DOI: 10.1007/s11099-013-0001-x PHOTOSYNTHETICA 51 (1): 85-94, 2013

Study of the senescence process in primary leaves of sunflower (*Helianthus annuus* L.) plants under two different light intensities

L. DE LA MATA, P. CABELLO, P. DE LA HABA, and E. AGÜERA+

*Departamento de Botánica, Ecología y Fisiología Vegetal, Área de Fisiología Vegetal, Facultad de Ciencias, Universidad de Córdoba, Campus de Rabanales. Edificio Celestino Mutis (C4), E-14071 Córdoba, Spain**

Abstract

Different parameters that vary during leaf development may be affected by light intensity. To study the influence of different light intensities on primary leaf senescence, sunflower (*Helianthus annuus* L.) plants were grown for 50 days under two photon flux density (PFD) conditions, namely high irradiance (HI) at 350 μmol(photon) m^{-2} s^{-1} and low irradiance (LI) at 125 μmol(photon) m^{-2} s^{-1}. Plants grown under HI exhibited greater specific leaf mass referred to dry mass, leaf area and soluble protein at the beginning of the leaf development. This might have resulted from the increased CO_2 fixation rate observed in HI plants, during early development of primary leaves. Chlorophyll *a* and *b* contents in HI plants were lower than in LI plants in young leaves. By contrast, the carotenoid content was significantly higher in HI plants. Glucose concentration increased with the leaf age in both treatments (HI and LI), while the starch content decreased sharply in HI plants, but only slightly in LI plants. Glucose contents were higher in HI plants than in LI plants; the differences were statistically significant ($p<0.05$) mainly at the beginning of the leaf senescence. On the other hand, starch contents were higher in HI plants than in LI plants, throughout the whole leaf development period. Nitrate reductase (NR) activity decreased with leaf ageing in both treatments. However, the NR activation state was higher during early leaf development and decreased more markedly in senescent leaves in plants grown under HI. GS activity also decreased during sunflower leaf ageing under both PFD conditions, but HI plants showed higher GS activities than LI plants. Aminating and deaminating activities of glutamate dehydrogenase (GDH) peaked at 50 days (senescent leaves). GDH deaminating activity increased 5-fold during the leaf development in HI plants, but only 2-fold in LI plants. The plants grown under HI exhibited considerable oxidative stress *in vivo* during the leaf senescence, as revealed by the substantial H_2O_2 accumulation and the sharply decrease in the antioxidant enzymes, catalase and ascorbate peroxidase, in comparison with LI plants. Probably, systemic signals triggered by a high PFD caused early senescence and diminished oxidative protection in primary leaves of sunflower plants as a result.

Additional key words: antioxidant enzymes; ascorbate peroxidase; catalase; glutamate dehydrogenase; glutamine synthetase; hexose; nitrite reductase; irradiance; nitrate reductase; plant; reactive oxygen species; senescence; sunflower; superoxide dismutase.

Introduction

Leaf senescence is a highly regulated and programmed degeneration process that is controlled by multiple developmental and environmental signals (Lim *et al*. 2003). Senescence is not only a degenerative process, but also a recycling process whereby nutrients are translocated from senescing cells to young leaves, developing seeds or storage tissues (Gan and Amasino 1997). Senescence is characterized mainly by cessation of photosynthesis, degeneration of cellular structures, intensive losses of chlorophylls (Chls), carotenoids (Car) and proteins, and

Received 24 January 2012, *accepted* 24 October 2012.
+Corresponding author; tel.: +34957218367, fax +34957211069, e-mail: vg1agbue@uco.es
Abbreviations: APX – ascorbate peroxidase; Car – carotenoids; CAT – catalase; Chl – chlorophyll; DTT – dithiothreitol; DM – dry mass; EDTA – ethylenediaminotetraacetic acid; FAD – flavin adenine dinucleotide; GDH – glutamate dehydrogenase; GOGAT – glutamate synthetase; g_s – stomatal conductance; GS – glutamine synthetase; GS1 – cytosolic glutamine synthetase; GS2 – chloroplastic glutamine synthetase; HI – high irradiance; LI – low irradiance; NiR – nitrite reductase; NR – nitrate reductase; PFD – photon flux density; P_N – net photosynthetic rate; ROS – reactive oxygen species; SLM – specific leaf mass; SOD – superoxide dismutase.
Acknowledgements. This work was funded by Junta de Andalucía (Grant P07-CVI-02627 and PAI group BIO-0159) and DGICYT (AGL2009-11290). The authors are grateful to Mr. A. Velasco Blanco for his valuable technical assistance and to Prof. Dr. J. Diz Pérez for the helpful advice on the statistical analysis.

dramatically increased lipid peroxidation (Srivalli and Khanna-Chopra 2004, Agüera *et al*. 2010). This process can start prematurely by effect of plant exposure to environmental stress or nutrient deprivation (Quirino *et al*. 2000, Lim *et al*. 2003, Lim *et al*. 2007, Wingler *et al*. 2009, Agüera *et al*. 2010).

Irradiance and duration of exposure to light are major factors influencing plant growth and development. A very high intensity (about 500–5,000 μmol m^{-2} s^{-1}) applied for an extended period leads to irreversible damage of PSII. This phenomenon inhibits photosynthesis and diminishes plant growth (Prasil *et al*. 1992, Melis 1999, Schansker and van Rensen 1999). To avoid photooxidative damage, plants possess highly efficient photoprotective systems that operate *via* two primary mechanisms. The first one involves dissipation of excess excitation energy as heat in the antenna pigment complexes of PSII. This process is related to the xanthophyll cycle, by which violaxanthin is de-epoxidized to antheraxanthin and zeaxanthin in the so-called "violaxanthin cycle" or "xanthophyll cycle". The second photoprotective mechanism involves antioxidant enzymes such as superoxide dismutase (SOD), which converts superoxide anions into H_2O_2, and catalase and APX, which detoxify the resulting peroxide (Asada 1999, Logan *et al*. 2006). Plant ageing increases oxidative stress and ROS levels, but this may also reduce antioxidant protection (Buchanan-Wollaston *et al*. 2003a, Zimmermann and Zentgraf 2005, Vanacker *et al*. 2006). High PFD exposure has been deemed one major cause of oxidative stress in plants (Dat *et al*. 2000). Some reports describe changes in activity and expression of antioxidant enzymes in response to high PFD stress, albeit to a varying extent depending on the particular plant material and treatment conditions (Hernández *et al*. 2006, Ariz *et al*. 2010). The equilibrium between ROS production and scavenging may be altered under different stress conditions (Srivalli and Khanna-Chopra 2009).

Almost all plant stress situations studied to date have similar effects to natural senescence, and reduce NR, NiR, GOGAT, total GS, and GS2, but increase GDH and GS1 transcripts, proteins, and activity levels (Masclaux *et al*. 2000, Masclaux-Daubresse *et al*. 2005, Pageau *et al*. 2006). NR activity in leaves is rapidly modulated by reversible phosphorylation of NR protein in response to light/dark transitions (Agüera *et al*. 1999, de la Haba *et al*. 2001). NR activity decreases under low irradiance in coffee plants (Carelli *et al*. 2006). According to Cabello *et al*. (2006), ageing induces oxidative stress in sunflower leaves, having an adverse impact on chloroplastic GS2 and photosynthetic pigments. Ever since GOGAT was discovered, the GS/GOGAT pathway has proved to be the most important process for ammonia assimilation into amino acids (Miflin and Lea 1980). Because of its low affinity for ammonia, GDH is assumed to act as a catabolic enzyme in the deamination of glutamate (Pahlich 1996, Lehmann and Ratajczak 2008). Masclaux-Daubresse *et al.* (2002) ascribed age-related induction of GDH in a range of plant tissues to either an increase in ammonia content or depletion of carbohydrates, both of which are usually observed during senescence.

The purpose of this work was to study the effect of two PFDs [125 and 350 μmol(photon) m^{-2} s^{-1}], with special emphasis on growth, sugar levels, regulation of nitrogen metabolism, enzyme activities, and photoprotection mechanisms during development in primary sunflower (*H. annuus* L.) leaves. Sunflower has a great agronomical and economical value since it is one of the five most important sources of edible oil in the world (Cantamutto and Poverene 2007). Sunflower oil is also a source of biodiesel (Arzamendi *et al.* 2008), sunflower plants have an ornamental value and are used for phytoremediation (Mani *et al.* 2007).

Materials and methods

Plant material and growth: Seeds of sunflower (*H. annuus* L.) from the isogenic cultivar HA-89 (Semillas Cargill SA, Sevilla, Spain) were surface-sterilized in 1% (v/v) hypochlorite solution for 15 min. After rinsing in distilled water, the seeds were imbibed for 3 h and then sown in plastic trays containing a mixture of perlite and vermiculite (1:1, v/v). All seeds were germinated and plants were incubated in a chamber under a 16/8 h light/dark cycle and a day/night regime of 25/19ºC temperature and 70/80% relative humidity. Plants were irrigated daily with a nutrient solution containing 10 mM KNO_3 (Hewitt 1966).

Two different PFDs (provided by *Sylvania F72T12/CW/VHO*, 160 W fluorescent lamps supplemented with *Mazda* 60 W incandescent bulbs and measured using a model *CRS068* portable infrared gas analyser governed *via* the software *CIRAS*), HI and LI, were applied over a period of 50 d. At different times (16, 22, 32, 42, and 50 d), primary leaf samples were collected 2 h after the photoperiod start. Entire leaves were excised and pooled in two groups: one was used to measure leaf area and specific leaf mass (SLM) referred to DM, and the other was frozen immediately in liquid nitrogen and stored at –80°C. The frozen plant material was ground in a mortar precooled with liquid N_2 and the powder distributed into small vials that were stored at –80°C until enzyme activity and metabolite determinations.

Net CO_2 fixation and stomatal conductance were measured on attached leaves, 2 h after the photoperiod start, using a model *CRS068* portable infrared gas analyser (MA, USA), governed *via* the software *CIRAS*. The instrument was adjusted to have inside the leaf chamber constant conditions of CO_2 concentration (360 μl l^{-1}), flow (150 cm^3 min^{-1}), leaf temperature (25ºC), relative humidity (80%) and the light intensities used in each treatment (HI and LI). Measurements were made on

primary leaf samples after the stabilization period, using several plants per treatment. Leaf samples were acclimated in the leaf chamber for 5–10 min and measurements were carried out during 3–5 min.

Extraction and activity measurement of NR, GS, and GDH: Frozen material was homogenized with cold extraction medium (4 ml g^{-1}) consisting of 100 mM Hepes-KOH (pH 7.5), 10% (v/v) glycerol, 1% (w/v) polyvinylpolypyrrolidone (PVPP), 0.1% (v/v) Triton, 6 mM DTT, 1 mM EDTA, 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 25 μM leupeptin, 20 μM flavin adenine dinucleotide (FAD) and 5 μM Na_2MoO_4. The homogenate was centrifuged at 8,000 × *g* at 4ºC for 2 min, and enzyme activity measured immediately in the clear extract.

NR (EC 1.6.6.1) activity was determined in the presence and absence of Mg^{2+}. The activation state of NR was calculated as the ratio of its activity in the presence and absence of Mg^{2+}, and expressed as a percentage, as described by Agüera *et al*. (2006). GS (EC 6.3.1.2) activity was measured with the transferase assay of de la Haba *et al*. (1992). Finally, GDH (EC 1.4.1.2) aminating and deaminating activities were determined spectrophotometrically at 340 nm according to Loyola-Vargas and Sánchez de Jiménez (1984).

Enzyme antioxidant activity: Enzyme extracts for determination of catalase (CAT, EC 1.11.1.6) and APX (EC 1.11.1.11) were prepared by freezing a weighed amount of leaf samples (g) in liquid nitrogen to prevent proteolytic activity, followed by grinding in 10 ml extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM ascorbic acid). The resulting homogenate was passed through 4 layers of gauze and the filtrate centrifuged at 15,000 × *g* for 20 min, the supernatant being used as enzyme source.

CAT activity was estimated according to Aebi (1983). The reaction mixture contained 50 mM potassium phosphate (pH 7) and 10 mM H_2O_2. After enzyme addition, H_2O_2 decomposition was monitored spectrophotometrically at 240 nm ($\varepsilon = 43.6\ mM^{-1}\ cm^{-1}$).

APX activity was measured according to Nakano and Asada (1981). The reaction mixture contained 50 mM phosphate buffer (pH 7), 1 mM sodium ascorbate and 25 mM H_2O_2. After adding of ascorbate to the mixture, the reaction was monitored at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

Carbohydrates were extracted from the powdered frozen tissue in successive steps with ethanol/water mixtures in different proportions according to Agüera *et al.* (2006). The supernatants from each centrifugation were collected and combined to determine soluble sugars, whereas the pellets were used to quantify starch. Sucrose, glucose, and fructose were determined according to Outlaw and Tarczynski (1984), Kunst *et al*. (1984) and Beutler (1984), respectively. The pellets were resuspended in water and incubated at 100ºC for 5 h. Glucose was then released by incubation with α-amylase and amyloglucosidase, and assayed enzymatically as described above.

Protein, pigment, and H_2O_2: Soluble protein was extracted in 50 mM Hepes-KOH (pH 7), 5 mM $MgCl_2$ and 1 mM EDTA, and determined with the *Bio-Rad* protein assay according to Bradford (1976). Pigments were determined by HPLC in the plant extracts according to Cabello *et al*. (1998). For H_2O_2 determination, 1 g of leaf material was ground with 10 ml of cooled acetone in a cold room, and filtered through *Whatman* filter paper. H_2O_2 was determined by formation of a titanium-hydroperoxide complex according to Mukherjee and Choudhuri (1983).

Statistical analysis: Values are given as the means ± SD of three separate experiments with duplicate determinations. All results were statistically analyzed in a bifactorial model, which considers the effect of the PFD and the cultivation time on the variables using the *ANOVA* and *Tukey*'s test and they were conducted at a significance level of 5% ($p<0.05$).

Results

Some growth parameters such as SLM referred to DM, leaf area and soluble protein were determined in primary leaves of sunflower plants grown for 50 d under two PFD regimes, LI and HI (Fig. 1). SLM referred to DM peaked at 22 d in plants grown under both regimes and then decreased by effect of ageing. HI plants exhibited higher SLM referred to DM values than LI plants at the beginning of the growth period (16 and 22 d, Fig. 1*A*). With both treatments (HI and LI), leaf area increased until day 32, but the values were 24% higher at the beginning of the growth period (16 d) in HI plants (Fig. 1*B*). In general, HI plants showed greater development than LI plants (data not shown). The soluble protein content exhibited a similar variation pattern in both HI and LI plants, decreasing about 50% during sunflower primary leaf ageing, but the protein levels were about 11% higher in HI plants than in LI plants at 22 and 32 d (Fig. 1*C*).

Leaf development had a negative effect on photosynthetic pigment content in both treatments. The chlorophylls (Chls) *a* and *b* contents were lower in HI plants than in LI plants throughout leaf development, with up to 30% decrease in young leaves (Fig. 2*A,B*). On the contrary, the carotenoid content in HI plants was statistically significantly higher than in LI plants during leaf development period (Fig. 2*C*).

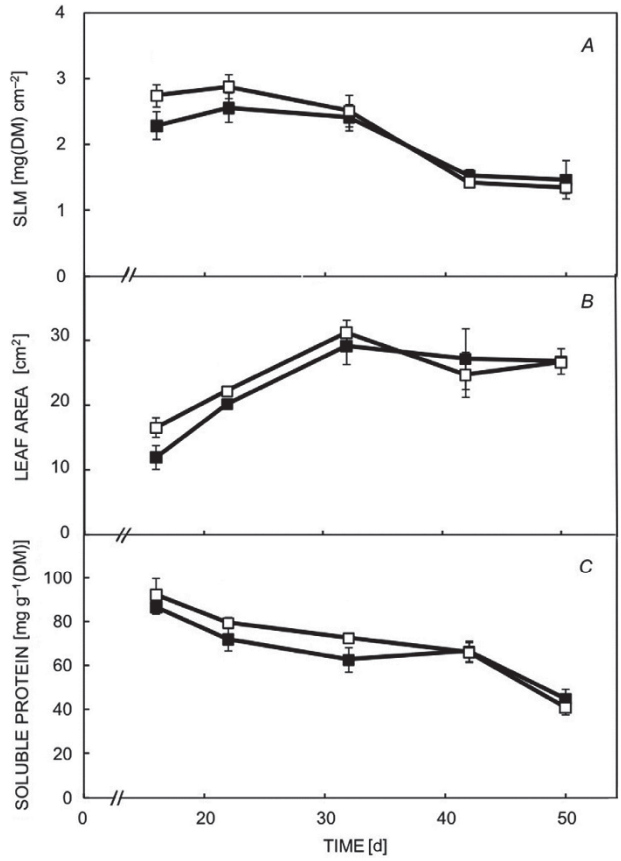

Fig. 1. Changes in specific leaf mass (SLM) referred to dry mass (DM), leaf area and soluble protein during sunflower primary leaf development under LI (■) and HI (□). Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test (p<0.05).

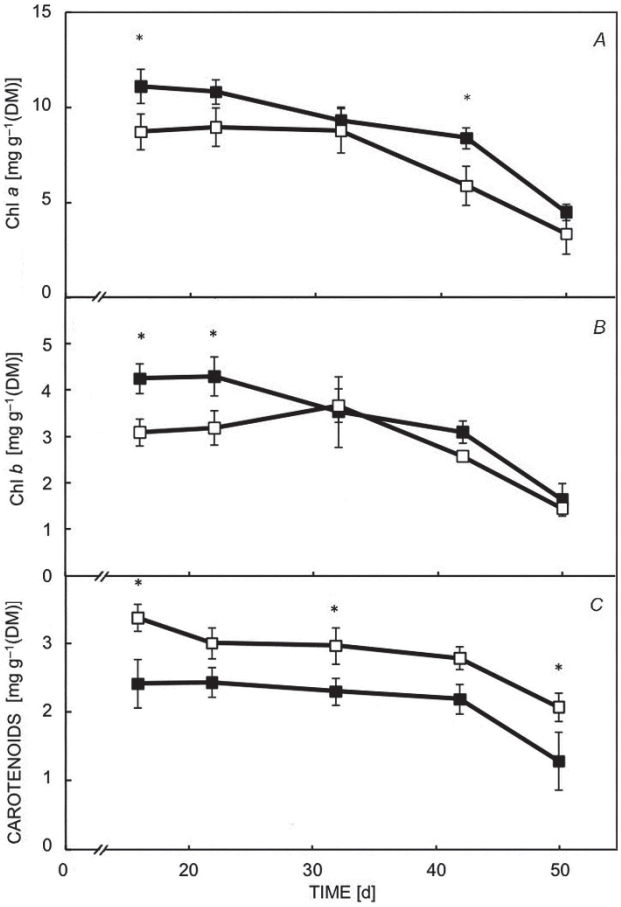

Fig. 2. Changes in the pigment contents during sunflower primary leaf development under LI (■) and HI (□). Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test (p<0.05). DM – dry mass; Chl – chlorophyll; Car – carotenoids.

The CO_2 fixation rate was negatively affected by leaf ageing in both treatments, but the loss of photosynthetic activity was more marked and occurred earlier in HI plants than in LI plants. Thus, under HI the CO_2 fixation rate decreased by about 90% between 22 and 50 d, whereas in LI plants it decreased only about 64% in the same period. Stomatal conductance decreased considerably after 22 d, but no statistically significantly differences were observed between both treatments (Table 1).

We also examined the changes in carbohydrate contents in sunflower primary leaves during ageing in order to identify a potential role as metabolic signals for senescence (Fig. 3). With both treatments (HI and LI), the concentrations of soluble sugars (glucose, fructose and sucrose) increased with the leaf age up to 42 d. By contrast, the starch content decreased very sharply in HI plants during leaf development, but only slightly in LI plants (Fig. 3). Glucose contents were higher in HI plants than in LI plants, these differences were statistically significant (p<0.05), mainly at the beginning of the leaf senescence (32 d) (Fig. 3*A*). On the other hand, starch contents were higher in HI plants than in LI plants, throughout the whole leaf development period (Fig. 3*D*). NR activity was determined in the presence and absence of Mg^{2+} in sunflower primary leaves, and the NR activation state was also estimated. As shown in Table 2, NR activity decreased during leaf development, both in the presence and absence of Mg^{2+}. The decrease was more evident under LI, while under HI it was noted only from 22 day. The NR activation state diminished in senescent leaves with both treatments. However, in the HI plants the NR activation state was higher, and it also decreased more sharply during leaf ageing, than in LI plants (Table 2).

GS activity also declined during leaf ageing, and it showed higher values in HI plants (Fig. 4). On the contrary, GDH aminating and deaminating activities (Table 3) peaked in senescent leaves (50 d) and higher values were noted in LI compared with HI. However, the GDH deaminating activity increased 5-fold during leaf

Table 1. Net photosynthetic rate (P_N) and stomatal conductance (g_s) during sunflower primary leaf development under LI and HI. Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test ($p<0.05$).

Time [d]	P_N [μmol(CO_2) m^{-2} s^{-1}]		g_s [mmol(H_2O) m^{-2} s^{-1}]	
	LI	HI	LI	HI
16	2.63 ± 0.25	5.06 ± 0.76*	570.25 ± 24.24	550.46 ± 28.87
22	3.40 ± 0.37	5.76 ± 0.80*	620.67 ± 2.42	615.50 ± 5.10
32	3.37 ± 0.30	2.00 ± 0.02*	351.67 ± 2.21	355.30 ± 0.25
42	1.43 ± 0.12	0.66 ± 0.06	30.67 ± 2.03	10.94 ± 0.20
50	1.23 ± 0.12	0.60 ± 0.36	16.56 ± 0.54	6.67 ± 0.77

development in HI plants, but only 2-fold in LI plants.

We also studied H_2O_2 production and the activity of CAT and APX in sunflower leaves. As shown in Fig. 5*A*, the production of H_2O_2 increased considerably with leaf ageing. After 22 d, the H_2O_2 levels in HI plants were higher than in LI plants, these differences were statistically significant ($p<0.05$). CAT and APX activities rose during early leaf development and decreased in senescent leaves (42 and 50 d). This decline in the activity of the antioxidant enzymes was more marked, about 40%, in HI plants (Fig. 5*B,C*).

Discussion

Our results showed that in primary leaves of sunflower (*H. annuus* L.) plants some metabolic processes, like carbon and nitrogen metabolism and susceptibility to oxidative stress, were sensitive to irradiance. In fact, we found HI to increase significantly leaf area, SLM referred to DM, and soluble protein content at the beginning of the leaf development (Fig. 1). This might be a result of the increased photosynthetic capacity observed in HI plants during early development of primary leaves. At later stages, the photosynthetic rate decreased faster in HI plants than in LI plants (Table 1). We have also found a decrease in soluble protein content during leaf development (Fig. 1*C*) that could be caused by strong degradation of chloroplast proteins during senescence, as reported by Martínez *et al.* (2008). On the other hand, changes in the protein content can reflect alterations in the distribution of N and C compounds as a consequence of more efficient N mobilization during senescence (Díaz *et al*. 2008).

Leaf development had a negative effect on photosynthetic pigment content of sunflower plants, in both treatments. The content of Chls *a* and *b* was lower in HI plants than in LI plants throughout leaf development, with up to 30% decrease in young leaves (Fig. 2*A,B*). Plants can avoid excessive light absorption by, *e.g*., reducing Chl production, adopting a steep inclination or reflecting incident light (Adams *et al*. 2004, Baig *et al*. 2005, Demmig-Adams and Adams 2006). The loss of Chls is typical of leaf senescence and may be used as an indicator of this phenomenon (Yoo *et al*. 2003, Guo and Gan 2005, Ougham *et al*. 2008). Astolfi *et al.* (2001) also observed a decrease in Chls content and suggested that high PFD induces premature senescence and increases the senescence rate. The CO_2 fixation rate in HI plants might have no correlation with Chl content. The higher net photosynthetic rate (P_N) observed in young leaves (16 and 22 d) in HI plants (Table 1), could be explained by a greater Rubisco content (Ariz *et al.* 2010) and also as a consequence of more efficient penetration of incident radiation into the leaves with lower Chls content (Radochová and Tichá 2008). The Car content of primary leaves in sunflower plants grown under both PFD used in this work revealed that HI plants contained more Car than LI plants (Fig. 2*C*). This suggests that plants can synthesize large amounts of Car as an adaptative strategy to protect their photosynthetic machinery when subjected to high PFD (Behera and Choudhury 2001, 2003; Lichtenthaler 2007).

The concentration of soluble sugars increased up to 42 d in both treatments, with a slight decrease in glucose and sucrose contents in more senescent leaves (50 d). On the other hand, the starch content decreased, especially in HI plants (Fig. 3). Interestingly, our results show a significant accumulation of glucose and starch in HI plants in comparison with LI plants. Glucose contents were statistically significant mainly at the beginning of the leaf senescence (Fig. 3*A*). The accumulation of glucose could not be directly related to photosynthetic activity because pigment contents and CO_2 fixation rates decreased during leaf ageing (Figs. 2, 3; Table 1). It could be rather due to starch hydrolysis, especially in HI plants (Fig. 3*D*). The increase in soluble sugars, especially glucose, might also be the result of senescence promoting a decline in the functional and structural integrity of cell membranes, thereby accelerating the membrane lipid catabolism which produces sugars by gluconeogenesis (Buchanan-Wollaston *et al*. 2003b, Lim *et al*. 2007). Accumulation of soluble sugars at the beginning of senescence has been described in various plants, but changes are not clearly associated with leaf development

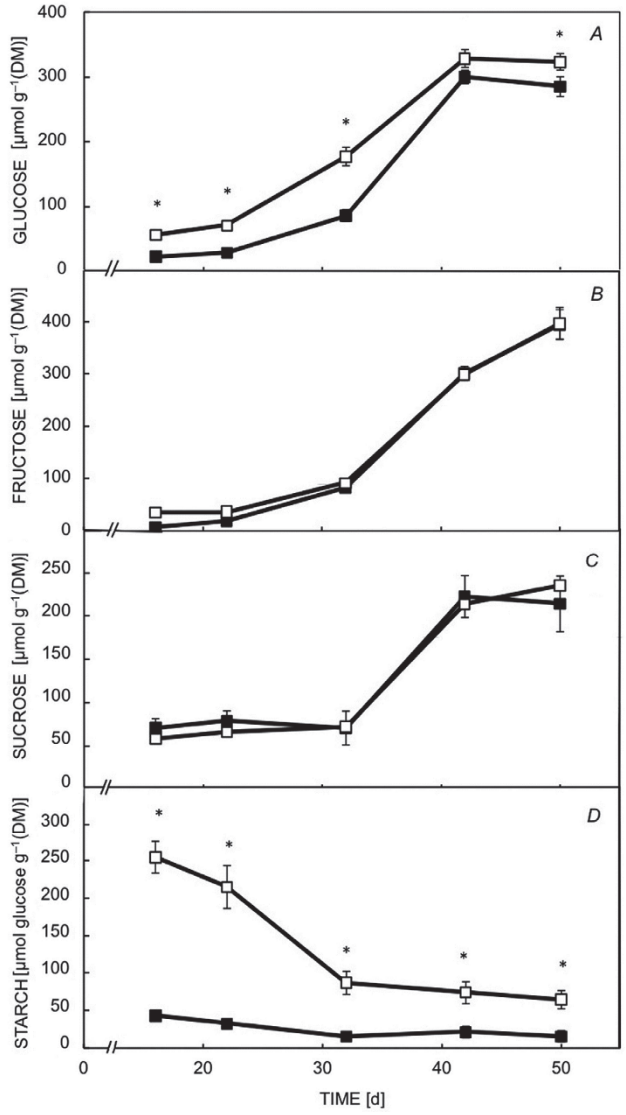

Fig. 3. Changes in the contents of glucose, fructose, sucrose and starch during sunflower primary leaf development under LI (■) and HI (□). Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test (p<0.05). DM – dry mass.

and may vary depending on the plants lines (Díaz *et al.* 2005, Wingler *et al.* 2006, Agüera *et al.* 2010). In fact, leaf senescence is a plastic process that can be triggered by a variety of external and internal factors (Buchanan-Wollaston *et al*. 2003a, Wingler *et al*. 2006, Masclaux-Daubresse *et al*. 2007,Wingler *et al.* 2009). Ono *et al*. (2001) have shown that shading leaves of sunflower or bean reduces their sugar contents and delays senescence, which suggests that the carbohydrate accumulation induces leaf senescence. It has been suggested a role of hexose accumulation in ageing leaves as a signal for either senescence initiation or acceleration in annual plants (Masclaux *et al.* 2000, Moore *et al*. 2003, Díaz *et al*. 2005, Masclaux-Daubresse *et al*. 2005, Parrot *et al*. 2005, Pourtau *et al*. 2006, van Doorn 2008, Wingler and Roitsch 2008, Agüera *et al*. 2010).

Nitrogen metabolism in old source leaves is characterized by progressive hydrolysis of stromal proteins and degradation of chloroplasts (Masclaux *et al.* 2000, Hörtensteiner and Feller 2002). We found that NR activity in the presence or absence of Mg^{2+}, as well as the activation state of NR, decreased during leaf ageing for both HI and LI plants. However, in HI plants, activation state of NR increased during early leaf development, and then decreased drastically during senescence (Table 2). De la Haba *et al*. (2001) previously found that both activity and activation state of NR in cucumber plants raise with high light intensity and diminish with darkness, and they ascribed this NR regulation effect to a potential phosphorylation/dephosphorylation mechanism. Since the main metabolic process in leaf senescence involves nutrient remobilization, ammonium should be rapidly assimilated into amino acids *via* GS/GOGAT to avoid deleterious effects and to supply nitrogenous forms suitable for source–sink transport (Masclaux-Daubresse *et al*. 2006). Our results revealed that GS activity decreased during sunflower leaf ageing under both irradiance regimes, and that HI plants showed the highest GS activities (Fig. 4). The effects of light on the expression of genes coding for chloroplastic isoform GS2 have been tested by Oliveira and Coruzzi (1999). They found that chloroplastic isoform GS2 is induced by light or by carbon metabolites such as sucrose. GS activity is known

Table 2. NR activity (assayed without and with Mg^{2+}) and activation state of NR during sunflower primary leaf development under LI and HI. Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test (p<0.05).

Time [d]	LI NR activity [µmol(NO_2^-) h^{-1} g^{-1}(DM)] Assay - Mg2+	Assay + Mg2+	LI NR activation state [%]	HI NR activity [µmol(NO_2^-) h^{-1} g^{-1}(DM)] Assay - Mg2+	Assay + Mg2+	HI NR activation state [%]
16	64.98 ± 0.21	24.88 ± 0.21	38.0	52.57 ± 2.82*	34.95 ± 0.88*	66.5
22	54.94 ± 1.04	18.05 ± 0.26	32.9	58.59 ± 0.67	38.76 ± 0.83*	66.2
32	47.29 ± 0.36	13.03 ± 0.36	27.5	23.88 ± 0.15*	6.60 ± 0.26*	27.7
42	14.17 ± 0.1	4.65 ± 0.05	32.8	21.45 ± 0.15*	5.53 ± 0.15*	24.9
50	11.18 ± 0.57	2.06 ± 0.11	18.5	4.02 ± 0.36*	0.55 ± 0.05*	13.7

Table 3. Glutamate dehydrogenase (GDH) aminating and deaminating activities during sunflower primary leaf development under LI and HI. Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test ($p<0.05$).

Time [d]	GDH activity [μmol h^{-1} g^{-1}(DM)]			
	Aminating		Deaminating	
	LI	HI	LI	HI
16	85.0 ± 4.6	48.5 ± 3.4*	99.5 ± 3.4	38.8 ± 3.7*
22	233.0 ± 15.5	80.1 ± 7.1*	128.6 ± 14.0	67.9 ± 3.7*
32	250.0 ± 37.8	135.9 ± 14.2*	182.0 ± 10.3	89.8 ± 3.8*
42	276.7 ± 14.3	240.2 ± 14.0	196.6 ± 3.4	169.9 ± 10.0*
50	327.7 ± 44.6	235.4 ± 15.2*	216.0 ± 10.3	194.1 ± 7.4

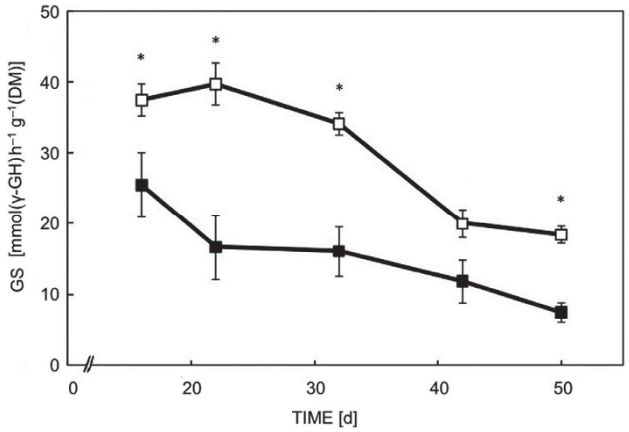

Fig. 4. Changes in the glutamine synthetase (GS) activity during sunflower primary leaf development under LI (■) and HI (□). Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the ANOVA and *Tukey*'s test ($p<0.05$). DM – dry mass.

to decrease during leaf senescence (Masclaux *et al*. 2000, Cabello *et al*. 2006). The loss of GS activity during leaf development must be mainly due to a progressive decline in activity and expression of the GS2 isoform since the cytosolic isoform GS1 increases during sunflower leaf ageing (Cabello *et al*. 2006). The greatest increase in GDH deaminating activity observed in HI plants during leaf development (5-fold in HI compared with only 2-fold in LI, Table 3) might be explained assuming that GDH does not play a role in ammonium assimilation, but rather it participates in glutamate catabolism (Miflin and Habash 2002, Masclaux-Daubresse *et al*. 2006). GDH activity is also induced in old leaves when nitrogen remobilization is maximal (Masclaux-Daubresse *et al*. 2006).

Plants grown under HI exhibited considerable oxidative stress *in vivo* at final stages of leaf development, as revealed by a significant increase in H_2O_2 accumulation and a more marked decrease in antioxidant enzyme (catalase and APX) activities, in comparison with LI plants (Fig. 5). Leaf senescence is an oxidative process that involves degradation of cellular and subcellular structures and macromolecules, and mobilization of the resulting degradation products to other plant parts (Vanacker *et al*. 2006). Oxidative stress during senescence may be caused or increased by the loss of antioxidant enzyme activities (Zimmermann and Zentgraf 2005, Zimmermann *et al*. 2006, Procházková and Wilhelmová 2007, Pompelli *et al*. 2010). Senescence is also accompanied by an increased ROS production,

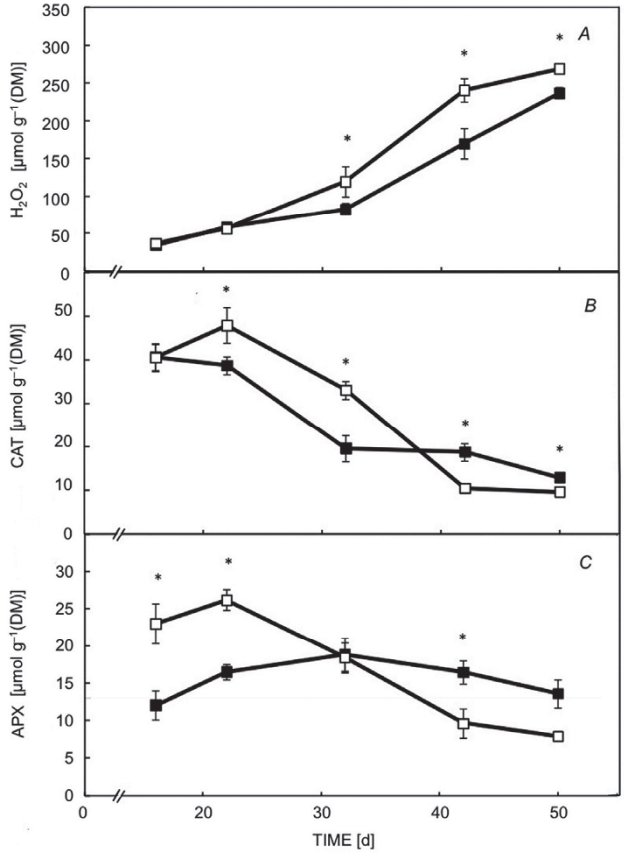

Fig. 5. H_2O_2 accumulation and enzymatic activities of catalase (CAT) and ascorbate peroxidase (APX) during sunflower primary leaf development under LI (■) and HI (□). Data are means ± SD of 3 separate experiments with duplicate determinations. * – statistically significant differences among the PFD treatments and the cultivation time according to the *ANOVA* and *Tukey*'s test ($p<0.05$).

and one of the reasons for this phenomenon is the imbalance between generation and consumption of electrons in the photosynthetic electron transport chain caused by preferential inhibition of stromal reactions relative to photosystem II photochemistry (Špundová *et al*. 2003). Inhibition of stromal reactions increases the electron flow to molecular oxygen, thereby causing ROS to accumulate and chloroplast components to be damaged as a result (Špundová *et al*. 2005, Couée *et al*. 2006). Susceptibility to oxidative stress depends on the overall balance between production of oxidants and the antioxidant capability of cells. High PFD regime was previously found to cause reversible photoinhibition of photosynthesis in pea chloroplasts and to increase ROS potentially regulating the accumulation of mRNA encoding antioxidant enzymes (Hernández *et al*. 2006.). Changes in an activity and expression of antioxidant enzymes in response to high PFD stress have been reported (Yoshimura *et al*. 2000, Hernández *et al*. 2004). High PFD causes early symptoms of senescence during leaf expansion in tobacco plants (Radochová and Tichá 2008). Our results suggest that HI accelerated senescence in the primary leaf of sunflower plants, probably in order to preserve the functionality of young leaves, and also that one of the reasons for accelerated senescence in HI plants might be the strong cellular oxidation and oxidative damage caused by an increased H_2O_2 accumulation, which might be partially due to an earlier decline of antioxidant enzyme activities in these plants.

In conclusion, our results showed that high PFD caused early senescence in sunflower (*H. annuus* L.) primary leaves by altering the CO_2 fixation rate and the Chl and sugar levels, the activity of key enzymes of nitrogen metabolism (NR, GS, and GDH), and the oxidation status of the plant (accumulation of H_2O_2 and loss of APX and CAT activities). Systemic signals triggered by a high PFD probably caused early senescence and diminished oxidative protection in primary leaves of sunflower plants as a result.

References

Adams, W.W., III, Zarter, C.R., Ebbert, V., Demmig-Adams, B.: Photoprotective strategies of overwintering evergreens. – BioScience **54**: 41-49, 2004.

Aebi, H.E.: Catalase. – In: Bergmeyer, H.U., Grassl, M. (ed.): Methods of Enzymatic Analysis. Pp. 273-286. Verlag Chemie, Weinheim 1983.

Agüera, E., Poblete, L., de la Haba, P., Maldonado, J. M.: Light modulation and *in vitro* effects of adenine nucleotides on leaf nitrate reductase activity in cucumber (*Cucumis sativus*). – Physiol. Plant. **105**: 218-223, 1999.

Agüera, E., Ruano, D., Cabello, P., de la Haba, P.: Impact of atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants. – J. Plant Physiol. **163**: 809-817, 2006.

Agüera, E., Cabello, P., de la Haba, P.: Induction of leaf senescence by low nitrogen nutrition in sunflower (*Helianthus annuus*) plants. – Physiol. Plant. **138**: 256-267, 2010.

Ariz, I., Esteban, R., García-Plazaola, J.I., Becerril, J.M. *et al*.: High irradiance induces photoprotective mechanisms and a positive effect on NH_4^+ stress in *Pisum sativum* L. – J. Plant Physiol. **167**: 1038-1045, 2010.

Arzamendi, G., Campo, I., Arguiñarena, E., Sánchez, M., Montes, M., Gandía, L.M.: Synthesis of biodiesel from sunflower oil with silica-supported NaOH catalysts. – J. Chem. Technol. Biotechnol. **83**: 862-870, 2008.

Asada, K.: The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. – Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 601-639, 1999.

Astolfi, S., De Biasi, M.G., Passera, C.: Effects of irradiance-sulphur interactions on enzymes of carbon, nitrogen and sulphur metabolism in maize plants. – Photosynthetica **39**: 177-181, 2001.

Baig, M.J., Anand, A., Mandal, P.K., Bhatt, R.K.: Irradiance influences contents of photosynthetic pigments and proteins in tropical grasses and legumes. – Photosynthetica **43**: 47-53, 2005.

Behera, R.K., Choudhury, N.K.: Photosynthetic characteristics of chloroplasts of primary wheat leaves grown under different irradiance. – Photosynthetica **39**: 11-15, 2001.

Behera, R.K., Choudhury, N.K.: High irradiance-induced changes in carotenoid composition and increase in non-photochemical quenching of Chl *a* fluorescence in primary wheat leaves. – J. Plant Physiol. **160**: 1141-1146, 2003.

Beutler, H.O.: D-Fructose. – In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Pp. 321-327. Verlag Chemie, Weinheim 1984.

Bradford, M.M.: A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. – Anal. Biochem. **72**: 248-254, 1976.

Buchanan-Wollaston, V., Earl, E., Harrison, E., Mathas, E., Navabpour, S., Page. T, Pink, D.: The molecular analysis of leaf senescence – a genomics approach. – Plant Biotechnol. J. **1**: 3-22, 2003a.

Buchanan-Wollaston, V., Wellesbourne, H.R.I., Warwick, U.K.: Senescence, leaves. – In: Encyclopedia of Applied Plant Sciences, Elsevier Academic Press. Pp. 808-816, 2003b.

Cabello, P., de la Haba, P., González-Fontes, A., Maldonado, J.M.: Induction of nitrate reductase, nitrite reductase, and glutamine synthetase isoforms in sunflower cotyledons as affected by nitrate, light, and plastid integrity. – Protoplasma **201**: 1-7, 1998.

Cabello, P., Agüera, E., de la Haba, P.: Metabolic changes during natural ageing in sunflower (*Helianthus annuus*) leaves: expression and activity of glutamine synthetase isoforms are regulated differently during senescence. – Physiol. Plant. **128**: 175-185, 2006.

Cantamutto, M., Poverene, M.: Genetically modified sunflower release: opportunities and risks. Field Crop. Res. **101**: 133-144, 2007.

Carelli, M.L.C., Fahl, J.I., Cochicho J.D.: Aspects of nitrogen metabolism in coffee plants. – Braz. J. Plant Physiol. **18**: 9-21, 2006.

Couée, I., Sulmon, C., Gouesbet, G., El Amrani, A.: Involvement of soluble sugars in reactive oxygen species balance and

responses to oxidative stress in plants. – J. Exp. Bot. **3**: 449-459, 2006.

Dat, J., Vandenabeele, S., Vranová, E., Van Montagu, M., Inzè, D., Van Breusegem, F.: Dual action of the active oxygen species during plant stress responses. – Cell. Mol. Life Sci. **57**: 779-795, 2000.

de la Haba, P., Cabello, P., Maldonado, J.M.: Glutamine-synthetase isoforms appearing in sunflower cotyledons during germination. Effects of light and nitrate. – Planta **186**: 577-581, 1992.

de la Haba, P., Agüera, E., Benítez, L., Maldonado, J.M.: Modulation of nitrate reductase activity in cucumber (*Cucumis sativus*) roots. – Plant Sci. **161**: 231-237, 2001.

Demmig-Adams, B., Adams, W.W., III: Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. – New Phytol. **172:** 11-21, 2006.

Díaz, C., Purdy, S., Christ, A., Morot-Gaudry, J.F., Wingler, A., Masclaux-Daubresse, C.: Characterization of markers to determine the extent and variability of leaf senescence in *Arabidopsis*. A metabolic profiling approach. – Plant Physiol. **138**: 898-908, 2005.

Díaz, C., Lemaître, T., Christ, A., *et al*.: Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in *Arabidopsis* under low nitrogen nutrition. – Plant Physiol. **147**: 1437-1449, 2008.

Gan, S., Amasino, R.M.: Making sense of senescence. Molecular genetic regulation and manipulation of leaf senescence. – Plant Physiol. **113**: 313-319, 1997.

Guo, Y., Gan, S.: Leaf senescence: signals, execution, and regulation. – Curr. Top. Develop. Biol. **71**: 83-112, 2005.

Hernández, J.A., Escobar, C., Creissen, G., Mullineaux, P.M.: Role of hydrogen peroxide and the redox state of ascorbate in the induction of antioxidant enzymes in pea leaves under excess light stress. – Funct. Plant Biol. **31**: 359-368, 2004.

Hernández, J.A., Escobar, C., Creissen, G., Mullineaux, P.M.: Antioxidant enzyme in pea plants under high irradiance. – Biol. Plant. **50**: 395-399, 2006.

Hewitt, E.J.: Sand and water culture methods used in the study of plant nutrition. – Commonwealth Bureau of Horticultural and Plantation Crops, East Malling. Tech. Commun. No 22, 1966.

Hörtensteiner, S., Feller, U.: Nitrogen metabolism and remobilization during senescence. – J. Exp. Bot. **53**: 927-937, 2002.

Kunst, A., Draeger, B., Ziegenhorn, J.: D-Glucose: UV-methods with hexokinase and glucose-6-phosphate dehydrogenase. – In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Pp. 163-172. Verlag Chemie, Weinheim 1984.

Lehmann, T., Ratajczak, L.: The pivotal role of glutamate dehydrogenase (GDH) in the mobilization of N and C from storage material to asparagine in germinating seeds of yellow lupine. – J. Plant Physiol. **165:** 149-158, 2008.

Lichtenthaler, H.K.: Biosynthesis, accumulation and emission of carotenoids, α-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance. – Photosynth. Res. **92**: 163-179, 2007.

Lim, P.O., Woo, H.R., Nam, H.G: Molecular genetics of leaf senescence in *Arabidopsis*. – Trends Plant Sci. **8**: 272-278, 2003.

Lim, P.O., Kim, H.J., Nam, H.G.: Leaf senescence. – Annu. Rev. Plant Biol. **58**: 115-136, 2007.

Logan, B.A. Kornyeyev, D., Hardison, J., Holaday, A.S.: The role of antioxidant enzymes in photoprotection. – Photosynth. Res. **88**: 119-132, 2006.

Loyola-Vargas, V.M., Sánchez de Jiménez, E.: Differential role of glutamate dehydrogenase in nitrogen metabolism of maize tissues. – Plant Physiol. **76**: 536-540, 1984.

Mani, D., Sharma, B., Kumar, C.: Phytoaccumulation, interaction, toxicity and remediation of cadmium from *Helianthus annuus* L. (sunflower). - Bull. Environ. Contam. Toxicol. **79**: 71-79, 2007.

Martínez, D.E., Costa, M.L., Guiamet, J.J.: Senescence-associated degradation of chloroplast proteins inside and outside the organelle. – Plant Biol. **10:** 15-22, 2008.

Masclaux, C., Valadier, M.H., Brugière, N., Morot-Gaudry, J.F., Hirel, B.: Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. – Planta **211**: 510-518, 2000.

Masclaux-Daubresse, C., Valadier, M.H., Carrayol, E., Reisdorf-Cren, M., Hirel, B.: Diurnal changes in the expression of glutamate dehydrogenase and nitrate reductase are involved in the C/N balance of tobacco source leaves. – Plant Cell Environ. **25**: 1451-1462, 2002.

Masclaux-Daubresse, C., Carrayol, E., Valadier, M.H.: The two nitrogen mobilisation- and senescence-associated *GS1* and *GDH* genes are controlled by C and N metabolites. – Planta **221**: 580-588, 2005.

Masclaux-Daubresse, C., Reisdorf-Cren, M., Pageau, K. *et al*.: Glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. – Plant Physiol. **140**: 444-456, 2006.

Masclaux-Daubresse, C., Purdy, S., Lemaître, T., Pourtau, N., Taconnat, L., Renou, J. P., Wingler, A.: Genetic variation suggests interaction between cold acclimation and metabolic regulation of leaf senescence. – Plant. Physiol. **143**: 434-446, 2007.

Melis, A.: Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage *in vivo*? – Trends Plant Sci. **4**: 130-135, 1999.

Miflin, B.J., Lea, P.J.: Ammonium assimilation. – In: Miflin, B.J. (ed.): The Biochemistry of Plants Amino Acids and Derivatives. Pp. 169-202. Academic Press. New York-London – Toronto – Sydney – San Francisco 1980.

Miflin, B.J., Habash, D.: The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. – J. Exp. Bot. **53**: 979-987, 2002.

Moore, B., Zhou, L., Rolland, F., *et al*.: Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light and hormonal signaling. – Science **300**: 332-336, 2003.

Mukherjee, S.P., Choudhuri, M.A.: Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. – Physiol. Plant. **58**: 166-170, 1983.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Oliveira, I.C., Coruzzi, G.M.: Carbon and amino acids reciprocally modulate the expression of glutamine syntethase in *Arabidopsis*. – Plant Physiol. **121**: 301-310, 1999.

Ono, K., Nishi, Y., Watanabe, A., Terashima, I.: Possible mechanisms of adaptive leaf senescence. – Plant Biol. **3**: 234-243, 2001.

Ougham, H., Hörtensteiner, S., Armstead, I., Donnison, I., King, I., Thomas, H., Mur, L.: The control of chlorophyll

catabolism and the status of yellowing as a biomarker of leaf senescence. – Plant Biol. **10**: 4-14, 2008.

Outlaw, W.H., Jr., Tarczynski, M.C.: Sucrose. – In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Pp. 96-103. Verlag Chemie, Weinheim 1984.

Pageau, K., Reisdorf-Cren, M., Morot-Gaudry, J.F., Masclaux-Daubresse, C.: The two senescence-related markers, GS1 (cytosolic glutamine synthetase) and GDH (glutamate dehydrogenase), involved in nitrogen mobilization, are differentially regulated during pathogen attack and by stress hormones and reactive oxygen species in *Nicotiana tabacum* L. leaves. – J. Exp. Bot. **57**: 547-557, 2006.

Pahlich, E.: Remarks concerning the dispute related to the function of plant glutamate dehydrogenase: commentary. – Can. J. Bot. **74**: 512-515, 1996.

Parrott, D., Yang, L., Shama, L., Fischer, A.M.: Senecence is accelerated, and several proteases are induced by carbon "feast" conditions in barley (*Hordeum vulgare* L.) leaves. – Planta **222**: 989-1000, 2005.

Pompelli, M.F., Martins, S.C.V., Antunes, W.C., Chaves, A.R.M., DaMatta, F.M.: Photosynthesis and photoprotection in coffee leaves is affected by nitrogen and light availabilities in winter conditions. – J. Plant Physiol. **167**: 1052-1060, 2010.

Pourtau, N., Jennings, R., Pelzer E. *et al.*: Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in *Arabidopsis*. – Planta **224**: 556-568, 2006.

Prášil, O., Adir, N., Ohad, I.: Dynamic of PSII: Mechanism of photoinhibition and recovery process. – In: Baber, J. (ed.): The Photosystems: Structures, Function and Molecular Biology. Pp 295-348. Elsevier, Amsterdam – London – New York – Tokyo 1992.

Procházková, D., Wilhelmová, N.: Leaf senescence and activities of the antioxidant enzymes. – Biol. Plant. **51**: 401-406, 2007.

Quirino, B.F., Noh, Y.S., Himelblau, E., Amasino, R.M.: Molecular aspects of leaf senescence. – Trends Plant Sci. **5**: 278-282, 2000.

Radochová, B., Tichá, I.: Excess irradiance causes early symptoms of senescence during leaf expansion in photoautotrophically *in vitro* grown tobacco plants. – Photosynthetica **46**: 471-475, 2008.

Schansker, G., van Rensen, J.J.S.: Performance of active Photosystem II centers in photoinhibited pea leaves. – Photosynth. Res. **62**:175-184, 1999.

Špundová, M., Popelková, H., Ilík, P., *et al.*: Ultra-structural and functional changes in the chloroplasts of detached barley leaves senescing under dark and light conditions. – J. Plant Physiol. **160**: 1051-1058, 2003.

Špundová, M., Slouková, K., Hunková, M., Nauš, J.: Plant shading increases lipid peroxidation and intensifies senescence-induced changes in photosynthesis and activities of ascorbate peroxidase and glutathione reductase in wheat. – Photosynthetica **43**: 403-409, 2005.

Srivalli, B., Khanna-Chopra, R.: The developing reproductive 'sink' induces oxidative stress to mediate nitrogen mobilization during monocarpic senescence in wheat. – Biochem. Biophys. Res. Commun. **325**: 198-202, 2004.

Srivalli, B., Khanna-Chopra, R.: Delayed wheat flag leaf senescence due to removal of spikelets is associated with increased activities of leaf antioxidant enzymes, reduced glutathione/oxidized glutathione ratio and oxidative damage to mitochondrial proteins. – Plant Physiol. Biochem. **47**: 663-670, 2009.

Vanacker, H., Sandalio, L.M., Jiménez, A., *et al.*: Roles for redox regulation in leaf senescence of pea plants grown on different sources of nitrogen nutrition. – J. Exp. Bot. **57**: 1735-1745, 2006.

van Doorn, W.G.: Is the onset of senescence in leaf cells of intact plants due to low or high sugar level? – J. Exp. Bot. **59**: 1963-1972, 2008.

Wingler, A., Masclaux-Daubresse, C., Fischer, A.M.: Sugars, senescence and ageing in plants and heterotrophic organisms. – J. Exp. Bot. **60**: 1063-1066, 2009.

Wingler, A., Purdy, S., MacLean, J.A., Pourtau, N.: The role of sugars in integrating environmental signals during the regulation of leaf senescence. – J. Exp. Bot. **57**: 391-399, 2006.

Wingler, A., Roitsch, T.: Metabolic regulation of leaf senescence: interactions of sugar signalling with biotic and abiotic stress responses. – Plant Biol. **10**: 50-62, 2008.

Yoo, S.D., Greer, D.H., Laing, W.A., McManus, M.T.: Changes in photosynthetic efficiency and carotenoid composition in leaves of white clover at different developmental stages. – Plant Physiol. Biochem. **41**: 887-893, 2003.

Yoshimura, K., Yabuta, Y., Ishikawa, T., Shigeoka, S.: Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. – Plant Physiol. **123**: 223-234, 2000.

Zimmermann, P., Zentgraf, U.: The correlation between oxidative stress and leaf senescence during plant development. – Cell. Mol. Biol. Lett. **10**: 515-534, 2005.

Zimmermann, P., Heinlein, C., Orendi, G., Zentgraf, U.: Senescence-specific regulation of catalases in *Arabidopsis thaliana* (L.) Heynh. – Plant Cell Environ. **29**: 1049-1060, 2006.