The effect of red and far-red light on proton secretion from mesophyll-cell protoplasts of *Vicia faba* L.

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**Abstract.** The influence of red and far-red irradiation on the transport of H⁺ and ^86^Rb⁺ through the plasmalemma was studied using parenchymal protoplasts isolated from *Vicia faba* leaves. The results indicate that red light stimulates H⁺ secretion and the uptake of ^86^Rb⁺. Moreover, it has been demonstrated that far-red irradiation acts antagonistically with respect to red light in both these processes.

**Key words:** Light (red, far red) – Mesophyll cell protoplast – Phytochrome – Proton secretion – *Vicia* (H⁺ secretion).

**Introduction**
Recently, some data have been published which indicate that light is involved in the transport of protons and other cations across the plasmalemma of the mesophyll cells of leaves. For instance, it has been demonstrated that under light conditions, protons are secreted by the protoplasts of mesophyll cells (Kelly 1983) as well as by the mesophyll cells (Bown 1982) and by the leaves (Gepstein 1982). Moreover, using ^86^Rb⁺, it was found that the protoplasts accumulated potassium much more intensively in light than in darkness (Rahat and Reinhold 1983; Leurs et al. 1982). These processes are probably linked, since in plants some uptake of K⁺ takes place in the antiport with H⁺ (Pitman and Lüttge 1983). It should be mentioned that Blakeley et al. (1983) demonstrated that the far-red-absorbing form of phytochrome (Pfr) was involved in the swelling of protoplasts from leaf mesophyll cells.

The investigations of Rahat and Reinhold (1983), Kelly (1983), Bown (1982) and Gepstein (1982) utilised visible light, not far-red irradiation, and Blakeley et al. (1983) did not attempt to prove the existence of a relationship between the phytochrome-dependent swelling of mesophyll-cell protoplasts and the secretion of H⁺ and-or K⁺ uptake. However, the existence of a relationship between the swelling of mesophyll-cell protoplasts and K⁺ uptake was very likely, judging by the investigations of other cell types, e.g. stomatal cells (Roth-Bejerano et al. 1985), motor cells (Satter and Galston 1981; Holmes and Klein 1985), or hypocotyl and subhypocotyl sections (Brownlee and Kendrick 1977).

These kinds of cells are responsible for the movement of other cells or organs. Thus it was considered of interest to investigate whether or not phytochrome controls the secretion of H⁺ and the uptake of K⁺ in cells which are not involved in movement, such as the mesophyll cells of leaves. The experimental confirmation of such a contention might be of great theoretical importance.

**Material and methods**

*Plant material.* Seeds of *Vicia faba* L. were soaked in water for 48 h, placed in plastic pots filled with garden soil, and the plants grown in artificial light from fluorescent tubes of the Flora type (energy fluence rate approx. 20 W·m⁻²). The photoperiod applied was 16 h light and 8 h darkness. The investigations were carried out on protoplasts isolated from the leaves of 20-d-old seedlings.

*Isolation of protoplasts.* Protoplasts were isolated from the third leaves of seedlings. After peeling off the lower epidermis the leaf was placed in a Petri dish on the surface of a solution containing 0.5 M sorbitol, 0.01 M CaCl₂, 1% cellulase R-10 and 1% macerozyme R-10, pH 5.7 ('Onozuka' Yakult Pharmaceutical Industry Co., Japan). The prepared dishes were then placed at a temperature of 27°C on a shaker which was switched on for about 2 s every 30 s. After 2 h the suspension of protoplasts was filtered through 40-µm-mesh nylon gauze and placed in test-tubes for centrifugation on a steplike iso-osmotic sucrose-sorbitol gradient (Piwowarczyk 1979).

*Measurements of pH.* The suspension of protoplasts (about 1·10⁶·ml⁻¹) was placed in a measuring vessel in 1 ml of the
incubation solution at pH = 7.2 (for details see Kelly 1983). Air was pumped through the suspension at constant velocity by means of a peristaltic pump connected to a hypodermic needle placed at the bottom of the vessel. The design of the vessel made it possible to illuminate the protoplast suspension.

Measurements of the effects of red and far-red light on pH changes in the protoplast suspension. The protoplast suspension was initially kept in a darkened measuring vessel for 25 min. Red light with a maximum transmission at about 660 nm and an irradiance of 5 W·m⁻² (filters used: RG 4 and C 805; C. Zeiss, Jena, GDR) was then switched on. After 10 min, the protoplasts were simultaneously illuminated for 5 min with red (660 nm) and far-red light (maximum about 730 nm; filters used: RG 8 and C 805).

The cycle applied was 5 min red light (5 W·m⁻²) and 5 min red (5 W·m⁻²)+far red (100 W·m⁻²). Changes in pH were recorded automatically.

Incorporation of ⁸⁶Rb⁺. The purified protoplasts were placed in 1 ml of incubation solution (for details, see Kelly 1983) to which 37 kBq ⁸⁶Rb⁺ was added.

Density gradients were prepared in four centrifuge tubes to each of which were added 6 ml of 125 mM KCl + 50 mM CaCl₂ in the incubation solution, then a layer of 1 ml of 37 kBq ⁸⁶Rb⁺ + 0.36 M sorbitol + 0.14 M sucrose in the incubation medium, and finally a layer of 0.2 ml protoplast suspension in the incubation medium containing 6.62 kBq ⁸⁶Rb⁺.

The protoplasts were first illuminated for 3 min with red light (filters RG 4 and C 805) with an irradiance of about 6 W·m⁻² and then for 3 min with far-red light (filters RG 8 and C 805) with an irradiance of about 6 W·m⁻². This cycle was then repeated. After each exposure to either red or far-red light, one test-tube was taken out under dim green light and placed in the horizontal rotor of the centrifuge. Ten minutes after the last test-tube was removed the protoplasts were centrifuged and the supernatant rejected. The amount of ⁸⁶Rb⁺ taken up by the protoplasts in the sediment was measured by means of a scintillation counter (US-2; Biuro Urzędów Techniki Jądrowej, Warsaw, Poland). On the basis of absorbancy in the 663- and 646-nm bands in 80% acetone the chlorophyll content in the centrifuged protoplasts was calculated, applying the formula of Lichtenthaler and Wellburn (1983).

The amount of absorbed potassium was calculated from the ratio ⁸⁶Rb⁺ to K⁺ in the incubation solution.

In the experiments, special attention was paid to obtaining high-quality protoplasts. When the preliminary purification in the iso-osmotic sucrose-sorbitol density gradient (Piwowarczyk 1979) was not applied the protoplasts lost a great deal of isotope when passing through the layer of CaCl₂ and KCl. It is not known whether this was caused by bursting of the protoplasts or by isotope escape through the plasmalemma. The loss of ⁸⁶Rb⁺ during centrifugation was especially high in protoplasts not subjected to preliminary purification and preilluminated with red light (data not shown).

Usually after light treatment the protoplasts were centrifuged in a gradient in which a solution of 125 mM KCl + 50 mM CaCl₂ (Leurs et al. 1982) was the bottom layer. When this solution was replaced by a saccharose-sorbitol one, an even more extensive escape of ⁸⁶Rb⁺ from the protoplasts was observed during centrifugation (data not shown).

Results

The effect of light absorbed by the phytochrome system on pH changes in the protoplast suspension. The protoplast suspension was illuminated successively with red light (maximum 660 nm) and red light together with far-red light (maximum 730 nm), and the pH changes in the medium were recorded.