Heterologous Expression of Arabidopsis Phytochrome B in Transgenic Potato Influences Photosynthetic Performance and Tuber Development

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Transgenic potato (Solanum tuberosum) plants expressing Arabidopsis phytochrome B were characterized morphologically and physiologically under white light in a greenhouse to explore their potential for improved photosynthesis and higher tuber yields. As expected, overexpression of functional phytochrome B caused pleiotropic effects such as semidwarfism, decreased apical dominance, a higher number of smaller but thicker leaves, and increased pigmentation. Because of increased numbers of chloroplasts in elongated palisade cells, photosynthesis per leaf area and in each individual plant increased. In addition, photosynthesis was less sensitive to photoinactivation under prolonged light stress. The beginning of senescence was not delayed, but deceleration of chlorophyll degradation extended the lifetime of photosynthetically active plants. Both the higher photosynthetic performance and the longer lifespan of the transgenic plants allowed greater biomass production, resulting in extended underground organs with increased tuber yields.

Abbreviations: CaMV, cauliflower mosaic virus; FR, far-red; phyA and phyB, phytochromes A and B, respectively.

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Plant Material

Plasmid pMAB316 (Wagner et al., 1991) encodes the Arabidopsis PHYB cDNA under the control of the CaMV 35S promoter. Transformation of potato (Solanum tuberosum cv Désirée) plants with pMAB316 was performed according to the method of Rocha-Sosa et al. (1989). We selected two lines with different expression levels on the basis of reduced internode elongation in white light. These lines were named “Dara” after the host cultivar (Désirée) and the donor species (Arabidopsis).

Growth Conditions

Plants cultivated in a sterile culture (Murashige and Skoog, 1962) in a controlled-environment chamber (CU 32-L, Percival Scientific, Boone, IA) at photon flux densities of approximately 0.1 mmol m^{-2} s^{-1} white light and at 24°C (day)/22°C (night) were transferred to soil (maximum pot diameter, 20 cm) and grown in a greenhouse at 0.15 to 0.5 mmol m^{-2} s^{-1} white light, 15°C to 35°C, and a RH of approximately 55%. After 2 weeks, all but the two strongest shoots were cut to obtain plants of comparable strength.

RNA Extraction and Northern Analysis

Preparation of poly(A\(^+\)) RNA from leaves and subsequent northern analysis were performed as described by Heyer and Gatz (1992a). Arabidopsis phyB transcripts were probed with a 3.9-kb KpnI fragment generated from pMAB316 and potato phyB transcripts with a 2.7-kb HpaI/KpnI fragment of a plasmid encoding the full-length cDNA (Ruddat et al., 1997). Probes against the potato ribosomal protein S4 (Devi et al., 1989) served to normalize for equal loading of poly(A\(^+\)) RNA. Radioactive signals were detected with a bio-imaging analyzer (BAS 1000, Fuji, Tokyo) and quantified with TINA 2.0 software (Raytest, Straubenhardt, Germany).

Protein Extraction and Immunoblot Analysis

Protein extraction and immunoblotting were performed as described by van Tuinen et al. (1995) with slight modifications. The protein pellet (corresponding to 0.3 g of leaf material) resulting from the ammonium sulfate precipitation was resuspended in 15 μL of 0.5 mL Tris buffer, pH 6.8. The protein concentration of the extract was determined (Bradford, 1976) and the extract was dissolved at 100°C for 2 min in 4× sample buffer (Laemmli, 1970). Equal amounts of protein (100 μg) were separated on a 7% SDS-PAGE gel and electroblotted onto a PVDF membrane (Millipore). Ponceau staining confirmed uniform protein loading and homogenous blotting. The membrane was incubated with either a mixture of three monoclonal antibodies raised against Arabidopsis phyB in a mouse (1:5000 dilution; Somers et al., 1991) or a polyclonal serum raised against potato phyB in a rabbit (1:400 dilution). After treatment with peroxidase-conjugated secondary antibody (1:1000 dilution, anti-mouse or anti-rabbit; Amersham), the enhanced chemiluminescence kit (Amersham) visualized phyB and Aida 2.0 software (Raytest) quantified it densitometrically. Separate tests confirmed that the phyB signal was proportional to the amount of protein loaded.

Phenotypic Analysis

Stem height was measured as the distance from the apex to the soil surface. For determination of the leaf-to-stem weight ratio, representative plants were cut above the soil and the leaf laminas were separated from the petioles and stems. Stem circumferences were measured 1 cm above the soil. Weighing leaf discs randomly cut from mature leaves determined the specific leaf fresh weight (milligrams per centimeter). We estimated total leaf area (centimeters square) per plant from the mean specific leaf weight and the total leaf weight per plant. We determined tuber yield by measuring tuber number, total tuber fresh weight per plant, and fresh weight per single tuber, considering only tubers of at least 1 g. The tubers were harvested after growth for approximately 5 months in two consecutive winters (until February 1998 and April 1997). In the summer plants aged much earlier, living only to a maximum age of 3 months, due to higher temperatures and stronger pathogen exposure. In addition, developing tubers that had grown during summer and winter were harvested from a few green plants at 3 months.
Chlorophyll Determination

Leaf discs (1.3 cm²) were ground in a mortar with liquid nitrogen; the chlorophyll was quantitatively extracted with 80% acetone in the presence of approximately 1 mg of NaCO₃. After the sample was centrifuged for 2 min at maximum speed, we determined the total chlorophyll content (chlorophyll a and b) of the supernatant photometrically (Uvicon 932, Kontron, Neuzahrn, Germany), according to the method of Lichtenthaler (1987). Chlorophyll contents were in terms of leaf area or leaf fresh weight.

Photosynthesis Measurements

We determined the CO₂ uptake per leaf area using IR spectroscopy with a transportable gas-exchange porometer (ADC, Hoddesdon, UK), consisting of a central LCA-3 analyzer and a PLC-3 leaf chamber for simultaneous recording of photon fluxes and endogenous chamber temperature. An integrated personal computer stored and processed the data. The terminal leaflets of leaves 6 to 8 (leaf 1 was the first leaf larger than 1 cm) of 32- to 37-d-old plants were used. Before measurement, the leaflet was fixed in the chamber and exposed to 50 to 500 μmol m⁻² s⁻¹ white light provided by a 150-lux lamp (Flexilux, Schölly Fiberoptik, Denzlingen, Germany) at 22°C to 25°C until CO₂ assimilation reached a maximum steady-state level (10–15 min).

High-Light Studies

To study light-stress sensitivity, we exposed attached leaves (leaves 6–8 of 32- to 37-d-old plants) for 1.8 mmol m⁻² s⁻¹ white light (Power Star HQIT N/E, 2000 W, Osram, Munich, Germany) at a maximum (fan cooled) leaf temperature of 38°C. We again determined CO₂ assimilation rates as described above. Additionally, leaf discs from three different plants were floated on water and exposed to the same light source at 25°C to 30°C for 5 h. The ratio of variable to maximum chlorophyll a fluorescence recorded with a fluorometer (PAM 101, Walz, Effeltrich, Germany; kindly provided by Dr. K. Raschke, Göttingen), after 10 min of dark adaptation, served as a measure of photosynthetic efficiency (Krause and Weis, 1991). High-light phenotypes were investigated by culti-vating plants under the same light source between 1.5 and 1.8 mmol m⁻² s⁻¹ white light (continuously fan cooled).

Preparation and Documentation of Light Micrographs

Cross-sections were cut from the center of mature, nonsenescent leaf lamina of 6-week-old plants. The preparation procedure followed the paraffin method (Gerlach, 1969). A microscope (DNLS, Leica) magnified and a camera (NPS48, Leica) photographed the cross-sections.

RESULTS

Determination of phyB Transcript and Protein Levels

Potato plants were transformed with the Arabidopsis phyB cDNA under the control of the CaMV 35S promoter. After regeneration of 12 independent transformants, we selected two plants exhibiting different degrees of stem elongation suppression in white light for further characterization. Northern analysis of poly(A⁺) RNA from leaves (Fig. 1) showed that the potato line Dara-5 expressed 0.2 relative units (the phyB signal divided by the S4 signal), whereas Dara-12 expressed 3.9 relative units of phyB mRNA. No signal was obtained in wild-type plants. Transcript of the endogenous PHYB was not significantly affected in the transgenic lines.

For determination of phyB protein levels, we used monoclonal antibodies raised against Arabidopsis phyB (Fig. 2; Somers et al., 1991) to perform immunoblots with protein extracts from leaves. Because the applied antibodies recognized equally Arabidopsis and potato phyB, the signal obtained with wild-type extracts represented the amount of endogenous potato phyB. Densitometric quantification of three blots indicated approximately 4-fold (Dara-5) and 20-fold (Dara-12) overexpression of phyB in the transgenic lines compared with the wild type. Low-temperature fluorescence measurements (Sinenshchekov et al., 1996) on etiolated sprouts served to prove spectral activity of the transgenic phytochrome. Dark-grown sprouts (lower stem part)
yielded 1.80 ± 0.49 relative fluorescence units in the wild type and 2.46 ± 0.44 relative fluorescence units in the transgenic Dara-12 plants. Irradiation for 3.5 h with red light depleted the phytochrome pool in wild-type plants to transgenic Dara-12 plants. Irradiation for 3.5 h with red

**Leaf Characteristics**

As shown in Figure 3c, leaves of the transgenic plants were considerably smaller than wild-type leaves. However, the number of leaves was higher, resulting in the same total leaf area per individual plant (Table I). Increased total fresh weight per leaf area (14% for Dara-5 and 23% for Dara-12, Table II) resulted from the increased thickness of the leaves. Light micrographs of leaf cross-sections (Fig. 4) revealed that the increased thickness was basically due to the increased length of palisade cells in the leaf mesophyll (19% more for Dara-5 and 30% more for Dara-12), whereas the dimensions of the spongy tissues remained largely unaffected (Fig. 4; Table II). The enlarged palisade cells contained more chloroplasts, resulting in strongly elevated chlorophyll contents on a leaf-area basis (Fig. 5) but not on the basis of leaf fresh weight (Table II). Despite the differences in chlorophyll content per leaf area, fractional red-light absorbance (Dr. G.H. Krause, Düsseldorf, Germany, personal communication) was very similar for all three lines (0.79 relative unit for wild type and 0.80 relative unit for the two transgenic plants). The impression of a darker pigmentation was intensified by slightly higher amounts of anthocyanins in the leaves (Fig. 3c), which increased considerably when the plants grew under higher light intensities (see Fig. 7).

**Table 1. Anatomical characteristics of Dara-5 and Dara-12 shoots compared with wild-type potato plants**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild Type</th>
<th>Dara-5</th>
<th>Dara-12</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem height (cm)</td>
<td>82 ± 6</td>
<td>30 ± 11.1</td>
<td>24 ± 4</td>
<td>10</td>
</tr>
<tr>
<td>Stem circumference (cm)*</td>
<td>2.5 ± 0.4</td>
<td>2.9 ± 0.4</td>
<td>4.8 ± 1.0</td>
<td>10</td>
</tr>
<tr>
<td>Leaf-to-stem wt ratio b</td>
<td>0.34 ± 0.03</td>
<td>0.48 ± 0.07</td>
<td>0.65 ± 0.04</td>
<td>5</td>
</tr>
<tr>
<td>Leaf fresh wt/plant (g)b</td>
<td>51.8 ± 3.2</td>
<td>62.0 ± 9.6</td>
<td>63.9 ± 11.5</td>
<td>5</td>
</tr>
<tr>
<td>Leaf area/plant (m²)b</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.04</td>
<td>5</td>
</tr>
</tbody>
</table>

* Measured 1 cm above the soil.  
  b Data are from representative plants; effects were reproduced with five to eight different plants at other developmental stages; SD as indicated.
from apical to basal leaflets were pooled, and chlorophyll was extracted and analyzed photometrically. The results are presented in Figure 5. The chlorophyll content per leaf area in the transgenic leaves began to rise within the first 20 d after transfer from sterile to soil culture. Soon after the chlorophyll accumulation per leaf area reached a maximum, senescence started simultaneously in both the transgenic and the wild-type leaves. Flowering, which initiates senescence, started at approximately the same time (d 47). The time needed for complete chlorophyll degradation, however, extended the lifetime of photosynthetically active transgenic plants by 3 to 4 weeks.

Photosynthetic Activity

Figure 6 presents the photosynthetic activity of mature wild-type and transgenic leaves. Young leaves from nonsenescent plants (32–37 d old, compare with Fig. 5) were used. Photon fluxes of more than approximately 250 μmol m⁻² s⁻¹ in the transgenic plants showed higher rates of leaf-area photosynthesis than did those in the wild-type plants (Fig. 6a). As the senescence-related breakdown of chlorophylls proceeded (Fig. 5), the advantages in the photosynthetic performance of the transgenic plants became even more pronounced (data not shown). We observed no difference in photosynthetic activity (Fig. 6b) when the chlorophyll content was normalized. The increased rates per unit leaf area can thus be attributed to thicker leaf cross-sections and elevated chlorophyll contents. Because the total amount of leaf area was unaffected, increases in photosynthetic rates per individual plant (23% and 30% for Dara-5 and Dara-12, respectively) were extrapolated from the mean rates of leaves 5 to 12.

No significant structural changes in the photosynthetic apparatus of the transgenic leaves were detected. HPLC analysis of ethanolic leaf extracts of 30- to 40-d-old plants (device kindly provided by Dr. P. Jahns, Düsseldorf; method modified from that of Gilmore and Yamamoto, 1991) revealed no changes in the carotenoid composition (data not shown). Also, the ratios of chlorophyll a to b were almost identical in wild-type and transgenic leaves (2.6 ± 0.3 for wild type and Dara-5 and 2.7 ± 0.4 for Dara-12). Finally, electron microscopy (kindly performed by Dr. S. Hillmer, Göttingen; data not shown) did not indicate substantial changes or deformations in the structure of the chloroplasts of the transgenic plants, as previously observed in oat phyA-overexpressing tobacco plants (Sharkey et al., 1991).

Light-Stress Experiments

Because the transgenic leaves revealed a sun leaf phenotype, we analyzed the susceptibility of the photosynthetic apparatus to strong irradiance using the nonsenescent leaves of plants grown under moderate white light (0.15–...
After 5 h at 1.8 mmol m$^{-2}$ s$^{-1}$ white light and 25°C to 30°C, maximum CO$_2$ assimilation had dropped to about 25% of that in the plants maintained under moderate light in the wild type and to only 50% (Dara-5) and 60% (Dara-12) in the transgenics (Fig. 7). During that period, the ratio of variable to maximum chlorophyll $a$ fluorescence, used as a measure of photosynthetic efficiency, declined from approximately 0.8 in the dark controls to approximately 0.2 in the wild-type leaves to 0.3 in the Dara-5 leaves and to 0.35 in the Dara-12 leaves. Both studies indicated a significantly lower sensitivity of the transgenic leaves to photoinhibition.

To study phenotypes under high irradiation, five plants per line were cultivated under artificial white light at 1.5 to 1.8 mmol m$^{-2}$ s$^{-1}$ in a greenhouse. Differences in internode length and leaf size were less pronounced under these conditions. However, the differences in leaf thickness and chlorophyll contents remained. The formation of anthocyanins in leaves (primarily in the lower epidermis, near veins, and in the petiole and rachis) and, to a smaller degree, also in stems (outer cortex cells) of the transgenic plants was strongly enhanced compared with plants grown under moderate light (compare Fig. 3c with Fig. 8).

**Tuber Yields and Underground Organ Growth**

The phenotypes of the underground organs were determined when the plants were totally senescent after approximately 5 months of growth in a greenhouse. Table III summarizes the yield data and Figure 9 shows representative underground organs of each line. Biomass allocation to underground organs (tubers and the root and underground shoot systems) increased in the transgenic lines. The number of tubers per plant was approximately 2-fold higher in Dara-5 and 3-fold higher in Dara-12 compared with the wild type. The tuber weight per plant in Dara-5 and Dara-12 increased by 56% and 30%, respectively, of the tuber weight of the wild type; and the average tuber size decreased to 65% and 43%, respectively, of the tuber size of the wild type. Relative dry weight and starch contents of the tubers (Dr. B. Marty, Golm, Germany, personal communication) were constant in the wild type, Dara-5, and

![Figure 6](image)

**Figure 6.** Photosynthesis rates of mature, non-senescent leaves (leaves 6–8) of 32- to 37-d-old wild-type, Dara-5, and Dara-12 plants. a, Rates of wild-type (○), Dara-5 (■), and Dara-12 (●) at different photon fluxes (50–500 μmol m$^{-2}$ s$^{-1}$) normalized to leaf area (μmol CO$_2$ m$^{-2}$ s$^{-1}$). Data points are means of measurements of 4 to 15 leaves from eight plants of each line. b, Rates at 500 μmol photons m$^{-2}$ s$^{-1}$ normalized to chlorophyll contents at site of the investigated leaf (μmol CO$_2$ g$^{-1}$ s$^{-1}$). In a, data points are for 4 to 15 leaves from eight plants of each line; in b data are from nine leaves of three plants of each line (SD = 0.5–2.6 μmol m$^{-2}$ s$^{-1}$ for Dara-5 or as indicated). chl, Chlorophyll.
Expression of Arabidopsis Phytochrome B in Potato

Table III. Tuber yield of Dara-5, Dara-12, and wild-type potato plants after decay of the shoots following growth for 5 months and after only 3 months

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild Type</th>
<th>Dara-5</th>
<th>Dara-12</th>
</tr>
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<tbody>
<tr>
<td>Tuber wt (g)/plant</td>
<td>122 ± 35</td>
<td>190 ± 52</td>
<td>158 ± 25</td>
</tr>
<tr>
<td>Tuber no./plant</td>
<td>9 ± 3</td>
<td>22 ± 7</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Average wt (g)/single tuber</td>
<td>14 ± 12</td>
<td>9 ± 8</td>
<td>6 ± 6</td>
</tr>
<tr>
<td>Tuber wt (g)/plant after 3 months</td>
<td>104 ± 36</td>
<td>92 ± 21</td>
<td>86 ± 29</td>
</tr>
</tbody>
</table>

*Data are from 3 representative plants; all others are from 12 plants; SD as indicated.

Dara-12 (22 ± 1 and 15 ± 1, respectively; n = 15). However, when harvested before 3 months (repeated several times on a small scale), no significant increases in organ expansion appeared and depressed tuber yields were even found in the transgenic lines. Table III shows the tuber weights of three representative plants per line, harvested at 3 months. At that age, yields were about 14% (Dara-5) and 23% (Dara-12) lower in the transgenics compared with the wild type.

DISCUSSION

Transgenic plants overexpressing different members of the phytochrome family have been extremely valuable for elucidating the specific functions of individual photoreceptors (McCormac et al., 1992, 1993). Moreover, they have also been used to map functional domains important for photosensory specificity, dimerization, signal transduction, and light-dependent degradation (for review, see Quail, 1997). Because of suppression of the shade-avoidance response, transgenic tobacco plants overexpressing oat phyA revealed an increased leaf-harvest index, opening new avenues for biotechnological applications of transgene technology (Robson et al., 1996). Being particularly interested in the latter application, we generated transgenic potato plants overexpressing phyB. Potato was chosen as a host to explore the possibility that improved harvest index could also be applied to tubers.

By choosing phyB we tried to circumvent the adverse effects of phyA on chloroplast structure (Sharkey et al., 1991). We preferred Arabidopsis phyB over potato phyB because it encodes an N-terminal hydrophobic extension that is missing in potato phyB (Heyer and Gatz, 1992b). Deletion of this extension decreased the sensitivity of the protein to red light (Wagner et al., 1996). Thus, we speculated that expression of Arabidopsis phyB might be more effective than expression of potato phyB. Transgenic potato plants transformed with the Arabidopsis phyB cDNA under the control of the CaMV 35S promoter supported the notion that phytochrome-overexpressing plants may have agricultural importance. We discuss the data presented here in the context of results previously obtained with other phyB- or phyA-overexpressing plants.

Transgenic potato plants overexpressing Arabidopsis phyB exhibited a semidwarf phenotype, with shorter and thicker stems, reduced apical dominance, smaller but thicker leaves, a higher leaf-to-stem weight ratio, increased chlorophyll content, higher rates of photosynthesis, prolonged timespan for photosynthesis, and, when harvested after the natural decay of the shoots, higher tuber numbers and yields. Physiological data from other Arabidopsis phyB-overexpressing plants under white light are limited. Transgenic Arabidopsis plants also revealed an increased chlorophyll content. In addition, a slightly reduced ratio of chlorophyll a to b was detected (Wester et al., 1994), a phenomenon not observed in transgenic potato plants. Like the transgenic potato plants described here, transgenic tobacco plants overexpressing phyB showed reduced stem extension (Halliday et al., 1997).

Transgenic tobacco plants overexpressing oat phyA have been studied in more detail. Like phyB-overexpressing potato plants, phyA-overexpressing tobacco plants are characterized by semidwarfism, reduced apical dominance, smaller but thicker leaves, increased chlorophyll content, and delayed senescence (Keller et al., 1989; Cherry et al., 1991). The elicitation of this “light-exaggerated” phenotype by overexpression of either phyA or phyB is consistent with the notion that a variety of developmental processes can be controlled by either phytochrome, with phyA being activated by FR light and phyB by red light (Quail et al., 1995). As white light contains red and FR light, both types of overexpressors contain elevated levels of Pfr under white light. The increased sensitivity of phyA-overexpressing plants to FR light has been well documented (McCormac et al., 1992; Robson et al., 1996). However, it remains to be shown that it is the red-light component of white light that is responsible for the light-exaggerated phenotype in the transgenic plants described here.

In terms of photosynthetic performance, phyB-overexpressing potato plants were superior to phyA overexpressors (Sharkey et al., 1991). Both types of phytochrome overexpressors had thicker leaves and higher chlorophyll content per leaf area. Light micrographs illustrate that leaf thickening was due to general enlargement of mesophyll cells in phyA-expressing tobacco (Sharkey et al., 1991), whereas only palisade cells were elongated in phyB-

![Figure 9. Expansion of underground organs and tuber yields of the wild type (Wt), Dara-5, and Dara-12 harvested upon decay of the aerial parts after 5 months of cultivation in a greenhouse. The transgenic lines showed enlarged underground organs and more but smaller tubers, resulting in increased total tuber weight per plant (Table III). Bar = 5 cm.](https://www.plantphysiol.org)
overexpressing potato (Fig. 4; Table II). Electron micro-
graphs from transgenic phyA overexpressors showed that
many of the chloroplasts were cup-shaped, with the middle
portions of the chloroplasts bowing away from the plas-
malemma (Sharkey et al., 1991). These abnormalities wors-
ened the photosynthetic performance of the plants at nor-
mal CO₂ concentrations.

Abnormalities of the chloroplasts were not detectable in
transgenic phyB-overexpressing potato plants. In contrast,
these plants displayed higher photosynthesis rates (Fig.
6a). This effect was proportional to the increased chloro-
phyll levels (Fig. 6b). The differences in photosynthetic
activity were even more pronounced in senescing leaves as
the relative differences in pigmentation increased. In addi-
tion, photosynthesis was less sensitive to photoinhibition
(Fig. 7), which may have been due to the higher chlorophyll
content per unit leaf area resulting in lower excitation of
pigments compared with wild-type leaves. Whether the
stimulated anthocyanin formation observed under high-
light conditions was also involved in protecting the trans-
genic photosynthesis apparatus (especially under high UV
radiation in the field) remains to be elucidated.

The enhanced responsiveness of the transgenics to white
light led not only to increased thickening of leaves, and
thus to higher photosynthetic performance and higher bio-
mass production of the aerial parts of the plants, but also to
a preferential allocation of assimilates to the leaves at the
expense of the stems (Table I). This is consistent with the
phenotype of transgenic tobacco plants overexpressing
phyA (Robson et al., 1996). phyA-overexpressing plants
also displayed this altered assimilate allocation but, in
contrast to plants overexpressing phyB, only in response to
low relative ratios of red to FR light.

Both phyA-overexpressing tobacco plants and phyB-
overexpressing potato plants stayed green longer. To ana-
lyze whether this apparently delayed senescence was due
to a delayed beginning or to a slower senescing process, we
monitored the chlorophyll contents over the life cycle of the
phyB-overexpressing potato plants (Fig. 5). Chlorophyll
levels of wild-type and transgenic plants increased at dif-
ferent rates, resulting in 35% more chlorophyll in the ma-
ture leaves of the transgenic lines. Chlorophyll contents
started to decrease at approximately d 40 in all of the plant
lines, indicating no difference in the start of senescence.
Also, the slope of the decline was almost identical. How-
ever, assuming a similar amount of chlorophyll per chlo-
roplast in the nonsenescent state (deduced from the con-
comitant increases in chloroplast number per cell and chlo-
rophyll level per leaf area), the rate of chlorophyll loss
per individual chloroplast decelerated; the time needed for
complete chlorophyll degradation was several weeks
longer in the transgenic plants. This result is consistent
with findings of Cherry et al. (1991), who also observed a
simultaneous start but a decelerated senescence process in
phyA-overexpressing plants.

Tuber yield is the essential parameter for estimating the
agricultural potential of transgenic potato plants. Keiller
and Smith (1989) have shown that increasing the ratio of
red to FR light for radish results in preferential assimilate
allocation to the storage organ at the expense of leaf petiole
extension. To our knowledge, similar effects have not been
reported in potato. By increasing phyB levels, and thus
presumably the sensitivity to red light, we explored the
possibility of stimulating photosynthesis and assimilating
allocation to tubers using one central regulator. When har-
vested after 3 months, tuber yields were lower than in
wild-type plants. phyB apparently delays tuber formation,
although we observed no effect on flowering. The delayed
tuber formation in phyB overexpressors corresponds well
with accelerated tuber formation in phyB antisense plants
(Jackson et al., 1996). In these plants tuber formation was
induced even under long-day conditions using the short-
day potato cv Andigena. Because the work presented here
was done with the potato cv Désirée, which is under less-
stringent photoperiodic control, these experiments are not
directly comparable. Once tuber induction was initiated,
the high photosynthetic performance of even senescing
plants led to storage of elevated amounts of biomass into
tubers, resulting in higher yields. In spite of higher yields,
average tuber size was smaller than in wild-type plants. It
may be speculated that decreased apical dominance of the
underground shoot leads to increased stolon formation.
Increased biomass allocation was not confined to tubers
but was also present in other underground organs such as
roots and shoots.

The results of this study support the idea that improving
agricultural performance by altering phyB levels is feasible.
Higher photosynthetic rates led to more biomass, which is
allocated into leaves but also into underground organs
such as tubers. Whether these features are maintained un-
der field conditions remains to be determined. Judging
from our experiments here, we believe that these plants are
potentially more productive, especially in areas with high
irradiation; therefore, reduced photoinhibition becomes
advantageous. As plants are able to assimilate CO₂ for a
longer time because of decelerated chlorophyll breakdown,
they might be preferentially valuable in areas with long
growing seasons.

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