IN VIVO ABSORPTION MEASUREMENT OF INTACT LEAVES — A SIMPLE AND USEFUL METHOD TO STUDY CHLOROPHYLL MUTANTS

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The applicability and usefulness of in vivo absorption measurement of intact leaves were tested using two chlorophyll mutants from Dr. Gottschalk's collection in comparison with the initial line (IL) 'Dippes Gelbe Viktoria'. Mutant 1206 A lacks chlorophyll b and has only 50% as much chlorophyll a as the IL. Mutant 29 is deficient in both chlorophyll a and chlorophyll b, having only 40% and 23% of the mother variety, respectively.

The opal glass method of Shibata (1) minimizes the scattering errors that occur when Intact leaves are irradiated by light. In this study, strips of opal plastic material (perspex) were used instead of opal glass. The plant material — whole etiolated leaves or pieces of green leaves — were affixed to the plastic strips by transparent adhesive tape. In vivo absorption spectra were measured in a Beckman model 25 spectrophotometer. Depending on the absorption of the leaves, one or two strips of opal perspex were placed into the spectrophotometer to serve as reference and also to reduce light intensity. The plastic strips themselves do not exhibit absorption, as indicated by the base line (dotted line, Fig. 1b-d).

In Fig. 1, in vivo absorption curves of mutants and the initial line are compared with the absorption of chlorophyll a extracted by acetone after separation by thin layer chromatography. The absorption maxima of in vivo spectra are situated at 678 nm wavelength, in contrast to 663 nm of chlorophyll a dissolved and measured in acetone. This spectral shift of the absorption maxima is caused by the interaction of the chlorophyll molecules with their protein parts in the chlorophyll-protein complexes integrated in the thylakoids of intact chloroplasts. Apart from the different absorption maxima, the in vivo spectrum of the chlorophyll b-less mutant 1206 A (Fig. 1b) exhibited the same spectrophotometric properties as chlorophyll a (Fig. 1a), i.e. characterized by the clearly visible minor band of chlorophyll a at 625 nm in the in vivo spectrum corresponding to 620 nm in the acetone spectrum.

The distinct minor peak at 625 nm disappears in the spectra of mutant 29 and the initial line. This phenomenon can be explained by the presence of chlorophyll b in the leaves of these genotypes. Because of the higher chloropohyll b content of the mother variety (chlorophyll a/b ratio: IL = 3.5:1, mutant 29 = 6.1:1), a shoulder at 654 nm is produced in the in vivo spectrum (Fig. 1d). Thus, this simple and rapid method .seems to be very useful in distinguishing mutants lacking chlorophyll b from other chlorophyll deficient genotypes according to their characteristic absorption curves.

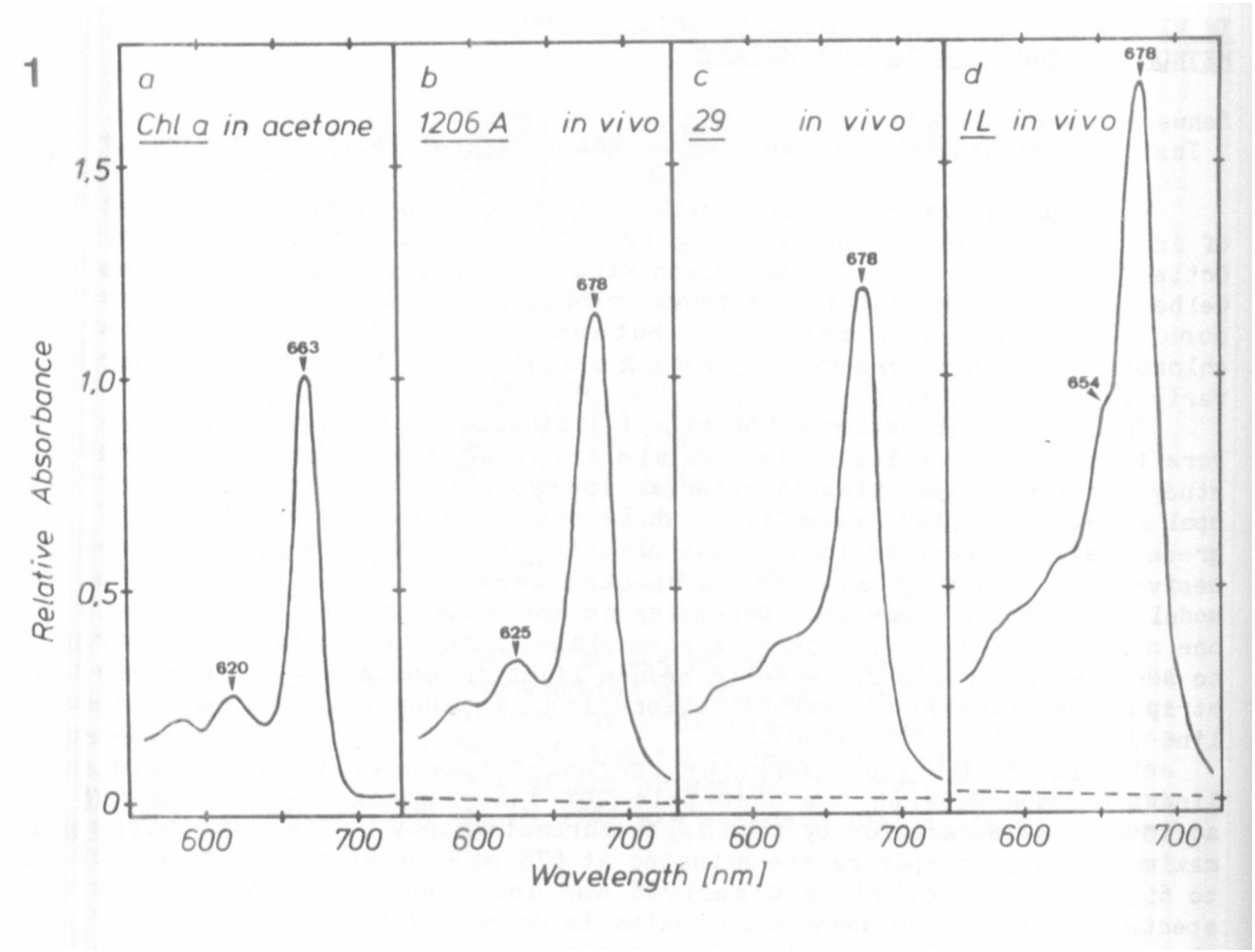

Fig. 1. In vivo absorption spectra of intact green leaves recorded at room temperature compared with the absorption of chlorophyll a dissolved in acetone.

The in vivo absorption method also allows one to obtain information about intact chlorophyll biosynthesis. Etiolated leaves of higher plants accumulate protochlorophyllide in the dark, which is reduced to chlorophyllide by light treatment only. As shown in Fig. 2a, protochlorophyllide is detectable in etiolated leaves of mutant 1 206 A by the absorption maximum at 650 nm. The photoreduction of protochlorophyllide to chlorophyllide induced by brief irradiation with white light (1 min, incandescent light, 100 W, 2500 lux) was evidenced by the decrease of the absorption at 650 nm and by a spectral shift of the absorption maximum from 650 nm to 680 nm. In the dark following the light pulse, a second shift (the Shibata shift) occurred, moving the absorption maximum back to 672 nm. After the Shibata shift, the absorption at 672 nm increased, indicating an accumulation of chlorophyll for a limited time. About 60 minutes after the light treatment, protochlorophyllide is detectable again in the dark leaves (Fig. 2a, arrow).

Mutant 29 and the initial line show the same spectrophotometric properties of etiolated leaves as mutant 1206 A. The increase of the absorption at 672 nm after the Shibata shift is similar for the IL and mutant 1206A. However, in mutant 29 the increase is slower (Fig. 2b). These in <u>vivo</u> absorption measurements suggest that the first steps of chlorophyll biosynthesis are not totally blocked in the mutants.

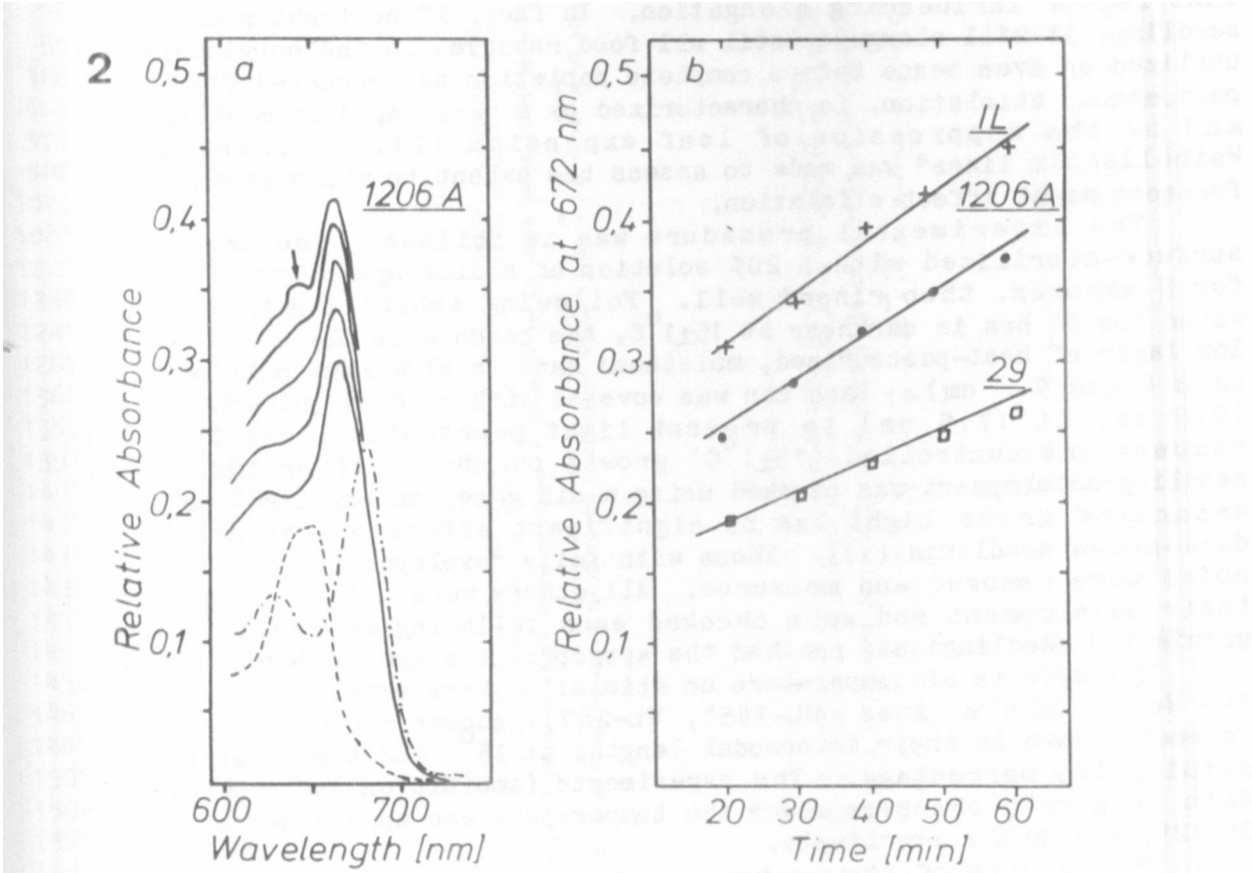

Fig. 2a <u>In vivo</u> absorption spectra of 8-day-old etiolated leaves measured in the dark (----), after a brief light illumination (-.-.-.), and after 20, 30, 40, 50, 60 minutes of darkness following the illumination (———).

Fig. 2b Graph of the relative absorption increase at 672 nm measured in the darkness after a brief illumination of etiolated leaves.

1. Shibata, K. 1957. J. Biochem. 44:147-173-