Identification of Mutants of Arabidopsis Defective in Acclimation of Photosynthesis to the Light Environment

Robin G. Walters, Freya Shephard, Jennifer J.M. Rogers, Stephen A. Rolfe, and Peter Horton

Department of Molecular Biology and Biotechnology (R.G.W., F.S., J.J.M.R., P.H.), and Department of Animal and Plant Sciences (S.A.R.), University of Sheffield, Western Bank, Sheffield S10 2TN, United Kingdom

In common with many other higher plant species, Arabidopsis undergoes photosynthetic acclimation, altering the composition of the photosynthetic apparatus in response to fluctuations in its growth environment. The changes in photosynthetic function that result from acclimation can be detected in a noninvasive manner by monitoring chlorophyll (Chl) fluorescence. This technique has been used to develop a screen that enables the rapid identification of plants defective at ACCLIMIATION OF PHOTOSYNTHESIS TO THE ENVIRONMENT (APE) loci. The application of this screen to a population of T-DNA-transformed Arabidopsis has successfully led to the identification of a number of mutant lines with altered Chl fluorescence characteristics. Analysis of photosynthesis and pigment composition in leaves from three such mutants showed that they had altered acclimation responses to the growth light environment, each having a distinct acclimation-defective phenotype, demonstrating that screening for mutants using Chl fluorescence is a viable strategy for the investigation of acclimation. Sequencing of the genomic DNA flanking the T-DNA elements showed that in the ape1 mutant, a gene was disrupted that encodes a protein of unknown function but that appears to be specific to photosynthetic organisms, whereas the ape2 mutant carries an insertion in the region of the TPT gene encoding the chloroplast inner envelope triose phosphate/phosphate translocator.

The ability of plants to modify their growth, development, and physiology according to variations in environmental factors (e.g. light, temperature, and nutrient availability) plays a crucial role in determining their tolerance to stress, their ability to compete with other plants, and the efficiency with which external inputs are used for growth and productivity (Anderson and Osmond, 1987; Murchie and Horton, 1997). Some of the clearest responses to such environmental factors involve major modifications to the photosynthetic apparatus, “photosynthetic acclimation”, which can serve, for instance, to improve the efficiency with which light energy is used in photosynthesis (Chow et al., 1990; Walters and Horton, 1995a) or to ameliorate the damaging effects of environmental extremes (Anderson and Osmond, 1987; Park et al., 1996; Savitch et al., 2000).

The changes in chloroplast composition that result from variations in the quantity of incident light have been particularly well-characterized. With increasing light availability during growth, the requirement for light-harvesting complexes (LHCs) to ensure efficient light capture is reduced, and there is increased demand for electron transport and carbon assimilation components to support higher rates of photosynthesis (Anderson and Osmond, 1987; Anderson et al., 1995). In low light (LL), there are accordingly high levels of chlorophyll (Chl) a/b-binding LHCs, particularly those associated with photosystem II (PSII), whereas growth in high light (HL) increases the levels of photosystems, the cytochrome b6/f electron transport complex, ATP synthase, and Calvin cycle enzymes, particularly the CO2-fixing enzyme Rubisco.

As a result of these changes in chloroplast composition, both the maximum rate of photosynthesis and the ratio of Chl a to Chl b (Chl a/b) increase as growth light irradiance increases. Because these parameters are both simple to measure and correlate strongly with the underlying changes in chloroplast composition, they are commonly used as indicators of photosynthetic acclimation. Moreover, changes in the relative rates at which absorbed light energy is used in photosynthesis or dissipated via other processes (as heat) are readily detected via changes in the yield of Chl fluorescence (for review, see Krause and Weis, 1991).

Despite the extensive characterization of acclimation in terms of the composition and function of the photosynthetic apparatus, little is known about the mechanisms by which it is regulated. Although significant progress has been made in dissecting the regulation in developing seedlings of gene expression by phytochrome and blue-light photoreceptors, our recent work indicates that the regulation of acclimation is...
largely independent of such photoreceptor-mediated light signals (Walters et al., 1999). There is growing evidence that acclimation depends instead on signals from photosynthetic metabolism, most notably the re- dox state of one or more electron carriers (Escoubas et al., 1995; Maxwell et al., 1995; Pfannschmidt et al., 1999, 2001). However, there is little understanding of these signals or of the mechanisms by which they are transduced.

The acclimation response is complex: It involves changes in the relative abundance of a large number of proteins encoded by both chloroplast and nuclear genomes; expression of some of these proteins also responds to altered spectral quality of light and is influenced by other environmental factors; and control of the levels of a number of photosynthetic proteins occurs at levels other than transcription (Flachmann and Kühlbrandt, 1995; Kim and Mayfield, 1997; Petracek et al., 1997), including degradation of particular pigment-protein complexes by specific proteases (Yang et al., 1998). Therefore, the regulation of acclimation potentially involves multiple signal transduction chains, with crosstalk between re- dox control and other pathways that control photosynthetic gene expression (Walters et al., 1999; Oswald et al., 2001). It is also important to note that acclimation occurs not only during growth under particular growth conditions, but also following a change in growth conditions: For instance, a transfer from LL to HL prompts rapid adjustments in photosynthesis and chloroplast composition.

Our recent work has established Arabidopsis as a model system for investigating photosynthetic acclimation. Variations in the levels of Rubisco, photosystems, and LHCs are observed for growth under a wide range of light environments, leading to differences in Pmax and Chl a/b (Walters and Horton, 1994, 1995a, 1995b, 1999; Walters et al., 1999; Bailey et al., 2001). This has opened up the possibility of using a genetic approach to investigate photosynthetic acclimation. Because acclimation leads directly to changes in photosynthesis and therefore also results in altered Chl fluorescence, visualization of Chl fluorescence provides a means of rapidly screening mutagenized populations of plants. Simple Chl fluorescence measurements have previously been used successfully in several mutant screens (Dinkins et al., 1994; Niyogi et al., 1997, 1998; Kruse et al., 1999; Peterson and Havir, 2000; Varotto et al., 2000). Here, we describe a novel Chl fluorescence-based screen designed to identify mutants that affect acclimation either directly by interfering with its regulation or indirectly as a result of changes in photosynthesis. A preliminary screen of the “Feldmann” T-DNA-transformed population (Feldmann, 1991) has identified several lines with apparently altered acclimation characteristics, demonstrating that this method can be used to quickly and simply identify such mutants.

RESULTS

Acclimation to Growth Irradiance in Arabidopsis cv Wassilewskija (Ws-2)

Figure 1 shows that the Ws-2 accession, the parental line for the Feldmann populations of T-DNA- transformed Arabidopsis (Feldmann, 1991), acclimates to growth in HL and LL conditions in a similar manner to that previously reported for the Landsberg erecta accession, although the scale of the response varies between the two ecotypes (compare with Walters and Horton, 1994; Walters et al., 1999). Clear differences between LL- and HL-grown plants were observed for Pmax, the maximum rate of O2 evolution in CO2-saturated conditions (Fig. 1A), and for Amax, the maximum rate of CO2 assimilation in air (Fig. 1B). As expected, the occurrence of photorespiration when the CO2 concentration was at ambient levels led to the capacity for CO2 assimilation being lower.

![Figure 1](image_url)

Figure 1. Acclimation of photosynthesis in Arabidopsis cv Ws-2. A, Light response curve for O2 evolution in saturating CO2 from leaf discs cut from 6- to 7-week-old plants grown under LL (○) or HL (●). B, Light response curve for CO2 consumption by attached leaves from 6- to 7-week-old plants in ambient (350 μmol quanta m-2 s-1) CO2, with parallel measurements of PSII photochemical efficiency ΦPSII (C) and the estimated linear electron transport rate calculated as 0.5 × ΦPSII × PFD (D). Data are means ± SE, n = 3. E, ΦPSII during illumination (250 μmol quanta m-2 s-1) of seedlings grown under LL (○) or HL (●), determined periodically during their development and following transfer of 14-d-old LL-grown seedlings to growth under HL (□). Data are means ± SE, n = 10.
than to the capacity for $O_2$ evolution in saturating CO$_2$. The substantially larger difference for HL-grown plants (a 50% reduction compared with 25% for LL plants) is readily explained by an increase in photorespiration: The intercellular CO$_2$ concentration was significantly lower ($138 \pm 8$ compared with $181 \pm 5 \mu$L$^{-1}$) at light-saturated rates of photosynthetic rate.

As photosynthesis approached its maximum rate with increasing light, the efficiency with which light was used was reduced. However, because of their higher maximum photosynthetic rate, HL-grown plants underwent this reduction in photosynthetic efficiency more slowly than LL-grown plants. This was reflected in differences in the Chl fluorescence parameter $\Phi_{PSII}$ (Fig. 1C), which is directly proportional to photosynthetic efficiency (Genty et al., 1989). Estimation of PSII electron transport rate using the data for $\Phi_{PSII}$ further illustrates that $W$s-2 acclimates strongly to growth irradiance (Fig. 1D). As indicated in Figure 1C, as irradiance increased above approximately 200 $\mu$mol quanta m$^{-2}$ s$^{-1}$, HL- and LL-acclimated plants could be distinguished on the basis of differences in photosynthetic efficiency as indicated by the value of $\Phi_{PSII}$. It should be stressed that this did not reflect a fundamental difference in the efficiency of photosynthesis (under low illumination, there were negligible differences between HL- and LL-grown plants) but was attributable to the higher maximum photosynthetic rate in HL-grown plants (i.e. in HL-grown plants, photosynthesis required higher illumination to reach saturation, so that quantum efficiency was greater in HL).

The ability to distinguish different acclimation states on the basis of Chl fluorescence characteristics was not restricted to mature plants but was also possible for developing seedlings (Fig. 1E). Different fluorescence characteristics were clearly observed in the cotyledons of HL- or LL-grown seedlings as young as 7 d old. Furthermore, LL-grown seedlings exhibited rapid changes in photosynthesis after transfer to HL growth conditions, indicating that even 14-d-old seedlings were capable of dynamic acclimation to varying environmental conditions. Although there appears to be incomplete acclimation to the change in growth conditions, suggesting that cotyledons may not have the same acclimation capability as true leaves, the extent of the change in $\Phi_{PSII}$ observed after 4 d is comparable with that achieved by mature plants; under these growth conditions, it takes approximately 10 d to fully acclimate to a LL to HL transfer (data not shown).

Identification of Mutants with Altered Acclimation

The ability to use Chl fluorescence measurements to distinguish between HL- and LL-acclimated plants at an early stage of development has been exploited in the design of a method for identifying mutants with altered acclimation characteristics. Monitoring Chl fluorescence by video imaging (Rolfe and Scholes, 1995) allowed many thousands of young seedlings from mutagenized populations to be rapidly screened in a non-destructive manner. The strategy involved growing plants under LL for 14 d and then transferring them to HL for a further 3 d. Before and after the period of HL growth, they were exposed to even illumination (approximately 250 $\mu$mol quanta m$^{-2}$ s$^{-1}$), and images of Chl fluorescence were captured under steady-state conditions and during application of a high intensity pulse of light. Mutants with altered chloroplast composition before and/or after the increase in growth light would be identified on the basis of the resulting changes in the maximum rate of electron transport, detected via changes in Chl fluorescence (see Fig. 1E).

This two-stage screen was tested using the Feldmann T-DNA-transformed families (Feldmann, 1991). Four hundred to 500 seeds from each family of 100 lines were sown on pairs of 9-cm petri dishes (see “Materials and Methods”) and grown under LL for 14 d. For the first part of the screen, each dish was illuminated for 15 min, and video images of Chl fluorescence were captured to allow calculation of $\Phi_{PSII}$. The seedlings were then moved to HL growth conditions for 3 d, after which a second set of fluorescence images were acquired. Figure 2 shows a typical pair of false color images of $\Phi_{PSII}$ generated from a single set of seedlings during the two stages of the screen. It is clear from the overall change in color of the images that $\Phi_{PSII}$ under the conditions of the screen increased after the transfer to HL growth. This agrees with the expectation that the majority of seedlings (i.e. those with wild-type acclimation) would exhibit an increase in photosynthetic capacity in response to the changed growth conditions.

Although individual seedlings could in many cases be clearly identified, there was frequently a degree of overcrowding that made it difficult to discern individual seedlings from the $\Phi_{PSII}$ images because of a combination of overlapping fluorescence signals and uneven illumination in crowded regions. Nevertheless, during both the first and second stages of the screen, seedlings were readily identifiable that had values for $\Phi_{PSII}$ that appeared different from those for the surrounding seedlings; one such seedling is highlighted in Figure 2C. In all, this trial of the screening strategy identified 51 individuals representing 28 separate families, out of a total of approximately 30,000 seedlings from 64 families of 100 transformed lines each. To confirm that these plants showed changes in $\Phi_{PSII}$ and to show that these were stably inherited, these plants were allowed to self-fertilize, and their progeny were rescanned by a protocol similar to that used for the initial screen, except that fluorescence measurements for individual seedlings were carried out using a portable fluorometer rather than by video imaging.
Figure 3A shows the results of this rescreening: Six independent lines were identified with fluorescence characteristics that were reproducibly distinct from the wild type. From these data, together with further fluorescence measurements taken 7 d after the transfer to HL (Fig. 3B), three separate classes of acclimation defect were identified: Lines 99-1 and 88-1 were indistinguishable from the wild type when grown under LL, but responded to the HL transfer to a lesser extent (99-1) or more slowly (88-1) than wild type; 56-1 and 22-1 had markedly reduced photosynthetic efficiency under the conditions of the screen after both LL growth and transfer to HL; whereas 6-1 and 3-3 showed incomplete acclimation to LL, having an increased $\Phi_{PSII}$ compared with the wild type, but both acclimated normally to HL.

Of these lines, 88-1, 56-1, and 3-3 were found to be kanamycin sensitive (Kn$^s$), suggesting that the phenotype was not associated with a T-DNA element. The absence of T-DNA sequences in these lines was confirmed by the failure of T-DNA left and right border probes to hybridize to Southern blots; conversely, hybridization of LB and RB sequences to genomic Southern blots confirmed that the three kanamycin-resistant (Kn$^r$) lines 99-1, 22-1, and 6-1 lines carried T-DNA sequences (not shown). The mutations carried by these lines were respectively denoted acclimation of photosynthesis to the environment (ape$^1$), ape$^2$, and ape$^3$, corresponding to genes designated APE1, APE2, and APE3, T-DNA insertions into which were postulated to be responsible for the acclimation-defective phenotypes.

Figure 4 shows that the differences in the Chl fluorescence characteristics of mutant and wild-type lines were sufficiently clear to provide markers for the ape$^1$ (99-1) and ape$^2$ (22-1) phenotypes that could be used in genetic analysis: $\Phi_{PSII}$ during steady-state photosynthesis under 250 $\mu$mol quanta m$^{-2}$ s$^{-1}$ actinic illumination was measured directly using a PAM2000 fluorometer. A, $\Phi_{PSII}(250)$ after 14 d in LL and after a further 4 d under HL plotted against each other for 51 candidate mutants ($\bullet$) and three sets of Ws-2 seedlings ($\square$), highlighting the six lines with the clearest difference from the parental line ($\bullet$). B, $\Phi_{PSII}(250)$ after 14 d in LL and 0 (black), 4 (hatched), and 7 (white) d under HL, for Ws-2 and the six selected lines. Data are means ± SE, $n = 10$. For comparative purposes, broken lines show $\Phi_{PSII}(250)$ for Ws-2 after 0, 4, and 7 d in HL. a, Significant difference from Ws-2 plants grown under the same conditions, $P < 10^{-4}$. 

Figure 3. Identification of ape mutants. The progeny of candidate mutants were analyzed alongside the parental Ws-2 line using a similar protocol to that used for the initial screen, except that $\Phi_{PSII}$ during steady-state photosynthesis under 250 $\mu$mol quanta m$^{-2}$ s$^{-1}$ actinic illumination was measured directly using a PAM2000 fluorometer. A, $\Phi_{PSII}(250)$ after 14 d in LL and after a further 4 d under HL plotted against each other for 51 candidate mutants ($\bullet$) and three sets of Ws-2 seedlings ($\square$), highlighting the six lines with the clearest difference from the parental line ($\bullet$). B, $\Phi_{PSII}(250)$ after 14 d in LL and 0 (black), 4 (hatched), and 7 (white) d under HL, for Ws-2 and the six selected lines. Data are means ± SE, $n = 10$. For comparative purposes, broken lines show $\Phi_{PSII}(250)$ for Ws-2 after 0, 4, and 7 d in HL. a, Significant difference from Ws-2 plants grown under the same conditions, $P < 10^{-4}$. 

Figure 2. Imaging of photosynthetic efficiency. Chl fluorescence images were determined for populations of T-DNA-transformed seedlings during steady-state photosynthesis under 250 $\mu$mol quanta m$^{-2}$ s$^{-1}$ actinic illumination and during application of a saturating light pulse. Sample images of the calculated $\Phi_{PSII}$ are shown in false color for the batch of seedlings containing the ape$^1$ mutant, line 99-1, after 14 d of growth under LL (A) and after a further 3 d under HL (B); C, the inset highlights the location of the mutant seedling.
Figure 4. Chl fluorescence measurements as markers for ape phenotypes. Mutant and Ws-2 lines were grown under LL (14 d) and transferred to HL for a further 7 d. The ability to distinguish the wild-type and mutant phenotypes on the basis of fluorescence measurements was tested by comparison of the distribution of the data for Ws-2 (white) and the mutants (black). A, $\Phi_{\text{PSII}}(250)$ data for ape1 and Ws-2 seedlings after 7 d at HL; B, $\Phi_{\text{PSII}}(250)$ data for ape2 and Ws-2 seedlings after 7 d at HL; C, $\Phi_{\text{PSII}}(250)$ data for LL-grown ape3 and Ws-2 seedlings. All data are for measurements from a minimum of 36 seedlings.

concluded that T-DNA sequences were inserted into the genome of ape1 plants at a single site and that the ape1 marker was in all probability attributable to insertional inactivation by a T-DNA element: It mapped to within 2.6 centiMorgans of the Kn’ marker ($P < 0.05$). An identical analysis of ape2 × Ws-2 crosses (five $F_1$ plants, 179 $F_2$ plants, and 124 $F_3$ populations) led to a similar conclusion: that T-DNA sequences were inserted into the nuclear genome of ape2 plants at a single site and that the ape2 marker mapped to within 3.4 centiMorgans of the Kn’ marker ($P < 0.05$).

In contrast to the ease with which Ws-2 plants could be distinguished from ape1 or ape2 mutants, there was considerable overlap between the $\Phi_{\text{PSII}}$ values for ape3 plants and the parental line, even under LL growth—the conditions giving the greatest difference between them (Fig. 4C); other differences (see below) were also insufficient to give a clear distinction between Ws-2 and ape3 seedlings or plants, so that we have thus far been unable to confirm whether or not the ape3 phenotype is attributable to the T-DNA element(s) present in this line. However, photosynthesis measurements carried out on the $F_1$ progeny of crosses between Ws-2 and 6-1 plants suggested that the ape3 mutation was recessive.

Altered Acclimation in ape Mutants

Characterization of the acclimation properties of the three Kn’ lines confirmed that the altered fluorescence characteristics of ape seedlings were reflected in changes in the composition and/or function of the photosynthetic apparatus in mature plants. Figure 5 shows measurements of photosynthetic $O_2$ evolution for leaf discs taken after HL or LL growth. From these results, it was clear that the changes in the observed $\Phi_{\text{PSII}}$ were correlated with altered maximum photosynthetic rate, $P_{\text{max}}$ for mature ape2 and ape3 plants: ape2 plants had reduced photosynthetic capacity whether grown under LL or HL, whereas LL-grown ape3 plants had increased $P_{\text{max}}$ compared with the Ws-2 parental line, with HL-grown ape3 plants being indistinguishable from wild type. In contrast, the ape1 mutant showed no significant change in $P_{\text{max}}$ for growth under either LL or HL, contrary to what might be predicted from the $\Phi_{\text{PSII}}$ observed in seedlings.

In wild-type Arabidopsis as with many other plants, changes in $P_{\text{max}}$ are strongly correlated with parallel changes in the Chl $a/b$ ratio, which reflect adjustments in the composition of the thylakoid membrane (Bailey et al., 2001). Compared with growth in LL, HL-grown plants increase their PSII content and decrease levels of LHCs, particularly LHCII (where most Chl $b$ is bound), the net result being the observed increase in Chl $a/b$. Table I shows the results of further analysis of acclimation in the three T-DNA-transformed lines. Chl $a/b$ ratio and PSII content were measured for leaves from LL- and HL-grown plants, and also for plants grown under LL and then transferred to HL growth for 7 d; care was taken that the leaves used for these latter measurements were already fully developed at the time of the LL-to-HL transfer, so that the measurements reflected dynamic acclimation of mature leaves and not the characteristics of leaves that developed under the new growth conditions. When grown under LL and HL, the parental Ws-2 line had Chl $a/b$ ratios and PSII levels similar to those previously reported for the Landsberg erecta line grown under identical conditions (Walters et al., 1999), and as expected, there was broad agreement between Chl $a/b$ and PSII measurements for all lines and growth conditions, i.e. higher values for Chl $a/b$ corresponded to higher levels of PSII.

The different ape lines exhibited different patterns for Chl $a/b$ and PSII, in some cases distinct from those observed for photosynthesis and Chl fluorescence.

Figure 5. Acclimation of photosynthesis in fully grown ape mutants. Oxygen evolution rates from leaf discs under CO$_2$-saturated conditions were measured during step-wise increases in actinic illumination. Leaf discs were cut from 5- to 7-week-old plants grown under LL (●) or HL (○). A, ape1; B, ape2; C, ape3. For comparison, the data for Ws-2 from Figure 1 are replotted as broken lines. Data are means ± se, $n \geq 3$.  

cence. Under LL growth, ape1 was unchanged compared with Ws-2, and under HL growth, ape1 had only a small (not statistically significant) decrease in Chl \(a/b\) and no change in PSII content. Because there was no change in photosynthesis (Fig. 5), there does not appear to be any acclimation defect in this line when it is grown under constant conditions. However, following an LL to HL transfer (a similar treatment to that used during the mutant screen) there was a clear difference between ape1 and wild-type plants: Whereas wild-type plants almost fully adjusted to the change in growth conditions within 7 d, in terms of both thylakoid composition (Table I) and photosynthesis (data not shown), ape1 plants showed much slower acclimation of the thylakoid membrane with smaller changes in both Chl \(a/b\) and PSII; in contrast, acclimation of \(P_{\text{max}}\) was unchanged compared with the wild type. It is thus clear that the acclimation defect in this mutant relates to changes in the acclimation response to HL in terms of thylakoid composition and/or function, and that these changes are what give rise to the altered Chl fluorescence characteristics by which this mutant was identified.

As for ape1, there is no evidence for an altered thylakoid composition compared with wild type in either LL- or HL-grown ape2 plants. However, this contrasts greatly with the dramatic reductions in \(P_{\text{max}}\) and represents a marked disruption of the strong correlation between \(P_{\text{max}}\) and thylakoid composition, which is displayed by wild-type plants (Bailey et al., 2001); the same correlation is also shown by numerous mutants in which there is an altered “acclimation midpoint” but which still show parallel changes in \(P_{\text{max}}\) and Chl \(a/b\) (Walters et al., 1999). It therefore appeared that, whereas ape2 plants retained the capacity to acclimate to their growth conditions, some aspect of the acclimation response was altered, which altered the relationship between Chl \(a/b\) and \(P_{\text{max}}\). Plants subjected to an increase in growth light showed a rapid increase in \(P_{\text{max}}\) comparable with that for the wild type, and although the measured Chl \(a/b\) of such plants was reduced (Table I), this appears to be an artifact associated with the rapid accumulation of appreciable (clearly visible) levels of anthocyanins in the leaves of these plants. We have found that anthocyanins affect measurement of the Chl \(a/b\) ratio: In 80% (v/v) acetone they absorb at the wavelengths used for Chl determination. It is notable that those few leaves that did not display significant levels of anthocyanins had measured Chl \(a/b\) ratios that were comparable with those of wild-type plants (data not shown).

For the ape3 mutant, the pattern for Chl \(a/b\) and PSII content was the same as that observed for photosynthetic rate and Chl fluorescence. HL-grown plants were indistinguishable from the wild type, but after LL growth, there were increases in both Chl \(a/b\) and PSII. The response to a LL to HL transfer was not statistically different from the wild type, for both Chl \(a/b\) (Table I) and \(P_{\text{max}}\) (data not shown). Thus all of the observed changes in the ape3 line were consistent with it having a specific defect in acclimation to LL—under which conditions both thylakoid composition and photosynthetic capacity were altered to what would be appropriate for a higher light level—but having normal acclimation to HL.

### Identification of the Basis for ape Mutants

Rapid identification of the basis for a mutant phenotype is frequently possible when the mutant has been selected from a population generated by random insertion of a DNA element into the genome, by cloning the genomic sequences flanking the insertion using the inserted sequence as an “anchor.” One such approach, thermal asymmetric interlaced PCR (Liu et al., 1995), was used to amplify sequences from the 99-1, 22-1, and 6-1 lines; in each case, two sets of primers were used that corresponded to the sequences of the left and right borders of the pGV3850: 1003 T-DNA element presumed to be present in these lines (Feldmann, 1991). For the 99-1 and 22-1 lines, sequences were amplified that defined genome/T-DNA junctions at single locations in the genome and that could be re-amplified using primers specific for the identified regions of the Arabidopsis genome. However, for the 6-1 line, all sequences amplified were either nonspecific or entirely T-DNA-derived.

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**Table 1. Acclimation in ape mutants**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Chl (a/b)</th>
<th>Photosystem II Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL</td>
<td>LL → HL</td>
</tr>
<tr>
<td>Wild type</td>
<td>3.27 ± 0.02</td>
<td>4.07 ± 0.03</td>
</tr>
<tr>
<td>ape1</td>
<td>3.30 ± 0.02</td>
<td>3.73 ± 0.06</td>
</tr>
<tr>
<td>ape2</td>
<td>3.26 ± 0.02</td>
<td>3.71 ± 0.08b</td>
</tr>
<tr>
<td>ape3</td>
<td>3.44 ± 0.01b</td>
<td>4.30 ± 0.10</td>
</tr>
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*\(aP < 0.05\), \(bP < 0.01\), \(cP < 0.002\).
and no success was achieved using other approaches such as inverse PCR and marker rescue.

Figure 6 illustrates the locations of the T-DNA insertions present in lines 99-1 and 22-1, both of which were located on chromosome 5, approximately 3.2 Mb apart. 99-1 carried an insertion within a gene encoding a protein of unknown function designated At5g38660 (Arabidopsis Genome Initiative, 2000). The insertion interrupted the fifth of eight predicted exons, and there was an additional 18-bp deletion of an exon-intron boundary at the site of the insertion; there were likely to be of a minimum of two elements in tandem at this site—T-DNA left-border sequences flanked the genome in both upstream and downstream directions. Database searches failed to identify any significant similarities to the predicted protein product, other than apparent orthologs in other oxygenic photosynthetic organisms. In contrast, the insertion carried by line 22-1 affected a gene with a well-established role in photosynthesis: the single-copy TPT gene encoding the putative triose-phosphate/phosphate translocator of the chloroplast inner envelope (At5g46110). The insertion was once again likely to be of at least two T-DNA elements and was located to leave intact only 23 bp upstream from the TPT structural gene; upstream from this point, there was a 14-bp inversion and a 13-bp deletion at the site of the insertion. Inspection of published expressed sequence tag and full-length cDNA data indicated that the insertion was downstream from TPT promoter sequences and that the deletion included the transcriptional start site; there was therefore likely to be a severe effect on TPT expression.

DISCUSSION

The current consensus is that the primary signal in acclimation is dependent on the redox state of the plastoquinone (PQ) pool, and that acclimation acts to return the redox poised of PQ to an “optimum” (Kim et al., 1993; Escoubas et al., 1995; Maxwell et al., 1995; Pfannschmidt et al., 1999, 2001). However, the evidence remains circumstantial, and whereas numerous studies have successfully correlated PQ redox state with acclimation response, there is as yet no direct evidence in support of this hypothesis. Furthermore, at least one additional signal (e.g. thioredoxin redox state) must be involved, because changes in the quantity or spectral quality of light can each alter the redox state of the photosynthetic electron transport chain, but with opposite effects in terms of chloroplast composition. PSII levels increase in response to HL, and decrease when PQ is reduced as a result of a change in spectral quality. It is notable that recent evidence suggests that redox signals on the acceptor sides of both PSII and PSI interact in the regulation of LHCII phosphorylation (Rintamaki et al., 2000).

The continuing absence of clear evidence to indicate the molecular mechanisms underlying acclimation demand that alternative approaches be adopted; one such approach is the identification of mutations that affect the acclimation process. The isolation and characterization of mutants has proved powerful in the dissection of numerous signal transduction pathways in Arabidopsis, such as phytochrome-mediated light perception (Smith, 2000); auxin, abscisic acid, and ethylene signaling (Leung and Giraudat, 1998;
Callis and Vierstra, 2000; Stepanova and Ecker, 2000) and sugar sensing (Pego et al., 2000). In this work, we have exploited the fact that Chl fluorescence provides a non-destructive quantitative probe of changes in photosynthetic efficiency and/or capacity and have developed a mutant screen based on analysis and imaging of Chl fluorescence. Although Chl fluorescence has been used to identify mutants in photosynthetic eukaryotes on a number of previous occasions (Dinkins et al., 1994; Niyogi et al., 1997, 1998; Kruse et al., 1999; Peterson and Havir, 2000; Varotto et al., 2000), these studies either have identified gross changes in fluorescence that reflect serious perturbations of photosynthesis or have focused on specific photosynthetic processes that are directly measured using Chl fluorescence.

An initial screen of a T-DNA-transformed population (Feldmann, 1991) identified a number of Arabidopsis mutants with altered Chl fluorescence characteristics. Further analysis of three of these lines showed that they had altered acclimation characteristics. The ape1 mutation carried by line 99-1 had a specific effect on the ability to alter thylakoid composition in response to a LL to HL transfer as shown by measurements of Chl a/b and PSII content, correlated with reductions in the photochemical efficiency of PSII as measured by Chl fluorescence; in contrast, there was no effect on acclimation of maximum photosynthetic rate $P_{\text{max}}$ to an increase in light. The increase in Chl a/b during acclimation of wild-type plants to an increase in growth irradiance results principally from proteolytic degradation of surplus LHClII, which binds a large proportion of total Chl b, together with parallel de novo synthesis of additional PSII reaction centers; one possibility is therefore that the ape1 mutant has a defect in this aspect of acclimation—perhaps in promoting the synthesis of additional PSII and/or related to the proteolytic degradation of surplus LHClII (Yang et al., 1998). Thus the Chl fluorescence screen has exposed a potential role in acclimation for a protein with no previously identified function either in photosynthesis or in the regulation of gene expression but that has been strongly conserved during evolution—for instance, the predicted APE1 protein product shows 39% identity, 55% similarity to the $sll0575$ open reading frame of Synechocystis sp. PCC 6803.

A different class of apparent acclimation defect was exhibited by line 22-1 carrying the ape2 mutation, with a significant reduction in $P_{\text{max}}$ under all growth conditions, although there was no change in PSII content or Chl a/b ratio. The identification of a likely TPT defect in this line is consistent with this finding—potato (Solanum tuberosum) and tobacco (Nicotiana tabacum) plants in which TPT expression was reduced in using antisense also showed reduction in the rate of photosynthesis under high CO$_2$ concentrations (Heineke et al., 1994; Häusler et al., 2000). However, the ape2 mutant showed a reduction in $\Phi_{\text{PSII}}$ and therefore an inferred reduction in maximum electron transport rate, under the conditions of the mutant screen and subsequent Chl fluorescence analysis. This contrasts with the previous studies—the antisense plants showed no measurable effect on photosynthetic electron transport under ambient CO$_2$ (Heineke et al., 1994; Häusler et al., 2000)—suggesting that the potentially more extreme reduction in TPT because of the ape2 mutation had qualitatively distinct consequences for the plant. In particular, the mutation appears to give rise to a restriction on electron transport and a resulting reduction in PSII photosynthetic efficiency, suggesting that export of photosynthesize from the chloroplast via the TPT is crucial for the maintenance of high rates of photosynthetic electron transport. Thus, although the basis for the ape2 phenotype was perhaps different from what might have been expected, the identification of this mutant nevertheless demonstrates that the screen successfully detects mutants with altered maximum photosynthetic rates, which is an expected consequence of certain types of acclimation defect.

Line 6-1, carrying the ape3 mutation, had a third type of acclimation defect. Numerous changes in the characteristics of LL-grown plants—increased photosynthetic efficiency during illumination with HL, increased $P_{\text{max}}$ increased Chl a/b, and increased PSII content—were in each case characteristic of growth under significantly higher irradiance. HL-grown plants, on the other hand, were indistinguishable from the Ws-2 parental line, indicating that the ape3 mutation did not affect light perception across the full irradiance range. The ape3 mutation therefore appears to limit the response to LL growth conditions, a similar acclimation phenotype to that observed for a number of photomorphogenic mutants: A det1 mutant completely lacked LL acclimation; a COP1/cop1 heterozygote was partially defective in LL acclimation; and a hy5 mutant had a restricted acclimation range, including a reduction in the extent of LL acclimation (Walters et al., 1999). Therefore, although ape3 plants were phenotypically distinct from each of these, having no obvious photomorphogenic phenotype (e.g., altered leaf development or hypocotyl length) or altered plant or seed viability (as for cop1 and det1 mutants), it is tempting to speculate that the ape3 mutation may define an acclimation-specific component of the COP/DET/ FUS regulatory network.

The identification of these mutant lines with changes characteristic of varying acclimation defects clearly demonstrates that imaging of Chl fluorescence is a viable strategy for the investigation of acclimation. Furthermore, the range of phenotypes exhibited by these lines also showed that the screen has the sensitivity and selectivity necessary to allow the identification of mutations affecting diverse aspects of this complex physiological process. We fully expect that a more systematic and methodical screen...
of this and other mutagenized populations of Arabidopsis will allow the identification of many more acclimation mutants. Furthermore, simple modifications to the screen protocol—e.g. changes in growth light spectral quality, changes in temperature, and changes in ambient CO₂—offer numerous possibilities for identifying mutants affecting other aspects of photosynthetic acclimation, finally opening this hitherto intractable biological problem to genetic analysis.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of Arabidopsis cv Ws-2 (N1601) and T-DNA-transformed populations derived from it (N2606–N2654 and N6481–N6496) were provided by the Nottingham Arabidopsis Stock Centre (Nottingham, UK). Plants were grown from seed in growth chambers with an 8-h photoperiod at a photon flux density of 100 µmol quanta m⁻² s⁻¹ (LL) or 400 µmol quanta m⁻² s⁻¹ (HL), as previously described (Walters et al., 1999). For the mutant screen and Chl fluorescence measurements, seeds were sown on sieved compost (Levington’s M2) in 9-cm petri dishes that had been perforated and lined with filter paper (to allow bottom watering), at a density of up to 150 seedlings per plate (equivalent to 150–300 seedlings per plate). After thorough watering, they were held in the light at 4°C for a minimum of 7 d and then transferred to LL or HL growth conditions as appropriate. Kn resistance was determined from the bleached/green phenotype of 2-week-old seedlings grown on 0.6% (w/v) agar containing Murashige and Skoog basal medium plus Gamborg’s vitamins (Sigma-Aldrich, St. Louis) plus 50 µg mL⁻¹ Kn.

Fluorescence Screen

Chl fluorescence imaging was performed essentially according to Rolfe and Scholes (1995). Petri dishes containing approximately 150 to 300 seedlings were pre-illuminated for 15 to 30 min at an irradiance of 250 µmol quanta m⁻² s⁻¹ provided by a metal-halide lamp fitted with a 2-mm polycarbonate heat filter and filtered through a sheet of Cineous 415 Peacock Blue (Strand Lighting, London) to produce a spectral quality similar to that used in the fluorescence imaging system. The seedlings were then placed under the imaging system and illuminated at an actinic irradiance of 250 µmol m⁻² s⁻¹ for a further 5 to 10 min so that steady-state photosynthesis was achieved. The petri dish was illuminated evenly using a custom-built 15-cm-diameter ring light (Volpi AG, Zurich) with two fiber-optic inputs, and the petri dish was illuminated evenly using a custom-built 15-cm-diameter ring light (Volpi AG, Zurich) with two fiber-optic inputs, provided by two KL1500 lamps (Schott, Mainz, Germany) via a 15-cm-diameter fiber optic ring light.

Thylakoid Composition

Assays of active PSI in leaf discs were carried out by measurement of the O₂ flash yield in the presence of background far-red light according to Chow et al. (1991). PSI was assayed spectrophotometrically after extraction of leaf discs in 80% (v/v) aceton, using extinction coefficients according to Porra et al. (1989).

Identification of T-DNA Insert Junctions

T-DNA insert junctions were initially amplified by thermal asymmetric interlaced PCR essentially according to Liu et al. (1995), using degenerate primer AD2 in combination with left border-specific primers TL-3 (5'-TCT GGG AAT GGC GTA ACA AAG GC-3'), TL-2 (5'-AAC TGT AAT GAC TCC GCG CAA TA-3'), and TL-1 (5'-CAG CCA ATT TTA GAC AAG TAT CA-3'). TL-1 in combination with gene-specific primers, designed using initial sequence information from the cloned amplification products, were then used to reamplify the T-DNA insert junctions.

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LITERATURE CITED


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