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Original Scientific Article

Changes in Photosystem II Photochemistry in Senescing Maple Leaves

Hrvoje Lepeduš,[a] Vlatka Jurković,[a] Ivna Štolfa,[b] Mirna Ćurković-Perica,[c] Hrvoje Fulgosi,[d] and Vera Cesar[b,*]

[a]*Agricultural Institute Osijek, Južno predgrađe 17, HR-31000 Osijek, Croatia*
[b]*Department of Biology, University of J. J. Strossmayer in Osijek, Trg Lj. Gaja 6, HR-31000 Osijek, Croatia*
[c]*Department of Botany, Faculty of Science, University of Zagreb, Roosevelteov trg 6, HR-10000 Zagreb, Croatia*
[d]*Ruđer Bošković Institute, Bijenička c. 54, HR-10000 Zagreb, Croatia*

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Abstract. Biochemical and functional changes in photosystem II (PSII) were investigated during natural senescence of maple (*Acer platanoides* L.) leaves. Three different types of senescing leaves were used: early senescent green (G), yellow green (YG) and yellow (Y). The main goal of this study was to investigate the dynamics of degradation of major photosynthetic proteins (D1, LHCII and Rubisco LSU) and how this would reflect on PSII photochemistry. It was revealed that D1 and LSU degrade faster than LHCII. Such differential degradation dynamics influenced PSII photochemistry. A decrease in the maximum (F_v/F_m) and effective ($\Delta F/F'_m$) quantum yields of PSII as well as a decrease in its capability for O_2 evolution were observed. Analysis of chlorophyll *a* fluorescence transient measurement (OJIP test) in senescent leaves showed striking decrease in performance index (PI_{ABS}), a measure of overall photosynthetic performance. Further, increased absorption, trapping and dissipation of excitation energy per reaction centre were found in senescent leaves. In addition, it was shown that a marked decrease in electron transport beyond the primary electron acceptor (Q_A) in Y leaves was due to reduced electron transfer ability from Q_A^- to Q_B. It can be concluded that molecular and functional modifications of a certain fraction of reaction centres from active to dissipative might be considered an important regulatory mechanism in chloroplast electron-transport chain redox poise control during natural senescence of maple leaves.

Keywords: senescence, photosystem II, protein degradation, chlorophyll fluorescence

INTRODUCTION

The natural senescence of leaves represents a genetically programmed developmental process, which ends with cell death and leaf abscission. During recent years, senescence has become appreciated as an actively ordered process that couples cell and /or organ metabolism with disassembly processes.[1] The degradation of cell structures and organelles that happens during senescence produces nutrients that are available for remobilization to other parts of the plant, such as young leaves or growing seeds. Chlorophyll degradation, seen as leaf yellowing, is the most prominent sign of senescence.[2] However, a decrease in chlorophyll content and the down-regulation of photosynthetic performance should not always be appreciated simply as a symptom, especially in cases of stress-induced aging of plant organs.[3] In addition, many nuclear transcription factors play a significant role in integration of senescence signals changing the expression of a significant number of SAG (senescence associated genes) (see Lim *et al.*[4] and refs therein). For example, it has recently been reported that leaf longevity is mediated by auxin response factor 2 (ARF2).[5]

The major regulatory component of photosynthetic membranes in chloroplasts of higher plants is photosystem II (PSII) which functions as the water-plastoquinone oxidoreductase and is composed of about 25 protein subunits.[6] Control mechanisms regulating its efficiency comprise a number of proteins which are distributed at different sites inside the chloroplast. The major proteins in the photosystems include light-harvesting proteins (LHC proteins) and reaction centre proteins such as D1 protein in the PSII reaction centre. Also, an important regulatory role in the photosynthesis process is attributed to enzymes of the Calvin-Benson cycle, especially Rubisco. Furthermore, there are numerous other proteins that take part in photosynthesis and may be designated as auxiliary proteins, such as different proteases, kinases, phosphatases, *etc*.

* Author to whom correspondence should be addressed. (E-mail: vcesarus@yahoo.com)

The available data on senescence mostly emerges from studies using different herbaceous plant species. However, less is known about this important physiological process in deciduous tree species. A recent study of free-growing aspen (*Populus tremula*) done by Keskitalo *et al.*[7] revealed that rapid transformation of leaf chloroplasts to gerontoplasts during senescence was accompanied by differentially orchestrated functional, biochemical and structural changes. They also suggested that photoperiod seemed to be the sole trigger for the onset of leaf senescence in aspen. The present investigation is part of our broader study on the regulatory mechanisms of photosynthetic performance in different maple species during natural senescence[8] and rearrangement of thylakoid membranes during seasonal course,[9] as well as their molecular organization and function in some *Arabidopsis thaliana* mutants that are deficient in important photosynthetic regulatory proteins.[10,11] It appeared that natural senescence of maple leaves was accompanied by a certain misbalance between light harvesting and electron transport processes, which was associated with increased superoxide dismutase (SOD; 1.15.1.1) activity as well as increased levels of lipid peroxidation and protein carbonyl content.[8] Also, strong photoinhibitory processes were shown to have more pronounced detrimental effects on thylakoid membrane proteins while lipids were less susceptible to degradation.[9]

Different approaches have been employed in the investigation of PSII photochemistry with the intention to probe photosynthetic performance *in vivo*.[12] Here we used two widespread techniques: the saturating pulse method and measurement of chlorophyll *a* fluorescence transient (OJIP test) that gave us the opportunity to follow the fate of excitation energy inside PSII. Among other approaches to the measurement of chlorophyll fluorescence, fluorescence imaging should be emphasized. This method has become very popular, since it allows for the identify of spatial heterogeneity of photosynthetic performance.

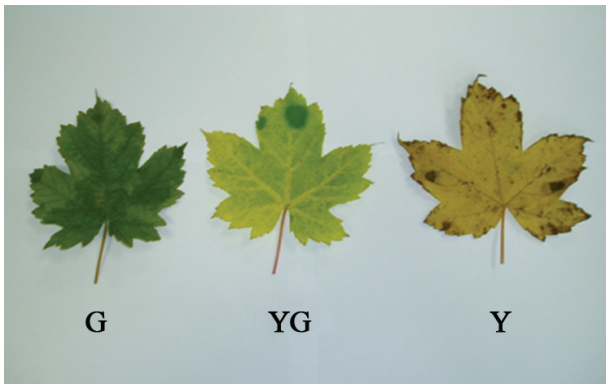

Figure 1. Three types of maple leaves used as a plant material: green (G), yellow-green (YG) and yellow (Y) leaves.

Here, we aimed to investigate to what extent some major photosynthetic proteins are degraded during leaf senescence and how this would reflect on PSII photochemistry. Also, we were interested in specific photochemical processes inside PSII such as light absorption (ABS) and trapping (TR_0), electron transport (ET_0) and energy dissipation (DI_0) per active reaction centre. Based on earlier investigation we hypothesized that a misbalance between light harvesting and electron transport processes would arise as a consequence of differential degradation dynamics of photosynthetic proteins which, in turn, cause changes in PSII photochemistry.

EXPERIMENTAL

Plant Material

Experiment 1

Three different types of senescing maple (*Acer platanoides* L.) leaves: early senescent green (G), yellow green (YG) and yellow (Y) (Figure 1) were harvested in October 2005 from three different trees cultivated in the city of Osijek (N 45° 33' 68'', E 18° 40' 51''). The approximate age of trees was 25 years. All three leaf types were found simultaneously on the same tree. A combined sample was made for each leaf type and five replicates were subsequently taken for every analysis. Sampling was done at 8:00 AM and leaves were put in a plastic bag, sealed and transported to the laboratory within half an hour. In all measurements leaf tissue was used after the main veins were removed.

Experiment 2

In order to investigate PSII photochemistry by chlorophyll *a* fluorescence transient measurement (OJIP test) leaves were randomly taken in October 2009 from five maple (*Acer platanoides* L.) trees cultivated in the city of Osijek (N 45° 32' 53'', E 18° 43' 49''). The approximate age of trees was 25 years. Leaves were categorized as described in experiment 1. Sampling was done at 8:00 AM and leaves were put in a plastic bag, sealed and transported to the laboratory within half an hour. All physiological measurements (chlorophyll fluorescence and oxygen evolution) were done in the laboratory. Twelve leaves of each category were used for measurement.

Photosynthetic Parameters Determination

For chlorophylls *a* and *b* as well as for total carotenoids determination, leaf tissue was macerated into a fine powder with liquid nitrogen using a pestle and mortar. Fine tissue powder was extracted with cold anhydrous acetone ($\rho = 0.79\ \mathrm{kg\ L^{-1}}$) and the absorbance was measured at different wavelengths ($\lambda = 661.6$ and 644.8 nm) using an Analytik Jena Specord 40 spectrophotometer.

Table 1. OJIP test parameters and expressions

OJIP TEST DATA AND PARAMETERS
F_0 – fluorescence intensity at 50 μs (O step)
F_{300} – fluorescence intensity at 300 μs
F_J – fluorescence intensity at 2 ms (J step)
F_I – fluorescence intensity at 30 ms (I step)
F_m – maximal fluorescence intensity (P step)
F_v – maximal variable fluorescence; $F_v = F_m - F_0$
V_J – variable fluorescence at J step; $V_J = (F_J - F_0) / (F_m - F_0)$
V_I – variable fluorescence at I step; $V_I = (F_I - F_0) / (F_m - F_0)$
S_m – normalized total complementary area above OJIP transient; S_m = AREA / $(F_m - F_0)$
N – turnover number; $N = S_m \times [(dV/dt)_0] / V_J$
RC / CS_0 – density of reaction centers; RC / $CS_0 = F_v / F_m \times (V_J / M_0) \times$ ABS / CS_0
ABS / RC – absorption per active reaction center; ABS / RC = $M_0 \times (1 / V_J) \times [1 / (F_v / F_m)]$
TR_0 / RC – trapping per active reaction center; TR_0 / RC = $M_0 \times (1 / V_J)$
ET_0 / RC – electron transport per active reaction center: ET_0 / RC = $M_0 \times (1 / V_J) \times (1 - V_J)$
DI_0 / RC – dissipation per active reaction center; DI_0 / RC = (ABS / RC) – (TR_0 / RC)
PI_{ABS} – performance index; PI = (RC / ABS) × (TR_0 / DI_0) × [ET_0 / (TR_0 / ET_0)]
RC / ABS – density of reaction centers on chlorophyll basis; RC / ABS = (RC / TR_0) × (TR_0 / ABS) = $[(F_J - F_0) / 4(F_{300} - F_0)] \times (F_v / F_m)$
TR_0 / DI_0 – flux ratio trapping per dissipation; TR_0 / $DI_0 = F_v / F_0$
ET_0 / (TR_0 / ET_0) – electron transport beyond Q_A^-; ET_0 / (TR_0 / ET_0) = $(F_m - F_J) / (F_J - F_0)$
F_0 – fluorescence intensity at 50 μs (O step)

The concentrations of chlorophyll *a*, chlorophyll *b* and total carotenoids were calculated according to Lichtenthaler.[13]

In vivo chlorophyll *a* fluorescence measurements in experiment 1 were performed using a pulse-amplitude-modulated photosynthesis yield analyser (Mini-PAM, Waltz). The plant material was dark-adapted for approximately 30 minutes before measurement. Minimal (F_0) and maximal (F_m) fluorescence yields were measured in the dark adapted leaves. The same parameters (F) and (F'_m) were measured upon light applications (150 and 500 μmol m^{-2} s^{-1}). The radiation was maintained until both, F and F'_m were stable. The unit μmol m^{-2} s^{-1} corresponds to the photosynthetically active photon flux density (PPFD) or the preferred term by some scientists, photon irradiance equivalent to PPFD. The maximum quantum yield of photosystem II (F_v / F_m) as well as the effective quantum yield of photosystem II ($\Delta F / F'_m$) were calculated according to Schreiber *et al.*[14]

In experiment 2, chlorophyll *a* fluorescence transient measurement (OJIP test) was done by Plant Efficiency Analyser (PEA, Hansatech). Plant materials were dark adapted for about 30 minutes before measurements. Chlorophyll fluorescence transient was induced by applying a pulse of saturating red light (peak at 650 nm, 3000 μmol m^{-2} s^{-1}). Changes in fluorescence were measured for 1s, starting from 50 μs after onset of illumination. During the first 2 ms changes were recorded every 10 μs and every 1 ms afterward. The obtained data were used in the OJIP test[15] in order to calculate several parameters of PSII photochemistry (Table 1).

Oxygen evolution was measured using a gas-phase Clark-type oxygen electrode (Hansatech). Leaf discs (2.5 cm^2) were placed in the reaction chamber and oxygen evolution was measured upon light applications (150 and 500 μmol $m^{-2}s^{-1}$, respectively). Respiration rate was measured in the dark. The temperature inside the reaction chamber was maintained at 25 °C.

SDS-PAGE and Immunodetection of D1, LHCII and Rubisco LSU

Plant material was powdered in liquid nitrogen and extracted with hot (80 °C) buffer containing Tris / HCl (c = 0.13 mol dm^{-3}, pH = 6.8), glycerol (vol. fraction, φ = 16 %), 4.6 g / 100 ml SDS and 0.59 g / 100 ml DTT. Protein concentrations were determined according to Bradford,[16] using bovine serum albumin (BSA) as a standard. Aliquots of each homogenate were mixed with corresponding volumes of de-naturing 0.065 mol dm^{-3} Tris-HCl buffer containing 6 g / 100 ml SDS, β-mercaptoethanol (vol. fraction, φ = 6 %), glycerol (vol. fraction, φ = 30 %), and 0.01 g / 100 ml bromphenol blue.[17] The extracts were boiled for 2 min. Constant protein amounts (30 μg total protein per lane) were analysed by SDS-PAGE (Mini-

gel-Twin, Biometra) and subsequent western blotting (Fastblot B43, Biometra). The resolving gel consisted of 10 % (T) polyacrylamide. Standard prestained proteins (Biorad) of known molecular weight were run in the same gel. The membranes were blocked with 10 % non-fat powdered milk solution made in PBS buffer (58 mmol dm^{-3} Na_2HPO_4, 17 mmol dm^{-3} NaH_2PO_4, 68 mmol dm^{-3} NaCl), pH = 7.4 containing Tween 20 (TPBS buffer; vol. fraction, $\varphi = 1$ %),. The membrane was incubated with rabbit monoclonal antibody raised against the pea D1, LHCII and Rubisco LSU (in a dilution of 1 : 1000 in TPBS buffer). The incubation was made overnight at 4 °C. The membrane was washed for 20 minutes three times with TPBS buffer. The secondary antibody was an alkaline phosphatase-anti-rabbit IgG (Sigma) diluted 1 : 2000. The membrane was developed with BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium). Membranes were scaned and relative intensity of the bands was measured using the Kodak1D software.

RESULTS AND DISCUSSION

Three substantially different stages of leaf senescence (green (G), yellow-green (YG) and yellow (Y) leaves) (Figure 1) were compared with respect to photochemical efficiency of PSII measured as maximum and effective quantum yields (F_v/F_m and $\Delta F/F'_m$, respectively), oxygen evolution and abundance of some crucial photosynthetic proteins (D1, Rubisco LSU and LHCII). The photochemical efficiency of PSII declined in YG and Y leaves when it was compared to G leaves (Figure 2a). The values of maximum quantum yield of PSII (F_v/F_m) were 0.77 ± 0.01, 0.69 ± 0.03 and 0.47 ± 0.08 in G, YG and Y leaves, respectively. Statistical evaluation showed no difference in effective quantum yield of PSII ($\Delta F/F'_m$) between YG and Y leaves at 500 µmol m^{-2} s^{-1}. The capacity of photosystem II (PSII) for oxygen evolution based on leaf area decreased in YG and Y leaves at both light levels applied, 150 and 500 µmol m^{-2} s^{-1}, respectively (Figure 2b). Also, Y leaves had a reduced PSII capacity for oxygen evolution compared to YG leaves, in spite of lacking significance in $\Delta F/F'_m$ at 500 µmol m^{-2} s^{-1} between them. Changes in relative abundance of D1, LSU and LHCII proteins are shown in immunoblots (Figure 3). The largest decrease in relative abundance was observed for D1 protein: 53 and 29 % in YG and Y leaves, respectively, compared to G leaves. Similar dynamics were shown for Rubisco LSU, with relative abundance of 65 and 40 % in YG and Y leaves, respectively. The smallest loss was observed for LHCII: values were 94 % in YG and 64 % in Y leaves. In a recent review done by Martinez *et al.*[18] they discuss pathways of chloroplast protein degradation and the proteases involved in those processes. While LCHII proteins are degraded by the FtsH6 protease, it seems that at least two proteases, DegP and FtsH, are cooperatively involved in degradation of D1 protein. Another chloroplast protease, CND41, is likely involved in Rubisco degradation. However, there is some evidence that Rubisco cannot be completely degraded inside chloroplasts,[19] but is subject to an extra-plastidic degradation pathway which includes the so called Rubisco-containing bodies (RCB). This process relies on vesicular export of stromal components and it is most likely enhanced during senescence-associated deterioration of chloroplasts.

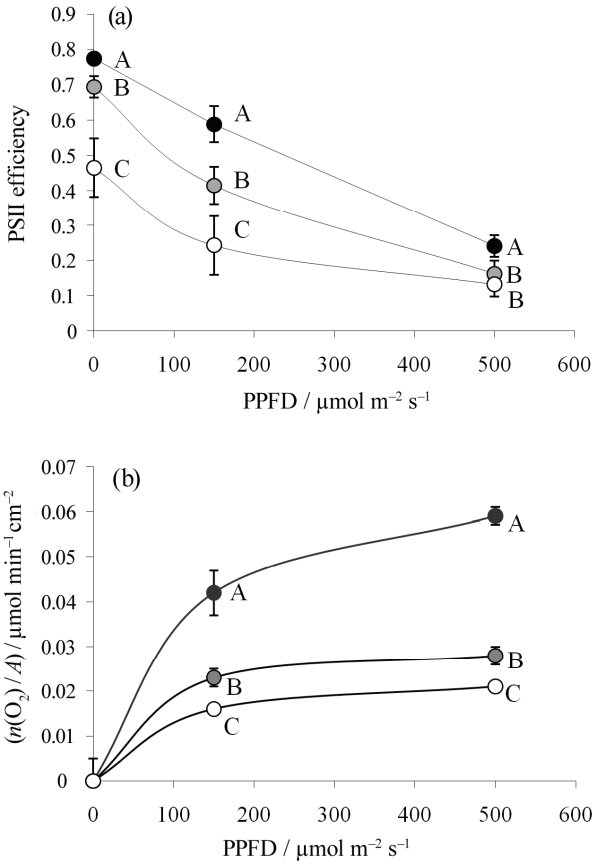

Figure 2. The aritmetic mean values and standard deviations of photosystem II efficiency (given as maximum quantum yield (F_v/F_m) at 0 PPFD and effective quantum yields ($\Delta F/F'_m$) at 150 and 500 PPFD's) (a) and of PSII capacity for oxygen evolution (b) in green (G, black circles), yellow-green (YG, grey circles) and yellow (Y, white circles) maple (*Acer platanoides* L.) leaves. Significant differences between G, YG and Y leaves were designated by different letters (A, B, C) placed near the circles. The statistical evluation of each light amount applied (PPFD) was done separately. Symbols: $n(O_2)$ - oxygen amount; A - area of leaf disc; PPFD - photosynthetically active photon flux density.

Although the maximum quantum yield of PSII (F_v/F_m) in YG leaves dropped below 0.75 (Figure 2a), which is considered to be a boundary value for fully

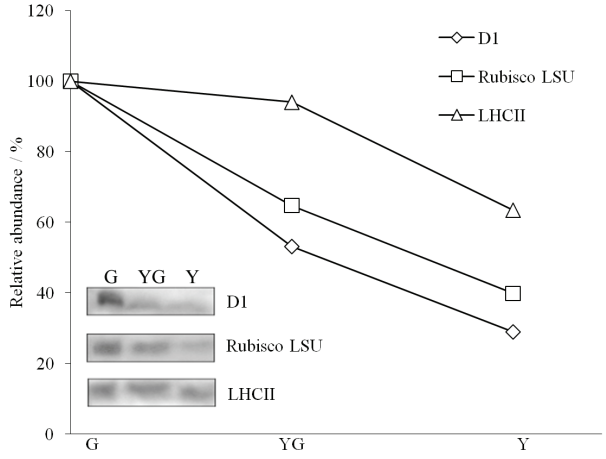

Figure 3. Relative abundance of D1, Rubisco LSU and LHCII proteins on imunoblots in green (G), yellow-green (YG) and yellow (Y) leaves of maple (*Acer platanoides* L.). The abundance of each protein in G leaves was taken as 100 %.

functional PSII,[20] it decreased less than its capacity for O_2 evolution at 500 μmol m^{-2} s^{-1} (Figure 2b). The relatively slow degradation of LHCII proteins with respect to D1 protein (Figure 3) would correlate with the observed better stability of PSII light harvesting than its oxygen evolving capability (Figure 2). Humbeck and Krupinska[21] reported that decreased oxygen evolution at saturating light intensity (2050 μmol m^{-2} s^{-1}), together with a decline in the level of D_1 protein, was noticed earlier than a decline of the F_v/F_m value and chlorophyll content in senescing barley (*Hordeum vulgare* L.). Also, the F_v/F_m ratio was shown to remian high and constant until very late senescence in aspen.[7] The major factor responsible for reduced overall photosynthetic capacity during senescence is a decline in Rubisco content.[22] It appeared that the small Rubisco subunit (SSU) was more stable that the large subunit (LSU) during the natural senescence of barley (*Hordeum vulgare* L. cv. Carina).[21] Our previous study[8] revealed that a decrease in relative electron transport rate in YG and Y maple leaves correlated with a fast and pronounced degradation of *rbc*L gene (a gene encoding the Rubisco LSU). Since *rbc*L is present in chloroplast DNA as a single copy, its abundance could be used to evaluate the abundance of total chloroplast DNA. Chloroplast DNA degradation was shown to be one of the first events to occur with the onset of leaf senescence,[23] so a reduced amount of *rbc*L could account for a decrease in template availability which in turn would cause its decreased transcriptional capability and consequently reduced biosynthesis of Rubisco LSU. Besides suppressed biosynthesis, a decrease in Rubisco abundance during senescence is likely to arise as a consequence of its degradation, as well.

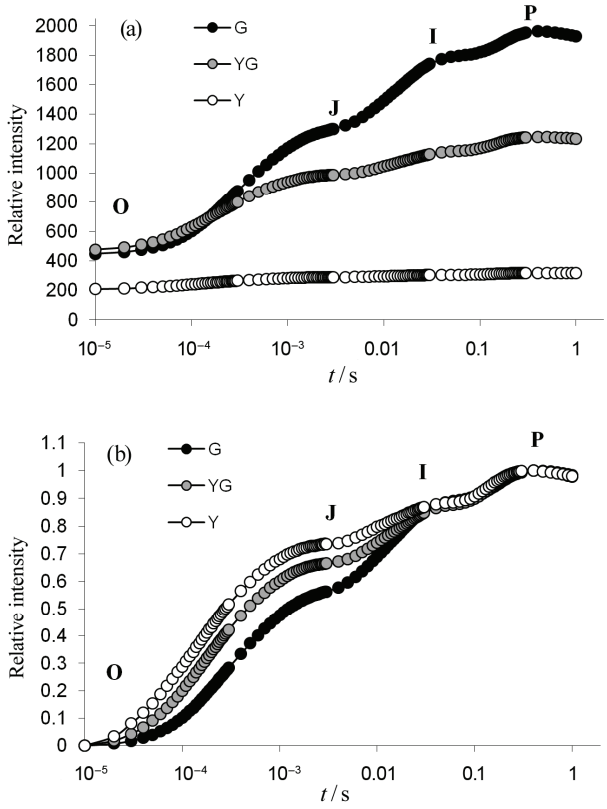

Figure 4. OJIP chlorophyll a fluorescence transients without normalization (a) and O-P normalized (b) in green (G; black circles), yellow-green (YG; grey circles) and yellow (Y; white circles) leaves of maple (*Acer platanoides* L.).

In order to dissect photochemical processes during natural senescence of maple leaves the measurement of chlorophyll a fluorescence transient, the so called OJIP test, was applied (experiment 2). Results of some OJIP parameters are shown in Tables 2 and 3 as well as in Figure 4. Polyphasic transient of chlorophyll a fluorescence in studied plants is shown in Figure 4a. G leaves revealed a characteristic OJIP curve shape, while YG and Y leaves had a substantially changed curve shape. YG leaves had the same level of F0 as G leaves but a significantly decreased Fm value, while Y leaves were characterized by a decrease in both F0 and Fm values (Table 2). The decrease in F0 was suggested to be an indication for energy dissipation in minor antenna.[24] O-P normalized curves revealed differences at O-J step between investigated senescent stages. This resulted in a substantial increase in variable fluorescence measured at 2 ms (V_J). No changes were observed in relative variable fluorescence at 30 ms (V_I) during leaf yellowing (Table. 2). It is the prevailing opinion that the O-J step corresponds to the reduction of primary electron acceptor (Q_A) and to the redox state of Mn-cluster in oxygen evolving complex (OEC). So, the observed decrease of PSII capacity for oxygen evolution based on leaf area

Table 2. The aritmetic mean values of chlorophyll *a* fluorescence parameters in green (G), yellow-green (YG) and yellow (Y) maple leaves

	G	YG	Y
F_0	413.17^A	492.83^A	201.33^B
F_m	1941.89^A	1306.74^B	254.56^C
V_J	0.55^C	0.69^B	0.82^A
V_I	0.85^A	0.85^A	0.86^A
S_m	25.00^A	22.89^A	8.74^B
N	53.44^A	61.26^A	25.71^B
RC/CS_0	152.16^A	112.20^B	13.05^C

All parameters are expressed in relative units. Significant differences between G, YG and Y leaves were designated by different letters (A, B, C). The statistical evaluation of each parameter was done separately.

Table 3. The aritmetic mean values of performace index (PI_{ABS}) and its components in green (G), yellow-green (YG) and yellow (Y) maple leaves

	G	YG	Y
PI_{ABS}	1.40^A	0.19^B	0.01^C
RC/ABS	0.37^A	0.23^B	0.05^C
TR_0/DI_0	3.70^A	1.62^B	0.18^C
$ET_0/(TR_0-ET_0)$	0.83^A	0.46^B	0.24^C

All parameters are expressed in relative units. Significant differences between G, YG and Y leaves were designated by different letters (A, B, C). The statistical evaluation of each parameter was done separately.

Table 4. The aritmetic mean values of specific fluxes or specific activities in green (G), yellow-green (YG) and yellow (Y) maple leaves

	G	YG	Y
ABS/RC	2.72^B	4.53^B	27.43^A
TR_0/RC	2.14^B	2.68^A	2.73^A
ET_0/RC	0.97^A	0.83^A	0.47^B
DI_0/RC	0.58^B	1.85^B	24.69^A

All parameters are expressed in relative units. Significant differences between G, YG and Y leaves were designated by different letters (A, B, C). The statistical evaluation of each parameter was done separately.

that we observed in experiment 1 (Figure 2b) might contribute to the increase in variable fluorescence at 2 ms (V_J) during leaf yellowing. The accumulation of Q_A^- was evident in other two important parameters: the turnover number (N) and normalized total complementary area above OJIP transient (S_m). N represents the number of Q_A reduction events between F_0 and F_m, while Sm corresponds to the energy needed to close all reaction centres.[25] Both, an N and S_m values appeared to decrease 2 and 3 times respectively, in Y leaves (Table 2), which indicated reduced transport from Q_A^- to Q_B.

The performance index (PI_{ABS}), the parameter that describes overall photosynthetic performance, appeared to be about 84 and 99 % decreased in YG and Y leaves, respectively (Table 3). PI_{ABS} was established by Strasser *et al.*[25] and it combines several parameters that describe the three main functional characteristics of the PSII reaction center, namely RC/ABS (density of reaction centres on chlorophyll basis), TR_0/DI_0 (ratio of trapping and dissipation fluxes) and $ET_0/(TR_0-ET_0)$ (efficiency of the conversion of excitation energy to electron transport). A gradual decrease in all of these parameters was observed during leaf yellowing (Table 3). In YG leaves the values of RC/ABS, TR_0/DI_0 and $ET_0/(TR_0-ET_0)$ were 37.84, 56.22 and 44.58 % lower than in G leaves, respectively. In Y leaves the most pronounced decrease was observed for TR_0/DI_0 (95.14 %), while RC/ABS and $ET_0/(TR_0-ET_0)$ were 86.49 and 71.08 lower in comparison to G leaves, respectively.

Further, the main photochemical processes (absorption (ABS), trapping (TR_0), electron transport (ET_0) and dissipation (DI_0)) per active reaction centre were analysed. The values of ABS/RC and DI_0/RC were not significantly different between G and YG leaves, while their multi-folded increase was observed in Y leaves (Table 4). The values of TR_0/RC increased already in YG leaves and remained unchanged in Y leaves, while ET_0/RC remained unchanged in YG leaves and then significantly decreased in Y leaves (Table 4). As reported by Okabe *et al.*[26] lower chlorophyll content corresponds to a smaller photosynthetic unit which is considered more efficient in the conversion of light energy. This would explain the increase in ABS/RC and TR_0/RC we observed here. Surprisingly, ET_0/RC remained at the same level in YG leaves. This would mean that the reduced capability of electron-transport chain components beyond the primary electron acceptor (Q_A) was maintained with the onset of senescence. Lu and Zhang[27] demonstrated that a decrease in photosynthetic efficiency during leaf senescence might be the consequence of an increase in the fraction of reaction centres that are unable to reduce plastoquinone (Q_B). Guided by this observation we analyzed the density of active reaction centres (RC/CS_0). It appeared that RC/CS_0 (Table 2) decreased during the leaf senescence process, with a drastic decrease in Y leaves. Further, the values of DI_0/RC were shown to increase to a great extent in Y leaves. This would correlate by the fact that the increase in ABS/RC was accompanied with the modification of a certain fraction of reaction centres in a way that their capability for dissipation of excess light energy absorbed by PSII was increased.[28] The increase

in DI_0/RC values along with leaves yellowing pointed to conformational changes in PSII that should be seen as a protection event in senescent leaves where the overall photosynthetic performance was severely impaired while the light-harvesting process was still functioning relatively well.

In conclusion, this study revealed functional and molecular changes in PSII that took place during natural senescence of maple leaves. Since all three considered leaf stages might be found simultaneously on the same tree, the present results might indicate dynamics in photochemical processes. Different dynamics in the degradation of the major photosynthetic proteins (LHCII, D1 and Rubisco LSU) caused functional disturbance in PSII photochemistry seen as a decrease in its photochemical efficiency and capability for oxygen production. A previously described misbalance between light harvesting and PSII driven relative electron transport rate in senescent maple leaves[8] is shown here to be primarily associated with an increased absorption, trapping and dissipation of excitation energy and later on combined with decreased electron transport beyond primary electron acceptor (Q_A). The major cause for decreased electron transport appears to be the reduced ability of electron transfer from Q_A^- to Q_B. Such molecular and functional modifications of a certain fraction of reaction centres from active to dissipative ones might be considered an important regulatory mechanism of chloroplast degradation during natural senescence of maple leaves.

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REFERENCES

1. L. D. Noodén, J. J. Guiamét, and I. John, *Physiol. Plant.* **101** (1997) 746–753.
2. P. Matile, S. Hortensteiner, H. Thomas, and B. Krautler, *Plant Physiol.* **112** (1996) 1403–1409.
3. H. Lepeduš, M. Viljevac, V. Cesar, and N. Ljubešić, *Russ. J. Plant Physiol.* **52** (2005) 165–170.
4. P. O. Lim, H. J. Kim, and H. G. Nam, *Annu. Rev. Plant Biol.* **58** (2007) 115–136.
5. P. O. Lim, I. C. Lee, J. Kim, H. J. Kim, J. S. Ryu, H. R. Woo, and H. G. Nam, *J. Exp. Bot.*, Advance Access published on February 17, 2010, doi:10.1093/jxb/erq010
6. J. Barber, J. Nield, E. P. Morris, D. Zheleva, and B. Hankamer, *Physiol. Plant.* **100** (1997) 817–827.
7. J. Keskitalo, G. Bergquist, P. Gardeström, and S. Jansson, *Plant Physiol.* **139** (2005) 1635–1548.
8. H. Lepeduš, I. Štolfa, S. Radić, M. Ćurković Perica, B. Pevalek-Kozlina, and V. Cesar, *Croat. Chem. Acta* **81** (2008) 97–103.
9. H. Fulgosi, S. Jurić, H. Lepeduš, K. Hazler-Pilepić, T. Prebeg, and N. Ljubešić, *Croat. Chem. Acta* **81** (2008) 97–103.
10. S. Jurić, K. Hazler-Pilepić, A. Tomašić, H. Lepeduš, B. Jeličić, S. Puthiyaveetil, T. Bionda, L. Vojta, J. F. Allen, E. Schleiff, and H. Fulgosi, *Plant J.* (2009) *in press*.
11. H. Lepeduš, B. A. Tomašić, S. Jurić, Z. Katanić, V. Cesar, and H. Fulgosi, *Food Technol. Biotechnol.* **47** (2009) 275–280.
12. N. Baker, *Annu. Rev. Plant Biol.* **59** (2008) 89–113.
13. H. K. Lichtenthaler, *Methods Enzymol.* **148** (1987) 350–382.
14. U. Schreiber, W. Bilger, and C. Neubauer, *Ecol. Stud.* **100** (1994) 49–70.
15. R. J. Strasser, A. Srivastava, and M. Tsimilli-Michael: *Analysis of the chlorophyll a fluorescence transient.* in: Papageorgiou G and Govindjee (Eds.), *Chlorophyll Fluorescence a Signature of Photosynthesis: Advances in Photosynthesis and Respiration, Vol. 19*, Kluwer Academic Publishers, The Netherlands, 2004, pp. 321–362.
16. M. M. Bradford, *Anal. Biochem.* **72** (1976) 248–254.
17. U. K. Laemlli, *Nature* **227** (1970) 680–685.
18. D. E. Martinez, M. L. Costa, and J. J. Guiamet, *Plant Biol.* **10** (2008) 15–22.
19. P. L. Gregersen, P. B. Holm, and K. Krupinska, *Plant Biol.* **10** (2008) 37–49.
20. H. R. Bolhàr-Nordenkampf, S. P. Long, N. R. Baker, G. Öuquist, U. Schreiber, and E. G. Lechner, *Funct. Ecol.* **3** (1989) 497–514.
21. K. Humbeck and K. Krupinska, *J. Photochem. Photobiol.* **36** (1996) 321–326.
22. K. Krupinska and K. Humbeck, *Photosynthesis and chlorophyll breakdown* in: L. D. Noodén (Ed.), *Plant Cell Death Processes*, Elsevier Academic Press, San Diego, 2004, pp. 169–187.
23. N. Inada, A. Sakai, H. Kuroiwa, and T. Kuroiwa, *Planta* **206** (1998) 585–597.
24. A. M. Gilmore, T. L. Hazlett, P. G. Debrunner, and Govindjee, *Photochem. Photobiol.* **64** (1996) 552–563.
25. R. J. Strasser, A. Srivastava, and M. Tsimilli-Michael: *The fluorescent transient as a tool to characterise and screen photosynthetic samples.* in: M. Yunus, U. Pathre, P. Mohanty (Eds.), *Probing Photosynthesis: Mechanisms, Regulation and Adaptation,* Taylor and Francis, London, 2000, pp. 445–483.
26. K. Okabe, G. S. Schmid, and J. Straub, *Plant Physiol.* **60** (1977) 150–156.
27. C. Lu and J. Zhang, *J. Exp. Bot.* **49** (1998) 1671–1679
28. M. Tsimilli-Michael, P. Eggenberg, B. Biro, K. Köves-Pechy, I. Vörös, and R. J. Strasser, *Applied Soil Ecology* **15** (2000) 169–182.

SAŽETAK

Promjene u fotokemiji fotosustava II senescentnih listova javora

Hrvoje Lepeduš,[a] Vlatka Jurković,[a] Ivna Štolfa,[b] Mirna Ćurković-Perica,[c] Hrvoje Fulgosi[d] i Vera Cesar[b]

[a]*Poljoprivredni institut Osijek, Južno predgrađe 17, HR-31000 Osijek, Hrvatska*
[b]*Odjel za biologiju, Sveučilište J. J. Strossmayera u Osijeku, Trg Lj. Gaja 6, HR-31000 Osijek, Hrvatska*
[c]*Botanički zavod, Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu, Roosevelteov trg 6, HR-10000 Zagreb, Hrvatska*
[d]*Institut Ruđer Bošković, Bijenička c. 54, HR-10000 Zagreb, Hrvatska*

Biokemijske i funkcionalne promjene fotosustava II (PSII) su istražene tijekom prirodne senescencije listova javora (*Acer platanoides* L.). Proučavana su tri različita tipa senescentnih listova: zeleni (G), žutozeleni (YG) i žuti (Y). Glavni cilj ovog istraživanja je istražiti dinamiku razgradnje glavnih fotosintetskih proteina (D1, LHCII i Rubisco LSU) i kako će se to odraziti na fotokemiju fotosustava II. Otkriveno je da se D1 i LSU razgrađuju brže od LHCII. Takva različita dinamika razgradnje utječe na fotokemiju fotosustava II. Primjećeno je smanjenje maksimalnog (F_v/F_m) i efektivnog ($\Delta F/F'_m$) prinosa kvanta fotosustava II kao i njegove sposobnosti produkcije kisika. Analizom mjerenja porasta fluorescencije klorofila *a* (OJIP test) u senescentnim listovima utvrđeno je izraženo smanjenje indeksa učinkovitosti (PI_{ABS}), koji predstavlja mjeru ukupne fotosintetske učinkovitosti. Nadalje, utvrđena je povećana apsorpcija, hvatanje i rasipanje pobudne energije po reakcijskom centru u senescentnim listovima. Također, utvrđeno je da značajno smanjenje elektronskog transporta iza primarnog akceptora elektrona (Q_A) u žutim listovima potječe od njihove smanjene sposobnosti elektronskog transporta od Q_A^- do Q_B. Iz ovoga se može zaključiti da se molekularne i funkcionalne promjene određenog udjela reakcijskih centara iz aktivnih u rasipajuće mogu smatrati važnim regulacijskim mehanizmom kontrole redoks stanja lanca transporta elektrona kloroplasta tijekom prirodne senescencije listova javora.