

*Full Length Research Paper*

## Effect of red and far-red light on inhibition of hypocotyl elongation in ecotypes of *Betula pendula* Roth

Berhanu A. Tsegay<sup>1\*</sup>, Jorunn E. Olsen<sup>2</sup>, and Olavi Junnttila<sup>3</sup>

<sup>1</sup>Department of Biology, Bahir Dar University, Ethiopia.

<sup>2</sup>Dept. of Biology and Nature Conservation, Agricultural University of Norway, N-1430 Ås.

<sup>3</sup>Department of Biology, University of Tromsø, Norway.

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Plants sense the quality, quantity, and duration of light signals and use them to optimise their growth and development. These signals are perceived by special light receptors of which the phytochrome pigment system is one of the most important for photomorphogenetic responses. Using special diodes that emit monochromatic light, we studied the effect of red (R), far-red (FR) and R+FR combinations on hypocotyl elongation of latitudinal ecotypes of *Betula pendula*. Continuous R and FR inhibited hypocotyl elongation equally, but inhibition was higher when seedlings were irradiated by continuous R+FR. In all cases, inhibition increased with increasing irradiance, from 0.75  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 76  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Moreover, seedlings treated by R or R+FR synthesised more anthocyanins than those exposed to FR. Accumulation of anthocyanins increased with increasing irradiance up to about 19  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

**Key words:** Anthocyanin, diode, ecotype, monochromatic, photomorphogenesis, photosynthetically active radiation, skotomorphogenesis.

### INTRODUCTION

Light, as a main environmental trigger, plays a central role in regulating plant development. Most terrestrial plants grow by selective absorption of natural light from the sun. The most effective components of the spectrum of light are red (R), far-red (FR), and blue. These lights are involved in the regulation of photosynthesis, pigment biosynthesis, photoperiodism, phototropism, and photomorphogenesis. The photomorphogenetic response of plants to light include, among others, seed germination, inhibition of hypocotyl elongation, cotyledon and leaf expansion, pigment (chlorophyll, anthocyanin) synthesis, stem elongation, and induction of flowering (Weller et al., 2000).

Seedling development in darkness and in light follows different programs, skotomorphogenesis and photomor-

phogenesis. In darkness, hypocotyl elongates rapidly after germination while the apical hook persists long and the cotyledons remain folded for weeks until the seedling gets light or dies. Proplast in the cotyledons become etioplasts and remain colourless until they are exposed to light and develop into chloroplasts. In light, seedling of a dicot species emerges from the seed coat with hypocotyl, apical hook, and two folded cotyledons. Within a short period of time, the hook starts to straighten, the cotyledons open and expand accompanied by chlorophyll development and greening. The hypocotyl becomes relatively stiff and keeps the cotyledons straight for optimal exposure to light. The apical tip undergoes active cell division and gives rise to the first true leaves. Under such conditions, the elongation of hypocotyls is inhibited. There are three possibilities by which hypocotyl elongation can be inhibited: by phytochrome A (phyA) in very low fluence (VLF) light, by phytochrome B (phyB) in low fluence (LF) light, or by phyA in high irradiance (HIR)

\*Corresponding author. E-Mail: [berhanutsegay@yahoo.com](mailto:berhanutsegay@yahoo.com).

Light (Shinomura et al., 2000; McNellis and Deng, 1995; von Arnim and Deng, 1996).

Once a seedling develops all the necessary organs and start to assimilate, its growth and maintenance requires an appropriate spectral quality for a specific period of time in a day. In Temperate Zone woody plants, short days (SD) induce growth cessation and bud dormancy. However, when SDs are extended with light extensions of appropriate spectral composition, plants perceive them as LDs. Such perception depends on the light requirement of those plants under consideration. Latitudinal ecotypes of northern tree species have different responses to light quality (Håbjørg, 1972; Junttila and Kaurin, 1985; Clapham et al., 1998), which could indicate differences in composition of their phytochrome systems. In experiments using light sources enriched in R or FR light, differences in FR light requirement by latitudinal ecotypes have been indicated. Such studies, however, have not been done on inhibition of hypocotyle elongation in *Betula pendula*. Moreover, although phytochrome genes have been cloned from both conifers and deciduous species, so far there is no information about possible differences between photoperiodic ecotypes at the phytochrome level (Olsen et al., 2002). This study aimed to compare hypocotyle growth responses of latitudinal ecotypes of *B. pendula* to monochromatic R and FR and to combinations of R and FR. Production of anthocyanins were also quantified from seedlings of the various treatments and compared.

## MATERIALS AND METHODS

### Light Treatments

Light emitting diodes (Q-BEAM 2200, Quantum Devices, Inc. 112 Orbison Barneveld, WI 53507, USA) were used as sources of R and FR lights. In these light sources, both irradiances and R: FR ratios could be changed independently. Photon fluence rates of R with maximum emission at about 667 nm were measured using a Datalogger (Model L<sub>1</sub> - 1000 Serial No. LDL 3527, USA) with the light sensor, LI-CDR Quantum, Q2012 attached to it. Fluence rates of FR at maximum emission of 739 nm were adjusted in relation to the quantified R. This was possible by switching off FR, turning on the radiometric control fully to R, and adjusting the required intensity. Then, after measuring the intensity of R, switching it off at a fixed intensity and turning FR on at the adjusted intensity gave the required irradiance of FR. These light treatments were established in trolleys with a size of 50 x 50 cm. The light source heads of the LEDs were mounted on a board at about the top-center of each trolley, providing almost uniform intensities at various parts of the trolleys. A fourth trolley was prepared for dark control. All trolleys were covered on all sides by curtain to prevent possible entrance of light beams from one trolley to the other. Effects of monochromatic R and FR, and R+FR were studied at 0.75, 9.5, 19.0, 37.5, and 76  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

### Hypocotyl elongation

Stratified seeds of the 3 ecotypes of *B. pendula* (northern, 67° N, intermediate, 64° N and southern, 59° N) were sown on peat: perlite and sand (6:3:1) in 10-cm pots. In order to enhance the germina-

tion, seeds were exposed briefly to white light, after which they were transferred to different light treatments (continuous R, FR, R+FR) or dark. Based on preliminary experiments and observation on time course of hypocotyl elongation, the experiments were terminated after 13 days from sowing (about a week after germination). Hypocotyls of about 30 longest seedlings were measured using a millimetre scale (square paper). The actual length measured was the distance between the root-hypocotyl transition (at soil surface) to the openings of the cotyledons or bases of apical hook.

### Analyses of anthocyanins

After measuring hypocotyl elongation, seedlings were collected in 15 ml of acidified (1% HCl) 80% methanol (Mancinelli and Rabino, 1975). They were extracted in dark for 3 - 4 days at about 0 - 0.5 °C and filtered. Vials and filter papers used were washed with 3 ml of 80% methanol. Absorbance of extracts was read at 530 nm (Weller, et al., 2000) in Microplate Spectrophotometer (Sunnyvale CA 94089, Molecular Devices, USA) using soft max software (BIO. RAD Smartspec 3000). Absorbance of dark grown seedlings was subtracted from each treatment. The possible contribution by chlorophylls and their acid degradation products (Burgin et al., 1999; Mancinelli and Rabino, 1975; Mancinelli, et al., 1975) was not assayed and subtracted. Results reported are extracts from about 30 seedlings of each ecotype.

### Statistical analyses

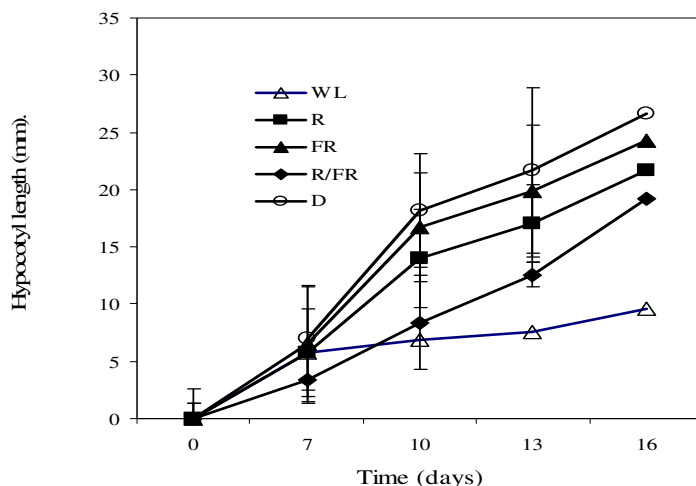
Statistical analyses were carried out using Abacus Concepts (Haycock et al., 1994), Stat View (Abacus Concepts, Inc., Berkeley, CA, 12994). PLSD<sub>5%</sub> or standard deviations or standard errors are presented.

## RESULTS

### Hypocotyl elongation

The appropriate age of seedlings for harvesting was determined on preliminary studies with the northern ecotype. Seedlings were grown for about 9 days after germination (16 days since sowing). Because some of the elongated seedlings started to collapse at day 16, measurements on subsequent experiments were done 13 days from sowing. Time course for hypocotyl elongation in the northern ecotype (at low fluence) showed that the magnitude of inhibition increased from FR through R to their mixture (R+FR), when compared to the dark control (Figure 1). As shown in Figure 1, combined treatments with R+FR resulted in a significant inhibition of hypocotyl elongation. Under white light (WL), hypocotyl elongation stopped already within about a week from sowing (Figure 1), but in darkness and under low irradiance treatments, elongation continued during the whole experiment. Response to white light may be, however, not only due to a different spectral composition but also due to a higher irradiance, 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  compared to about 1  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in R, FR, and R+FR treatments.

In the experiment shown in Figure 2, seedlings were grown under different intensities of continuous R, FR, or



**Figure 1.** Time course of hypocotyl elongation in the northern ecotype of *B. pendula*.

**Table 1.** Effect of ecotype and light quality (average from irradiances of 0.75, 9.5, 25.0, and 76.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) on elongation of hypocotyls of latitudinal ecotypes of *B. pendula*.

Ecotype	Hypocotyl length (mm)				
	Dark	FR	R	R+FR	Mean $\pm$ SD
Southern	27.2	20.8	20.0	16.8	19.2 $\pm$ 2.0
Intermediate	22.6	17.9	18.1	14.6	16.9 $\pm$ 1.9
Northern	28.1	22.8	22.7	17.9	21.2 $\pm$ 2.7
Mean	26.0	20.5	20.3	16.4	20.8 $\pm$ 3.9
Relative Effect (%)	100	78.6	77.9	63.1	

R+FR for 13 days. At lowest intensity used (0.75  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), there was less inhibition of hypocotyl growth by FR than by R or R+FR in the northern and southern ecotypes. However, the effect of FR equated to that of R at higher irradiances. The declining rates of hypocotyl elongation due to the monochromatic lights (R and FR) were statistically similar throughout the irradiances tested. Inhibition of elongation increased with increasing irradiance in all treatments (Figure 2A), but it was much higher when seedlings were grown in increasing irradiances of continuous R+FR than in the monochromatic lights. For example, R+FR at 0.75  $\mu\text{mol m}^{-2} \text{s}^{-1}$  inhibited elongation by about 24, 25, and 27%, while at 76  $\mu\text{mol m}^{-2} \text{s}^{-1}$  the percent inhibition was increased to 51, 55, and 45% in the southern, the intermediate and the northern ecotype, respectively (Figure 2B).

The mean hypocotyl length of all treatments (average) was 19.2, 16.9, 21.2 mm for the southern, the intermediate and the northern ecotype, respectively (Table 1). The differences of these means are significant at 95% confidence level with a critical difference of 1.01 in the southern and the northern ecotypes. In darkness, hypocotyl elongation of the intermediate ecotype was

significantly shorter than the southern and the northern. R, FR, and R+FR inhibited hypocotyl elongation of all ecotypes compared to their growth in dark (Table 1). However, combined irradiation (R+FR) affected hypocotyl elongation significantly more than R or FR alone (critical difference 3.37 at  $p = 0.05$ ).

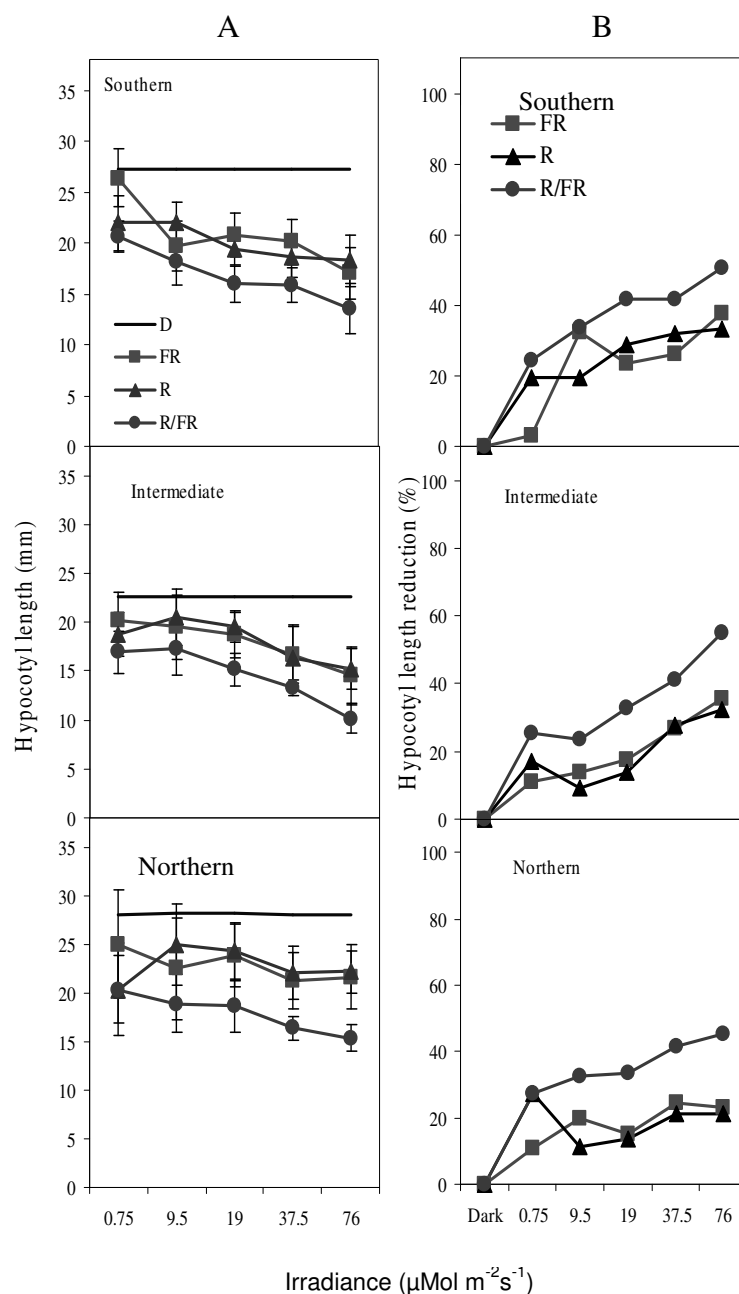
There was a significant interaction between ecotype and irradiation as well as between light quality and irradiation (Table 2). This interaction was due to the results at the two lowest irradiances (Figure 2). As shown in Figure 2, results at 0.75  $\mu\text{mol m}^{-2} \text{s}^{-1}$  appear to deviate from the general trends, and this deviation could be caused by unknown experimental errors.

Linear regressions against irradiances of R, FR or R+FR were significant for the southern and the intermediate ecotypes:

(Y= Hypocotyl length, mm; and X = Irradiances,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ):

Southern ecotype:

FR	$Y = 84.3 - 0.30X$	$R^2 = 0.424$	$P = 0.04$
R	$Y = 78.8 - 0.19$	$R^2 = 0.549$	$P = 0.01$
R + FR	$Y = 70.6 - 0.31$	$R^2 = 0.705$	$P = 0.002$



**Figure 2.** Effect of R and FR light on elongation of hypocotyls in latitudinal ecotypes of *B. pendula*.

**Table 2.** Analysis of the variance of hypocotyl elongation latitudinal ecotypes of *B. pendula* showing the main effects of ecotype irradiance and light quality, and the interactions between them.

Source of variation	DF	Sum of Squares	Mean Square	F-Value	P-Value
Irradiance	5	998.3	199.7	71.392	<0.0001
Ecotype	2	312.3	156.2	55.834	<0.0001
Light Quality	2	230.5	115.2	41.200	<0.0001
Irradiance * ecotype	10	83.7	8.4	2.991	0.0045
Irradiance * LightQuality	10	130.5	13.1	4.665	<0.0001
Ecotype * Light Quality	4	11.5	2.9	1.032	0.3993
Irradiance *ecotype*Light Quality	20	39.0	2.0	0.697	0.8111
Residual	54	151.0	2.8		

Intermediate ecotype:

$$\begin{array}{llll} \text{FR} & Y = 87.9 - 0.33X & R^2 = 0.656 & P = 0.005 \\ \text{R} & Y = 87.9 - 0.28X & R^2 = 0.728 & P = 0.002 \\ \text{R + FR} & Y = 76.1 - 0.43X & R^2 = 0.793 & P = 0.0006 \end{array}$$

The differences between the regressions of the two ecotypes against R and FR were so small that the total data for the two treatments at all irradiances gave a highly significant regression:

$$Y = 84.7 - 0.28X \quad R^2 = 0.515; \quad P < 0.0001$$

Similarly, combined data of the southern and the intermediate ecotypes for R+FR treatment gave a highly significant correlation between irradiance and hypocotyls length.

$$Y = 73.3 - 0.37X; \quad R^2 = 0.735 \quad P < 0.0001$$

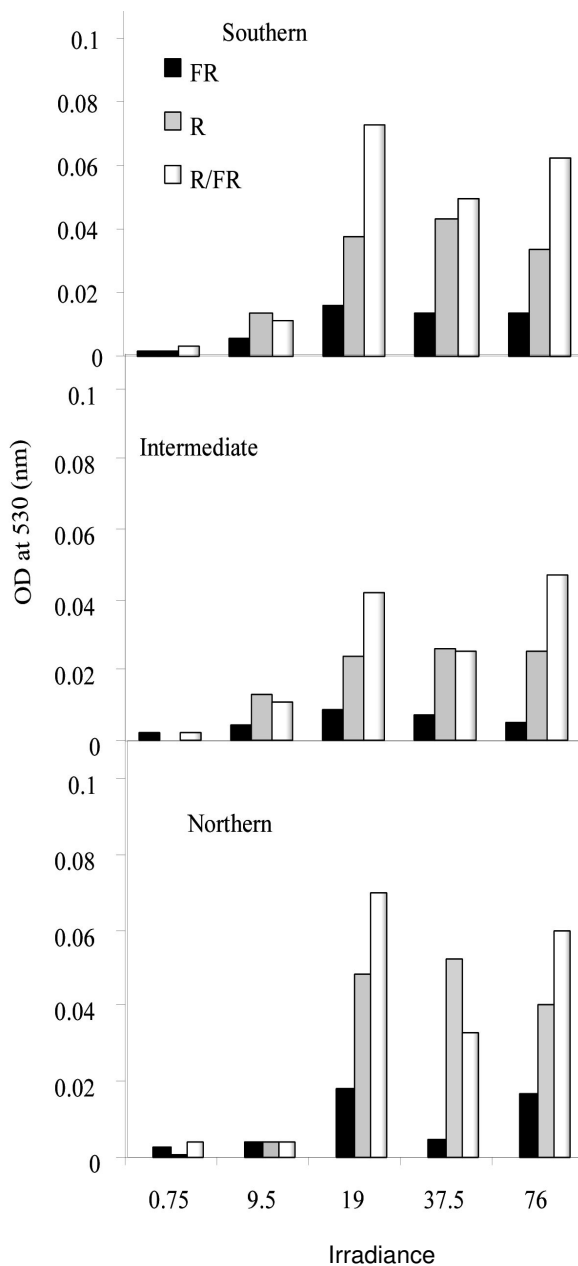
On the other hand, linear regression between hypocotyl length of the northern ecotype and irradiances was insignificant for R ( $P=0.84$ ) and for FR ( $P=0.79$ ), but significant for R+FR:

$$Y = 70.5 - 0.23X \quad R^2 = 0.675 \quad P = 0.004$$

These analyses indicate that the northern ecotype responded differently than the two ecotypes.

## Anthocyanin production

Anthocyanin synthesis in *B. pendula* ecotypes was dependent on both light quality and irradiance (Figure 3). Accumulation of anthocyanin increased when seedlings were grown under R, while FR had a minor effect. However, the mixture of the monochromatic R and FR was in some cases more effective than R alone. Accumulation of anthocyanins in R and R+FR treatments increased with increasing irradiance up to  $19 \mu\text{mol m}^{-2} \text{s}^{-1}$ . At irradiances of  $19 \mu\text{mol m}^{-2} \text{s}^{-1}$  and above there was more than two-fold accumulation of anthocyanin compared to lower irradiances ( $0.75 - 9.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Irradiances above  $19 \mu\text{mol m}^{-2} \text{s}^{-1}$  did not appear to enhance anthocyanin production (Figure 3). Comparison between the ecotypes showed that the average pigment level at irradiances of 19.0, 37.5, and  $76.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  was less in the intermediate ecotype than in southern and northern ecotypes. Means of the three highest irradiances were 0.038, 0.023, and 0.038 for the southern, intermediate, and the northern ecotype, respectively. However, the trend of anthocyanin accumulation was similar in all three ecotypes. There was an indication of an effect of R and R+FR even at an irradiation of  $9.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the southern and the intermediate ecotypes but not in the northern one. As shown in Figure 3, the threshold level for induction of



**Figure 3.** Effect of R and FR on the levels of anthocyanins in hypocotyls of three latitudinal ecotypes of *B. pendula* seedlings. Anthocyanins were extracted for three to four days in acidified methanol from about 30 seedlings and absorbance at 530 nm was recorded. OD of dark controls (0.028, 0.03, and 0.03 for the southern, the intermediate and the northern ecotype, respectively) have been subtracted.

anthocyanin synthesis in *B. pendula* hypocotyls was between  $9.5$  and  $19 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

## DISCUSSION

Although the inhibition of hypocotyl elongation by R, FR, or R+FR at lower light intensities was not more than 50%

of the dark control, all light treated seedlings were de-etiolated. However, there were marked differences in morphology and pigmentation. Seedlings treated by LDs of PAR light (white light, WL) were deep green with their cotyledons fully opened and elongation of their hypocotyls fully inhibited, being short, brown, and stiff. They had well-developed roots and also development of new true leaves was initiated. Seedlings grown in R+FR light were also fully de-etiolated. The opened cotyledons were not as deep green as in the plants grown under continuous WL. While the cotyledons of seedlings grown in R or in FR were also opened, the R-grown seedlings were pale green and the FR-grown ones yellowish. Compared to WL-grown seedlings, R-grown and FR-grown ones had less developed roots. On the other hand, seedlings grown in darkness were fully etiolated with very rudimentary rooting. In general, root growth seemed to increase from dark through FR, R to R+FR and, finally to WL treatments. These morphological observations suggest possible influence of photoperiod and/or light quality not only on inhibition of hypocotyl elongation, but also on root growth. Correll and Kiss (2002) discussed the interaction of photoperiod and gravity on development of plant organs: shoot, root, and hypocotyls.

Inhibition of hypocotyl elongation in all ecotypes was higher under combined R+FR treatments compared to corresponding treatments with monochromatic lights (Figure 2). This is reasonable assuming the involvement of both phytochromes, phyA and phyB, in mediating light effects on hypocotyl growth. Continuous FR and continuous R are mediated predominantly by phyA and phyB, respectively. There are two possible ways for these phytochromes to mediate the inhibition of hypocotyl elongation. One way is by acting independently. When phyA and phyB are irradiated with narrow bands of FR and R, respectively, their role in inhibiting hypocotyl elongation might not interfere with each other (Shinomura et al., 2000). On the other hand, if broad bands of R and FR are irradiated simultaneously, it is possible that both phytochromes can be functional in both lights and as a result, interaction and "interdependence" may occur. Such an interaction (in broad light bands) between phyA and phyB components ( $B_1$  and  $B_2$ ) in the process of anthocyanin accumulation in tomato seedlings have been reported by Weller et al. (2000). In this work, the light bands used ranged between 620 to 700 nm with maximum at 667 nm for R, and 690 to 770 nm with maximum at 739 for FR. Hence, the present results indicate that R and FR affected hypocotyl elongation in an additive manner at the tested irradiances. This was clearly shown in Table 1 where the average inhibition of elongation by monochromatic R or FR was about 21% as compared to the 37% average inhibition of elongation by R+FR. Moreover, the joint effect of multiple light receptors in inhibiting hypocotyl elongation is well illustrated in LD WL-grown seedlings (Figure 1). In these seedlings, in addition to the R+FR, blue light and possibly

UV-A might also have played a great role in inhibiting elongation of the hypocotyls to such an extent. Inhibition of hypocotyl elongation increased with increasing doses of R, FR, and R+FR (Figure 2). Likewise, accumulation of anthocyanins generally increased with increasing intensities of FR, R, and R+FR, but the effect of FR light was very small (Figure 3).

Light signals cause the conformational change of Pr to Pfr and vice versa, and the active phytochrome generates signals that repress the constitutive photomorphogenetic repressors (for example, COP, DET, FUS) (Von-Arnim and Deng, 1996; Kircher, et al., 1999; Gill et al, 2000; Wang et al., 2001). Consequently, repression of gene expression by dark is removed and genes involved in the inhibition of hypocotyl elongation, and syntheses of anthocyanins are expressed. The fact that inhibition of hypocotyl elongation and accumulation of anthocyanins increased with increasing irradiance might entail that more fluence is required for higher rate of turnover between Pr and Pfr leading to continuous production of signals and persistent de-location of the photomorphogenetic repressors to cause the required responses.

In conclusion, R and FR inhibited hypocotyl elongation equally, but much less compared to their combined effect. The higher inhibition of hypocotyl elongation in seedlings by R+FR clearly indicated the combined effect of phyA and phyB in all *B. pendula* ecotypes. On the other hand, FR had little influence on the accumulation of anthocyanins when compared with R or R+FR. Moreover, accumulation of anthocyanin increased when the intensities of R and R+FR increased from 0.75 to 19  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

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