High CO_2-mediated down-regulation of photosynthetic gene transcripts is caused by accelerated leaf senescence rather than sugar accumulation

Frank Ludewig[1], Uwe Sonnewald*

Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstraße 3, 06466 Gatersleben, Germany

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Abstract The influence of elevated atmospheric CO_2 on transcript levels of photosynthetic genes was investigated in leaves of *Nicotiana tabacum* cv. SamsunNN and cv. Wisconsin38 plants. Plants were grown under ambient (400 ppm) and elevated (800/1000 ppm) atmospheric CO_2, and transcript levels were determined in leaves of different age. Down-regulation of photosynthetic gene transcripts was apparent in senescing leaves only. A correlation between transcript levels and leaf contents of soluble sugars could not be found. To investigate whether a shift in leaf ontogeny would be involved in the regulation of photosynthetic genes transgenic tobacco plants expressing either the *gus* or *ipt* gene under control of the senescence-specific SAG-12 promoter [Gan, S. and Amasino, R.M. (1995) Science 270, 1986–1988] were included in our studies. As expected SAG-12-driven GUS activity increased with leaf age. This increase of GUS activity was stimulated by elevated atmospheric CO_2, accompanied by a loss of chlorophyll and the down-regulation of photosynthetic genes, verifying that high CO_2 accelerates leaf ontogeny. Senescence as well as down-regulation of photosynthetic genes could be delayed by *ipt* expression. Levels of soluble sugars were indistinguishable from wild type or even slightly elevated in *ipt* transgenic plants. Therefore, sugar accumulation as a cause for down-regulation of photosynthetic genes under high CO_2 can be excluded. It appears more likely that the high CO_2-mediated decline in photosynthetic gene transcripts is due to a temporal shift in leaf ontogeny.

Key words: Carbon dioxide; Photosynthetic gene; Sugar regulation; Senescence; Transgenic plant; *Nicotiana tabacum*

1. Introduction

Plant responses to an elevated partial pressure of atmospheric CO_2 have been studied in great detail. Theoretically, elevated levels of atmospheric CO_2 should lead to enhanced photosynthetic CO_2 uptake for at least two reasons: (1) photorespiration will be repressed and (2) substrate binding of Rubisco will be enhanced. However, this gain is often counteracted by a decreased photosynthetic capacity termed acclimation or down-regulation of photosynthesis (for review see [2]). Acclimation is accompanied by a reduced accumulation of Rubisco protein, which is assumed to reflect changes in gene expression. An often observed increase of soluble sugars under elevated CO_2 has been claimed to be responsible for the regulation of photosynthetic gene expression and thereby the down-regulation of photosynthesis [3,4]. In agreement with this hypothesis several genes have been shown to be regulated by external sugars (for recent review see [5–9]). Depending on their response to sugars plant genes can be grouped into three classes: 'famine', 'feast' and non-responding genes. Expression of 'famine' genes is increased by sugar depletion, whereas expression of 'feast' genes is enhanced by sugar abundance (definition in [5]). Photosynthetic genes belong to the first (sugar-repressed) class, which is in agreement with the above mentioned hypothesis. Although found in many investigations [10,11] a negative correlation between assimilate content and leaf photosynthetic rates has not always been found. Ludewig et al. [12] studied the regulation of photosynthetic genes in transgenic potato plants with a reduced leaf-starch content. The starchless phenotype was achieved by leaf-specific antisense inhibition of ADP-glucose pyrophosphorylase. As a consequence of reduced starch content soluble sugars increased in leaves. Interestingly, expression of photosynthetic genes did not decline under elevated atmospheric CO_2 although the level of soluble sugars increased. These data could be confirmed by Sun et al. [13] using starchless *Arabidopsis thaliana* mutants.

These and other studies indicate that soluble sugars are unlikely to be responsible for the observed down-regulation of photosynthetic rates under elevated atmospheric CO_2. Therefore, an alternative explanation is required to explain the phenomena of acclimation. An attractive hypothesis has been put forward by Miller et al. [14]. Typically, photosynthetic rates of dicot leaves undergo two distinct phases during ontogeny. During leaf development photosynthetic rates increase, which correlates with leaf expansion. The second phase is characterised by the decline of photosynthetic rates, which is paralleled by leaf senescence. Studying the ontogenetic development of tobacco leaves under ambient and elevated CO_2 Miller et al. [14] observed that the pattern of photosynthetic rates over time was similar between both treatments. However, under high CO_2 leaf senescence and the decline of photosynthetic rates was accelerated. Therefore, the authors speculated that the observed down-regulation of photosynthetic rates is caused by an accelerated senescence rather than accumulation of soluble sugars. Unfortunately, the leaf soluble sugar content was not determined in this study.

Senescence is regulated by several factors including plant growth regulators such as ABA, ethylene, and cytokinin (for review see [15]). ABA and ethylene have been shown to promote senescence whereas cytokinin application inhibits leaf senescence. To manipulate leaf senescence Gan and Amasino [1] created transgenic tobacco plants expressing the *ipt* gene

*Corresponding author. Fax: (49)-39482-5515.
E-mail: sonnewal@ipk-gatersleben.de

[1] Present address: Institut für Botanik, Universität Köln, Gyrhofstrasse 15, 50931 Cologne, Germany.

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under control of the senescence-specific SAG-12 promoter [16]. The *ipt* gene encodes a bacterial isopentenyl transferase which catalyses the rate limiting step in cytokinin biosynthesis. As shown by GUS-fusion experiments the SAG-12 promoter is activated at the onset of senescence, leading to the synthesis of cytokinin. Increased levels of cytokinin will inhibit senescence and thereby attenuate SAG-12-driven expression. This prevents overproduction of cytokinin that otherwise would interfere with normal plant development.

The aim of the present work was to determine (1) whether elevated atmospheric CO_2 leads to an accelerated leaf senescence and (2) whether this coincides with down-regulation of photosynthetic genes. To this end transgenic plants expressing either the GUS reporter or the *ipt* gene under control of the SAG-12 promoter were cultivated under ambient or elevated CO_2. Subsequently, leaf samples were taken during leaf development and analysed for sugar content, GUS activity, and expression of photosynthetic genes. This study provides evidence that CO_2-mediated down-regulation of photosynthetic genes coincides with accelerated leaf senescence.

2. Materials and methods

2.1. Plant material and growth conditions

(a) Seeds of tobacco (*Nicotiana tabacum* cv. SamsunNN) plants were sown in soil and grown under ambient CO_2. 10 days after germination seedlings were cultivated either under 400 ppm (ambient) or 800 ppm (elevated) CO_2, with supplementary light in a 16 h/8 h day/night cycle at ca. 20°C in 7.5 l pots at harvest under green house conditions. Plants were grown in a 2:1 mixture of soil and 'Substrat 2' (Klasmann, Geeste-Groß-Hesepe, Germany) and watered daily. (b) Seeds of SAG12-GUS and SAG12-IPT transgenic tobacco (*N. tabacum* cv. Wisconsin 38) were treated in a similar way with the exception that seedlings were transferred to growth chambers. Growth chamber conditions were at 400 ppm (ambient) and 1000 ppm (elevated) CO_2, respectively, with a light intensity of 450 μE in a 12 h day/night cycle at 24°C (in light) and 20°C (in dark) with 60% relative humidity in 2.5 l pots during the whole growth period. Plants were grown in 'Substrat 1' (Klasmann, Geeste-Groß-Hesepe, Germany), watered daily and fertilised weekly with 500 ml of a 5‰ solution of 'HaKaPhos blau' (Compo, Münster, Germany) containing 15% N (4% as NO_3^-, 11% as NH_4^+), 10% P_2O_5, 15% K_2O, 2% MgO and 0.01% B, Cu*, Zn*, 0.05% Fe*, Mn* and 0,001% Mo. Asterisks indicate the ions were chelated with EDTA.

2.2. RNA isolation and Northern blot analysis

Tobacco leaves were harvested at the indicated times and RNA was isolated essentially as described in [17] with the following modifications. Aqueous RNA solutions were diluted with DEPC-treated water to a final volume of 0.5 ml. One volume 5 M LiCl (in DEPC-treated water) was added, mixed and incubated for 30 min at 12°C. During this time RNA precipitates, whereas contaminating genomic DNA and high molecular weight polysaccharides remain soluble. After 15 min. centrifugation at 15000 rpm the pellet was washed with 3 M Na-acetate pH 5.2 (in DEPC-treated water) and 75% ethanol (in DEPC-treated water). Purified RNA was then solved in an appropriate volume of DEPC-treated water and 20 μg total RNA was separated on 1.5% agarose gels containing formaldehyde and blotted on nylon membranes (GeneScreen, NEN, Boston, MA, USA). Radioactive labelling of cDNA fragments was carried out using the High Prime kit (Boehringer, Mannheim, Germany) and [α-^{32}P]dCTP. Hybridisation was performed as described in [18], and detection was carried out on an imaging analyser (Fuji BAS 2000, Fuji, Tokyo).

2.3. Physiological measurements

Samples for the determination of leaf contents of soluble sugars and starch were harvested from the same leaves taken for RNA preparation. Samples for the determination of chlorophyll, protein and β-glucuronidase activity were taken from different developmental leaf stages as indicated. Soluble sugars, starch and chlorophyll were determined as in [19]. The protein content was analysed according to [20] and β-glucuronidase activity was measured according to [21] using the 'SpectraFluor' fluorimeter (Tecan, Crailsheim, Germany).

3. Results and discussion

3.1. Influence of atmospheric CO_2 on gene expression during tobacco leaf development

Molecular mechanisms underlying acclimation responses to elevated CO_2 are largely unknown. Miller et al. [14] reported that elevated CO_2 results in an earlier onset of the natural decline in photosynthetic rates associated with leaf senescence. Analysing the influence of sugars on transcript levels of photosynthetic leaves Van Oosten et al. [3,4] proposed that nuclear genes encoding chloroplast proteins would be repressed by accumulation of photosynthetic end-products which results in photosynthetic acclimation to high CO_2. To study the relative importance of leaf senescence and/or sugar levels on gene expression in leaves tobacco plants (*N. tabacum* cv. SamsunNN) were cultivated under ambient (400 ppm) and elevated (800 ppm) CO_2. 70 days after germination leaf samples were taken for RNA, chlorophyll, and sugar analysis. At harvest plants had developed 26 leaves (size of leaf number 1 was 5 cm in length). Starting from leaf 6 (from top) always three successive leaves were combined for RNA, chlorophyll, and sugar analysis. As expected the leaf chlorophyll content decreased with leaf age and ranged from 452.0 ± 8.7 to 212.1 ± 12.7 mg m^{-2} (mean ± S.E.M., $n = 4$) under ambient and from 414.2 ± 29.4 to 151.0 ± 12.1 mg m^{-2} (mean ± S.E.M.,

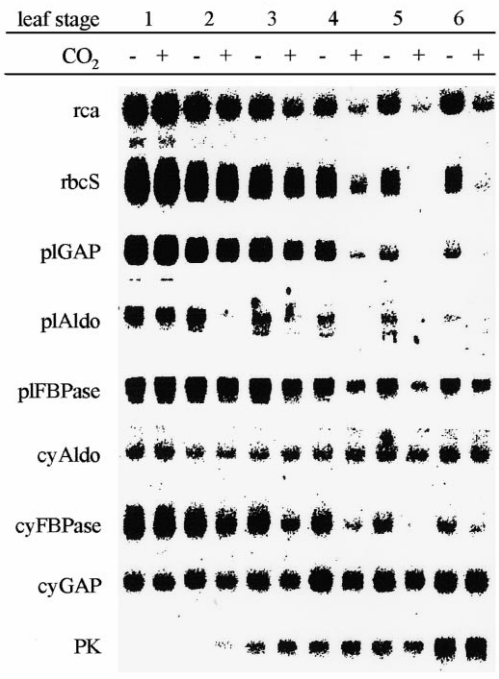

Fig. 1. Northern blot analysis of tobacco plants grown under either ambient (−) or elevated (+) atmospheric CO_2 for 70 days. Samples were taken from upper (1) to lower (6) source leaf stages 6 h after illumination. 20 μg of total RNA was loaded in each lane. Probes were cDNAs for rca, accession number: Z14981; rbcS, [22]; plGAP, accession number: M14417, of tobacco; plAldo, accession number: Y10380; plFBPase, [23]; cyAldo, Badur and Zrenner, unpublished; cyFBPase, accession number: X76946; cyGAP, accession number: M14419 and PK, accession number: X53688 of potato.

$n = 4$) under elevated atmospheric CO_2 conditions. As shown in Fig. 1 transcripts of the photosynthetic genes Rubisco activase (rca), the small subunit of Rubisco (rbcS), plastidic glyceraldehyde-3-phosphate dehydrogenase (plGAP), plastidic aldolase (plAldo) and plastidic fructose-1,6-bisphosphatase (plFBPase) were found to be coordinately down-regulated during leaf development (from leaf stage 1–6; Fig. 1). As has been found for photosynthetic rates the pattern was similar between ambient and high CO_2 conditions. However, the decrease was found to be accelerated under high CO_2. In parallel to photosynthetic gene transcripts cytosolic fructose-1,6-bisphosphatase (cyFBPase) mRNA levels decreased with leaf age. This co-regulation supports the notion that cyFBPase is involved in sucrose biosynthesis during the light period, whereas at night sucrose synthesis occurs via an cyFBPase independent pathway. This assumption is supported by results obtained from transgenic plants characterised by decreased levels of either triose-phosphate translocator [24] or cytosolic FBPase [25]. In these plants hexose and/or hexose-phosphates are exported from chloroplasts to feed the sucrose biosynthetic pathway at night.

In contrast, transcript levels of genes encoding glycolytic enzymes are either not influenced (cytosolic aldolase, cyAldo; cytosolic glyceraldehyde-3-phosphate dehydrogenase, cyGAP) or increase during leaf development (pyruvate kinase, PK). Expression of these genes was not regulated by atmospheric CO_2.

3.2. *Accumulation of photosynthetic end-products in leaves does not correlate with the down-regulation of photosynthetic gene transcripts under elevated CO_2 conditions*

Depending on plant species and investigation a wide variation in the accumulation of soluble sugars in leaves of plants grown under elevated CO_2 has been reported. In leaves of *Glycine max* (soybean) and *Triticum aestivum* (wheat) sucrose accumulated [26,27], in *A. thaliana* hexose levels increased [28], and in *Solanum tuberosum* (potato) both sucrose and hexose content increased under elevated CO_2 conditions [29].

To analyse high CO_2-mediated changes in sugar content during tobacco leaf development glucose, fructose, sucrose, and starch contents were determined in leaves which had been used to determine transcript levels (Fig. 1). As shown in Fig. 2 elevated CO_2 did not lead to accumulation of soluble sugars (glucose, fructose and sucrose) in tobacco leaves. During development the leaf content of sucrose declined whereas hexose levels slightly increased in older (senescing) leaves. The total amount of soluble sugars, however, did not increase during leaf development. Therefore, soluble sugars as end-products of photosynthesis are unlikely to be responsible for the observed down-regulation of photosynthetic genes in leaves of tobacco plants. This is in contradiction to observations from Van Oosten and co-workers [30,31] who observed a correlation between soluble sugars and decreased levels of photosynthetic gene transcripts. In contrast to soluble sugars a clear induction of leaf starch content was visible under elevated CO_2 conditions (Fig. 2). This increase in leaf starch content, however, did not parallel changes in the repression of photosynthetic gene transcripts either. Whereas down-regulation of photosynthetic gene transcripts became visible in leaf stage 4 (Fig. 1), starch accumulation was observed throughout leaf development (Fig. 2).

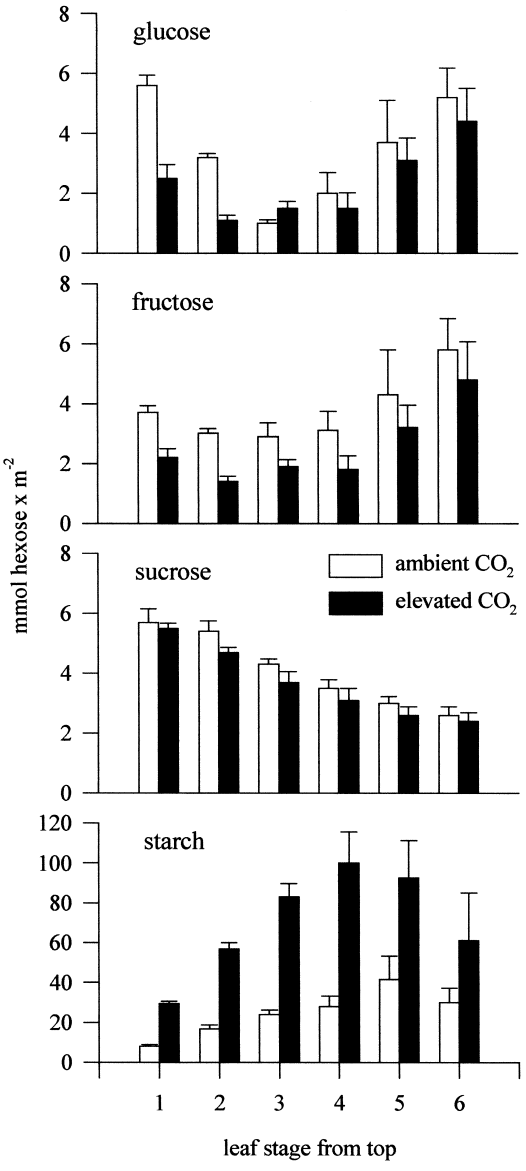

Fig. 2. Glucose, fructose, sucrose and starch contents of upper (1) to lower (6) source leaf stages of 70 days old tobacco plants. Samples were taken 6 h after illumination. Results are given as mean ± S.E.M. ($n = 5$). Plants were grown under either ambient (open bars) or elevated (filled bars) atmospheric CO_2.

3.3. *Elevated atmospheric CO_2 led to an accelerated leaf ontogeny*

Screening for senescence-associated genes (SAGs) from *A. thaliana*, Amasino and co-workers observed that expression of SAG12 was highly senescence specific [1]. Thus, transgenic plants expressing the GUS reporter gene under control of the SAG12 promoter are powerful tools to quantify the senescence state of leaves by analyzing their β-glucuronidase activity. To prove that the leaf ontogeny of plants grown under elevated atmospheric CO_2 was accelerated transgenic tobacco (*N. tabacum* cv. Wisconsin 38) plants expressing the GUS reporter gene under control of the SAG12 promoter (kindly provided by Richard M. Amasino) were used in our studies.

These plants were grown for 56 days under ambient (400 ppm) and elevated (1000 ppm) CO_2, and leaves of different age were analysed for β-glucuronidase activity. Plants used for

biochemical analysis had reached the 23 leaf stage. Leaf numbers given below are counted from the top of a tobacco plant with leaf number 1 being 5 cm in length. Under both growth conditions β-glucuronidase activity increased with leaf age. Under elevated CO_2, β-glucuronidase activity became visible in leaf number 17 (from the top), whereas under ambient CO_2 conditions β-glucuronidase activity was detectable starting from leaf number 20 (Fig. 3). Comparing elevated with ambient CO_2 it is obvious that the β-glucuronidase activity is dramatically increased under high CO_2 conditions. These results demonstrate that plant growth under elevated atmospheric CO_2 led to an accelerated leaf senescence. This is in agreement with the conclusion drawn by Miller et al. [14] and with the observation that *Lycopersicon esculentum* (tomato) plants grown under high CO_2 conditions produce more ethylene [32] which is paralleled by an accelerated flower formation and fruit ripening [33] indicating an accelerated ontogeny under elevated CO_2.

3.4. SAG12-driven expression of isopentenyl transferase led to a delayed chlorophyll loss in leaves of high CO_2-cultivated tobacco plants

To further analyse whether accelerated senescence is responsible for the repression of photosynthetic genes transgenic tobacco plants expressing the *ipt* gene under control of the SAG12 promoter (SAG12-IPT, [1]) were included in our study. The *ipt* gene encodes a bacterial isopentenyl transferase catalysing the rate limiting step in cytokinin formation. These plants have been demonstrated to be delayed in leaf senescence [15]. As one criteria for leaf senescence the chlorophyll content can be considered. Usually, the chlorophyll content declines during leaf age. Therefore, the chlorophyll content of different leaf stages of 56 days old SAG12-GUS and SAG12-IPT plants was determined under ambient and elevated atmospheric CO_2. In SAG12-GUS control plants a continuous decline of the chlorophyll content with increasing leaf age (upper panel, from left to right; Fig. 4) was found under ambient and elevated atmospheric CO_2. This decline in chlorophyll content was strongly accelerated under high CO_2 conditions. In contrast to GUS control plants the chlorophyll content of SAG12-IPT plants decreased only slightly in older leaves (Fig. 4, lower panel). Although, elevated CO_2 led to a reduced chlorophyll content in older leaves of SAG12-IPT plants, the decrease in chlorophyll content was much less pronounced as compared to GUS control plants. These results indicate that the CO_2-mediated acceleration of leaf senescence was delayed in SAG12-IPT plants.

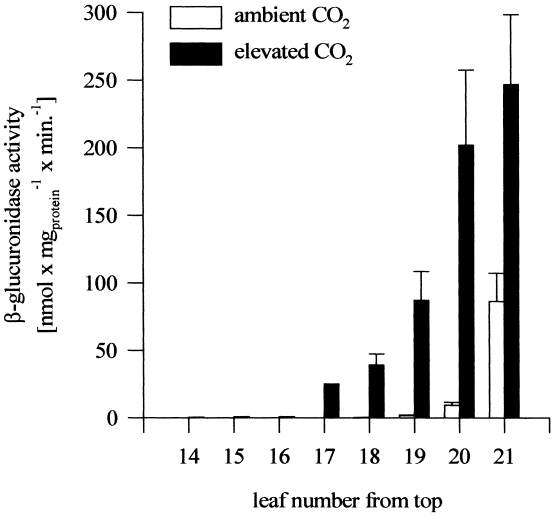

Fig. 3. β-Glucuronidase activity in upper (14) to lower (21) source leaves of 56 days old SAG12-GUS tobacco plants. At harvest leaf number 1 was 5 cm in length. Samples were taken 3 h after illumination. Results are given as mean ± S.E.M. ($n = 3$ or 4). Plants were grown under either ambient (open bars) or elevated (filled bars) atmospheric CO_2.

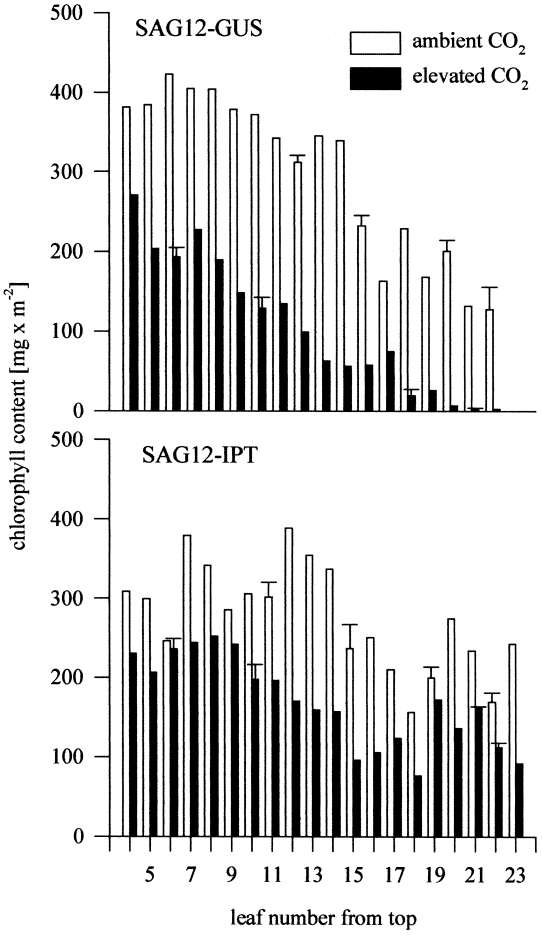

Fig. 4. Chlorophyll content of upper (4) to lower (23) source leaves of 56 days old SAG12-GUS and SAG12-IPT tobacco plants. At harvest leaf number 1 was 5 cm in length. Samples were taken 3 h after illumination. Results are given as mean ± S.E.M. ($n = 4$, indicated by an error bar) or as single values. Plants were grown under either ambient (open bars) or elevated (filled bars) atmospheric CO_2.

3.5. SAG12-driven expression of isopentenyl transferase delays down-regulation of photosynthetic gene transcripts under elevated CO_2 conditions

As discussed in the previous sections several lines of evidences suggest that the frequently observed phenomena of acclimation to elevated CO_2 conditions is a consequence of accelerated plant ontogeny. Therefore, analysis of the regulation of photosynthetic genes in transgenic plants with delayed leaf senescence should allow to address the question whether senescence is the main factor regulating expression of these genes under elevated CO_2. To this end leaf RNA from 56 days old GUS control (Fig. 5, lanes 1–6) and *ipt* expressing tobacco

plants (Fig. 5, lanes 7–12) was isolated and probed for the presence of photosynthetic gene transcripts. As expected from data shown in Fig. 1, transcript levels of rbcS, plAldo and plGAP declined with leaf age. Under elevated CO_2 conditions (Fig. 5, lanes 4–6) this decline was strongly enhanced as compared to ambient CO_2 (Fig. 5, lanes 1–3). This age-dependent decline in photosynthetic gene transcripts could be overcome by expression of the *ipt* gene (Fig. 5, lanes 7–12) which also suppressed the CO_2-mediated down-regulation of photosynthetic gene transcripts (Fig. 5, lanes 10–12). As has been observed for wild type tobacco plants (Fig. 1) cyGAP-specific transcripts are not influenced by leaf age or CO_2 conditions. This result could be confirmed in GUS reporter and *ipt* expressing plants. As discussed earlier ethylene evolution has been shown to be stimulated by high CO_2 [32]. Since ethylene induces senescence and negatively regulates expression of photosynthetic genes, it could be involved in down-regulation of photosynthetic genes during senescence. To study possible changes in the capability of ipt expressing plants to synthesise ethylene, expression of Acc-oxidase and Acc-synthase was analysed by Northern blotting. As expected expression of both genes was slightly induced under elevated CO_2 conditions in control plants (Fig. 5, lanes 4–6). Interestingly this induction was not altered in ipt expressing plants (Fig. 5, lanes 10–12) ruling out a prominent role of ethylene in down-regulation of photosynthetic genes under elevated CO_2 conditions.

Wingler et al. [34] used the SAG12-IPT plants to investigate interactions between cytokinin, sugar repression and light in the senescence-related decline of photosynthetic enzymes of leaves under ambient CO_2 conditions. In *ipt* expressing plants increased amounts of photosynthetic proteins (Rubisco, plFBPase, plAldo and plGAP) were observed in older leaves as compared to wild type plants. Furthermore, the decline of chlorophyll content with leaf age was diminished. These findings matched our results, albeit differences between control and SAG12-IPT plants in Northern experiments were much more pronounced under elevated CO_2. Thus, preventing leaf senescence gives rise to a longer persistence of photosynthetic gene transcripts and enzymes within tobacco leaves.

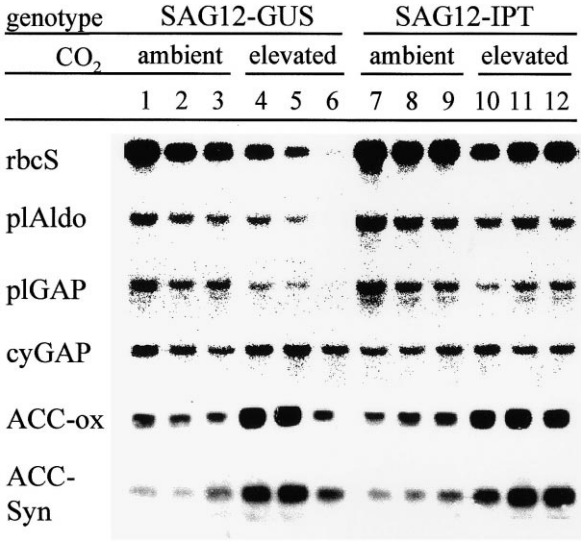

Fig. 5. Northern blot analysis of SAG12-GUS and SAG12-IPT tobacco plants grown under either ambient or elevated atmospheric CO_2 for 56 days. Samples were taken from upper (8–10 from top; lanes 1, 4, 7, 10), intermediate (13–15 from top; lanes 2, 5, 8, 11) and lower (18–20 from top; lanes 3, 6, 9, 12) source leaves 3 h after illumination. 20 μg of total RNA was loaded in each lane. Probes were cDNAs for rbcS, plGAP, ACC-oxidase (ACC-ox, accession number: AB012857), and ACC-synthase (ACC-syn, accession number: X98492) of tobacco and plAldo, and cyGAP of potato.

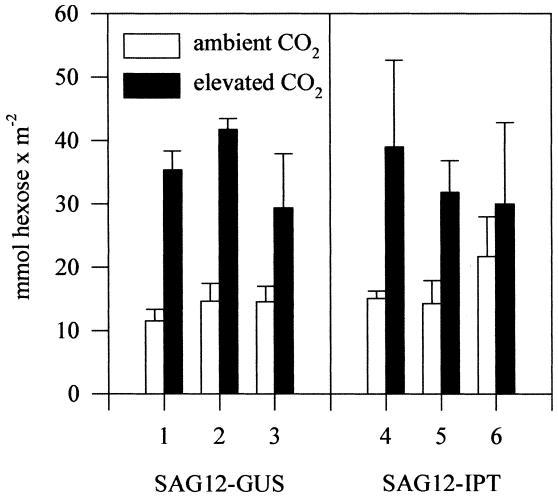

Fig. 6. Soluble sugar (sum of glucose, fructose and sucrose) content of upper (8–10 from top; 1, 4), intermediate (13–15 from top; 2, 5) and lower (18–20 from top; 3, 6) source leaves of 56 days old SAG12-GUS and SAG12-IPT tobacco plants. Samples were taken 3 h after illumination. Results are given as mean ± S.E.M. ($n = 4$). Plants were grown under either ambient (open bars) or elevated (filled bars) atmospheric CO_2.

To exclude that the observed suppression of down-regulation of photosynthetic gene transcripts in old leaves of SAG12-IPT plants was due to a reduced accumulation of soluble sugars, the leaf content of glucose, fructose and sucrose was determined (Fig. 6) in the same leaves which had been used for Northern analyses. As can be seen in Fig. 6, there is no significant difference in the accumulation of soluble sugars between SAG12-GUS (left part of Fig. 6) and SAG12-IPT (right part of Fig. 6) plants. In this experiment we observed a strong increase of soluble sugars in leaves under elevated atmospheric CO_2 conditions (filled bars, Fig. 6). This is in contrast to data shown in Fig. 2. The reason for this discrepancy most likely resides in the different growth conditions (indicated in Section 2). Irrespective of these differences sugar levels in GUS or *ipt* expressing plants do not correlate with the expression of photosynthetic gene transcripts. This strongly argues against a role of soluble sugars in the regulation of photosynthetic gene transcripts under elevated atmospheric CO_2. The age-dependent regulation of photosynthetic gene transcripts has been analysed by Jiang and Rodermel [35] using different leaf stages of tobacco (*N. tabacum*) plants grown under ambient CO_2 conditions and Bate et al. [36] using source leaves of *Phaseolus vulgaris* (bean) plants of different age. In both cases rbcS (as a photosynthetic gene) transcripts were down-regulated with increasing age.

3.6. Conclusion

Careful analysis of transgenic tobacco plants delayed in leaf senescence supports the hypothesis that acclimation is the result of an earlier onset of the natural occurring down-regulation of photosynthesis which is associated with senescence. In tobacco plants no evidence for the involvement of photosynthetic end-products in the down-regulation of photosyn-

thesis could be obtained. The mechanism underlying induction of senescence under elevated CO_2 is unknown. However, elevated levels of CO_2 may accelerate senescence by increasing the flow of sugars through hexokinase. This view is supported by the observation that overexpression of hexokinase leads to accelerated senescence of transgenic tomato plants [37].

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References

[1] Gan, S. and Amasino, R.M. (1995) Science 270, 1986–1988.
[2] Webber, A.N., Nie, G.-Y. and Long, S.P. (1994) Photosynth. Res. 39, 413–425.
[3] Van Oosten, J.J. and Besford, R.T. (1994) J. Plant Physiol. 143, 306–312.
[4] Van Oosten, J.J., Wilkins, D. and Besford, R.T. (1994) Plant Cell Environ. 17, 913–923.
[5] Koch, K.E. (1996) Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 509–540.
[6] Smeekens, S. (1998) Curr. Opin. Plant Biol. 3, 230–234.
[7] Yu, S.-M. (1999) Plant Physiol. 121, 687–693.
[8] Sheen, J., Zhou, L. and Jang, J.-C. (1999) Curr. Opin. Plant Biol. 2, 410–418.
[9] Roitsch, T. (1999) Curr. Opin. Plant Biol. 2, 198–206.
[10] Goldschmidt, E.E. and Huber, S.C. (1992) Plant Physiol. 99, 1443–1448.
[11] Martinez-Carrasco, R., Cervantes, E., Perez, P., Morcuende, R. and del Molino, I.M.M. (1993) Physiol. Plant. 89, 453–459.
[12] Ludewig, F., Sonnewald, U., Kauder, F., Heineke, D., Geiger, M., Stitt, M., Müller-Röber, B.T., Gillissen, B., Kühn, C. and Frommer, W.B. (1998) FEBS Lett. 429, 147–151.
[13] Sun, J., Okita, T.W. and Edwards, G.E. (1999) Plant Physiol. 119, 267–276.
[14] Miller, A., Tsai, C.H., Hemphill, D., Rodermel, S. and Spalding, M. (1997) Plant Physiol. 115, 1195–1200.
[15] Gan, S. and Amasino, R.M. (1997) Plant Physiol. 113, 313–319.
[16] Lohmann, K.N., Gan, S., John, M.C. and Amasino, R.M. (1994) Physiol. Plant. 92, 322–328.
[17] Logemann, J., Schell, J. and Willmitzer, L. (1987) Anal. Biochem. 163, 16–20.
[18] Herbers, K., Mönke, G., Badur, R. and Sonnewald, U. (1995) Plant Mol. Biol. 29, 1027–1038.
[19] Sonnewald, U. (1992) Plant J. 2, 571–581.
[20] Bradford, M.M. (1976) Anal. Biochem. 72, 243–254.
[21] Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) EMBO J. 6, 3901–3907.
[22] Muller, K.-D., Salnikow, J. and Vater, J. (1983) Biochim. Biophys. Acta 742, 78–83.
[23] Koßmann, J., Müller-Röber, B., Dyer, T.A., Raines, C.A., Sonnewald, U. and Willmitzer, L. (1992) Planta 188, 7–12.
[24] Heineke, D., Kruse, A., Flügge, U.-I., Frommer, W.B., Riesmeier, J.W., Willmitzer, L. and Heldt, H.W. (1994) Planta 193, 174–180.
[25] Zrenner, R., Krause, K.-P., Apel, P. and Sonnewald, U. (1996) Plant J. 9, 671–681.
[26] Sicher, R.C., Kremer, D.F. and Bunce, J.A. (1995) Photosynth. Res. 46, 409–417.
[27] Nie, G., Hendrix, D.L., Webber, A.N., Kimball, B.A. and Long, S.P. (1995) Plant Physiol. 108, 975–983.
[28] Cheng, S.-H., Moore, B.D. and Seemann, J.R. (1998) Plant Physiol. 116, 715–723.
[29] Kauder, F., Ludewig, F. and Heineke, D. (2000) J. Exp. Bot. 51, 429–437.
[30] Van Oosten, J.J. and Besford, R.T. (1995) Plant Cell Environ. 18, 1253–1266.
[31] Van Oosten, J.J. and Besford, R.T. (1996) Photosynth. Res. 48, 353–365.
[32] Woodrow, L. and Grodzinski, B. (1993) J. Exp. Bot. 44, 471–480.
[33] Micallef, B.J., Haskins, K.A., Vanderveer, P.J., Roh, K.-S., Shewmaker, C.K. and Sharkey, T.D. (1995) Planta 196, 327–334.
[34] Wingler, A., von Schaewen, A., Leegood, R.C., Lea, P.J. and Quick, W.P. (1998) Plant Physiol. 116, 329–335.
[35] Jiang, C.-Z. and Rodermel, S.R. (1995) Plant Physiol. 107, 215–224.
[36] Bate, N.J., Rothstein, S.J. and Thompson, J.E. (1991) J. Exp. Bot. 42, 801–811.
[37] Dai, N., Schaffer, A., Petreikov, M., Shahak, Y., Giller, Y., Ratner, K., Levine, A. and Granot, D. (1999) Plant Cell 11, 1253–1266.