RUNNING TITLE: Auxin control of pea stem growth in darkness and light

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TITLE: The involvement of indole-3-acetic acid in the control of stem elongation in dark- and light-grown pea (*Pisum sativum*) seedlings.

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Abstract

We investigated the role of auxin on stem elongation in pea (Pisum sativum L.) grown for 10 d in continuous darkness or under low irradiance blue, red, far red and white light. The third internode of treated seedlings was peeled and the tissues (epidermis and cortex + central cylinder) were separately analysed for the concentration of free and conjugated indole-3-acetic acid (IAA). Under red, far red and white light internode elongation was linearly related with the free IAA content of all internode tissues, suggesting that phytochrome-dependent inhibition of stem growth may be mediated by a decrease of free IAA levels in pea seedlings. The correlation between IAA and internode elongation, however, did not hold for blue light-grown seedlings. The hypotesis that the growth response under low irradiance blue light might be correlated with the lack of phytochrome B signalling and changes in gibberellin metabolism is discussed in view of current knowledge on hormonal control of stem growth.

Abbreviations: ABA, abscisic acid; B, Blue light; cry1, Cryptochrome 1; cry2, Chryptochrome 2; D, Darkness; DAS, Days after sowing; FR, Far red light; FW, Fresh weight; GA, gibberellin; IAA, indole-3-acetic acid; phyA, Phytochrome A; phyB, Phytochrome B; R, Red light; W, White light

Introduction

Growth and development of higher plants are complex phenomena, which are regulated by the concerted action of several environmental factors. Among these, light plays a crucial role, being able to induce both quantitative changes in growth and differentiation of cells and organs (Jones, 1992). Irradiance and spectral composition of light influence the developmental pattern. White light induces a decrease in stem growth rate of seedlings. Blue, R and FR are responsible for inhibition of cell elogation, with the corresponding photoreceptors initiating a signalling cascade that transduces the light signal (Fankhauser and Staiger, 2002). Activation of phytochromes and cryptochromes dramatically reduces the rate of hypocotyl elongation when an Arabidopsis (*Arabidopsis thaliana*

(L.) Heynh.) seedling grown in darkness is illuminated. Extensive research on the subject has led to the general view that phyB is responsible for inhibiting hypocotyl elongation in R, whereas FR-induced inhibition is mediated by phyA. Under high-irradiance B growth is inhibited mostly by cry1, while the related cry2 receptor functions similarly in low-irradiance conditions (Parks et al., 2001). However, much more complexity underlies the proposed scheme and several evidences demonstrate that the action of the main photoreceptors may vary, depending on plant species, stage of development, light wavelength, irradiance, duration and timing of illumination. In pea (*Pisum sativum* L.), phyA contributes significantly to R responses and phytochromes also function in B signalling: phyA is the major photoreceptor controlling de-etiolation responses to low-irradiance B, whereas both phyA and cry1 play a role under high-irradiance B. There is also a strong contribution of phyB to de-etiolation under high-irradiance B, while cry2 is unlikely to play a major role in de-etiolation of pea seedlings (Platten et al., 2005). Experiments with photoreceptor mutants of pea have highlighted a somewhat anomalous role for phyA, which may interfere with cry1 to reduce the effectiveness of high-irradiance B for inhibition of stem elongation (Weller et al., 2001). Such behaviour has not been described in other species.

It is widely believed that light perception by photoreceptors and their complex mutual interactions are transduced through signalling pathways involving plant growth regulators. In pea seedlings, the rapid inhibition of stem elongation, which is part of the de-etiolation process, is accompanied by a dramatic fall in the levels of the bioactive gibberellin GA_1, while in a later phase reduced elongation rates appear to be maintained by a reduced response to the hormone (O'Neill et al., 2000; Symons and Reid, 2003). While GA_1 is regarded as the prime regulator of stem height in pea plants (Reid, 1987), IAA is known to be active in promoting the growth of isolated stem segments. Nonetheless, in a range of pea genotypes differing in height, there is a close correlation between height and IAA level, even in slender peas that are ultratall regardless of their GA content (Law and Davies, 1990). Beyond its role in shoot elongation, the potential of auxin to mediate phytochrome responses has received considerable attention. In etiolated *Avena*, *Zea* and *Oryza*

seedlings, R inhibits mesocotyl growth and this correlates with a reduction in the supply of auxin from the coleoptile (Iino, 1982). A correlation between the levels of IAA and the phytochrome-mediated changes in stem elongation rate has been also observed in *Nicotiana plumbaginifolia* Viv. (Kraepiel et al., 1995), while Volmaro et al. (1998) suggested that hypocotyl elongation in etiolated seedlings of *Lactuca sativa* L. is controlled, under B, by a balance of hormones, namely IAA, GAs and ABA. Detailed studies on dwarf (*le* mutants) and tall pea seedlings (Behringer and Davies, 1992; Behringer et al., 1992a; Behringer et al., 1992b) suggest that phytochrome may influence stem growth, at least in part, by regulating IAA levels in the epidermis. This tissue is generally considered a control point for stem elongation (Kutschera, 1989), but there are evidences in striking contrast with such conclusion (Cleland, 1991; Rayle et al., 1991). Some questions are still open about the long-term effects of light treatments on pea stem growth and on IAA levels and metabolism in elongating tissues, and on the relationship between B signal and IAA. Our investigation was aimed at studying the effects on stem elongation of continuous D, different monochromatic lights (B, R and FR) or W at low irradiances and at studying the role of IAA in mediating the growth responses of pea seedlings, throughout a relatively prolonged period (10 d). Growth measurements and hormonal analyses were carried out on internode tissues, because the elongation responses were more clearly observed on these stem sections (Morelli and Ruberti, 2000). We separately analyzed epidermal tissues and the resulting peeled stems to probe the role of the former tissue as a target for auxin action and as a controller of internode elongation, thus allowing comparisons to be made with published data. Both free and bound IAA were quantified, because conjugation may be a major mechanism by which light regulates IAA levels (Bandurski et al., 1977). This experimental approach may allow to gain more insight into the dynamics of IAA and its distribution in tissues.

Materials and methods

Plant material and light treatments

Seeds of pea cv. ‘Rondo’ (Royal Sluis, Enkhuizen, The Netherlands) were sown in vermiculite and grown at 20-22 °C under constant lights of different wavelengths or in continuous D. White light was supplied by Philips TL 40W/33RS fluorescent tubes (Philips, Eindhoven, The Netherlands), with an irradiance of 8 μmol m^{-2} s^{-1}. Red light was provided by filtering the light from Philips TL 20W/15 fluorescent tubes through a 27 Roscolux film (Rosco, Stamford, Connecticut, USA), with an irradiance of 4.1 μmol m^{-2} s^{-1}. The light from Philips TL 20W/18 fluorescent tubes was filtered through a 83 Roscolux film, thus yielding B with an irradiance of 4.1 μmol m^{-2} s^{-1}. According to Platten et al. (2005) this irradiance is sufficient for B to expose the action of cry1 and cry2. Far red light was supplied by filtering the light from Sylvania VHO F40T12/232 fluorescent tubes (Osram-Sylvania, Danvers, Massachusetts, USA) through a 823 Roscolux film, with an irradiance of 4.1 μmol m^{-2} s^{-1}. Irradiances were measured by a LiCor 1800 spectroradiometer (LiCor, Lincoln, NB, USA). The spectral distribution of the different light sources is given in Lercari et al. (1999). Control seedlings were grown in D. Manipulation of dark-grown plants was carried out under dim green light. Growth measurements started 3 DAS and were repeated at 24 h interval. The last set of data was collected 10 DAS. All measurements started at 9 A.M. of each sampling day. Immediately after the last measurement (10 DAS) the third internode from all seedlings was cut and the epidermal tissues were separated from the inner cortical and central cylinder tissues by gently peeling by jeweller’s forceps. According to Behringer and Davies (1992) we assumed that, by that time, the third internode of pea seedlings was still growing, therefore it was still light-responsive. The isolated tissues were weighed and frozen in liquid nitrogen until auxin analysis. The experiments were carried out in triplicate: at each time point 3x20 seedlings were measured for each light treatment and the corresponding results were averaged. The three mean values calculated per thesis were analyzed by one-way ANOVA ($P < 0.05$) to compare growth data among light treatments, for each sampling time. Mean separation was performed by Tukey's multiple comparison test.

Extraction and purification of free IAA

Frozen epidermal peels (an average of 0.4 g FW per treatment) and cortical plus central cylinder tissues (an average of 1 g FW per treatment) were grounded with mortar and pestle and extracted with cold 80 % (v/v) aqueous acetone (1:10 w/v). The homogenates were supplemented with a suitable amount of [^{13}C]$_6$IAA (Cambridge Isotope Laboratories, Andover, MA, USA) as internal standard, then stirred for 12 h at 4 °C and centrifuged at 4,000*g* for 15 min. The residues were re-extracetd twice, the supernatants were pooled and the pellets temporarily stored at –20 °C. Supernatants were reduced to aqueous phase under vacuum at 35 °C; they were adjusted to pH = 2.8 with hydrochloric acid and partitioned 5 times against equal volumes of peroxide-free diethyl ether. The extracted aqueous phases were stored at 4 °C for a short time (about 1 h). The pooled diethyl ether extracts were kept at –20 °C overnight, to freeze and separate traces of water. Diethyl ether was then evaporated under vacuum and the dried samples were resuspended in a small volume of 0.5 % acetic acid in 20 % acetonitrile and purified by HPLC.

Hydrolysis of conjugates

The aqueous phases after diethyl ether partitions were pooled with the extracted pellets and hydrolyzed in 1 mol L^{-1} sodium hydroxide following the addition of a suitable amount of [^{13}C]$_6$IAA as internal standard. Hydrolysis was continued for 1 h at 27 °C (Nowacki and Bandurski, 1980) in a capped vial continuously purged with He. To prevent the formation of artifactual IAA by the conversion of indolic compounds to IAA (Bialek and Cohen, 1989), a gas purifier (Supelco, Bellefonte, Pennsylvania, USA) was inserted on the gas line to trap any residual O_2. Following hydrolysis, samples were centrifuged at 13,000*g* for 30 min at 4 °C. The supernatants were acidified with hydrochloric acid to pH = 2.8 and partitioned against diethyl ether as previously described. The residual aqueous phases were pooled with the centrifugation pellet and hydrolyzed for 4 h at 100 °C in 7 mol L^{-1} sodium hydroxide (Bialek and Cohen, 1989), following the addition of a new

aliquot of [^{13}C]$_6$IAA as internal standard. At the end of this hydrolysis, samples were processed as described above (*i.e.* they were centrifuged and partitioned with ether) to separate free IAA.

HPLC

Samples were purified by reverse phase HPLC using a Waters 501 (Waters, Milford, MA, USA) instrument equipped with a SpectroMonitor 3200 UV detector (LDC Analytical, Riviera Beach, Florida, USA) operating at 280 nm wavelength. An ODS column (Hypersil, Runcorn, Cheshire, UK) 150 x 4.5 mm ID particle size 5 μm, eluted at a flow rate of 1 mL min^{-1}, was used. Samples were applied to the column and fractions were collected while the column was being eluted with 0.5 % acetic acid in 20 % acetonitrile. The fractions corresponding to the elution volume of standard IAA were reduced to a small volume and transferred to capillary tubes, dried thoroughly and silylated with bis(trimethylsilyl)trifluoroacetamide containing 1 % trimethylchlorosilane (Pierce, Rockford, Illinois, USA).

GC-MS

GC-MS analysis was performed on a Saturn 2200 quadrupole ion trap mass spectrometer coupled to a CP-3800 gas chromatograph (Varian) equipped with a CP-Sil 8 CB Low Bleed/MS capillary column (25 m x 0.25 mm ID x 0.25 μm film thickness) coated with 5% phenyl, 95% dimethylpolysiloxane (Varian). Samples were introduced in the instrument by split/splitless injection at 250 °C. The temperature of the transfer line between the gas chromatograph and the mass spectrometer was set at 250 °C. Full scan mass spectra were obtained in EI$^+$ mode with an emission current of 30 μA, an axial modulation of 4 V and the electron multiplier set at –1300 V. Data acquisition was from 70 to 350 Da at a speed of 1.4 scan s^{-1}. The following ions were monitored for IAA analysis: m\z 202 and 319 for IAA, and 208 and 325 for ^{13}C-labelled internal standard. Identification and quantitation of the analytes were confirmed by tandem MS, by simultaneous dissociation of the ions 202 for IAA and 208 for ^{13}C-labelled internal standard under a

resonant waveform with an excitation amplitude of 0.6 V. Quantitation of IAA was carried out by reference to a calibration plot obtained from the GC-MS analysis of a series of mixtures of the standard hormone with its labelled form. Each individual sample was run three times on the GC-MS and the corresponding values were averaged. As described above, the experiments were carried out in triplicate. For each light treatment, one sample was taken from each replication (each made of 20 seedlings) and separately extracted and analyzed. Thus, 3 values per thesis were obtained and analyzed by one-way ANOVA. Means were compared by Tukey's multiple comparison test. The statistical analyses were separately performed on data of free, ester- and amide-conjugated IAA; within each of these groups, data of the whole internode were compared separately from those of the epidermis. Hormonal results were reported as concentrations of free and conjugated IAA in the epidermis and in the whole internode. The latter data were obtained by pooling the results of the analyses separately performed on epidermal and on cortical plus central cylinder tissues.

Results

Seedling growth

The effect of D and of different light wavelengths on the growth of pea seedlings was investigated throughout a period of 10 DAS. Plant growth was strongly influenced by both D and light wavelength. Figure 1 shows the time course of total stem length. Seedlings grown in D scored the highest value, albeit only at the end of the experiment. Until 6 DAS, stem elongation of etiolated seedlings was not significantly different from that of B-grown ones. By the same time, FR-, R- and W-grown plantlets had elongated to a similar extent, although significantly less than D- and B-grown plantlets. The final seedling growth response showed that the treatments with W and R were the most effective in lowering stem elongation and the extent of their induced inhibition was not significantly different from each other. Far red light was statistically less effective than R and W for growth inhibition, yet FR-grown seedlings were significantly shorter than B-grown ones at the end of the experiment. The growth response of the third internode from treated seedlings is shown in

Fig. 2. Measurements of this stem segment first became possible 5 DAS. The third internode of D-grown seedlings showed the greatest final length, although the effect of etiolation on the growth of this part of the stem was not evident just 2 d earlier, *i.e.* at d 8. Statistically, B was the least effective light treatment for inhibiting the elongation of the third internode and this also became evident in the last 2 d of the experiment. Overall, at 10 DAS the elongation of the third internode showed the same rank as that of the whole stem with respect to the treatment administered and the statistical analysis confirmed this correspondance. Therefore, the growth of the third internode may be considered representative of the growth response of the entire stem.

Auxin analyses

The epidermis and the cortical + central cylinder tissues were separately analyzed for their auxin content. From these data, we also calculated the auxin concentrations in the whole internode, by pooling the hormone levels of epidermis + cortical tissues + central cylinder, in order to highlight any putative correlation between internode growth response and its overall auxin content, with respect to the light regime administered. As the elongation of the third internode 10 DAS was representative of that of the whole stem, the comparison between the growth response and the auxin levels was carried out only on the data relative to the third internode. The concentration of free IAA in the epidermis 10 DAS was higher in etiolated seedlings (Table 1), that also were more elongated than light-treated ones (Fig. 2). Despite this, we could not observe any correlation between the free auxin levels in the epidermis and the growth response of the third internode in light-grown seedlings: for instance, the third internode of R-treated peas showed the highest level of free IAA, but its elongation was limited. The relative contribution of the epidermis to the free IAA concentration of the whole internode was varying and there was not a clear relationship with the light regime. In D and R nearly 80 % of the entire internode free IAA was found in the epidermal peels, while in B, FR and W such value ranged from 47 % to 56 % (Table 1). A different picture emerges when comparing the growth response of the third internode with changes of free IAA in

the whole internode, instead of the sole epidermis. In this way, a correlation was found between internode final length and its free auxin level: the r^2 value was 0.9806 ($P < 0.01$) when the analysis did not take into account B-grown plants. Hormone concentrations ranked in the same order as growth data (Table 1 and Fig. 2), albeit a notable exception exists: seedlings grown under B were characterized by the lowest free IAA levels in both the epidermis and the whole internode, despite they were the most elongated among the light-treated ones. Conjugated IAA was analyzed by releasing IAA molecules from their conjugate moieties. This was achieved by two consecutive basic hydrolyses: the hydrolysis with 1 mol L^{-1} sodium hydroxide released IAA linked to oligosaccharides and to low molecular mass compounds (ester linked IAA), while that performed in 7 mol L^{-1} sodium hydroxide released IAA linked to amino acids and peptides (amide linked IAA). Ester conjugates were less abundant than free IAA and their changes were limited, with the sole exception of the whole internode from W-grown seedlings, whose ester linked IAA concentration was considerably lower than that of the other theses. Amide conjugates were generally more abundant than esters and the percent ratio between amide linked and free IAA showed marked changes under all light regimes (Table 1). The most remarkable data concerned D- and B-grown seedlings. In the third internode of etiolated plants such ratio was nearly 26-27 %, whereas in B-treated seedlings the amide IAA concentration exceeded that of the free auxin. Therefore, conjugation of the hormone must have proceeded at a notably higher rate under B than under D.

Discussion

We investigated the effects of D and of different light wavelengths on pea seedlings grown for 10 d under constant conditions and the role of auxin in mediating the response of pea seedlings to light. Immediately following the last growth measurement, the third internode of seedlings was excised for auxin analysis. At this age the growth response of the third internode to the different light treatments was reproducible and could be considered representative of the whole stem. As expected, etiolated seedlings (D) were the most elongated, while FR showed a relatively limited

action on growth inhibition, at least when the response to this wavelength was compared with that to R and W. It was somehow surprising that B was the least effective wavelength for growth inhibition.

The results of auxin analyses highlighted a positive correlation between free IAA concentration of the whole third internode and its elongation in response to the various treatments, suggesting that the different growth-inhibiting activity of the administered wavelengths may be mediated, at least partially, by changes in auxin levels. Activation of the specific photoreceptors may lower to a different extent the IAA concentration in the internode, thereby regulating its elongation and, ultimately, that of the whole stem. Such correlation was previously observed in studies on de-etiolation of several species under R (Morelli and Ruberti, 2002). Our results support the hypothesis that IAA is a key factor in mediating the growth response to continuous light in pea and basically agree with those of Behringer and Davies (1992) and Behringer et al. (1992a; b), in that they suggest for IAA a pivotal role in photomorphogenesis. Nevertheless, a major difference is represented by the distribution pattern of auxin in the stem. According to the aforesaid authors, R and FR control stem elongation through changes of IAA levels in the epidermis, which is considered the target tissue for auxin action in photomorphogenesis. Conversely, our data suggest that it is the overall IAA content of all internode tissues the primary factor mediating the growth response to light. This discrepancy may be attributed to the substantial differences in the experimental procedures. We studied the growth and hormone responses of pea seedlings to prolonged and continuous illumination and our auxin analyses were performed on samples taken from 10-d-old plantlets. Behringer's research particularly dealt with de-etiolation and was carried out on seedlings that had been exposed to light for a few hours before being sampled for auxin analyses. Under the latter conditions, the epidermis appeared to be the major target for auxin action. In our experiments, pea seedlings underwent light treatments for several days, that elicited a growth response whose regulation may be different from that induced by de-etiolation. We suggest that, 10 DAS, a sustained stem growth is only possible if the inner tissues are auxin-stimulated, in addition

to epidermis. This might explain why we found a correlation between the free IAA concentration in the whole internode and its elongation. The sole exception to the proposed mechanism is represented by B-grown seedlings: beside the marked decrease of free IAA observed under B in both the epidermis and the whole internode, this wavelength did not strongly inhibit elongation. The lack of correlation between IAA level and growth inhibition in B-grown seedlings might be partly ascribed to the operation of a signalling pathway involving GAs.

The reduction in stem elongation under B is thought to be driven by phyA and cry1, acting through the regulation of both the levels of GA_1 in the shoot, and the sensitivity of the stem tissues to this bioactive GA (Foo et al., 2006). As suggested by Weller et al. (1997; 2001), under this wavelength phyA antagonizes the growth inhibiting action of cry1, whose modest restriction of stem growth would ultimately arise from the inhability of lowering GA_1 levels and/or tissue sensitivity to the hormone. Further photoreceptors might have contributed to the observed response to continuous B. We assume that the different light regimes have affected phyB response to varying degrees. In D, of course, there was no phyB activation, while under relatively low B-irradiance (4.1 $\mu mol\ m^{-2}\ s^{-1}$) the operation of the phyB signalling should have been negligible and under FR it should have been weak (Parks et al., 2001; Platten et al., 2005). Therefore, the effectiveness of the different treatments toward growth inhibition might be correlated with the varying degrees of phyB activation. In most cases, phyB might have functioned as a regulator of IAA levels, maybe concurrently with phyA and/or cry1. In B-grown seedlings however, stem elongation was not correlated to free auxin concentration, as the third internode was greatly elongated despite its low auxin levels. This response might be related to the lack of phyB signalling under B irradiance and to its reported depression of tissue sensitivity to GA_1 in pea seedlings (O'Neill et al., 2000). Abscisic acid may also be involved in growth responses of B-grown pea, being an inhibitor of internode elongation. Under B irradiance, ABA concentration is increased in lettuce hypocotyls (Volmaro et al., 1998) while in tomato, hypocotyl responsiveness to the hormone is enhanced (Fellner and Sawhney, 2002). As a working hypothesis, we suggest that, in pea seedlings grown under B light,

the growth inhibition exerted by the decline of IAA and possibly by the increase of ABA concentration/responsiveness is overcome by a strong growth stimulation induced by GA_1, the last effect being the result of the higher tissue responsiveness to GAs in the absence of phyB signalling.

Metabolic reactions are of fundamental importance for regulating hormone levels. Conjugation may contribute to modulate IAA action by converting the hormone into a storage, inactive form, which may be subsequently reversed to the active molecule. Moreover, some kind of conjugation reactions may commit IAA to terminal catabolism (Woodward and Bartel, 2005). In pea seedlings conjugation with amide moieties gave rise to an abundant pool of IAA (see Table 1), thus decisively influencing free IAA levels. A relatively high concentration of amide IAA was found in B-grown seedlings, whose free hormone levels were accordingly low, although, as previously observed, this did not directly affect stem growth. Conversely, D-grown seedlings showed a very low concentration of amide IAA, which coincided with high levels of the free hormone. Hence, the regulation of free auxin levels, and consequently of stem elongation, by photoreceptors seems also to rely on hormone conjugation.

Pea seedlings respond to continuous D or illumination by changing the IAA levels in the stem and no particular tissue appears to be a major target for auxin action. Whether this hormone can ultimately affect stem elongation may depend on the interactions among the photoreceptors, differentially activated under various light wavelengths and irradiances. Although the role of changes in IAA responsiveness should not be overlooked (Symons and Reid, 2003), under conditions that are known to activate phyB it appears that stem growth is directly regulated through the modulation of IAA levels.

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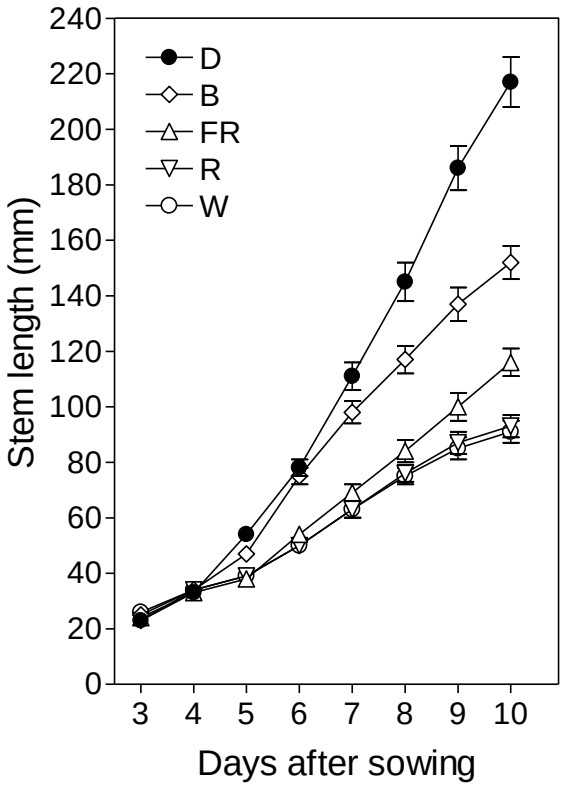
Legends of figures

Fig. 1. Elongation of the stem of pea seedlings grown under continuous darkness or different light wavelengths. Each point represents the mean ± SD of 3 replications, each made of 20 seedlings.

Fig. 2. Elongation of the third internode from stems of pea seedlings grown under continuous darkness or different light wavelengths. Each point represents the mean ± SD of 3 replications, each made of 20 seedlings.


Stem length (mm)
Days after sowing
D
B
FR
R
W
0
20
40
60
80
100
120
140
160
180
200
220
240
3
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10

Fig. 1

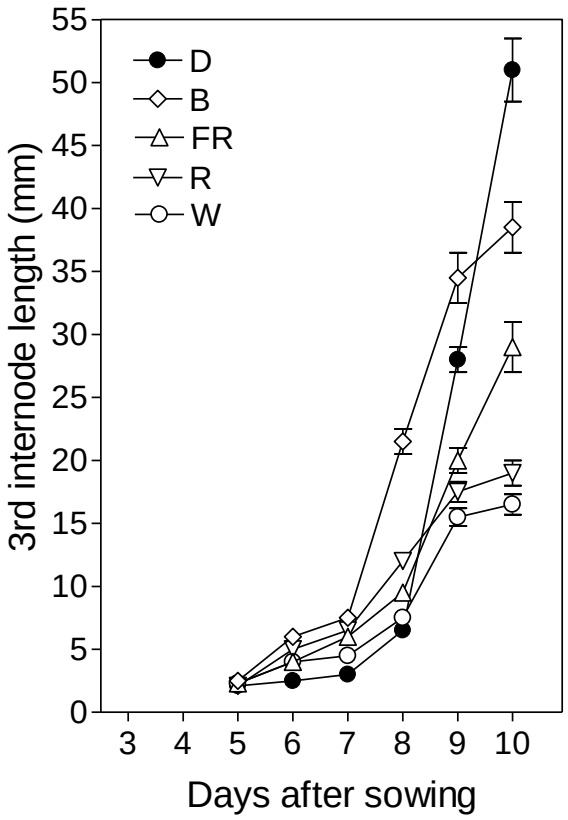
3rd internode length (mm)
Days after sowing
D
B
FR
R
W
0
5
10
15
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Fig. 2

Table 1. Concentration of free and conjugated IAA in the entire internode and the epidermis of pea seedlings grown under continuous darkness or different light wavelengths.

Treatment	Sample	IAA ng g^{-1} FW				
		Free IAA	E/WI	Ester IAA	Amide IAA	A/F
Darkness	Whole internode	414.5^{a}		34.3^{a}	113.7^{a}	27.4 %
	Epidermis	341.6^{v}	82 %	24.1^{v}	89.4^{v}	26.2 %
Blue	Whole internode	103.7^{b}		36.2^{ab}	122.4^{b}	118 %
	Epidermis	51.3^{w}	49 %	18.2^{w}	95.8^{w}	186.7 %
Far red	Whole internode	197.9^{c}		43.8^{b}	152.4^{c}	77 %
	Epidermis	93.5^{x}	47 %	28.3^{v}	36.5^{x}	39 %
Red	Whole internode	154.2^{d}		40.1^{b}	132.1^{d}	85.7 %
	Epidermis	124.7^{y}	80 %	14.8^{wx}	67.4^{y}	54 %
White	Whole internode	129.1^{e}		22.5^{c}	57.4^{e}	44.5 %
	Epidermis	72.8^{z}	56 %	12.1^{x}	30.8^{z}	42.3 %

Values in the column E/WI are the percent ratios between the mean free IAA concentration in the epidermis and that of the whole internode, for each treatment. The column A/F reports the percent ratios between amide IAA and free IAA mean concentrations. Each concentration value represents the mean of 3 independent experiments. The data were analyzed by one-way ANOVA and mean comparison was performed by Tukey's test. Statistical analyses were carried out separately for each auxin form and for each kind of tissue. Different letters indicate values statistically different for $P < 0.05$. The average standard error was ± 6.5 %.