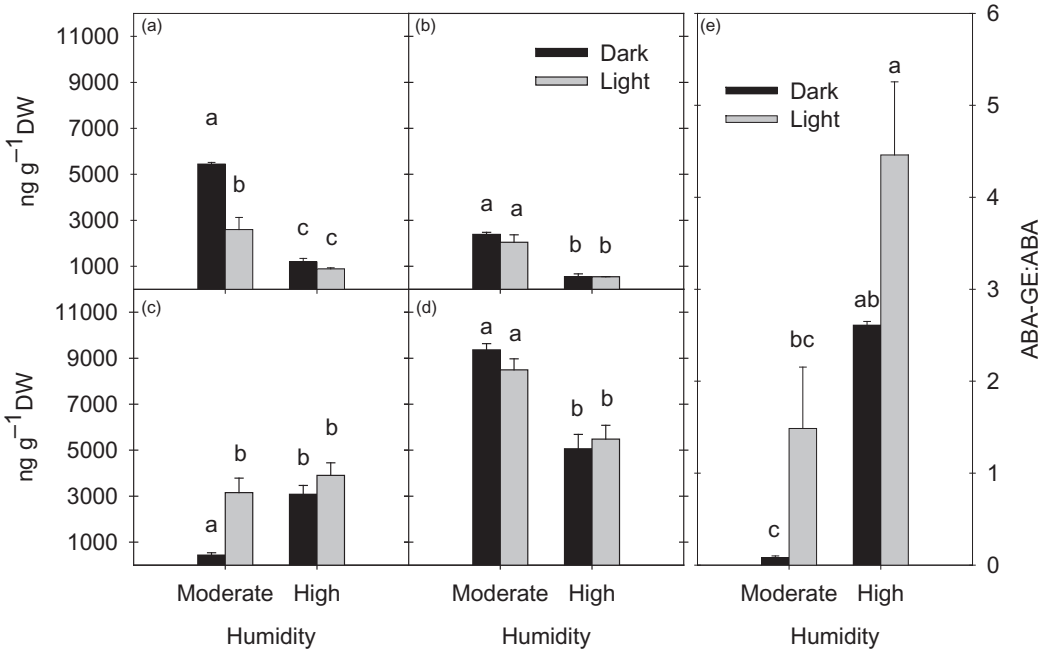

Figure 4. The effect of moderate (60%) and high (90%) relative air humidity on amounts of abscisic acid (ABA) (a) and its metabolites phaseic acid (PA) (b), and ABA-β-D-glucosyl ester (ABA-GE) (c), the total combined amount of ABA and its metabolites (d), and the ABA-GE:ABA ratio in leaves of rose plants (e). Measurements were done in the middle of the photoperiod and in the middle of the dark period. Different letters within each figure indicate significantly different values ($P < 0.05$). Mean ± SE. $n = 3$. Each sample consisted of 5–6 leaves from a single plant.

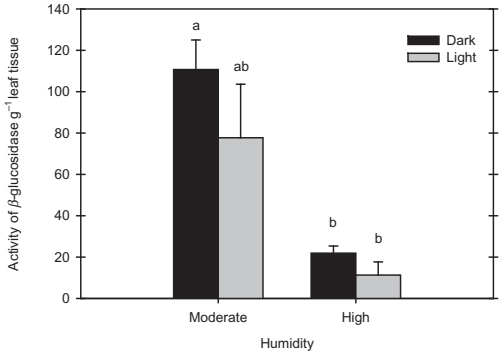

Figure 5. The activity of β-glucosidase in the middle of the light and dark periods in leaves of rose plants growing in high (60%) and moderate (90%) relative air humidity. Different letters indicate significantly different values ($P < 0.05$). Mean ± SE. $n = 3$. Each sample consisted of 5–6 leaves from a single plant.

treatments during light (Fig. 4c). However, as the level of ABA was increased [2800 ng g^{-1} dry weight (DW)] during dark in moderate RH, the level of ABA-GE was decreased similarly (2700 ng g^{-1} DW, $P = 0.013$), but remained unchanged in high RH. ABA-GE might therefore be the source of the increased ABA levels.

In moderate RH, the amount of ABA was similar or higher than that of ABA-GE, while in high RH the amount of ABA was lower than that of ABA-GE (Fig. 4e). This is consistent with the measured activity of β-glucosidase, converting ABA-GE into ABA. The activity of β-glucosidase was about fivefold higher in plants grown in moderate RH, than in plants grown in high RH ($P = 0.001$, Fig. 5). This indicates that more ABA-GE was converted to ABA in leaves developed under moderate RH. However, there was no significant difference between light and dark within either of the treatments, only a slight tendency of higher activity during the dark in both treatments.

When ABA is inactivated by oxidation, it is converted into 8′-hydroxy ABA and PA by the enzyme 8′-hydroxylase (Cutler & Krochko 1999). The amount of PA was significantly higher in plants from moderate RH ($P < 0.001$), where the amount of ABA was higher as well, but there was no difference in PA between light and dark in either treatment (Fig. 4b). The ratio between ABA and PA was not significantly different in either treatment or in light or darkness. Thus, inactivation of ABA to PA was similar in light and dark, and the relative amount of ABA being inactivated to PA was the same in both moderate and high RH.

Continuous light reduces the desiccation tolerance and affects the ABA regulation

Extension of the natural daylength with artificial light is important to increase productivity in greenhouses in periods with low natural irradiance. However, a combination of very long days and high RH significantly decreases post-harvest life of roses (Mortensen & Gislerød 1999). To further understand the effect of daylength and the importance of a dark period in stomatal development, we studied the effects of RH under continuous lighting.

During the desiccation test, plants developed under continuous light and high RH exhibited significantly higher water loss at the end compared with plants developed under continuous light and moderate RH ($P = 0.004$, Fig. 6a). Thus, the leaves developed under continuous lighting showed a similar behaviour as the leaves developed under 20 h photoperiod. However, during the first hour of desiccation, the plants developed under continuous lighting showed significantly higher ($P < 0.040$) water loss than their counterparts developed under 20 h photoperiod. On the other hand, during the last 2 h of desiccation, this difference was reduced and not statistically significant.

To get a better understanding of the increased water loss in continuous light, we examined the ABA levels. In general, most of the levels of ABA and its metabolites in continuous light were more similar to the levels from high RH in 20 h photoperiod and lower than the levels from moderate RH in 20 h photoperiod. There were also no interaction effects between photoperiod and RH, except in PA, indicating that continuous lighting has the same effect irrespective of the RH.

The levels of the combined amount of ABA and its metabolites in continuous light was significantly higher in moderate RH, compared to high RH ($P = 0.036$, Fig. 6f). However, when only the ABA levels were considered, there was no significant difference between the two treatments (Fig. 6c). Compared with the 20 h light treatment, these ABA values were not significantly different from the high RH values. On the other hand, the concentrations in high RH and continuous light were significantly lower than the moderate RH values from 20 h light ($P = 0.0336$). Similarly, the ABA concentrations from moderate RH in continuous light appeared slightly, although statistically insignificantly ($P = 0.085$) lower than the values from moderate RH with 20 h photoperiod.

The ABA-GE concentrations in continuous light were similar in both humidity regimes (Fig. 6e). There was no significant difference between any of these values and those in the 20 h light experiment. The amount of ABA-GE converted back to ABA by β-glucosidase, was also similar in both humidity treatments in continuous light (Fig. 6b).

The PA concentrations in continuous light were higher in moderate RH than in high RH ($P = 0.003$, Fig. 6d). However, there was no significant difference between these values and those in high RH in 20 h light. However, the PA levels in both treatments in continuous light were significantly lower than those from moderate RH and 20 h light ($P < 0.0008$).

Overall, the plants developed under continuous light behaved similarly as plants developed under a 20 h photoperiod. However, they had lower desiccation tolerance, with increased water loss the first hour of desiccation than their counterparts growing in 20 h photoperiod. Also, their ABA

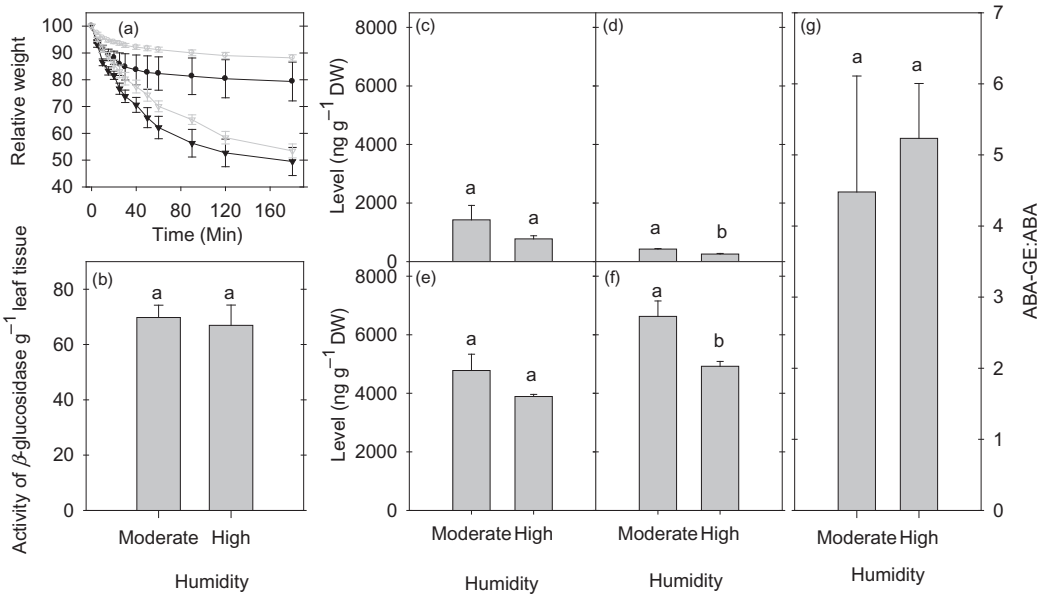

Figure 6. Relative weight during a 3 h desiccation (a) and levels of abscisic acid (ABA) and its metabolites in leaves of plants developed under moderate (60%, circles) and high (90%, triangles) relative air humidity (RH) in continuous light (dark lines). For comparison, in (a) the results from 20 h light (above) moderate (circles) and high (triangles) RH is added and shown in grey. (b–g) The amount of β-glucosidase (b), ABA (c), phaseic acid (PA) (d), ABA-β-D-glucosyl ester (ABA-GE) (e), the total combined amount of ABA and its metabolites (f), and ABA-GE:ABA ratio (g). Different letters within each figure indicate significantly different values. Mean ± SE. $n = 10$ (a), 5 (b), 3 (c–g). Each sample consisted of 5–6 leaves from a single plant (b–g) and were taken between 1700 and 1800 h.

levels were more similar to those of plants developed under high RH and 20 h photoperiod.

DISCUSSION

High RH induces malfunctional stomata in different growing systems and several different plant species (Santamaria *et al*. 1993; Mortensen 2000; Torre & Fjeld 2001; Nejad & Van Meeteren 2005). Most studies in this respect has been performed on roses (*Rosa x hybrida*), which are among the economically most important greenhouse-grown ornamental plants. Malfunctional stomata result in rapid post-harvest water loss and highly reduced stress tolerance, thus strongly reduced plant quality. ABA application and ABA quantification in plants from different RH regimes have suggested an involvement of ABA (Nejad & Van Meeteren 2007; Okamoto *et al*. 2009; Fanourakis *et al*. 2011), but the effect of RH on ABA regulation has not been studied. In this study, we demonstrate for the first time that plants growing under constant high RH regulate the ABA levels differently than plants grown at constant moderate RH.

High RH affects stomata morphology and physiology in roses

The diurnal response and desiccation test confirmed that roses developed under high RH have higher transpiration rate than roses developed under moderate RH (Fig. 2). Previous studies have shown similar results in several other species, such as *T. virginiana*, *Begonia x cheimantha*, *Euphorbia pulcherrima*, *Kalanchoe blossfeldiana* and *Chrysanthemum morifolium*, indicating that uncontrolled water loss induced by high RH during development is a universal response in plants (Mortensen 2000; Torre & Fjeld 2001; Nejad & Van Meeteren 2005). The uncontrolled loss of water has been suggested to either be due to alteration in stomata morphology or physiology, which causes the stomata to remain open when subjected to stimuli normally inducing closing.

The plants developed under high RH had larger stomatal length and aperture, which in turn resulted in a larger stomatal area (Table 1). This is similar to previous studies in a number of species (Fordham *et al*. 2001; Torre *et al*. 2003; Karbulkova *et al*. 2008). Furthermore, we found that the stomata of plants developed under high RH did not respond to darkness or drought, but remained open. In contrast, the stomata of plants developed under moderate RH responded by closing their stomata when subjected to darkness or drought. Several studies have indicated that stomata of *in vitro* grown plants, which also experience high RH, fail to close fully in response to ABA (Santamaria *et al*. 1993), low leaf water potential (Fordham *et al*. 2001) and darkness (Ziv, Schwartz & Fleminger 1987). In addition,

plants developed under high RH have higher stomatal density (Torre *et al*. 2003; Nejad & Van Meeteren 2005). The increased area of the stomatal pore in combination with higher number of stomata might explain at least parts of the increased transpiration found in plants developed under high RH. It has previously been discussed if the large stomata found in plants developed under high RH are a result of low endogenous ABA levels. Supporting this is a study on *Populus x canescens* where it was found that ABA insensitive plants had larger stomata (Arend *et al*. 2009).

High RH during growth in 20 h photoperiod affects the ABA content in light and darkness

ABA is an important stress hormone that induces stomatal closure. The regulation of ABA is well known and its two main pathways of inactivation result in formation of PA and ABA-GE (Nambara & Marion-Poll 2005). It has been suggested that the lack of stomatal response in plants developed under high RH is partly due to low ABA concentrations. In this study, we measured the ABA concentrations in leaves of plants developed under high and moderate RH under a 20 h photoperiod, in light and darkness, and compared the conversion rates of ABA with PA and ABA-GE with ABA.

The amount of ABA and the catabolite PA was significantly lower in plants developed under high RH, compared with moderate RH (Fig. 4). The lower PA levels in high RH are apparently a result of the lower ABA levels and a constant inactivation rate of ABA to PA. Previous studies on *T. virginiana* and *A. thaliana* also found lower ABA concentrations in plants developed under high RH or when moved from low to high RH (Zeevaart 1974; Nejad & Van Meeteren 2007, 2008; Okamoto *et al*. 2009). It is believed that the decrease in ABA levels when plants are moved from low to high RH is a result of increased inactivation and not altered biosynthesis (Okamoto *et al*. 2009).

In our study, the amount of ABA increased in the dark in moderate RH, while it remained unchanged in high RH (Fig. 4). The increased ABA concentration during dark in moderate RH is believed to act as a signal for stomatal closure during darkness (Tallman 2004; Novakova *et al*. 2005). Plants from high RH did not increase their ABA levels, and the stomata thus lack a signal for closure during darkness. A previous study on the diurnal variation of ABA in *N. tabaccum* demonstrated a peak in ABA concentration after 3 h of darkness, before decreasing, and remaining low throughout the rest of the dark period (Novakova *et al*. 2005). Similarly, a study of *S. oleracea* showed decreased levels of ABA at the end of an 8 h dark period compared with at the onset of darkness (Zeevaart 1974).

The amount of ABA-GE during light was similar in the two humidity treatments under 20 h photoperiod. However, it was reduced during dark in moderate RH (Fig. 4). Several studies, including this one, have hypothesized that ABA-GE is a storage form of ABA and can be converted back to ABA when needed (Dietz *et al*. 2000; Sauter *et al*. 2002). The increased levels of ABA and reduced levels of ABA-GE in moderate humidity during dark support this, indicating that the increased ABA levels have been converted from ABA-GE.

β-glucosidase has a central role in ABA regulation in moderate RH

The constant amount of ABA metabolized to PA during light and dark in both RH treatments indicates that the changes in ABA levels are due to either conversion to ABA-GE or increased biosynthesis. A study on *Nicotiana plumbaginifolia* showed that light regulates the ABA concentration through affecting degradation rate and not biosynthesis (Kraepiel *et al*. 1994). Although the regulation of ABA through biosynthesis has not been quantified, the combined amount of ABA and its metabolites did not differ between light and dark (Fig. 4). This indicates that there is no difference in the biosynthesis or degradation by oxidation between light and dark (Fig. 5). The β-glucosidase assay also showed that there are higher concentrations of enzyme converting ABA-GE into ABA in moderate humidity, while there was very little of this enzyme in high humidity. In a previous study, the amount of β-glucosidase was highest during light, and after an initial peak during darkness, remained low the rest of the dark period (Novakova *et al*. 2005). Thus, it appears very probable that the increased levels of ABA during darkness arise from ABA-GE. To increase the amount of enzymes in the ABA biosynthesis during drought stress, turgor has to decrease significantly (Liu *et al*. 2005). In this study, there was no loss of turgor in the leaves during darkness in either of the treatments, indicating that β-glucosidase does not require a large reduction of turgor before converting ABA-GE to ABA. The release of ABA from ABA-GE is therefore a quicker response to stress than an increase in biosynthesis. However, although the enzyme has been found to be most active at hydrolyzing ABA-GE, it is presumed that it has a broader substrate specificity, hydrolyzing also other hormones (Dietz *et al*. 2000; Minic 2008).

The lack of change in the amount of ABA and ABA-GE between light and dark in plants developed under high RH might be due to the extremely favourable conditions for rapid growth in high RH and long photoperiod (20 h). These conditions might make it unnecessary for the plants to close the stomata during darkness and reduce the amount of β-glucosidase, reducing the plant's ability to convert ABA-GE to ABA. It is also possible that plants developed under high RH are insensitive to or not receiving the signals inducing the production of β-glucosidase.

In another study of *Arabidopsis*, we have shown that the ABA-GE concentration is very low (Arve *et al*. unpublished results) in both moderate and high RH. The regulation of ABA through biosynthesis and degradation to PA therefore appears more important in *Arabidopsis* (Okamoto *et al*. 2009). In light of this, it can be hypothesized that the pathways of ABA regulation might be different in different species.

A dark period and reduced RH (<85%) are important for the development of functional stomata

As shown here, stomata developed under continuous light are poorer at retaining water than those developed under a 20 h photoperiod, regardless of RH treatment (Fig. 6). This is consistent with previous studies on roses (Mortensen & Gislerød 1999). The ABA content in plants developed under continuous light was also lower than in plants developed in moderate RH under 20 h photoperiod and more similar to that of the plants developed in high RH under 20 h photoperiod. This shows that the dark period is important for the development of fully functional stomata in rose plants. The lack of stomatal closure during dark in high RH is an indication that high RH overrides the signals from the dark period. In a study on six temperate deciduous tree species, the leaf water potential was found to be the most important signal for stomatal responses, and responses to air humidity dominated over responses to photosynthetic signals (Aasamaa & Sober 2011).

One possibility is that the higher ABA concentrations during dark in moderate RH are important in stomatal development. Plants developed under high RH have never experienced high ABA content, which might be necessary to produce functional stomata. Applications of ABA during growth or transfer from high to moderate RH before the leaves are fully expanded, have been shown to result in production of functional stomata (Fanourakis *et al*. 2011). It is also possible that it is not the high ABA concentrations *per se* that are important, but the fact that the stomata are closing. If so, the stomata must open and close during development to be able to close properly when they are fully developed. ABA application in high RH has been shown to produce functional stomata (Fanourakis *et al*. 2011), but it is not known whether it is the ABA in itself that changes the stomatal development or whether it is an indirect effect of the closing stimulus.

CONCLUSION

The present study confirms that rose plants developed under high RH have higher transpiration both during growth and during desiccation stress, and that there is no stomatal response to darkness. We have further shown that in moderate RH, the amount of ABA was increased during darkness and that there was simultaneously a similar decrease in ABA-GE, indicating that the increased levels of ABA is due to conversion of ABA-GE. In contrast, plants developed under high RH show no increase in ABA levels during dark, and have very little β-glucosidase activity converting ABA-GE to ABA. As ABA is a signal for stomatal closure, the low ABA levels in high RH might explain some of the lack of stomatal closure during darkness and development of malfunctioning stomata. However, further study is still needed to fully understand the lack of stomatal responses in plants developed under high RH.

Further, in plants developed under continuous lighting, the β-glucosidase activity was low irrespective of the RH, indicating that a dark period is essential to activate this enzyme. On the other hand, as stomata developed under high RH lack dark-induced closure mechanisms, it can be hypothesized that high RH overrides the signals and importance of the dark period. Thus, these data provide new insight into the regulation of ABA under high RH and continuous lighting. The results clearly show that β-glucosidase has a central role and is a key enzyme in regulating the ABA pool in rose plants and that β-glucosidase does not require a large reduction in turgor before releasing ABA.

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PAPER IV

Meseret Tesema Terfa, Madhu S. Poudel, Hans Ragnar Gislerød, Jorunn Elisabeth Olsen, Sissel Torre (2013). **Blue light improves stomata function and dark-induced stomata closure of rose leaves (*Rosa* x *hybrida* cv. Toril) grown at high air humidity.** (Manuscript)

Blue light improves stomata function and dark-induced stomata closure of rose leaves (*Rosa* x *hybrida* cv. Toril) grown at high air humidity

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Abstract

Stomata opening and closing is regulated by several environmental stimuli. Blue (B) light is among the numerous environmental stimuli affecting stomatal aperture and hence stomatal conductance. To conserve water plants close their stomata in response to increased abscisic acid (ABA) and ABA signaling is thought to predominate over B light signaling in guard cells. Plants developed under constant high (>85%) relative air humidity (RH) have larger stomata that are unable to close completely in response to closing stimuli. To test the effect of B light on stomata function under high RH conditions, we investigated the ability of rose leaves developed under continuous high RH or moderate RH to close their stomata in response to increased B light proportion. Moreover, the content and regulation of ABA in light and darkness in relation to B light was studied. Our results show that increased B light proportion improved stomata function and dark-induced stomata closure under high RH conditions that correlated with increased ABA content in general and a dynamic ABA peak during dark. This increase in ABA was associated with the presence of high β-glucosidase activity. This indicates that B light is important as a signal to activate the β-glucosidase enzyme. Altogether, the improved stomata function and reduced transpiration in combination with increased ABA level indicate that B light plays an important role in governing stomata functionality and ABA homeostasis under high RH.

Key-words: Abscisic acid; Blue light; Darkness; Relative air humidity (RH), Stomata

Introduction

The opening and closing of stomata is regulating not only the photosynthetic carbon assimilation but also the leaf water status by controlling water release through transpiration. Among the numerous environmental stimuli affecting stomatal aperture and hence stomatal conductance for water vapour (g_{sw}), light plays a critical role in governing the stomata function and adaptability. Stomatal apertures vary over the diurnal cycle, and stomata tend to be open during the day in response to light and closed at night in response to dark (Schroeder *et al.*, 2001; Tallman, 2004). In response to blue (B) light stimuli, stomata open through activation of an H^+ pump in guard cells. This creates an inside-negative electrical potential across the plasma membrane and drives K^+ uptake through voltage-gated inward-rectifying K^+ channels (Assmann *et al.,* 1985, Shimazaki *et al.,* 1986; Schroeder *et al.,* 1987). The B/UVA light-absorbing cryptochromes, zeaxanthin and phototropins are suggested receptors for B light stomata responses. Phototropins (phot1 and phot2) have been identified as B light receptors mediating the H^+-ATPase activation in the plasma membrane (Zeiger and Zhu, 1998; Kinoshita *et al.,* 2001, Briggs and Christie 2002, Kinoshita *et al.,* 2003; Mao *et al.*, 2005; Shimazaki *et al.*, 2007).

ABA signaling is thought to predominate over B light signaling in guard cells, since it is important for plants to prevent water loss under drought stress (Shimazaki *et al.,* 2007; Kim *et al.,* 2010). The endogenous level of ABA in plant tissues is dynamically regulated by the balance between the biosynthesis and inactivation of the hormone (Zeevaart, 1980; Cutler and Krochko, 1999). The biosynthesis of ABA involves series of complex steps and enzymes from early steps of carotenoid precursor synthesis in plastids to later stages in cytosol where xanthoxin is converted to ABA (Cutler and Krochko, 1999; Seo and Koshiba, 2002). The inactivation of free ABA involves either hydroxylation of ABA to the ABA catabolites phaseic acid (PA) and dihydrophaseic acid (DPA) or conjugation of ABA with glucose, creating ABA-glucose ester (ABA-GE) (Lim *et al.*, 2005; Priest *et al.*, 2006). ABA-GE is believed to be a storage form of ABA, and can be stored in the vacuoles and hydrolyzed to free ABA when required (Dietz *et al*., 2000, Arve *et al.,* 2012). In many plant species it has been shown that ABA-GE is hydrolyzed in response to water stress and darkness by β-glucosidase, leading to an increase in the pool of active ABA (Dietz *et al*., 2000; Sauter *et al.*, 2002; Lee *et al*., 2006; Arve *et al.,* 2012).

Despite ABA playing an important role in many environmental stress responses, the biosynthesis and catabolism of ABA itself is also regulated by many environmental factors like drought, salt, air humidity, light and suboptimal temperatures (Luan, 2002; Zhu, 2002; Nejad and Van Meeteren, 2007; Okamoto *et al*., 2009; Reynolds-Henne *et al*., 2010). There are numerous reports that light may influence the levels of different hormones (Novakova *et al.,* 2005). Light regulates ABA biosynthesis and degradation directly or indirectly (Xiong and Zhu, 2003; Tallman, 2004; Novakova *et al.,* 2005; Arve *et al.,* 2012). Specific light qualities like B light are also reported to regulate endogenous ABA levels during different developmental

processes (Fellner and Sawhney, 2002). These authors showed that B light improved osmotic stress tolerance of wild-type tomato seeds by inhibiting germination, and this was accompanied by increased ABA level. Furthermore, the diurnal pattern of stomata movements is affected by the diurnal alterations in metabolism of endogenous ABA, which might be associated with the effect of light on ABA precursors (Tallman, 2004). The ABA biosynthesis in guard cells restricted by the removal of the ABA precursor, violaxanthin, through light-driven xanthophyll cycling, which converts violaxanthin to zeaxanthin (Eskling *et al.*, 1997). Zeaxanthin is proposed to be the B-light-specific photoreceptor of guard cells (Zeiger and Zhu, 1998; Frechilla *et al.*, 1999; Talbott *et al.*, 2003) and conversion of violaxanthin to zeaxanthin is part of a mechanism regulating endogenous guard cell ABA turnover. During the dark period conditions favoring ABA biosynthesis in guard cells prevail, maintaining stomata in closed position. Zeaxanthin accumulated in guard cells during the day will then start to be converted to violaxanthin, and this favors ABA biosynthesis, indicating a cross talk between ABA and light (Tallman, 2004). In addition, low fluences of B light inhibits the biosynthesis of the active isomer of ABA (cis-(+)-S-ABA) by causing photo-isomerization of the ABA carotenoid precursors 9-cis-neoxanthin, 9-cisviolaxanthin, and/or cis-xanthoxin to their trans isomers (Schwartz *et al.*, 2003). Metabolism of these trans isomers yields physiologically inactive trans-ABA instead of the active cis-(+)-S-ABA (Schwartz *et al.*, 2003). However, these accumulated inactive isomers will be converted back to their respective active forms at absence of B light or when the plants are in urgency for free ABA.

Moreover, environmental factors such as drought and relative air humidity (RH) are also known to affect the endogenous ABA levels of plants. For instance, in *Spinacia oleracea* (spinach), *Tradescantia virginiana* (Virginia spiderwort) *Arabidopsis thaliana* and *Rosa hybrida* (roses), ABA levels were lower in leaves developed under high compared with moderate RH (Zeevaart, 1974; Nejad and Van Meeteren, 2007; Okamoto *et al*., 2009; Arve *et al.*, 2012). Besides, continuous growth under high RH (> 85 %) causes the development of malfunctioning stomata leading to poorer water control in dry air and lack of dark-induced closure. The poorer stomata water control leads to low postharvest stress tolerance and lower survival rate (Torre *et al*., 2003). It has been shown that daily ABA applications could overcome the negative effect of high RH and produce functional stomata in roses (Fanourakis *et al*., 2011). Arve *et al.* (2012) also recently reported that the dramatic increase in ABA level during dark in plants grown in moderate RH, was absent in rose leaves developed at high RH, indicating a difference in the regulation of the diurnal ABA-pool under different RH regimes. Besides, this increase in ABA during dark for moderate RH-grown roses was accompanied by a decrease in ABA-GE content and an increase in the activity of β-glucosidase, indicating that conjugated ABA plays an important role in changing the ABA pool throughout the day and night in moderate RH-grown roses (Arve *et al.*, 2012). However, the activity of the β-glucosidase enzyme was low during both day and night at high RH (Arve *et al.*, 2012).

Darkness is thought to be one of the strong signals in stomata closure but the degree to which stomata closes differs among species (Caird *et al.*, 2007; Dawson *et al.*, 2007; Kavanagh *et al.*, 2007). The magnitude of water loss occurring during the night depends on both the stomata conductance (g_{sw}) during the night and the vapor pressure difference (VPD) between leaves and the air, as well as canopy structure and atmospheric mixing (Caird *et al.*, 2007). Furthermore, daytime growth conditions such as RH, photoperiod, light quality and irradiance can affect the rate and degree to which stomata close in the dark time g_{sw} (Blom-Zandstra *et al.*, 1995; Caird *et al.*, 2007; Fanourakis *et al.*, 2012; Arve *et al.*, 2012). Arve *et al.* (2012) discussed that plants grown at high RH lack stomata closure and continue to transpire during dark, indicating that high RH overrides darkness as a signal for closure as compared to moderate RH. Fanourakis *et al.* (2012) have also showed that the rates of transpiration during the dark period was five-fold higher in cut flowers of roses grown at elevated RH, compared with those grown at moderate RH. These roses were, therefore, largely unable to recover during darkness from the water stress because of lack of stomata closure.

In greenhouse production systems the RH can exceed 90% in certain periods of the year when heating costs are high and ventilating of humid air is avoided to save energy. To increase the productivity in greenhouse systems, supplementary lighting is common in the periods when the natural irradiance is low. The light is mainly applied by gas-discharge lamp-types like high pressure sodium (HPS) lamps, which have a high radiant emission, high photosynthetic active radiation (PAR) emission and a high electrical efficiency but only 5% B light. In comparison, the natural solar radiation contains more than 18% B light. In another study with roses, the combination of high RH and HPS as a light source resulted in higher transpiration and postharvest water loss of rose leaves compared to a light source with a higher portion of B light, indicating poor stomata function (Terfa *et al.*, 2012a). In spite of the progress in research on light regulation of stomata movement, there is a lack of information on the interaction between light quality and other environmental factors like RH. This study was hence aimed to investigate the role of B light in stomata function and dark-induced stomata closure under different RH conditions. Further, since ABA is an important signal in stomata closure of roses, its content and regulation in light and darkness in relation to B light was studied.

Material and Methods

Plant materials and growing conditions

Rosa x hybrida, cv. Toril plants were 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 electrical conductivity (EC) level were 5.7 and 1.75, respectively, in all experiments (Superba: NPK 9-5-25+Mg+S+Mikro and calcinit, Yara, Oslo, Norway). During pre-cultivation, the plants were kept in a greenhouse compartment (glass roof and polycarbonate walls) at a temperature of 21°C, and average daily relative air humidity (RH) of 70% (corresponding to a water vapour deficit (vpd) of 0.74 KPa), at the Center for plant research in controlled climate at the Norwegian University of Life Sciences, Ås, Norway (N 59° 40.120', E 10° 46.232'). Supplementary light by high pressure sodium lamps (HPS, Osram NAVT- 400W, Munich, Germany) was given 20 h every day at a photon flux density of 100 (±10) µmol m^{-2} s^{-1} at 400-700 nm (measured with a Li-Cor, Model L1-185, quantum sensor, Li-Cor Inc., Lincoln, NE, USA). The pre-cultivation ended when the plants had 1-1.5 cm long shoots. Thereafter, the plants were transferred to different air humidity treatments in growth chambers.

Two different experiments were carried out in controlled growth chambers and both experiments were repeated twice. The temperature set point was 20±0.5°C, for all experiments during the experimental period. The RH in the growth chambers was either 60±3% (moderate RH, vpd: 0.7 KPa) or 90±2% (high RH, vpd: 0.23 KPa) and the CO_2 concentration was 400 ppm in all experiments. In *experiment I* plants were exposed to 100 µmol m^{-2} s^{-1} irradiance in a 20 h photoperiod provided either by light emitting diode (LED) lamps (round LED-light with 3 chains, Sola-co, Guangdong, China) containing 80% red (R; peak wavelength at 630 nm) and 20% blue (B) light (peak wavelength at 465 nm) or HPS lamps containing 5% B light (HPS, Osram NAVT- 400W) in high (90%) and moderate (60%) RH (Figure 1). In *experiment II,* the plants were grown at high RH (90%) and subjected to 100 µmol m^{-2} s^{-1} irradiance in a 20 h photoperiod provided by a mixture of B and R LEDs (round LED-light with 3 chains, SoLa-Co) with different proportions of B and R with dominant wavelength peaks at 465 nm and 630 nm, respectively. Three different spectral treatments expressed as the B light percentage 5% B, 20% B, and 100% B were given while the remaining percentage was R. The spectra of the lamps were measured with an OceanOptics SD2000 spectrometer (Fig. 1) (model SD2000, OceanOptics, Eerbeek, The Netherlands). The spectrometer was calibrated against a NIST-traceable calibration lamp (model LS-1-CAL, OceanOptics).

For analysis of ABA, β-glucosidase activity and stomata imprints, fully developed leaves were sampled in the middle of the light and dark period for all treatments after 6 weeks of

treatment when the plants had 1-3 open flowers. The leaf samples were immediately frozen in liquid nitrogen and stored at -80°C prior to extraction for ABA and β-glucosidase quantification.

Stomata Morphology Analysis

To study stomata morphology, impressions of the epidermal layer 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 previously (Tanaka *et al.*, 2005). Samples were taken interveinally close to the mid-rib on the abaxial side of the leaf from the first fully developed leaves of each plant from each air humidity and light quality treatment during both light and dark. The SUMP imprints were observed under a light microscope (Leitz, Labolux K, Type 0.2, Wetzlar, Germany) and stomata images were obtained with a Leica camera (Leica DC200, Heerbrugg, Switzerland). Stomatal morphology (length, width and area) and density were measured with the use of UTHSCSA ImageTool for windows version 3.00 (The University of Texas Health Science center, San Antonio, Texas, USA). The experiment was repeated twice and twelve imprints were made from each experiment and three images were taken from each imprint for image analysis. Since the trends in responses were similar, the data were presented as average of the experimental repeats.

Measurement of stomata conductance, water usage and test for desiccation tolerance

To study the diurnal pattern of stomata conductance, measurements were done on intact fully expanded leaves for 24 h in experiment I using a CIRAS-2 Portable Photosynthesis System with PLC6 (U) Automatic Universal Leaf Cuvette (PP Systems, 2001, Amesbury, MA, USA). During all measurements, the RH and light in the leaf cuvette were the same as in the growth chamber, the CO_2 concentration was 400 mmol mol^{-1}, the airflow 250 mmol s^{-1} and the temperature 22°C. Measurements were taken every 15 min for 24 h. The experiment was repeated twice and the measurement was taken from three plants in each repeat. Since the trends in responses were similar, the data were merged and presented as average of the experimental repeats.

To analyze the water usage capacity, plants with intact roots were transferred from the different treatments to a test chamber with 40-50% RH, 100 µmol m^{-2} s^{-1} irradiance for 20 h photoperiod provided by Mercury lamps (Osram NAV T-400W, Munich, Germany), and a temperature of 20±0.5°C. The pots were covered with plastic bags to prevent water loss through evaporation from the soil. The pots were then weighed right before dark and right after the dark period for three consecutive days. After the measurements the leaf area was determined with a leaf area meter (LI-COR, LI-3100). Leaf stomata conductance of these plants was also measured on intact fully expanded leaves using an AP4 leaf porometer (Delta-T devices LTD, Cambridge, UK). The rate of water loss (transpiration rate) per leaf area per hour was calculated as per the following equation:

$$water\ usage = \frac{Change\ in\ plant\ weight\ before\ and\ after\ dark\ period}{leaf\ area * hour}$$

Desiccation tests were also done to study the stomata response to dehydration. Detached upper leaves from eight plants grown under different RH and light quality treatments were tested in a test room with 50% RH, an irradiance of 15 µmol m^{-2} s^{-1} and 22°C. The leaves were weighed after 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120 and 180 min after detachment and their relative weight after the desiccation test were determined. The experiments were repeated twice and eight samples were taken from each repeat, and since the trends in responses were similar, the data were presented as average of the experimental repeats.

ABA and β-glucosidase quantification

Frozen leaf tissue was freeze-dried and finely grounded and extracted in distilled deionized water with an extraction ratio of 1:70 (g dry weight: ml water) overnight at 5°C. ABA concentrations of the extract were determined using a radioimmunoassay technique as previously described (Quarrie *et al*., 1988).

β-glucosidase analysis was done based on the procedure described by Arve *et al.* (2012). The leaf samples were taken from the freezer (-80°C) and immediately homogenized in liquid nitrogen using a mortar and pestle. 700 mg samples were extracted for 1.5 hours at 4°C in 10 ml 100 mM citrate buffer, containing 5% (w/v) PVPP, 1 mM EDTA, 14 mM mercaptoethanol and 10% (w/v) glycerol. Samples were then centrifuged at 1000 rpm for 4 min (Eppendorf 5810 centrifuge, Hamburg, Germany). 100 µl of the supernatant was mixed with 1 ml 100 mM citrate buffer containing 4 mM p-nitrophenol-*β*-D-glucopyranoside (pNPG) and incubated at 37°C for 60 min (Termaks B 8054 Incubator, Bergen, Norway). The reaction was then terminated with 2 ml 1M Na_2CO_3 and the amount of liberated p-nitrophenol was measured spectrophotometrically at 405 nm (Helios Alpha Spectrophotometer, Thermo Scientific, Surrey, UK). The concentration was calculated using the Beer-Lambert law, Absorbance=ε*length*concentration, and the molar extinction coefficient for p-nitrophenol ε=18300 (Dietz *et al.*, 2000). 1 unit of enzyme is then defined as the amount of enzyme needed to yield 1 nmol of p-nitrophenol per hour at 37°C. The samples were collected from five plants at the middle of the light and dark periods. Each sample consisted of 5-6 young and mature leaves from a single plant.

Statistical analyses

Both experiments (*Experiment I and II)* were repeated twice and since the trends of the results in the experiments were similar the data are presented as an average of the experimental repeats unless otherwise. Significant differences between means were tested for normally distributed

general linear models (GLM) and Tukey's test. Differences with $p < 0.05$ were considered significantly different. All statistical tests were performed in Minitab 16.1.1 (Minitab 16.1.1, windows version, State College, PA, USA).

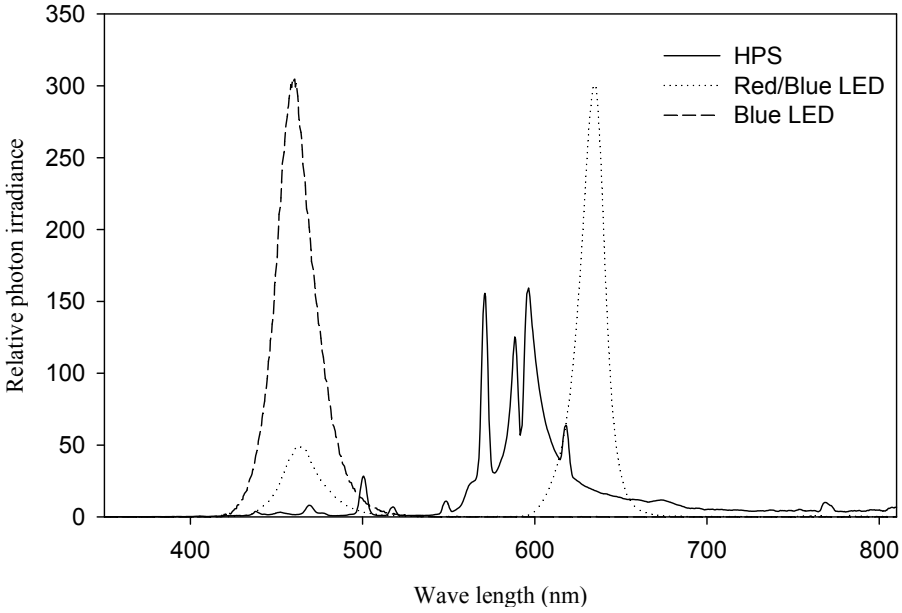

Figure 1. Relative spectra of the lamps used in the experiments: High pressure sodium (HPS) lamps Osram NAV T-400W (Solid lines), Light emitting diode (LED) lamps (Round LED-light 92W with 3 chains, SoLa-Co) (Dotted lines) and pure blue LED (Round LED-light 92W with three chains, SoLa-Co) (Dashed lines)

Results

LED (20% B and 80%R) reduces the transpiration rate and improves stomata closure in plants grown under high RH

RH and light quality (LED; 20% B and HPS; 5% B) during growth significantly affected the diurnal stomata conductance (g_{sw}) of rose leaves (Fig. 2A, B; $P < 0.01$). The diurnal g_{sw} throughout day and night was higher for leaves of rose plants grown under high RH as compared to moderate RH (Fig. 2A, B). However, if plants were grown under LED with a high B light proportion (20% B) at high RH, they had 10% lower g_{sw} throughout the day and reached their average lowest g_{sw} (30 $mmol^{-2}\ s^{-1}$) during the dark as compared to plants grown under HPS with

a lower B proportion (5% B), which still had higher average g_{sw} (48 mol^{-2} s^{-1}) during dark (Fig. 2A; Table 1). In the case of moderate RH, even though there was no statistically significant difference in g_{sw} between plants grown under LED and HPS, still a trend of slightly lower g_{sw} was measured throughout the day in plants grown under LED (Fig. 2A, Table 1). The percent reduction in g_{sw} between light and dark for LED-grown plants at high RH was higher (20%) as compared to HPS-grown plants, which was 9.6% only (Fig. 2A; Table 1). In addition, the calculated day: night ratio of g_{sw} was higher (1.7) for LED-grown plants than HPS (1.2). To understand if the change in g_{sw} was due to the change in stomata aperture and/or stomata number, we analyzed the ratio of stomata size between light and dark. In high RH the change in aperture was much higher (37.6%) in LED-grown plants than HPS where there was no significant difference (Fig. 3). The reduced aperture in dark in LED-plants partly explains the decrease in transpiration during dark, entailing an improvement in stomata function due to the prevailing environmental condition during the day.

Stomata response to desiccation was tested to further analyze the degree to which the detached leaves close their stomata and retain water during a 3 h dehydration test. The test showed that plants grown at moderate RH closed their stomata during the first 30 min and lost only 10% of their weight irrespective of the light quality (Fig. 2B). However, after 3 h of desiccation, plants grown at high RH at both light qualities had lost much of their weight (40-57%), and showed a continuous transpiration throughout the testing hours as compared to moderate RH. Nevertheless, a better stomata closure and water retaining ability was observed for the plants grown under LED. They showed about 20% less weight loss as compared to HPS-grown plants (Fig. 2B).

Table 1. Average stomata conductance (mmol m^{-2} s^{-1}) measured by CIRAS-2 during dark and light periods for rose leaves grown under different relative air humidity (RH) regimes (moderate RH, 60% and high RH, 90%) and light qualities provided by light emitting diodes (LED; 20% B) or high pressure sodium (HPS; 5%B) lamps. Data are the mean values ± SE of measurements from two experimental repeats with three replications in each and three sampling points per hour (n=18). Different superscript letters indicate significant differences (P<0.05).

	Moderate RH (60%)		**High RH (90%)**	
	LED	HPS	LED	HPS
Light	21.9^{c}±0.4	26.5^{c}±0.4	50.5^{b}±1.0	60.5^{a}±0.9
Dark	12.0^{c}±1.5	15.4^{c}±1.7	30.1^{b}±1.5	48.6^{a}±1.5
Light/Dark ratio	1.8^{a}±0.8	1.7^{a}±0.9	1.7^{a}±0.7	1.2^{b}±0.7

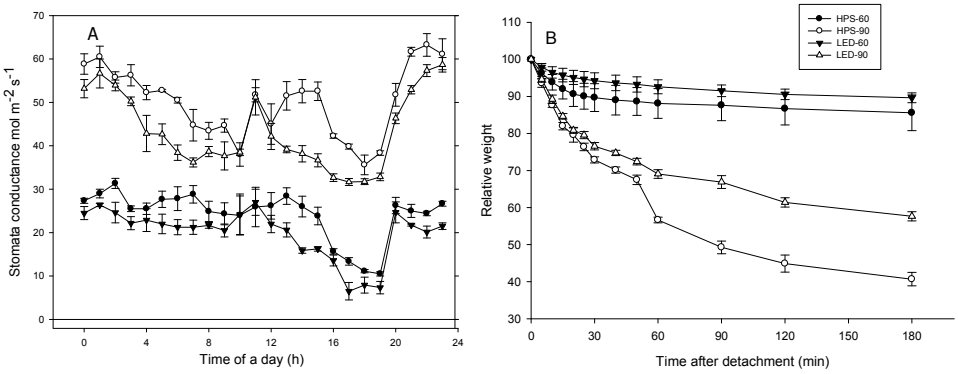

Figure 2. Diurnal stomata conductance (A) and change in relative weight (%) of detached leaves during 3 h of desiccation test (B) of rose leaves grown under different relative air humidity (RH) (moderate RH; 60% and high RH; 90%) and blue (B) light proportions provided by light emitting diodes (LED; 20% B) and high pressure sodium (HPS; 5% B) lamps. Data points and error bars indicate: A: Stomata conductance: mean values ± SE of measurements from two experimental repeats with three replications from each repeat (n=6). B: desiccation test: mean ± SE from two experimental repeats with eight replications from each repeat (n=16).

Stomata imprints of rose leaves were made during the light and dark periods to further study the effects of different RH treatments and light quality on stomata morphology and response to dark as a signal for closure. Generally, the stomata pore length and aperture were significantly larger at high RH than moderate RH, regardless of the light quality treatments (Fig. 3 and Table 2; $P<0.05$). The stomata pore length and aperture of high RH- grown plants was on average 1.8 and 1.7 times higher than in moderate RH-grown plants, and this affected the stomata area too (Fig. 3 and Table 2). However, for the high RH-grown plants these stomata characteristics were much smaller for LED-grown plants than HPS (Fig. 3 and Table 2). The stomata pore length and aperture were even smaller during dark for plants grown under LED compared to HPS. The length of the stomata pore of plants grown at high RH and HPS was 1.2 and 1.6 times larger during light and dark, respectively, compared to those of LED-grown plants (Table 2). Correspondingly, the stomata aperture of plants grown under HPS was even 2.4 and 1.7 times larger than those of LED-grown plants during light and dark, respectively (Fig. 3). Consequently, this led to a larger pore area for plants grown at high RH under HPS light than LED-grown plants (Table 2). The stomata aperture was smaller during dark in LED-plants while there was no significant change in the size of the aperture for HPS-grown plants. However, plants grown under LED had a higher number of stomata per area as compared to HPS-grown plants (Table 2). This indicates that, in spite of the higher number of stomata in a high B light proportion, the stomata then have a better capability to close in response to dark.

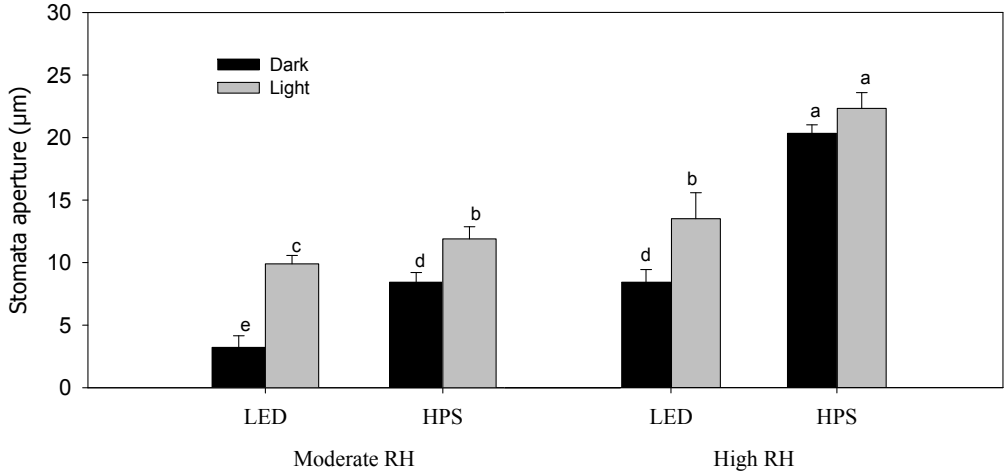

Figure 3. Stomata aperture of rose leaves grown under different relative air humidity (RH) regimes (moderate RH, 60% and high RH, 90%) and light qualities provided by light emitting diodes (LED; 20 % Blue) and high pressure sodium (HPS; 5% Blue) lamps. Data are the mean values ± SE from two experimental repeats with five imprint samples from each repeat and three images from each sample (n=30). Different letters indicate significant differences ($p<0.05$).

Table 2. Stomata characteristics of rose leaves grown under different relative humidities (RH) (moderate RH; 60% and High RH; 90%) and blue (B) light proportions provided by light emitting diodes LED (20% B) and high pressure sodium HPS (5% B) lamps. Data are the mean values ± SE from two experimental repeats with five imprint samples from each repeat and three images from each sample (n=30). Different letters indicate significant differences ($P<0.05$).

		Moderate RH (60%)		**High RH (90%)**	
		LED	HPS	LED	HPS
Pore length (µm)	Light	26.2 ± 1.0^{d}	32.8 ± 1.6^{c}	40.1 ± 0.4^{b}	48.2 ± 0.7^{a}
	Dark	21.5 ± 0.3^{e}	24.4 ± 0.3^{d}	34.2 ± 0.9^{c}	42.9 ± 1.3^{b}
Pore area (µm^2)	Light	176.7 ± 9.2^{d}	267.3 ± 8.4^{c}	325.0 ± 12.7^{b}	373.1 ± 18^{a}
	Dark	111.3 ± 3.5^{e}	166.7 ± 11.8^{d}	234.5 ± 5.6^{c}	370.7 ± 10.9^{a}
Stomata number (µm^{-2})		75 ± 2.5^{b}	60 ± 3.4^{c}	85 ± 5.2^{a}	70 ± 3.1^{b}

The ABA content and β-glucosidase activity is highly affected by RH and light quality

In HPS-grown plants the amount of ABA was significantly higher in plants from moderate RH compared to high RH (Fig. 4A; $P= 0.011$). In moderate RH the highest ABA level was measured during dark in both HPS and LED-grown plants (Fig. 4A). However, in high RH the

highest level of ABA was measured in LED-grown plants, which had 40% higher total ABA level as compared to HPS-grown plants (Fig. 4A; P= 0.001). This situation for the LED-grown plants from high RH was very much comparable to the amount of ABA measured in moderate RH (Fig. 4A). Nevertheless, there was no significant change in ABA level between light and dark in high RH for any of the light treatments, except a slight trend of higher ABA level during the night under LED. Hence, for plants grown under high RH and LED this slight change in ABA during light and dark might be an important signal to induce closure in the dark.

To further study if the increase in ABA level during dark was related to ABA conjugation, we quantified the β-glucosidase activity during dark. In our recent work with roses grown under moderate and high RH it was shown that ABA-GE, which is degraded by β-glucosidase, is the main catabolite playing a major role in affecting the diurnal ABA pool turnover (Arve *et al.*, 2012). The level of β-glucosidase activity was significantly higher in moderate RH compared to high RH-grown plants irrespective of the light quality difference (Fig. 4B; P<0.05). Furthermore, at high RH the activity of this enzyme was significantly higher under LED compared to HPS (Fig. 4B). This partly explains the slight trend of an increase in the level of ABA in the night under LED.

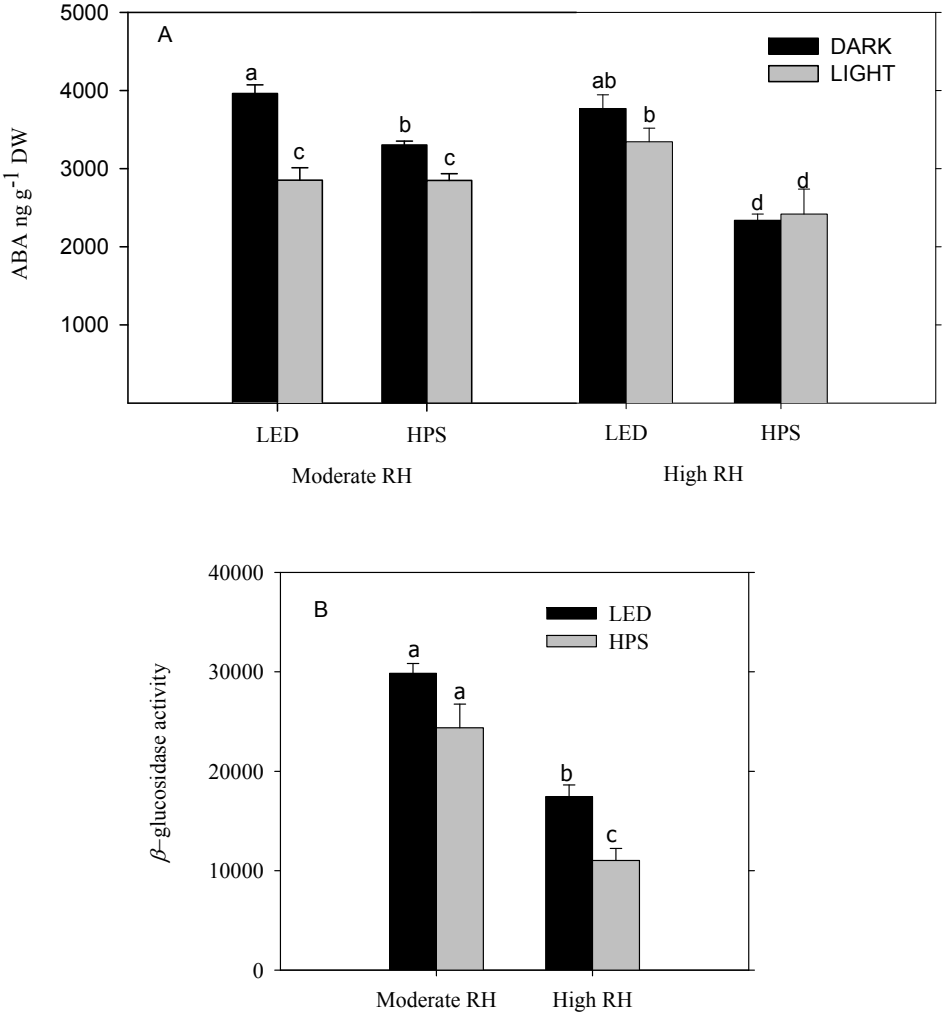

Figure 4. The effect of different relative air humidity (RH; Moderate RH, 60% and High RH; 90%) and blue (B) light proportions provided by light emitting diodes (LED; 20% B) and high pressure sodium (HPS; 5% B) lamps on the abscisic acid (ABA) content (A) and *β*-glucosidase activity (B) of freeze-dried rose leaves. For ABA quantification the samples were collected and measurements were done in the middle of the light and dark period. For *β*-glucosidase quantification, only samples from dark period were used. For both ABA and *β*-glucosidase analysis data are the mean values ± SE of 5 samples, each consisting of 5–6 leaves from each of five plants. Different letters within each figure indicate significantly different values ($P< 0.05$).

Transpiration rate, water usage and stomata function is improved as B light proportion is increased

Since HPS contains other wave lengths like yellow and orange in addition to R and B, another experiment (experiment II) was carried out to elucidate the effect of light quality on stomata function. Plants grown under high RH were then exposed to pure B or different B light proportions using LED lamps with distinct wavelengths of B and R light only (Fig 1).

Stomata morphology and water relations of rose leaves grown at high RH were notably affected by different B light proportions (Fig. 5 and 6; Table 3, $P<0.01$). The transpiration during the light period did not differ significantly between the different B light proportions, only a slight, insignificant trend for higher transpiration in the highest B proportion was observed However, during the dark period the transpiration decreased significantly with lower g_{sw} (165 mmol on average) at 20% B and 100% B compared to 5% B (Fig. 5A; $P<0.05$). The transpiration ratio between day and night under 20% B or 100% B were 2.01 and 2.23 respectively, and this was higher than for plants grown under 5% B, which was only 1.2. This indicates a reduction in transpiration during night due to improved stomatal dark closure ability in the two highest B light proportions.

The porometer data was correlated with water usage measured gravimetrically right before and after the dark period (Fig. 5B). Plants grown under 5% B showed 49% and 45% higher weight loss during the dark period as compared to 20 and 100% B, respectively, indicating higher transpiration rate under 5% B even during the dark (Fig. 5B). Thus, the data on water usage as well as transpiration suggested improvement in stomata sensitivity during darkness in response to increased B proportions. To further verify this, we analyzed the stomata imprints of rose plants grown at high RH with different B proportions (Fig. 6 and Table 3). In all treatments the plants had open stomata during the light period and tended to close their stomata during dark. During the light the stomata aperture and length were larger for plants grown under 5% B than 20% B (Fig. 6 and Table 3). Although no significant difference in stomata aperture or pore length during light in 100% B compared to 5 %B was observed, pore area was significantly lower in 100% B than 5% B. This trend continued during the night where the smallest stomata aperture and length were recorded for plants grown under 20% B and 100% B (Fig. 6 and Table 3). The maximum percent reduction in the stomata size was recorded for plants grown under 100% B (50.1%), followed by 20% B (46.8%) and the smallest reduction was observed under 5% B (25%). This lead to smaller stomata pore area for leaves grown under 20% B, followed by 100% B, whereas the largest area was recorded for leaves of 5% B-grown plants. However, the highest number of stomata per area was counted when plants were grown under more B light (Table 3). Plants grown under 100% B had 1.6 and 1.3 time higher number of stomata per area than 5% B and 20% B, respectively. This might explain the slight (although insignificant) trend of higher transpiration during day. However, the interesting point is the ability of these stomata

to close during dark so that the transpiration was reduced by more than 50% in plants grown under 20% B or more.

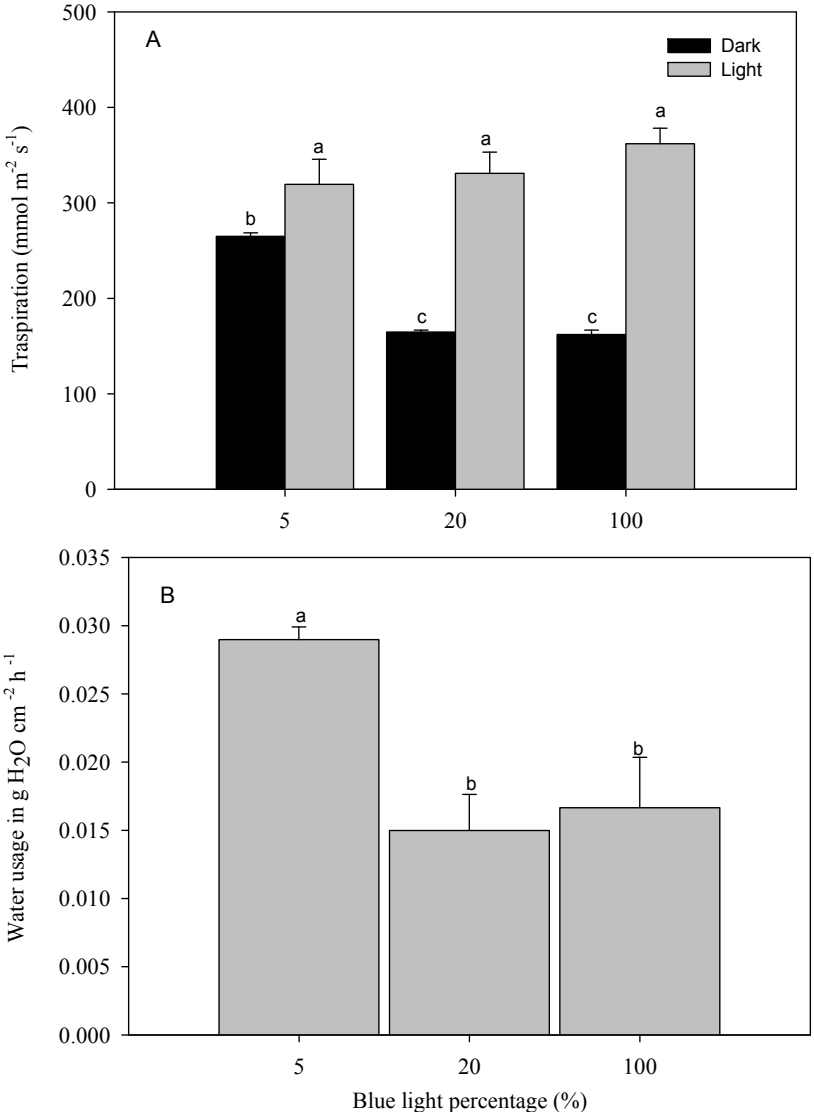

Figure 5. Transpiration rate during the light and dark cycle (A; measured by porometer) and water usage (B; measured gravimetrically right before and after the dark period, and data are put as a reduction in weight) of rose plants grown under high relative air humidity (RH; 90 %) with

different blue (B) light proportions provided by light emitting diodes (LED). The transpiration rate and water usage were measured for three consecutive days after plants were moved to 40 - 50% RH and 100 µmol m^{-2} s^{-1} irradiance for 20 h photoperiod. The data are mean ± SE of measurements on the first fully developed leaf from ten plants. Different letters within each subfigure indicate significant differences (P<0.05).

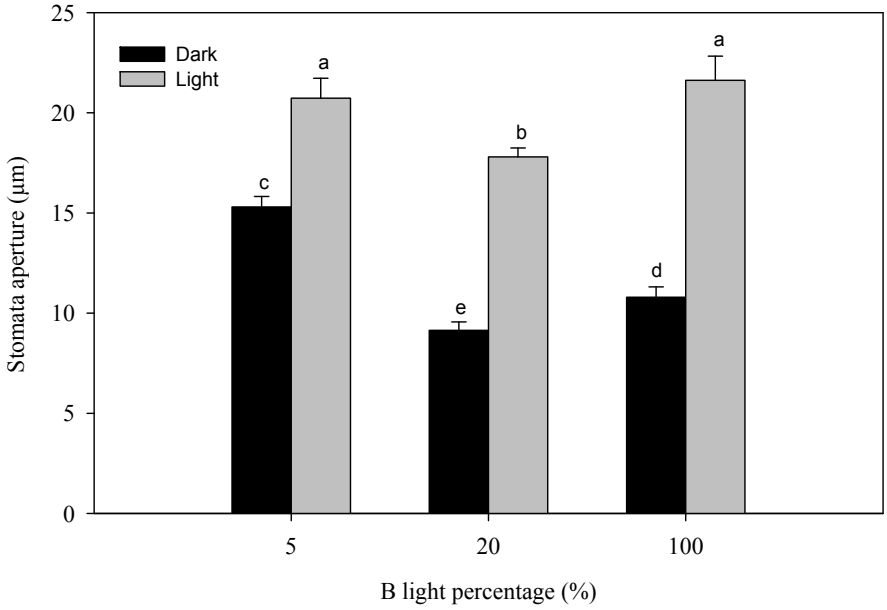

Figure 6. Stomata pore aperture of rose leaves grown under and high relative air humidity (RH) with different blue (B) light proportions. Data are the mean values ± SE from two experimental repeats with five imprint samples from each repeat and three images from each sample (n=30). Different letters indicate significant differences (P<0.05).

Table 3. Stomata pore characteristics of rose leaves grown under high relative air humidity (RH; 90%) and different B light proportions. Data are the mean values ± SE from two experimental repeats with five imprint samples from each repeat and three images from each sample (n=30). Different letters indicate significant differences (P<0.05).

		B light proportion (%)		
Stomata characteristics		5	20	100
Pore Length (µm)	Light	42.9±1.4^{a}	35.9±1.5^{c}	43.6±0.6^{a}
	Dark	40.7±1.6^{ab}	30.1±0.7^{d}	35.1±1.1^{c}
Pore Area (µm^2)	Light	378.6±21.5^{a}	287.1±9.4^{c}	332.8±12.2^{b}
	Dark	265.0±4.8c^{d}	196.6±7.2^{e}	195.4±8.2^{e}

Stomata Number (μm^{-2})	70±7.1 [c]	85±5.1[b]	113±9.2[a]

ABA level during dark is increased as B light proportion increases for plants grown under high humidity

It is known that the biosynthesis and degradation of ABA is affected by light quality (Xiong and Zhu, 2003; Tallman, 2004). A significant difference was observed in the level of ABA in rose leaves grown at high RH under the different B light proportions (Fig. 7; $P<0.05$). The highest level of ABA was recorded during the dark period when plants had grown at 20% B and 100% B, which was 1.2 times higher than for plants grown under 5% B. While the lowest measurement was recorded during the light period for plants grown at 100% B, there was no significant difference in the amount of ABA between 5% B and 20%B ($P<0.05$) during light period (Fig. 7A). However, similar to plants grown under HPS (experiment 1, Fig. 4), there was no significant change in the level of ABA between light and dark for plants grown at 5% B (Fig. 7A). The change in diurnal ABA level between light and dark for plants grown under 20% B and 100% B suggests change in the ABA pool either by altered biosynthesis or release from conjugated ABA. To verify this we quantified the β-glucosidase activity during dark (Fig. 7B). Hence, the quantified level of β-glucosidase activity was significantly higher in plants grown under 20% B or more (Fig. 7B). β-glucosidase activity in 20% B and 100% B plants was 1.5 and 1.6 fold higher than that of 5% B-grown plants, respectively. This correlates with the increase in the level of ABA during dark in plants grown under 20% B and 100% B.

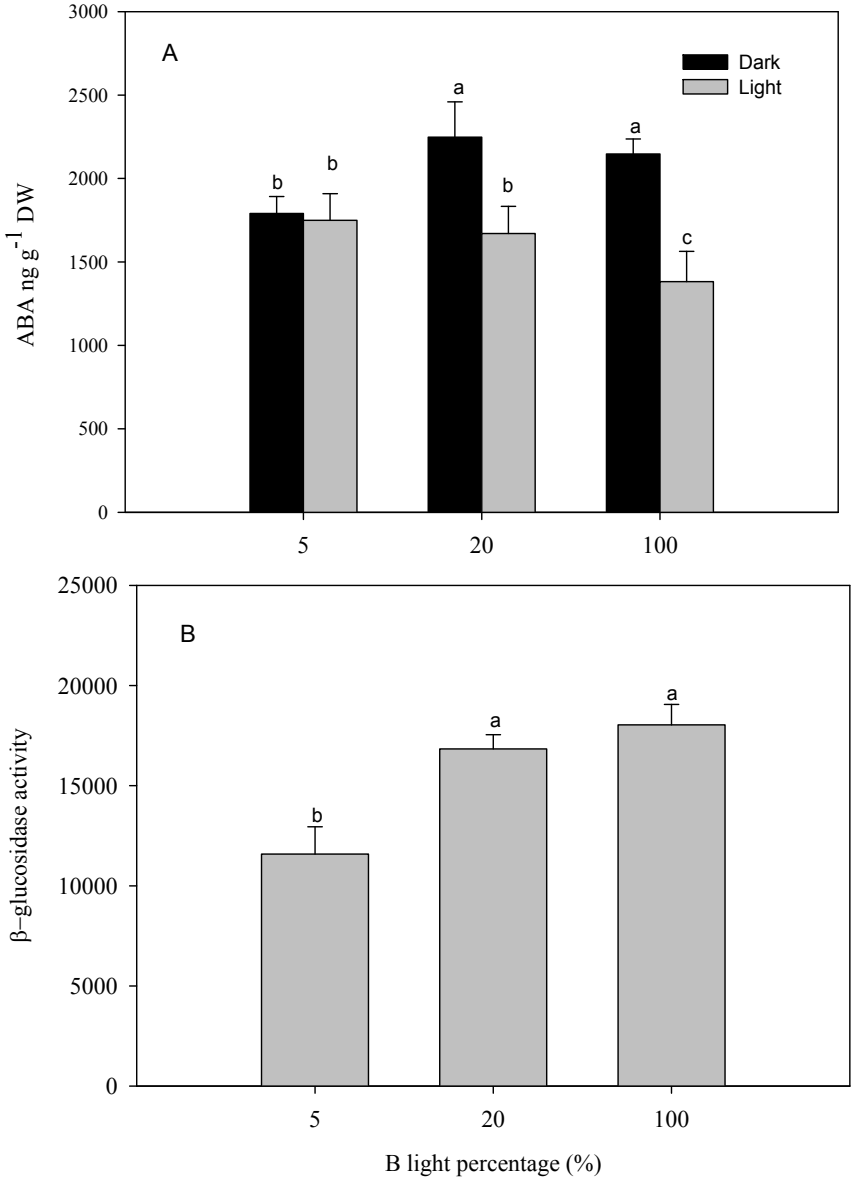

Figure 7. The effect of different blue (B) light proportions supplemented by light emitting diodes (LED) on the abscisic acid (ABA) content (A) and β-glucosidase (B) activity of freeze-dried rose leaves grown under high relative air humidity (RH; 90%). For ABA quantification the

samples were collected in the middle of the light and dark period. For β-glucosidase quantification, only samples from dark period were used. Data are the mean values ± SE of 5 samples each consisting of 5–6 leaves from each of plants. Different letters within each figure indicate significantly different values ($P< 0.05$).

Discussion

Uncontrolled water loss from plants grown at high RH is common in several plant species especially for leafy cuttings, in vitro propagated materials and ornamentals like cut flowers and plants produced in greenhouses (Santamaria *et al*., 1993; Fordham *et al.*, 2001; Torre & Fjeld, 2001; Fanourakis *et al*., 2011; Arve *et al.*, 2012). ABA application and ABA quantification in plants from different RH regimes have suggested an involvement of ABA in responses of stomata to RH (Nejad and Van Meeteren, 2007; Okamoto *et al*., 2009; Waterland *et al.*, 2010; Fanourakis *et al*., 2011; Arve e*t al.*, 2012). In greenhouses RH is usually high and the use of light from HPS lamps containing a low B light proportion as the common supplementary light source is making the scenario worse (Terfa et al., 2012a). It is well known that B light plays a critical role in stomata opening while ABA signaling under drought is thought to predominate over B light signaling in guard cells (Kinoshita *et al.*, 2001; Tallman, 2004, Shimazaki *et al.,* 2007; Kim *et al.,* 2010). Furthermore, the diurnal pattern of stomata response is dependent on the existing environmental cues during the light phase (Blom-Zandstra *et al.*, 1995; Tallman, 2004; Arve *et al.*, 2012; Fanourakis *et al*., 2012) and these cues in turn are influencing the diurnal variation in ABA pool (Tallman, 2004; Novakova *et al.,* 2005; Arve *et al.*, 2012). Darkness is a strong stimulus for stomata closure. Stomata developed normally are expected to close in response to darkness, but this trend might be disrupted by environmental cues prevailing during the day. Environmental factors like RH, photoperiod, light quality and irradiance can affect the speed and degree to which stomata close in the dark (Blom-Zandstra *et al.*, 1995; Caird *et al.*, 2007; Fanourakis *et al.*, 2012; Arve *et al.*, 2012). In this study we showed that a high portion of B light (≥20%) can improve the stomata function and induce a better dark closure of rose plants grown under high RH conditions.

Blue light reduces the transpiration and improves the stomatal function of roses grown under high RH

A constant high RH level during leaf development clearly resulted in a poor stomatal closure in response to dehydration and affected stomata morphology of roses similar to what found earlier (Torre *et al.*, 2003; Fanourakis *et al.*, 2011; Arve *et al.*, 2012). The rates of transpiration during both the light and dark periods were, respectively, three- and five-fold higher in cut rose flowers grown at elevated RH, compared with those grown at moderate RH (Fanourakis *et al.*,

2012). In this study it was found that the stomata characteristics and transpiration rates were significantly improved if plants were grown under 20% B provided by LED as compared to under HPS (5% B) (Fig 1 and 2; Table 1 and 2; $P<0.05$). The diurnal stomata conductance and desiccation test further confirmed that roses grown at high RH under 20% B (LED) had lower transpiration rate and improved desiccation tolerance (Fig. 1) as compared to plants grown under 5% B provided by HPS lamps. Thus, the transpiration and the stomata function under high RH were highly dependent on light quality.

As a response to stomata-closing stimuli, the stomata of rose plants grown at high RH and 20% B responded strongly to darkness by closing and eventually reducing transpiration. To further verify if the B light has a role and to separate the effects of the other wave lengths provided by HPS lamps, like orange and yellow, an experiment was carried out with distinct wavelengths of only B or R light. This showed similar B light-induced improvement in stomata function and reduced water usage and transpiration during dark (Fig. 5). The transpiration rate was highest during the light period for all the treatments but the rate was decreased during dark by 50 and 55%, respectively, if plants were grown at 20% B and 100% B as compared to those grown at 5% B (Fig. 4A). Accordingly, the water usage recorded during the dark, when stomata are expected to close, was lower for plants grown at 20% B and 100% B (Fig. 5B). The higher transpiration rate during the day for all treatments and a significant reduction in transpiration rate during the dark period for plants grown under a higher B light proportion indicate B light-induced improvement in dark closure of stomata. This suggests that stomata of plants grown under a higher B proportion have a better control of unnecessary water loss when subjected to stimuli that normally induces stomata closure. This is similar to Blom-Zandstra *et al.* (1995) showing that there was a significant reduction in water usage and g_{sw} during dark for rose plants grown at moderate RH (70% RH) and monochromatic supplementary B light than those grown under orange light.

Moreover, in general, rose plants grown under high RH had larger stomata length and aperture, which in turn resulted in a larger stomata area as compared to moderate RH (Fig. 2, Table 2).This is similar to previous studies in a number of species (Fordham *et al*., 2001; Torre *et al*., 2003; Karbulkova *et al*., 2008; Arve *et al*., 2012). Nevertheless, plants developed at high RH and higher B proportion had smaller stomata aperture and length, which in turn resulted in smaller stomata area as compared to those developed under HPS (Fig. 2 and 5, Table 2 and 3). The decreased stomata area and aperture in plants grown under 20% B and 100% B might partly explain the reduced transpiration rates and better water control, which eventually reduce the water usage. Furthermore, we found that the stomata of plants developed under high RH and 20% and 100% B responded by closing their stomata when subjected to darkness or desiccation as compared to those grown under 5% B. However, plants grown at higher B light proportion (20% B and 100% B) had higher number of stomata as compared to 5% B (Table 2 and 3). This correlates with our previous work with roses where a higher B light proportion increased the

number of stomata per area as compared to under HPS (Terfa *et al.*, 2012b). Interestingly, although plants grown under higher B light proportion had higher number of stomata which correlated with higher day time transpiration; they were able to close their stomata better during the dark period, avoiding further water loss (Table 1; Fig. 1 and 5). The ability of the leaves of plants grown under 20% B and more to close their stomata when subjected to darkness or desiccation shows the improved stomata ability to quickly adapt and respond to the prevailing environmental stimuli.

Blue light increases the activity of β-glucosidase under high RH conditions and affects the diurnal level of ABA

It has been suggested that the lack of stomatal response in plants developed under high RH is partly due to low ABA concentrations and/or ABA insensitivity. Long-term application of exogenous ABA during leaf expansion helped to overcome the lack of proper stomatal response to dehydration in *Rosa x hybrida* and *Tradescantia virginiana* grown at high RH (Nejad and van Meeteren, 2007; Fanourakis *et al*., 2011). Besides, a recent study showed that plants growing under constant high RH regulate their ABA levels differently than plants grown at constant moderate RH (Arve *et al.*, 2012).

In this study we also measured higher levels of total ABA in plants grown under a higher B light proportion (20% B and more) regardless of the humidity regime. However, the highest concentration was measured during the dark period. The increased ABA concentration during dark in moderate RH is believed to act as a signal for stomatal closure during darkness (Tallman, 2004; Novakova *et al*., 2005; Arve *et al.*, 2012). A previous study on the diurnal variation of ABA in *Nicotiana tabaccum* showed an increase in ABA concentration after 3 h of darkness (Novakova *et al*., 2005). Similarly, our previous study on *Rosa x hybrida* also demonstrated an increase in ABA level during the dark as compared to light period in moderate RH-grown plants, but there was no change in the level of ABA for high RH-grown plants (Arve *et al.*, 2012). However, in this experiment we observed a significant increase in the level of ABA during dark in high RH if plants were grown in a higher B light proportion (20% B and more), showing that the light quality during the day is influencing the ABA levels at night (Fig. 4 and 7). This increase in ABA during dark might arise from either increased biosynthesis of ABA or release from stored ABA-conjugate. This ABA might also have been transported from the root as a long distance chemical signal (Wilkinson and Davies, 2002) or from leaf cells (as short distance signal) (Cutler & Krochko, 1999).

Light has been shown to regulate ABA biosynthesis and degradation directly or indirectly (Xiong and Zhu, 2003; Tallman, 2004; Novakova *et al.,* 2005; Arve *et al.,* 2012). Specific light qualities like B light are also reported to regulate endogenous ABA levels during different

developmental processes (Fellner and Sawhney, 2002). The diurnal pattern of stomata movements are affected by the altering endogenous ABA metabolism during the day (Tallman, 2004). The author also discussed that light is one of the factors affecting sources of ABA during the day. The observed lower ABA level during light and a sharp increase during dark in the leaves of rose plants grown under 20% B or more in this study also indicate a dynamic turnover of the ABA pool either by degradation, biosynthesis or conjugation. Tallman (2004) indicated that guard cell ABA biosynthesis would be restricted by the removal of the ABA precursor, violaxanthin through light-driven xanthophyll cycling which converts violaxanthin to zeaxanthin (Eskling *et al.*, 1997). While zeaxanthin is proposed to be the B-light-specific photoreceptor of guard cells (Frechilla *et al.*, 1999; Talbott *et al.*, 2003), conversion of violaxanthin to zeaxanthin as a part of a mechanism to regulate endogenous guard cell ABA turnover partly explains the lower ABA level during light in plants grown under 20% B or more. Furthermore, during the dark period conditions favoring endogenous guard cell ABA biosynthesis would prevail once again to maintain stomata in closed position. Hence, the accumulated zeaxanthin during day as a result of light (B light photoreception) might start to convert to violaxanthin, which would favor endogenous ABA biosynthesis by guard cells (Tallman, 2004).

In the case of high RH, it has been shown that it is mainly the ABA degradation which is affected rather than the biosynthesis (Okamoto *et al.*, 2009; Arve *et al.*, 2012). In previous work with roses we have found that the ABA metabolite ABA-GE is the predominant factor in changing the ABA pool during light and dark in different RH regimes (Arve et *al*., 2012). Based on this knowledge, we assayed the *β*-glucosidase-activity to get insight if there is any inter-conversion between ABA and ABA-GE under the different light qualities. Clearly, the *β*-glucosidase assay showed a high activity of the enzyme in moderate RH compared to high RH, similarly to what was found in Arve et al. (2012). Interestingly, even though plants grown at high RH generally had lower *β*-glucosidase activity, those grown under 20% B and more had a higher activity of this enzyme as compared to those grown with a lower proportion of B light (HPS). Also a clear correlation between the enzyme activity and increased ABA during dark was observed. This pattern with high *β*-glucosidase activity was observed in both the experiment with HPS and LED (exp 1) and the experiment with different B-proportions provided by LED (exp 2), which points out that the increase in levels of ABA during dark might arise from ABA-GE conversion. In addition, the dramatic decrease in transpiration and stomata size during dark for plants grown under high RH and 20% B was correlated with increased ABA level which improved stomata closure during dark. This increase in ABA might arise from root. However, it is also logical to assume that the stored ABA (in the form of ABA-GE) is a rapid and easy way to regulate available ABA in guard cells to quickly respond to stomata-closing stimuli, whereas the bulk of transported ABA from the root might help to keep the stomata in closed position throughout the dark period /until stomata receives a signal inducing opening.

B light improves dark-induced stomata closure

Lack of dark-induced closure is commonly found in many different plant species (Caird et al., 2007) and can result in substantial water loss at times when photosynthetic carbon gain is not occurring or under postharvest conditions. Thus, night transpiration is potentially an important factor affecting whole-plant water balance and water use efficiency. Attention has also been placed on energy consumption in greenhouses because the lack of dark-induced closure may lead to evaporative heat loss at night. Besides, when the product continues to transpire during dark storage and during sale the water loss may lead to a shorter postharvest life (Waterland *et al.*, 2010).

The magnitude of water loss occurring during the night depends on both g_{sw} night and the vapor pressure difference (VPD) between leaves and the air, as well as canopy structure and atmospheric mixing (Caird *et al.*, 2007). However, there are variations in night time transpiration among and within species (Caird *et al.*, 2007; Dawson *et al.*, 2007; Kavanagh *et al.*, 2007). Furthermore, daytime growth conditions such as humidity, photoperiod length, light quality and irradiance can affect the rate and degree to which stomata close in the dark (Blom-Zandstra *et al.*, 1995; Caird *et al.*, 2007; Fanourakis *et al.*, 2012; Arve *et al.*, 2012). Higher irradiance during the day resulted in faster stomatal closure upon transition to darkness in roses, although closure was still incomplete (Blom-Zandstra *et al.*, 1995). The spectrum of the low intensity supplementary light (25 µmol m^{-2} s^{-1}) also affected g_{sw} at night, with orange supplementary light resulted in 100% and 50% higher g_{sw} as compared to control and B light, respectively (Blom-Zandstra *et al.*, 1995). In the present study, we found that plants grown under elevated RH had higher night time transpiration as compared to those grown under moderate RH regardless of the difference in the light quality (Fig. 1A and 5). Nonetheless, for plants grown under high RH, dark transpiration and water usage was reduced by 50% if the plants were grown under 20% B or more (Fig. 1A and 5). This significant reduction in transpiration and water usage complies with decreases in stomata aperture and area during dark (Fig. 2 and 6; Table 1 and 2).

The role of ABA in the lack of dark-induced closure in high RH is not well understood. However, the higher ABA level in general, and specifically the increase in amount during the dark for plants grown in high B light proportion (20% B or more) indicate a role of ABA in stomata closure (Fig. 3A and 7). Further, application of ABA during growth or transfer from high to moderate RH before the leaves are fully expanded, has been shown to result in improved stomata functionality and further elucidate the importance of ABA as one of the major stimuli in stomata closure (Nejad and van Meeteren, 2007; Fanourakis *et al*., 2011). However, in most studies and particularly in this study, the differences in ABA levels between the treatments are rather small and they all contain physiologically sufficient levels of ABA to have a normal stomata function. This points out that the distribution of ABA in the leaf cells and the amount

available in guard cells seem to be very important in governing closure. Hence, where the ABA is localized in the leaf might be more important than the total amount of ABA in the leaf.

The increased level of ABA concentrations during dark in higher B proportion might be important in the development of functional stomata. It has been shown that, the variation in the diurnal ABA might be important to develop functional stomata, similar to a daily spray with ABA (Tallman, 2004; Fanourakis *et al*., 2011). In our previous (Arve *et al.*, 2012) and present studies it has been shown that the absence of a significant change in ABA level during light and dark period in high RH grown plants correlated with absence of stomata closure. Hence, this absence of a dynamic ABA peak at the beginning of the dark period in high RH-grown plants might make the guard cells less sensitive to closing stimuli, and vice versa for moderate RH-grown plants. In another study we observed that detached fully developed leaves from roses ('Toril') produced under high RH did not respond exogenous ABA treatment (100µM) while the moderate RH leaves responded and closed their stomata (Carvalho et al., unpublished). This shows that the sensitivity to ABA is reduced when leaves are developed under high RH. Furthermore, the fact that B light itself plays a major role in stomata opening and closing, the stomata that are functional and normally adapted to regular opening and closing during development hence would be able to close properly when they are subjected to signals in stomata closure such as darkness, drought or ABA. Thereby, it can be suggested that both increased level of ABA and B light in combination or individually would contribute to the improved stomata functionality directly or indirectly.

Conclusion

The present study shows that B light improves the stomata function under high RH conditions. The ABA content was higher in darkness under a light source with a high B light proportion and this correlates with the presence of high β-glucosidase activity and an improved dark-induced closure under high RH. We conclude that B light is important as a signal to activate the β-glucosidase enzyme. Taken together, the improved stomata function and less transpiration in combination with increased ABA level, indicate that B light plays an important role in governing stomata functionality and ABA homeostasis under high RH. This result has both practical and experimental implication in such a way that, lighting sources in greenhouse production systems can be improved by adding extra B to reduce the water usage during growth and to minimize postharvest losses due to an improved water balance. Further, it is important to be aware of the impact of the light sources, and especially B light on stomata behavior when performing plant experiments in closed growth chambers or climate regulated rooms without natural light.

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PAPER V

Meseret Tesema Terfa, Amsalu Gobena Roro, Jorunn Elisabeth Olsen and Sissel Torre (2013). **Effects of UV radiation on growth and postharvest characteristics of three pot rose cultivars grown at different altitudes.** (Manuscript)

Effects of UV radiation on growth and postharvest characteristics of three pot rose cultivars grown at different altitudes

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Abstract

The ultra violet (UV) radiation reaching the ground is classified as UV-B (315-280) and UV- A (315-400 nm) and the levels vary with altitude and latitude. Numerous studies have shown that UV-B has various effects on morphology, biochemical composition and molecular responses of different species. It is well known that the climate conditions during growth also affect how plants behave after harvest. However, less is known about the effect of UV radiation during growth on postharvest characteristics of ornamentals, and especially the role of UV-B. In this study we investigated the effect of natural levels of UV radiation at different altitudes (2794 ma.s.l. (High altitude) and 1700 ma.s.l. (Low altitude)) on growth responses like morphology and flowering, postharvest water usage and shelf life of three pot rose cultivars ('Cygein', 'Snow White', 'Tom Tom'). Plants were grown under UV-transmitting or UV-blocking films at different altitudes. The results showed that UV radiation significantly reduced growth at both altitudes; however the effect was more prominent at lower altitude. Besides, higher level of solar UV radiation also delayed flowering by 7-10 days. Postharvest life and water usage were not significantly affected by UV radiation but rather by the altitude and plants produced at high altitude had a better control of water loss and a longer postharvest life compared to lower altitude-grown plants. In conclusion, UV radiation mainly affected morphology and development of the plants. However, stomata conductance, postharvest water usage and characteristics were rather affected by altitude differences than UV radiation.

Key words: Altitude; Growth; Postharvest life; UV radiation; Water usage

1. INTRODUCTION

Ultraviolet radiation (UV) is a part of the non-ionizing region of the electromagnetic spectrum and comprises approximately 8–9% of the total solar radiation (Hollosy, 2002). UV is traditionally divided into three wavelength ranges: UV-C (200–280 nm) is extremely harmful to organisms, but not relevant under natural conditions of solar irradiation since it does not reach the ground due to efficient filtration by stratospheric ozone layers; UV-B (280–315 nm) represents only approximately 1.5% of the total spectrum, but is of particular interest since it can induce a variety of effects in plants; UV-A (315–400 nm) represents approximately 6.3% of the incoming solar radiation and is the least hazardous part of UV radiation (Hollosy, 2002).

UV-B has various effects on morphology, biochemical composition and molecular responses of different species. However, the responses depend on species, cultivar, experimental conditions, levels of UV-B and the interaction with other climate factors like temperature and photosynthetically active radiation (PAR) (Frohnmeyer and Staiger, 2003; Reddy et al., 2004; Brown et al., 2005; Berli et al., 2012). Even though UV-B effects on vegetative growth and morphology of plants are variable, reductions in shoot length and leaf expansion were found to be the most common effects (Mark et al., 1996; Caldwell, 2003; Zhao et al., 2003). Besides, extended exposure of plants to UV-B ration results in higher accumulation of phenolic compound to absorb UV-B and reduce its penetration and cellular damage (Lois, 1994; Jansen et al., 1998; Caldwell, 2003). Accumulation of such secondary metabolites and reduction in leaf area are part of the strategy by which plants adapt and escape from harmful UV-B radiation, through reduction in its transmittance (Jansen et al., 1998).

Furthermore, there are many reports showing significant reduction in total plant biomass and photosynthetic capacity due to damages to the photosynthetic pigments and chloroplast structure (Teramura and Sullivan, 1994; Kakani et al., 2003), as well as inhibition of photosystem II (Ziska et al., 1993; Allen et al., 1997). Additionally, photosynthesis could be indirectly affected through reductions in stomata conductance (gs) (Day and Vogelmann, 1995; Zeuthen et al., 1997). There have been contradictory results on the responses of UV-B regarding gs and stomata characteristics. It has been indicated that elevated levels of UV-B radiation might decrease gas exchange through enhancement of stomata closing (Dai et al., 1995; Keiller and Holmes, 2001; Berli et al., 2012) but in some plants UV-B has also been shown to induce stomata opening (Musil and Wand, 1993).

Pre-harvest environmental conditions have an enormous effect on the shelf life of ornamentals like cut flowers, bedding plants and pot plants. Ornamentals are mainly grown in protected production systems and the environmental conditions during growth such as light, photoperiod (Mortensen and Gislerød, 1999; Fjeld et al., 1994), day and night temperatures (Moe, 1975; Hamrick, 2003), carbon dioxide levels (Dole and Wilkins, 1999) and relative air humidity (Torre et al., 2001; Pettersen et al., 2007; Fanourakis et al., 2012) are all shown to

affect the postharvest shelf life (for review see, Halevy and Mayak, 1979a, 1979b). Stomatal behavior and water relations are one of the main factors determining the potential postharvest life, especially for cut flowers, but also for some pot- and bedding plants (Torre and Fjeld, 2001; van Doorn, 1997; Waterland et al., 2010a, 2010b). Studies have shown that the stomata behavior in response to conditions of the cultivation environment, such as relative air humidity (Torre and Fjeld, 2001; Fanourakis et al., 2012), light quality (Terfa et al., 2012), and photoperiod (Mortensen and Gislerød, 1999), will persist also after harvest. Thus, the postharvest water loss might be dependent on the environment during growth.

UV-B can induce a range of specific plant responses, some of which are particularly desirable from a horticultural perspective. However, less is known about the effect of UV radiation during growth on postharvest characteristics of ornamentals, and especially the role of UV-B (280–315 nm). Although UV-B was earlier mainly considered a plant stressor and a potential source for damage, currently an ambient or ecological dose of UV-B is believed to be an important signal for plants rather than a stressor (Jansen et al., 1998; Searles et al., 2001; Jordan, 2002; Jenkins, 2009; Jansen et al., 2012). Novel technologies to manipulate UV levels are emerging and e.g. by using different selective plastic films, either UV-blocking or UV-transparent, specific parts of the UV spectrum can be manipulated. This provides new opportunities in protected crop production (Jansen et al., 2012).

Since UV-B at ground level varies with altitude and latitude, UV-B exposure of plants will depend on the specific growing site. Close to the equator commercial plant cultivation is possible also at high altitudes. E.g. in Ethiopia highland areas have a mild climate for ornamental and other crops production. Ethiopia, is currently the second largest exporter of cut flowers in Africa (Gebreeyesus and Iizuka, 2012), and roses are produced in protected production systems under plastic coverings but without heating. The two main locations where the commercial rose productions are intensively under way in Ethiopia are highlands (2,400-2,600 ma.s.l) around the capital, Addis Ababa, where the climate is characterized by high daily temperatures and cool nights, and Ziway (mainly characterized as lowland; 1,100-1,800 ma.s.l) where the temperatures are higher (25°C in average). The UV radiation reaching the highland region of Ethiopia is higher compared to lowland due to the increase in solar UV radiation with altitude (Sullivan et al., 1992; Schmucki and Philipona, 2002). Obviously, there is also a huge difference in daily mean temperature and day and night temperatures between highland and lowland. However, the expected difference in UV-B at the two altitudes may also have a role in postharvest behavior either directly or indirectly by affecting stomata function and eventually postharvest water usage. Thus, the aim of this study was to test the role of natural levels of UV radiation at different altitudes in Ethiopia in growth responses like morphology and flowering, postharvest water usage and shelf life of different cultivars of pot-roses. These pot roses were grown under UV-transmitting and UV-blocking films at different altitudes.

2. MATERIALS AND METHODS

2.1. Study Area and Planting Material

Field experiments covered with different plastic films (see below; Fig 1) were carried out in the southern part of Ethiopia at two different locations commonly described as highland (Hagereselam) and lowland (Hawassa). Hawassa (7°3'N 38°28E) is located at an altitude of 1700 m.a.s.l and Hagereselam (6°27'N 38°27'E) at an altitude of 2794 m.a.s.l. During the experiments climatic parameters at the experimental sites were recorded every hour by a thermo hygrometer data logger (Testo 174H, Testo comfort software basic, Version 5.0.2564.18771, Lenzkirch, Germany) hanged on the top of the plant canopy (Table 1). Three pot rose (*Rosa x hybrida*) cultivars collected from a commercial rose grower near Addis Ababa (Ethio Plants PLC, Alemgena, Ethiopia) were used in the experiments; 'Snow white' (white petals), 'Tom-Tom' (pink petals) and 'Cygein' (red petals).

Table 1. Climate data sampled during the experimental period (April-July, 2012) at both research sites: Higher altitude (2794 m.a.s.l) and lower altitude (1700 m.a.s.l). The temperature (T), relative air humidity (RH) and calculated water vapour pressure deficit (VPD) were sampled by a thermo hygromerter data logger hanged on the top of plant canopy inside each plastic film cover during the growing periods. While UV-B (mW $m^{-2}s^{-1}$), UV-A (mW $m^{-2}s^{-1}$) and PAR (µmol m^{-2} s^{-1}) were measured two times every hour on a clear sky day from 6 a.m. – 6 p.m. The climate data are the mean values of recordings from two experimental repeats.

Altitude	Plastic films	T_{mean} (°C)	RH_{mean} (%)	VPD kPa	PAR	UV-B	UV-A	UV-B/ UV-A
High altitude	-UV	16.6b	76.7a	0.45	825.4a	35.5c	1722c	0.02c
	+UV	16.5b	77.7a	0.41	889.6a	885.4a	11970a	0.08a
Low altitude	-UV	24.6a	65.8b	1.12	599.8b	36.8c	1397c	0.03c
	+UV	25.3a	63.4b	1.16	675.8b	557.b	8612b	0.07b
p-Values								
Altitude		0.001	0.001	0.001	0.012	0.04	0.01	0.03
Film		0.973	0.845	0.082	0.15	0.01	0.001	0.01
Altitude x Film		0.670	0.589	0.749	0.20	0.03	0.20	0.09

2.2. Pre-cultivation and growth condition

Plants from the three pot rose cultivars were grown from a single node stem segment with one mature leaf. Cuttings were made from the middle and lower position of fully developed stems with open flowers and rooted in pots with coconut peat rooting medium (Galuku

Lankaexport PVt. Ltd, Kurunegala, Sri Lanka) for 3 weeks. During the rooting the plants were kept under plastic cover to keep the air humidity high. After rooting the plants were transferred to a 15 cm new pot with fertilized coconut peat (Nitrogen-Phosphorus-Potassium (NPK) 12-7.5-28 ppm) and kept in shade house in Hawassa for about 10-12 days. The climate under the shade house was $20^{o}C \pm 5$ temperature, 70% relative humidity and 12/12 h of light/dark. Natural light was used during the experimental period. When the plants had 1-2 cm long shoots they were transferred to a structure made of UV-blocking plastic covers (selectively cut-off UV-B below 350 nm radiation; Solar EVA- 5 High Diffuse opaque polyethylene film with 0.20 mm thick and 3 m wide, Revora plastic, The Netherlands), and UV-transmitting white polyethylene sheet (transmits all solar spectrum beyond 250 nm; 0.2 mm polyethylene sheet, Addis Ababa, Ethiopian) (Fig 1).

The structure was 3 m x 3 m wide and 2 m high with the bottom and top sides (15 cm above ground and 15 cm below roof) left open to allow air ventilation. It was constructed in the North–South direction over the treatment plot to ensure the solar radiation reaching the plants only after passing through the filter as the sun moves from East to West. The main climate factors recorded inside the structure during growth were temperature, relative air humidity (RH), and UV-B distribution (Table 1 and Fig. 2). The photosynthetically active radiation (PAR) passing through the UV-blocking and UV-transmitting films was about 80% and 75%, respectively, compared with unfiltered radiation (Fig. 2). Hereafter plants growing under plastic film blocking UV-B and short UV-A radiation will be referred to as minus UV (-UV), and those grown under white transparent plastic film transmitting UV-B and UV-A radiation will be referred to as plus UV (+UV). The solar irradiance was measured using a PAR quantum sensor (Skye quantum sensor, Skye Instruments Ltd, Llandrindod Wells, UK), in ($\mu molm^{-2}\ s^{-1}$). The amount of UV-A and UV-B were quantified by a UV-A and UV-B Sensor (Skye UV-A and UV-B sensor, Skye Instruments Ltd, Llandrindod Wells, UK) in $mW\ m^{-2}\ s^{-1}$.

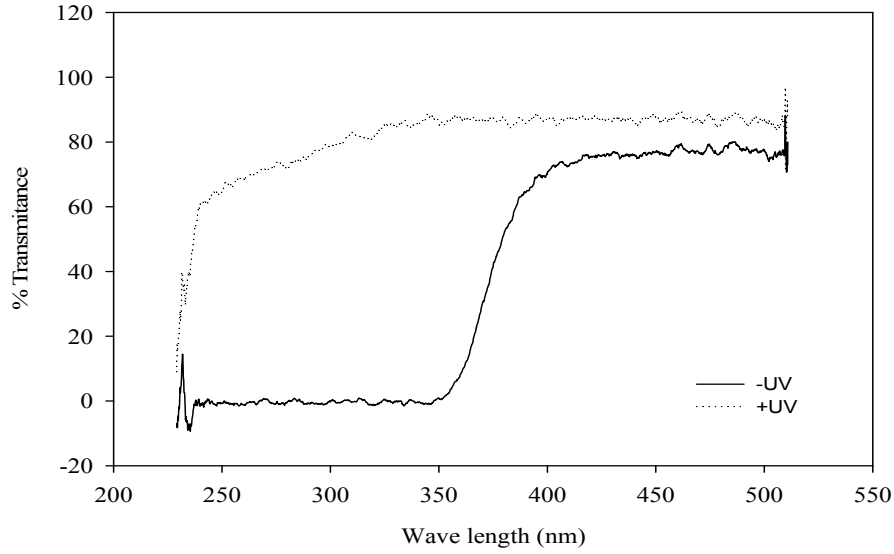

Figure 1. Solar spectrum transmission of polyethylene films used in the growth experiment: UV-blocking polyethylene film (-UV) (solid line; blocks UV-B (280-315) and the short wavelengths of UV-A; Solar EVA- 5 0.20 mm thick high diffuse opaque polyethylene film, Revora plastic, The Netherlands) and UV-transmitting polyethylene film (+UV) (dotted line; transmits all solar spectrum beyond 250 nm; 0.2 mm polyethylene sheet, Addis Ababa, Ethiopian).

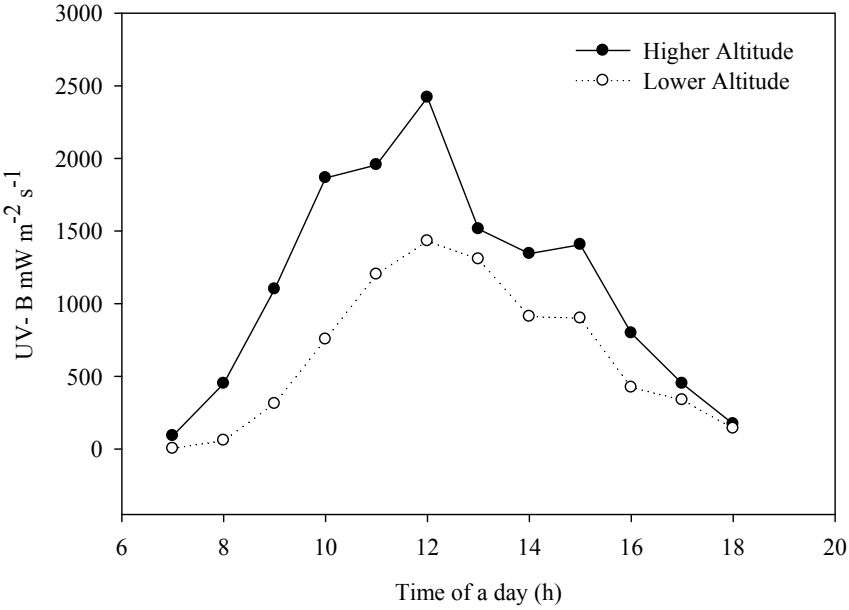

Figure 2. UV-B distribution throughout the day at clear sky during the wet season (April-July, 2012) at higher altitude (solid line; 2794 m.a.s.l, Hagereselam) and lower altitude (dotted line; 1700 m.a.s.l, Hawassa).

2.3. Growth parameter measurements

Plant growth parameters such as shoot length, average leaf area (LA), leaf number, leaf and shoot dry weight (DW) were analyzed when plants were at the commercial stage of sale with fully developed leaves and 1-3 open flowers. LA was measured with a LI-3100 leaf area meter (LI-COR, Inc., Lincoln, Nebraska, USA). DW of the leaves and shoots was determined after drying the leaves and stems for 5 days at 70°C. Twice a week flowering status was recorded in order to calculate number of days until open flower.

2.4. Stomata conductance and fluorescence

Stomata conductance (gs) was measured at local noon time (between 10:00 a.m. – 3:00 p.m.) on intact first fully expanded leaves of five plants per treatment in each experiment using an open system LCA-4 ADC portable infrared gas analyzer (Analytical development company, Hoddeson, England). During the measurements the calibrations/adjustment in the leaf cuvette

and gas analyzer was: leaf surface area 2. 5 cm^2, ambient carbon dioxide concentration (C_{ref}) 340 µmol mol^{-1}, temperature of leaf chamber (T_{ch}) varied from 22 to 25^oC, leaf chamber molar gas flow rate (U) 410 µmol/s, ambient pressure (p) 828 mbar and PAR (Q) at leaf surface was maximum up to 1500 µmol $m^{-2}s^{-1}$. The average leaf temperature during measurements varied between the locations. The leaf temperature for plants grown at the lower altitude varied between 30 and 32°C while it was between 20-22°C for plants at the higher altitude. Measurements were taken every five minutes for 30 minutes in each plant. The maximum efficiency of PSII photochemistry Fv/Fm was measured in the same time period by a plant efficiency analyzer Handy-*PEA* (Hansatech, Kings Lynn, UK).

2.5. Postharvest characters and measurements

To analyze postharvest characteristics six plants with intact roots were transferred from each treatment to a common test room in Hawassa University. The climate during testing were 58±5% RH (corresponding vapour pressure deficit (VPD) was 1.2 kPa), irradiance 35±5 µmol m^{-2} s^{-1} supplied by fluorescent tubes (Osram NAV T-400W, Munich, Germany) as 12 h/12 h of light/dark, and a temperature of 23±1.5°C. Three control pots with no plants and only soil were also placed in the room to estimate the water loss through evaporation from the soil. All the pots were then weighed every day from the first day (D_0) until the end of the postharvest life duration for every plant. At the end of the postharvest life the leaf area of the plants was determined by a leaf area meter (LI-COR, LI-3100). Then rate of water loss (transpiration rate) per leaf area per day (H_2O cm^{-2} day^{-1}) was calculated. Assessment of the postharvest life duration was done visually according to a standard procedure (Association of Dutch Flower Auctions (VBN), 2005). The postharvest life of a plant was considered terminated when 50% of either one or more of the postharvest symptoms were visible. The visual symptoms taken into account were petal wilting, petal necrosis, leaf wilting and drying.

2.6. Statistical analysis

At both locations the experiment was repeated twice with the same experimental layout during the wet season (April-July, 2012). Since the trends of the results in the experiments were similar the data are presented as an average of the experimental repeats unless otherwise mentioned. Significant differences between means were tested for by applying normally distributed general linear models (GLM). Differences with $p \leq 0.05$ were considered significantly different. All statistical tests were performed in Minitab 16.1.1 (Minitab 16.1.1, windows version, State College, Pennsylvania, USA).

3. RESULTS

3.1. Plant growth and development

Number of days to flower opening was significantly affected by altitude and UV radiation in all cultivars. In general, plants grown at high altitude required 2-3 more weeks to get visible flower buds compared to low altitude (Table 2). Plants grown under –UV radiation flowered 7-10 days earlier in both altitudes as compared to +UV radiation (Table 2). There was no significant interaction between altitude and UV radiation in days to flowering. In addition, UV radiation caused petal blackening in the red color cultivar ('Cygein') and brown spots the petals on the white petal color cultivar ('Snow white').

The shoot length and leaf number were significantly affected by altitude and UV radiation in all the cultivars (Table 1). In all the cultivars higher altitude-grown plants had 9-10 cm longer shoots than those grown under lower altitude regardless of the UV radiation (Table 2). However, the internode number and number of leaves were 1.3 and 2 times higher respectively, in lower altitude than high altitude. UV radiation also significantly affected shoot length and number of leaves in all cultivars in both altitudes (Table 2). The reduction in shoot length and leaf number due to UV radiation was 25-35% and 15-19%, respectively, for all cultivars regardless of altitude. However, the reduction was more pronounced at low altitude and plants were on average 10% shorter than high altitudes plants in all cultivars (Table 2). Even though both altitude and UV radiation had a significant effect on shoot length, leaf number and internode number, the strongest reduction in all growth parameters was mainly due to altitude rather than UV radiation. There was a significant interaction between altitude and solar UV radiation on average leaf area (LA) and leaf dry weight (LDW) (Table 2). LA was reduced by 25-30% by +UV radiation, in both altitudes and the effect was more pronounced at low altitude. This was correlated with LDW, which was slightly affected by both altitude and UV radiation (Table 2).

Table 2. Growth and morphology of *Rosa* x *hybrida* cultivars grown at different altitudes under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (-UV). Data are the mean values of measurements from two experimental repeats with ten replications in each (n=20; $p<0.05$).

Altitude	Plastic film	Cultivar	Shoot length	Leaf number	Internode number	Average leaf area	Leaf DW	Shoot DW	Days to flowering (weeks)
High altitude	+UV	'Cygein'	16.0	6.2	7.5	140.8	0.9	0.8	8.0
		'Tom-Tom'	19.9	5.7	7.8	218.4	1.7	0.9	7.5
		'Snow white'	16.8	5.0	6.3	98.6	0.8	0.6	8.0
	-UV	'Cygein'	21.7	9.3	7.0	252.3	1.9	1.8	6.5
		'Tom-Tom'	23.5	8.8	7.5	325.5	2.6	1.6	6.5
		'Snow white'	20.3	8.0	6.9	134.7	1.8	1.7	6.0
Low altitude	+UV	'Cygein'	6.7	13.2	9.0	67.0	0.6	0.4	4.0
		'Tom-Tom'	8.8	10.8	9.8	139.4	1.5	0.7	4.5
		'Snow white'	8.5	11.2	8.2	65.9	0.6	0.8	4.0
	-UV	'Cygein'	10.3	16.3	9.6	139.8	1.6	1.6	3.0
		'Tom-Tom'	12.4	12.8	9.5	177.4	2.4	1.4	3.5
		'Snow white'	11.5	14.8	8.8	90.7	1.7	1.4	3.0
p-Values									
	Altitude		0.003	0.001	0.001	0.01	0.325	0.205	0.001
	Film		0.001	0.001	0.621	0.01	0.021	0.001	0.002
	Cultivar		0.042	0.032	0.050	0.001	0.011	0.052	0.356
	Altitude x Film		0.653	0.147	0.172	0.032	0.051	0.903	0.547
	Altitude x Cultivar		0.493	0.65	0.280	0.428	0.502	0.295	0.256
	Film x Cultivar		0.634	0.337	0.143	0.703	0.707	0.654	0.432
	Film x Cultivar x Altitude		0.923	0.567	0.584	0.893	0.725	0.561	0.982

Table 3. Stomata conductance (gs) and Fv/Fm (maximal dark-adapted photosystem II efficiency) during growth of *Rosa* x *hybrida* cultivars grown under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (-UV) at different altitudes. Data are the mean values of measurements from two experimental repeats with five replications in each (n=10; $p<0.05$).

Altitude	Plastic film	Cultivar	Stomata conductance (mmol m^{-2} s^{-1})	Fv/Fm
High altitude	+UV	'Cygein'	149.7	0.79
		'Tom-Tom'	151.3	0.78
		'Snow white'	152.3	0.78
	-UV	'Cygein'	150.0	0.79
		'Tom-Tom'	152.0	0.79
		'Snow white'	154.0	0.79
Low altitude	+UV	'Cygein'	98.7	0.80
		'Tom-Tom'	96.7	0.81
		'Snow white'	92.3	0.80
	-UV	'Cygein'	100.0	0.81
		'Tom-Tom'	98.0	0.81
		'Snow white'	95.3	0.81
p-Values				
	Altitude		0.014	0.052
	Film		0.152	0.132
	Cultivar		0.05	0.329
	Altitude x Film		0.703	0.654
	Altitude x Cultivar		0.283	0.206
	Film x Cultivar		0.769	0.908
	Altitude x Film x Cultivar		0.823	0.709

3.2. Stomata conductance (gs) and fluorescence

Stomata conductance (gs) was significantly affected by altitude but not UV radiation and no interaction between altitude and UV radiation was found (Table 3). In general, plants (all cultivars) grown at high altitude had higher gs as compared to lower altitude during growth (Table 3; $p \leq 0.05$). The gs of plants were on average 1.8 times higher in high altitude as

compared to lower altitude regardless of the UV radiation (Table 3). Fv/Fm (maximal dark-adapted photosystem II efficiency; indicates plant stress) was slightly affected by the altitude difference, where plants grown at high altitude showed a slightly lower average value of Fv/Fm (0.785) than those grown at lower altitude (Fv/Fm= 0.80) (Table 3). Fv/Fm was not affected by UV radiation in any of the cultivars.

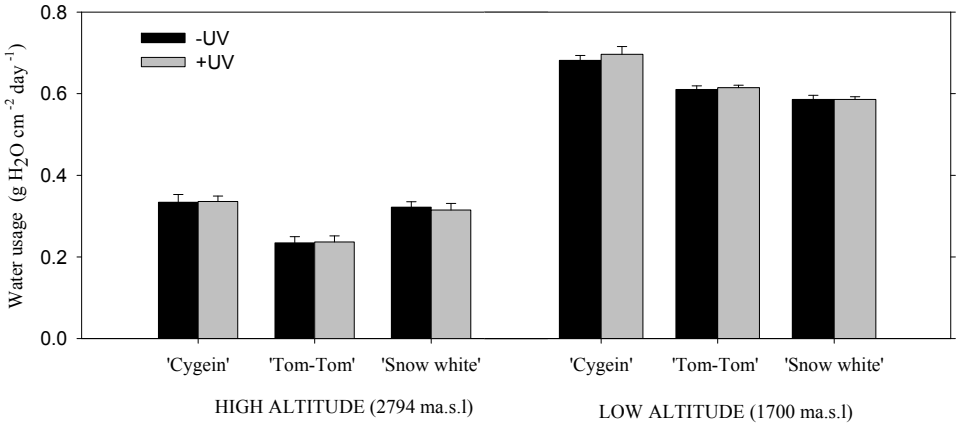

Figure 4. Postharvest water usage of *Rosa* x *hybrida* cultivars grown under different plastic coverings transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (-UV) at different altitudes. The water usage was measured gravimetrically every morning until the end of the postharvest life after plants from different treatment were moved to a common postharvest room with 58±5% RH (corresponding vapour pressure deficit (VPD) is 1.2 kPa), irradiance 35±5 µmol m^{-2} s^{-1} supplied by fluorescent tubes as 12 h/12 h of light/dark, and a temperature of 23±1.5°C. The error bars indicate the mean values of measurements from two experimental repeats with six replications in each (n=12).

3.3. Postharvest characters and water usage

Postharvest water usage was significantly higher in plants grown at low compared to high altitude; however the water usage was not affected by the UV radiation (Fig. 4). Plants grown at the lower altitude had twice as high water consumption than high altitude-grown plants (Fig. 4). There was also a significant difference in water consumption between cultivars; the cultivar Cygein used more water as compared to the other two cultivars at low altitude (Fig. 4). In line with this, in general, compared to lower altitude-grown plants, plants grown at high altitude had a longer postharvest life that also correlated with the postharvest symptoms recorded (Table 4).

Postharvest symptoms such as petal wilting and leaf drying were more prominent in low altitude-grown plants than high altitude (Table 4).

Table 4. Postharvest characteristics of *Rosa* x *hybrida* cultivars grown at different altitudes under different plastic coverings; transmitting UV-A and UV-B (+UV) or blocking UV-B and short UV-A (-UV). Postharvest life terminated when 50% of the leaves or petals showed the mentioned symptoms individually or in combination. Data are the mean values of measurements from two experimental repeats with six replications in each (n=12; $p<0.05$).

Altitude	Plastic film	Cultivars	Petal wilting (% of total)	Leaf wilting (% of total)	Petal necrosis (% of total)	Leaf drying (% of total)	Postharvest life (days)
High altitude	+UV	'Cygein'	63.1	41.2	25.1	57	11.3
		'Tom-Tom'	62.2	43.1	29.2	60	13.5
		'Snow white'	64.7	40.4	32.8	61	12.5
	-UV	'Cygein'	62.5	40.2	23	58	12.2
		'Tom-Tom'	65.2	41.3	28	65	14
		'Snow white'	66.5	42	30	62	13
Low altitude	+UV	'Cygein'	71.2	56.8	37.2	69.4	8.5
		'Tom-Tom'	72.5	56.2	39.5	68.5	9.6
		'Snow white'	71.3	59.3	41.3	69.2	8.3
	-UV	'Cygein'	70.2	55.2	35.5	68.2	8.0
		'Tom-Tom'	73.5	56.1	38.1	66.3	9.1
		'Snow white'	70.5	57.2	39.5	67.8	8.3
p-Values							
	Altitude		0.014	0.012	0.003	0.021	0.001
	Film		0.452	0.132	0.329	0.536	0.324
	Cultivar		0.245	0.329	0.042	0.482	0.568
	Altitude x Film		0.603	0.654	0.367	0.357	0.413
	Altitude x Cultivar		0.383	0.206	0.529	0.423	0.583
	Film x Cultivar		0.569	0.908	0.843	0.703	0.703
	Altitude x Film x Cultivar		0.348	0.706	0.809	0.349	0.708

4. DISCUSSION

Under natural conditions plants are exposed to different levels of UV radiation, especially UV-B, depending on geographic location, cloud cover, and solar altitude (Estupiñán et al., 1996; Rozema et al., 1997; Diffey, 2002). Even at the same geographical location and season the amount of UV-B reaching the ground varies with time of the day and time of the year and also depends on the interaction between UV-B and other climatic factors. In the present study we investigated the effect of UV radiation at different altitudes on growth, development and postharvest characteristics of pot roses. The UV-blocking film used in the experiment blocked all UV up to 350 (all UV-B and the short UV-A) while the +UV film transmitted the full UV range. Thus, the main difference between the two films is in the UV-B region (280-320) and the short UV-A (Fig 1).

UV-B radiation is a key environmental signal that regulates diverse processes in a range of organisms including plant morphology (Jansen, 2002; Jenkins, 2009). In the present study, UV radiation affected most of the vegetative growth variables at both altitudes. A 30-40% reduction in shoot length and LA were found under the UV-transmitting film compared with the treatment where UV was blocked (Table 2). The reduction in shoot length and vegetative growth is a typical UV-B response found in many different species e.g. such as lettuce, mung bean, maize, cucumber, grapevine and *Arabidopsis thaliana* (Krizek et al., 1997, Pal et al., 1997, Krizek et al., 1998; Jansen, 2002; Wargent et al., 2009; Berli et al., 2010; 2012). From this study, it is clear that all the rose cultivars tested responded similarly to UV radiation. The compact and shorter plants in +UV radiation was due to shorter internodes since the number of internodes was not affected by UV radiation (Table 2).

It has been demonstrated that LA is very sensitive growth parameters that easily respond to elevated UV-B due to reduced leaf formation and leaf expansion (Nogu´es et al., 1998, Zhao et al., 2003). Ballaré et al. (1995) and Grant (1999) also found that when plants were exposed to UV-B, the LA was lower because of both smaller leaves and a lower number of leaves. These morphogenic responses are possibly a part of the photomorphogenic acclimatization mechanism of the plants to reduce the interception of the UV-B (Jansen, 2002; Jenkins, 2009). Besides, according to Hectors et al. (2010), UV treatment did not affect cell number, cell shape, cell area variation, or stomata formation, rather the reduction in leaf size was solely due to smaller pavement cells, because of impaired cell expansion at an early stage of leaf development.

Number of days to flower opening was significantly affected by altitude and solar UV radiation in all cultivars. The longest flowering time (2 -3 weeks) was recorded at high altitude regardless of the UV radiation (Table 2). It is known that flower induction in roses is autonomous however flower development is promoted by increasing temperature and irradiance (Zieslin and Halevy, 1975; Mortensen et al., 1992). Temperature is well known to facilitate

flowering in many plant species (see review by van Doorn and van Meeteren, 2003). Shin et al. (2001) showed that in roses the number of days from bud break to flowering increased from 21.6 to 63.0 days as temperature decreased from 30 to 15°C. The number of days to flower was primarily influenced by the temperature after formation of a visible bud. This suggests that the temperature after visible bud formation significantly affects the rate of flower development and opening. Plants grown at higher altitude experienced lower temperature during development and this might have delayed flowering. Furthermore, plants grown under –UV radiation flowered 7-10 days earlier in both altitudes as compared to +UV radiation (Table 2). However, the flower induction might have occurred earlier in +UV radiation since they had fewer number of leaves at flower opening. The delay in flowering might be an indirect effect of UV radiation, because of reduced leaf area resulting in lower light capturing and lower dry matter accumulation. Carbohydrates are essential to flowering of plants (Bernier et al., 1993) and an important energy source facilitating flower opening (Ho and Nichols, 1977, Marissen and LaBrijn, 1995). In most species, the mobilization of storage carbohydrates and/or the import of sucrose accompanies flower opening (van Doorn and van Meeteren, 2003).

The effect of UV radiation on growth was more prominent at low altitude (with higher temperature) where the reduction in shoot length and LA was 10 -15 % higher than at high altitude, despite significantly higher UV-B level at high altitude (with lower temperature) (Table 1 and 2). This might be due to the interaction of UV-B with other climatic factor such as temperature. Temperature is one of climate factors known to affect shoot elongation and cell expansion (Moe and Heins, 1990; Berghage and Heins, 1991). The interactive effect of temperature and UV-B has been shown to affect plant growth in many species (Mark and Tevini, 1996; Kakani et al., 2003; Reddy et al., 2004). However, some studies showed contradictory responses of these interactive effects. Nedunchezhian and Kulandaivelu (1996) showed that in cowpea plants, UV-B damage was greater for plants grown at 30°C than for plants grown at 20°C. In contrast, the UV-B induced reduction in seedling growth of maize and sun flower was alleviated by 4°C increase in temperature from 28°C to 32°C (Mark and Tevini, 1996). Plants from high altitude and high latitude ecosystems where UV-B and cold temperatures are naturally simultaneous or subsequent stresses, are less sensitive to enhanced UV-B than plants from low UV-B locations (van de Staaij et al.,1995; Binder and L'-Hirondelle, 1999; Chalker-Scott and Scott, 2004). This is partly due to increased tolerance towards UV-B as a result of low temperature. There are evidences about cross-tolerance between different stressors such as UV-B, low temperature and drought (Manetas et al., 1997; Chalker-Scott and Scott, 2004; Poulson et al., 2006), where plants showed increased tolerance against UV-B, low temperature or drought because of increased acclimation to the other stressor (Chalker-Scott and Scott, 2004; Poulson et al., 2006). The Fv/Fm values measured in our experiment show that UV radiation has no significant effect on Fv/Fm, and no indication of stressed plant. The values at both altitudes are within the Fv/Fm value range of 0.8 ± 0.05 shown for healthy and sun adapted leaves (Critchley, 1998) (Table 3).

Altitude rather than the UV radiation affected gs in all the cultivars (Table 3). In general, plants (all cultivars) grown at high altitude on average had 1.8 and 1.3 times higher gs as compared to at lower altitude, regardless of the UV radiation (Table 3). The gs was measured in the middle of the day when the temperature and irradiance reach their highest levels. The higher transpiration at high altitude and vice versa in low altitude might be due to effect of other climatic factors such as RH (VPD) and temperature. The VPD and temperature measured at the high altitude were lower than at the lower altitude (Table 1). The lower gs measured at low altitude might thus be due to the higher VPD and higher air and leaf temperature that increase the transpirational flux, forcing the plants to close their stomata in order to conserve water. Plants developed under higher VPD (low RH) are well known to have low gs during growth (Arve et al., 2012). Although plant surface area and density of stomata per leaf area are the major factors influencing the rate of water loss in plant, it has also been reported that gs is also related to altitude or difference in air pressure in addition to VPD (Smith and Geller, 1979; Leuschner, 2000; Gale, 2004; Körner, 2007). There are also reports indicating that with increasing altitude stomata density also increases, which positively correlates with increased gs (Holland and Richardson, 2009).

In the present experiment the postharvest water usage and postharvest life was significantly affected by altitude but not UV radiation (Table 4; Fig 4). The water usage was related to the postharvest life and characteristics measured. Leaf wilting and leaf drying are typical postharvest characteristics for water stressed plants (Torre and Fjeld, 2001). Hence, plants grown at the lower altitude showed higher percentage of leaf drying and wilting which might be due to water stress because of high transpiration rate. This led to shorter postharvest life for low altitude plants as compared high altitude. The efficiency of plants water usage is dependent on the gs and assimilation rate (Beer et al., 2009). Lower altitude-grown plants lost more water per area through transpiration during postharvest storage than high altitude grown plants. It has also been shown that environmental conditions during cultivation influence postharvest quality of roses by affecting the ability to control postharvest water loss (Halevy and Mayak, 1979a, 1979b). Stomata behavior and water relations are one of the main factors demining the potential postharvest life of cut flowers as well as for some bedding and pot plants (Waterland et al., 2010a, 2010b). Also, the postharvest water loss can be dependent on the stomata behavior during growth e.g. RH (Torre et al., 2001; Fanourakis et al., 2012). For plants grown at high altitude, during growth they have been experiencing lower VPD (0.4 kPa) as compared to the lower altitude-grown plants (Table 1). However, when they were moved to an environment where the VPD is very high (VPD in postharvest room: 1.2 kPa) they probably respond by closing their stomata to avoid water loss. However, for plants developed at the lower altitude, there was no significant change in VPD during growth (VPD: 1.12 kPa) and postharvest (VPD: 1.2 kPa). Since these plants did not sense any stimuli to close their stomata after transfer to the postharvest test room they continued to transpire as usual. Dynamic physiological adjustments are required to respond to sudden environmental changes, for example by dynamically controlling stomatal

conductance, plants can effectively regulate long-distance water flow and water potential over short term (Hacke and Sauter, 1995; Laur and Hacke, 2013). Hence, in this experiment the ability of plants grown at high altitude to easily sense the changing environment and dynamically adapt to it by keeping their water balance and avoiding unnecessary water loss was a key factor for a better postharvest life.

In conclusion, UV radiation reduced shoot length and LA in both altitudes. However, stomata conductance, postharvest water usage and characteristics were rather affected by altitude differences than UV radiation. Hence, plants grown at higher altitude had a better control of water loss and a longer postharvest life than lower altitude-grown plants. UV radiation can induce a range of specific plant responses, some of which are particularly desirable from a horticultural perspective. However, from this particular study it is not recommended to use UV-transmitting plastic coverings during rose cultivation either at highland or lowland since it reduced the growth, increased discoloration of petals and delayed the flowering without improving the postharvest shelf life.

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