Indole-3-acetic acid levels after phytochrome-mediated changes in the stem elongation rate of dark- and light-grown *Pisum* seedlings

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**Abstract.** The effect of red (R) and far-red (FR) light on stem elongation and indole-3-acetic acid (IAA) levels was examined in dwarf and tall *Pisum sativum* L. seedlings. Red light reduced the extension-growth rate of etiolated seedlings by 70–90% after 3 h, and this inhibition was reversible by FR. Inhibition occurred throughout the growing zone. After 3 h of R, the level of extractable IAA in whole stem sections from the growing zone of etiolated plants either increased or showed no change. By contrast, extractable IAA from epidermal peels consistently decreased 3 h after R treatments. Decreases of 40% were observed for epidermal peels from the top 1 cm of tall plants receiving 3 h R. Brief R treatments resulted in smaller decreases in epidermal IAA levels and these decreases were not as great when FR followed R. In light-grown plants, end-of-day FR stimulated growth during the following dark period in a photoreversible manner. The uppermost 1 cm of expanding third internodes was most responsive to the FR. Extractable IAA from epidermal peels from the upper 1 cm of third internodes increased by 30% or more 5 h after FR. When R followed the FR the increases were smaller. Levels of IAA in whole stem sections did not change and were twofold greater than in dark-grown plants. In both dark- and light-grown tall plants, IAA levels were lower in epidermal peels than in whole stem segments. These results provide evidence that IAA is compartmentalized at the tissue level within the growing stem and that phytochrome regulation of stem elongation rates may be partly based on modulating the level of IAA within the epidermis.

**Key words:** Auxin and stem growth – Epidermis and stem growth – *Pisum* (stem growth) – Phytochrome stem growth – Stem elongation

**Introduction**

The quality and quantity of light can dramatically affect stem elongation through the perception of light by photomorphogenic pigments (for review see Cosgrove 1986). Red (R) and far-red (FR) light, acting through phytochrome, alter stem elongation in dark-grown (e.g. Downs 1955; Lockhart 1960) and light-grown (e.g. Downs et al. 1957; Smith 1982) plants. However, beyond the recognition that phytochrome plays a central role as a phototransducer, little is known about the transduction pathway that leads to increases or decreases in stem 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 inhibition correlates with a reduction in the supply of auxin from the coleoptile (Iino 1982b). Reduced transport (Furuya et al. 1969), increased conjugation (Bandurski et al. 1977) and reduced synthesis (Iino 1982a) may all account for the drop in IAA in the mesocotyl. Whereas the coleoptile and mesocotyl of grasses have been widely used to study auxin in relation to phytochrome effects on elongation growth, this relationship has not been explored fully in dicotyledonous plants. Fletcher and Zalik (1964) suggested that auxin levels in etiolated *Phaseolus* seedlings decreased after R, and they attributed this to increased metabolism (Fletcher and Zalik 1965). However, it is difficult to reconcile these conclusions with the observations that auxin levels in light-grown plants are higher than in dark-grown plants (Scott and Briggs 1963; Tillberg 1974; von Guttenberg and Zetsche 1956).

We investigated the relationship between the levels of IAA and phytochrome-mediated growth-rate changes with the aim of examining the role, if any, that changes in auxin levels play as a mediator of changes in stem elongation in *Pisum* seedlings. In order to correlate auxin measurements with R-induced changes in stem growth as precisely as possible, position transducers were used to provide a continuous recording of stem-growth rate. The
kinetic analysis indicated when and where R inhibited stem growth and was used to optimize the harvest of tissue for auxin analysis. We also examined IAA levels in the epidermis since this tissue has been suggested as a site of control of stem elongation (see Kutschera 1989, for review).

Materials and methods

Plant materials. The genetic lines of *Pisum sativum* L. NGB1771 (wild-type, tall) and L203 (dwarf, lele genotype) were obtained from Dr. J.B. Reid, Department of Plant Science, University of Tasmania, Hobart, Australia. The lele genotype results in a dwarf habit by limiting the synthesis of gibberellin A1 (Ingram et al. 1984). Seeds of the cultivar Alaska were obtained from Burpee Seed Co., Warminster, Penn., USA.

Seeds were sown in grade 3 vermiculite (Horticultural Products, W.R. Grace and Co., Cambridge, Mass., USA) and grown under well-watered conditions at 20-22°C in either darkness or under continuous light from fluorescent lamps at 60-80 µmol photons m⁻² s⁻¹ until the light treatments were given. Plants for growth studies were grown singly in 80-cm³ pots, while plants for auxin measurements were grown 50 per 20 × 9 × 28 cm³ tray. The seedlings were used for experiments when the third internodes were expanding but less than 50% of their final length (between days 8 and 9 after sowing for dark-grown and 7-8 d for light-grown plants).

Growth measurements. Growth of intact seedlings was recorded at 1-mm intervals using angular-position transducers which were interfaced with a microcomputer, and growth rates were computed from the growth curves as previously described (Behringer et al. 1990).

To determine elongation growth of different intervals along the growing zone of the stem, extension growth was measured at set distances from the top of expanding third internodes. For dark-grown plants the top of the internode was taken as the point where the stem begins to swell. Average growth rates were computed for 5-mm intervals for dark-grown plants and 10-mm intervals for light-grown plants. Neonate sections were used for auxin analysis. We also examined IAA levels in the epidermis since this tissue has been suggested as a site of control of stem elongation (see Kutschera 1989, for review).

Quantitation of extractable IAA. Indole-3-acetic acid was extracted from stem sections (referred to as whole stem sections), peeled stem sections or epidermal strips. When plants were growing in darkness at the time of sample harvest, segments were harvested under the green safelight. Whole stem sections were immediately frozen in liquid nitrogen. Epidermal peels were obtained by gripping the epidermis at the basal end of a stem section with fine jewelers forceps and gently peeling. The majority of the epidermis could be removed from a section in three peels, and the peels were frozen in liquid nitrogen within 15 s unless otherwise indicated. The sections were briefly illuminated by weak light from fluorescent lamps during the removal of the epidermis. The harvested material was stored at −80°C until the time of extraction.

For the analysis of whole stem tissue, 20-30 1-cm-long sections (appro. 0.5–1.0 g) or 10–20 2-cm-long sections (appro. 0.7–1.5 g) were ground to a fine powder in liquid nitrogen with a mortar and pestle. The frozen powder was transferred to 100% methanol which contained 1-[¹⁴C]IAA (Amersham Corp., Arlington Heights, Ill., USA) as a tracer and [¹³C]IAA (99% ¹³C; Cambridge Isotope Laboratories, Woburn, Mass., USA) as an internal standard (Cohen et al. 1986). The amount of radioactive tracer added was typically 20–25% of the endogenous IAA. For the analysis of epidermal peels, 0.250–0.450 g of tissue were extracted. The radioactive tracer was omitted from these samples because the amount of [¹⁴C]IAA required as an effective tracer would have been several times greater than the amount of endogenous IAA and would have resulted in an unreliable calculation of the endogenous IAA.

The IAA was purified as described by Law and Davies (1990). In brief, samples were filtered and evaporated to approx. 0.5 ml by rotary film evaporation. The sample was fractionated using reverse-phase high-performance liquid chromatography (HPLC) and the IAA was collected by a radioactivity detector (Model 7140; Packard) or manually based on retention time. The samples were dried and methylated (Diazald; Aldrich Chemical Co., Milwaukee, Wis., USA), injected onto a reverse-phase HPLC column, and collected as described above. Samples were dried for storage until mass-spectral analysis.

Quantitation was performed using a Hewlett-Packard gas chromatograph (Model 5890A) and mass spectrometer (Model 5970B) as described by Law and Davies (1990).

Histological analysis. Epidermal peels were fixed in 2% glutaraldehyde (pH 6.5) overnight. The tissue was dehydrated in an ethanol series and embedded in Spurr resin (Electron Microscopy Sciences, Fort Washington, Penn., USA). One-μm sections were cut approximately midway along the length of the peels with a glass knife, mounted on glass slides, and stained with a 1% toluidine blue. 1% sodium borate solution. Photography was performed with a Zeiss Axioscope (Zeiss, Oberkochen, FRG) and an FX–35WA camera (Nikon, Tokyo, Japan).

Statistical analysis. The standard error of the mean and two sample *t*-tests were computed using Minitab software (Minitab, State College, Penn., USA).