

## CHLOROPHYLLS IN ORGANS OF SEEDS AND DARK GROWN SEEDLINGS OF PEA

C. W. RAVEN

Laboratorium voor Plantenfysiologisch Onderzoek, Landbouwhogeschool, Wageningen<sup>1)</sup>

### SUMMARY

Leaves of dark grown seedlings of *Pisum sativum* L. cv. 'Krombek' contain both chlorophyll-*a* (Chl-*a*) and Chl-*b* in significant amounts. These pigments are not synthesized during seedling growth in darkness, but are already present in the embryonic axis. A comparison of different pea cultivars showed a positive correlation between the Chl content of the seeds and that of their embryonic axes. We have not attempted, however, to determine in which way chlorophylls are accumulated during the development of the embryonic stage.

### 1. INTRODUCTION

In Angiosperms light is supposed to be required for the transformation of protochlorophyllide (Pchlde) to chlorophyllide-*a* (Chlide-*a*), since greening of dark grown seedlings will only occur in continuous light of suitable wavelengths and intensities (KIRK 1967).

Several representatives of Gymnosperms, ferns, mosses, and algae, on the contrary, possess the ability to synthesize appreciable amounts of chlorophylls in the dark (KIRK 1967). Nevertheless, some reports in the literature indicate the presence of small amounts of chlorophylls in seedlings of higher plants grown in complete darkness. In 1947 GOODWIN & OWENS demonstrated this for dark grown oat seedlings. This observation was confirmed by SEYBOLD (1948) for wheat and by RÖBBELEN (1956) for *Arabidopsis thaliana*. It has also been reported that cotyledons of citrus and seedlings of water lotus (*Nelumbo*) can develop chlorophylls in the absence of light (MEYER *et al.* 1960). These findings indicate that the ability for chlorophyll (Chl) formation in darkness is not uncommon in flowering plants. The fact that only small quantities of Chl are found in seedlings grown in the dark has been explained by RÖBBELEN (1956) as resulting from an equilibrium between dark-synthesis and dark-destruction of chlorophylls. Experimental evidence for the existence of dark-destruction of Chl-*a* was obtained by FRANK & KENNEY (1955).

During a study of the rates of Pchlde regeneration in several species of higher plants we detected in dark grown pea seedlings significant amounts of a substance spectroscopically similar to Chl-*a* (SPRUIT & RAVEN 1970), confirming an earlier observation (SPRUIT 1966). We suggested as a possible explanation a transport of Chl from the green cotyledons to the embryonic axis during the early stages of development. In this article we intend to discuss the results of a

<sup>1)</sup> 315th Communication of the Laboratory of Plant Physiological Research

comparative study of three pea cultivars with widely divergent Chl contents of their seeds.

## 2. MATERIAL AND METHODS

### 2.1. Plant material

Seeds of *Pisum sativum* L. cv. 'Krombek', cv. 'Zoete Eminent', and cv. 'Meidopper' were obtained from Nunhems Zaden N.V. (Haelen, Holland). Seeds were soaked in water in complete darkness for a few hours. They were then sown in pasteurized soil by only a weak green safelight (SPRUIT & RAVEN 1970). Subsequent growth was in absolute darkness, as well as all further manipulations, such as watering and harvesting of the leaf material. When parts of pea embryos were to be used, they were excised under dim daylight conditions after the soaked seeds had first been submerged in boiling water for a few minutes in the dark.

### 2.2. Pigment estimations

Weighing and extraction was done under green safelight. Pigments were estimated spectrophotometrically in 80% acetone-water extracts in a Zeiss spectrophotometer model PMQ II equipped with a grating monochromator M20. Absorption spectra of the extracts were run in a Cary model 14 spectrophotometer. When the extracts were not perfectly clear, they were first dried over anhydrous sodium sulphate before spectrophotometer readings were carried out.

### 2.3. Calculation of pigment concentrations

We have adopted the molar absorption coefficients determined by MACKINNEY (1941) for calculation of Chl-*a* and Chl-*b* concentrations. Molar absorption coefficients given by ANDERSON & BOARDMAN (1964) were used for calculations when a mixture of Pchl, Chl-*a*, and Chl-*b* was present in the extracts.

## 3. RESULTS

*Fig. 1* gives the absorption spectra of extracts in 80% acetone of whole seeds of the three pea cultivars. It can be concluded from the height of the absorption peak at 664 nm that seeds of cv. 'Krombek' and of cv. 'Zoete Eminent' are quite rich in Chl-*a*, seeds of cv. 'Meidopper' are almost completely devoid of Chl. The absorption spectrum of the extract of this cultivar can almost fully be ascribed to yellow pigments, most probably carotenoids.

The extracts also contain Chl-*b*. We have found a Chl-*a* to Chl-*b* ratio of 2.6 for cv. 'Krombek', 1.9 for cv. 'Zoete Eminent', and of 1.0 for cv. 'Meidopper'. The latter value, however, is of doubtful significance, due to the extremely low Chl content of the seeds of this cultivar.

Differences between the cultivars in the position of the main absorption peak and shoulders in the blue wavelength region of the spectrum may be contributed

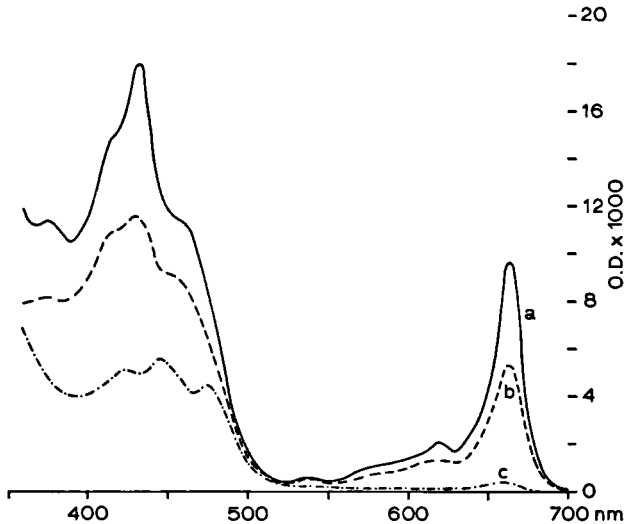


Fig. 1. Absorption spectra of extracts of seeds of different pea cultivars in 80% acetone. Absorption cell 5 cm, 2 g seeds in 50 ml acetone.

- a. cv. 'Krombek'.
- b. cv. 'Zoete Eminent'.
- c. cv. 'Meidopper'.

to concomitant absorption of varying amounts of chlorophylls and carotenoids.

We have carefully separated embryonic axes from the soaked seeds in order to estimate their pigment content (*fig. 2*). The embryonic axis of both green-seeded cultivars 'Krombek' and 'Zoete Eminent' contained significant amounts of chlorophylls. The Chl-*a* to Chl-*b* ratio was 1.5 for extracts of embryonic axes of cv. 'Krombek' and 1.3 for extracts of embryonic axes of cv. 'Zoete Eminent'. However, between these cultivars a remarkable difference in the distribution pattern of chlorophylls in the embryonic axis could be observed: In the embryonic axis of cv. 'Krombek', chlorophylls were evenly distributed among plumula (stem and leaves) and radicula (root), whereas in the embryonic axis of cv. 'Zoete Eminent' chlorophylls were mainly restricted to the radicula (*fig. 3* and *fig. 4*).

When seedlings were raised in complete darkness for 7 days, an appreciable absorption at 664 nm was observed only in leaf extracts of cv. 'Krombek' (*fig. 5*).

The ratio of Chl-*a* to Chl-*b* was 1.3. The Chl content of this cultivar, calculated for a constant number of leaves, remained fairly constant throughout development from the embryonic stage (*fig. 3*) to the 7 day old dark grown seedling. Extracts of leaves of dark grown seedlings of cv. 'Zoete Eminent' contained only trace amounts of chlorophylls, whereas in leaves of cv. 'Meidopper' no Chl could be detected. These observations are also in good agreement with the

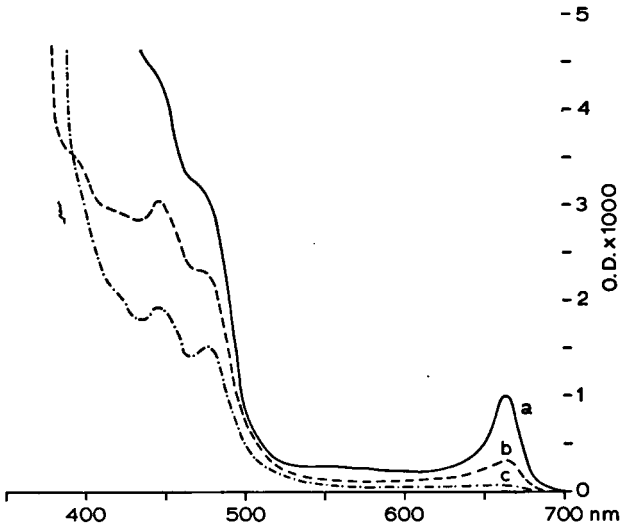


Fig. 2. Absorption spectra of extracts of whole embryonic axes of different pea cultivars in 80% acetone. Absorption cell 5 cm, 150 embryonic axes in 25 ml acetone.

- a. cv. 'Krombek'.  
 b. cv. 'Zoete Eminent'.  
 c. cv. 'Meidopper'.

observed absence of chlorophylls in the plumula of the embryonic axis of cv. 'Zoete Eminent' (*fig. 3*) and in the plumula and radicle of cv. 'Meidopper' (*fig. 2*), respectively.

#### 4. DISCUSSION

The observation that the quantity of Chl per individual is constant during development of the embryonic axis to the seedling supports the hypothesis that the pigment existed preformed in the resting embryonic axis and was accumulated there during the ripening of the seed.

It may be concluded from the mutual proportion of the height of the Chl-*a* absorption peaks at 664 nm (shown in *fig. 1* and *fig. 2*) that, in the different pea cultivars, a positive correlation exists between Chl content of the seeds (*fig. 1*) and of their embryonic axes (*fig. 2*). However, this does not prove that the Chl in the embryonic axes of cv. 'Krombek' and 'Zoete Eminent' is derived from the cotyledons by transport to those parts during the early stages of their development.

Formation of Chl *in situ* in the embryonic axis during its development in the seed is also a possible explanation, but no conclusion can be drawn from our data with respect to this problem. At any rate, it is likely that the chlorophylls

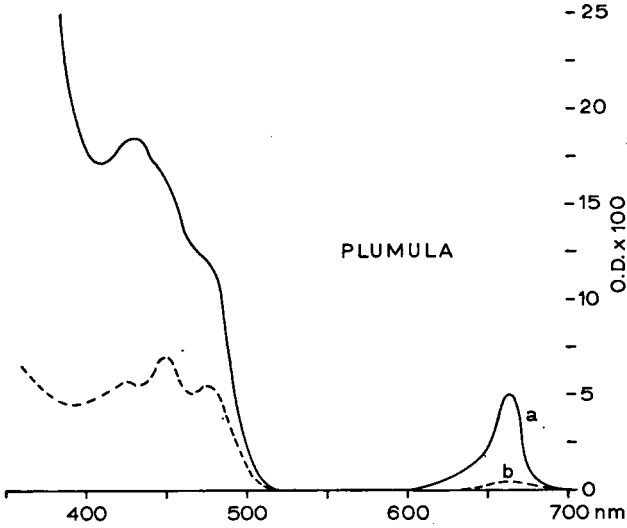


Fig. 3. Absorption spectra of extracts of stem and leaves (= plumula) of embryonic axes of different pea cultivars in 80% acetone. Absorption cell 5 cm, 150 plumulae in 25 ml acetone.  
a. cv. 'Krombek'.  
b. cv. 'Zoete Eminent'.

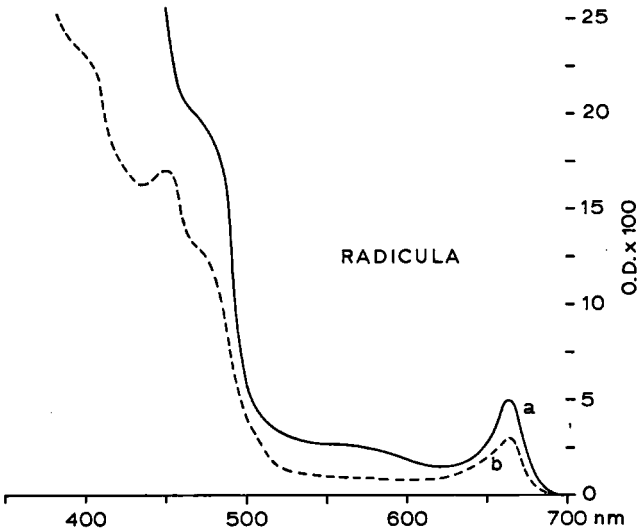


Fig. 4. Absorption spectra of extracts of roots (= radicula) of embryonic axes of different pea cultivars in 80% acetone. Absorption cell 5 cm, 150 radiculae in 25 ml acetone.  
a. cv. 'Krombek'.  
b. cv. 'Zoete Eminent'.

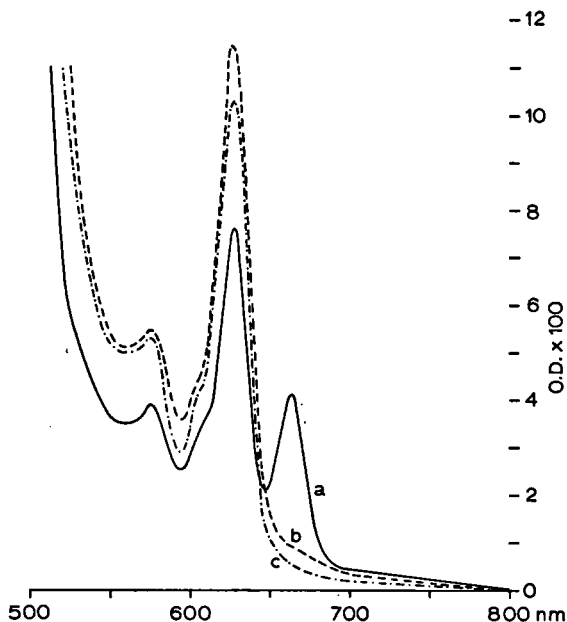


Fig. 5. Absorption spectra of extracts of leaves of 7 day old dark grown pea seedlings in 80% acetone. Absorption cell 5 cm, 150 'plumules' in 25 ml acetone.  
 a. cv. 'Krombek'.  
 b. cv. 'Zoete Eminent'.  
 c. cv. 'Meidopper'.

we have observed in leaves of dark grown seedlings of pea cv. 'Krombek' originate from the embryonic stage of development, and that they are not synthesized during seedling growth in darkness.

#### REFERENCES

- ANDERSEN, J. M. & N. K. BOARDMAN (1964): Studies on the greening of dark-grown bean plants. II. Development of photochemical activity. *Aust. J. Biol. Sci.* **17**: 93-101.
- FRANK, S. & A. L. KENNEY (1955): Chlorophyll and carotenoid destruction in the absence of light in seedlings of *Zea mays* L. *Plant Physiol.* **30**: 413-418.
- GOODWIN, R. H. & O. V. H. OWENS (1947): The formation of chlorophyll a in etiolated oat seedlings. *Plant Physiol.* **22**: 197-200.
- KIRK, J. T. O. (1967): In J. T. O. Kirk & R. A. E. Tilney-Bassett, *The plastids*. W. H. Freeman, London & San Francisco, pp. 402-476.
- MACKINNEY, G. (1941): Absorption of light by chlorophyll solutions. *J. Biol. Chem.* **140**: 315-322.
- MEYER, B. S., D. B. ANDERSON & R. H. BÖHNING (1960): *Introduction to Plant Physiology*. D. Van Nostrand Company, New York, p. 183.
- RÖBBELEN, G. (1956) Über die Protochlorophyllreduktion in einer Mutante von *Arabidopsis thaliana* (L) Heynh. *Planta* **47**: 532-546.
- SEYBOLD, A. (1948): Zur Kenntnis des Protochlorophylls. III. *Planta* **36**: 371-388.
- SPRUIT, C. J. P. (1966): Spectral observations related to the red-far red antagonism in plants. In: *Currents in photosynthesis*. Proc. Sec. West. Eur. Conf. on Photosynthesis, Woudschoten, Zeist, 1965. A. Donker, Rotterdam, pp. 67-74.
- & C. W. RAVEN (1970): Regeneration of protochlorophyll in dark grown seedlings following illumination with red and far red light. *Acta Bot. Neerl.* **19**: 165-174.