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VARIABILITY OF CHLOROPHYLL CONTENT UNDER FLUCTUATING ENVIRONMENT

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Summary

A leaf chlorophyll concentration is strongly affected by numerous external factors. Therefore, their quantitative determination, in different investigation objects, is specially recommended. In present work we demonstrated effect of different light intensity regimes and water supply on the chlorophyll content development. This work did not confirm fully the available informations about the chlorophyll content dynamics and chlorophyll *a/b* ratio decrease in shaded plants. In field of water stress, our results, except to chlorophyll *a/b* ratio, are consistant with many authors.

Keywords: chlorophyll content, chlorophyll *a/b* ratio, light regimes, drought, barley

Introduction

Chlorophylls of different form play an important role as a part of photosynthetic apparatus of all phototrophic organisms. Higher plants contain chlorophyll a (the major, yellow-green pigment), chlorophyll b (blue-green), accessory pigments and several additional forms of chlorophyll. Both chlorophyll a and b pigments are associated with light harvesting processes at the antenna. Moreover, chlorophyll a takes a part in the electric charge separation (Procházka *et al.,* 1998). Numerous authors demonstrate a rapid chlorophyll content response to internal as well as external factors. Here are several examples of the ambient ones.

It is known for a longer time that shaded plants exhibit a higher chlorophyll pigments content per dry mass weight unit but a lower chlorophyll a/b ratio (about 2,5 - 2,9), whereas sun plants ca 3,2 - 4 (Lichtenthaler, 1987). On the other hand, excessive light intensity has a destructive effect on photosynthetic pigments leading to inhibition of photosynthesis. Haspelová (1981) discusses importance of sufficient water supply of maturing leaves to form and develop new leaves and allocate the chlorophylls. Furthermore, it was concluded that the chlorophyll content changes correlate with water stress intensity. Recent works with subtropic trees demonstrate the chlorophyll content dynamics within dry and wet seasons (Montagu, Woo, 1999). During dry period, decreases in phyllode chlorophyll (at the end of dry season it was about 73%) were accompanied by a decrease in stomatal conductance. Four weeks after the water stress was acting, photosynthetic rate recovered to 70 - 95% what corresponded also with chlorophyll content restoration. Ramalho, Lauriano, Nunes (2000) add information on lower values of chlorophyll a/b ratio at the end of summer (2,6) than obtained in the spring (3,6). Relationship between plant nutrition and chlorophyll content values is very complicated and strongly modified by other internal and external conditions. The N- and chlorophyll concentration dynamics are studied very detail. Dhir *et al.* (1999) show the impact of air pollutants from a thermal power station on chlorophyll content. Also soybean *(Glycine max* Merrill.*)* and maize *(Zea mays* L.*)* plants exposed to herbicide diquat led to chlorophyll destruction (Milivojevic, Nikolic, 1998). Nowadays, the quantitative determination of chlorophyll in different experimental plant material and investigation objects is specially recommended as a valuable characteristic of light harvesting capacity under stress. In the present work we verify the effect of different light intensity regimes and water supply on chlorophyll content development in juvenile to mature barley *(Hordeum vulgare* L.*)* plants.

Materials and methods

Plants of spring barley *(Hordeum vulgare* L.*)* cv. Kompakt (Slovakia) were grown under external conditions in 10-litre pots with soil substrate. Elementary fertilization and water supply were applied. Since the first leaf emergency, the plants were treated by different growth light intensity regimes - sunny regime and shading regime (25% of natural PAR values). First two measurable leaves were cut and the chlorophyll concentration (Lichtenthaler, 1987) was determined. The examination of sunny plants continued to the end of vegetation (once a week). The irrigation was interrupted in the DC 45 - 49 stage of development.

The chlorophyll content determination

Firstly, we cut ten leaf tissue segments and ground with a mortar and pestle in a presence of a little sea sand, 0,2 - 0,5 g $MgSO_4$ and ca 0,5 ml 100% acetone (Šesták, Čatský 1966). We added 2 -5 ml of 80% acetone to the fine powder and decanted the homogenate into centrifugation tube through 2 x 5 ml 80% acetone (Cholvadová, Erdelský, Masarovičová, 1999). Centrifugation at 2500 rpm for 2 min separated solid compound elements. Measured absorption values was used for chlorophyll content calculation according to Lichtenthaler (1987):

chl. *a* (mg.l^{-1}) = $(12{,}25 \cdot A_{663} - 2{,}79 \cdot A_{647}) \cdot D$
chl. *b* (mg.l^{-1}) = $(21{,}5 \cdot A_{647} - 5{,}1 \cdot A_{663}) \cdot D$
chl. *a+b* (mg.l^{-1}) = $(7{,}15 \cdot A_{663} + 18{,}71 \cdot A_{647}) \cdot D$,
where: A is absorption at given wavelengths, D - thickness of the used cuvette (cm).

Expression of results in leaf area units:

PC (mg.m^{-2}) = (V/1000*1/A)*PC (mg.l^{-1}),

where: PC is the pigment concentration, V - 80% acetone volume used, A - leaf tissue segments area (m^2).
To get a higher accuracy, adjustment of sample absorbancy values (-A_{750}) is needed (Cholvadová, Erdelský, Masarovičová, 1999).

In comparison with widely used expression method (Šesták, Čatský 1966, Arnon, 1949) we did not achieve any significant differences, but the used procedure is less pretentious and more exact results are obtained due to more exact equations (Figure 1).

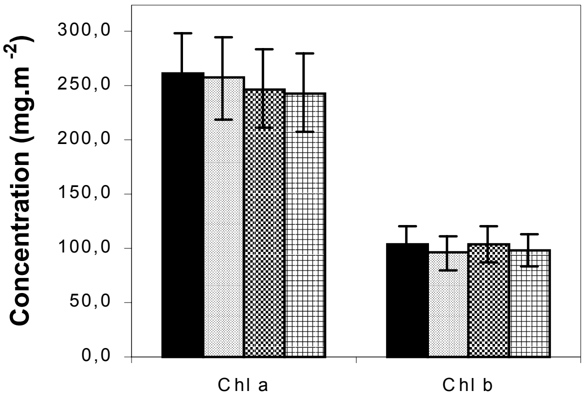

Figure 1: Comparison of chlorophyll content by two methods in maize example (Arnon, Arnon – adjusted, Lichtenthaler, Lichtenthaler – adjusted)

Results and discussion

Shaded leaves exhibit a lower chlorophyll content. As we suppose, this disproportion with many authors (Lichtenthaler, 1987, Larcher, 1988, Gilmore, 1997) could arise in relationship to pigment content expression units (mg.g^{-1} dry matter / mg.m^{-2} leaf area). As very interesting seems to be the chlorophyll a/b ratio in second shaded leaf which might be caused by different in physiological age of both sun and shade leaves. Generally, pigment ratio in sun leaves indicates no real sun effect (Lichtenthaler, 1987).

Table 1

Variant	Chlorophyll a content (mg.m^{-2})	Chlorophyll b content (mg.m^{-2})	Chlorophyll a/b ratio
Shade, 1st leaf	223,64	108,73	2,06
Shade, 2nd leaf	234,97	108,11	2,17
Sun, 1st leaf	396,55	192,64	2,06
Sun, 2nd leaf	343,49	180,2	1,91

1st leaf – onthogenetically older

Differences between individual leaves position in sun plants are in favour of the first leaf that may indicate an unfinished development of the second leaf, whereas in shade plants both leaves seem to be alreeady developed.

Leaf pigment content dynamics was strongly affected by stress (Figure 2). This result is consistant with Fernandéz-Conde (1998). Similar conclusions demonstrate Montagu, Woo (1999). Nilsen, Orcutt (1996) confirm the degradation effect of water stress on pigment content. Lower values of chlorophyll b content in the 6th leaf during first stages of their function were probably caused by cloudy and cold weather. Stress conditions had no effect on chlorophyll a/b ratio, what is in oposition to Ramalho, Lauriano, Nunes (2000).

We may conclude that fluctuating environment influenced chlorophyll content more than chlorophyll a/b ratio.

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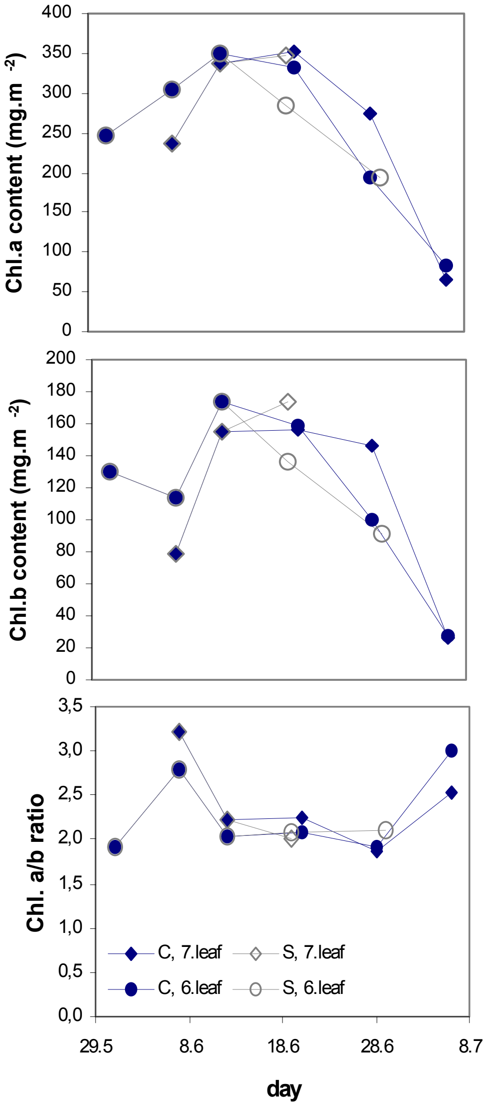

Figure 2: Development of leaf chlorophyll content and chlorophyll a/b ratio in stressed (S) and control (C) plants, 7th leaf – penultimate leaf.