The Influence of Phytochrome in the Water Exchange of Epidermal Cells of Taraxacum officinale

It has been proposed that one of the first modifications in plant cells, following a change in the form of phytochrome, may be a change in permeability¹. JAFFE² has measured the Pfr effect on the H⁺ efflux to H⁺ ions. Water permeability might also be affected by the Pfr level, and such a change could be of importance for the cell activity.

We report here the results from measurements of the effect of red and far red light on 3 parameters related to permeability: a) Plasmolysis initiation time (TPL); time for separation of the protoplast from the cell wall when the external concentration changes from distilled water to manitol 1.0M solution. b) Deplasmolysis initiation time (TDEPL); time for the first visible increase in protoplast length when the external solution changes from 1.0 to 0.6M manitol. c) Time course for the increase in protoplast length during deplasmolysis in 0.6 M. Strips of abaxial petiole epidermis from Taraxacum officinale were used for the experiments. The strips were taken from plants kept at 20°C, 95% RH, 10 h/day fluorescent light with an irradiance of 7000 µW cm⁻² (400-700 nm). Each strip was used only for 2 measurements, 1 of them after 20 min of red light (RL), the other after 10 min of far red light (FRL), both measurements in the same strip have been made on the same cell. The RL and FRL irradiations were always given with the tissue in equilibrium with distilled water. In half of the experiments, the first irradiation was with red light, in the other half with far red light. In total 20 measurements of the 3 parameters have been made after red and 20 after far red light. The measurements were made using a perfusion chamber³, where the external solution can be changed in a few seconds, and the procedure was the same as described by Stadelmann⁴. The protoplast length was measured with an ocular micrometer under green light. All the experiments were carried out in an airconditioned room at 20 °C ± 1 °C. Handling of the material, changes of solutions and measurements were done with dim green safe light. The source of FRL was an incandescent lamp of 500 W, and a Schott RGN9 glass filter; the transmission was between 700 and 850 nm. The irradiance on the plane where the perfusion chamber was located was $0.25~\mu\mathrm{W}~\mathrm{cm}^{-2}~\mathrm{nm}^{-1}$ at 730 nm. The red light source was a bank of 3 red fluorescent tubes Philip Tl 15 40 W with emission within 600 and 700 nm and maximum at 670 nm; the irradiance was of 460 μW cm⁻² nm⁻¹. The green light source in the microscope was a 6 V/20 W incandescent lamp and a band between 520-570 nm was isolated with a Wratten 74 gelatin filter plus 2 layers of green acetate, the irradiance at sample level was 0.15 $\mu\mathrm{W}$ cm⁻² nm⁻¹ at 550 nm. Light quality and intensities were checked with an ISCO SR spectroradiometer.

Both plasmolysis and deplasmolysis initiation time were significantly decreased by previous irradiation with FRL,

Effect of red and far red light on plasmolysis, deplasmolysis and Kw in cells of petiole epidermis of Taraxacum officinale

	Plasmolysis initiation time TPL (sec)	Deplasmolysis initiation time TDEPL (sec)	Constant of permeability Kw (μsec^{-1})
Red light Far red light	41.9±7.1	42.7±5.0	36.0 ± 6.8
	32.9±4.0	35.5±3.4	49.6 ± 10.0

Mean values for 20 cells. The difference between red and far red values are significant at 0.02 < P < 0.05 level, Kw = permeability constant for water.

irrespectively of the order in which the irradiations were given (Table). The differences are significant at the P < 0.05 level.

If during the first or second plasmolysis-deplasmolysis cycle some damage and/or change in the response of the system had taken place, it could be expected that the order in which the light irradiations were given would be important. An analysis of variance of our data showed that the sequence of measurements had no significant influence on the results, which only depend on the light quality. Therefore such damage or change in response seems unlikely. Moreover, the protoplast lengths observed at equilibrium with either 1.0 or $0.6\,M$ were the same in the first and second plasmolysis-deplasmolysis cycle, and this fact gives further support to the precedent assumption.

The rate of water uptake when the external concentration changed from 1.0 to 0.6M manitol was increased by FRL irradiation. This is evidenced by the constant of permeability Kw, as defined by STADELMANN⁴. The values for Kw (Table) were calculated from a graph recording change in protoplast length over time.

The results show that the RL and FRL irraiations influenced the water exchange through the system plasmalemma-cytoplasm-tonoplast. As the light energy doses were low, and the effect of RL and FRL is reversible, phytochrome seems to be involved. When the Pfr level was low, TPL and TDEPL decreased and Kw ncreased. The observed effects of Pfr level could, in a first approach, be thought of as mediated by a change in permeability to water, which could explain the shorter time required to observe a change in protoplast length when the external solution changes, and the faster course of deplasmolysis.

The water exchange might also be affected by modifications in the water potential of the vacuole; but since the volume of the vacuole at equilibrium with solutions of the same osmotic potential was repeatedly found to be the same, irrespective of the light treatment; we think it is unlikely that RL or FRL affected the water potential of the vacuole in our experiments. As is known, other factors, such as the reflection coefficient and the elasticity modulus may influence the water exchange in the cell, but no attempt has been made to analyze the extent to which these factors could be involved, in the RL-FRL modifications of the water exchange that we have measured 5.

Résumé. La qualité de la lumière a modifié le passage de l'eau dans les cellules épidermiques des pétioles de Taraxacum officinale. L'irradiation par du rouge sombre a augmenté la rapidité avec laquelle l'eau entre dans la cellule et la lumière rouge clair a renversé cet effet.

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- 5 This work was supported by a grant of the Consejo Nacional de Investigaciones Científicas y Técnicas.