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Violaxanthin Cycle Pigment Contents in Potato and Tobacco Plants with Genetically Reduced Photosynthetic Capacity[1]

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The influence of photosynthetic activity on the light-dependent adaptation of the pool size of the violaxanthin cycle pigments (violaxanthin + antheraxanthin + zeaxanthin) was studied in leaves of wild-type and transgenic potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.) plants. The genetically manipulated plants expressed an antisense mRNA coding for the chloroplastic fructose-bisphosphatase. Chl fluorescence quenching analysis revealed that the transformed plants exhibited a greatly impaired electron transport capacity. Light-limited and light-saturated nonphotochemical quenching was strongly enhanced in the mRNA antisense potato plants. After 7 d of adaptation at various high photosynthetic photon flux densities (PPFDs), the violaxanthin cycle pool size increased, with a progressive elevation in PPFD. The pool size was higher for transgenic potatoes than for wild-type plants at all PPFDs. This difference vanished when pool size was correlated with the PPFD in excess of photosynthesis, as indicated by the epoxidation state of the violaxanthin cycle. Contrasting results were obtained for tobacco; in this species, photosynthetic activity did not affect the pool size. We conclude that regulatory mechanisms exist in potato, by which photosynthetic activity can influence the violaxanthin cycle pool size. Furthermore, evidence is provided that this adaptation of the pool size may contribute to an improved photoprotection of the photosynthetic apparatus under high-light conditions. However, tobacco plants seem to regulate their pool size independently of photosynthetic activity.

Plants growing in full sunlight often receive and absorb more light than they are able to use for photosynthesis. This excess absorbed energy may cause photoinhibition or even photobleaching (Baker and Bowyer, 1994; Long et al., 1994). During evolution, plants have adapted to this type of stress and have developed a variety of protective mechanisms (Björkman and Demmig-Adams, 1994), e.g. the regulated increase of radiationless dissipation of absorbed light in the antenna or at the reaction center of PSII, which causes qN-quenching of Chl fluorescence (for reviews, see Krause and Weis, 1991; Demmig-Adams and Adams, 1992a; Horton et al., 1994).

Although the exact molecular mechanism of Z action is not yet resolved, evidence has accumulated that the carotenoid Z is involved in the regulation of nonradiative energy dissipation (Demmig-Adams, 1990; Pfündel and Bilger, 1994). Under high-light conditions, Z can be synthesized rapidly by the deepoxidation of the carotenoid V via the intermediate A. Since under low light Z is epoxidized again to V, this sequence of reactions has been termed the V cycle. It has been demonstrated that a large proportion of qN-quenching depends on the presence of both Z and a ΔpH across the thylakoid membrane (Gilmore and Yamamoto, 1992, 1993; Rees et al., 1992; Bilger and Björkman, 1994). In particular, when Z is limiting, qN-quenching quantified by the Stern-Volmer formalism is linearly correlated with the Z content of leaves (Demmig-Adams, 1990; Bilger and Björkman, 1991).

Additional evidence for a photoprotective action of Z has been deduced from the xanthophyll cycle pool size, i.e. the total content of V, Z, and the intermediate A in leaves. A strong dependence of the pool size on the PPFD during growth has been demonstrated (Hager, 1957; Thayer and Björkman, 1990; Demmig-Adams and Adams, 1992b, 1994; Johnson et al., 1993a). Irrespective of expression on a leaf area or a Chl basis, sun leaves contained four to five times as much V cycle pigments as shade leaves (Thayer and Björkman, 1990). This adaptation was reported to be of a highly dynamic nature. Shade leaves transferred to the sun adjusted their pool size within 5 to 6 d, and the reverse effect was seen when sun leaves were shaded (Björkman and Demmig-Adams, 1994). Such a strong regulation seems to emphasize the adaptive significance of the V cycle pool size. It was suggested that this acclimation reflects the photoprotective nature of Z, since an enhanced pool size will allow increased Z formation (Thayer and Björkman, 1990; Demmig-Adams and Adams, 1994).

Thayer and Björkman (1990) observed that the pool size of several sun-grown plants was inversely related to photosynthetic capacity. They speculated that an elevated ca-

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Abbreviations: A, antheraxanthin; cp-FBPase, chloroplastic fructose-bisphosphatase; ΔF, $F_M' - F$; EPS, epoxidation state; F, stationary fluorescence yield; F_M, F_M', maximal fluorescence yield with all reaction centers closed in the dark and in the light-adapted state, respectively; qN-quenching, nonphotochemical quenching; V, violaxanthin; Z, zeaxanthin.

pacity for Z formation in plants with lower photosynthetic capacity could be related to the increased demand for radiationless dissipation of excessive absorbed light. Schöner and Krause (1990) found that leaves of spinach plants, which where acclimated to low temperature (1°C) and thus impaired in photosynthetic energy conversion, contained a larger V cycle pool compared to leaves that developed at 20°C. On the other hand, Demmig-Adams and Adams (1992b) reported that in sun-grown crop plants with a high photosynthetic rate, the V cycle pool was larger than in a group of perennial shrubs and vines grown under comparable conditions that had lower maximal photosynthetic rates. Hence, it remains unclear if the adaptation of the pool size served a photoprotective role.

In these early studies, a variety of species was used that differed with respect to leaf anatomy and genetic potential. This complicates a comparison of plants with low and high photosynthetic rates. Therefore, we used plants of a single species, with varying photosynthetic capacities. Recently, transgenic potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.) plants have been constructed that express an antisense mRNA to cp-FBPase, a key enzyme of the reductive pentose phosphate cycle (Kossmann et al., 1994). These plants exhibit a strongly reduced light-saturated rate of O_2 evolution (Kossmann et al., 1994). Depending on the extent of antisense mRNA expression, the genetically manipulated lines show high qN-quenching, even at low light intensities (Fisahn et al., 1995). When grown at low or moderate PPFD, they have an almost indistinguishable phenotype.

Using these transgenic and wild-type plants, it was possible to expose genetically very similar plants to identical PPFDs. However, since photosynthetic capacity differed between wild-type and transgenic plants, they were at the same time exposed to different degrees of excess PPFD, i.e. that PPFD that could not be utilized for photosynthetic electron transport. This protocol enabled us to test the hypothesis that the adaptation of the V cycle pool size to the PPFD is in response to excessive PPFD.

MATERIALS AND METHODS

Experimental Plants

Potato (*Solanum tuberosum* L. cv Désirée) and tobacco (*Nicotiana tabacum* L. cv Samsun) were transformed as described by Kossmann et al. (1994). In the lines of transgenic potato plants used in our experiments, the residual expression of cp-FBPase was between 12 and 15% of the wild type. For the tobacco plants used, the expression was not determined. Expression varies between 10 and 90% in the lines of genetically manipulated tobacco plants (J. Kossmann, unpublished data). The reduction of light-saturated electron transport through PSII, as determined from Chl fluorescence analysis in transgenic plants used for our experiments, was approximately 70 and 50% in potato and tobacco, respectively.

Plants were grown from cell cultures and kept in a greenhouse at an average PPFD of 50 to 200 μmol m^{-2} s^{-1} and at air temperatures of 22°C. For the experiments, five transgenic and wild-type potato and tobacco plants were transferred to a phytocell (Type PB 3000, Brown-Boveri-York, Mannheim, Germany). The distance of the plants from the lamps was varied so that the leaves were exposed to a range of PPFDs between 100 and 1700 μmol m^{-2} s^{-1}. Irradiation was provided by an array of halogen lamps (HQI TS WD 400, Osram, Berlin, Germany). The photoperiod was 15 h at air temperatures of 24°C in the light and 20°C in darkness. After 7 d, leaf discs were punched with a corkborer (diameter, 9 mm) and frozen in liquid nitrogen within 20 s. Incident PPFD was determined for each sample, using a Li-Cor quantum meter (model 185B, Li-Cor, Lincoln, NE). The experiments were repeated at least three times, with independent plant lines and under slightly different conditions in other phytocells, and essentially the same results were obtained.

Chl Fluorescence Measurements

In each experiment, PSII quantum yield and qN-quenching were determined from leaves of greenhouse-grown plants, using a PAM-2000 Chl fluorometer (Walz, Effeltrich, Germany). After at least 2 h of dark adaptation, the attached leaves were fixed horizontally with thin, transparent fishing rope in a dark room. Subsequently, they were illuminated by a halogen cold-light source (Osram Xenophot HLX 64 634, 150 W) that was positioned at a distance of about 0.2 m and at an angle of 60° to the leaf. Long-wavelength radiation was reduced by an IR filter (Calflex X, Balzers, Liechtenstein) and PPFD was adjusted by neutral density filters (NG series, Schott, Mainz, Germany). Starting at darkness, the PPFD was increased stepwise to allow at least 15 min of adaptation at each PPFD. Then fluorescence was measured at three to four different locations on the leaf with the PAM-2000 fluorometer in conjunction with a leaf clip holder (model 2030B, Walz).

The fluorescence measurements were repeated at each PPFD on the previous location. Before irradiation and at various PPFDs after fluorescence determination, leaf discs (diameter, 9 mm) were punched with a corkborer and rapidly frozen in liquid nitrogen for pigment determination. Subsequent fluorescence measurements at increased PPFDs were continued at a position in close vicinity to the one sampled previously. To remain at conditions in which Z formation was limiting qN-quenching, it was necessary to raise PPFD slowly and regularly. Therefore, it was not possible to turn off the light source to measure dark relaxation of the fluorescence yield. Due to variation in the distance to the lamp, the PPFD was not homogeneous over the leaf but was measured for each fluorescence determination separately by the microquantum sensor of the leaf clip. This detector was calibrated with a Li-Cor quantum sensor (190B). PSII quantum yield was calculated according to Genty et al. (1989). qN-quenching was quantified using the formula $F_M/F_M' - 1$ (Bilger and Björkman, 1990). For each spot, $F_M/F_M' - 1$ was determined separately, although the F_M values differed only slightly over the leaf.

Pigment Determination

Pigments were determined by HPLC, applying the method of Gilmore and Yamamoto (1991) with slight modifications. The HPLC system consisted of two pumps (type 501 and 510, Waters Associates), an injector (model 7125, Rheodyne, Cotati, CA), an automated gradient controller (Waters), and a photodiode array detector (type 1000S, Applied Biosystems) connected to an integrator (data module 745, Waters) for peak area determination. A Spherisorb ODS-1 column (5-μm particle size, 250 mm × 4.6 mm i.d., Alltech, Deerfield, IL) and a direct-connect cartridge guard column (Alltech) filled with the same material were used. Column temperature was maintained at 20 ± 0.5°C by a column thermostat (Gynkotek, München, Germany). Solvents were degassed by a degasser (type ERC-3312, ERMA CR, Inc., Tokyo, Japan) and pumped at a flow rate of 2 mL/min. Solvent A was a mixture of acetonitrile, methanol, and Tris-HCl buffer (0.1 M, pH 8.0) (72:8:4, v/v/v), and solvent B was a mixture of methanol and *n*-hexane (7:1, v/v). The following gradient program was used: 6 min in A, a 4-min linear gradient to B, 6 min in B, a 2-min linear gradient to A, and a 10-min equilibration in A before the next injection. Pigments were calibrated with standards prepared by TLC (Demmig et al., 1987; neoxanthin, V, A, and lutein) or commercially purchased (Chl *a*, Chl *b*, and Z from Roth [Karlsruhe, Germany]; β-carotene from Sigma). Pigment concentrations in the standard solutions were determined spectrophotometrically (Uvikon 930, Kontron, München, Germany) using extinction coefficients from Davies (1976) and Porra et al. (1989) for carotenoids and Chls, respectively. The pigments were extracted from the stored samples according to Thayer and Björkman (1990). EPS was calculated as in Thayer and Björkman (1990).

RESULTS

Characterization of the Transgenic Plants

Chl fluorescence quenching analysis was used to assess the apparent electron transport rates and the quantum efficiency of PSII. Figure 1A depicts the light dependence of PSII quantum yield ($\Delta F/F_M'$) of potato plants grown in a greenhouse at a maximal PPFD of approximately 150 μmol m^{-2} s^{-1}. In the dark-adapted state and at low PPFD, the quantum yield was identical for wild-type and transgenic plants, but with increasing PPFD the decline was more pronounced in the transgenic plants. The difference between the two plant types became even more obvious when apparent relative electron transport rates were calculated by multiplication of the quantum yield with incident PPFD (Fig. 1B). After an almost linear increase at low PPFD, the electron transport rates of the transgenic and wild-type plants saturated at PPFDs of 200 and 450 μmol m^{-2} s^{-1}, respectively. In the genetically manipulated plants not only the PPFD at saturation was lower, but also the maximal apparent rate was reduced by a factor of 3. Calculation of apparent electron transport rate, according to absorbed PPFD, would have further exaggerated this difference because the leaves of the wild-type plant contained more Chl. The data clearly indicate that the transgenic plants examined in our experiments exhibited a strong inhibition of photosynthetic capacity, and accordingly, PSII experienced a heavier burden of excess PPFD.

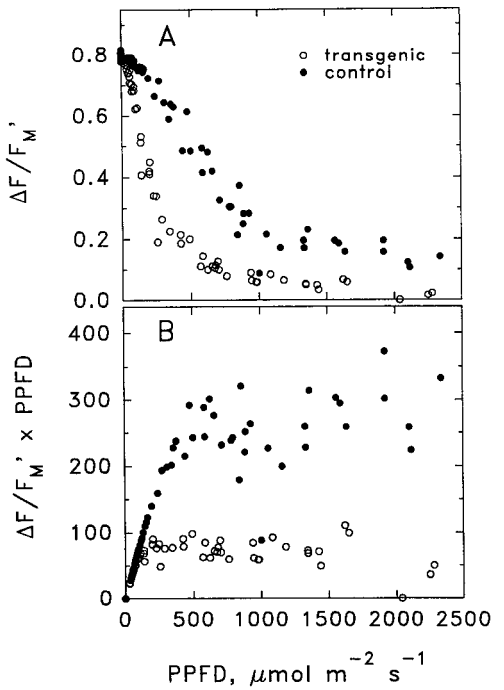

Figure 1. Dependence of the quantum yield of PSII, $\Delta F/F_M'$ (A), and of the apparent relative electron transport rate, $\Delta F/F_M' \times$ PPFD (μmol m^{-2} s^{-1}) (B), of potato leaves on PPFD. Data points were collected from different leaflets of a single leaf of a transgenic (○) and a wild-type plant (●). Measurements were performed in air; the leaf temperature varied between 20 and 25°C.

Concomitant with a reduced electron transport efficiency, one would expect increased nonradiative dissipation of light energy. Indeed, qN-quenching was higher in the transgenic plants (Fig. 2A). qN-quenching was not only elevated at low PPFD, it was also saturated at a higher level compared to the wild-type plants. For experimental reasons, the dark reversibility of qN-quenching was not determined (see "Materials and Methods").

A large part of qN-quenching is thought to depend on the presence of Z. As expected, the Z content of the transgenic plants was significantly enhanced (Fig. 2B). This was caused in part by an increased relative conversion of V but also by a larger total pool of the V cycle pigments per Chl in the transgenic plants, which averaged 101.9 (±13.1 SD) mmol/mol Chl *a*+*b* compared to 54.4 (±2.8 SD) mmol/mol Chl *a*+*b* in control plants.

When $F_M/F_M' - 1$ was plotted as a function of Z content, a linear relationship was obtained with a high regression coefficient (r^2 = 0.963; Fig. 2C, circles). Gilmore and Yamamoto (1993) proposed that A might have a function in energy dissipation similar to that of Z. However, application of the same analysis for Z + A yielded a reduced correlation coefficient of 0.900 (Fig. 2C, triangles).

V is de-epoxidized when light becomes excessive for photosynthesis, or, in other words, when the quantum

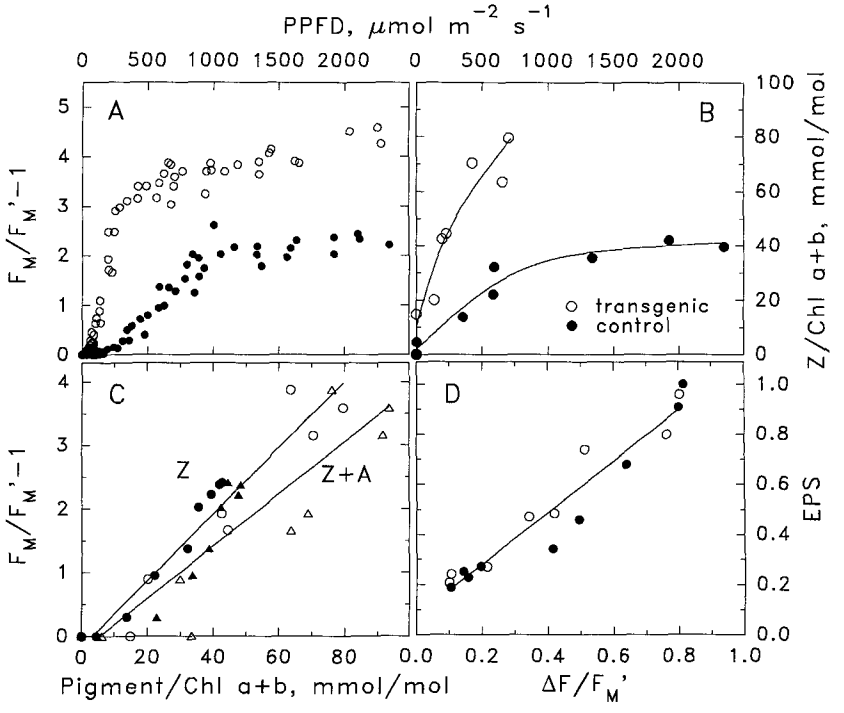

Figure 2. Dependence of $F_M/F_M' - 1$ (A) and the Z content (B) in leaves of a transgenic potato plant (○) and a wild-type plant (●) on PPFD. C, Relationship between $F_M/F_M' - 1$ and the Chl-related Z (circles) or Z + A content (triangles). The lines were drawn by linear regression. For r^2, see text. Open symbols denote transgenic potato plants, and closed symbols denote wild-type potato plants. D, Relationship between EPS of the V cycle and PSII quantum yield for the same samples used for B. The line was drawn by linear regression (r^2 = 0.967). All data are from the same experiment shown in Figure 1. (V + A + Z)/Chl $a+b$ was 101.9 (±13.1 SD) and 54.4 (±2.8 SD) mmol/mol in the transgenic and control plant, respectively. Chl $a+b$ contents were 173.1 (±16.3 SD) and 378.9 (±22.4 SD) μmol m^{-2}, in the same order.

yield of photosynthesis decreases. This also took place in our experiments, leading to a close relationship between the epoxidation state of the V cycle pool and the PSII quantum yield (Fig. 2D). For both plant types the relationship was very similar, despite the large difference in the absolute amount of Z formed. These data suggest that EPS may be used as an indicator for excess PPFD.

Pigment Contents after Adaptation to High PPFD

To test for enhanced pool size in the leaves of transgenic plants adapted to the same PPFD as controls, we transferred transgenic and wild-type plants to a phytocell and exposed them to a variety of PPFDs that were constant throughout the light period. Samples were collected daily from a single leaf of a potato plant transferred from a PPFD of 200 to 1000 μmol m^{-2} s^{-1}. Pigment analysis of these samples showed that the pool size had reached saturation within 5 d (data not shown). After 7 d, samples were taken in the middle of the photoperiod from leaves subjected to a wide range of PPFDs and the pigment contents were determined. The EPS of the V cycle pool in these samples is shown in Figure 3. EPS was significantly lower in the transgenic plants at all PPFDs. This indicates the persistence of the reduction in PSII quantum efficiency. No significant adaptation of photosynthetic capacity had occurred in the genetically manipulated plants during acclimation.

As expected, the size of the V cycle pool was strongly correlated with incident PPFD in both wild-type and transgenic plants (Fig. 4A). However, at all PPFDs the pool size was elevated in the mRNA antisense plants. Since the PPFD in the phytocell was constant during the light period, the PPFD experienced by the leaves at the time of sampling was identical to the PPFD that the leaves had been exposed to for 7 d.

These results suggest that it was not exclusively the PPFD experienced by the leaf that caused the increase in the pool size, but that the photosynthetic efficiency played an important role. Comparison of the pool size of the leaves and the EPS further corroborated this assumption. In short-term light dependencies, a linear correlation between EPS and quantum efficiency of PSII had been observed that was similar for transgenic and wild-type plants (Fig. 2D). The dependence of the V cycle pool size on EPS revealed no difference between wild-type and transgenic plants (Fig. 4B). Under the assumption that the relation between EPS and PSII quantum yield was the same in the long-term treatment, the data indicate that in both plant types the pool size adapted similarly according to PPFD in excess for photosynthesis.

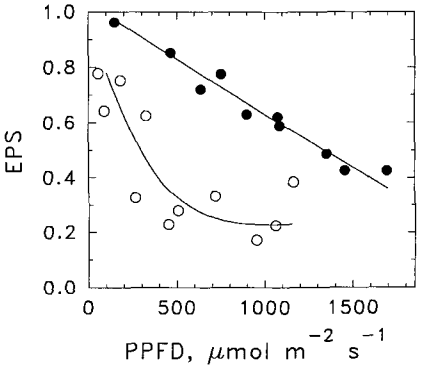

Figure 3. EPS in different leaves of transgenic (○) and wild-type (●) potato plants after exposure for 7 d to the PPFD indicated on the abscissa. For exposure conditions, see "Materials and Methods."

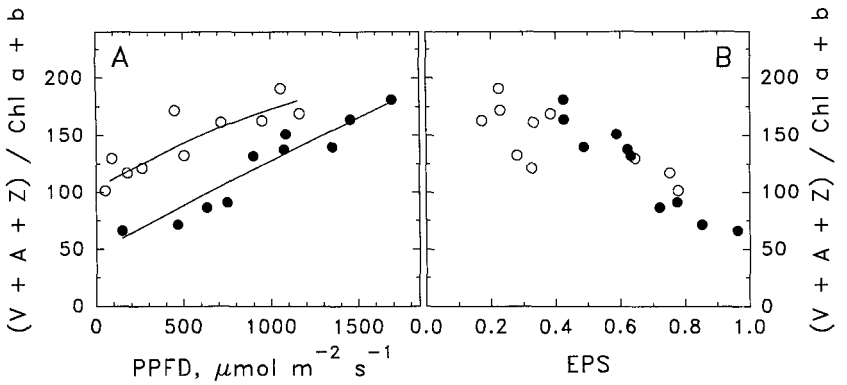

Figure 4. A, Dependence of the pool size of the V cycle pigments in leaves of transgenic (○) and wild-type (●) potato plants after exposure for 7 d to the PPFD indicated on the abscissa. B, Relationship between the pool size and EPS in the same samples. The data are from the same samples as shown in Figure 3. The pool size is given as mmol pigments per mol Chl *a*+*b*.

Concomitant with the adaptation of the V cycle pigments was a loss in Chl *a* and *b* (Fig. 5A). With increasing PPFD, Chl contents declined in control plants, whereas the mRNA antisense plants showed reduced contents already at low PPFD. The Chl *a*/*b* ratio was not different between the two types of plants and did not change significantly with PPFD (r^2 = 0.27; Fig. 5B). Also, the Chl-related contents of other carotenoids were the same in control and transgenic plants. This is shown for lutein and neoxanthin in Figure 5C, and the same trend was observed for β-carotene (data not shown). Lutein contents rose by approximately 15% over the entire PPFD range in accordance with earlier observations (Thayer and Björkman, 1990; Demmig-Adams and Adams, 1992b; Johnson et al., 1993a). From these data it can also be deduced that the ratio of the pool of V cycle pigments in relation to lutein or to the sum of carotenoids was increased in the mRNA antisense compared to control plants.

All experiments were also performed on mRNA antisense and wild-type plants of tobacco. However, for the tobacco plants we did not obtain the same result as for potato. Although after adaptation to the different PPFDs, EPS was strongly reduced in the transgenic tobacco (Fig. 6A), and although the pool sizes of the plants rose with increasing PPFD, no differences appeared in the pool sizes of transgenic and wild-type plants (Fig. 6B). The same

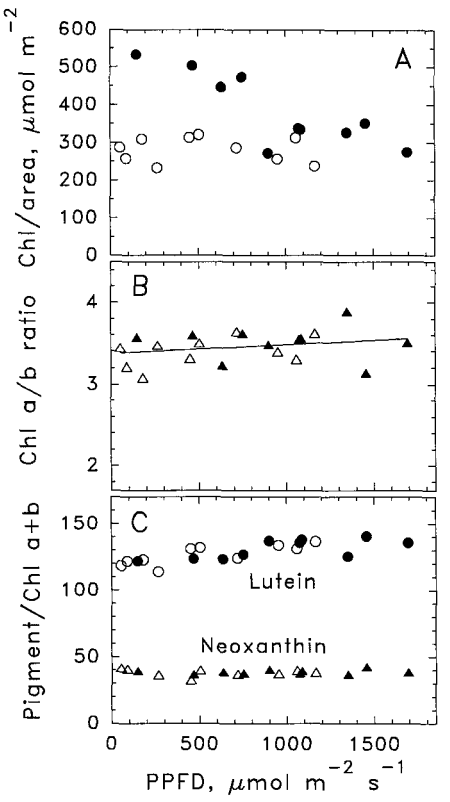

Figure 5. Pigment contents of leaves of transgenic (open symbols) and wild-type (closed symbols) potato plants in dependence of the PPFD to which the leaves were exposed for 7 d. A, Leaf area-related Chl *a*+*b* content. B, Chl *a*/*b* ratio. C, Chl-related lutein (circles) and neoxanthin (triangles) content. The data are from the same experiment shown in Figures 3 and 4.

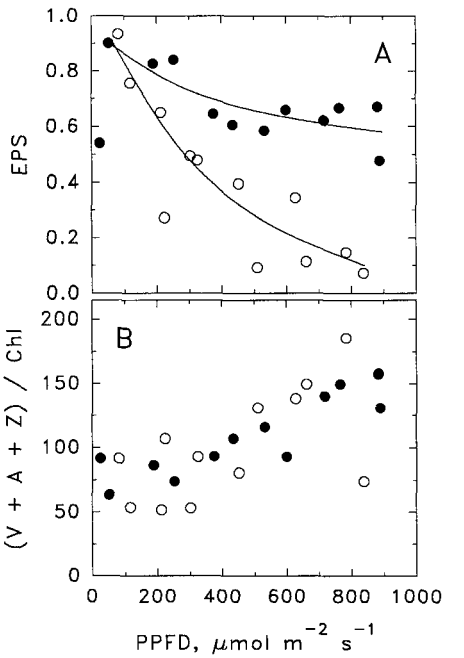

Figure 6. Dependence of EPS (A) and the pool size of the V cycle pigments (B) in leaves of transgenic (○) and wild-type (●) tobacco plants on exposure to PPFD after 7 d of adaptation. The pool size is given as mmol pigments per mol Chl *a*+*b*.

result was obtained when the pool sizes were related to lutein or total carotenoids. From the data shown in Figure 6, A and B, one can conclude that the relation between pool size and EPS was not identical for transgenic and control tobacco plants, in contrast to the findings with potato. We repeated the experiment with tobacco three times with the same outcome.

DISCUSSION

Our experiments were designed to examine the effect of photosynthetic efficiency on the pool size of the V cycle pigments. Therefore, we analyzed plants with genetically reduced cp-FBPase to inhibit photosynthetic activity. It has been shown that the FBPase antisense plants exhibited a decreased rate of O_2 evolution at saturating CO_2 (Kossmann et al., 1994). These results are corroborated by our Chl fluorescence measurements (Fig. 1), since the light-saturated electron transport through PSII in transgenic potato plants was only one-third of that in wild-type plants. In transformed tobacco, this reduction amounted to approximately 50%. Although the two types of plants, with high and low photosynthetic efficiency, are genetically very similar, in contrast to other plants used for an analysis of the V cycle pool size adaptation, the possibility that the genetic manipulation induced secondary effects apart from the reduction of Calvin cycle activity cannot be excluded a priori. In particular, the insertion of the antisense gene into the plant genome could affect the function of a gene located close to this site. However, to eliminate such artifacts, we used various lines of transgenic plants, which where generated independently. The results obtained from these lines were always consistent. In addition, we deliberately selected plants with almost identical appearance of transgenic and wild-type phenotypes. Therefore, secondary effects of the expression of the antisense gene apart from photosynthetic efficiency on the V cycle pool size appear highly unlikely.

Under identical conditions, a comparison of the pool sizes of transgenic with wild-type plants revealed different results in tobacco and potato plants. The leaves of transgenic potato plants contained elevated V cycle pigment contents at all PPFDs, compared to wild-type plants. No increase was observed in tobacco plant lines. Therefore, we will discuss the results obtained for tobacco and potato plant lines separately.

One could argue that the increased V cycle pool size of transgenic plants was caused by their reduced Chl content, since this was used as a basis for the expression of xanthophyll contents. The Chl content of a given leaf may change because of a reduction of the number of chloroplasts, or a reduction of thylakoid membrane area per chloroplast, or a diminished amount of pigment protein complexes per membrane area. In all cases one would expect that carotenoid contents closely followed Chl contents. Hence, Chl should be the appropriate reference. Indeed, we found that lutein and neoxanthin contents related to Chl were identical between genetically manipulated and wild-type plants (Fig. 5C). Furthermore, within each type of plant, at high PPFD the V cycle pool increased more than Chl contents declined. Therefore, the observed differences in the pool size appear not to be caused by choosing Chl as a reference; rather, they reflect a response to excess PPFD.

We used EPS as an indicator for excess PPFD at the particular environmental conditions at which the pigment samples were taken. EPS seems to be an especially well-suited internal standard for excess PPFD, since it is not only influenced by the external light and temperature conditions but also by the variation in photosynthetic capacity between different leaves. The latter is unavoidably caused by small differences, e.g. in leaf age, fertilization, or gene expression. In Figure 2D it is apparent that the close relationship between EPS and PSII quantum yield is only slightly influenced by the V cycle pool size. Therefore, the fact that the relationship between pool size and EPS was identical for transgenic and wild-type potato plants supports the idea that the quantum efficiency of photosynthesis may exert control on the signal chain leading to increased pigment synthesis.

Such a control would be consistent with the proposal that an increase in the capacity for Z formation is related to enhanced photoprotection (Thayer and Björkman, 1990; Demmig-Adams and Adams, 1992a). Accordingly, a higher capacity for nonradiative dissipation should be expected in plants with a larger pool size. For the potato plants, we observed an explicit saturation of qN-quenching at high PPFD and an increased maximal qN-quenching associated with an enhanced pool size (Fig. 2A). The relation between $F_M/F_M' - 1$ and Z content was identical for both plant types, and the increment in $F_M/F_M' - 1$ was matched by the increment in the Z content of the transgenic potato plant (Fig. 2C).

Recently, interspecific variation of light-saturated qN-quenching that correlated with habitat preference was observed by Johnson et al. (1993b) (see also Björkman and Demmig-Adams, 1994; Demmig-Adams and Adams, 1994). However, these authors did not find intraspecific variation for *Digitalis purpurea* and *Chenopodium album* when these plants were grown at different light intensities (Johnson et al., 1993b). On the other hand, Demmig-Adams and Adams (1994) found an increased capacity for qN-quenching in *Monstera* plants, which was induced by acclimation to sunlight. In this and other plants they demonstrated a positive correlation between V cycle pool size and capacity for qN-quenching. A different result was obtained by Brugnoli et al. (1994). These authors reported a higher capacity for qN-quenching in sun leaves of *Ligustrum ovalifolium* compared to shade-adapted leaves. They found an identical correlation between qN-quenching and EPS in sun and shade leaves, and the increased maximal qN-quenching in sun leaves was related to increased potential for V deepoxidation. Since the two types of leaves contained different pool sizes, these data exclude a correlation between $F_M/F_M' - 1$ and Chl-related Z content. Obviously, more work is necessary to determine if qN-quenching is related to the absolute or the relative amount of Z present and to elucidate by which mechanism the capacity for nonradiative dissipation is regulated in plants. A major drawback of all these studies, including ours, is that one cannot exclude secondary effects, when relying solely on correlations.

However, if the observed difference in qN-quenching between wild-type and transgenic plants were due to the specific potential for Z formation, this effect may be explained in the framework of a model for the action of Z that involves direct quenching of excited singlet Chl by Z (Demmig-Adams, 1990). Recent results have supported this hypothesis (Owens et al., 1992; Frank et al., 1994; Owens, 1994). In particular, it was found that the energy level of the first excited singlet state of the Z molecule was low enough to enable energy transfer from excited singlet Chl to Z. Since the probability of energy transfer to Z should depend on the ratio between Z and Chl molecules, our results obtained from potato and a photoprotective function of the pool size adaptation are in line with this model.

According to an alternative hypothesis that describes Z-regulated aggregation of the light-harvesting complex II (Horton and Ruban, 1992; Horton et al., 1994), nonradiative dissipation would depend on the ratio between V and Z. Although adaptation of the V cycle pool size is difficult to understand in this model, one cannot exclude the possibility that the aggregation of the light-harvesting complex II and the regulation of this process are affected by the amount of V cycle pigments present. Although investigations on the V cycle pool size may provide clues to the molecular mechanism of Z functioning, understanding of the latter has to come from experiments addressing this mechanism directly. Rather, a better knowledge of the events at the molecular level relating Z formation to nonradiative dissipation would help to interpret the PPFD-dependent adaptation of the V cycle pool size.

In tobacco, photosynthetic activity did not affect the V cycle pool size (Fig. 6). The difference between potato and tobacco is especially obvious when the relation between pool size and EPS is considered (Figs. 4B and 6, A and B). At present we have no explanation for this result. The electron transport capacity was less reduced in transgenic tobacco compared to transgenic potato, which may have caused a reduction in excess PPFD. However, the EPS values obtained from the tobacco plants demonstrate that the transgenic plants experienced strong, excess PPFD (Fig. 6A). This gives rise to the assumption that two different types of regulation of the V cycle pool size exist. In one type, photosynthesis exerts a control on the xanthophyll cycle pool size, whereas no effect occurs in the other type. The simultaneous existence of "potato-like" and "tobacco-like" plants could resolve some of the contradictory results discussed in the literature (Demmig-Adams and Adams, 1992b, 1994; Johnson et al., 1993b).

The present study cannot directly address the question of the molecular mechanism involved in the regulation of the V cycle pool size. The data obtained for tobacco indicate a regulation independent of photosynthesis. This component might also be present in potato, but modulated by photosynthetic efficiency. Mechanisms that serve to induce the synthesis of carotenoids or enzymes involved in carotenogenesis include oxidative stress, the blue-light receptor, and the phytochrome system (Bartley et al., 1994; Rau, 1985). Although the latter receptor systems are independent of photosynthetic activity, oxidative stress is enhanced with a reduction in photosynthesis. The observed loss of Chl in the transgenic plants and in control plants at high light might not be solely a regulatory response but might indicate the occurrence of photooxidative processes. Further investigations are required to examine the contribution of these regulatory mechanisms to the light-dependent adaptation of the V cycle pool size.

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