Photoinhibition and loss of photosystem II reaction centre proteins during senescence of soybean leaves. Enhancement of photoinhibition by the ‘stay-green’ mutation cytG

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Received 7 May 2001; revised 7 January 2002

The ‘stay-green’ mutation cytG in soybean (Glycine max) partially inhibits the degradation of the light-harvesting complex II (LHClI) and the associated chlorophyll during monocarpic senescence. cytG did not alter the breakdown of the cytochrome b6/f complex, thylakoid ATP synthase or components of Photosystem I. In contrast, cytG accelerated the loss of oxygen evolution activity and PSII reaction-centre proteins. These data suggest that LHClI and other thylakoid components are degraded by separate pathways. In leaves induced to senesce by darkness, cytG inhibited the breakdown of LHClI and chlorophyll, but it did not enhance the loss of PSII-core components, indicating that the accelerated degradation of PSII reaction centre proteins in cytG was light dependent. Illumination of mature and senescent leaves of wild-type soybean in the presence of an inhibitor (lincomycin) of chloroplast protein synthesis revealed that senescence per se did not affect the rate of photoinhibition in leaves. Likewise, mature leaves of the cytG mutant did not show more photoinhibition than wild-type leaves. However, in senescent cytG leaves, photoinhibition proceeded more rapidly than in the wild-type. We conclude that the cytG mutation enhances photoinhibition in senescing leaves, and photoinhibition causes the rapid loss of PSII reaction-centre proteins during senescence in cytG.

Introduction

The breakdown of thylakoid membranes and stromal proteins of the chloroplasts during senescence causes the photosynthetic activity of senescing leaves to decline to very low levels (Gepstein 1988, Noodeén 1988). The dismantling of thylakoid membranes is characterized by chlorophyll (Chl) degradation and loss of photosynthetic electron transport activity (Holloway et al. 1983, Gepstein 1988). Initially, the decline in photosynthetic electron transport during senescence is due to the loss of the cyt b6/f complex, which is followed by a decrease in Photosystem I (PSI) and Photosystem II (PSII) activities and, later, ATP synthase (Holloway et al. 1983, Gepstein 1988). The decrease in the activity of the photosynthetic light reactions in the thylakoids correlates with the degradation of the protein components of each complex, but the mechanism and regulation of thylakoid protein catabolism are poorly understood.

The decline of photosystem activity during senescence may be caused in part by photodamage. Susceptibility to photodamage increases in excised rye leaves during low-light-induced senescence (Kar et al. 1993). Photoprotective mechanisms might decline during senescence and, later, ATP synthase (Holloway et al. 1983, Gepstein 1988). The decrease in the activity of the photosynthetic light reactions in the thylakoids correlates with the degradation of the protein components of each complex, but the mechanism and regulation of thylakoid protein catabolism are poorly understood.

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ence, since senescent leaves may contain lower levels of zeaxanthin and other xanthophyll cycle carotenoids (Afitlhile et al. 1993). Senescence-related changes in leaf orientation of the flag leaf in rice may also increase light exposure at midday, thereby accelerating photoinhibition under field conditions (Murchie et al. 1999). These connections between senescence and photodamage prompted us to study the susceptibility of senescing leaves to photoinhibition of PSI.

Several mutations interfere with Chl degradation and cause the retention of Chl in senescent leaves of various species (Thomas and Smart 1993, Bachmann et al. 1994, Noorden and Guiamet 1996). Chl retention in these 'stay-green' mutants correlates with the preservation of Chl-binding proteins and certain other protein components of the thylakoid membranes. In soybean, the maternally inherited mutation cytG (Bernard and Weiss 1973) blocks the degradation of the light-harvesting complex of PSI and its associated pigments (Guiamet et al. 1991, Chao et al. 1995). cytG appears to act on Chl degradation rather than synthesis (Canfield et al. 1995) suggesting that at least one non-nuclear gene is involved in thylakoid membrane disassembly during senescence. The retention of excess LHClI in senescent leaves of cytG may increase the effective size of light-harvesting antenna in PSI with no concomitant increase in light energy utilization for CO2 fixation in the mutant (Guiamet et al. 1990). This might favour photodamage of PSI reaction centres in senescent leaves of the mutant.

The main objective of this work was to determine if photodamage increases during senescence of soybean leaves, and if there are differences in rate of photoinhibition between wild-type and cytG leaves. Additionally, we report on the effects of cytG on the degradation of thylakoid proteins besides LHClI in senescing leaves. In this process, we have also characterized the normal breakdown of the photosynthetic apparatus.

Materials and methods

Plant material and growing conditions

Soybean (Glycine max [L.] Merr.) seeds of wild-type cv. Clark, and a near-isogenic line carrying the maternally inherited mutation cytG were obtained from Dr R. Bernard, Department of Agronomy, University of Illinois, USA. Soybeans were cultured as described previously (Guiamet et al. 1990) in pots with soil, watered daily and fertilized regularly with N-P-K (20:20:20, w/w). Plants were initially grown under long days (16 h, 350 μmol m−2 s−1 of photosynthetically active radiation) at 27/21°C day/night temperature. After 2–3 weeks (unifoliate leaves fully expanded), they were shifted to short days (10 h, 27/21°C day/night temperature) for the duration of the experiment. In some experiments, leaf senescence was induced by darkness. These plants were grown under long days for 3 weeks until the unifoliate leaves were completely expanded and then transferred to continuous darkness at 25°C. Thereafter, all manipulations (e.g. watering, sampling) were performed under a dim (<1 μmol m−2 s−1) green safelight.

Sampling

For studies with plants undergoing monocarpic senescence, samples of the uppermost fully expanded leaf at flowering were taken at different stages of development, from leaf maturity to abscission. Measurements involving dark-induced senescence were performed on the unifoliate leaves. Since there were small differences in the timing of the degradation of thylakoid proteins between different batches of soybeans, we have indicated the changes in Chl content in wild-type for each particular experiment shown.

Thylakoid isolation

Thylakoids from mature and senescent leaves of both wild-type leaves (cv. Clark) and from cytG leaves were isolated using a method modified from Camm and Green (1980). Leaves were harvested and homogenized in chilled buffer (50 mM HEPES, pH 7.5, 0.4 M sucrose, 2 mM MgCl2, 1 mM EDTA, 0.2% w/v bovine serum albumin) with a Waring blender. The homogenate was filtered through 4 layers of cheesecloth and a 10-μm pore nylon mesh, and centrifuged at 3000 g for 5 min at 4°C. The resultant pellet was resuspended in wash buffer (50 mM HEPES, pH 7.5, 10 mM NaCl) and centrifuged again as above. This second pellet was resuspended in a small volume of wash buffer containing glycerol (10% v/v) and stored at −70°C until further analysis. For measurements of senescence-induced changes in photosystem II activity, thylakoids were prepared essentially as described above, except that the homogenization buffer contained 0.4 M NaCl instead of sucrose, the wash buffer was 50 mM HEPES, pH 7.5, 0.25 M NaCl, 5 mM MgCl2, and thylakoids were resuspended and frozen in 50 mM HEPES, pH 7.5, 0.4 M sucrose, 15 mM NaCl, and 0.2% w/v bovine serum albumin. For photoinhibition experiments, thylakoids were isolated according to Patsikká et al. (1998).

Photoinhibition experiments

For in vivo photoinhibition measurements, mature and senescing leaves of wild-type cv. Clark and cytG were incubated overnight under very dim light with their petals immersed in a solution of lincomycin (1 g l−1). The leaves were then illuminated at a PPF D of 560 ± 40 μmol m−2s−1 at 30°C. After 0, 2 and 4 h of illumination, thylakoids were isolated and oxygen evolution activity was measured immediately. The rate constant of photoinhibition was calculated from first-order fits (Tyyystjärvi and Aro 1996) of the loss of oxygen evolution activity. An aliquot of each thylakoid sample was used for determination of the D1 protein content by Western blotting. In vitro photoinhibition was measured in thylakoids
isolated from both attached, normally senescing leaves at 20, 45 and 55 DAF, and from detached, dark-senescent leaves. To obtain the dark senescent material for in vitro photoinhibition, leaves of cv. Clark and cytG were allowed to senesce in darkness for 15 days until the Chl concentration of the wild-type leaves dropped to 26% and that of cytG to 49% of the initial value. Thylakoids isolated from control and senescent leaves were suspended in 50 mM HEPES (pH 7.6), 0.3 M sorbitol, 20 mM NaCl and 5 mM MgCl₂ at a Chl concentration of 50 μg ml⁻¹ and illuminated at 20°C with a heat-filtered 250 W projector lamp delivering 1000 μmol m⁻² s⁻¹ of photosynthetically active radiation at the level of the thylakoid suspension. A liquids of thylakoids were taken at regular intervals for PSII activity measurements as described below.

Protein electrophoresis

For SDS-PAGE, thylakoids were solubilized with Laemmli’s solubilization buffer (Laemmli 1970). Each well was loaded either on the basis of Chl content or leaf area, as indicated in each figure. Proteins were separated in either 13% (w/v) or 11–16% (w/v) acrylamide concentration gradient gels.

Western blot analysis

Proteins separated by SDS-PAGE were electro-transferred to nitrocellulose membranes at 70 V for 3 h, or at 200 mA overnight, in a buffer containing 48 mM Tris, 39 mM glycine, 0.037% w/v SDS and 20% v/v methanol. Nitrocellulose membranes were blocked, incubated with antibodies and developed as described earlier (Guiamet and Giannibelli 1996) or with a chemiluminescent detection system based on horseradish peroxidase.

Heme peroxidase staining of cytochrome f

Mildly denaturing electrophoresis was performed as described by Camm and Green (1989). Cytochrome f was detected in mildly denaturing gels by staining for the peroxidase activity of its heme group as described in Thomas et al. (1976). After soaking the gels for 2 h in a solution of 1.9 mM tetramethyl benzidine (TM BZ) in 30% (v/v) methanol, 175 mM Na-acetate buffer (pH 5), the reaction was initiated by adding hydrogen peroxide to a final concentration of 30 mM. Band intensity was quantified by laser densitometry at 600 nm. Since staining intensity was linear for loads between 5 and 15 μg Chl and up to 15 min of incubation, gels were routinely loaded with 7.5–10 μg of Chl and developed for 15 min. All operations were performed in darkness or under subdued light.

Northern blot analysis

Leaves were harvested at midday and RNA was extracted as in John et al. (1995). RNA was electrophoresed in denaturing gels containing formaldehyde under standard conditions (Sambrook et al. 1989) and transferred overnight to nylon membranes (Gene Screen, Du Pont, USA) by capillary transfer. Blots were UV crosslinked at 1200 J. Blotted RNA was pre-hybridized at 42°C for 4 h in formamide (50% v/v), 6× SSC (1% v/v), 1 M NaCl, dextran sulphate (10% w/v) and denatured salmon sperm DNA (0.1 mg ml⁻¹). Radiolabelled probes were added to this solution and hybridized overnight at 42°C. Membranes were washed twice for 5 min each with 2× SSC (1× SSC is 0.15 M NaCl, 0.15 M sodium citrate, pH 7.0) plus SDS (0.1% w/v) and twice for 15 min each with 0.1× SSC plus SDS (0.1% w/v).

Plasmids containing the soybean 18S rRNA gene and the barley psbA gene were digested with EcoRI/BamHI (18S rRNA) and EcoRI/HincII (psbA), and the inserts were radiolabelled with a random primed labelling kit (Boehringer Mannheim) following the manufacturer’s instructions. Blots were hybridized first with the psbA probe, then the probe was stripped and the same blot was hybridized with the soybean 18S rRNA probe. Levels of the psbA and 18S rRNA mRNAs were quantified with a Bio-Rad Molecular Imager System using a BI Imager Screen (Bio-Rad Laboratories, Hercules, CA, USA).

D1 synthesis in vivo

Eight leaf disks (12-mm diameter) were incubated for 1 h at 560 ± 40 μmol m⁻² s⁻¹ and 30°C in 4 ml of distilled water containing 0.37 MBq of ³⁵S-methionine (37.0 M Bq μmol⁻¹). After this feeding period, thylakoids were isolated, polypeptides were solubilized with Laemmli’s solubilization buffer (Laemmli 1970), separated by SDS-PAGE, and transferred to a nitrocellulose membrane as described above, and autoradiograms were developed using Kodak X-OMAT AR film (SIGMA Chemical Co., St. Louis, MO, USA).

Photosystem II activity

For photoinhibition experiments, light-saturated oxygen-evolution activity of PSII was measured polarographically from isolated thylakoids as described in Pätsikkä et al. (1998). 2,6-dichloro-p-benzoquinone was used as electron acceptor. Changes in PSII activity during senescence were measured according to Kim et al. (1994).

Chlorophyll a fluorescence measurements

The ratio of variable to maximum Chl a fluorescence (Fv/Fm), and the photochemical (qP) and non-photochemical (qN) components of fluorescence quenching were measured in attached leaves with an OS-500 M oculated Fluorometer (Opti-Sciences Inc., Tyngsboro, MA, USA). Leaves were dark adapted for 15 min before measuring F₀ (fluorescence with all PSII centres open) and Fm (fluorescence under a saturating flash of light).
For the analysis of the quenching parameters, leaves were illuminated continuously with an actinic light source (350 μmol m⁻² s⁻¹), and saturating flashes of light (10 mmol m⁻² s⁻¹, duration 0.8 s) were given every 20 s. At the end of each run, the quenched F₀ level (Fₒ) was measured with the actinic light off and illuminating the leaf with a far-red source. The coefficients qᵦ and qₑ were calculated according to van Kooten and Snel 1990).

Chlorophyll content

Leaf disks were extracted overnight with dimethyl formamide and Chl content was calculated from the absorbances at 647 and 664.5 nm using the equations of Inskoop and Bloom (1985). The chlorophyll content of thylakoids was measured with the method of Porra et al. (1989).

Results

Senescence-associated changes in thylakoid membrane composition

In wild-type leaves, the degradation of most thylakoid proteins on a leaf area basis occurred in parallel with the loss of Chl. Western blots showed that the amounts of light-harvesting complexes of PSI (LHCII) and PSI (LHCI) as well as PSI core proteins D1, CP47 and the PSII inner antenna protein CP26 declined at roughly similar rates after 26 days after flowering (DAF) (maximum Chl content, Fig. 1, lane 2) and reached non-detectable levels in abscising leaves of the wild-type, which contained less than 5% of the peak Chl content. Since the anti-CP26 antibody used in this work also recognizes CP29 in some species (Falbel and Staehelin 1992) the band labelled CP26 in Fig. 1 is actually a doublet of a CP26 band and slower migrating CP 29 band. The loss of the β-subunit of the ATP synthase was less rapid than other thylakoid proteins, and abscising leaves still retained significant amounts of this protein (Fig. 1). Immunoblots probed against cyt f and the Rieske Fe-S protein of the cyt b6/f complex, and quantification of cyt f in gels stained for the peroxidase activity of its heme group, show that in wild-type leaves the amounts of these proteins started to decline at 18 DAF (Fig. 2) before Chl content reached its maximum at 26 DAF (data not shown).

CytG retarded the degradation of Chl, particularly the degradation of Chl b, during monocarpic senescence causing a decline in the Chl a/b ratio (data not shown). At abscission, the cytG leaves retained about 30% of their Chl. Overall, this pattern is very similar to that observed earlier (Guiamet et al. 1991). Immunoblots confirmed earlier findings based on Coomassie-blue staining that senescing leaves of the cytG mutant retain the two main LHCII polypeptides. The cytG mutation did not cause retention of LHCI, the β-subunit of ATP synthase or the cyt b6/f complex (Figs 1 and 2). By contrast to LHCII proteins, the PSII core proteins D1 and CP47 as well as PSII inner antenna proteins CP26 and CP29 disappeared faster in cytG than in wild-type (Fig. 1).

Photosystem II activity and Fᵥ/Fₘ

PSII activity measurements confirmed the accelerated degradation of PSII reaction centre proteins in senescing leaves of the cytG mutant (Fig 3A). Likewise, the ratio of variable to maximum fluorescence (Fᵥ/Fₘ) of attached leaves (Fig 3B) remained fairly constant during senescence in the wild-type, except for a late decline of about 25%, but Fᵥ/Fₘ decreased substantially to very low values during senescence in leaves of cytG, suggesting accelerated loss of photochemically active PSII centres.

Degradation of PSII and thylakoid membrane proteins in darkness

The accelerated degradation of PSII proteins and decline of Fᵥ/Fₘ during senescence in cytG might be due to increased photodamage to PSII. Since this photodamage would not occur during senescence in darkness, the degradation of thylakoid proteins and loss of PSII activity was followed in leaves induced to senesce in continu-

Fig. 1. Immunoblot analysis of the changes in steady-state levels of representative thylakoid proteins during senescence. Lane 1: 18 DAF; lane 2: 26 DAF; lane 3: 42 DAF; lane 4: 46 DAF; lane 5: 52 DAF; lane 6: 58 DAF (leaf abscission). For wild-type (cv. Clark), these stages represent: full leaf expansion (18 DAF), maximum Chl content (26 DAF), 25% (42 DAF), 50% (46 DAF) and 75% (52 DAF) of the Chl degraded, and leaf abscission (58 DAF). Each lane was loaded with an amount of solubilized thylakoids equivalent to 0.05 cm² of leaf. The anti-CP26 antibody used in this work was made against a 13-amino acid synthetic peptide, but it also recognizes CP29 in some species (Falbel and Staehelin 1992). In our blots, this can be seen as a slightly diffuse doublet, the slower-migrating band corresponding to CP29 and the faster one to CP26.
ous darkness. As with leaves undergoing monocarpic senescence in a normal photoperiod, cytG retarded the dark-induced loss of Chl, particularly of Chl b; therefore, the Chl a/b ratio decreased in leaves of cytG senescing in darkness (data not shown). Most thylakoid proteins were degraded to a similar extent in wild-type and cytG leaves incubated in darkness (Fig. 4A), with the exception of LHCI, which was lost more slowly in cytG. The immunoblot and activity measurements of PSII in Fig. 4B,C show that there was no difference in the rates of decline of PSII activity or in the loss of the D1 protein between wild-type and cytG leaves senescing in darkness, indicating that the accelerated degradation of PSII reaction centre proteins in cytG leaves senescing under a normal photoperiod is light-driven.

**Senescent, lincomycin-treated leaves of cytG are more susceptible to photoinhibition in vivo**

The light-accelerated loss of PSII reaction centre proteins in senescing leaves of cytG might be due to greater susceptibility to photoinhibition in the mutant. The rate of photoinhibition (i.e. light-dependent decrease of PSII activity) was measured in vivo during normal (monocarpic) senescence in wild-type cv. Clark and cytG soybeans. For the photoinhibition studies, we chose fully

![Image](https://via.placeholder.com/150)

**Fig. 2.** Changes in the levels of key proteins of the cyt b6f complex during senescence in wild-type (cv. Clark) and cytG. Panel A – immunoblot analysis of cytochrome f and the Rieske Fe-S protein of the cyt b6f complex. Lane 1: 18 DAF; lane 2: 26 DAF; lane 3: 42 DAF; lane 4: 46 DAF; lane 5: 52 DAF; lane 6: 58 DAF (leaf abscission). For wild-type, these stages represent: full expansion (18 DAF), maximum Chl content (26 DAF), 26% (42 DAF), 60% (48 DAF) and 81% (52 DAF) of the Chl degraded, and leaf abscission (58 DAF). Each lane was loaded with an amount of solubilized thylakoids corresponding to 0.05 cm² of leaf. Panel B – quantification of the changes in cyt f by staining for its heme-peroxidase activity in mildly denaturing gels. Samples were taken when wild-type leaves reached full expansion (18 DAF), maximum Chl content (26 DAF), 26% (42 DAF), 60% (48 DAF) and 81% (52 DAF) of the Chl degraded. Each point is the average of three replicate measurements, and vertical bars represent the standard error of the mean.

![Image](https://via.placeholder.com/150)

**Fig. 3.** A – Photosystem II activity of thylakoid membranes isolated from leaves of wild-type (cv. Clark) or cytG at different stages of senescence. The samples were taken at 18, 26, 42, 48 and 52 days after flowering. For wild-type, this represents: full expansion (18 DAF), maximum Chl content (26 DAF), 26% (42 DAF), 60% (48 DAF) and 81% (52 DAF) of the Chl degraded. Each point is the average of three replicate measurements, and vertical bars represent the standard error of the mean. B – The ratio of variable to maximum fluorescence in dark-adapted leaves of wild-type cv. Clark and cytG soybeans. For the wild-type, these stages represent maximum Chl content (19 DAF), 19% (28 DAF), 30% (35 DAF), 64% (40 DAF) and 92% (46 DAF) Chl loss. Each point is the average of five replicate measurements, and vertical bars represent the standard error of the mean.
expanded mature leaves (total Chl 65.3 ± 15.9 μg cm⁻²; Chl a/b 3.2 ± 0.14) and senescing leaves (total Chl 40.3 ± 0.68 μg cm⁻²; Chl a/b 3.3 ± 0.10) of the wild-type. The leaves were treated with lincomycin, which inhibits chloroplast protein synthesis and thus blocks the concurrent recovery of PSII from photoinhibition (Aro et al. 1993). Subsequent illumination caused a decline in PSII activity with nearly identical time-courses in mature and senescing leaves (Fig. 5A), indicating that in vivo the rate of photoinhibition does not change in wild-type leaves during the early phases of senescence. The PPFD used for the photoinhibitory illumination of the intact leaves was only slightly higher than used during growth, which ensures that the conclusions drawn from the photoinhibition experiments can be applied to the growth conditions.

While mature leaves of cytG (total Chl 68 ± 6.8 μg cm⁻²; Chl a/b 2.7 ± 0.13) had photoinhibition rate constants similar to those of the wild-type, the rate constant of photoinhibition was 1.6 times higher in senescent (total Chl 39.0 ± 2.2 μg cm⁻²; Chl a/b 1.8 ± 0.11) than in mature leaves of the mutant (Fig. 5A). Furthermore, in the presence of the chloroplast protein synthesis inhibitor lincomycin, the high-light-induced degradation of the D1 protein occurred more rapidly in senescent leaves of cytG than in the wild-type (Fig. 5C).

The higher rate of photoinhibition in intact leaves of cytG prompted us to compare in vitro photoinhibition in thylakoids isolated from untreated, senescent leaves of wild-type and the cytG mutant. Values for the in vitro rate constant of photoinhibition measured in thylakoids isolated from control and senescent leaves of both cv. Clark and cytG were similar (Table 1). Because differences in the composition of the starting material might affect this comparison, we repeated the experiment with thylakoids isolated from leaves induced to senesce in the dark, so as to have equal amounts of PSII reaction centre and D1 protein in both the wild-type and cytG thylakoids before the photoinhibition treatment. Likewise, in this experiment, thylakoids isolated from mature and senescent leaves of both the mutant and wild-type showed no difference in the rate constant of PSII inactivation under illumination in vitro (Fig. 5B).

**Photochemical and non-photochemical quenching in wild-type and cytG leaves**

The greater rate of photoinhibition in cytG leaves could be due to increased excitation pressure on PSII (i.e. decreased photochemical quenching, qP) caused by its larger light-harvesting capacity (Park et al. 1997). Photochemical quenching (qP) in wild-type leaves did not change much during the early stages of senescence (up to 30% Chl loss in wild-type cv. Clark) and declined only slightly thereafter (Fig. 6A). The changes in qP of cytG leaves were similar to those in the wild-type at all stages studied, and therefore, differences in qP, i.e. in excitation pressure, do not explain the faster photoinhibition of PSII in the mutant. Since non-photochemical quenching (qN) has been claimed to protect PSII against photoinhibition (Park et al. 1997), another possible explanation for the enhanced photoinhibition-susceptibility of cytG may be that inefficient non-photochemical quenching of excitation energy renders senescing cytG leaves more sensitive to photodamage. However, in wild-type leaves, qN declined markedly during senescence (Fig. 6B), but in cytG, qN remained relatively constant, and therefore senescing leaves of the mutant maintained a higher qN than the wild-type. Thus, non-photochemical quenching afforded cytG leaves a larger capacity than the wild-type to dissipate excitation energy absorbed by the light-harvesting pigments. We do not know whether the sustained capacity for...
qN quenching in cytG is caused by retention of leaf xanthophylls, by a better capacity to maintain the transthy-
lakoid pH gradient during senescence in the mutant, or by some other factor.

Synthesis of the D1 protein of PSII in senescing leaves of cytG

The D1 protein of the PSII reaction centre was degraded quite rapidly during photoinhibition of PSII activity
(Fig.5B), and indeed D1 protein levels appeared to decline faster than those of other PSII proteins, e.g. CP26
and CP47, in senescing leaves of cytG (Fig.1). To exclude the possibility that the rapid loss of the D1 protein
is caused by a reduced capacity for de novo protein synthesis, we measured the changes in translation activity
of D1 protein and the steady-state levels of the psbA mRNA during senescence (Fig.7). The results showed
that the D1 protein was actively translated in senescent leaves of both wild-type and cytG (Fig.7A). For reasons
explained elsewhere (Noodén et al. 1997), we believe normalization on the basis of FW (Fig.7D) better re-
fects the concentration of mRNAs in senescing tissues, and this decreases in senescing leaves. The amounts
of psbA mRNA were similar in both genotypes during senescence (Fig.7B,C,D); however, the concentrations
of psbA expressed on the basis of FW were slightly higher
in cytG (Fig.7D).

Discussion

Breakdown of thylakoid proteins during senescence: alterations caused by the ‘stay-green’ mutation cytG

In wild-type genotypes of higher plants, the breakdown of the thylakoid membranes starts with the degradation
of the cyt b6/f complex, and that is followed by the roughly simultaneous loss of Chl and decline of the pro-

Table 1. Photoinhibition rate constants (h⁻¹) of thylakoids isolated from non-treated leaves and subjected to photoinhibitory illumi-
nation (PPFD 1000 μmol m⁻² s⁻¹) at 20°C. Oxygen evolution activ-
ity was measured periodically during the illumination and the rate
 constants were calculated from first-order fits of the decrease of the activity. Wild-type and cytG thylakoids were isolated from leaves harvested at different stages of senescence. For the wild-type cv.
Clark, these stages represent: maximum Chl content (20 DAF), 38
(45 DAF) and 70% Chl loss (55 DAF). Figures in parenthesis repre-
sent standard errors.

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<td>2.1 (0.3)</td>
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Fig. 6. Photochemical (A) and non-photochemical quenching (B) of Chl a fluorescence in leaves of wild-type cv. Clark and cytG soybeans at different stages during senescence. Measurements were made at 19, 28, 35, 40 and 46 DAF. For the wild-type, these stages represent maximum Chl content (19 DAF), 19% (28 DAF), 30% (35 DAF), 64% (40 DAF) and 92% (46 DAF) Chl loss. The fluorescence kinetics in cytG at 46 DAF precluded the accurate determination of qP and qN and therefore qP and qN for cytG at 46 DAF are not shown. Vertical bars represent the standard error of the mean.

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presence of DCMU indicate that the antenna size of PSII increases in senescing thylakoids of cytG (data not shown). Thus, the fact that cytG thylakoids are not more susceptible to photoinhibition confirms that the antenna size of PSII plays no significant role in susceptibility to photoinhibition (Tyystjärvi et al. 1992, 1994). Likewise, the photochemical capacity of PSII (Fv/Фm) declines at similar rates during senescence of wild-type and the sidG Festuca mutant, in spite of retention of LHCII in sidG (Kington-Smith et al. 1997), which lends support to our conclusion that retention of LHCII alone is not the reason for faster photoinhibition in cytG.

Fluorescence quenching in cytG

Several mechanisms dissipate energy absorbed in excess of that needed for CO₂ fixation (Horton et al. 1996), presumably helping to prevent the formation of active oxygen species that could impair photosystem function (Foyer et al. 1994). Photochemical and non-photochemical quenching phenomena have been shown to affect the balance between photoinhibitory damage and repair of PSII in several species (Park et al. 1997).

Photochemical quenching may decrease during senescence (Šesták and Šiffel 1997), but in leaves of wild-type soybeans qP remained relatively constant until quite late in senescence. Photochemical quenching in cytG was similar to the wild-type at all stages of senescence. Non-photochemical quenching may increase (Šesták and Šiffel 1997, Murchie et al. 1999) or decline (Fig. 6B) in senescing leaves, probably depending on environmental conditions. Interestingly, while non-photochemical quenching declined during senescence of wild-type leaves, qN remained constant in senescing leaves of the cytG mutant. The higher qN of senescing cytG leaves may have helped to maintain a high qP in spite of an increasing antenna size of PSII (data not shown). In any event, senescing cytG leaves appeared to dissipate absorbed energy more efficiently than the wild-type cv. Clark, reinforcing the conclusion that the light-accelerated loss of PSII in cytG is not caused by absorption of excess energy by the retained LHCII.

Repair of photosystem II in cytG

Photoinhibition occurs at low photon yield in illuminated leaves such as those used here (Tyystjärvi and Aro 1996). The resulting photodamage to the PSII reaction centre is repaired by degradation of the damaged D1 protein and re-synthesis of a new copy of D1 (Aro et al. 1993, Okada et al. 1996). Since we measured photoinhibition in the presence of the translation inhibitor lincomycin, the enhanced rate of photoinhibition in senescing leaves of cytG is not primarily due to inefficient repair of damaged PSII centres.

However, since PSII core proteins and many chloroplast ribosomal proteins are chloroplast-encoded, and cytG is a maternally inherited mutation, we tested whether the cytG mutation has an additional deleterious effect on chloroplast protein synthesis and therefore on the PSII repair cycle. The levels of the psbA mRNA in cytG soybeans were equal to or greater than those in the wild-type (Fig. 7B,C,D) suggesting that the accelerated loss of the D1 protein in cytG is not due to decreased
transcription of the psbA gene. Moreover, D1 is synthesized at a similar rate by senescing leaves of wild-type and cytG soybeans (Fig. 7A). Thus, the accelerated loss of PSII in cytG is due to a higher rate ofphotodamage, rather than to decreased synthesis of the D1 protein.

In conclusion, susceptibility to photoinhibition does not change during senescence of wild-type soybean leaves. However, photoinhibition in senescing leaves is increased by the cytG mutation, even though fluorescence- quenching mechanisms are quite active in the mutant. This enhanced photodamage accelerates degradation of the D1 protein, which in turn may enhance the breakdown of other PSII polypeptides in the mutant. Since the rate of photoinhibition is not altered in isolated thylakoids of cytG, a stromal or cytosolic factor, rather than absorption of excess light by the retained LHCII, appears to be responsible for the faster photoinhibition in cytG. The nature of this factor remains to be elucidated, but it may be possible that the same factor conferring enhanced susceptibility to photoinhibition is responsible for the retention of LHCII in cytG.

Acknowledgements – We thank Drs S. Gepstein, B. R., Green, M. Kimokka, A. M. Attoo, R. M. Cartth, J. Mellet, R. Sayre, B. Sears and A. Staehelin for the gift of antibodies and clones used in this work. We thank Dr C. F. Yocum for the use of his O2 electrode and Dr J. Flore for the OS-500 Chlorophyll Fluorescence Meter. This work was financed partially by a Biotechnology Career Fellowship from The Rockefeller Foundation to J. G. and by Foundation An- torchias (Argentina). J. G. is a researcher at C. I. C., Pcia de Buenos Aires, Argentina. E. T., T. T. and M. K. were supported by a fellowship from Academy of Finland.

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Edited by V. Hurry

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