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1 **The impact of photosynthesis on initiation of leaf senescence**

2

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12

13

14 **Abbreviations**

15 ABA, abscisic acid; CP, chlorophyll binding protein; Cyt, cytochrome; Fd, ferredoxin; FNR, Fd-
16 NADP⁺ reductase; JA, jasmonic acid; LHC, light-harvesting complex; PS, photosystem; NDH,
17 NAD(P)H dehydrogenase; PQ, plastoquinone; PTOX, plastid terminal oxidase; ROS, reactive oxygen
18 species; SA, salicylic acid; SOD, superoxide dismutase

19

20

21 **Abstract**

22 Senescence is the last stage of leaf development preceding the death of the organ, and it is important
23 for nutrient remobilization and for feeding sink tissues. There are many reports on leaf senescence but
24 the mechanisms initiating leaf senescence are still poorly understood. Leaf senescence is affected by
25 many environmental factors and seems to vary in different species and even varieties of plants, which
26 makes it difficult to generalize the mechanism. Here, we give an overview on studies reporting about
27 alterations in the composition of the photosynthetic electron transport chain in chloroplasts during
28 senescence. We hypothesize that alternative electron flow and related generation of the proton motive
29 force required for ATP synthesis become increasingly important during progression of senescence. We
30 address the generation of reactive oxygen species (ROS) in chloroplasts in the initiation of senescence,
31 retrograde signaling from the chloroplast to the nucleus and ROS-dependent signaling associated with
32 leaf senescence. Finally, differences between natural senescence and dark-induced senescence are
33 pointed out and a few ideas for increasing crop yields by increasing the chloroplast lifespan are
34 presented.

35

36 **1. Introduction**

37 Photosynthesis is the basis for primary biomass production, and photosynthesis in all green
38 tissues is needed to feed sink tissues like roots, developing organs or specialized storage organs.
39 Senescence is the last stage of leaf development preceding the death of the organ. During senescence
40 valuable compounds are remobilized and nutrients are transported into sink tissues. In crops,
41 photosynthesis especially in flag leaves is known to substantially contribute to the filling of grains and
42 hence to the productivity of cereals (Stoy, 1963; Stamp and Herzog, 1976; Gregersen et al. 2013).
43 Chloroplasts are the main source of carbon- and/or nitrogen-recycling in plants because they contain
44 the majority of nutrients; chloroplastic proteins account for 75% to 80% of total the leaf nitrogen in C₃
45 plants (Makino, 2003). Senescence is hence of pivotal importance for plant yields, and premature
46 senescence during stress situations limits yield. The onset of senescence is modulated by external and
47 internal factors like day length (Keskitalo et al. 2005; Fracheboud et al. 2009), temperature
48 (Fracheboud et al. 2009), metabolic status of the leaf, redox homeostasis, epigenetic modifications
49 etc.; and is induced or accompanied by changes in gene expression, especially in the expression of
50 genes encoding transcription factors together with post-transcriptional and post-translational
51 modifications. Senescence is accompanied by a decline of chlorophyll, modifications of the

52 photosynthetic apparatus and the generation of reactive oxygen species (ROS) which may be an
53 important trigger for the initiation of specific senescence-relevant processes.

54 Although it is obvious that chloroplasts are not only the major targets of senescence associated
55 degradation processes, and the declines in photosynthetic activity is an early event in senescence
56 (Krupinska, Humbeck, 2004), the importance of chloroplast signalling in regulation of senescence has
57 been hitherto underestimated in research on senescence regulation. Studies on the sequence of
58 senescence associated process with the model plant *Arabidopsis thaliana* (Hensel et al. 1993) and
59 with flag leaves of barley collected in the field (Humbeck et al. 1996) clearly showed that the decline
60 in photosynthetic activity precedes the upregulation of the expression of major senescence-associated
61 genes. This suggests, as it has been proposed already in the early 1990th by Hensel and co-workers
62 (Hensel et al. 1993), that nuclear gene expression is controlled by signals originating from
63 chloroplasts, indicating a retrograde control over senescence regulation.

64 The disassembly of the photosynthetic apparatus during senescence is a developmental phase
65 with a high probability of ROS production caused by changes of the organization of the photosynthetic
66 complexes and changes in electron transport pathways. Although there is very good evidence for the
67 importance of ROS during senescence in many species (Pintó-Marijuan and Munné-Bosch, 2014), the
68 exact sites and mechanisms of light-dependent ROS generation are unknown. In the light, the
69 chloroplast is recognized as the most important site of ROS generation (Laloi and Havaux, 2015).
70 Beside the chloroplasts, other cell compartments like mitochondria, peroxisomes and specialized
71 enzymes, like *e.g.* the NADPH oxidase at the plasma membrane, also contribute to the overall ROS
72 load.

73 In this review, we focus mainly on changes in the photosynthetic apparatus and electron
74 transport during early stages of senescence and on related ROS generation in the chloroplast. Dark-
75 induced senescence is not covered.

76

77 **2. Alteration of photosynthesis during senescence**

78 In leaves photosynthetic activity is highest at the mature stage of the leaf and declines
79 gradually during senescence (Stoddart and Thomas, 1982; Fischer and Feller, 1994). Loss of
80 chlorophyll is the most obvious parameter of senescence although the loss of individual compounds of
81 the photosynthetic electron transport chain has been shown to precede chlorophyll loss (see *e.g.*
82 Humbeck et al. 1996; Schöttler, Tóth, 2014). In other words, one characteristic feature of senescence
83 is the remodeling of the photosynthetic electron transport chain induced by changes in the protein

84 composition of the photosynthetic apparatus (Park et al. 2007; Sakuraba et al. 2012a; Nath et al.
85 2013; Schöttler et al. 2017).

86

87 **2.a The photosynthetic apparatus**

88 In mature leaves, the photosynthetic apparatus is situated in the thylakoid membrane of the
89 chloroplasts and it is composed of a number of pigment/protein complexes (Fig. 1). Two photosystems
90 (PSII and PSI) are central for the photosynthetic electron transport on the thylakoid membrane in
91 chloroplasts. The PSII reaction center is often referred to as the complex of the cofactor carrying
92 subunits, D1 and D2, together with cytochrome (Cyt) *b*₅₅₉, which associates with the other large core
93 subunits PsbB (CP47) and PsbC (CP43). The PSII core complex is associated with the light-harvesting
94 complex (LHC) of PSII, i.e. LHCII, trimers, contains altogether about 27 subunits and is mainly
95 located in the internal part of the grana stacks of the thylakoid membranes. Additionally, the
96 monomeric LHCB is located between the core subunits of PSII and the LHCII trimers (Caffarri et al.
97 2014). Different to the PSII-LHCII complex, PSI is located in the stroma-exposed membranes, which
98 are the last layers of the grana (margins) and the stroma lamellae. Based on the model by Caffarri et al.
99 (2014), PSI is composed of ~15 subunits, including the PsaA and PsaB, the core subunits forming the
100 PSI reaction center, and the monomeric LHCA. Light energy absorbed by the antenna chlorophyll in
101 PSII and PSI is transferred to reaction center chlorophylls (P680 in PSII and P700 in PSI) where
102 charge separation takes place resulting in the formation of the primary charge pair and initiating
103 electron transport reactions (Fig. 1A). At the oxygen evolving complex (Mn₄CaO₅ cluster) on the
104 donor side of PSII water is oxidized and oxygen is released. At the acceptor side of PSII plastoquinone
105 (PQ) is reduced to plastoquinol diffuses to the PQ pool and is replaced at PSII by an oxidized PQ.
106 Plastoquinol is oxidized by the Cyt *b*₆*f* complex which reduces in the so-called high potential chain
107 plastocyanin, the electron donor of PSI, and which pumps protons via the so-called low potential chain
108 and the Q-cycle. At the acceptor side of PSI ferredoxin is reduced that is the substrate of the
109 ferredoxin NADP⁺ oxidoreductase (FNR) reducing NADP⁺ to NADPH. During linear electron
110 transport a proton gradient is established across the thylakoid membrane which provides the driving
111 force for ATP synthesis by the chloroplast ATP synthase. In the chloroplast stroma s, ribulose 1,5-
112 bisphosphate (RuBP) carboxylase/oxygenase (Rubisco), catalyzes the carboxylation or oxygenation of
113 RuBP to produce 3-phosphoglycerate, which drives the Calvin-Benson cycle using NADPH and ATP
114 produced by photosynthetic electron transport. Additionally, 2-phosphoglycolate generated in the

115 oxygenation reaction of Rubisco is metabolized to RuBP in photorespiration using both reduced
116 ferredoxin and ATP provided by photosynthetic electron transport.

117 Beside linear electron transport, alternative electron transport pathways do exist (Fig. 1B).
118 Reverse electron flow can take place via reduced ferredoxin using the Cyt *b₆f* complex or the NDH
119 complex as electron input module. Cyclic electron flow can take place via two distinct pathways, the
120 first one involving a ferredoxin quinone oxidoreductase, the second one the chloroplast NAD(P)H
121 dehydrogenase (NDH) complex, a homolog of the NADH dehydrogenase (complex I) in the
122 respiratory chain in mitochondria. Cyclic electron flow around PSI permits additional proton pumping
123 and is required to optimize the NADPH/ATP ratio. Similar to cyclic electron flow the so-called
124 Mehler reaction, using oxygen as electron acceptor at the acceptor side of PSI, leads to additional
125 proton pumping (Allen 2003). In the chlororespiratory pathway, a light-independent pathway, electron
126 transport takes place from NAD(P)H via the NDH complex or the Cyt *b₆f* complex and reduces
127 plastoquinone to plastoquinol which is then oxidized by the plastid terminal oxidase (PTOX), a non-
128 heme diiron carboxylate enzyme (Bennoun 2005). In the mature leaf, alternative electron pathways are
129 secondary, but they may become more important during the dismantling of the photosynthetic
130 apparatus during senescence. During senescence the structural alterations of the photosynthetic
131 apparatus, the reorganization of the membrane system within the plastid and the transport processes
132 required for nutrient remobilization are highly energy demanding so that alternative electron transport
133 processes leading to the generation of a proton motif force and ATP synthesis may be of pivotal
134 importance.

135

136 **2.b Dismantling of the antenna**

137 Different scenarios, that may be species-dependent, have been reported for the dismantling of
138 LHC during senescence. In common to all described events is a loss of the chlorophyll content during
139 senescence. In many species a decrease in the chlorophyll *a/b* ratio, indicating earlier loss of the
140 reaction centers than of LHCII which dominantly harbors chlorophyll *b*, has been observed, e.g. for
141 rice (*Oryza sativa* L. cv. Sasanishiki; Hidema et al. 1991), *Arabidopsis thaliana* (Nath et al. 2013),
142 aspen (*Populus tremula*; Keskitalo et al. 2005), tobacco (*Nicotiana tabacum*; Schöttler et al. 2017). In
143 contrast, in barley (*Hordeum vulgare*), cv. Lomerit, the chlorophyll *a/b* ratio consistently increased
144 during the progressing of senescence, and the LHCII proteins were upon the first proteins that were
145 degraded during senescence (Krupinska et al. 2012). These examples show that the order of
146 dismantling of the antenna in the processes during senescence may differ between species and that it is

147 impossible, at least at the current state of knowledge, to put into a universal scheme of events that
148 occur in a definite order during senescence. Further, in barley the abundances of LHCB4 (CP29) and
149 LHCA2 (LHCI) are controlled by light and decrease in response to high light during senescence in the
150 field or under controlled environmental conditions. When during senescence the irradiance was high,
151 abundance was low. When, the senescence process was reversed/retarded by low irradiance (rainy
152 days), abundance of the complexes was observed to be high again (Humbeck and Krupinska 2003).
153 The monomeric LHCB are proposed to play a pivotal role in protection of PSII by regulating energy
154 flow from the peripheral antenna system to the core subunits (De Bianchi et al. 2011). The minor
155 light-harvesting complexes such as LHCB4 located between the reaction center of photosystem II and
156 the LHC trimers (Caffarri et al. 2014), were proposed to play a pivotal role in protection of the
157 photosystem by regulating energy flow from the peripheral antenna system to the reactions centers (De
158 Bianchi et al. 2011). CP29 is reversibly phosphorylated in high light. Hence, a decline in the abundance
159 of LHCB4 during exposure to high irradiance may serve in prevention of photo-oxidative damage of
160 PSII (Humbeck and Krupinska 2003). These studies reveal that reorganization of LHC is involved in
161 the modulation of senescence in response to changes in the light intensity in barley.

162 In several mutant plants the stay-green character of the plants positively correlates with the
163 retained chlorophyll *b* content, implying an important role of the degradation of LHC for the initiation
164 of senescence. The construction and destruction of LHC is regulated by three enzymes of the
165 chlorophyll cycle, i.e. chlorophyll *a* oxygenase, chlorophyll *b* reductase and 7-hydroxymethyl
166 chlorophyll reductase (Tanaka and Tanaka 2011). When the chlorophyll *a/b* ratio in plants is lowered
167 by overexpression of chlorophyll *a* oxidase or its catalytic domains only, chlorophyll *b* was
168 incorporated into the core complexes of the photosystems (Hirashima et al. 2006) with a stay-green
169 phenotype both in light and in darkness (Sakuraba et al. 2012b). Additionally, Kusaba et al. (2007)
170 found that the rice mutant defective in the gene for a chlorophyll *b* reductase (*NYCI*) retained LHCII
171 and grana during senescence. In the mutant of *Arabidopsis thaliana* impaired in *NYCI* gene in the
172 stay-green phenotype occurs not only during senescence in the light but also in the dark as is the case
173 in the chlorophyll *a* oxygenase overexpressing plants (Jibrán et al. 2015). From these studies, it has
174 been discussed that excessive chlorophyll *b* leads to activation of a signaling pathway connecting the
175 chloroplast status and the nuclear gene expression (Sakuraba et al. 2012b). A much more likely
176 explanation for the stay-green phenotype is the stabilization of the photosystems by exchange of
177 chlorophyll *a* by chlorophyll *b* within originally only chlorophyll *a* binding pigment protein
178 complexes such as CP43, CP47 and PSI reaction centre (Hirashima et al. 2006; Sakuraba et al. 2010).

179 This may be explained by the fact that detachment of chlorophyll *b* from the protein requires more
180 energy than detachment of chlorophyll *a* (Horn et al. 2007). Finally, Voitsekhovskaja and Tyutereva
181 (2015) proposed in their review that stabilization of pigment protein complexes by chlorophyll *b* can
182 change the level of signaling molecules necessary for senescence induction.

183 Gregersen et al. (2013) proposed that the light-dependent degradation of the minor LHC
184 (Humbeck and Krupinska 2003) is related to enhanced synthesis of abscisic acid known to accelerate
185 the senescence process (Gepstein and Thimann 1980). Indeed, neoxanthin can dissociate from LHCII
186 upon temperature increase providing a substrate pool for synthesis of abscisic acid as part of a heat
187 and a drought response (Hobe et al. 2006). Further, the minor LHCs bind a high amount of the
188 xanthophylls that can be utilized as substrates for biosynthesis of abscisic acid (Seo and Koshiba 2011;
189 Caffarri et al. 2014). It is hence possible that the synthesis of abscisic acid as result of degradation
190 processes in the antenna system could be a specific mechanism for retrograde control of nuclear gene
191 expression by plastids being degraded during senescence as reported by Pfannschmidt and Munné-
192 Bosch (2013). Intriguingly, plants overexpressing LHC genes showed enhanced stomata
193 responsiveness to abscisic acid and drought resistance, while disruption of any apoprotein of LHC in
194 *Arabidopsis thaliana* was observed to increase drought sensitivity of the mutants (Xu et al. 2012).
195 Accordingly, chlorophyll *b* less mutants were reported to be less resistant towards drought
196 (Voitsekhovskaja and Tyutereva 2015) pinpointing to common features in stress response and
197 senescence.

198

199 **2.c Degradation of photosystems**

200 Many studies show preferential loss of either PSI or PSII or of both in parallel during leaf
201 senescence. A unique strategy of adjustment of the photosystems to the electron transport capacity
202 seems not to exist. The different strategies observed may depend on the nitrogen status or on the
203 environmental conditions before and during the onset of senescence. No systematic differences
204 between monocotyledons and dicotyledons were reported. Further, the difference between the stability
205 of PSII compared to PSI seems to genetically depend on species and varieties. An early loss of PSI
206 activity compared to PSII activity was observed in barley, cv. Steffi (Miersch et al. 2000), in rice, cv.
207 Ilyou 129 and Shangyou 63 (Tang et al. 2005), in oak (*Quercus bicolor* Wild.) and in sugar maple
208 (*Acer saccharum* Marsh.; Moy et al. 2015), while a high stability of PSI was found in *Festuca*
209 *pratensis*, cv. Rossa (Hilditch et al. 1989), in rice, cv. Nipponbare (Yamazaki et al. 1999), in
210 *Arabidopsis thaliana* (Nath et al. 2013) and in tobacco (*Nicotiana tabacum* cv. Petit Havana

211 (Schöttler et al. 2017). Krupinska et al. (2012) reported for two different varieties of barley grown in
212 the same year in the field differences in the stability of PSII compared to PSI and in changes of the
213 chloroplast ultrastructure during chloroplast to gerontoplast transition. In flag leaves of cv. Lomerit
214 PSII activity declined earlier than PSI activity while in cv. Carina the opposite was observed (Krieger-
215 Liskay et al. 2015). In cv. Lomerit the level of LHCII proteins declined very early upon the onset of
216 senescence and grana stacks were reduced which is in accordance with an early degradation of LHCII
217 and PSII. In contrast to cv. Lomerit cv. Carina showed the typical structure of gerontoplasts with
218 loosened grana stacks and with reduced interconnecting stroma thylakoids (Mulisch and Krupinska
219 2013). This goes hand in hand with an early loss of stroma thylakoids compared to grana stacks during
220 senescence (Krupinska et al. 2012). This indicates that differences in the stability of the photosynthetic
221 complexes during senescence can vary within one species (Krieger-Liskay et al. 2015). It is an open
222 question whether these differences are in general genetically determined, as it seems to be the case at
223 first glance in the two barley varieties, or whether preferential degradation of PSII compared to PSI or
224 vice versa depends on environmental factors like light intensity and quality (UV dose), temperature or
225 nitrogen availability. With barley plants grown in the field it has been shown that the level of the D1
226 protein declines before Cyt *b₆f* complex (Humbeck et al. 1996). This is most likely a consequence of
227 the high light intensity and UV plants are exposed to in the field. However, in a controlled
228 environment with maximal irradiance of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ the D1 protein was more stable than the
229 rapidly declining FNR (Kucharewicz et al. 2017) catalyzing the transfer of electrons from PSI to the
230 terminal acceptor NADP^+ of the electron transport chain.

231 Possibly, the net loss of PSII during senescence is just the accumulation of oxidative damage
232 to a degree that the repair system cannot take care any longer about it. It is well known that after
233 photo-oxidative damage to PSII, i.e. PSII photoinhibition, the core subunit D1 protein is fast degraded
234 by Deg and FtsH proteases and fast resynthesized. The half-time in the light of the D1 protein was
235 estimated to be 2.5 h (Nelson et al. 2014; Li et al. 2017). The D1 resynthesis and the repair of PSII in
236 the so-called damage-repair cycle (Aro et al. 1993) are very efficient so that no net loss of D1/PSII is
237 seen over a wide range of light intensities in mature plant leaves (Edelman and Mattoo, 2008).
238 Contrary, the susceptibility of PSII to photo-oxidative damage was shown to increase with senescence
239 in detached leaves of rye (*Secale cereal* L. cv. Halo), owing to a decline of the repair capacity of PSII
240 (Kar et al. 1993). Wang et al. (2016) proposed that both degradation and resynthesis of the D1 protein
241 are declined during senescence in flag leaves of rice, cv. Zhehui7954. Increased vulnerability to
242 photoinhibition has also been attributed to an imbalance in the photosynthetic apparatus caused by

243 differential rates of decline during senescence, e.g. the decreases in Cyt *b₆f* complex, plastocyanin and
244 Rubisco as described below, resulting in a greater capacity for light capture than required for carbon
245 reduction (Hodgkinson 1974; Allahverdiyeva et al. 2005).

246

247 **2.d Decrease in Cyt *b₆f* complex and plastocyanin**

248 In respect to the photosynthetic electron transport chain, it has been reported that in tobacco
249 Cyt *b₆f* complex is the first protein complex that is degraded which leads to a decrease of the activity
250 of photosynthetic linear electron flow (Ben-David et al. 1983; Roberts et al. 1987; Schöttler and Tóth
251 2014; Schöttler et al. 2017). Similar early loss of Cyt *b₆f* complex together with a loss of the
252 chloroplast ATP synthase has been observed in rice, cv. Sasanishiki (Hidema et al. 1991). Under
253 optimal conditions in mature chloroplasts, Cyt *b₆f* complex is the rate determining step of the linear
254 electron transport chain. A reduction in the amount of this complex relative to the photosystems exerts
255 a control effect on photosynthetic electron transport. A loss or a reduction in the amount of Cyt *b₆f*
256 complex compared to PSII and/or PSI reduces linear electron flow. Not only linear flow but also
257 cyclic electron flow around PSI can depend also on Cyt *b₆f* complex. However, cyclic electron flow
258 may require a supercomplex formation of Cyt *b₆f* complex with PSI, LHCI, LHCII, FNR and proton
259 gradient regulation 5-like 1 protein (PGRL1; Iwai et al. 2010) or with PSI and NDH complex (Yadav
260 et al. 2017; Kato et al. 2018). The proportion of PSI involved in these complexes is minor compared to
261 PSI participating in linear electron flow. Thus, a reduction in the ratio Cyt *b₆f*/PSI may not affect these
262 supercomplexes but mainly linear electron flow.

263 In addition to a decrease in the level of Cyt *b₆f* complex a decrease in the level of plastocyanin
264 has been observed upon onset of senescence and electron transport in tobacco, cv. Samsun NN
265 (Schöttler et al. 2014; 2017). In senescing tobacco leaves plastocyanin reduction by Cyt *f* is delayed
266 and the equilibration between plastocyanin and Cyt *f* is disturbed. Reoxidation of Cyt *f* by plastocyanin
267 limits under these conditions electron flow and not, like in non-senescent leaves, the plastoquinol-
268 oxidase activity of Cyt *b₆f* complex (Schöttler et al. 2004). A correlation between plastocyanin content
269 and electron transport rate has also been reported for barley (Burkey 1994). In *Arabidopsis thaliana*,
270 however, 90% decrease in plastocyanin content did not limit electron flow under conditions of non-
271 senescent plants grown under standard conditions (Pesaresi et al. 2009). However, when different
272 developmental stages were tested, the mutant *pete2-1* that contains drastically less plastocyanin
273 (Pesaresi et al. 2009) showed the highest peak in chlorophyll content three days earlier than the
274 corresponding wild type, indicative of an earlier senescence start (Wang et al. 2015). Although less

275 intensively studied as Cyt *b₆f* complex, changes in plastocyanin content and reduction kinetics may be
276 the key element that controls a decrease of linear electron flow upon onset of senescence. The other
277 diffusible electron carrier, plastoquinone that shuttles electrons between PSII and Cyt *b₆f* complex,
278 does not change in content during senescence (Schöttler et al. 2004). If plastocyanin/Cyt *b₆f* control
279 the photosynthetic electron transport rate, the content of PSII and PSI are possibly adjusted to the
280 maximal capacity of electron transport during senescence, or alternatively an excess of photosystems
281 might serve for specific electron pathways or for signaling events.

282 Isotopic labelling coupled with mass spectrometry analyses has enabled the determination of
283 degradation rates and half-lives of hundreds of individual proteins (Li et al. 2017; Li et al. 2018),
284 indicating that the subunit PetD of Cyt *b₆f* complex shows a comparably high degradation rate as the
285 D1 protein while the other subunits of the photosynthetic protein/pigment complexes were more stable.
286 If the synthesis rate of the PetD protein is low in senescing leaves, this could explain the overall loss
287 of Cyt *b₆f* complex upon the initiation of senescence. Altogether more than 20 different proteases have
288 been discovered that degrade chloroplast proteins, some of which are associated with senescence
289 (Gepstein et al. 2003; Martínez et al. 2008; for a recent review see Nishimura et al. 2016). It has been
290 suggested that ClpP protease functions in the degradation of chloroplast proteins, including Cyt *b₆f*
291 complex (Majeran et al. 2000). However, the amount of the ClpP transcript decreases with leaf
292 senescence in field-grown barley, cv. Trixi (Humbeck and Krupinska 1996), indicating that the
293 degradation of the PetD protein during senescence is independent on the ClpP-dependent protein
294 degradation. Overall, the mechanism of the decline of Cyt *b₆f* complex is still unclear.

295

296 **2.e Alternative electron transport pathways in gerontoplasts**

297 When, upon the onset of senescence, the capacity of linear electron transport decreases
298 because of a reduction of plastocyanin, Cyt *b₆f* content and changes in the electron transport between
299 plastocyanin and Cyt *b₆f* complex, alternative electron pathways may become more important, for
300 example in gerontoplast in senescing leaves, than it is the case in mature leaves (Fig. 2). The
301 reorganization of the thylakoid membrane system and the export of nitrogen, carbon, metals etc. from
302 chloroplasts to sink tissue is a highly energy demanding process requiring ATP. ATP in chloroplasts
303 with a disrupted linear electron transport chain can be generated via cyclic electron flow or via
304 chlororespiratory pathways. Cyclic electron flow may persist if the fraction of Cyt *b₆f* complex and
305 plastocyanin involved in supercomplex formation is less degraded during senescence than the main
306 fraction participating in linear electron flow. Plants that keep PSI until a late stage of development

307 may profit from cyclic electron flow to generate ATP. Plants that lose PSI during early stages of
308 senescence may rely more on chlororespiratory electron flow via the NDH complex and plastid
309 terminal oxidase (PTOX). In non-green plastids electron transport generating a proton motif force and
310 ATP has been described. In chromoplasts isolated from tomato (*Solanum lycopersicum* cv. Micro-
311 Tom; Renato et al. 2014), generation of a proton motif force by electron transport involving the NDH
312 complex, plastoquinone and PTOX has been shown to take place. The same is the case in etioplasts
313 (Kambakam et al. 2016). However, to be efficient, the levels of NDH and PTOX, both minor
314 compounds of the thylakoid membrane in mature chloroplasts (Lennon et al. 2003), have to be
315 significantly increased upon the start of senescence. Indeed, the amount and activity of the NDH
316 complex was observed to increase during senescence in tobacco, cv. Petit Havana (Zapata et al. 2005).
317 Additionally, larger amounts of NDH and PTOX have been detected in senescent leaves than in
318 mature leaves of oat (*Avena sativa* L., cv. Prevision; Tallón and Quiles 2007). Furthermore, hydrogen
319 peroxide (H₂O₂), a ROS generated upon the onset of senescence, can activate the NDH-dependent
320 cyclic flow and render enzymes of the Calvin-Benson cycle inactive (Strand et al. 2015).

321

322 **2.f Loss of Rubisco**

323 Alteration of photosynthesis during senescence depends not only on the photosynthetic
324 electron transport but also on the Calvin-Benson cycle. Rubisco, the enzyme initiating photosynthetic
325 CO₂ assimilation and photorespiration, is also one of the important photosynthetic components
326 degraded during leaf senescence. Approximately half of the nitrogen in chloroplasts is occupied by
327 Rubisco (Hörtensteiner and Feller 2002), showing that Rubisco degradation plays a large role for the
328 remobilization of nitrogen. Senescing leaves of the C₃ plant rice (cv. Sasanishiki) showed an earlier
329 degradation of Rubisco than of chlorophyll at the early stage of senescence (Hidema et al. 1991).
330 However, the degradation of Rubisco has often been reported to be slower than that of photosynthetic
331 electron transport components, including PSII, PSI, and Cyt *b₆f* (see for example Krupinska et al.
332 2012). However, during natural senescence in *Arabidopsis thaliana* leaves, a decrease in Rubisco is
333 observed along with rapid decreases in PSII and Cyt *f* (Nath et al. 2013). A decrease in Rubisco during
334 senescence limits photosynthetic activity under high light. In low light and/or low available nitrogen,
335 where low levels of Rubisco are required to maintain a maximal assimilation rate, Rubisco is degraded
336 as fast as Cyt *f* in Morning glory (*Ipomoea tricolor* Cav. cv. Heavenly Blue; Hikosaka 1996). The
337 affinity of Rubisco for CO₂ and O₂, respectively, may also vary during senescence. A decrease in
338 photorespiration relative to CO₂ assimilation has been observed in the senescence of cotyledons of

339 sunflower (*Helianthus annuus* L. cv. Spanners Allzweck; Betsche and Eising 1989), but there is little
340 report on the role of photorespiration in leaf senescence.

341 Rubisco is digested by specialized autophagic bodies and even whole chloroplasts can be
342 digested by autophagy (Ishida et al. 2014). Rubisco alone accounts for about 50% of total soluble
343 proteins in the leaf nitrogen in C₃ plants. Autophagy is induced by energy limitation due to
344 interruption of photosynthetic carbon fixation. Inhibition of photosynthetic electron transport by
345 DCMU, a herbicide which binds to the acceptor side of photosystem II and blocks linear electron
346 transport, stimulates the initiation of autophagy (Izumi et al. 2015; for recent reviews on autophagy
347 see Havé et al. 2017; Izumi and Nakamura 2018). Photoinhibition by visible or UV light also induces
348 autophagy (Izumi and Nakamura 2017), suggesting strongly that an impairment of the photosynthetic
349 electron transport chain and concomitant ROS production are triggers for autophagy initiation and
350 extra plastidic degradation pathways such as the ubiquitin-proteasome system (Ling et al. 2012;
351 Woodson 2016).

352 On the other hand, it has been shown that the cysteine protease targeted to plastids is involved
353 in the degradation of Rubisco during senescence. Compelling evidence for chloroplast degradation by
354 plastid protease is the observation that isolated chloroplasts kept in the light accumulate a 44 kDa
355 cleavage product of the large subunit of Rubisco (Feller et al. 2008a, b). In barley *HvPAP14* encoding
356 a cysteine protease is highly expressed during senescence (Hollmann et al. 2014). The cysteine
357 protease HvPAP14 is targeted to chloroplasts where it is in association with the thylakoid membrane,
358 and thus can be activated by low pH of the lumen. Additionally, the overexpression mutant of
359 *HvPAP14* accumulates specific degradation products of the large subunit of Rubisco (Frank et al.
360 2018, submitted to the Plant Journal). Many cysteine proteases associated with senescence are
361 predicted to be synthesized at the endoplasmic reticulum and to be active in the vacuole (Callis 1995).
362 Searches for senescence associated proteases revealed that only few of them are targeted to plastids
363 (Gepstein et al. 2003). Hence, final degradation of chloroplast proteins, including Rubisco, is likely
364 occurring in the vacuolar compartment (Feller et al. 2008a; Martínez et al. 2008).

365

366 **3. Generation of reactive oxygen species in chloroplasts upon initiation of senescence**

367 It has been recognized since many years that plant leaves during senescence have increased
368 ROS levels, in particular H₂O₂ (Dhindsa et al. 1981; Kar and Feierabend 1984), and it has been
369 proposed that ROS are involved in the initiation of senescence (for reviews Zimmermann and Zentgraf
370 2005; Juvany et al. 2013). Indeed, many genes upregulated during senescence are also upregulated in

371 response to H₂O₂ (Sabater and Martín 2013). Increased levels of ROS during senescence have been
372 reported to coincide with enhanced lipid peroxidation in chloroplasts (Dhindsa et al. 1982; Hopkins et
373 al. 2007) and oxidized proteins (Mehta et al. 1992; Vanacker et al. 2006). Furthermore, singlet oxygen
374 (¹O₂) has been shown to induce autophagy and extra plastid degradation pathways involving the
375 ubiquitin/E3 ligase system (Ling et al. 2012; Woodson 2016).

376 Although there is no doubt about the involvement of ROS in initiation and execution of
377 senescence, the cellular sources of the senescence associated ROS are hitherto not known, except for
378 the clear evidence for H₂O₂ production in peroxisomes (Pastori and del Rio 1997). During senescence
379 the photosynthetic electron transport chain altering as described above might become another major
380 source for ROS such as singlet oxygen (¹O₂), superoxide anion radicals (O₂^{•-}) and also H₂O₂ (Apel
381 and Hirt 2004; Dietz et al. 2016). The exact site and mechanism of ROS production in senescing
382 chloroplasts has, however, not been identified so far at the molecular level.

383

384 **3.a Singlet oxygen (¹O₂)**

385 When excited chlorophyll in its triplet state (³Chl) reacts with molecular oxygen (³O₂) ¹O₂ is
386 generated. The triplet state of chlorophyll is generated by charge recombination reactions in the PSII
387 reaction centre (for review see Rutherford and Krieger-Liszkay, 2001; Krieger-Liszkay 2005) and in
388 LHCII when it is damaged (Rinalducci et al. 2004; for review see Fischer et al. 2013), and probably
389 also when it starts to be degraded during senescence. In the functional intact LHCII the formation of
390 the triplet state of chlorophyll is avoided by the close distance between carotenoid and chlorophyll
391 molecules (Edge and Truscott 2010).

392 Experiments on two barley varieties, cv. Carina and cv. Lomerit, revealed that these two
393 varieties employ different strategies of senescence in respect to ROS generation in plastids (Krieger-
394 Liszkay et al. 2015). Lomerit showed an early loss of PSII activity and LHCII (Krupinska et al. 2012)
395 and an increase in both, ¹O₂ and O₂^{•-} during senescence while Carina showed an early loss of PSI
396 activity compared to PSII, a similar increase in O₂^{•-} like Lomerit but, different to Lomerit, a constant
397 low level of ¹O₂ production. No increase in ¹O₂ generation in Carina together with a high stability of
398 PSII and LHCII indicate that in Lomerit either degradation of PSII or LHCII are responsible for the
399 observed increase in ¹O₂. When forward electron transport is blocked, due to the decrease in Cyt *b₆f*
400 complex and plastocyanin, charge recombination reactions in PSII are favored and the probability of
401 charge recombination reactions leading to the formation of the triplet state of the reaction center
402 chlorophyll and of ¹O₂ is increased. Dismantling of the PSII reaction center, the monomeric LHCB

403 such as CP29 (Humbeck and Krupinska 2003) and LHCII may also increase $^1\text{O}_2$ generation when the
404 close distance between chlorophyll and neighboring carotenoids is not any longer conserved or when
405 free chlorophyll or chlorophyll breakdown products accumulate which act as photosensitizers.

406 The $^1\text{O}_2$ amount can be modulated by the level of carotenoids quenching $^1\text{O}_2$. $^1\text{O}_2$ can be
407 scavenged also by α -tocopherol that is re-reduced by ascorbate. As shown in a previous study with
408 pea, a decline in the ascorbate pool is a good marker of the onset of leaf senescence (Vanacker et al.
409 2006). The contents of carotenoids are suggested to be usually stable relative to those of chlorophyll
410 during leaf senescence (Yoo et al. 2003). Lutein, β -carotene, and xanthophyll cycle pigments: i.e.
411 violaxanthin, antheraxanthin, and zeaxanthin decline, whereas the amount of α -tocopherol is increased
412 during leaf senescence of wheat (*Hordeum vulgare* L. cv. Dyan; Afithile et al. 1993), pea (*Pisum*
413 *sativum* L. cv. Phoenix; Dertinger et al. 2003), in field-grown *Salvia officinalis* L. (Elizabeth and Sergi
414 2007) and α - and γ -tocopherol in tobacco (Falk et al. 2003). Therefore, tocopherol becomes an
415 important antioxidant in senescing leaves. These alterations may be important for the initiation of
416 senescence through the modulation of ROS concentrations.

417

418 **3.b Superoxide anion radical ($\text{O}_2^{\bullet-}$) and hydrogen peroxide (H_2O_2)**

419 Both $\text{O}_2^{\bullet-}$ and H_2O_2 can be generated by the reduction of O_2 in photosynthetic electron
420 transport in a number of different reactions. First, O_2 can act as a terminal electron acceptor in the
421 Mehler reaction at the acceptor side of PSI (Asada et al. 1974). Second, it can be reduced also by
422 semiplastoquinones in the thylakoid membrane (Mubarakshina and Ivanov 2010). Third, O_2 can be
423 reduced to $\text{O}_2^{\bullet-}$ at the acceptor side of PSII (Pospíšil et al. 2004). During photosynthetic linear electron
424 flow these reductions of O_2 to $\text{O}_2^{\bullet-}$ can occur under conditions of limited electron acceptor availability
425 other than O_2 . Finally, alternative electron transport may also be involved in the productions of $\text{O}_2^{\bullet-}$
426 and H_2O_2 . A mutant of tobacco (cv. Petit Havana) lacking NDH complex, that is homologous to
427 complex I in mitochondria, was shown to have retarded senescence (Zapata et al. 2005), and it has
428 been suggested that NDH complex, is responsible for increased $\text{O}_2^{\bullet-}$ and H_2O_2 production during
429 senescence. In mitochondria, indeed complex I besides complex III is regarded as the main source of
430 these ROS (Chen et al. 2003; Murphy 2009). PTOX can also generate $\text{O}_2^{\bullet-}$ in a side reaction
431 depending on substrate availability (Yu et al. 2014) although this enzyme catalyzes as main reaction
432 the reduction of O_2 directly to H_2O . Two molecules of $\text{O}_2^{\bullet-}$ are converted to H_2O_2 and O_2 in
433 disproportionation, either spontaneously or catalyzed by superoxide dismutase (SOD). In the presence
434 of reduced transition metal ions such as Fe^{2+} , H_2O_2 gives rise to the highly reactive hydroxyl radical

435 (HO[•]) supported by the reduction of Fe³⁺ by O₂^{•-} (Haber-Weiss reaction). Upon the initiation of
436 senescence linear electron transport declines (see above) and therefore also the production of ROS as
437 byproduct of photosynthetic electron transport is expected to decline. This has been shown to be the
438 case for the generation of O₂^{•-} and H₂O₂ in thylakoids isolated from barley, cv. Golden Promise, while,
439 however, the level of lipid peroxidation products, mainly generated by ROS, increased during
440 senescence (Jajić et al. 2015). Furthermore, during senescence the capacity of O₂^{•-} production was
441 shown to increase in thylakoid membranes isolated from flag leaves of field-grown barley (cv.
442 Lomerit and cv. Carina; Krieger-Liszky et al. 2015). In leaves of *Arabidopsis thaliana*, H₂O₂ levels
443 have also been shown to increase with the initiation of senescence and this increase correlated with a
444 loss of CAT2, the main isoform of catalase in leaves, localized in peroxisomes and ascorbate
445 peroxidase activity (Zimmermann et al. 2006). These facts suggest that the generation of O₂^{•-} and
446 H₂O₂ in chloroplasts during senescence is affected also by the alterations of the scavenging systems.

447 Alteration of ROS-scavenging enzymes can be observed during leaf senescence, often
448 combined with those of the photosynthetic apparatus and the generation of O₂^{•-} and H₂O₂. In
449 chloroplasts, O₂^{•-} is rapidly dismutated by SOD into H₂O₂ which is itself detoxified, in chloroplasts, by
450 peroxidases such as ascorbate peroxidase from the ascorbate-glutathione cycle (Noctor and Foyer
451 1998). In this cycle, oxidized ascorbate and glutathione are re-reduced by enzymes using NADPH as
452 cofactor. Therefore, the regeneration of these cofactors is coupled with photosynthetic electron
453 transport (Miyake and Asada 1992). Other peroxidases, such as peroxiredoxins such as 2 Cys-Prx and
454 Prx-Q are important in chloroplast ROS detoxification (Dietz 2011). In the C₃ plants tobacco
455 (*Nicotiana tabacum* L. cv. Samsun) and pea (*Pisum sativum* L. cv. Phoenix) the amounts of ascorbate
456 and glutathione decrease during leaf senescence, and thereafter the activities of ascorbate peroxidase
457 and glutathione reductase declined with a little retardation (Dertinger et al. 2003; Vanacker et al.
458 2006). Other antioxidative enzymes, including SOD, catalase, dehydroascorbate reductase, and
459 monodehydroascorbate reductase also decrease with leaf senescence (Dertinger et al. 2003; Vanacker
460 et al. 2006), indicating that antioxidative systems totally decline during leaf senescence. The induction
461 of SOD gene expression in response to stress becomes progressively impaired when leaves enter
462 senescence (Casano et al. 1994; Kurepa et al. 1997; Ohe et al. 2005; Procházková and Wilhelmová
463 2007). Interestingly, Vanacker et al. (2006) showed the transient increase in these enzyme activities at
464 the early stage of senescence, which possibly regulates the ROS level in the cells. This may play a role
465 to retard senescence and to prolong the lifespan of the cell. On the other hand, it should be also noted

466 that the changing capacities of these ROS-scavenging enzymes with increasing age has been suggested
467 not to be the cause but a consequence of leaf senescence in plants (Dertinger et al. 2003).

468

469 **4. Retrograde signalling in the control of senescence**

470 Photosynthetic activity is highly susceptible to environmental cues such as irradiance,
471 temperature and water supply and this feature makes chloroplasts excellent sensors of environmental
472 information. Moreover, chloroplasts permanently transmit information about the functional state of the
473 photosynthetic apparatus to the nucleus by various signalling molecules/factors, such as ROS,
474 carotenoid-derived oxidation products with signalling function (Ramel et al. 2012; Shumbe et al.
475 2017), and hormones synthesized in chloroplasts (Bouvier et al. 2009). Responses in the nucleus
476 ensure that plants acclimate to the changing environmental situation and avoid oxidative stress
477 (operational control) (Pogson et al. 2008). To ensure a coordinate dismantling of chloroplasts during
478 senescence and thereby an efficient remobilization of the nutrients, retrograde signalling has been
479 proposed to be also of importance also during this phase of development (degradational control)
480 (Pfanschmidt and Munné-Bosch 2013).

481 The decline in photosynthetic activity during senescence likely results in an excess light situation in
482 the dismantling chloroplasts, i.e. light is in excess for the remaining photosynthetic units diminishing
483 in number during senescence. In order to allow for a controlled and efficient remobilization of
484 nutrients from chloroplasts it is important that the remaining photosynthetic units work efficiently and
485 oxidative stress is prevented. Indeed, even in the field the decline in Fv/Fm occurs much later than the
486 declines in photosynthetic gas exchange and chlorophyll content indicating that the residual
487 photosynthetic units still work efficiently (Humbeck et al. 1996, Krupinska et al. 2012). This indicates
488 that for a certain time plants are capable to cope with the excess energy situation resulting from the
489 dismantling of the photosynthetic apparatus. It is well-known that SA being produced by chloroplasts
490 is responsible for the plants' acclimation to high light by altering gene expression in the nucleus
491 (Szechynska-Hebda and Karpinski 2013). It is hence likely that SA involved in retrograde signaling
492 from chloroplasts is also important during senescence. ROS generated during disassembly of the
493 photosynthetic apparatus may be implicated in changes of the hormone status during senescence.

494 Although high levels of ROS could induce premature cell death and hence are detrimental for
495 efficient remobilization of nutrients from the senescing leaves to other still growing leaves and to
496 seeds (Juvany et al. 2013), ROS are also required as signals for induction of stress responses,
497 development and senescence (Apel and Hirt 2004; Li et al. 2009; Foyer and Noctor 2013; Dietz et al.

498 2018). Different ROS, such as $^1\text{O}_2$ and $\text{O}_2^{\bullet-}/\text{H}_2\text{O}_2$ induce different signalling cascades. $^1\text{O}_2$ is not only
499 a very potent oxidant leading to oxidative damage of nucleic acids, lipids and proteins but it is also
500 triggering a signalling pathway distinct from $\text{O}_2^{\bullet-}/\text{H}_2\text{O}_2$ mediated signaling (Galvez-Valdivieso and
501 Mullineaux 2010; Fischer et al. 2013; Laloi et al. 2007; Laloi and Havaux 2015). Senescence
502 associated declines in the levels of antioxidative systems have been reported and could be crucial for
503 ROS dependent initiation of senescence (Procházková and Wilhelmová 2007). Throughout senescence
504 ROS, especially H_2O_2 , are believed to play a pivotal role in signalling and molecule degradation
505 (Zentgraf et al. 2012). Many senescence-associated TFs are highly responsive to H_2O_2 in *Arabidopsis*
506 *thaliana* including, for example, *WRKY53* (Miao et al. 2004), *ORS1* (Balazadeh et al. 2011), *JUB1*
507 (Wu et al. 2012) and *ATAF1* (Garapati et al. 2015). Efficient signal transduction by ROS requires that
508 the levels of ROS are kept under a tight control that is governed by enzymatic and non-enzymatic
509 ROS-scavenging systems.

510 SA besides JA and ABA, accumulates during natural senescence (Morris et al. 2000) and is
511 involved in the control of a large set of nuclear genes upregulated during senescence (Morris et al.
512 2000; Buchanan-Wollaston et al. 2005). Comparing gene expression during natural/developmental
513 senescence and dark/starvation induced senescence revealed that SA is involved in control of natural
514 senescence in the light rather than in the regulation of dark-induced senescence (Buchanan-Wollaston
515 et al. 2005). A subset of the SA-dependent senescence associated genes is also expressed during
516 pathogen responses (PR) and cell death (Buchanan-Wollaston et al. 2005). Since a higher SA content
517 has been also measured in plants after treatment with UV-C radiation (Yalpani et al. 1994) it is likely
518 that SA is of utmost importance for senescence and plant productivity under field conditions.

519 The DNA binding protein WHIRLY1 which is located in both chloroplasts and nucleus of the
520 same cell is an interesting candidate for transducing signals from chloroplasts to the nucleus
521 (Grabowski et al. 2008). It had been first identified as a transcription factor inducing expression of PR
522 genes in a salicylic acid dependent manner (Desveaux et al. 2004) making it an ideal candidate for
523 retrograde signaling during senescence. Barley plants with an RNAi mediated *WHIRLY1* knockdown
524 are compromised in responding to environmental changes due to a reduced capability to synthesize
525 microRNAs (Swida-Barteczka et al. 2018). When exposed to high irradiance the WHIRLY1 deficient
526 plants show retarded senescence in comparison to the wild type that is able to accelerate senescence
527 processes in response to environmental cues such as high light (Kucharewicz et al. 2017).
528 Interestingly, the level of salicylic acid is higher in the WHIRLY1 deficient plants than in the wild
529 type (Kucharewicz et al. 2017) clearly indicating that WHIRLY1 acts downstream of salicylic acid. It

530 has been proposed that SA induces a conformational change of oligomeric WHIRLY1 yielding
531 monomeric WHIRLY1 that is able to translocate from chloroplasts to the nucleus (Foyer et al. 2014).
532 In the WHIRLY1 deficient plants retarded senescence was shown to coincide with a reduced thousand
533 grain weight (Kucharewicz and Krupinska, unpublished data) indicating that an appropriate reaction to
534 the environment is important for the senescence associated nutrient remobilization and productivity of
535 crop plants. Interestingly, the WHIRLY1 deficient plants are not affected in dark-induced senescence
536 (Kucharewicz et al. 2017) underpinning the importance of the photosynthetic apparatus in determining
537 onset and progression of senescence under natural conditions.

538

539 **5. Conclusions and Perspectives**

540 In recent years leaf senescence has been mainly investigated in the model plant *Arabidopsis thaliana*
541 under controlled environmental conditions including darkness as a tool for induction of senescence.
542 Considering that leaf senescence is of high importance for crop yields, there is an urgent need for more
543 studies with various crop plants in the field (Gregersen et al. 2013) or under controlled growth
544 conditions allowing the simulation of real climate conditions (temperature, irradiance, humidity)
545 (Franzaring et al. 2011). Characterization of leaf senescence under field conditions revealed that the
546 sequence of events leading to the dismantling of the photosynthetic apparatus differs from that under
547 controlled conditions in growth chambers (e.g. D1 degradation). Relevant senescence modifying
548 factors identified in the field should be furthermore systematically tested under controlled climatic,
549 soil and plant canopy conditions. These studies should monitor both senescence associated changes in
550 the photosynthetic apparatus and in remobilization of nitrogen and other nutrients. Premature
551 senescence due to adverse environmental situation is known to decrease crop productivity. Future
552 studies should be conducted using different light intensities with varying proportions of UV as well as
553 fluctuating light in combination with a variety of temperatures to obtain results that are relevant for
554 improvement of nutrient recycling and productivity of crop plants in the field.

555 In order to better control senescence associated processes with regard to an enhancement of
556 plant productivity, the relationship between photosynthesis originating ROS production and the
557 production of senescence associated hormones by chloroplast metabolism needs to be elucidated.
558 Projects aiming to improve crop yields would have to include the objective to prolong the
559 photosynthetic/functional life span of the chloroplast without compromising recycling processes.
560 Comparative field studies on a few selected crop species should focus on the primary events in respect
561 to alterations of the photosynthetic apparatus to identify the key players (e.g. limiting step in electron

562 transport, poisoning of the chloroplast redox state, activity of proteases) in controlling the lifespan of the
563 chloroplasts. Such studies will also permit to clarify which event(s) is/are indeed the primary factor/s
564 initiating leaf senescence and which photosynthetic reaction(s) would be needed to become improved
565 for enhancing crop yield. As described in the present review, the existing literature does neither allow
566 to identify a general senescence strategy (if existing) nor to elucidate the most important event/s
567 leading to an initiation of senescence.

568

569

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575

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944 Figure Legends

945 Fig. 1.

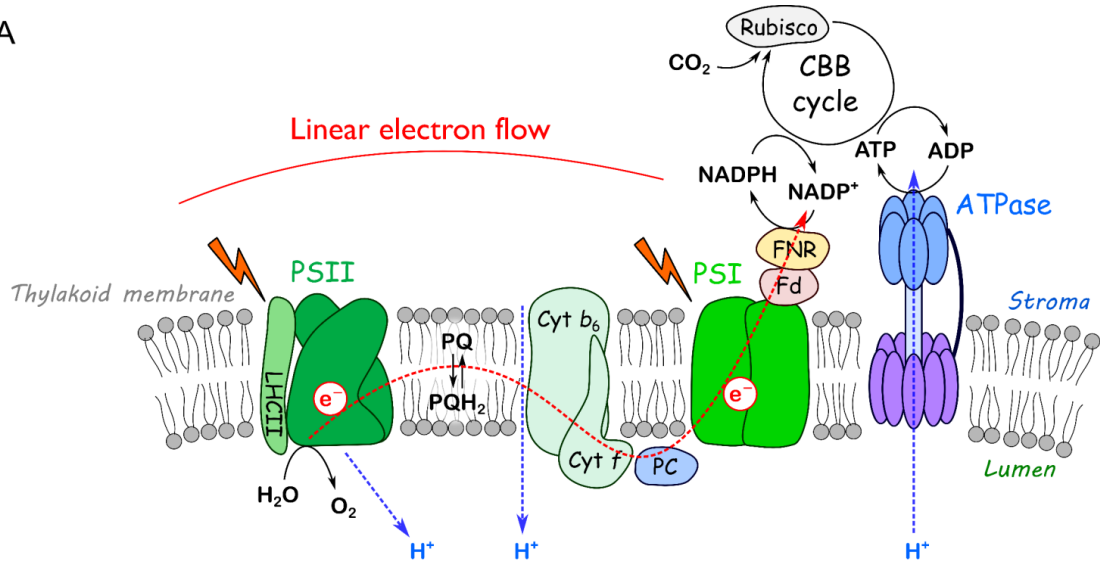
946 Photosynthetic electron transport system in C₃ plants. (A) Overview of photosynthetic linear electron
947 flow (dashed red arrow). Electrons from H₂O are transported from photosystem II (PSII) to
948 photosystem I (PSI) via plastoquinone (PQ) pool, cytochrome (Cyt) *b₆/f* complex and plastocyanin,
949 generating NADPH. Coupled with electron transport a proton gradient is built up that is used to
950 produce ATP. (B) Overview showing three different alternative electron transport pathways: (1) cyclic
951 electron flow via the protein Proton Gradient Regulation 5 (PGR5) and PGR5-like protein 1 (PGRL1;
952 dashed pink arrow); (2) via chloroplast NAD(P)H dehydrogenase (NDH) complex (dashed purple
953 arrow); and (3) chlororespiration through NDH complex and plastidial terminal oxidase (PTOX;
954 dashed green arrow). Dashed blue arrows indicate the transport of protons. The other abbreviations are
955 shown as follows: LHCII, light-harvesting complex in PSII; Fd, ferredoxin; FNR, Fd-NADP⁺
956 reductase; CBB cycle, the Calvin-Benson cycle; ATPase, chloroplast ATP synthase.

957

958 Fig. 2

959 Hypothetical alternative electron transport pathways in senescing leaves. (A) Photosynthetic linear
960 electron flow in non-senescing leaves. (B) Cyclic electron flow around photosystem I (PSI) in
961 senescing leaves with few or no photosystem II (PSII). (C) O₂-dependent alternative electron transport
962 via plastidial terminal oxidase (PTOX) in senescing leaves with few or no PSI. (D) Chlororespiration
963 via chloroplast NAD(P)H dehydrogenase (NDH) complex and PTOX in senescing leaves with few or
964 no PSII and PSI. Red and blue arrows indicate the electron and proton transport, respectively. The
965 other abbreviations are shown as follows: PQ, plastoquinone; Cyt *b₆/f*, cytochrome *b₆/f* complex;
966 ATPase, chloroplast ATP synthase

A



B

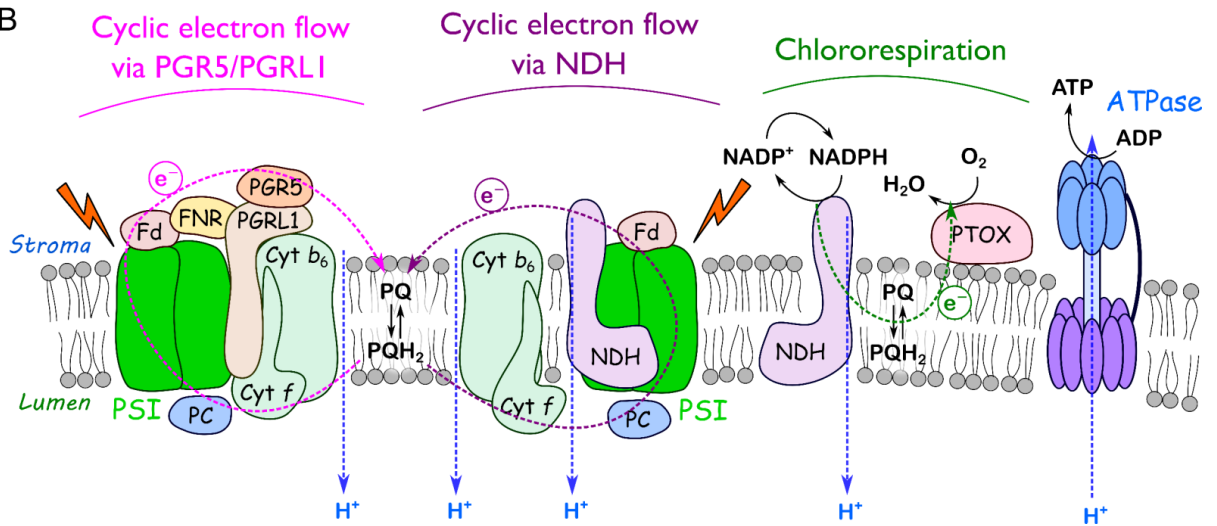


Fig. 1. Photosynthetic electron transport system in C_3 plants. (A) Overview of photosynthetic linear electron flow (dashed red arrow). Electrons from H_2O are transported from photosystem II (PSII) to photosystem I (PSI) via plastoquinone (PQ) pool, cytochrome (Cyt) b_6/f complex and plastocyanin, generating NADPH. Coupled with electron transport a proton gradient is built up that is used to produce ATP. (B) Overview showing three different alternative electron transport pathways: (1) cyclic electron flow via the protein Proton Gradient Regulation 5 (PGR5) and PGR5-like protein 1 (PGRL1; dashed pink arrow); (2) via chloroplast NAD(P)H dehydrogenase (NDH) complex (dashed purple arrow); and (3) chlororespiration through NDH complex and plastidial terminal oxidase (PTOX; dashed green arrow). Dashed blue arrows indicate the transport of protons. The other abbreviations are shown as follows: LHCII, light-harvesting complex in PSII; Fd, ferredoxin; FNR, Fd-NADP⁺ reductase; CBB cycle, the Calvin-Benson cycle; ATPase, chloroplast ATP synthase.

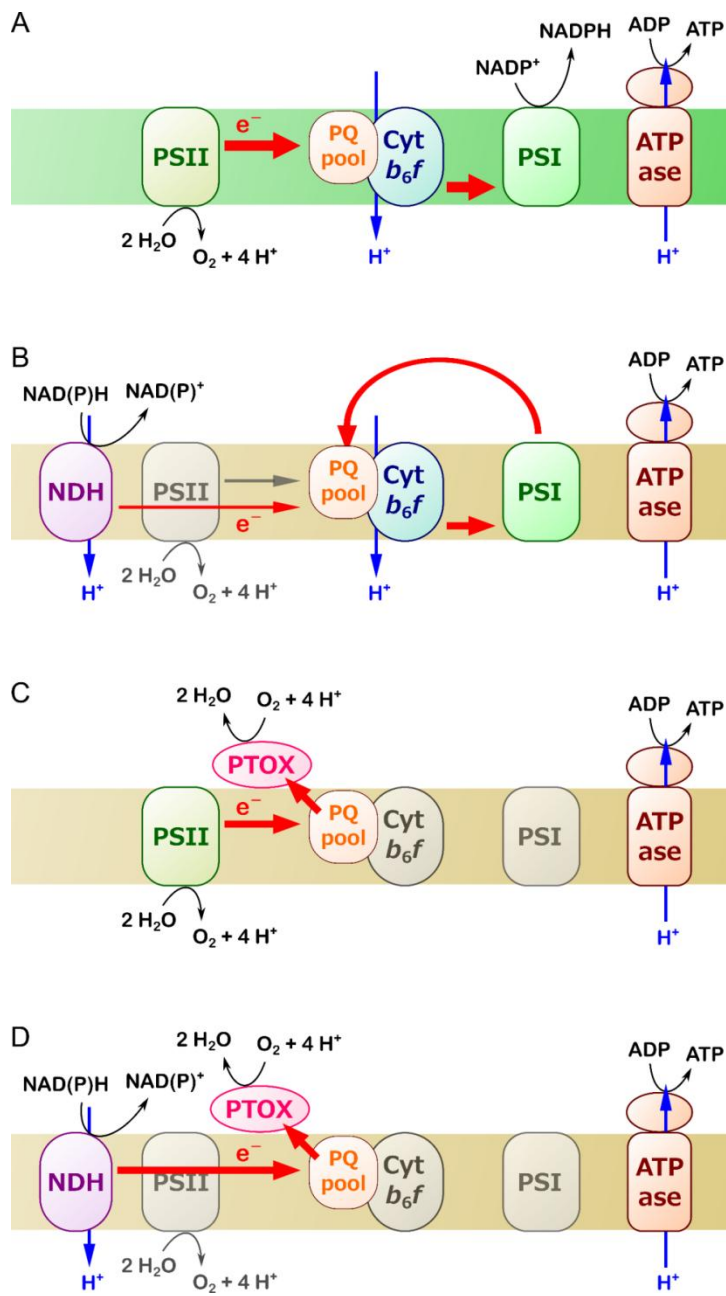


Fig. 2. Hypothetical alternative electron transport pathways in senescing leaves. (A) Photosynthetic linear electron flow in non-senescing leaves. (B) Cyclic electron flow around photosystem I (PSI) in senescing leaves with few or no photosystem II (PSII). (C) O_2 -dependent alternative electron transport via plastidial terminal oxidase (PTOX) in senescing leaves with few or no PSI. (D) Chlororespiration via chloroplast NAD(P)H dehydrogenase (NDH) complex and PTOX in senescing leaves with few or no PSII and PSI. Red and blue arrows indicate the electron and proton transport, respectively. The other abbreviations are shown as follows: PQ, plastoquinone; Cyt b_6/f , cytochrome b_6/f complex; ATPase, chloroplast ATP synthase.