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PIGMENT CHANGES IN LEAVES OF *HABERLEA RHODOPENSIS* (FRIV) AFTER PROLONGED LIGHT DEPRIVATION

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ABSTRACT. *Haberlea rhodopensis* (Friv.) is known as a plant capable to withstand harsh environmental conditions. Here we examined changes of photosynthetic pigments contents in plants subjected to prolonged light deprivation. For this purpose plants were kept for 5 weeks at constant temperature and humidity and in complete darkness. According to our data the pigment contents in light-deprived plants, measured as mg per gram dry weight (DW), were higher than those in light-grown plants. On the other hand, DW of leaves compared to the fresh weights (FW) decreased during first two weeks probably due to the usage of assimilates as energy sources. So we can explain the rapid initial increase in pigment contents with the decrease of DW. This explanation however did not correlate with observed changes in Chl *a* content, which continued to increase during the first three weeks. We found that excised leaves from plants deprived from light for two weeks and incubated in darkness in 5-aminolevolinic acid solution accumulated both protochlorophyllide and chlorophyllide. Therefore the observed changes in Chl *a* contents could be explained if we accept that *Haberlea* leaves continue to synthesize limited amounts of chlorophyll(ide) *a* in dankness.

INTRODUCTION

After light-to-dark transitions, the leaves show loss of their photosynthetic capacity (Biswal 1997). This effect is depended on duration of dark treatment. Initially darkness induces inhibition of photosynthesis including inactivation of key enzymes in Calvin cycle (Anderson 1999) and related changes in activity of thioredoxin system (Buchanan 1978). Prolonged development under dark conditions caused irreversible changes in photosynthetic machinery including considerable

pigment losses in mature leaves after 3-4 days dark (Lu and Zhang 1998), thylakoid membranes destruction (Biswal 1997; Smart et al., 1995), degradation of lipids and proteins, changes in membrane transport (Brouquisse et al. 1998) etc.

The Balkan endemic plants *Haberlea rhodopensis* Friv. (*Gesneriacea*) is tertiary paleophytic relict that is able to withstand harsh environmental conditions during vegetative growth. This is due to the ability of the plant for biosis-anabiosis and vice versa transitions under these conditions. Changes during these transitions with respect to photosynthesis and transpiration (Markovska et al., 1994), lipid and sterol composition of leaves (Stefanov et al., 1992), sugar content (Müler et al., 1997) and chloroplast ultrastructure (Minkov, 1976; Markovska et al., 1995) have been characterized. Air-dried *Haberlea* leaves preserve up to 80% of their chorophylls (Kimenov and Jordanov, 1974, Markovska et al. 1994).

Here we examined changes of photosynthetic pigments contents in plants subjected to prolonged light deprivation.

MATERIAL AND METHODS

Plants of *Haberlea rhodopensis* Friv. were collected from shady rocks on northern slopes of The Rhodopi Mountain. The plants were adapted for several weeks in a greenhouse at temperatures 22-25ºC with 14/10 h light/dark photoperiod. The control plants were kept under these conditions. 10 experimental plants were kept in a dark incubator under constant humidity and temperature of 25º C for 5 weeks. Samples in five replicas were taken every 7 days by punching small disks (surface area 1.5 sq. cm) from the middle parts of the leaf. Every disk was divided in two equal (by fresh weight) halves – one of the halves was dried at 110 ^{o}C in order to determine the absolute dry weight.

The other half was grinded with liquid nitrogen and the total pigments were extracted with 1 ml 85% acetone buffered with 0.1 M $NaHCO_3$. Extract was purified from cell and tissue debris by centrifugation at 10 000 g for 5 min. The amounts of chlorophylls (Chl – Chl *a* and Chl *b*) and carotenoids were calculated using their light absorption according to MacKinney (1941). Chlorophyllide (Chlide) and protochlorophyllide (Pchlide) were isolated from the acetone extracts by the following procedure: 2 ml of total acetone extracts were mixed three successive times with 1 ml hexane, which extracted chlorophylls and carotenoids. Further the Pchlide and Chlide were extracted by triple treatment of acetone solutions with 1 ml diethyl ether. The diethyl ether from these extracts was evaporated under nitrogen steam to nearly dry and the pigments were solubilized in 90% acetone. The amounts of Pchlide and Chlide were determined by their fluorescence at 636 nm and 670 nm resp. after excitation at 440 nm according to Kahn (1980). All the extractions and measurements were performed under room temperature and green dim light.

Slices from the middle part of *Heberlea* leaves, approximately 1.5 mm wide and 2 cm long, were placed in optical glass tubes and frozen at 77 K. Their low temperature fluorescence spectra were recorded between 600 and 780 nm with excitation wavelength 440 nm.

All data were statistically processed by Excel software.

RESULTS AND DISCUSSION

The changes in Chl *a*, Chl *b* and carotenoids amounts in the plants after light deprivation are presented on Fig.1. According to our data the pigment contents in light-deprived plants were higher than those in light-grown plants. Judging by trend-lines two tendencies could be seen: The first tendency was seen in changes of Chl *a* content, which was increasing during 3 weeks after beginning of light deprivation. The coefficient of reliably R^2 of this tendency was 0.94. The second tendency was observed in changes in Chl *b* and carotenoids. The initial increase in content of these pigments during the first week of light deprivation was followed by a decrease until the end of experiment (fifth week). The reliably coefficients of these tendencies were 0.98 for Chl *b* and 0.96 for carotenoids.

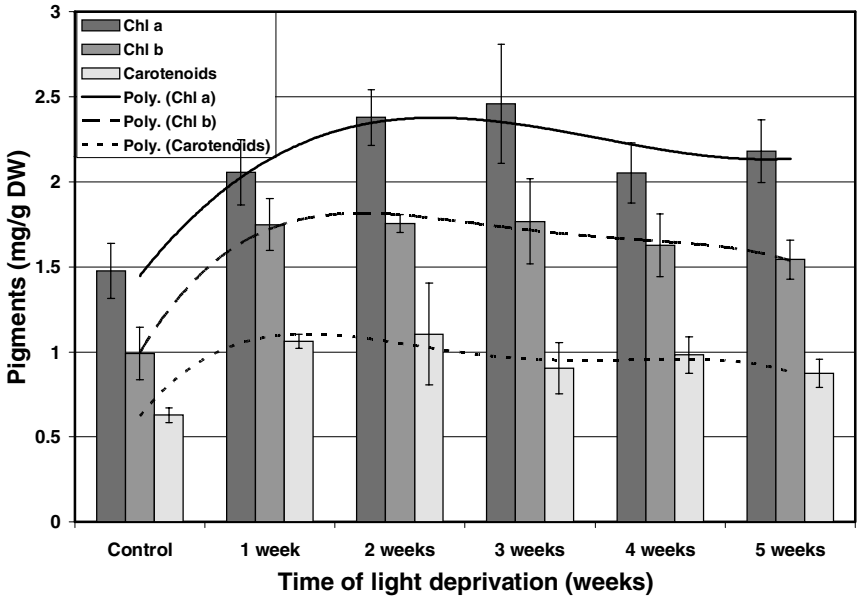

Fig. 1. *Changes in main pigments amounts during continuous light deprivation period. The bars represent the measured amounts of pigments at the time points, while the tendencies in their changes are presented as fourth order polynomial trend-lines*

It is known that under partial light deficiency pigment contents increase in many plants. However when plants were kept for prolonged period (usually 3-4 days) in complete darkness a high degree of pigment breakdown was reported (Lu and Zhang, 1998). *Haberlea* plants showed unusual changes in pigment contents during prolonged light deprivation (Fig. 1).

One possible explanation could be found in the pattern of changes in ratio DW/FW (Fig. 2).

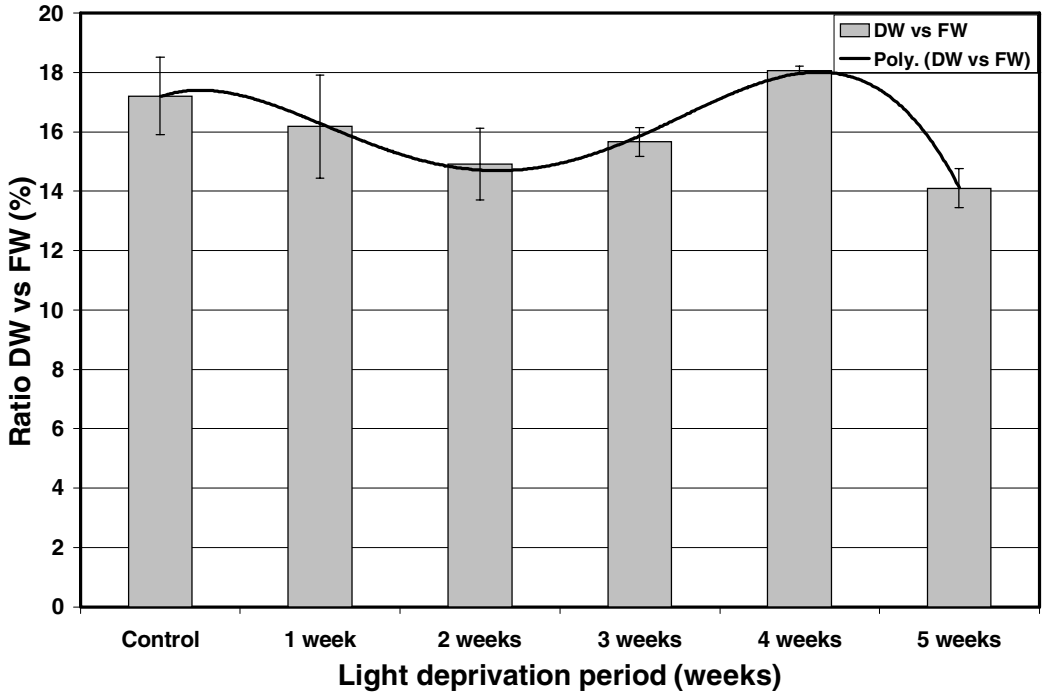

Fig 2. *Changes of ratio absolute dry weight vs. fresh weight during prolonged period of light deprivation. The bars represent the calculated ratios while the tendencies in their changes are presented as fourth order polynomial trend-lines*

During initial two weeks of light deprivation the leaves dry mass compared with their fresh mass decreased. Further, until the fourth week of light depravation the percentage of dry mass increased and during the last fifth week it decreased again. In this case, the statistic reliably coefficient of this tendency was 0.99. A possible explanation of the observed oscillatory pattern of DW/FW ratios could be that after the light depravation the plants continued to maintain their water balance at levels close to that in plants grown under normal light conditions. They probably used as energy sources starch and other end products accumulated in leaves. When these energy sources were exhausted (after second week), the plants probably started to lose water and dry weight/fresh weight ratio started to increase. It is difficult to explain the new relative decrease in the dry mass at the fifth week unless it was related somehow to the ability of plant to switch its metabolism from biosis to anabiosis. So, we can explain the rapid increase in pigments content during the first week of light deprivation with the decrease in total dry mass..

This explanation however did not correlate with observed changes in Chl *a* content, which continued to increase during the first three weeks. The changes in Chl(ide) ***a*** contents could possibly be explained if we accept that *Haberlea* continue to synthesize limited amounts of chlorophyll(ide) *a* in dankness.

The presented on Fig. 3A and B spectra demonstrated that *Haberlea* leaves accumulated small amounts of Pchlide, but we still needed to investigate whether a part of the Pchlide was converted to Chlide in the darkness.

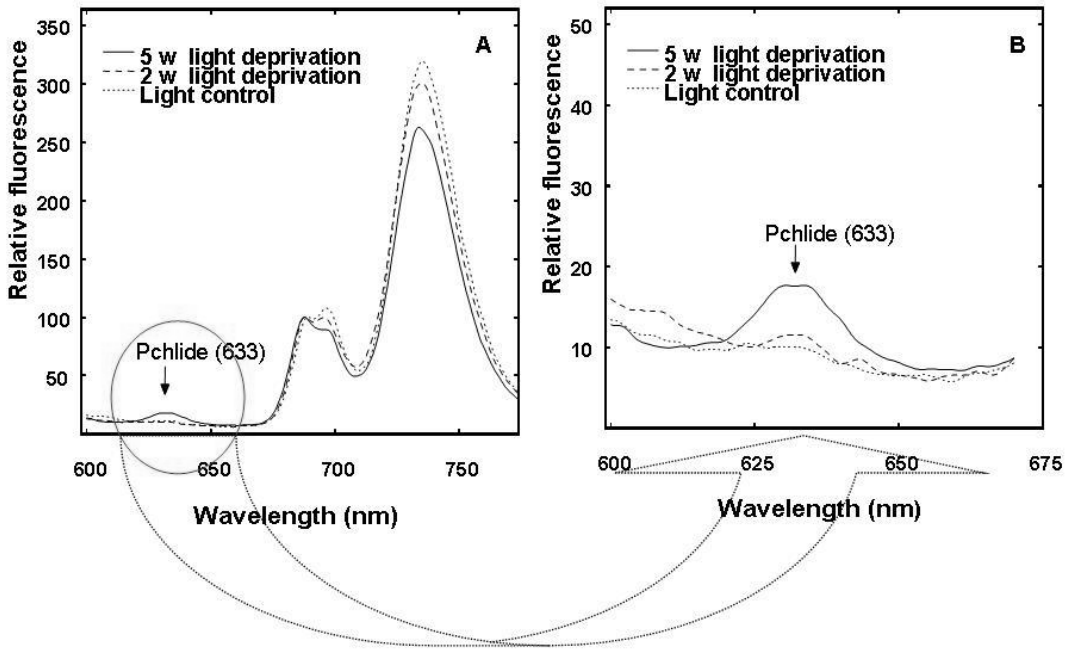

Fig. 3. *Low temperature spectra of leaf slices taken from plants grown under normal light conditions (photoperiod 14 h light/10 h darkness) and kept for 2 and 5 weeks in darkness*

For these reasons, incubation of individual *Haberlea* leaves for 32 h in 5 mM 5-aminolevolinic acid (5-ALA) was conducted. The amount of non-esterified pigments was measured every 8 hours. The results are presented on Fig. 4.

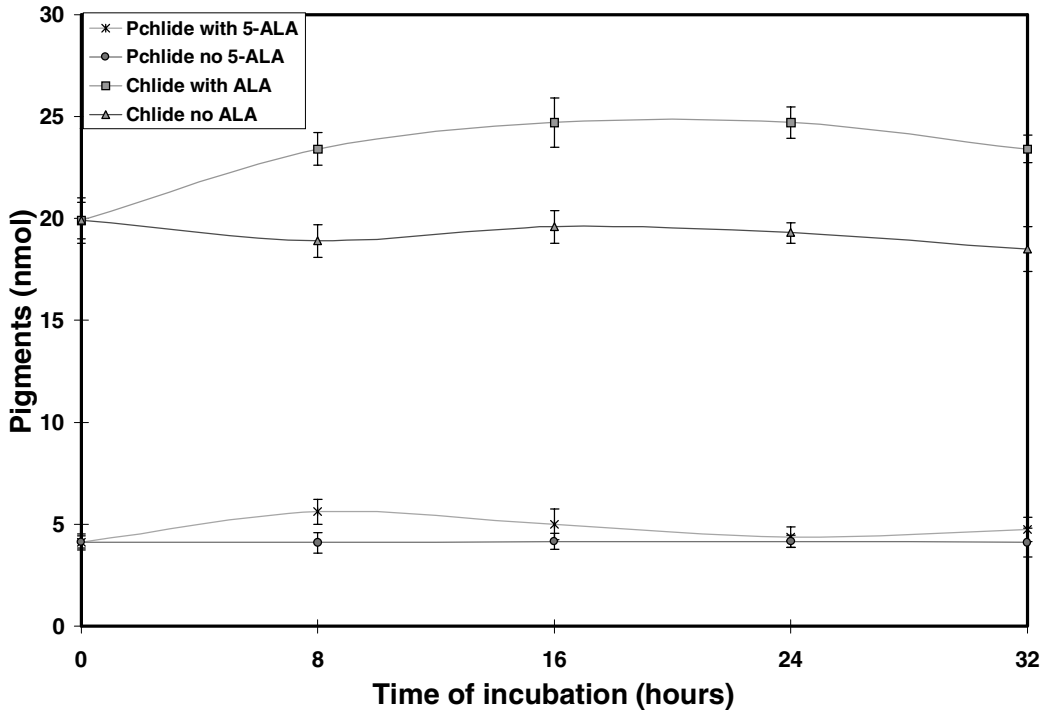

Fig. 4. *Time course of the accumulation of Pchlide and Chlide in deprived for two weeks Haberlea leaves supplied with 5 mM exogenic 5-ALA.*

In conclusion, the results indicated that *Haberlea* leaves deprived from light for more than two weeks contained significant amounts of Chlide. An addition of exogenic 5-ALA caused small increase in Pchlide content but much significant increase in Chlide. These results could be an indication for existence of light-independent chlorophyll biosynthetic pathway in *Haberlea* leaves that is observed very clearly by means of our experimental system. However future investigations with labeled 5-ALA could prove such assumption.

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