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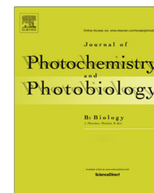

Light piping activates chlorophyll biosynthesis in the under-soil hypocotyl section of bean seedlings

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ABSTRACT

Protochlorophyllide (Pchlide), protochlorophyll (Pchl) and chlorophyll (Chl) contents, their distribution and native arrangements were studied in under-soil hypocotyl segments of 4-, 7- and 14-day-old bean (*Phaseolus vulgaris* L. cv. Magnum) seedlings. The plants were grown in general potting soil under natural illumination conditions in pots. For sample collection, the pots were transferred into dark-room where all manipulations were done under dim green light. The pigments were extracted with acetone; phase separation was used to identify the Pchl contents. Fluorescence microscopic studies were done and 77 K fluorescence emission spectra were recorded. Using a special setup of a spectrofluorometer, the vertical light piping properties of the above-soil shoots were measured. The segments in the 5–7 cm deep soil region contained Pchlide and Pchl in 4- and 7-day-old seedlings and the segments towards the soil surface contained Chl in increasing amounts. In parallel with the pith degradation of hypocotyls, the Chl content of elder seedlings increased in the deeper under-soil segments. These results prove that the tissue structure of the shoot ensures light piping thus greening process and chloroplast formation can take place even in under-soil organs not directly exposed to light.

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1. Introduction

The occurrence of etiolated tissues containing etioplasts and the chlorophyll (Chl) biosynthesis precursor protochlorophyllide (Pchlide) in the dark are well-known phenomena. The characterization of the etioplast structures and the native arrangement of Pchlide complexes, as well as their changes upon illumination i.e. the greening process has been studied mainly on laboratory plants germinated in full darkness. In the past few years, however, the occurrence of etiolated tissues has been described in shaded tissues of light-grown plants such as in innermost leaf primordial of cabbage [1,2], buds [3–5], fruits [6] and seeds [7]. In addition, etiolation symptoms were reported in under-soil epicotyl segments of light-grown pea plants [8]. Important results of these latter studies are that shaded tissues of light-grown plants contain etioplasts with similar structures and Pchlide spectral forms having similar photochemical properties as the tissues of plants grown in total darkness. The etioplasts usually contain prolamellar bodies (PLBs) and prothylakoids (PTs) however, the inner membranes are less organized in shaded tissues than in etiolated plants and resemble proplastids [1]. In addition, the relative amounts of the Pchlide complexes (forms) often differ from those of etiolated leaves. The shaded tissues often contain mainly short-wavelength forms with emission maxima at 629, 633 or 636 nm which are considered as monomer forms containing only a single Pchlide or Pchl molecule [9]. The 644 and 655 nm emitting Pchlide forms, which are considered as dimers or oligomers of this pigment [10], are present only in small amounts while they are characteristic for leaves of etiolated seedlings [11].

The dominance of the Pchl or Pchlide monomers have special importance since their illumination provokes photo-oxidation and thus photodamage [12,13] rather than photoreduction of Pchlide into chlorophyllide (Chlide) and thus Chl biosynthesis processes. When discussing the possibilities of these reactions in the under-soil shoot segments of plants grown under natural illumination conditions, one must consider the optical properties of the soil. Soil particles can function as optical filters, their color and size control how deep light penetrates into the soil [14]. Spectroreflectometric [15] and spectrophotometric [16] measurements showed that less than 0.01% of the natural light reaches a 4 mm soil depth regardless to the soil type and thus influence the germination of light sensitive seeds [15]. Obviously, other abiotic (water supply, drought) and biotic factors can change the illumination conditions thus they have combined photomorphogenetic effects on seedlings [17].

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In addition to the optical properties of the soil, light transmittance or light piping of the plant tissues may regulate Chl biosynthesis in under-soil shoot tissues. Results were reported mainly about tissue layers of the leaves: the characteristics of the epidermis, i.e. the cuticle or wax structure and thickness as well as the presence of hairs or crystals is very important [18–20] and also the parenchyma structure has a basic role in the absorbance and reflectance of leaves [21,22].

Investigations on the effect of incident light on etiolated tissues revealed that the quantity and quality of the entering light depends on the angle of incidence and the shape of the tissue-creating cells [23–26]. As the tissue becomes thicker the intensity of the incident red and far-red light decreases [27,28]. Measurements on the optical properties of stems and roots of woody plants showed axial light piping: the most effective cells (vessels, tracheids and fibers) conducting far-red light are located in the bundles [29]. Herbaceous plants have more diverse anatomical structure thus their optical characteristics show great variety; for example the light-conducting role of the pith and the cortex is species-specific [30]. These above described investigations were performed on detached organs, stem segments or tissues, and provided information about the possibilities of light conductance only of certain plant tissues. However, no results were reported about the light conduction of whole intact shoots.

The aim of this study was to examine if photons were piped by the above-soil shoot into the under-soil hypocotyl segments of bean into depths of soil where otherwise no light can penetrate. Different under-soil hypocotyl segments were collected in the dark from 4-, 7- and 14-day-old light-grown bean seedlings, the pigment contents, the 77 K fluorescence spectra and fluorescence microscopic images of which were studied. In other experiments, whole shoots or under-soil segment of the hypocotyls were fixed into the spectrofluorometer and the light piping was directly measured using a special setup and application of the fluorometer.

2. Materials and methods

2.1. Plant material and cultivation

Seeds of bush bean (*Phaseolus vulgaris* L. cv. Magnum, Rédei Kertimag Ltd., Réde, Hungary) were soaked in tap water for 2 h and pre-germinated on wet filter paper in Petri-dishes for 3 days in darkness. The seedlings with about 2 cm radicle were sown into pots using general potting soil (Biovital, pH 6.6–6.8, Szentendre, Hungary) under dim green light which was tested and caused no phototransformation of Pchlide into Chlide in etiolated seedlings. The recommended planting depth (4 cm) was used. The bean seedlings were grown in the laboratory window at room temperature under natural illumination conditions with an average photon flux density (PFD) of 690 μmol photon m^{-2} s^{-1} (the maximum value was 1450 μmol photon m^{-2} s^{-1}). The plants were watered at the bottom of the pots every second day with tap water. The 4, 7 and 14 days old plants (counted from sowing) were transferred into dark-room for sampling. The hypocotyl was labeled at the soil surface level and the seedlings or plants were removed from the soil at the above mentioned dim green light. The under-soil hypocotyl part of each plant was sectioned as follows:

The whole bodies of the 4 days old seedlings were still covered by the soil. They had 4 cm long hypocotyls which were dissected into 3 pieces: a 1-cm long segment under the hypocotyl hook (US-1), a 2-cm long section from the middle (US-2) and a 1-cm long part above the root (US-3). The 7 and 14 days old plants emerged over the soil surface, their under-soil hypocotyls were 7–8 cm long. Samples were taken from the following sections: a 1-cm long segment directly under the soil surface (US-1), a 2-cm long region between 2 and 5 cm depth (US-2) and a 2-cm long segment between 5 and 7 cm depth (US-3). In order to correlate the chlorophyll contents of the under-soil and above-soil shoot parts, the first cm of the above-soil hypocotyls of 7 and 14 days old plants were collected and measured.

These segments were used for fluorescence spectroscopy, pigment determination and microscopic works. For each variant, 7 parallels were studied.

Several plants were decapitated just above their root necks (at their transitional zones); these plants were used for the light piping measurements.

2.2. Photon flux density (PFD) measurements

The PFD values were measured with a Delta Ohm HD 2102.1 photo-radiometer (Delta Ohm, Padova, Italy).

2.3. Fluorescence spectroscopy

The 77 K fluorescence emission spectra were recorded with a Fluoromax-3 (Jobin Yvon-Horiba, Paris, France) spectrofluorometer equipped with a low temperature accessory. The excitation wavelengths were 440 and 460 nm, the integration time was 0.1 s; 2 nm excitation and 5 nm emission optical slits were used. The data collection frequency was 0.5 nm. Each sample was recorded three times and their mean was automatically calculated. The SPSERV V3.14 software (© Csaba Bagyinka, Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary) was used for computer analysis of the spectra: 5 point linear smoothing was used which does not modify the structure of the spectra. Baseline correction was done to correct the distortion caused by light scattering. The spectra were corrected for the wavelength-dependent sensitivity of the spectrofluorometer.

2.4. Light piping measurements

The optical characteristics of the shoots were measured with the Fluoromax-3 (Jobin Yvon-Horiba, Paris, France) spectrofluorometer the sample compartment of which was modified as follows: the cuvette holder stage was replaced with a plate on which a concave mirror was fixed in the position where it projected the vertically arriving light beam to the emission window of the fluorometer (Fig. 1). The shoot was fixed into the cover of the sample compartment; the soil surface level of the hypocotyl was exactly at the upper surface of the cover. The roots were removed before placing the shoot into the compartment. The bottom of the sagging sample was about 1 cm above the center the mirror. The excitation light shutter was closed during the measurements. The shoot segment emerging from the sample compartment was optically isolated from the sagging part with black plasticine. The shoot was illuminated with Tungsten lamp, its PFD value was 50 μmol photon m^{-2} s^{-1}. The lamp was located 10 cm from the plant; the angle of incidence was 60°. Each measurement was performed in 2-min interval thus the heating effect of the lamp could not prevail. After recording the spectrum of the intact plant the above-soil shoot part was cut and the light piping ability of the under-soil hypocotyl was measured in the next experiment. The spectrum of the Tungsten lamp was recorded through the hole of the sample compartment cover using the same setup. The emission spectra were recorded in the 380–780 nm region with 1 nm sampling frequency. The spectra of the transmitted light (*T*%) were calculated by the following equation:

$$T\% = (b/a) * 100$$

where a = amplitude value of the lamp at a given wavelength; b = amplitude of the light measured with the sample.

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