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# Photosynthetic Activities in the *Petunia* Corolla[1]

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## ABSTRACT

**Pink *Petunia hybrida* (cv Hit Parade Rosa) corollas were found to contain photosynthetically active chloroplasts. The corolla chloroplasts were similar to those of green leaves in size and structure. The chlorophyll (Chl) content of *Petunia* corollas increased during early stages of flower development, reaching a maximum just before anthesis. Chloroplasts isolated from corollas at this stage, carried out photosystem I-dependent electron transport at rates which were two-thirds of those measured in chloroplasts from green leaves, but full chain electron transport at only one-quarter of the rate carried out by chloroplasts from green leaves. Both the light saturated rate and the quantum yield for electron transport were lower in corolla chloroplasts, which also required lower intensities for light saturation. Reduced efficiency of photosystem II photoreactions in the corolla was also indicated by the ratio between variable and constant components of Chl fluorescence, which was lower in corollas compared to green leaves. The induction time of Chl fluorescence was at least three times shorter in corollas compared to green leaves, indicating a smaller number of functional photosystem II centers (per Chl) in the corolla. It is suggested that corolla chloroplasts of *Petunia* might have a role in flower developmental processes.**

Petals of most plants are green during early developmental stages, apparently due to the presence of Chl. This is rarely the case in mature petals where the presence of Chl is not visibly evident; it is either absent or masked by other pigments. Chloroplasts present in petals in early stages of flower development were shown to deteriorate into chromoplasts at later stages (4, 7, 14, 17), and it is generally assumed that mature white or colored corollas are devoid of chloroplasts. There are, with that, a few reports of the presence of chloroplasts in flower petals (13, 15, 16), but rather scarce information is available on photosynthetic processes in such chloroplasts and on their contribution to flower development. Dueker and Arditti (6) reported that green *Cymbidium* flowers are capable of $CO_2$ fixation both in the light and in the dark. Vu *et al.* (15) have similarly shown that light and dark $CO_2$ fixation occurs in young orange flower buds when petals are still green. We are unaware, however, of a report of significant photosynthetic activity in mature nongreen petals.

Preliminary experiments with *Petunia* flowers have shown, contrary to our expectation, that the Chl content of corollas increased during development, reaching maximum in expanded pink-colored corollas. The question was raised whether the corolla chloroplasts were photosynthetically active and whether they played any specific role in flower development. The present work addresses some of these questions, comparing photosynthetic activities of *Petunia* corollas to those of green leaves.

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## MATERIALS AND METHODS

**Plant Material.** Seeds of $F_1$ hybrid of *Petunia hybrida* (cv Hit Parade Rosa) were sown in 'speedling' trays filled with a peat:vermiculite mixture (1:1), and germinated in a greenhouse (18/27 night/day temperatures). Uniform seedlings at the 10 leaf stage were transplanted into 15 cm pots filled with peat:volcanic-scoria mixture (1:1) and grown in a similar greenhouse (18/29 night/day). Plants were irrigated thrice a day, with fertilization being supplied via the irrigation system. Flowers not used for experiments were removed from the plants twice a week, and plants were trimmed monthly so as to keep them compact. They were discarded after 1 year.

**Chl Determination.** Two hundred mg of leaf or corolla tissue was extracted with 5 ml of dimethylformamide for 48 h at 10°C in the dark. Chl concentration in the extract was determined spectroscopically (1). The presence of anthocyanin in corolla extracts did not interfere with Chl determination.

**Anthocyanin Determination.** Anthocyanins were extracted from fresh *Petunia* corollas using 1% HCl in methanol. Concentrations were determined by absorbance measurements at 530 nm and 657 nm, using the formula $A_{530} - 0.25 \times A_{657}$, to correct for the Chl and its degradation products present in the extract (10).

**Electron Micrographs.** Thin leaf or corolla slices (0.5 × 4 mm) were fixed for 4 h in a phosphate buffer containing 4% glutaraldehyde, washed several times in a phosphate buffer, and postfixed in 2% $OsO_4$ for 2 h. The slices were dehydrated in an ascending acetone series, embedded in an epon mixture and cured overnight at 60°C. The blocks were cut in an LKB ultramicrotome, and ultrathin sections were collected on 400-mesh grids. The sections were stained in a saturated uranyl acetate solution and in 0.2% lead citrate and were observed in a Joel Jem 100 CX EM.

**Chloroplast Isolation.** Chloroplasts were isolated from 15 to 30 g batches of either green leaves or corollas of petunia. Corollas were picked and used for chloroplast isolation just prior to anthesis. The leaves were homogenized for 20 s, using a blender, in 100 ml of medium containing 0.4 M sucrose, 0.01 M NaCl, 0.01 M tricine (pH 8.0), 0.04 M sodium ascorbate, and 1 mg/ml of bovine serum albumin. The homogenate was filtered through four layers of medical gauze and centrifuged briefly: the centrifuge was accelerated to 1,500*g* and then decelerated to a full stop. The pellet was discarded and the supernatant was centrifuged at 1,500*g* for 7 min. The supernatant was discarded, and the pellet was washed in 35 ml of 0.4 M sucrose, 0.01 M NaCl, 0.01 M tricine (pH 8.0) and was sedimented again by centrifugation at 12,000*g* for 7 min. The washing step was repeated, and the final pellet was resuspended in 4 ml of the same medium.

**Assay of Electron Transport.** Light-induced electron transport in chloroplast suspensions was estimated by measuring $O_2$ evolution or uptake with an $O_2$ monitor equipped with a Clark-type $O_2$ electrode. A light beam from a 150 W projector lamp (Sylvania G6,35-15) was passed through 5 cm of 1% $CuSO_4$, providing 650 $\mu E\ m^{-2}\ s^{-1}$ at the position of the suspension. The reaction

mixture contained 50 mM NaCl, 20 mM tricine (pH 8.0), 0.1 mM methyl viologen, 2 mM $NaN_3$, and chloroplasts equivalent to 30 $\mu$g of Chl per ml. For assays of PSI activity, the reaction mixture contained in addition 60 $\mu$M DCMU, 2 mM sodium ascorbate, and 0.1 mM 2,6 dichlorophenolindophenol.

**Chl Fluorescence Measurements.** Fluorescence induction curves of intact leaves were recorded with a laboratory-built apparatus. The actinic beam for fluorescence excitation was supplied by a 150 W projector lamp (Sylvania G6,35-15) equipped with its own heat filter and a Corning CS 4-96 filter. This provided a broadband blue light (400–500 nm) with a PPFD[2] of 450 $\mu$E $m^{-2}$ $s^{-1}$ at the position of the sample. The beam was aimed at an area of 5 × 5 mm, which in corollas was in the region joining the tube and the limb. The onset of illumination was controlled by an electronic shutter (Uniblitz SD 122B, Vincent Associates, Rochester, NY). The opening time was approximately 1 ms. Chl fluorescence emission was detected at a 90° angle through a Corning CS 2-64 red filter and a 685 nm interference filter, with a photomultiplier (R136, Hamamatsu TV Co., Hamamatsu, Japan). Transient signals were detected and stored with a model 200 Nicolet digital oscilloscope (Nicolet Instrument, Madison, WI), and permanent copies of the data were plotted on an *x-y* recorder. The fluorescence induction time ($t$), was measured essentially as described by Malkin *et al.* (8, 9).

## RESULTS

**Chl and Chloroplasts in the *Petunia* Corolla.** Figure 1 shows several developmental stages of the *Petunia* flower, starting with a small bud (stage 1) and ending with a fully developed corolla (stage 7). Buds at stages 1 to 3 were typically green, their anthocyanin content was negligible, and they exhibited a slow growth rate. The transition from stage 3 to stage 4 was characterized by the advent of anthocyanin accumulation and by a sharp increase in growth rate. Accumulation of anthocyanins in corolla stages 4 to 7 occurred largely in their upper part (the limb) which became pink, while the lower part (the tube) was white with a light greenish tint.

The Chl content of the *Petunia* corolla was found to increase during early developmental stages, reaching a maximum just before anthesis (stage 6). At this stage, the anthocyanin content of the corolla also reached maximum (Fig. 2A). On the other hand, Chl synthesis did not keep up with the corolla growth rate, so that its average concentration per gram fresh weight of corollas started to decrease at quite an early stage (Fig. 2B). The maximal Chl concentration, achieved at an early developmental stage, was about 40% of that of green leaves. It is interesting to note that at anthesis the Chl concentration in the pink limb was twice that measured in the greenish tube.


FIG. 1. Different developmental stages of the *Petunia* flower, starting from the first appearance of the corolla (stage 1) and ending with the fully expanded flower (stage 7). Sepals were removed from one side of each flower before taking this picture to expose the corolla.

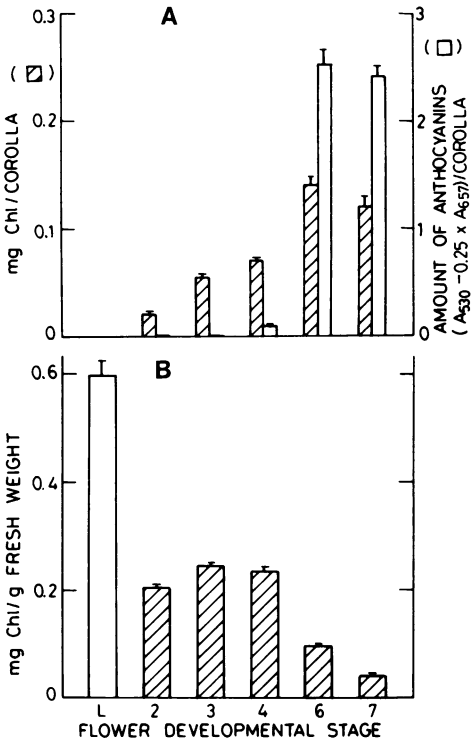


FIG. 2. Chl and anthocyanin levels in *Petunia* corolla during the different developmental stages. A, Chl and anthocyanin content per corolla; B, average Chl concentration per g fresh weight. The column marked L in frame B, shows for comparison the Chl concentration in fully expanded green leaves.

Electron micrographs of thin sections made in petunia corollas (in the region joining the limb and tube) show the presence of chloroplasts during all developmental stages. Chloroplast growth and development largely paralleled the flower developmental stages. At anthesis the corolla chloroplasts were similar to those observed in green leaves in size and number of grana and contained starch granules of considerable size (Fig. 3).

**Electron Transport in Chloroplasts.** The rate of light-induced electron transport with methyl viologen as acceptor in chloroplasts isolated from *Petunia* corollas amounted to ~24% of that measured in chloroplasts from green leaves (Table I). Similar results were obtained in the presence of 3 mM $NH_4Cl$, and spectrophotometric determinations of the photoreduction of potassium ferricyanide confirmed the measurements carried out with the $O_2$ electrode (data not shown). Electron transport in these experiments required the simultaneous operation of PSI and PSII. On the other hand, electron transport from reduced dichlorophenolindophenol to methyl viologen, which involved only PSI, amounted in corolla chloroplasts to 65% of the rate in chloroplasts from green leaves. These results seem to indicate that the reduced rates of the whole chain process were due to reduced PSII activity in the corolla chloroplasts.

A lower photon flux density was necessary for light-saturation in corolla chloroplasts, and with that they were less efficient in utilizing light energy for electron transport, compared to chloroplasts from green leaves. This can be seen, qualitatively, in a plot of V—the rate of whole-chain electron transport, *versus*

[2] Abbreviations: PPFD, photosynthetic photon flux density.

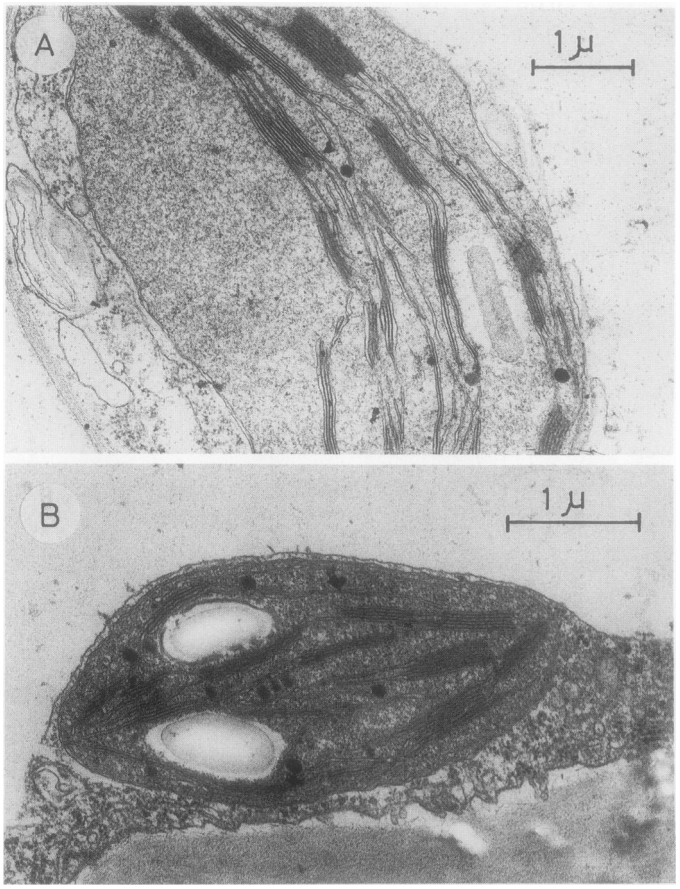


FIG. 3. Electron micrographs of thin sections in a *Petunia* green leaf (A) and in a corolla (B). The corolla was obtained from a flower in stage 6.

Table I. *Electron Transport in Isolated Chloroplasts from Petunia Corollas and Green Leaves*

Means of 15 replications ± SE. The reaction conditions are described under "Materials and Methods."

| Reaction | Electron Transport | | |
|---|---|---|---|
| | Green leaf | Corolla | Corolla/leaf |
| | *μeq mg Chl*$^{-1}$ *h*$^{-1}$ | | |
| PSI | 341 ± 11 | 220 ± 10 | 0.65 |
| PSII + I | 106 ± 5 | 25 ± 3 | 0.24 |

PPFD (Fig. 4A). The reduction in quantum yield is evident as a lower slope of the corolla curve at low PPFD levels. For quantitative determination of these effects, we take advantage of the fact that the plots in Figure 4A have the form of rectangular hyperbolas. Electron transport is accordingly given by Schönfeld *et al* (12),

$$V = \frac{\text{PPFD} \times V_{max}}{K_i + \text{PPFD}} \quad (1)$$

where $V_{max}$ stands for the maximal rate under light saturating conditions, and $K_i$ is the light intensity necessary to induce half the maximal rate. Eq. 1 can be rewritten in a form that permits the results to be plotted as straight lines (compare to equivalent analysis of enzyme catalyzed reactions; *e.g.* Ref 5).

$$\frac{\text{PPFD}}{V} = \frac{K_i}{V_{max}} + \frac{\text{PPFD}}{V_{max}} \quad (2)$$

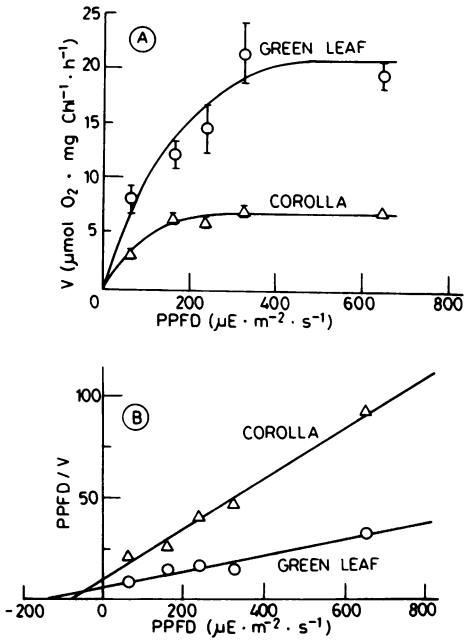


FIG. 4. Effect of PPFD on the rate of electron transport (V). The data in A were linearly transformed and replotted in B. See the text for details.

Table II. *Chl Fluorescence in the Presence of DCMU*

Means of 10 replications ± SE.

| | Green Leaf | Corolla |
|---|---|---|
| $1\text{-}F_0/F_m$ | 0.76 ± 0.04 | 0.61 ± 0.06 |
| Induction time (ms) | 38 ± 3 | 13 ± 1 |

A plot of PPFD/$V$ *versus* PPFD should yield a straight line with a slope of $1/V_{max}$, and an $x$-axis intercept of $-K_i$. The $y$-axis intercept, *i.e.* PPFD/$V$ at PPFD = 0, is a measure for the quantum yield of electron transport. Figure 4B, in which the data of Figure 4A were replotted, shows that the relative quantum yield for electron transport was some 40% lower in corolla compared to green-leaf chloroplasts. The light-saturated rate ($V_{max}$) of electron transport in corolla chloroplasts was one-third of that measured in green-leaf chloroplasts, and the light intensity required for half the maximal rate in corolla chloroplasts was half of the value obtained for green-leaf chloroplasts.

The three parameters determined via the light dependence of electron transport in isolated chloroplasts (Fig. 4) are not completely independent of each other. The relationship between $V_{max}$, $K_i$, and $Q_y$ (the relative quantum yield), is given by: $K_i = V_{max}/Q_y$ (12). The three-fold difference in $V_{max}$ between corolla and green-leaf chloroplasts (which, as indicated below, was probably due to a difference in size between the PSII photosynthetic units in the two organs) was partially offset by the reduced quantum yield in the corollas and was therefore associated with only a two-fold difference in $K_i$.

A reduction in quantum yield of PSII photochemical reactions in *Petunia* corollas compared to green leaves was also indicated by Chl *a* fluorescence measurements in intact organs (Table II). Illumination of dark-adapted green leaves or corollas, after infiltration with DCMU, resulted in a rapid rise of fluorescence from the initial (or constant) level, $F_o$, to the maximal level, $F_m$. The value of $1\text{-}F_o/F_m$, which is a measure for the quantum yield of photochemical conversion (8), was 20% lower for petunia corollas compared to that for green leaves. The rate of fluorescence rise, from $F_o$ to $F_m$, in corollas was faster than in green leaves. Table II shows that the induction time in corollas was one-third of that measured in green leaves. The induction time is defined as the time needed for the completion of the process, if proceed-

ing with a constant rate equal to its initial rate. The shorter induction time in corollas might indicate that the PSII photosynthetic unit in corollas was larger than in green leaves, *i.e.* that corolla chloroplasts contained a smaller concentration of PSII centers, relative to total Chl.

Basically similar results were obtained in the absence of DCMU (Table III). $F_v/F_o$, *i.e.* the ratio between the variable and constant portions of the Chl fluorescence signal was about 30% lower in corollas compared to green leaves. The induction time in corollas in the absence of DCMU was one-sixth of the time in green leaves. In addition, the intermediate fluorescence, $F_I$, level in corollas was significantly higher. The $F_I$ level, evident as a shoulder in the Chl induction curve (Fig. 5), has been used as a measure for the rate of electron transport from $Q_A$ to $Q_B$: the primary and secondary acceptors of PSII (2). The increase in the $F_I$ level seems to indicate a decrease in this electron transfer step in the corolla.

The Chl fluorescence induction in *Petunia* corollas differed from that measured in green leaves also in its dependence on the photon flux density (Fig. 6). The $F_v/F_o$ ratio increased in green leaves with the photon flux density until light-saturation was achieved. A much lower flux density was required to achieve the maximal level, and further increases resulted in progressive inhibition. The moderately high flux density (450 $\mu$E $m^{-2}$ $sec^{-1}$) used in the experiments described above (Tables II and III; Fig. 5) was evidently inhibitory for the corollas but not for the leaves. The $F_v/F_o$ ratio, measured for corollas at the optimum flux density, was not significantly different from that measured for leaves at the maximal PPFD.

## DISCUSSION

The results presented in this paper, which indicate that the Chl content did not decline with the change in pigmentation of *Petunia* corolla (from light green to dark pink), were a surprising finding. This is contrary to what is generally accepted as the regular developmental pathway of petal plastids, from chloroplasts to carotenoid-containing chromoplasts (7, 17). Chloroplasts were shown to deteriorate into chromoplast-like organelles during flower development even in flowers where anthocyanins are the predominant pigments (17). At variance with these results, the change in the *Petunia* corolla coloration did not stem from Chl degradation but was due to synthesis of epidermal anthocyanins, masking the presence of green chloroplasts in the cell layers underneath. It is not clear at this point how frequent this phenomenon is among anthocyanin-containing flowers of other species.

Table III. *A Comparison of Chl a Fluorescence Parameters in Petunia Corolla and Green leaves (in the absence of DCMU)*

Means of 20 replications ± SE.

| | Green Leaves | Corollas |
|---|---|---|
| $F_v/F_0$ | 2.2 ± 0.1 | 1.6 ± 0.1 |
| Induction time (s) | 0.55 ± 0.05 | 0.09 ± 0.01 |
| $(F_I - F_0)/F_0$ | 0.29 ± 0.01 | 0.76 ± 0.04 |

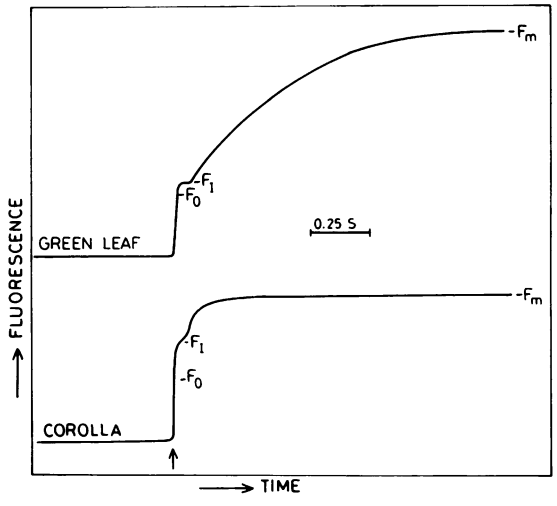


FIG. 5. Chl fluorescence induction curves of a *Petunia* green leaf and a corolla (in absence of DCMU). The initial fluorescence level—$F_o$, the intermediate level—$F_I$, and maximal level—$F_m$ are indicated. Fluorescence is in relative units.

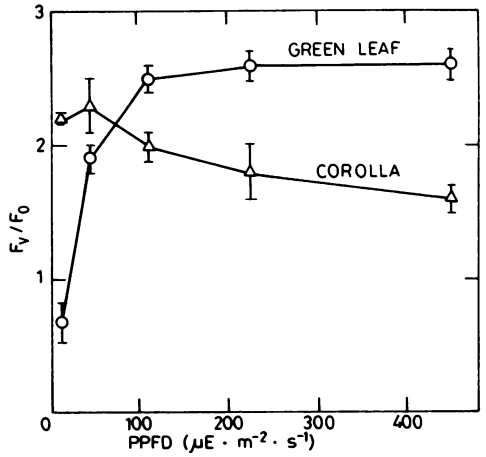


FIG. 6. Effect of PPFD on the ratio between the variable and constant portions of Chl fluorescence ($F_v/F_o$) in *Petunia* corolla and in a green leaf.

The high Chl content in mature colored *Petunia* corollas, the chloroplast ultrastructure resembling that of chloroplasts from green leaves, and the correspondence between flower and chloroplast development all seem to hint at the occurrence of specific functions for the corolla chloroplasts. These data seem also to rule out the possibility of the chloroplasts functioning only at early stages and slowly deteriorating after that. Chloroplasts in the *Petunia* corolla were not observed to change into chromoplasts.

The presence of starch granules in *Petunia* corolla chloroplasts (Fig. 3), the Chl fluorescence induction pattern in the intact corolla (Fig. 5), and direct measurements of electron transport (Table I; Fig. 4), all attest to the photochemical activity of corolla chloroplasts. We have found, however, a large difference between PSI and PSII potencies in the corolla chloroplasts, which should limit the overall rate of photosynthesis to that of PSII. The higher activity of PSI might indicate a special role for its products in the corolla development and pigmentation (11). Substantiation of this suggestion requires further experimental verification.

The low light intensity needed to saturate full-chain electron transport in chloroplasts isolated from *Petunia* corollas is similar to that observed in shade plants (3). Such plants are characterized by relatively large photosynthetic units or, in other words, by low concentrations of reaction centers on a Chl basis. The fluorescence 'induction time' can be used as a measure for the size of PSII photosynthetic unit (8): the shorter the time the larger is the unit. Indeed, the average induction time in corollas was 3 to 6 times shorter than in green leaves, indicating a significantly larger photosynthetic unit in the corolla. A need for a large photosynthetic unit may arise at an early stage of flower development, if operation of the photosynthetic apparatus of corolla chloroplasts is required when the corolla is still largely covered by the sepals. The light intensity reaching the corolla 'in the shade' of the sepals will, of course, be significantly reduced.

Measurements of the light dependence of electron transport in isolated chloroplasts and Chl fluorescence induction patterns in intact organs indicated reduced efficiency of light utilization by PSII in the *Petunia* corolla, as compared to green leaves. The increased $F_I$ fluorescence level, which was previously interpreted as indicating reduced electron transfer rates from $Q_A$ to $Q_B$ (2), also points out that PSII functions are modified in the corolla. The nature of these modifications and their physiological significance are not known at this stage.

The results presented in this study demonstrate the presence of photochemically active chloroplasts in expanded pink *Petunia* corollas. The photosynthetic apparatus in corolla chloroplasts differed from that in green leaves in having an apparently larger photosynthetic unit and in modified functions of PSII, including reduced quantum yield. The role of corolla chloroplasts in *Petunia hybrida* is still to be determined. Results to be presented elsewhere indicated that the photosynthetic apparatus of corolla chloroplasts might be involved in the corolla development and pigmentation. Growth and pigmentation of detached corollas incubated in the light in a sugar-containing medium were inhibited by DCMU. The contribution of the corolla chloroplasts evidently extends beyond carbohydrate supply, but further experiments are required to determine the nature of the products involved and their physiological significance.

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