A SHORT METHOD TO DEMONSTRATE THE LACK OR REDUCTION OF CHLOROPHYLL b IN DIFFERENT MUTANTS OF PISUM SATIVUM

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Chlorophyll b is integrated in several pigment protein complexes of the thylakoid membranes of higher plants. The light harvesting chlorophyll a/b complex has a great amount of this pigment. To test the pigment composition of the mutants and of special isolated complexes the spectra of acetone soluble pigments were measured.

The absorption spectra in acetone from thylakoid membrane preparation of the initial line (IL: 'Dippes Gelbe Viktoria') and of mutants 1206C (chlorophyll b-less) and 333 (reduced amount of chlorophyll b) are shown in Table 1.

Table 1. Chlorophyll a/ chlorophyll b ratio of the genotypes and of the isolated complex.
- IL, 1206C, 333 : TMP-solution
- LHCP II : light harvesting chlorophyll a/b complex from IL.

Genotypes	Chlorophyll a / chlorophyll b
IL	2.69
1206C	-
333	5.28
LHCP II	1.19

Chlorophyll b has absorption maxima at 650 nm and 460 nm. But in acetone extracts of thylakoid membranes the absorption spectrum of chlorophyll a (maxima at 663 nm and 433 nm) overlaps that of chlorophyll b. The spectra of the genotypes (Fig. 1) show only a deviation in the area of 450-470 nm. To demonstrate a loss or a reduction of chlorophyll b it is necessary to eliminate the absorption of chlorophyll a. Therefore the absorption of the thylakoid membrane solution was adjusted to 0.5 at 663 nm and difference spectra were recorded.

The difference spectrum from the chlorophyll b-less mutant 1206C versus the initial line shows two maxima in the area of **650** nm and 460 nm. The absorption of chlorophyll b in the IL solution cannot be compensated by the mutant pigment. Therefore, the difference spectrum shows more or less the spectrum of chlorophyll b.

Comparable results are shown in the difference spectrum of the mutant 333 (reduced amount of chlorophyll b) versus IL. The maxima have lower peaks because the mutant has a little chlorophyll b.

The light harvesting chlorophyll a/b complex (LHCP II) has a large amount of chlorophyll b, which can be seen in the spectrum (Fig. 2). There are two maxima in the area between 400-500 nm

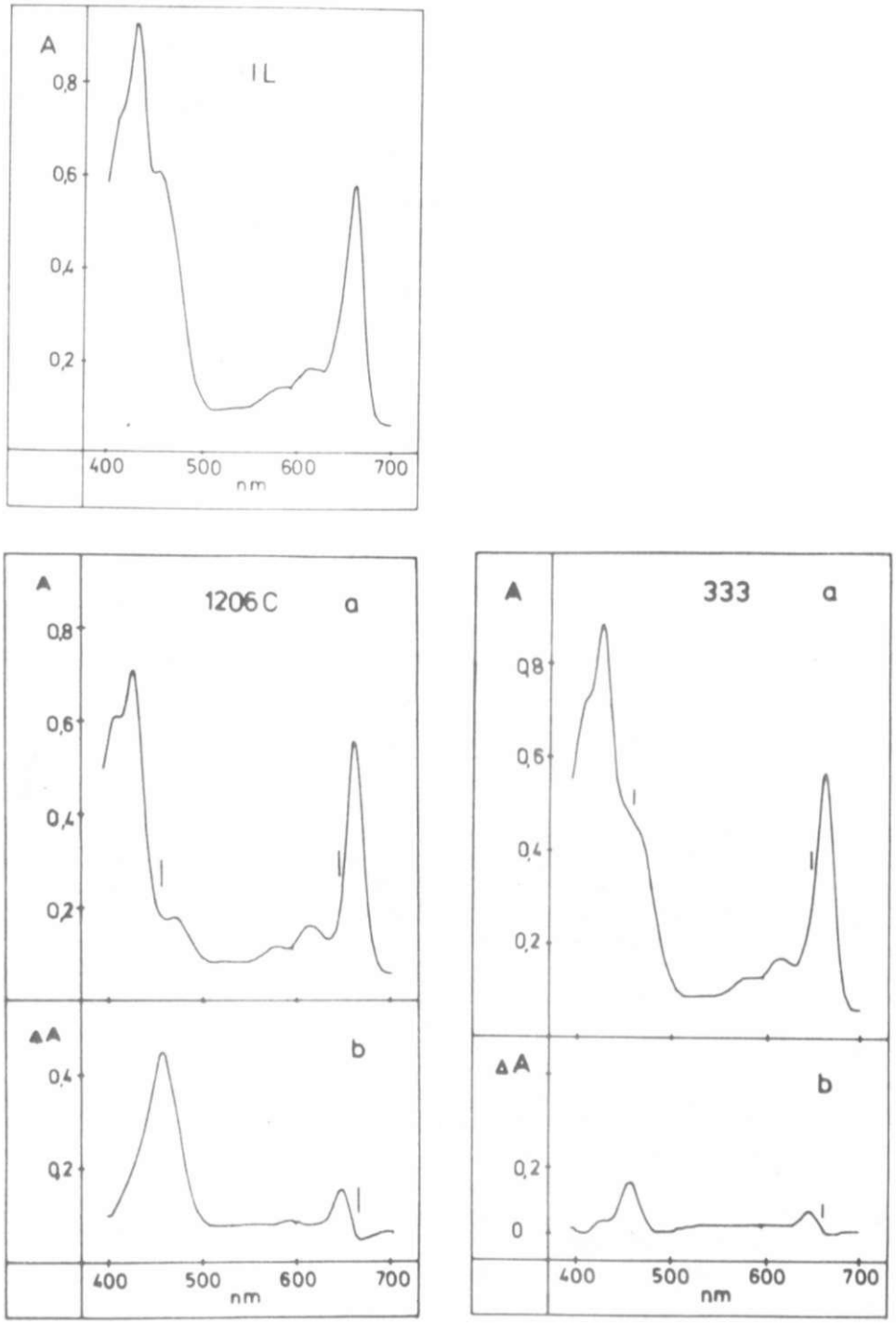

Fig. 1. Absorption spectra and difference spectra from Thylakoid membrane preparations of different genotypes, a) Absorption spectra from TMP-preparation, solubilized and measured against 80% acetone, b) Difference spectra from TMP preparations: - reference cuvette contains mutant solution, - sample cuvette contains mutant solution. The chlorophyll a absorption (663 nm) of both solutions have the same values of about 0.5.

which are caused by the absorption of chlorophyll a and chlorophyll b. The difference spectrum shows a higher peak in the area of 450-470 nm, as expected.

•The absorption spectra and especially the difference spectra are fast methods to characterize chlorophyll mutants and isolated pigment protein complexes.

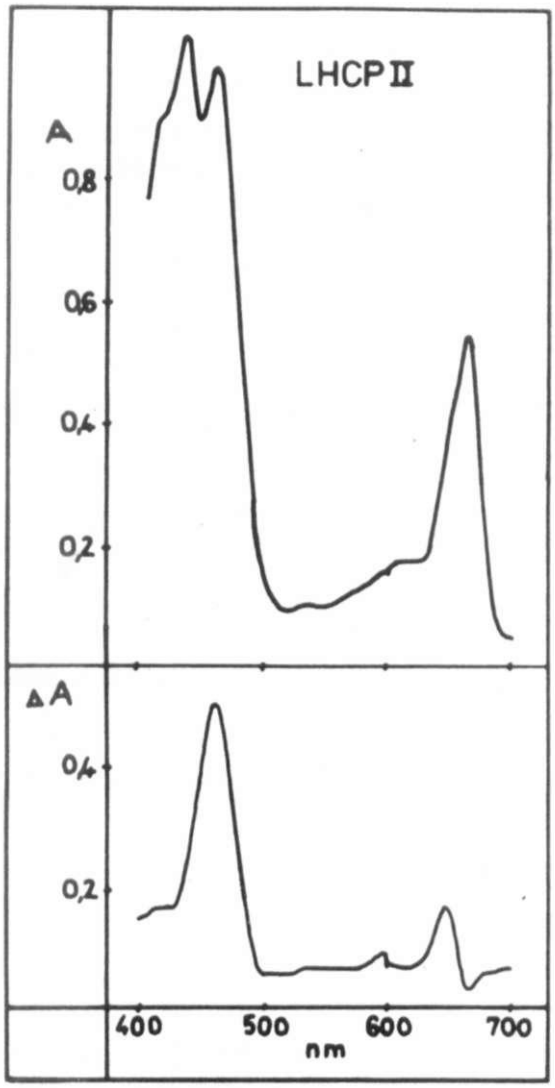

Fig. 2. Absorption spectrum and difference spectrum of the light harvesting chlorophyll a/b - protein complex of the 1L. a) absorption specti-um of LHCP II, solubilized and measured against 80% acetone, b) difference spectrum from LHCP II complex and TMP preparation of the IL: reference cuvette: TMP (IL), -sample c

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