PHOTOSYNTHETICA 34 (3): 377-391, 1997

Changes in French bean cotyledon composition associated with modulated life-span

N. WILHELMOVÁ*,+, J. WILHELM**, J. KUTÍK*** and D. HAISEL*

*Institute of Experiental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, 160 00 Praha 6, Czech Republic**
*2nd Medical School, Charles University, Plzeňská 221, 150 00 Praha 5, Czech Republic***
*Faculty of Sciences, Charles University, Viničná 5, 128 44 Praha 2, Czech Republic****

Abstract

The onset of *Phaseolus vulgaris* L. cotyledon senescence and its characteristics were modulated by irradiance (higher or lower than standard) and by epicotyl decapitation. The cotyledon life-span of 16 d was not influenced by irradiance while decapitation prolonged the life-span to 28 d. The fresh mass of cotyledons, an indicator of organ viability, decreased in a similar manner in all non-decapitated plants, though it was relatively slower in plants grown under a low irradiance (LI). Three days after decapitation the fresh mass of cotyledons increased by one third, a slight decrease was observed on the 21st d, and it lasted until the end of the life span. Deducing from the fall of chlorophyll (Chl) concentration expressed per unit protein, senescence started after the 10th day in non-decapitated plants. Decapitation postponed the onset of senescence until the 21st day. Expression of Chl amount per unit dry mass did not detect any changes in LI plants, hence this parameter can not be used for the assessment of senescence. The measurements of Chl *a* and *b* concentrations indicated that the light-harvesting complexes (LHCs) proliferated during ageing and were rapidly destroyed at the onset of senescence. Changes of the concentrations of carotenoids supported the hypothesis of free radicals involvement in senescence. The bean cotyledons responded to free radical production induced under higher irradiance by increased β-carotene synthesis. Oxidative damage to galactolipids during senescence was documented by fluorescence measurements. The changes in cotyledon composition were correlated to morphologic changes observed by electron microscopy.

Received 14 August 1996, *accepted* 10 June 1997.
+Fax: (420-2) 234 10 113, e-mail: scstak@site.cas.cz
Dedicated to Prof. Dr. P. Hoffmann from the Humboldt University of Berlin on the occasion of his 65th birthday.

Abbreviations: Chl, chlorophyll; DGDG, digalactosyldiacylglycerol; HI - grown under high irradiance; HPTLC, high performance thin layer chromatography; LHC - light-harvesting complex; LI - grown under low irradiance; MGDG, monogalactosyldiacylglycerol.

Acknowledgement: We thank Dr. D. Hulínská and Ing. M. Doubek from the State Institute of Public Health, Prague for enabling us to work with the electron microscope and for good advice. The skilled assistance of Ms. M. Koubová is highly appreciated. This work was financially supported by the Grant Agency of the Czech Republic (Project No. 206/95/1302).

Additional key words: ageing; carotenoids; chlorophyll; chloroplast; electron microscopy; fluorescence; galactolipids; lipid peroxidation; *Phaseolus vulgaris*; senescence.

Introduction

Ageing in plants leads to the terminal stage called senescence. At the level of whole plant it is regulated by various hormones responding to changes of external conditions such as the length of daylight or change in temperature (Thomas and Stoddart 1980). This implies the possibility of manipulation of life-span by modulating such factors as irradiance or decapitation of the apexes, where the production of regulatory hormones takes place (Dei 1978, Kutáček 1991).

A key to the senescence of leaf consists in the deteriorative processes in chloroplasts (Matile 1992). These can be of both qualitative and quantitative nature and comprise the loss of photosynthetic pigments, disorganisation of thylakoid membranes and stromal components, and a decline in the activities of photochemical reactions (Kutík 1985, Šesták 1985). Finally, chloroplasts are transformed into gerontoplasts, destined for destruction (Matile 1992).

The most conspicuous feature of chloroplast senescence is the change in composition of photosynthetic pigments (Šesták 1977a,b, 1978, 1981). Chl degradation has been considered synonymous with the syndrome of leaf senescence (Thimann 1980, Matile 1992, Hillman *et al.* 1994), and therefore it can be used to trace the course of ageing at the level of whole leaf.

Lipid composition of thylakoid membranes qualitatively changes during senescence as well. The major lipid components in the thylakoid membranes are galactolipids containing one or two galactose molecules attached to glycerol backbone. Their content decreases preferentially, over the other lipids, during senescence of bean leaves (Novitskaya *et al.* 1977). As galactolipids specifically occur in thylakoids, they can be used as a marker of chloroplast changes assayed in whole tissue preparations.

Carotenoids play an important role: they are relatively more stable than Chl (Biswal and Mohanty 1976, Matile 1992), and probably protect Chl molecules from the damage induced by excessive irradiance and ensuing free radical reactions (Demmig-Adams 1990, Young and Britton 1990).

A suitable object for the developmental studies are cotyledons due to their short and distinct life span (Tsukaya *et al.* 1994). In the present study we attempted to modulate the life span of bean cotyledons by increased or reduced irradiance, or by removing the epicotyl. Among the parameters we studied throughout the whole life span were the changes in fresh and dry masses of the cotyledons, in concentrations of Chl and carotenoids, and the oxidative damage to galactolipids. The results were correlated to morphological changes studied by electron microscopy.

Materials and methods

Plants: Control bean plants (*Phaseolus vulgaris* L. cv. Jantar) were grown in sand in a growth chamber at ambient temperature 18/16 °C, air humidity 60/80 %, and irradiance of 200/0 μmol(PAR) m^{-2} s^{-1}, day/night, respectively. The plants were supplied twice a week with an IBP nutrient solution. Life-span was modulated as follows: in the "decapitated" plants, the whole shoot above cotyledons was first excised when the plant raised up (7^{th} d), and then whenever needed. The "low irradiance" (LI) plants were grown under an irradiance of 5 μmol(PAR) m^{-2} s^{-1}. The "high irradiance" (HI) plants were grown at 400 μmol(PAR) m^{-2} s^{-1}.

Pigment contents: Fresh cotyledons were homogenised in a mortar with pestle in the presence of sand, and after that 85 % acetone was added. The Chl and carotenoid contents were determined after double centrifugation at 5000×*g* for 10 min according to Lichtenthaler (1987). Detailed pigment analysis in freeze-dried cotyledons was carried out using high performance liquid chromatography (HPLC). The cotyledons were homogenised in the same way as above. Pigments were separated in a HPLC system (*Spectra-Physics*, San Jose, CA, USA) using a reverse phase column (*Sepharon SGX C18*, 5 μm particle size, 150×3 mm, *Tessek*, Praha). The solvent systems for the combined isocratic and gradient separation were acetonitrile/methanol/water (80:12:6) followed by 100 % methanol, and the gradient was run from 6 to 10 min. The flow rate was 16.67 mm^3 s^{-1}, the detection wavelength was 445 nm. Calibration was made using individual pigments separated by high performance thin layer chromatography (silica gel plates 5×10 cm, *Merck*, Darmstadt, Germany; solvent mixture petroleum ether - acetone, 4:1). Each pigment was dissolved after scraping off from the plate with 100 % acetone, and then used as a standard for HPLC. Their concentrations were determined spectrophotometrically

Protein content was determined in the sediments after Chl extraction. The sediments were solubilized at room temperature overnight in 0.5 M NaOH. Then the method of Miller (1959) was followed as described in Wilhelmová and Kutík (1995). All measurements were taken in triplicate.

Galactolipid content: Freeze-dried cotyledons were homogenised in a mortar in a nitrogen-flushed chloroform/methanol mixture (1:2, v/v). The samples were evaporated in vacuum and dissolved with 165 mm^3 of chloroform. Then 15 mm^3 aliquots were applied in one zone for the quantitative determination of galactose on silica gel HPTLC plates (10×10 cm, *Merck*, Darmstadt, Germany). Remaining 150 mm^3 were applied in another zone for fluorescence detection. The plates were placed in an enclosed spotting chamber filled with nitrogen. Then they were developed in a chloroform/methanol/water mixture (65:25:4) for a 45 min period. Chromatograms were removed and dried in a stream of nitrogen. The 15 mm^3 portion bands were excised and sprayed with orcinol (*Sigma*, St. Louis, MIS, USA) for the galactolipid assay. The sprayed strips were developed at 85 °C for 10 min, scraped off, and finally dissolved in 1 mm^3 of methanol/water mixture (1:1). The absorbance was measured at λ_{max}. As for the bands of the 150 mm^3 sample portion, those were scraped off the plates, and dissolved in 3 cm^3 of nitrogen-flushed chloroform.

Electron microscopy: Samples were taken from the interior of the middle part of a cotyledon. Cotyledons of plants 7-d-old and fully senescent were compared for each cultivation type. Standard transmission electron (*Philips EM 300*) and light microscopy procedures were used (see Wilhelmová and Kutík 1995).

Fluorescence spectra of galactolipids: Excitation and emission characteristics of purified galactolipid fractions were investigated using tridimensional spectral arrays measured on a *Perkin Elmer LS5* fluorometer. Emission spectra were measured in the wavelength range of 385-500 nm after excitation which was increasing with a step of 5 nm between 250 and 380 nm. Excitation spectra were measured in the range of 250-400 nm for the emission between 370-480 nm adjusted at the step of 10 nm. The software for organizing the spectra in 3D arrays was developed in our laboratory.

Statistical analysis: The values reported are means of three measurements. Analysis of variance was done by using a statistical analysis program ANOVA (*StatView*, *Abacus Concepts*, Berkeley, CA, USA). Statistical significance of the observed differences was evaluated by the post-hoc test of Scheffé's F procedure.

Results

The plants were exposed to changed irradiance immediately after sowing. The first samples were analysed after the cotyledons fully developed (on the 7th d after sowing). The water content of cotyledons, reflected in fresh mass of harvested cotyledons, served as an indicator of organ viability (Fig. 1*A*): this parameter was decreasing continuously with time in all experimental groups except the decapitated plants. In the control group, the fresh mass was significantly decreased starting from the day 12 after sowing ($p < 0.001$). The loss of fresh mass in LI cotyledons was generally slower than in other non-decapitated groups, being about twice as high as that of the other non-decapitated groups at the end of the life-span. The time course of fresh mass of LI cotyledons differed significantly ($p < 0.005$) from that of all other groups. There was no statistically significant difference in the time course between control plants and the HI group. The time course of fresh mass changes in the plants decapitated on the 7th d differed from the control plants ($p < 0.0001$) as well as the HI plants ($p < 0.0001$), however, it did not differ from the LI plants.

The dry mass of cotyledons (Fig. 1*B*) decreased very rapidly in all experimental groups on the 10th d, indicating a uniform consumption of storage materials of the seed. No statistically significant difference between individual groups was observed. In the non-decapitated plants the dry mass of the cotyledons then remained practically constant until the end of life span. The decapitated plants had a higher dry cotyledon mass than the other groups up to the 16th d, however, it was not statistically significant.

The content of Chl ($a + b$) related to dry mass (Fig. 2*B*) indicated that senescence began after the 14th d in controls and in HI plants because this ratio started to decrease sharply in this period. In the LI plants the total concentration of Chl remained constant throughout the whole life-span. A statistically significant

difference was observed only for the time course in decapitated plants compared to controls ($p < 0.04$) and HI plants ($p < 0.006$).

When the concentration of Chl was expressed per unit protein mass (Fig. 2*A*), statistically significant differences were not observed between controls and both HI and LI plants. In the LI plants, the difference in the Chl time course, related either to dry mass or to unit protein concentration in the cotyledons, indicated changes in the protein content per dry mass. Senescence apparently started in the decapitated plants from the 21st d when a rapid decrease in Chl content was observed. The decreased Chl content on the 28th d was not significant when compared to the 7th d, but it was significant relative to the content observed on the 21st d ($p < 0.0007$).

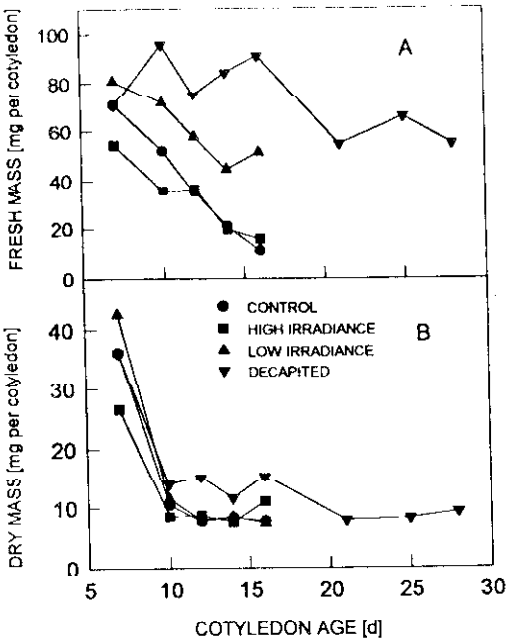

Fig. 1. Fresh (*A*) and dry (*B*) masses of one bean cotyledon during its life span at different cultivations. Points represent means of three measurements. The s.d. was between 10-15 % in all cases.

Changes in the ratio of Chl *a*/*b* (Fig. 2*C*) were observed between the controls and the LI plants ($p < 0.03$), and between the decapitated plants and the HI ($p < 0.01$) or LI ($p < 0.0001$) plants. In the controls, the decrease relative to the 7th d was significant on the days 10, 14, and 16 (all with $p < 0.0001$). This change was caused by an increase in Chl *b* concentration. In the HI plants the concentration of Chl *b* was slowly decreasing up to the 14th d while the concentration of Chl *a* was roughly constant. In the decapitated plants the ratio of Chl *a*/*b* reached the minimum on the 14th d due to a higher concentration of Chl *b*, and then it started to increase with decreasing concentration of Chl *b*, reaching its maximum on the 28th d ($p < 0.0001$).

As concerns carotenoids (Fig. 3), the time course of β-carotene content (Fig. 3*A*) was significantly different from controls in both HI ($p < 0.01$) and LI ($p < 0.002$) plants. No significant difference was observed between control and decapitated plants, however, decapitated plants differed both from the HI ($p < 0.002$) and LI ($p < 0.0002$) plants.

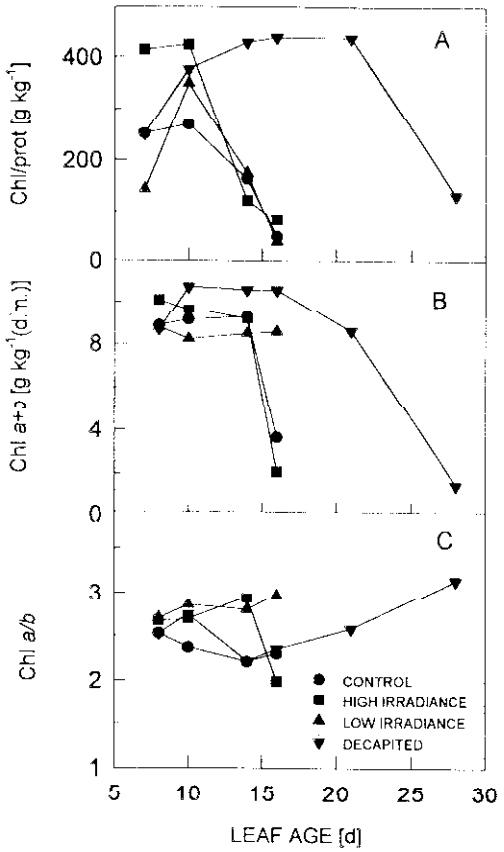

Fig. 2. Chlorophyll (*a*+*b*) content per protein amount (*A*) or per dry mass (*B*), and chlorophyll *a*/*b* ratio (*C*) during life span of bean cotyledons at different cultivations.

In lutein concentrations (Fig. 3*B*) the only significant difference was found between the HI plants and the decapitated plants. In the decapitated plants, the concentration of lutein increased on the 10th d ($p < 0.002$), stayed at this level until the 21st d, and afterwards a sharp decrease was observed ($p < 0.0002$).

Neoxanthin was quantitatively less abundant than lutein, and it had different time course during ageing. There was a significant difference between control and LI plants ($p < 0.001$), and between control and decapitated plants ($p < 0.0006$). The time course of neoxanthin concentrations during ageing did not differ significantly from the controls. The content in decapitated plants increased to a maximum in 14 d plants and then continuously declined

Violaxanthin, antheraxanthin, and zeaxanthin form the so-called xanthophyll cycle, the role of which has been widely studied. Violaxanthin (Fig. 3*D*) represents the initial part of the cycle. In relation to the control plants, its time course differed only in the HI cotyledons ($p < 0.0001$). With the exception of LI plants (a continuous decline), a small initial increase in violaxanthin content was followed by a slow decline in all experimental groups. The time course of antheraxanthin concentration

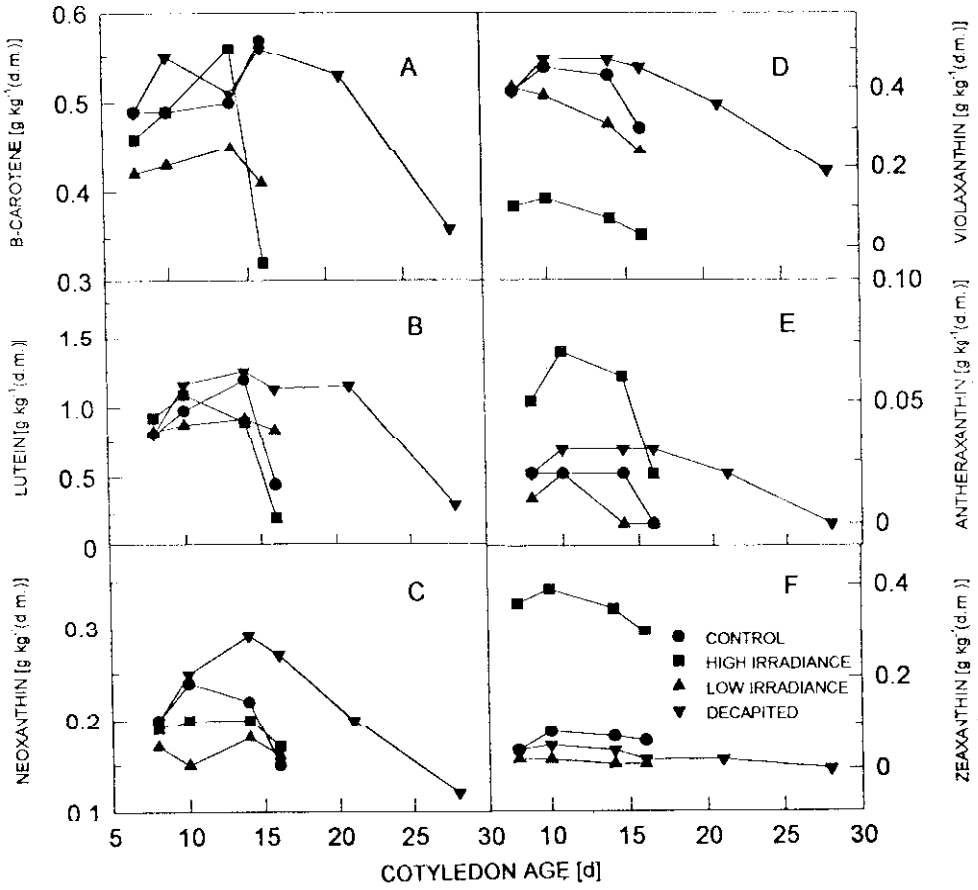

Fig. 3. β carotene (*A*), lutein (*B*), neoxanthin (*C*), violaxanthin (*D*), antheraxanthin (*E*), and zeaxanthin (*F*) contents per unit dry mass during life span of bean cotyledons at different cultivations.

(Fig. 3*E*) was significantly different from control in both HI ($p < 0.0001$) and LI ($p < 0.02$) cotyledons, but not in the decapitated plants. The concentration of antheraxanthin in HI cotyledons was about twofold of that in the controls, and it further increased on the 10th d ($p < 0.01$). Zeaxanthin concentrations (Fig. 3*F*) had a different time course only in the HI cotyledons ($p < 0.0001$, about ten fold content than in other groups), the contents in other experimental groups usually did not statistically differ.

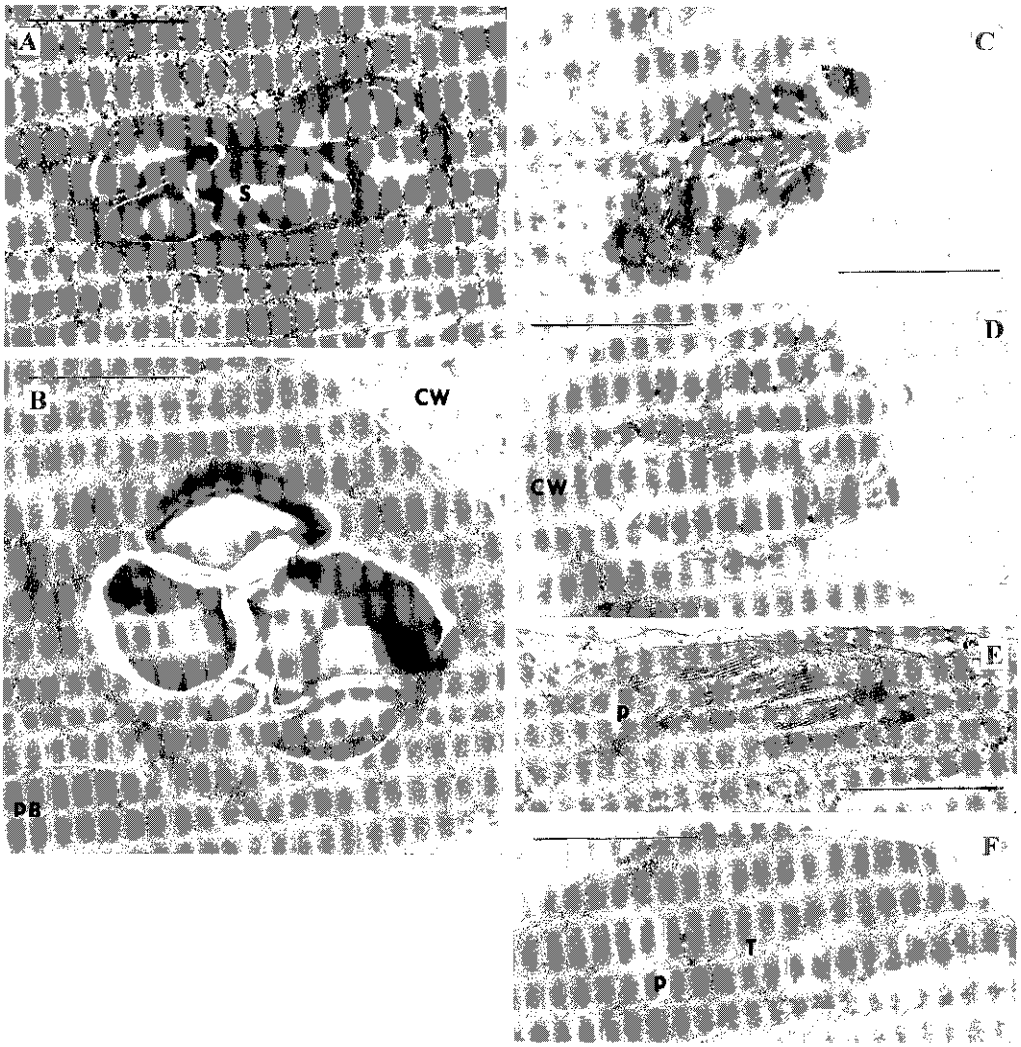

Fig. 4. Chloroamyloplast with large starch inclusions (S) in a cotyledon mesophyll cell of 7-d-old control (*A*) and LI (*B*) bean cotyledon, gerontoplast from 16-d-old control (*C*) and LI plant (*D*) cotyledon, chloroplast with well developed system of thylakoids from 16-d-old decapitated cotyledon (*E*), and senescent chloroplast from 24-d-old decapitated cotyledon (*F*). Bar = 1 μm. CW - cell wall, P - plastoglobulus, PB - prolamellar body, T - weakly contrasted thylakoids.

Electron microscopy pictures (Fig. 4*A-F*) enabled comparison of changes in chloroplast composition with changes in cell ultrastructure. For easier comparison, all figures have the same magnification. In 7-d-old control plants the cells were characterized by a dense cytoplasm containing chloroamyloplasts of various size and other organelles (Fig. 7*A*): there were large starch inclusions (labelled S). 7-d-old LI cotyledons contained developed chloroamyloplasts and many protein bodies (Fig. 7*B*). Senescent control plants on the day 16 (Fig. 7*C*) contained seriously altered gerontoplasts with very few plastoglobules. The other cellular organelles were highly diminished. The cotyledons of 16 d-old LI plants (Fig. 7*D*) had better preserved gerontoplasts than the corresponding controls, and the plastoglobules were more apparent. However, the overall situation in cytoplasm was similar. The 16-d-old decapitated plants (Fig. 4*F*) still had chloroplasts with well-developed thylakoids and with plastoglobuli. The cytoplasm contained decreased amount of other organelles, especially mitochondria. Senescing decapitated plants contained on the 24th d strongly senescent chloroplasts with weakly contrasted thylakoids. Plastoglobuli were pronounced, but other cellular organelles were lacking (Fig. 4*F*).

Fluorescence of monogalactolipids and digalactolipids isolated by thin layer chromatography was measured in order to detect the presence of peroxidized fatty acids. The measurements were organized into tridimensional spectral arrays which represent a "fingerprint" for a given compound(s), and therefore can serve for the detection of even subtle changes in the sample composition. The spectra of monogalactolipids isolated from 7- and 16-d-old control cotyledons were compared (Fig. 5). In the fluorescence emission spectra of 7-d-old plants, a maximum at 413 nm, typical for peroxidized fatty acids (Fig. 5*AI*), was found. On the 16th day, maximum emisson was at 416 nm (Fig. 5*AII*). Main excitation maxima were at 266 and 350 nm (Fig. 5*BI*) for both age groups, but in the 16-d-old cotyledons the maximum at 266 nm and the shoulder at 313 nm were more expressed (Fig. 5*BII*). The overall shapes of spectral array indicated modifications of chemical composition of the monogalactolipid fraction.

As concerns digalactolipids from cotyledons of these age groups, their emission maxima were at shorter wavelength (410 nm) in 7-d-old plants (Fig. 6*AI*) and at a much longer wavelength (426 nm) in 16-d-old plants (Fig. 6*AII*) than those of monogalactolipids. The main maxima of excitation spectra for digalactolipids were at 266 and 350 nm, similarly as for monogalactolipids, but their height ratio was much changed: the height of the 266 nm peak was increased in relation to that at 350 nm (Fig. 6*BI* and 6*BII*). These differences indicated large modification of this fraction during cotyledon ageing.

Discussion

In the present study we showed that increased or reduced plant irradiance did not influence the life-span of 16-d-old bean cotyledons, while it modulated several parameters characterizing the viability of cotyledons. On the other hand, decapitation of the epicotyl prolonged the life-span of the cotyledons up to 28 d.

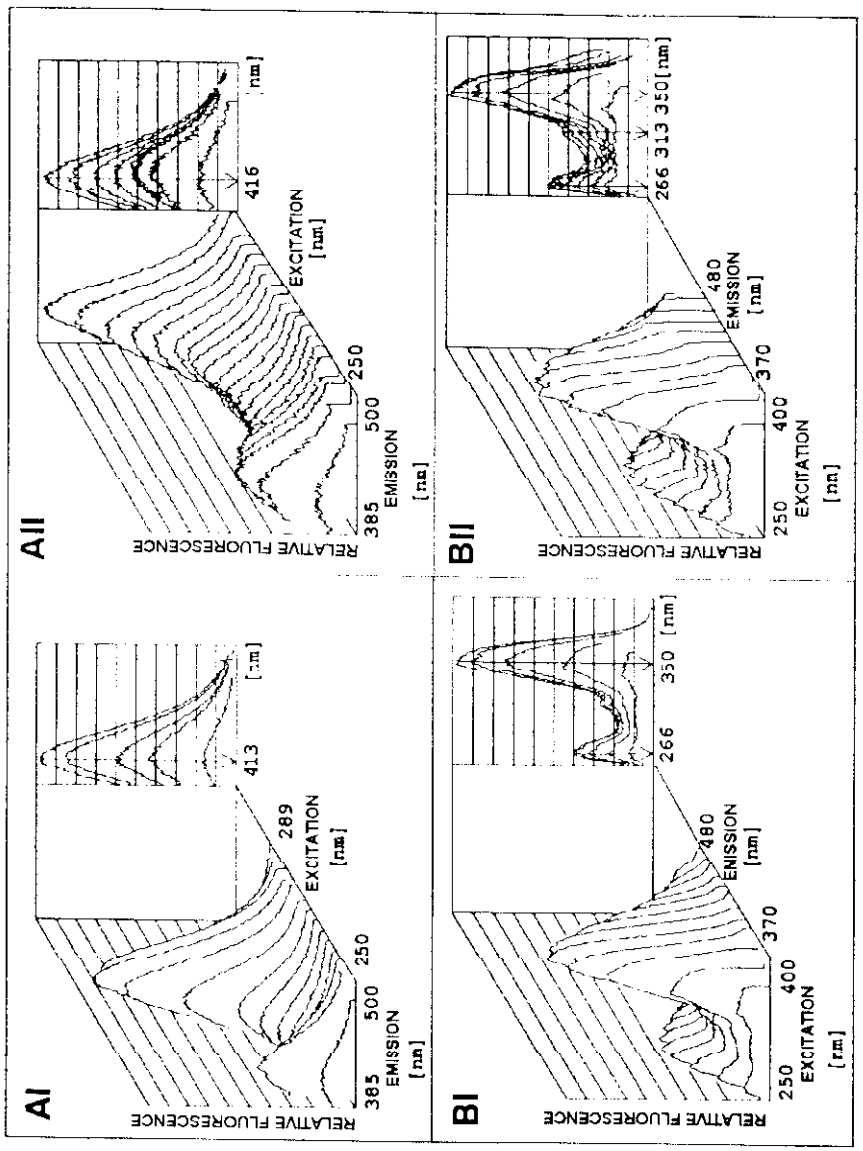

Fig. 5. Emission (*A*) and excitation (*B*) fluorescence spectra of monogalactolipids from 7-d-old (*I*) and 16-d-old (*II*) control bean cotyledons. Emission was measured in a range of 385-510 nm after excitation with growing wavelength from 250 to 390 nm (a step of 5 nm).

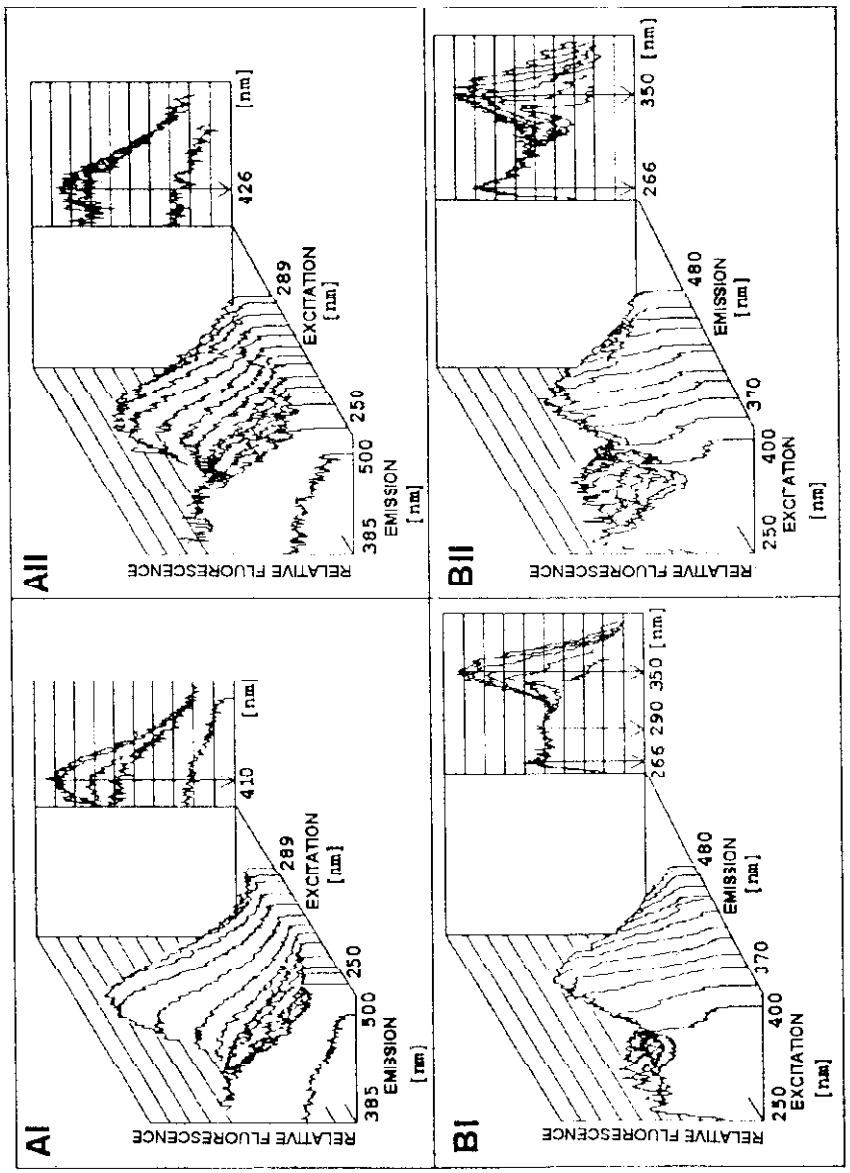

Fig. 6. Emission (*A*) and excitation (*B*) fluorescence spectra of digalactolipids from 7-d-old (*I*) and 16-d-old (*II*) control bean plant cotyledons. Emission was measured in a range of 385-510 nm after excitation with growing wavelength from 250 to 390 nm (a step of 5 nm).

The basic characteristic of cotyledon viability is its fresh mass. Its continuous decrease during ageing was found in cucumber cotyledons (Ferguson and Simon 1973) and primary bean leaves (Fong and Heath 1977). We observed the same trend, but the loss of fresh mass was smaller in the LI plants, and the decapitation even caused an increase in fresh mass which lasted until the 16th d after sowing. The fresh mass at the end of life span of the decapitated plant cotyledons did not differ from the initial value. On the other hand, the time course of the dry mass followed the same pattern in all experimental groups, indicating that the basic degradative/synthetic processes were not influenced by the treatments.

Chl content is a generally used marker of leaf senescence (Thimann 1980, Matile 1992, Hillman *et al.* 1994). According to this view, cotyledon senescence was initiated after the 14th d after sowing. Until this period, the Chl concentration per unit dry mass remained constant, and a sharp decrease was observed on the 16th day. This situation was observed in the control group and in the HI plants. However, no change in the Chl concentration was observed in the LI plants. The electron microscopic observations documented better preserved gerontoplasts in the LI group, though the overall degeneration of cellular content was similar as in the other groups. This implies that the expression of Chl concentration per unit dry mass is not a suitable parameter for the assessment of senescence. A more useful expression is the Chl concentration per unit protein. This parameter started to decrease after the 10th d in all experimental groups except in decapitated plants where it was postponed until the 21st d. On the 16th d, the end of life span of cotyledons of other groups, the decapitated plants had higher Chl concentration than on the 7th d. The good state of chloroplasts from 16-d-old decapitated plants was confirmed by electron microscopy, which also proved an intracellular disorganization on the 24th d.

The decrease of Chl *a*/*b* ratio in the course of ageing was caused by an increase in Chl *b* concentration in the control group up to the 14th d, while the Chl *a* content remained almost constant. This indicates that the LHCs are proliferating during ageing. The sharp fall of Chl concentration on the 16th d was higher for Chl *b* than Chl *a* which was reflected in a relative increase in the Chl *a*/*b* ratio. The antenna complex of PS2 is usually broken down at the beginning of senescence. As the complex contains equimolar amounts of Chl *a* and *b*, its disappearance results in a shift in the Chl *a*/*b* ratio in favour of Chl *a* (Hilditch 1986). No dramatic changes in the Chl *a*/*b* ratio were observed in the LI plants, the slight increase in this ratio relative to the initial value was caused by the higher concentration of Chl *a*. The final fall of Chl concentration was not observed in this plant group, indicating a different mechanism of senescence. In the decapitated plants, Chl *b* concentration increased until the 14th d similarly as in controls. Then followed a phase of Chl *b* degradation, which was, however, much longer than in control plants. Thus the mechanism of ageing and senescence in the decapitated plants may be similar as in controls, but the degenerative changes are postponed or slowed down under the influence of root produced-cytokinins that are induced by plant decapitation (Dei 1978).

Carotenoids are relatively more stable than Chl (Biswal and Mohanty 1976, Matile 1991), and they may protect Chl against oxidative damage induced by excessive radiant energy or by free radicals (Demmig-Adams 1990, Young and Britton 1990).

The content of β-carotene, that is present in reaction centres, remained constant in the control group up to the 14th d, and then increased on the 16th d. The unicellular green alga *Dunaliella bardawil* responds to a high irradiance and other stresses by massive accumulation of β-carotene (Lers *et al.* 1991), and a similar effect is produced by reactive oxygen species (Shaish *et al.* 1993). This could be a general response of the photosynthetic apparatus exposed to high concentrations of free radicals. This view was supported by the continuous increase in β-carotene concentration in HI plants up to the 14th d. The sharp fall of β-carotene concentration in this group during senescence might mean that combined effects of free radicals produced during senescence and the photooxidative damage overwhelmed the antioxidant capacity of the cell. The low content of β-carotene in the LI plants was in accordance with the above mentioned concept.

Lutein is a xantophyll found in LHC1 and LHC2 (Yamamoto and Bassi 1996). Its time-course during ageing was similar to that of β-carotene, except that it was not induced by HI, and its final decrease was observed also in the control group. This means that lutein is not inducible by free radicals as β-carotene. Neoxanthin had a similar distribution as lutein, but it reacted differently to the experimental treatments. This might be an illustration of a different control of individual carotenoids.

Violaxanthin is a precursor for the synthesis of antheraxanthin which is an intermediate in the production of zeaxanthin (for review see, *e.g.*, Gilmore 1997). Zeaxanthin stabilizes the reaction centre by dissipating the excess of excitation energy (Foyer *et al.* 1994). During cotyledon ageing we observed a decrease in violaxanthin concentration in all experimental groups, but only in the HI plants we found an increase in zeaxanthin concentration. This could indicate that violaxanthin was consumed during ageing for other purposes, *e.g.* for the synthesis of abscisic acid (Kutáček 1991). According to the studies of Demmig-Adams (1990) and Britton (1993), zeaxanthin concentration increased in the HI plants. Nevertheless, the violaxanthin cycle modulations depend on interaction of irradiance and temperature (Stefanov *et al.* 1996). The violaxanthhin cycle carotenoids may also modulate aggregation capability of the LHCPs (Hagen *et al.* 1996). The slow decrease of zeaxanthin concentration during ageing might represent the loss of its protective effect.

By measurement of fluorescence emission and excitation spectral arrays, we detected qualitative changes in the galactolipid fractions isolated from aged cotyledons. Especially the fluorescence excitation spectra, which are analogical to absorption spectra and thus reveal changes in chemical composition, indicated the modifications characteristic for peroxidized fatty acids. This oxidative modifications of galactolipids during ageing could be used for further characterization of free radicals production during senescence.

References

Biswal, U.C., Mohanty, P.: Changes in the ability of photophosphorylation and activities of surface-bound adenosine triphosphatase and ribulose diphosphate carboxylase of chloroplasts isolated from the barley leaves senescing in darkness. - Physiol. Plant. **44**: 127-133, 1978.

Britton, G.: Carotenoids in chloroplast pigment-protein complexes. - In: Sundqvist, C., Ryberg, M. (ed.): Pigment-Protein Complexes in Plastids: Synthesis and Assembly. Pp. 447-485. Academic Press, San Diego - New York - Boston - London - Sydney - Tokyo - Toronto 1993.

Dei, M.: Inter-organ control of greening in etiolated cucumber cotyledons. - Physiol. Plant. **43**: 94-98, 1978.

Demmig-Adams, B.: Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. - Biochim. biophys. Acta **1020**: 1-24, 1990.

Ferguson, C.H.R., Simon, E.W.: Membrane lipids in senescing green tissues. - J. exp. Bot. **24**: 307-316, 1973.

Fong, F., Heath, R.: Age dependent changes in phospholipids and galactolipids in primary bean leaves (*Phaseolus vulgaris*). - Phytochemistry **16**: 215-217, 1977.

Foyer, C.H., Lelandais, M., Kunert, K.J.: Photooxidative stress in plants. - Plant Physiol. **92**: 696-717, 1994.

Gilmore, J.A.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. - Physiol. Plant. **99**: 197-209, 1997.

Hagen, C., Pascal, A.A., Horton, P., Inoue, Y.: Influence of structural changes in the photosynthetic antenna system on the thermoluminescence Z-band. - Photosynthetica **32**: 529-543, 1996.

Hillman, J.R., Glidewell, S.M., Deighton, N.: The senescence syndrome in plants: an overview of phytogerontology. - Proc. roy. Soc. Edinburgh 102B: 447-458, 1994.

Hilditch, P.: Immunological quantification of the chlorophyll *a/b* binding protein in senescing leaves of *Festuca pratensis* Huds. - Plant Sci. **45**: 95-99, 1986.

Huber, D.J., Newman, D.W.: Relationships between lipid changes and plastid ultrastructural changes in senescing and regreening soybean cotyledons. - J. exp. Bot. **27**: 490-511, 1976.

Kutáček, M.: Plant hormones as factors of morphogenesis and structural integrity in plants. - In: Šebánek, J., Sladký, Z., Procházka, S. *et al.* (ed.) Experimental Morphogenesis and Integration of Plants. Pp. 13-56. Academia, Praha 1991.

Kutík, J.: Chloroplast development. - In: Šesták, Z. (ed.): Photosynthesis During Leaf Development. Pp. 51-75. Academia, Praha, Dr W. Junk Publ., Dordrecht - Boston - Lancaster 1985.

Lers, A., Levy, H., Zamir, A.: Co-regulation of a gene homologous to early light-induced genes in higher plants and β-carotene biosynthesis in the alga *Dunaliella bardawil*. - J. biol. Chem. **266**: 13698-13705, 1991.

Lichtenthaler, H.K.: Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. - In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. 148. Pp. 350-382. Academic Press, San Diego New York Berkeley Boston London - Sydney - Tokyo - Toronto 1987.

Matile, P.: Chloroplast senescence. - In: Baker, N.R., Thomas, H. (ed.): Crop Photosynthesis: Spatial and Temporal Determinants. Pp. 413-440. Elsevier, Amsterdam 1992.

Miller, G.L.: Protein determination for large numbers of samples. - Anal. Chem. 3: 964, 1959.

Novitskaya, G.V., Rutskaya, L.A., Molotkovskiĭ, Yu.G.: [Changes in lipid composition and the activity of broad bean chloroplast membranes with ageing.] - Fiziol. Rast. **24**: 35-43, 1977. [In Russ.]

Rascio, N., Dalla Vecchia, F., Casadoro, G.: Behaviour of cotyledonal plastids during seed germination in some chloroembryophytes. - Cytobios **64**: 53-64, 1990.

Sagisaka, S., Akita, T.: Occurrence of a putative precursor to the amyloplast, the "amyloplast initial" in developing stolons of the potato. - Biosci. Biotech. Biochem. **58**: 1077-1079, 1994.

Šesták, Z.: Photosynthetic characteristics during ontogenesis of leaves. 1. Chlorophylls. - Photosynthetica **11**: 367-448, 1977a.

Šesták, Z.: Photosynthetic characteristics during ontogenesis of leaves. 2. Photosystems, components of electron transport chain, and photophosphorylation. - Photosynthetica **11**: 449-474, 1977b.

Šesták, Z.: Photosynthetic characteristics during ontogenesis of leaves. 3. Carotenoids. - Photosynthetica **12**: 89-109, 1978.

Šesták, Z.: Leaf ontogeny and photosynthesis. - In: Johnson, C.B. (ed.): Physiological Processes Limiting Plant Productivity. Pp. 147-158. Butterworths, London - Boston - Sydney - Wellington - Durban - Toronto 1981.

Šesták, Z. (ed.): Photosynthesis During Leaf Development. - Academia, Praha, Dr W. Junk Publ., Dordrecht - Boston - Lancaster 1985.

Shaish, A., Avron, M., Pick, U., Ben-Amotz, A.: Are active oxygen species involved in induction of β -carotene in *Dunaliella bardawil*? - Planta **190**: 363-368, 1993.

Stefanov, D., Yordanov, I., Tsonev, T.: Effect of thermal stress combined with different irradiance on some photosynthetic characteristics of barley (*Hordeum vulgare* L.) plants. - Photosynthetica **32**: 171-180, 1996.

Thimann, K.V.: The senescence of leaves. - In: Thimann, K.V. (ed.): Senescence in Plants. Pp. 85-115. CRC Press, Boca Raton 1980.

Thomas, H., Stoddart, J.L.: Leaf senescence. - Annu. Rev. Plant Physiol. **31**: 83-111, 1980.

Tsukaya, H., Tsuge T., Uchimiya, H.: The cotyledon: A superior system for studies of leaf development. - Planta **195**: 309-312, 1994.

Wilhelmová, N., Kutík, J.: Influence of exogenously applied 6-benzylaminopurine on the structure of chloroplasts and arrangement of their membranes. - Photosynthetica **31**: 559-570, 1995.

Yamamoto, H.Y., Bassi, R.: Carotenoids: localization and function. - In: Ort, D.R., Yocum, C.F. (ed.): Oxygenic Photosynthesis: The Light Reactions. Pp. 539-563. Kluwer Academic Publishers, Dordrecht - Boston - London 1996.

Young, A., Britton, G.: Carotenoids and stress. - In: Alscher, R.G., Cumming, J.R. (ed.): Stress Responses in Plants. Pp. 87-112. Willey-Liss, New York 1990.