



RESEARCH PAPER

Internal axial light conduction in the stems and roots of herbaceous plants

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Abstract

In order to reveal any roles played by stems and roots of herbaceous plants in responding to the surrounding light environment, the optical properties of the stem and root tissues of 18 herbaceous species were investigated. It was found that light was able to penetrate through to the interior of the stem and was then conducted towards the roots. Light conduction was carried out within the internodes and across the nodes of the stem, and then in the roots from the tap root to lateral roots. Light conduction in both the stem and root occurred in the vascular tissue, usually with fibres and vessels serving as the most efficient axial light conductors. The pith and cortex in many cases were also involved in axial light conduction. Investigation of the spectral properties of the conducted light made it clear that only the spectral region between 710 nm and 940 nm (i.e. far-red and near infra-red light) was the most efficiently conducted in both the stem and the root. It was also found that there were light gradients in the axial direction of the stem or root, and the light intensity generally exhibited a linear attenuation in accord with the distance of conduction. These results revealed that tissues of the stem and root are bathed in an internal light environment enriched in far-red light, which may be involved in phytochrome-mediated metabolic activities. Thus, it appears that light signals from above-ground directly contribute to the regulation of the growth and development of underground roots via an internal light-conducting system from the stem to the roots.

Key words: Axial light conduction, far-red light, herbaceous dicotyledons, light gradients, monocotyledons, optical proper-

ties of stem and root tissues, photomorphogenesis, phytochromes.

Introduction

The light-related metabolic activities of plant tissues are not directly dependent upon the external light environment of plants but rather upon an internal one, in which tissues and cells are actually bathed. The construction of the internal light environment is derived from diverse modifications by the plant tissues themselves of the incident light (Walter-Shea and Norman, 1991; Vogelmann, 1993, 1994). A detailed investigation of the optical properties of plant tissues is therefore necessary to understand the characteristics of the internal light environment and, furthermore, to understand its exact, functional significance.

Investigations of the influences of plant tissues on incident light have so far been restricted to leaves and seedlings (especially etiolated seedlings). In leaves, the epidermis (Poulson and Vogelmann, 1990; Kolb *et al.*, 2001), palisade parenchyma (McClendon and Fukshansky, 1990; Fukshansky *et al.*, 1992; Vogelmann and Han, 2000), spongy parenchyma (Terashima and Saeki, 1983; Cui *et al.*, 1991), and sclerenchyma (Karabourniotis *et al.*, 1994, 2000) have attracted the most attention to date. The chief roles of the leaf tissues in modifying the light passing through them are to contribute to the formation of an intensity-attenuated, diffuse, and green and far-red rich internal light environment, which is believed to have adaptive significance for leaf photosynthesis (Bone *et al.*, 1985; Fukshansky *et al.*, 1991; Myers *et al.*, 1994). In the etiolated seedlings, investigations of the optical properties of tissues have focused on cotyledons (Seyfried and

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Schäfer, 1983; Turunen *et al.*, 1999), mesocotyls (Mandoli and Briggs, 1982; Kunzelmann and Schäfer, 1985), and coleoptiles (Vogelmann and Haupt, 1985; Kunzelmann *et al.*, 1988). Light intensity gradients across both the transverse and axial directions of the seedling organs (Seyfried and Fukshansky, 1983; Parks and Poff, 1985; Vogelmann and Haupt, 1985) have been revealed, and are believed to be involved in the observed phototropic responses of seedlings (Piening and Poff, 1988; Iino, 1990) and in regulating the elongation of the coleoptiles and mesocotyls (Mandoli and Briggs, 1982, 1984*a, b*; Parks and Poff, 1986), respectively. These studies have shown that the tissues of both leaves and seedling can change the incident light in quality, quantity, and direction of conduction to form a characteristic internal light environment, which is of crucial physiological significance for light-related metabolic activities.

Stems and roots are important organs of plants, and are best characterized for two functions: mechanical support and substance transport (Esau, 1977; Fahn, 1990). Prior to this research, there have been few reports on the direct roles of these organs in dealing with the external light environment. There has been some research on stem photosynthesis which investigated the internal light environment of woody stems and its relationship to stem photosynthesis (Pfan, 1999; Pfan and Aschan, 2001, Pfan *et al.*, 2002). However, this study addressed the light environment mainly within bark, especially in the region directly under the epidermis or periderm where chlorenchyma cells are abundant. In the roots, any direct relations between them and the external light environment above-ground have yet to be investigated. In previous studies on the optical properties of the stems and roots of woody plants, it became clear that light can not only enter the interior of a woody stem but it is also conducted towards the roots along the axis (Sun *et al.*, 2003, 2004). Certain elements of vascular tissue (fibres, tracheids, and vessels) are involved in axial light conduction, and the living tissues in the xylem and phloem of stems and roots are bathed in an internal light environment, comprised mostly of far-red light, which is probably correlated with the photomorphogenic processes in the stems and roots of woody plants.

The stems and roots of herbaceous plants differ greatly from those of woody plants not only in habit, pattern of development, and period of growth, but also in morphological structures and even in certain functional aspects etc. (Esau, 1977; Fahn, 1990). Little is known about the roles of herbaceous stems and roots in dealing with the surrounding light environment and the internal light milieu. The present study therefore investigated the ability of these tissues to modify incident light. The aim was to clarify the internal light-conducting paths, the variations in light intensity and spectral properties in the course of conduction, and the possible functional significance of the internal light environment in the stems and roots of herbaceous plants.

Materials and methods

Species investigated and sample preparation

Eighteen herbaceous species were used in the present study (Table 1). The selection for these species was based on differences in the phylogenetic groups of angiosperms (dicotyledons and monocotyledons) and the diversity of structural characteristics representative of the stems and roots of herbaceous plants. Samples of these species were collected from the Botanical Garden, Tohoku University (38°15'23" N, 140°51'00" E), or the national woodland near the Photodynamics Research Center, RIKEN (38°14'46" N, 140°49'34" E), Sendai, Japan.

Five to twelve well-grown plants of each species were carefully dug out of the ground with a certain amount of soil around their root system, and immediately placed in a bucket with the root system immersed in water. After soil around the root system was carefully washed off with water, each plant was divided into two lengths by transversely cutting the stem with a sharp razor at a height of 5–10 cm above the ground surface. The upper stem length was used immediately for investigations of the light conduction properties of the stem. To the lower stem–root length, another transverse cut was made at the tap root (or the adventitious root in certain species), a primary or secondary lateral root, dividing the length into two parts: stem–root transition length and root length, respectively. These two lengths were trimmed to a length of 5–10 cm and then used for analysis of the internal light conduction from the stem to the root.

Observations and measurements of light conduction in stems and roots

The experimental set-up details have been described in previous work (Sun *et al.*, 2003). In brief, each prepared stem, stem–root or root length was inserted with the downward cut end into a dark box through a hole at the top, and then sealed with black gum to maintain integrity of the box from light entering from the outside. Set directly under the cut end surface of the plant length was a microscope which was attached to a far red-sensitive monochrome CCD image sensor (Wat-902H, Watec Corp., Japan). The latter was connected to a personal computer with image processing software (Argus-20,

Table 1. *Herbaceous species used in this study*

| Species name |
|---|
| Dicotyledons with herbaceous stems and roots |
| <i>Medicago polymorpha</i> L. |
| <i>Persicaria filiformis</i> (Thumb.) Nakai ex W.T. Lee ^a |
| <i>Fallopia japonica</i> (Houtt.) Ronse Decr. var. <i>uzenensis</i> (Honda) Yonek. Et H. Ohashi |
| <i>Boehmeria macrophylla</i> Hornem. |
| <i>Leucanthemum vulgare</i> Lam. |
| <i>Trifolium pratense</i> L. |
| Dicotyledons with woody stems and roots |
| <i>Pueraria lobata</i> (Willd.) Ohwi |
| <i>Hedera helix</i> L. |
| <i>Cosmos bipinnatus</i> Cav. |
| <i>Cucumis sativus</i> L. |
| <i>Artemisia princeps</i> Pamp. |
| <i>Arachis hypogaea</i> L. |
| <i>Ipomoea batatas</i> Lam. var. <i>edulis</i> O. Kuntze |
| Monocotyledons |
| <i>Polygonatum macranthum</i> (Maxim.) Koidz |
| <i>Epipremnum aureum</i> Bunting |
| <i>Phyllostachys bambusoides</i> Sieb. Et Zucc. ^a |
| <i>Zea mays</i> L. |
| <i>Carex idzuroei</i> |

^a Species in which stems only were investigated for optical properties

Hamamatsu Photonics K. K., Japan) and image-acquisition and analysis software (Aquacosmos version 1.10, Hamamatsu Photonics K. K.). The incident light used in the investigation of the light-conducting tissues or cells was from a microscopic halogen light source or from solar radiation. By using the halogen light source through a light guide, unilateral illumination was presented to the protruding part of the plant length. Because sunlight reaches the stem surface at different angles according to the time of day, the angles of unilateral oblique illumination included 90°, 60°, 40°, and 20° to the plant axis, respectively. The distribution of light conducted in the tissues on the cut end surface was recorded by the CCD image sensor after enlargement through a microscopic objective lens. The images acquired were then processed by the personal computer to clarify the efficiency of light conduction in different cell types of the stem and root tissues. For further investigation, the illumination site was kept constant while 2–3 mm lengths were progressively cut back from the lower cut end. After every cut, the image from the newly made end surface was obtained for comparison of the distribution of transmitted light in the tissues in order to verify the tissue- and cell-dependent light conduction in the stem and root further. The same investigation on these plant lengths was also carried out under sunlight, instead of the halogen light source, to clarify the internal light conduction of the stem and root in the natural light environment.

The same apparatus was used to investigate the spectral properties of the conducted light by replacing the microscope with the detector of a photonic multi-channel analyser (PMA-11, Hamamatsu Photonics K. K.). Spectra of the transmitted light were measured under the above-mentioned different illumination conditions of the halogen light source and sunlight. The relative ratios of transmission were obtained by dividing the spectra of light transmitted by those of the corresponding incident light across the spectrum from 400 nm to 950 nm, and are presented as logarithmic values.

Using similar methods and apparatus, certain other stem, stem–root or root lengths were taken to investigate the internal light attenuation under axial illumination of the halogen light source. For each plant length, a transverse cut was made from the lower cut end to produce progressively shorter lengths of 50 mm, 40 mm, 30 mm, 20 mm, and 10 mm. The upper cut end was illuminated axially after every cut and the spectrum of light transmitted from the lower cut end was measured. The relative ratios of transmission from 400 nm to 950 nm were then obtained and compared in the same way as mentioned above.

In order to clarify whether the light conducted in stems and roots is part of the incident light or was fluorescence of the plant tissues, experiments were conducted with monochromatic light as the incident light. Monochromatic light was obtained by inserting an interference filter (half-band width 8–12 nm) between the light guide from the halogen light source and the sample. The relative intensities were matched by adjusting the iris and/or voltage of the light source. Monochromatic incident light from 400 nm to 950 nm with an interval of 10–20 nm was applied axially to illuminate a 1–2 cm long stem or root length. The spectral properties of the transmitted light were measured and compared with those of their corresponding monochromatic incident light to verify the origin of the light transmitted by stems or roots.

All these observations and measurements of the samples were performed rapidly so as to avoid any drying effects of the cut end. Routine tissue sectioning was also made after the measurements to identify the cell types involved in the light conduction within the stem and root.

Results

Stem tissues of herbaceous plants are involved in internal axial light conduction

The distribution patterns of vascular tissue and the presence or absence of secondary growth are two characteristic

structural differences in the stems of herbaceous angiosperm plants (Esau, 1977; Fahn, 1990). A vascular cylinder (Fig. 1A, B) and scattered vascular bundles (Fig. 1C) are two common distribution patterns of the vascular tissue in the stems of dicotyledons and monocotyledons, respectively. There is usually no secondary growth derived from the vascular cambium in stems with either a vascular cylinder or scattered vascular bundles (classified as ‘herbaceous stems’). However, in some species, secondary growth does occur to some extent so as to form secondary xylem and secondary phloem in stems with a vascular cylinder, especially in the later stages of development (classified as ‘woody stems’) (Fig. 1D).

As well as the above-mentioned structural differences in the stems of the herbaceous plants investigated, it was observed that external light penetrated into the interior and was conducted axially (Figs 1, 2). This axial light conduction occurred not only in the internodes and across the nodes in the stem, but also from the stem above-ground to the roots underground (Fig. 3). This internal light conduction occurred whatever the illumination angle of the incident light.

Different stem structures, however, exhibited differences in the efficiency of the axial conduction of light. Epidermis was a poor axial light conductor in all the stems investigated (Fig. 2B, D). Cortex and pith (in stems with a vascular cylinder), and ground tissue (in stems with scattered vascular bundles) in general conducted light axially via the lumina of their cells. This conduction was relatively efficient in many cases, especially in species that develop large axially elongated cells and which contain smaller amounts of pigments (Figs 1B, 2E). Less efficient light conduction by these same tissues was observed in the stems of other species (Figs 1A, C; 2A, F). In the cortex of certain species, several layers of collenchyma cells develop on the inner side of the epidermis, and efficient axial light conduction occurs within their thick cell walls (Fig. 2C).

The vascular tissue in the stems investigated, whatever the distribution pattern or whether there was a presence or absence of secondary growth, was always efficiently involved in axial light conduction (Fig. 1). However, the cells or tissues involved exhibited certain differences in terms of the distribution pattern and secondary growth of vascular tissue (Fig. 2). Fibres are a major component of stem vascular tissue. In the vascular cylinder without or with only weak secondary growth (herbaceous stems), phloem fibres, and vascular bundle sheath fibres in certain species as well, were the most efficient axial light conductors, but xylem fibres did not conduct light as efficiently (Fig. 2A, D). In the vascular cylinder with secondary growth (woody stems), in addition to primary phloem fibres, secondary xylem fibres and secondary phloem fibres were also efficient in conducting light axially (Figs 1D; 2G–I). In the scattered vascular bundles, vascular bundle sheath fibres with thick lateral walls were observed to be efficient axial light conductors (Figs 1C, 2F). Whatever the type of fibre, light

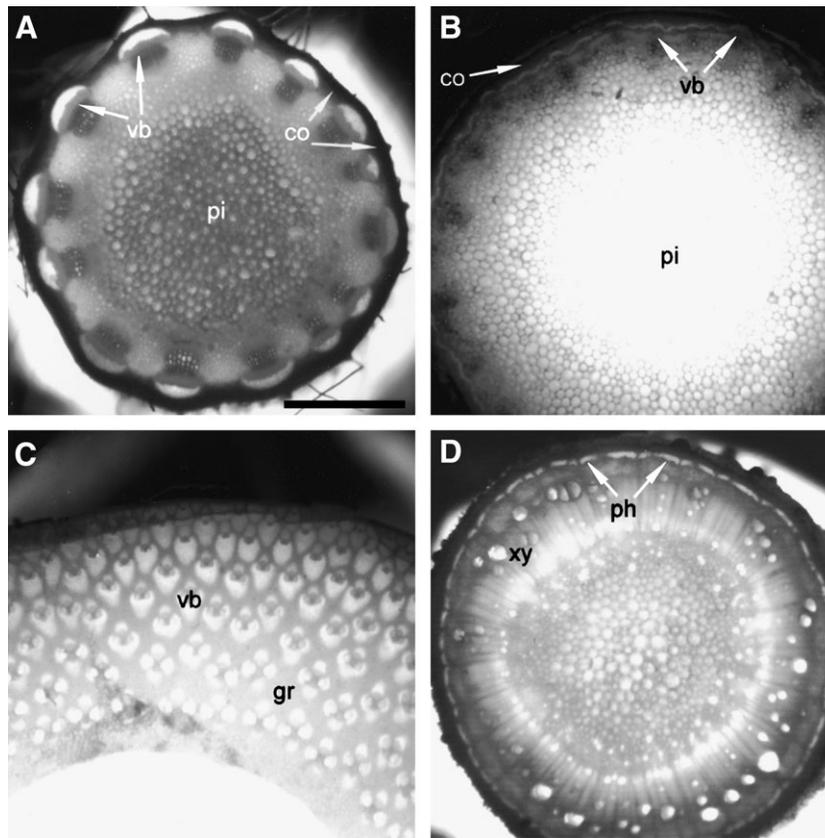


Fig. 1. Images of light transmitted from the transverse end of stems with different structural characteristics under oblique illumination (a halogen light source) of stems (incident angle at 60° to the stem axis). (A), (B) Stems with cylindrically arranged vascular bundles. (A) *Trifolium pratense*. Vascular bundles (vb) are involved in axial light conduction more efficiently than cortex (co) and pith (pi). (B) *Fallopia japonica* var. *uzemensis*. Pith (pi) conducts light axially more efficiently than vascular bundles (vb) and cortex (co). (C) *Phyllostachys bambusoides*. The vascular bundles (vb) scattered in ground tissue (gr) are more efficient axial light conductors than the ground tissue. (D) Stem with secondary growth in *Pueraria lobata*. Vascular tissue including secondary xylem (xy) and secondary phloem (ph) is most efficiently involved in axial light conduction. The scale bar for (A–D) is in (A) and equals 1 cm.

conduction within it proceeded via the cell walls, and fibre lumina were not involved in axial light conduction (Fig. 2B, F, H, I).

Vessels are another main constituent of xylem. In the vascular cylinder of herbaceous stems, primary xylem vessels varied among species in the efficiency of axial light conduction, but generally did not conduct light as efficiently as phloem fibres (Fig. 2A, D). However, in the vascular cylinder of woody stems, vessels in the secondary xylem were involved in efficient axial light conduction. In the scattered vascular bundles, the larger vessels were observed to conduct light efficiently (Fig. 2F). Light conduction by vessels took place via the large lumina. Other types of stem tissues or cells (such as sieve tubes, companion cells, phloem parenchyma cells, and xylem parenchyma cells) were generally poor axial light conductors (Fig. 2A, D, F, G).

Root tissues of herbaceous plants are also involved in internal axial light conduction

The present investigation dealt with both the tap root and fibrous root systems, which together represent the character-

istic root systems of herbaceous dicotyledons and monocotyledons, respectively (Esau, 1977; Fahn, 1990). The tap root system investigated usually included a tap root, primary lateral roots, and secondary lateral roots. The most obvious structural differences among these roots were in the vascular cylinder, where the tap root and thick primary lateral roots generally had undergone secondary growth to form a large amount of secondary xylem (Fig. 3A). The thin primary lateral roots tended to include less secondary structure (Fig. 3B), but the much thinner secondary lateral roots developed only primary structure (Fig. 3C). In the fibrous root system of the monocotyledonous species investigated, there was no secondary growth found in the vascular cylinder, but a large pith was often observed within it (Fig. 3D). Light was conducted from the stem into the roots, whatever the type of root system, and was conducted axially from the stem to the tap root, the tap root to the primary lateral roots, and thence to the secondary lateral roots (Figs 3, 4). Light conduction in roots took place whatever the illumination angle of the incident light.

Different structures in roots showed differences in the efficiency of axial light conduction. The epidermis was

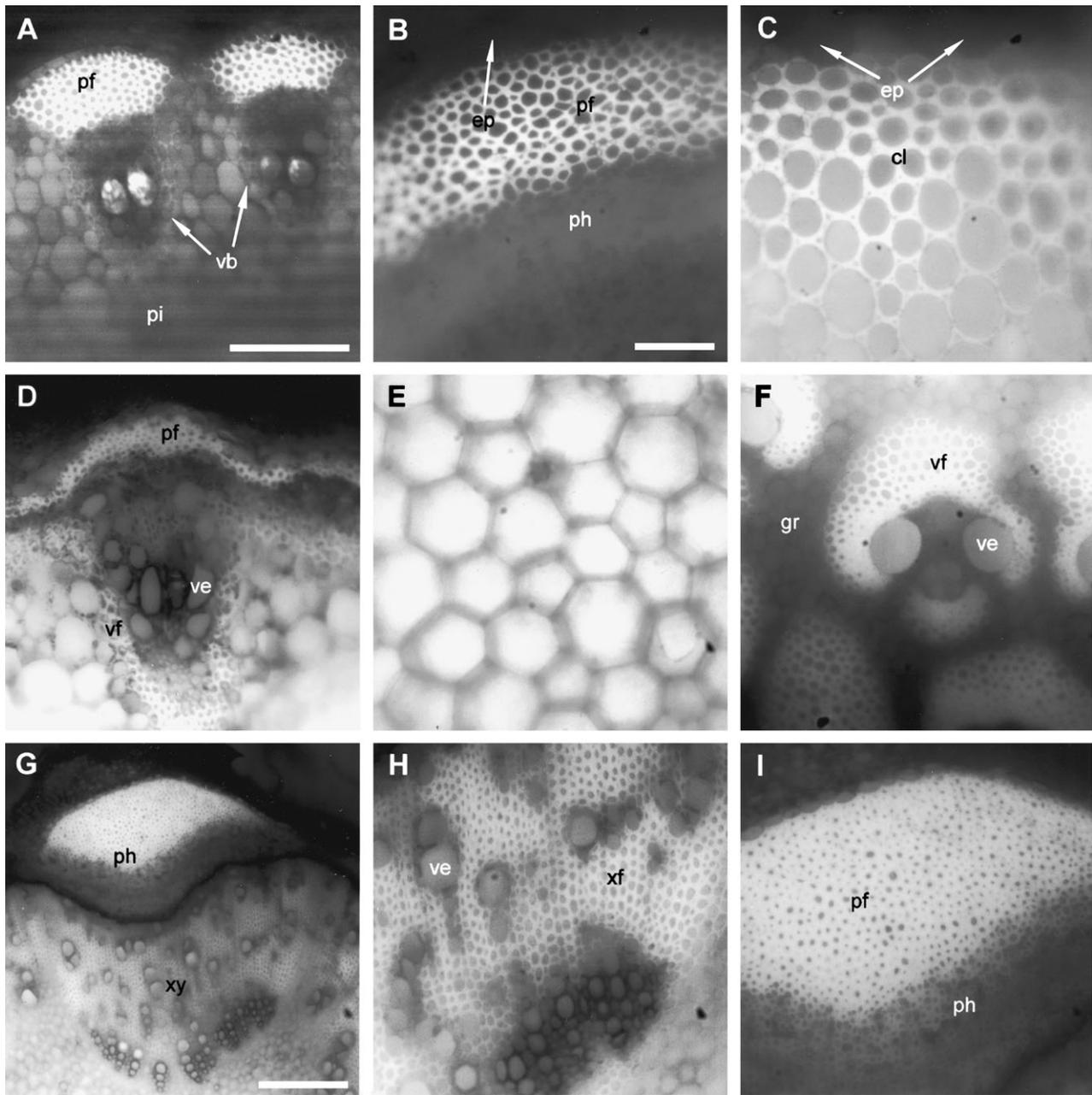


Fig. 2. Cells and tissues involved in axial light conduction in stems with different structural characteristics under oblique illumination (a halogen light source) of stems (incident angle at 60° to the stem axis). (A–E) Stems with cylindrically arranged vascular bundles. (F) Stem with scattered vascular bundles. (G–I) Stem with secondary thickening of *Artemisia princeps*. (A) *Leucanthemum vulgare*. Phloem fibres (pf) in vascular bundles (vb) are efficient axial light conductors, but pith (pi) is poor in conducting light. (B) *Trifolium pratense*. Phloem fibres (pf) of phloem (ph) on the inner side of epidermis (ep) conduct light, via the cell walls. (C) *T. pratense*. Collenchyma cells (cl) on the inner side of epidermis (ep) can conduct light via their cell walls. (D) *Fallopia japonica* var. *uzenensis*. Vascular bundle conducts light with the involvement of phloem fibres (pf), vascular bundle sheath fibres (vf) and vessels (ve). (E) *Boehmeria macrophylla*. Parenchyma cells in pith conduct light via the lumen. (F) *Phyllostachys bambusoides*. Fibres (vf) of the vascular bundle sheath and bundle sheath extension and vessels (ve) conduct light more efficiently than ground tissue (gr). (G) The vascular bundle with secondary growth, including xylem (xy) and phloem (ph), is efficiently involved in axial light conduction. (H) Fibres (xf) and vessels (ve) in secondary xylem are efficient axial light conductors. (I) Phloem fibres (pf) in phloem (ph) axially conduct light efficiently via the thick cell walls. The scale bar for (A), (D–F), (H) and (I) is in (A), and equals 150 μm ; that for (B) and (C) in (B), is 50 μm ; and that in (G) is 300 μm .

found to be a poor axial light conductor. Cortex tissues were generally not involved in efficient light conduction in most cases (Fig. 3B, D). Pith (whether the pith tissues or pith cavity), when present, always conducted light efficiently (Figs 3A, D; 4C, E, G). The vascular cylinder

always exhibited efficient axial light conduction in all types of roots investigated (Figs 3, 4).

The vascular cylinder of roots includes both phloem and xylem. The region composed of the phloem was much less efficient in the axial conduction of light than the xylem

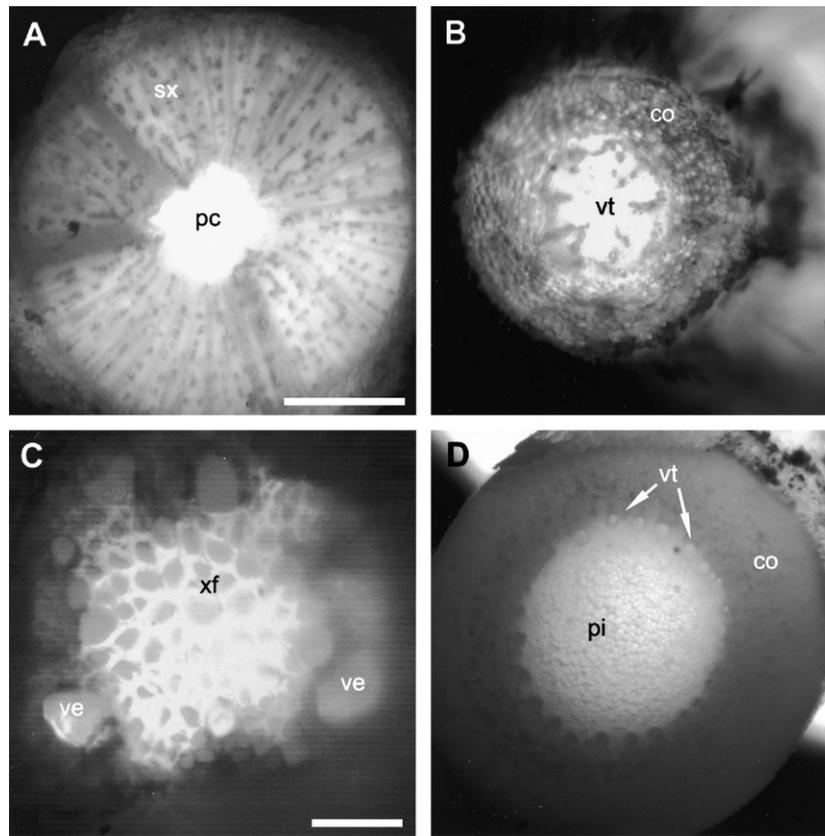


Fig. 3. Images of light transmitted from the transverse end of different types of root under oblique illumination (a halogen light source) of stem-root lengths (incident angle at 60° to the stem axis). (A) Tap root of *Cosmos bipinnatus*. The secondary xylem (sx) and pith cavity (pc) are involved in axial light conduction relatively efficiently. (B) Primary lateral root of *Artemisia princeps*. Vascular tissue (vt) conducts light more efficiently than cortex (co). (C) Secondary lateral root of *C. bipinnatus*. Xylem fibres (xf) and vessels (ve) in the primary xylem are more efficient axial light conductors, especially in the cell walls of xylem fibres and the lumina of vessels. (D) Adventitious root of *Zea mays*. Vascular tissue (vt) and pith (pi) are more efficient in axial light conduction than cortex (co). The scale bar for (A), (B) and (D) is in (A), and equals 1 cm. The scale bar in (C) equals 50 μm .

(Fig. 4C, D). Xylem tissues and cells involved in axial light conduction varied according to the xylem structure in the various types of roots (Fig. 4). In the vascular cylinder with obvious secondary growth (usually in the tap roots and thick primary lateral roots of certain species investigated), the secondary xylem was more efficiently involved in axial conduction than the primary xylem (Fig. 3A). In the secondary xylem, only fibres and vessels were efficient light conductors, while axial parenchyma cells and ray cells were generally relatively poor in the conduction of light (Fig. 4A, B). In the vascular cylinder with weak secondary growth (usually thin primary lateral roots or thick secondary lateral roots in the species investigated), the primary xylem played a major role in conducting light axially, and its fibres were always efficient light conductors, conducting light even more efficiently than its vessels in some cases (Fig. 4E, F, H, I). In the vascular cylinder without secondary growth (thin secondary lateral roots, adventitious roots, and fibrous roots), the primary xylem was involved in axial light conduction, but only fibres and vessels were efficient light conductors (Fig. 3C). As in the stems, in the roots light conduction by xylem fibres was carried out via the cell walls

(Figs 3C; 4B, D, I), while in vessels and pith parenchyma cells light was conducted through the lumina (Figs 3C; 4B, F, G). In the stems or roots, the structural components involved in axial light conduction remained the same whatever the illumination angle of the incident light.

Far-red light is always conducted most efficiently by the stem and root of herbaceous plants

The significance of light in regulating the metabolic activities of plant tissues is related to its spectral properties. Comparing the light transmitted from the stem or root (Fig. 5B) with the incident light (Fig. 5A) can reveal the role of the stem or root tissues in dealing with the surrounding light environment as well as the spectral properties of the light conducted in the stem or root. This investigation found that both stem and root tissues exhibited a marked difference in transmission intensity at certain wavelengths within the spectral range 400–950 nm (Fig. 5D–I). The wavelengths transmitted most efficiently by the stems or roots were generally between 710 nm and 940 nm, regardless of the absence (Fig. 5D, F, G) or presence (Fig. 5E, H, I) of

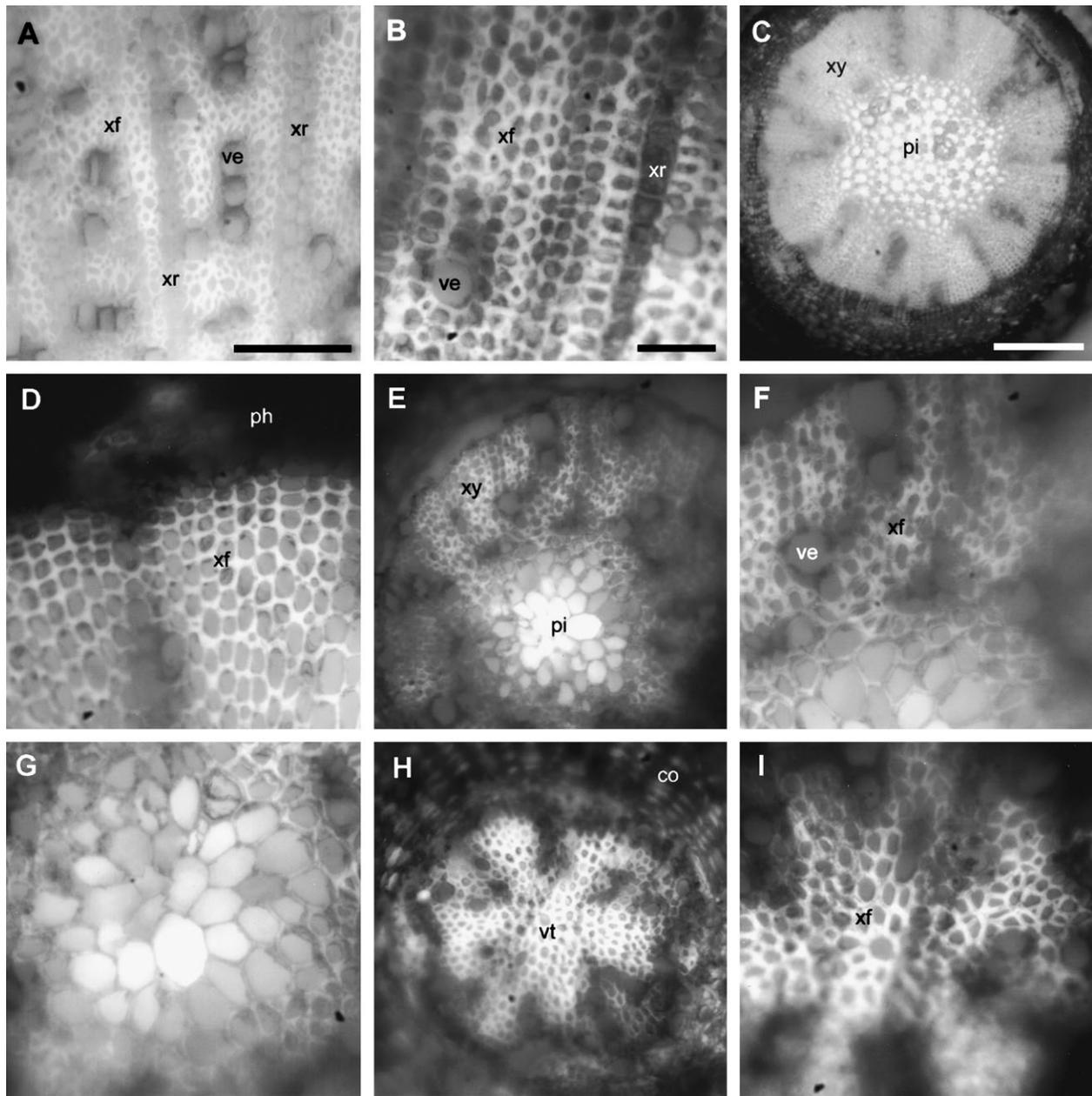


Fig. 4. Cells and tissues involved in axial light conduction in tap roots and primary lateral roots under oblique illumination (a halogen light source) of stem–root lengths (incident angle at 40° to the stem axis). (A) Secondary xylem in a tap root of *Cosmos bipinnatus*. The cell walls of xylem fibres (xf) and lumina of vessels (ve) conduct light more efficiently than xylem ray cells (xr). (B) Secondary xylem in a taproot of *Artemisia princeps*. Xylem fibres (xf) and vessels are more axial light conductors than xylem ray cells (xr). (C) Primary lateral root of *A. princeps*. Xylem (xy) and pith (pi) are efficient light conductors. (D) A portion of (C). Xylem fibres conduct light via the cell walls; phloem (ph) is a poor light conductor. (E) Primary lateral root (xy: xylem) with pith (pi) of *C. bipinnatus*. (F) A portion of (E). Xylem fibres (xf) and vessels (ve) conduct light the most efficiently. (G) Another portion of (E). Parenchyma cells in the pith are involved in axial light conduction. (H) Primary lateral root of *A. princeps* without pith. Vascular tissue (vt) conducts light more efficiently than cortex (co). (I) A portion of (H). Fibres in primary xylem (xf) conduct light via the cell walls. Scale bar for (A), (E) and (H) is in (A) and equals 150 μm ; that for (B), (D), (F), (G) and (I) is in (B) and equals 50 μm ; and that in (C) equals 300 μm .

secondary growth. There was some additional transmission at 520–600 nm in the stems and roots of a small number of species, but it was much less than in the region 710–940 nm (Fig. 5D, E, G, H). Outside this region, there was no significant transmission in either stems or roots. It is also verified that this spectral region is most efficiently conducted in the stem and root under natural light (Fig. 5C).

The spectral properties of the light conducted in the stem or root at different oblique illumination angles on the stem surface remained the same, and there was no change compared with those under axial illumination of the cut end of the stem (Fig. 5).

There are various pigments and other light-absorbing substances in plant tissues that can absorb certain

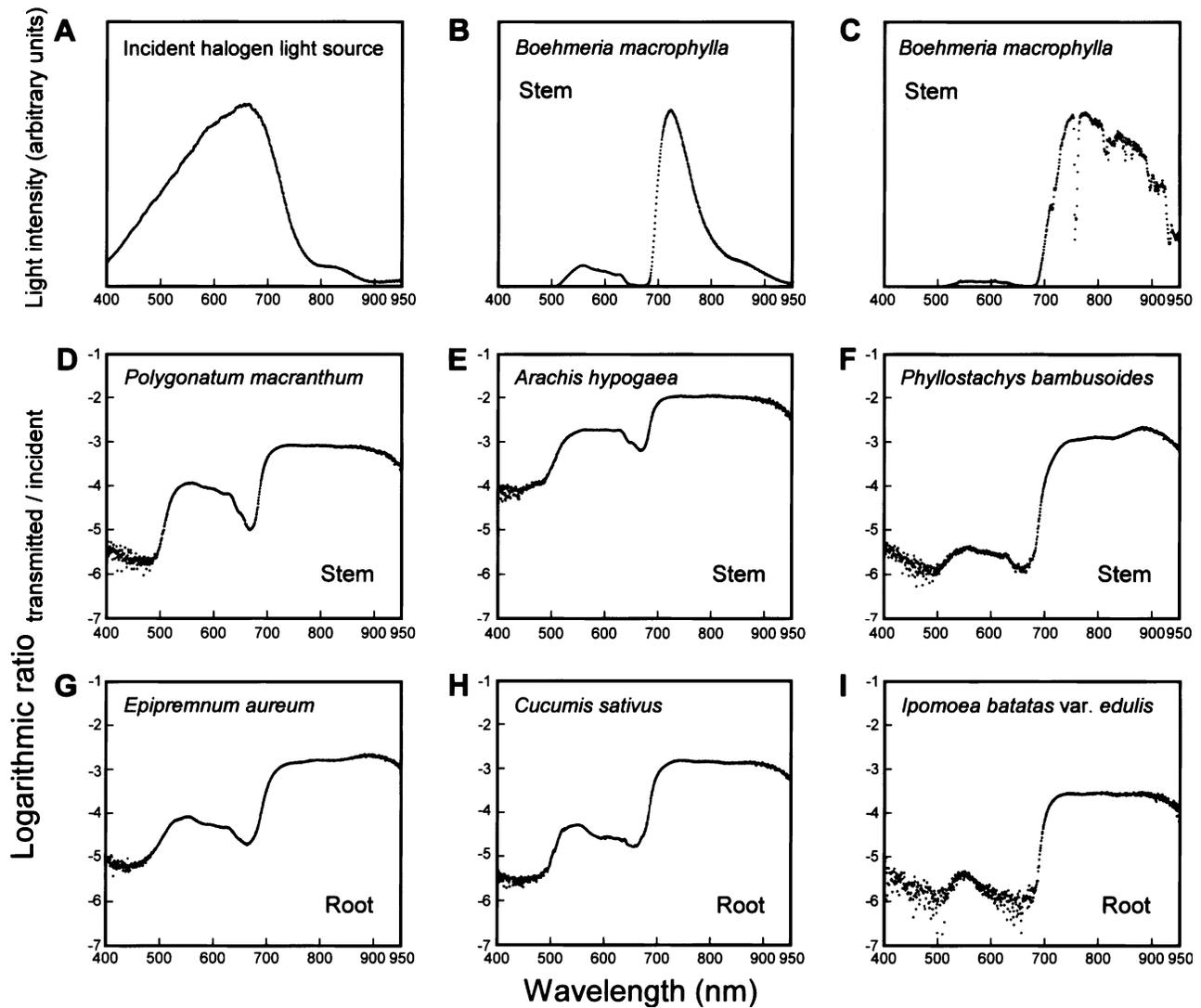


Fig. 5. Examples of light conduction characteristics at different wavelengths in 2 cm long lengths of stems and roots of representative species under different conditions of illumination. (A) Spectrum of incident light (a halogen light source). (B) Transmission spectrum of a stem under oblique illumination with the incident light (A) (incident angle at 60° to the axis). (C) Transmission spectrum of a stem under sunlight. (D–I) The relative ratios (logarithmic values) of transmission in three stems (D–F) and three roots (G–I) to the incident light (A), showing the differences of their axial light-conducting efficiency across the spectrum. (D) Stem of a monocotyledon without secondary growth. (E) Stem of a dicotyledon with secondary growth. (F) Stem of a monocotyledon without secondary growth. (G) Root of a monocotyledon without secondary growth. (H) Root of a dicotyledon with secondary growth. (I) Root of a dicotyledon with secondary growth. In (B), (D), (E), (G) and (H), the incident light (A) was applied to axially illuminate the upper cut end surface of a plant length. In (C), (F) and (I), sunlight or the incident light (A) was applied obliquely to illuminate the lateral surface of a plant length (incident angle at 40° to the axis).

wavelengths of light and subsequently emit fluorescence at a longer wavelength (Vogelmann and Han, 2000). To determine the effects of fluorescence, monochromatic light at wavelengths from 400 nm to 950 nm were applied, and the spectral composition of the emitted light transmitted by the stem or root was recorded. No obvious signals were detected when monochromatic light outside the most efficiently-conducting spectral region of the stem or root was applied as the incident light (Fig. 6A, C). When the incident light was within the spectral region conducted most efficiently, only the spectrum of the corresponding

transmitted light (with a transmission peak and half bandwidth closely similar to the monochromatic incident light) was detected (Fig. 6B, D, E). In these transmission spectra (Fig. 6B, D, E), the transmission ratios among the different wavelengths generally remained consistent with those shown in Fig. 5D–I. This result indicates that the light conducted within the stem and root of herbaceous plants is directly derived from the optical and filtering properties, and that any fluorescence derived from the stem and root tissues does not significantly contribute to the light conducted internally.

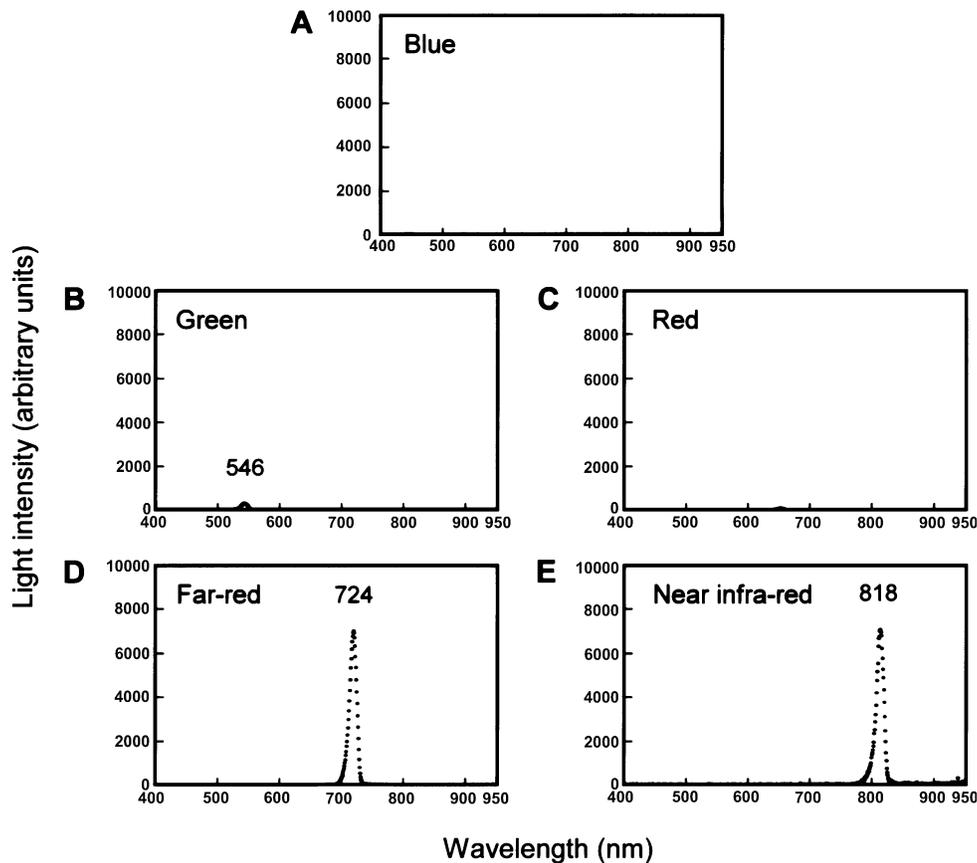


Fig. 6. Examples of the transmission characteristics of certain selected monochromatic lights by a 2 cm long stem length of *Epipremnum aureum* under oblique illumination with a halogen light source (incident angle at 40° to the stem axis). Monochromatic incident light was obtained from narrow band pass interference filters and matched for intensity. (A) Blue light as the incident light (using a 453 nm interference filter). (B) Green light as the incident light (using a 550 nm interference filter). (C) Red light as the incident light (using a 660 nm interference filter). (D) Far-red light as the incident light (using a 727 nm interference filter). (E) Near infra-red light as the incident light (using an 820 nm interference filter). The figures shown on the graphs are wavelengths with peak transmission data.

The intensity of the light conducted by stem and root tissues at each wavelength decreases linearly with axial distance of conduction

The efficiency of light conduction in a stem or root can be indicated by the slope of the light gradient in the direction of conduction. Attenuation of light was present in all the stems and roots investigated but it occurred to different extents, depending not only on the species but also on the structure measured (stem or root) (Figs 5D–I; 7). In most stems (Fig. 7A, C) or roots (Fig. 7B, D), including those with and without secondary growth, transmission intensity across the spectrum (400–950 nm) generally decreased in direct proportion to the distance of conduction. Light attenuation at specific wavelengths showed a negative linear relationship between the intensity of the light conducted and the distance of axial conduction at each wavelength over 500 nm (there was background noise to measurement taken below 500 nm because of the weak transmission intensity for a longer stem or root length in this region of the spectrum) (Fig. 8). Correlation coefficients for the regression lines of the stems and roots varied in a range, usually

between -0.99 and -0.92 across the spectral region. The slope of the regression line is an indicator of the extent of light attenuation per centimetre. The slope value varied at different wavelengths within the same stem or root, between the stem and root, and among species, but generally lay between -1.421 and -0.574 .

Discussion

Differences in internal stem and root light conduction between herbaceous and woody species

The present paper helps to clarify the characteristics of axial light conduction in the stems and roots of herbaceous species. These findings also identify certain differences between the structural components involved in light conduction in herbaceous and woody species. In woody species (Sun *et al.*, 2003, 2004), vascular tissue (consisting mainly of secondary xylem and secondary phloem) comprises the majority of the stem and root structure, and the vessels, fibres, and tracheids were the only efficient axial

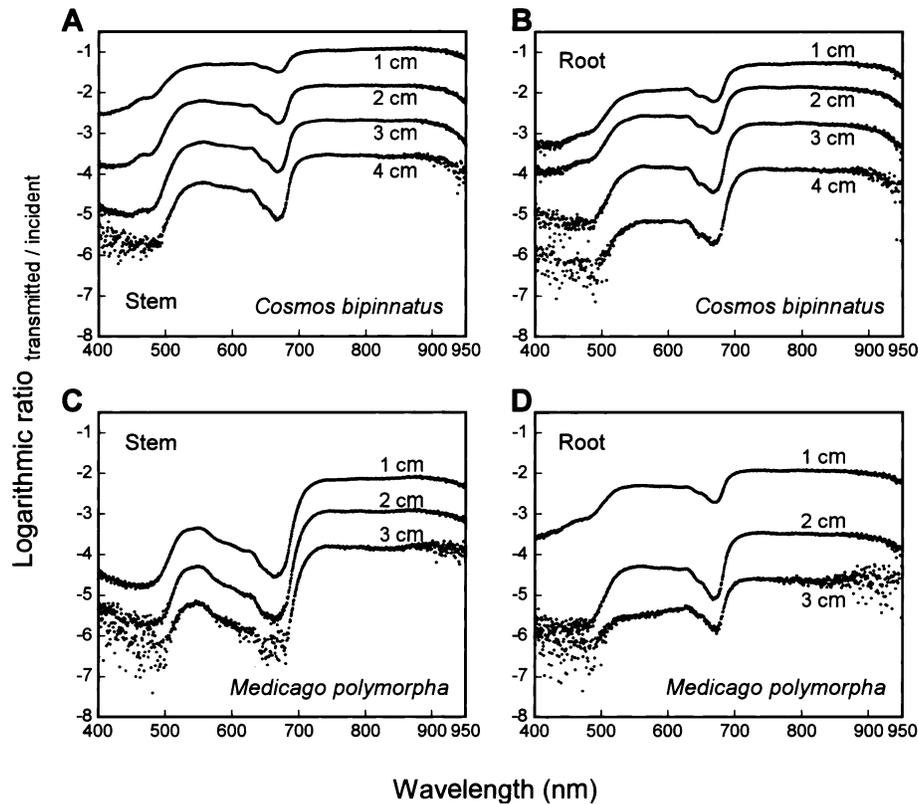


Fig. 7. Examples of the efficiency of axial light conduction in stems and roots of different lengths. Stem (A) and root (B) with secondary growth, showing light attenuation for lengths of 1 cm intervals under axial illumination with a halogen light source. Stem (C) and root (D) without secondary growth, showing light attenuation for lengths of 1 cm intervals under oblique illumination with a halogen light source (incident angle at 40° to the axis). Transmission ratios are presented in logarithmic values. The figures in the graphs indicate the lengths of stems or roots through which light travelled. The blurring at the ends of the spectrum is due to background noise in measuring the attenuation across longer axial distances with the subsequently relatively low intensities.

light conductors. Other tissues, such as the cortex and pith, consisting mainly of parenchyma cells, were generally poor light conductors. However, in herbaceous species, the light-conducting structural components of the stem and root were more diverse. In the stems and roots undergoing secondary growth, light conduction was relatively consistent with that in woody species (Figs 2G, H; 4A, B); while in the stems and roots without or prior to secondary growth, the vascular tissue involved in efficient light conduction occupied only a small portion of the structure in many of the species investigated, and light conduction via this route was usually overwhelmed by that occurring in the cortex, pith, and ground tissues (Figs 1B; 2E; 3D; 4G). Thus, in contrast to woody species, in herbaceous species the parenchyma of the cortex, pith, and ground tissues also contributed much to axial light conduction. The collenchyma in the cortex were also efficient light conductors (Fig. 2C).

The extent of interspecies variations in axial light attenuation of the stem and root is another important difference between herbaceous and woody species. In both groups, light intensity was attenuated linearly across the spectrum in the axial conduction (Figs 7, 8) (Sun *et al.*, 2003). However, the degree of light attenuation (steepness

of the slope in Fig. 8) among species differed between woody and herbaceous species. Among woody species, there was less interspecies difference in the extent of light attenuation; that is, the stem and root of different species tend to be more consistent in attenuating the conducted light (Sun *et al.*, 2003). However, among herbaceous species, interspecies differences in axial attenuation were much greater (Figs 5D–I; 7).

The reason for these observed differences is probably related to differences between the structure and pigments of the stems and roots in woody and herbaceous species. Light attenuation is related not only to the light-conducting character of the light conductors themselves, but also to their quantity and the pigment content in both the stems and roots. The presence of a larger ratio of efficient light conductors presumably contributes to a higher efficiency of axial light conduction. In the stems or roots of woody plants, light-conducting elements (fibres, vessels or tracheids of vascular tissues) form the majority portion of the cross-sectional area, and generally display a relative uniformity of structure in different species, which explains the relative lack of interspecies differences in terms of the extent of light attenuation. However, in the stems or roots of

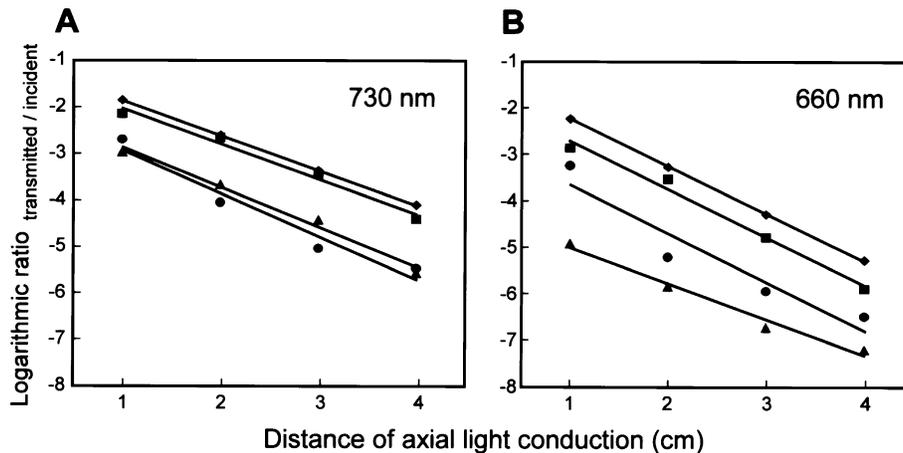


Fig. 8. Examples of axial light attenuation gradients at different wavelengths in stems and roots. Transmission ratios are presented in logarithmic values. Attenuation for the stems (filled diamonds) and roots (filled squares) with secondary growth (*Cosmos bipinnatus*) was measured under axial incident illumination, but that for the stems (filled triangles) and roots (filled circles) without secondary growth (*Medicago polymorpha*) was measured under oblique incident illumination (incident angle at 40° to the axis). (A) Axial attenuation at 730 nm. Each data point represents a mean of three measurements. The regression lines for the stems and roots of *C. bipinnatus*, and the stems and roots of *M. polymorpha* were as follows: $y = -0.857x - 0.125$, $R^2 = 1$; $y = -0.864x - 0.319$, $R^2 = 0.981$; $y = -0.976x - 1.163$, $R^2 = 0.985$; $y = -1.060x - 1.139$, $R^2 = 0.952$. (B) Axial attenuation at 660 nm. The regression lines for the stems and roots of *C. bipinnatus*, and the stems and roots of *M. polymorpha* were as follows: $y = -1.159x - 0.255$, $R^2 = 0.999$; $y = -1.181x - 0.773$, $R^2 = 0.986$; $y = -0.883x - 3.689$, $R^2 = 0.981$; $y = -1.198x - 1.820$, $R^2 = 0.907$.

herbaceous plants, efficient light conductors, such as fibres and vessels, are different in terms of both quantity and arrangement among these species. The contents and kinds of pigments in the parenchyma cells of pith, cortex, and ground tissue relevant to the efficiency of light conduction vary greatly with species, producing a wider range of interspecies differences in light attenuation observed in their stem and root.

Functional significance of the internal light environment of stems and roots of herbaceous plants

The present investigation has made it clear that light at wavelengths between 710 nm and 940 nm is conducted most efficiently by the stems and roots of herbaceous species (Fig. 5D–I), and that the spectral region rich in far-red light is only the composition in their stem and root in the natural light environment because of the spectral properties of sunlight (Fig. 5C). Thus, herbaceous species are similar to woody ones in terms of the spectral properties of the light conducted by the stem and root tissues (Sun *et al.*, 2003).

In the stems and roots of herbaceous plants, the structural components involved in axial light conduction include the parenchyma cells of cortex, pith, and ground tissue, and the fibres and vessels in vascular tissue. Among these, the fibres and vessels die upon maturation. As indicated previously (Sun *et al.*, 2003, 2004), the light conducted by fibres and vessels is not restricted to the light conductors only, but in fact leaks out to the surrounding living tissues during axial conduction. This has been verified in recent investigations of the spectral properties of specific tissues and cells, and even specific parts of a tissue or cell, by means of a fine light-guide detector (Q Sun and H Suzuki, unpublished data). Thus, tissues in the stems and roots of

herbaceous plants are bathed in an internal light environment rich in far-red light.

Plant tissues can perceive light signals by intrinsic photoreceptors so as to regulate the processes of their growth and development. To date, several kinds of photoreceptors have been identified which respond to specific wavelengths: five phytochromes (PhyA–E; Smith, 2000), two cryptochromes (Cry1 and Cry2; Cashmore *et al.*, 1999) and two phototropins (Phot1 and Phot2; Briggs *et al.*, 2001). The phytochromes are a family of photoreceptors that respond mainly to red and far-red light (Quail, 2002). The far-red responses of the phytochromes depend on the molecular species (Whitelam and Delvin, 1997), and are also related to the energetic levels of far-red light, including high irradiance response (HIR; Hartmann, 1966; Shinomura *et al.*, 2000; Cerdán and Chory, 2003), low fluence response (LFR; Fankhauser, 2001), and very low fluence response (VLFR; Botto *et al.*, 1996; Shinomura *et al.*, 1996). The present investigation indicates that the light from the surrounding environment of herbaceous plants enters the interior of the stem, and via an internal light-conducting system the far-red light is conducted axially towards the roots underground. This characteristic internal light environment is of crucial importance for the phytochrome-regulated metabolic activities of plant stems and roots. Attenuation of the axially conducted light along the root indicates that responses involving HIR, LFR, and VLFR probably happen in roots at different depths underground. Our primary investigation into *Arabidopsis* has verified that the presence or absence of far-red light can change the expression levels of many genes in the roots (K Sato-Nara, unpublished data). Thus, when analysing the metabolic activities of roots underground, direct influences

derived from the light environment surrounding the plants above-ground should be taken into consideration.

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References

- Bone RA, Lee DW, Norman JM.** 1985. Epidermal cells functioning as lenses in leaves of tropical rain-forest shade plants. *Applied Optics* **24**, 1408–1412.
- Botto JF, Sanchez RA, Whitelam GC, Casal JJ.** 1996. Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in *Arabidopsis*. *Plant Physiology* **110**, 439–444.
- Briggs WR, Beck CF, Cashmore AR, et al.** 2001. The phototropin family of photoreceptors. *The Plant Cell* **13**, 993–997.
- Cerdán PD, Chory J.** 2003. Regulation of flowering time by light quality. *Nature* **423**, 881–885.
- Cashmore AR, Jarillo JA, Wu YJ, Liu D.** 1999. Cryptochromes: blue light receptors for plants and animals. *Science* **284**, 760–765.
- Cui M, Vogelmann TC, Smith WK.** 1991. Chlorophyll and light gradients in sun and shade leaves of *Spinacia oleracea*. *Plant, Cell and Environment* **14**, 493–500.
- Esau K.** 1977. *Anatomy of seed plants*, 2nd edn. New York: J Wiley & Sons, 61–181.
- Fahn A.** 1990. *Plant anatomy*, 4th edn. Oxford: Butterworth-Heinemann, 185–307.
- Fankhauser C.** 2001. The phytochromes, a family of red/far-red absorbing photoreceptors. *Journal of Biological Chemistry* **276**, 11453–11456.
- Fukshansky L, Fukshansky-Kazarinova N, Martinez von RA.** 1991. Estimation of optical parameters in a living tissue by solving the inverse problem of the multiflux radiative transfer. *Applied Optics* **30**, 3145–3153.
- Fukshansky L, Martinez von RA, McClendon J, Ritterbusch A, Richter T, Mohr H.** 1992. Absorption spectra of leaves corrected for scattering and distributional error: a radiative transfer and absorption statistics treatment. *Photochemistry and Photobiology* **55**, 857–869.
- Hartmann KM.** 1966. A general hypothesis to interpret 'high energy phenomena' of photomorphogenesis on the basis of phytochrome. *Photochemistry and Photobiology* **5**, 349–366.
- Iino M.** 1990. Phototropism: mechanisms and ecological implications. *Plant, Cell and Environment* **13**, 633–650.
- Karabourniotis G, Bornman JF, Nikolopoulos D.** 2000. A possible optical role of the bundle sheath extensions of the heterobaric leaves of *Vitis vinifera* and *Quercus coccifera*. *Plant, Cell and Environment* **23**, 423–430.
- Karabourniotis G, Papastergiou N, Kabanopoulou E, Fasseas C.** 1994. Foliar sclereids of *Olea europaea* may function as optical fibres. *Canadian Journal of Botany* **72**, 330–336.
- Kolb CA, Käser MA, Kopecký J, Zotz G, Riederer M, Pfündel EE.** 2001. Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. *Plant Physiology* **127**, 863–875.
- Kunzelmann P, Iino M, Schäfer E.** 1988. Phototropism of maize coleoptiles. Influence of light gradients. *Planta* **176**, 212–220.
- Kunzelmann P, Schäfer E.** 1985. Phytochrome-mediated phototropism in maize mesocotyls. Relation between light and P_{fr} gradients, light growth response, and phototropism. *Planta* **165**, 424–429.
- Mandoli DF, Briggs WR.** 1982. Optical properties of etiolated plant tissues. *Proceedings of the National Academy of Sciences, USA* **79**, 2902–2906.
- Mandoli DF, Briggs WR.** 1984a. Fibre-optic plant tissues: spectral dependence in dark-grown and green tissues. *Photochemistry and Photobiology* **39**, 419–424.
- Mandoli DF, Briggs WR.** 1984b. Fibre optics in plants. *Scientific American* **251**, 90–98.
- McClendon JH, Fukshansky L.** 1990. On the interpretation of absorption spectra of leaves. I. Introduction and the correction of leaf spectra for surface reflection. *Photochemistry and Photobiology* **51**, 203–210.
- Myers DA, Vogelmann TC, Bornman JF.** 1994. Epidermal focussing and effects on light utilization in *Oxalis acetosella*. *Physiologia Plantarum* **91**, 651–656.
- Parks BM, Poff KL.** 1985. Phytochrome conversion as an *in situ* assay for effective light gradients in etiolated seedlings of *Zea mays*. *Photochemistry and Photobiology* **41**, 317–322.
- Parks BM, Poff KL.** 1986. Altering the axial light gradient affects photomorphogenesis in emerging seedlings of *Zea mays* L. *Plant Physiology* **81**, 75–80.
- Pfanz H.** 1999. Photosynthetic performance of twigs and stems of trees with and without stress. *Phyton* **39**, 29–33.
- Pfanz H, Aschan G.** 2001. The existence of bark and stem photosynthesis in woody plants and its significance for the overall carbon gain. *Progress in Botany* **62**, 477–510.
- Pfanz H, Aschan G, Langenfeld-Heyser R, Wittmann C, Loose M.** 2002. Ecology and ecophysiology of tree stem photosynthesis: cortical and wood photosynthesis. *Naturwissenschaften* **89**, 147–162.
- Piening CJ, Poff KL.** 1988. Mechanism of detecting light direction in first positive phototropism in *Zea mays* L. *Plant, Cell and Environment* **11**, 143–146.
- Poulson ME, Vogelmann TC.** 1990. Epidermal focussing and photosynthetic light harvesting in leaves of *Oxalis*. *Plant, Cell and Environment* **13**, 803–811.
- Quail PH.** 2002. Phytochrome photosensory signalling networks. *Nature Reviews Molecular Cell Biology* **3**, 85–93.
- Seyfried M, Fukshansky L.** 1983. Light gradients in plant tissue. *Applied Optics* **22**, 1402–1408.
- Seyfried M, Schäfer E.** 1983. Changes in the optical properties of cotyledons of *Cucurbita pepo* during the first seven days of development. *Plant, Cell and Environment* **6**, 633–640.
- Shinomura T, Nagatani A, Hanzawa H, Kubota M, Watanabe W, Furuya M.** 1996. Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **93**, 8129–8133.
- Shinomura T, Uchida K, Furuya M.** 2000. Elementary processes of photoperception by phytochrome A for high-irradiance response of hypocotyls elongation in *Arabidopsis*. *Plant Physiology* **122**, 147–156.
- Smith H.** 2000. Phytochromes and light signal perception by plants—an emerging synthesis. *Nature* **407**, 585–591.
- Sun Q, Yoda K, Suzuki M, Suzuki H.** 2003. Vascular tissue in the stem and roots of woody plants can conduct light. *Journal of Experimental Botany* **54**, 1627–1635.
- Sun Q, Yoda K, Suzuki H.** 2004. Spectral properties of light conducted in stems of woody plants show marked seasonal differences suggesting a close relationship with photomorphogenesis. *International Association of Wood Anatomists Journal* **25**, 91–101.

- Terashima I, Saeki T.** 1983. Light environment within a leaf. I. Optical properties of paradermal sections of *Camellia* leaves with special reference to differences in the optical properties of palisade and spongy tissues. *Plant and Cell Physiology* **24**, 1493–1501.
- Turunen MT, Vogelmann TC, Smith WK.** 1999. UV screening in lodgepole pine (*Pinus contorta* ssp. *latifolia*) cotyledons and needles. *International Journal of Plant Science* **160**, 315–320.
- Vogelmann TC.** 1993. Plant tissue optics. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**, 231–251.
- Vogelmann TC.** 1994. Light within the plant. In: Kendrick RE, Kronenberg GHM, eds. *Photomorphogenesis in plants*, 2nd edn. Netherlands: Kluwer Academic Publishers, 491–535.
- Vogelmann TC, Han T.** 2000. Measurement of gradients of absorbed light in spinach leaves from chlorophyll fluorescence profiles. *Plant, Cell and Environment* **23**, 1303–1311.
- Vogelmann TC, Haupt W.** 1985. The blue light gradient in unilaterally irradiated maize coleoptiles: measurement with a fibre optic probe. *Photochemistry and Photobiology* **41**, 569–576.
- Walter-Shea EA, Norman JM.** 1991. Leaf optical properties. In: Myneni RB, Ross J, eds. *Photon-vegetation interactions*. Berlin: Springer-Verlag, 229–251.
- Whitelam GC, Delvin PF.** 1997. Roles of different phytochromes in *Arabidopsis* photomorphogenesis. *Plant, Cell and Environment* **20**, 752–758.