Altered photosynthetic performance of a natural Arabidopsis accession is associated with atrazine resistance

Mohamed E. El-Lithy1,2, Gustavo C. Rodrigues2,*, Jack J. S. van Rensen2, Jan F. H. Snel3, Hans J. H. A. Dassen2, Maarten Koornneef1, Marcel A. K. Jansen4, Mark G. M. Aarts1 and Dick Vreugdenhil2,†

1 Laboratory of Genetics, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands
2 Laboratory of Plant Physiology, Wageningen University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands
3 Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands
4 Department of Plant Sciences (ZEPS), University College Cork, Distillery Field, North Mall, Cork, Ireland

Received 18 January 2005; Accepted 10 March 2005

Abstract

Natural variation for photosynthetic traits was studied by determining chlorophyll fluorescence parameters in a collection of Arabidopsis accessions. This screen revealed only one single accession (Ely), exhibiting photosynthetic characteristics markedly different from all others, while a few lines showed small but significant variation. Detailed genetic and physiological analyses showed reduced fitness for Ely compared with the standard laboratory strain Ler for various growth parameters. At low temperature (15 °C), Ely had a higher electron transport rate than Ler, indicating increased photosystem II efficiency under this condition, while at high temperature (30 °C) the opposite was observed. Ely had a high sensitivity to UV-B radiation compared with Ler and was atrazine resistant. This atrazine-resistance and related chlorophyll fluorescence traits were maternally inherited, pointing towards chloroplast-located gene(s). Definite proof that Ely is atrazine-resistant was obtained by sequencing the psbA gene, encoding the D1 protein of photosystem II, revealing a point mutation causing the same amino acid change as found in other atrazine-resistant species. Additional nuclear encoded genetic variation was also present, as was concluded from the small but significant differences in phenotype between Ely and its reciprocal crosses with Ler. It was concluded that the photosynthetic yield is highly conserved and that only severe selection pressure results in marked variations in photosynthetic performance.

Key words: Arabidopsis thaliana, atrazine, chlorophyll fluorescence, oxygen evolution, photoinhibition, temperature, UV-B.

Introduction

Photosynthesis is a complex chloroplast-located process, controlled by both nuclear and plastidic genes and considered as a central step in determining plant growth and productivity. Photosynthetic efficiency can be assayed in a non-destructive way by measuring chlorophyll fluorescence (ChlF) (Maxwell and Johnson, 2000). This is an integrative trait, reflecting both light and dark reactions of photosynthesis.

Physiological differentiation among populations showed that evolutionary divergence in photosynthetic traits is common within species. This implies that selection has influenced photosynthetic traits in some way (Arntz and Delph, 2001). Natural variation within a plant species may provide an interesting source of genetic variation to be used for the unravelling of gene functions ( Tanksley and McCouch, 1997). Within-species natural variation is the basis for QTL analysis, which has been shown to be useful.
for the genetic unravelling of complex plant traits (Koornneef et al., 2004). Intraspecific variations in photosynthetic traits may be direct, or indirect via changes in non-photosynthetic traits, and emphasize the importance of viewing the phenotype as an integrated function of growth, morphology, life-history, and physiology (Arntz and Delph, 2001). In a previous paper, substantial variation in growth characteristics has been described among a collection of accessions, based on overall growth characteristics, namely, plant size, relative growth rate, and flowering-related traits (El-Lithy et al., 2004). In the present study the genetic variation for photosynthetic efficiency has been investigated between Arabidopsis accessions. Remarkably, this screening of a large set of Arabidopsis accessions, using chlorophyll a fluorometry, showed that just one single accession exhibited photosynthetic characteristics substantially different from all others. Detailed genetic and physiological analyses showed that the deviating photosynthetic characteristics of this single ‘natural variant’ present in the collection, was caused by a point mutation in the chloroplast psbA gene, also leading to atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) resistance. Atrazine resistance is a trait thought to result from the extreme selection pressure exerted by man-made triazine herbicides at the site of collection. It was concluded that the kinetics of photosynthetic yield is highly conserved within Arabidopsis and that only severe selection pressure results in marked variations in photosynthetic performance.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana accessions were obtained from the Arabidopsis stock centres ABRC, NASC, and Sendai (www.arabidopsis.org), supplemented with accessions recently collected by members of the Laboratory of Genetics at Wageningen University and currently deposited at ABRC and NASC. Details of all the 127 accessions used in this study are given as supplementary material and can be found at JXB online. The Ely accession (CS6088), provided by ABRC was collected by Dr Paul Williams (University of Wisconsin, Madison, USA) at the railway station of Ely (UK) in 1988 (P Williams, personal communication).

All Arabidopsis seeds were pre-sown in Petri dishes on water-saturated filter paper, followed by cold treatment for 4 d at 4 °C, and then transferred to a climate room at 25 °C and 16 h light for 2 d before planting in 7 cm pots with standard soil. In all the descriptions of the experiments, time is referred to as days after planting. The plants were grown in an air-conditioned greenhouse with 70% relative humidity, supplemented with additional light (model SON-T 822–25, Philips, Eindhoven, The Netherlands) providing a day/night ratio of 12 h light/12 h darkness, and a light intensity of 125 μmol m−2 s−1, and maintained at a temperature between 22–25 °C (day) and 18 °C (night). For each accession, three plants were selected to measure their photosynthetic yield (Y). Heritability (broad sense) was estimated as the proportion of variance explained by between-line differences using the general linear model module of the statistical package of SPSS version 11.0.1 (SPSS Inc., Chicago, IL). Y and rosette radius were measured on day 20 for 24 plants for each of the parents (Ely and Ler) and the F1s of the different crosses, while 120 F2 plants were averaged for the same traits. For measuring electron transport rate (ETR), UV-B effect, chlorophyll a fluorescence rise curves, and oxygen evolution, plants of Ler, Ely, and the F2BC4 were grown under controlled conditions in a growth cabinet, with 70% relative humidity, 22 °C, 12 h day (short day), and a light intensity of 125 μmol m−2 s−1.

The F2BC4 plants were obtained by crossing Ely (female parent)× Ler (male parent). Resulting F2 plants were crossed again with Ler as the male parent to get the 2nd back cross (BC). The same was done for two further generations until 4th BC plants were obtained. These plants were selfed to get F3 plants of the 4th BC (F2BC4). The aim was to obtain plants with Ler nuclear DNA and Ely cytoplasm. Plants were placed on carts and the carts were shuffled daily to avoid an effect of minor local condition differences within the growth cabinet.

DNA isolation and sequencing

DNA was isolated from greenhouse-grown plants, one plant per genotype. The Bernatzky and Tanksley (1986) DNA isolation protocol was adapted for rapid extraction of small quantities. Flower buds were harvested in liquid nitrogen and ground in 330 μl of a preheated (65 °C) extraction solution [125 μl extraction buffer: 0.35 M sorbitol, 100 mM TRIS, 5 mM EDTA, pH 7.5 (HCl)] together with 175 μl lysis buffer: 200 mM TRIS, 50 mM EDTA, 2 M NaCl, 2% (w/v) cetrimethyl-ammonium bromide, to which 30 μl sarkosyl (10% w/v) was added. The mixture of crude plant material and extraction solution was incubated for 30 min at 65 °C; during this period occasional shaking was applied. Hereafter a solution of 400 μl chlorform/isoamyl alcohol (24:1, v/v) was added and vortexed. After centrifuging for 5 min at maximum speed in an Eppendorf centrifuge the water phase was transferred to a new tube. An equal amount of cold isopropanol was added to precipitate the DNA by carefully inverting the tube several times. After 10 min centrifugation at maximum speed in an Eppendorf centrifuge the water–alcohol mixture was discarded and the pellet washed with 70% cold ethanol. The pellet was left to dry and dissolved in water containing RNase A and incubated for 30 min at 37 °C. Thereafter it was stored at 4 °C.

The PCR amplification for the psbA gene was carried out using the forward primer 5′-GTCGGCTTGGGAGGATCCCTGATA-3′, and a reverse primer 5′-TATTCTAAGAGGCTTATATGTCGCTGTT-3′. The PCR product was re-PCRed using another primer combination (forward primer 5′-CTATGCATGCTTCCTGGAACCTC-3′, reverse primer 5′-CGGATGCCTAAACCTCATAATCA-3′) flanking the expected point mutation to get a fragment of 400 bp. This fragment was purified for both Ely and Ler using a PCR purification kit (Roche Diagnostics Corporation Indianapolis, IN, USA). Both strands of each fragment were sequenced using the same primers. For PCR a protocol of 30 s at 94 °C, 30 s at 50 °C, and 30 s or 60 s at 72 °C (35 cycles), was used.

Chlorophyll fluorescence and electron transport rate measurements

ChlF as a non-destructive marker of photosynthetic efficiency was measured, based on three independent measurements (one measurement/plant), as quantum yield (Y) using a MINI-PAM (Walz Mess- und Regeltechnik, Effeltrich, Germany). The effective photosystem II (PSII) quantum yield of photosynthetic energy conversion was calculated as $Y = F_a/F_m = (F_a - F_o)/F_m$ for dark-adapted and non-adapted leaves (Van Kooten and Snel, 1990). The Y data of the 127 accessions are given as supplementary material at JXB online. ETR was measured using the same MINI-PAM at two different temperatures, 15 °C and 30 °C, in a controlled growth cabinet. Plants were temperature adapted for 10 min before the measurement. Six plants were used for each genotype with one measurement per plant.
UV-B treatment

For UV treatments leaf discs (10–14 independent leaves, 1 leaf plant−1) were exposed to UV-B radiation, generated by Philips TL12 fluorescent tubes (λmax 315 nm). The light emitted by the bulb was filtered through a single layer of cellulose acetate. Exposure times and irradiance conditions were set to obtain a measurable decrease in PSII activity. Leaf discs were exposed for up to 5 h at 11.4 μmol m−2 s−1. The irradiance level represents radiation in the spectral range between 295 nm and 345 nm. Discs were floated on distilled water, with their adaxial side facing the UV-source. The decrease in photosynthetic activity was attributed to the UV-B wavelengths since the low level of UV-A radiation is ineffective in decreasing PSII activity (Jansen et al., 1998). A low level (12 μmol m−2 s−1) of additional PAR was applied during the UV-treatments. UV-levels were measured using an optometer (United Detector Technology Inc., Hawthorn, USA) equipped with a probe specific for UV-wavelengths. The photosynthetic efficiency of PSII was determined by the saturating pulse applied during the UV-treatments. UV-levels were measured with the Plant Efficiency Analyzer fluorometer (PEA, Hansatech Instruments Ltd, King’s Lynn, Norfolk, UK). The minimal fluorescence (F0), maximal fluorescence (Fm), and the variable fluorescence (FV=Fm−F0) were all measured according to Van Kooten and Snel (1990). The photochemical yield of open PSII reaction centres, commonly known as the relative variable fluorescence, was calculated as Fv/Fm. It reflects the maximal efficiency of PSII that was measured in tissue dark-adapted for at least 20 min.

Chlorophyll a fluorescence rise curves

The fast chlorophyll a fluorescence rise curves of dark-adapted leaves were measured with the Plant Efficiency Analyzer fluorometer (PEA, Hansatech Instruments Ltd, King’s Lynn, Norfolk, UK). The measurements were performed at room temperature with a 3 s excitation pulse of 100% light intensity, which corresponds to about 600 W m−2 of light with a peak at 650 nm (approximately 3000 μmol m−2 s−1). After a 10 min dark adaptation, data were recorded for 3 s to generate a fluorescence induction curve. The fluorescence signal at 50 μs, the earliest measurement free of any artefacts related to the electronics of the instrument (Haldimann and Strasser, 1999), was considered as F0. Of the so-called OJIP fluorescence rise curve the J-level was considered at 1–2 ms, the I-level at about 30 ms, and the P-level at about 500 ms. The curves were viewed and averaged with the instrument’s software (WinPea). The curves presented are the averages of measurements on six different leaves from three individual plants.

Infiltration of leaves

Detached leaves (four leaves from four different plants) were vacuum-infiltrated under 0.8 bar with DCMU (diquorin) [3-(3,4-dichlorophenyl)-1,1-dimethylurea] and atrazine (both from Sigma Chemical Co., St Louis, USA), using a concentration of 50 μM in 0.5% (v/v) ethanol. Control leaves were infiltrated with 0.5% (v/v) ethanol only. After infiltration, the leaves were dark-adapted at room temperature for 1 h in Petri dishes lined with filter paper saturated with the infiltrated solution.

Isolation of thylakoids

Details of the isolation of thylakoids were described previously (Van Rensen et al., 1977). Leaves from Arabidopsis plants were homogenized using a Sorvall Omnimixer in isolation medium containing 0.4 M sorbitol, 20 mM Tricine-NaOH (pH 7.8), 10 mM NaCl, 5 mM MgCl2, 2 mM sodium ascorbate, and 2 mg ml−1 bovine serum albumin. After squeezing through three layers of nylon cloth the chloroplasts were collected by centrifugation for 30 s at 3000 g, washed once in 50 mM sodium phosphate buffer (pH 7.8) to obtain broken chloroplasts collected by centrifugation for 5 min at 1000 g.

The chlorophyll content was measured according to Bruinsma (1963), and the chlorophyll concentration adjusted to 2 mg Chl ml−1.

Measurement of photosynthetic electron transport

Details of the measurement of photosynthetic electron transport activity were as described earlier (Van Rensen et al., 1977, 1978). Electron transport was estimated as oxygen evolution, which was measured with a Gilson oxygraph provided with a Clark oxygen electrode, at a temperature of 25 °C and at saturating white light. The isolated thylakoids were suspended in 2 ml reaction medium containing 0.3 M sorbitol, 50 mM Tricine-NaOH (pH 7.6), 5 mM MgCl2, 5 mM NH4Cl, 1 mM ferricyanide, and thylakoids containing 50 μg chlorophyll.

Results

The identification and genetic analysis of differences in photosynthetic yield

A collection of 127 Arabidopsis accessions was screened for their ChlF, measured as yield (Y=ΔF/Fm), where ΔF=variable fluorescence and Fm=fluorescence yield at zero photochemical and non-photochemical quenching for dark non-adapted leaves). This screening revealed that Ely (CS 6088) was the only accession with a considerably lower yield (Y=0.54), while the Y for the other accessions ranged from 0.63 to 0.71 with a mean Y of 0.69 (±0.02) (Fig. 1). Five other accessions, An-1, Pak-2, Pak-3, Labal, and Ws-1, showed reduced photosynthetic Y (Table 1), significantly different from both Ely and the rest of the accessions (P= 0.05). The first three accessions showed early senescence.

Fig. 1. Frequency distribution of the chlorophyll fluorescence measured as yield (Y=ΔF/Fm) for 127 Arabidopsis accessions, showing one clearly deviating accession (Ely) with low ChlF. The average value for Ely and Ler is indicated with an arrow, and the horizontal bars represent the SE for these accessions (based on three independent measurements, one measurement/plant). ChlF was measured at 125 μmol m−2 s−1 light intensity, 70% relative humidity, and 22 °C.
that might affect the photosynthetic capacity, although the measurements were carried out on (visually) healthy non-senescent leaves. The estimated heritability for all accesses (0.71 and 0.60 with and without Ely, respectively) indicated that despite the small differences for Y, genetic variation for this trait was present.

To determine if nuclear or chloroplast genes control this phenotype, Ely was reciprocally crossed to the laboratory reference accession Ler. Whenever Ely was used as the seed-bearing parent, the progeny had a low Y (Y=0.5–0.55), while progeny derived from Ler as the seed-bearing parent had a high Y (Y=0.67–0.71) (Fig. 2). The F2 progeny of either F1 did not segregate for Y, with a low Y in the case of Ely maternity and a high Y in the case of Ler maternity. The differences between both two groups were highly significant (P=4.94E−13). The differences in Y between plants were maintained after repeated back crossing (BC) of either progeny with Ler as pollen donor. These findings indicated maternal inheritance of the Y that is probably mitochondrial or chloroplast encoded.

The Y of Ely was significantly lower than that of the F1 or F2 plants derived from crosses with Ely as female parent (0.002 > P > 4.94E−13). Moreover, the coefficient of variation (standard deviation as percentage of the mean value) was slightly larger (2.12) among the F2 plants than among the parent lines, F1 (Ely×Ler) or F1 (Ely×Ler)×Ler (1.38, 0.88, and 1.19, respectively) suggesting a segregation of nuclear genes with additional minor effects on Y.

Correlation between photosynthetic yield and plant growth

To quantify the differences observed between the two maternal groups further, the rosette radius of plants was measured on day 20 as a non-destructive way of measuring growth (Fig. 3). Plants with low Y had a smaller rosette radius (ranging from 16.6–19.4 mm) compared with plants with a high Y (radius ranging from 21.3–27.3 mm). The differences between both maternal groups were significant at P <0.01 (Fig. 3). Similar differences have been observed when fresh and dry weights, rosette area, and relative growth rate of Ler and Ely were compared (El-Lithy et al., 2004). The rosette radius of F1 (Ler×Ely) was significantly larger than that of both parents, suggesting a hybrid vigour effect as shown by Barth et al. (2003). However, the F1 of the reciprocal cross (Ely×Ler) did not differ significantly from both parents (Fig. 3), which might be due to the low maternally inherited Y of Ely.

Physiological characteristics of the Ely accession and its back cross line

To investigate the differences between the two maternal genotypes, a number of experiments were performed in which photosynthetic characteristics were determined while varying environmental factors known to affect ChlF. The experiments were performed with Ler, Ely, and the progeny of the hybrid (F2BC4) for which Ler was always used as the pollen donor. This BC is expected to have mainly Ler nuclear DNA and Ely cytoplasm.

Effect of temperature on electron transport rate: Chlorophyll fluorescence analysis can be used to monitor the effects of low and high temperatures on photosynthesis, for example, at low temperature increased electron transport to alternative electron sinks was found in maize (Fryer et al., 1998). In this study it was found that, at high temperature (30°C), Ler had a significantly higher ETR than both Ely and the
Chl \textit{a} fluorescence rise curves of Ely, F2BC4, and Ler

Chlorophyll \textit{a} fluorescence, in general, is a useful indicator to monitor a wide variety of photosynthetic events. Determining the fluorescence rise curve (from microseconds to a few s), by exposing dark-adapted leaves to saturated light pulses, gives specific information on photosynthesis (Strasser \textit{et al.}, 1995). Chl \textit{a} fluorescence rise curves, and the transients of Ely, F2BC4, and Ler are presented in Fig. 6. The Ler curve had the typical OJIP characteristics as previously described by Strasser \textit{et al.} (1995, 2000); transients at about 1 ms (J), at about 20 ms (I), and a P-level at about 500 ms. The Ely curve was different: the \( F_o \) (chlorophyll fluorescence at origin in dark-adapted reaction centres with maximal photochemical quenching) was slightly higher and the J-level was also increased (Fig. 6A, B). The curve of the F2BC4 was similar to the one of Ely.

Table 2 illustrates details of the fluorescence measurements. In Ely and the F2BC4, \( F_o \) was slightly higher and the J-level was increased, while there was little difference in the I-transient and in the \( F_m \) compared with Ler. The \( F_o/F_m \) values were lower for Ely and F2BC4, as compared with Ler (Table 2), confirming the previous differences found in the UV-B analysis.

Effects of atrazine

The physiological characteristics of Ely and its F2BC4 progeny, namely, low \( Y_l \), low ETR at elevated temperatures, increased UV-B sensitivity, and altered OJIP characteristics were all found to be maternally inherited, suggesting that this is a chloroplast-encoded trait. A point mutation in the \textit{psbA} gene, which encodes the D1-protein of PSII, is known to cause similar physiological effects in many other species. This point mutation in the \textit{psbA} gene confers atrazine-resistance to the plant (Botterman and Leemans, 1988). This prompted an investigation to find if Ely is resistant to atrazine by measuring Chl \textit{a} fluorescence rise curves in the
presence of atrazine and another herbicide DCMU. DCMU inhibits photosynthesis in a similar way as atrazine, but there is no cross-resistance to DCMU in atrazine-resistant plants. The effect was also determined of various concentrations of atrazine on the PSII electron flow in isolated thylakoids as measured by oxygen evolution.

The normalized curves for Chl a fluorescence as a function of time are shown in Fig. 7 for leaf material infiltrated with DCMU, atrazine, or the solvent 0.5% ethanol as control. Low concentrations of ethanol have very little effect on the curves (Figs 6B, 7A). Infiltration with DCMU caused complete inhibition of electron flow at the acceptor side of PSII, leading to a very fast rise of the fluorescence to the P-level. This increase was equally fast in all three genotypes. However, after infiltration with atrazine this fast rise occurred only in Ler and not in Ely or the F2BC4, indicating that the latter two genotypes were resistant to atrazine, in contrast to Ler. The effect of various concentrations of atrazine on PSII electron flow in isolated thylakoids as measured by oxygen evolution are shown for Ler and Ely in Fig. 8. In Ler, oxygen evolution was inhibited for 50% by 3 μM atrazine, while in Ely a more than ten times higher concentration of atrazine (40 μM) was required to achieve a similar level of inhibition.

In many species atrazine-resistance is due to a specific nucleotide difference altering codon 264 of the chloroplast psbA gene encoding the D1 protein (Oettmeier, 1999). Therefore, the psbA gene was PCR-amplified from both Ler and Ely and the DNA sequence of the PCR fragments was determined. Only one nucleotide difference was found between both accessions, changing the sequence of codon 264 from AGT to GGT, thus changing the predicted amino acid from 264Ser into 264Gly. This conversion is the typical atrazine-resistance conferring mutation found in many other plant species (Botterman and Leemans, 1988; Gronwald, 1994; Sibony and Rubin, 2003).

Discussion

In general, there is ample evidence of genetic variation among and within natural plant populations for photosynthetic traits, although some studies report little or no genetic variation (Arntz and Delph, 2001). The variation found among Arabidopsis accessions for Y was very small. Despite the considerable variation in growth rate characteristics among accessions (El-Lithy et al., 2004), it was found that there is only very limited variation in the underlying photosynthetic reactions. These data are consistent with others, which show strong conservation of the sequences of key PSII proteins (Botterman and Leemans, 1988; Gronwald, 1994; Sibony and Rubin, 2003) and of the kinetics of electron transport near PSII (Jansen and Pfister, 1990) among a wide variety of photosynthetic organisms. A range of man-made PSII mutants has been produced over the last couple of years, some of which were non-photosynthetic while others were only slightly affected in terms of photosynthetic efficiency (Kless et al., 1994; Niyogi et al., 1998; Wu et al., 1999; Keilty et al., 2000; Varotto et al., 2001).

Table 2. Fluorescence at origin (F_o), fluorescence after 2 ms and 30 ms of excitation (F_2 and F_30, respectively), maximum fluorescence (F_m), and potential photochemical yield of PSII (F_v/F_m) measured in dark-adapted leaves of Arabidopsis genotypes Ler, Ely, and the F2BC4

<table>
<thead>
<tr>
<th>Fluorescence parameter</th>
<th>Ler</th>
<th>Ely</th>
<th>F2BC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_o</td>
<td>235 (9)</td>
<td>261 (12)</td>
<td>278 (10)</td>
</tr>
<tr>
<td>F_2</td>
<td>821 (26)</td>
<td>907 (30)</td>
<td>959 (35)</td>
</tr>
<tr>
<td>F_30</td>
<td>1169 (28)</td>
<td>1065 (38)</td>
<td>1116 (43)</td>
</tr>
<tr>
<td>F_m</td>
<td>1357 (40)</td>
<td>1242 (44)</td>
<td>1315 (57)</td>
</tr>
<tr>
<td>F_v/F_m</td>
<td>0.828 (0.004)</td>
<td>0.788 (0.004)</td>
<td>0.789 (0.004)</td>
</tr>
</tbody>
</table>

The standard deviations are given between brackets.
et al., 2000; Walters et al., 2003). The data clearly shows that photosynthesis appears to be subjected to strong natural selection allowing very few suboptimal genotypes to be maintained in a population. Ely was the only significantly distinct accession within a collection of 127 accessions. Ely showed both reduced photosynthetic yield (described here) and reduced growth (El-Lithy et al., 2004) indicating the penalty for a reduction of photosynthetic yield.

The difference in the photosynthetic traits observed between Ely and Ler was concluded to be related to the fact that the D1 protein of Ely has been altered because of the psbA mutation leading to atrazine-resistance. This conclusion is based on a series of experiments giving direct and indirect evidence.

Reduced ChlF is maternally inherited

The pattern of differences in the ChlF between the reciprocal crosses of Ler and Ely showed that this trait is maternally inherited and likely to be controlled by (a) chloroplast gene(s). This is in agreement with genetic analysis of barley where atrazine tolerance also inherited maternally (Rios et al., 2003).

Fig. 7. Fast fluorescence rise upon excitation of dark-adapted infiltrated leaves of Ely, F2BC4, and Ler, plotted on a log time scale. The leaves were infiltrated with 0.5% ethanol (control, A), DCMU in 0.5% ethanol (B), or atrazine in 0.5% ethanol (C). Data are plotted as the relative variable fluorescence ($F_v/F_m$). Each curve is the average of four infiltrated leaves, one leaf/plant.

Reduced photosynthetic yield

Reduced ChlF is maternally inherited

The pattern of differences in the ChlF between the reciprocal crosses of Ler and Ely showed that this trait is maternally inherited and likely to be controlled by (a) chloroplast gene(s). This is in agreement with genetic analysis of barley where atrazine tolerance also inherited maternally (Rios et al., 2003).

Reduced growth

Reduced growth of atrazine-resistant genotypes has been described in other species (Gressel, 2000), and has been attributed to the reduced PSII electron transfer efficiency caused by the psbA mutant allele (Holt et al., 1993). Earlier, it was reported that Ely is among the slow-growing Arabidopsis accessions (El-Lithy et al., 2004). These findings are confirmed here, and moreover, comparing the
standard laboratory strain Ler with the F2BC4 backcross population (having Ler nuclear genome and Ely chloroplasts) shows that the low Y trait is linked with significantly reduced growth.

**Temperature affects electron transport rate differently in Ler and Ely**

The sensitive *Arabidopsis* accession (Ler) has a higher ETR than the resistant accession (Ely) at 30 °C, while at 15 °C Ely, surprisingly, has a better ETR than Ler. Similar results were found for several other species of which triazine-resistance biotypes have been collected (*Polygonum lapathifolium*: Darmency and Gasquez, 1982; *Brassica rapa*: Plowman and Richards, 1997). Triazine-resistant PSII reaction centres were found to be much more sensitive to temperatures above 35 °C (Ducruet and Lemoine, 1985; Ducruet and Ort, 1988; Havaux, 1989; Fuks et al., 1992). This implies that the yield penalty observed in the greenhouse and climate chamber conditions used here may have been of less importance in the spring climate of Cambridgeshire (UK) where Ely was found and that these plants might not have a reduced fitness compared with atrazine-sensitive genotypes under those conditions.

**Ely is more susceptible to UV-B stress**

A lower PSII quantum yield is due to the slower electron transfer between QA and QB (Jursinic and Pearcy, 1988). It has been speculated that changes in the redox state of PSII directly affect the sensitivity of PSII to UV-radiation (Jansen et al., 1998; GC Rodrigues et al., unpublished results). It was found that UV-B radiation, when given in the presence of a low background intensity of PAR, resulted in a severe decrease in Fv/Fm in atrazine-resistant genotypes Ely and F2BC4, while the atrazine-sensitive genotype (Ler) was significantly less affected. Similar findings were reported by Olsson et al. (2000) for an atrazine-resistant cultivar of *Brassica napus*.

**Chlorophyll a fluorescence rise curve of Ely is typical for atrazine-resistant genotypes**

Chlorophyll a fluorescence data show that Ely and also F2BC4 are atrazine-resistant, since the characteristic OJIP curves of these genotypes differ from the one of the atrazine-sensitive biotype (Ler). Similar results were obtained by Kohno et al. (2000), for wild-type and triazine-resistant *Chenopodium album*. From Fig. 6 it is clear that the curves for Ely and F2BC4 are nearly identical, again indicating that the effect was mainly due to differences in the non-nuclear, presumably chloroplastic genome. Moreover, while all three tested genotypes are sensitive to DCMU, only Ler was inhibited by atrazine (Fig. 7).

**Oxygen evolution in Ely thylakoids is resistant to atrazine**

Chlorophyll fluorescence rise curves were determined using atrazine infiltrated leaf discs and differences in atrazine-resistance between genotypes might thus be due to differences in uptake of the herbicide. Therefore, oxygen evolution in isolated thylakoids and the effect of atrazine was also measured. This revealed that 50% inhibition required about 10 times higher concentration of atrazine in Ely compared with Ler (Fig. 8).

The gene from Ely showed the typical atrazine-resistance mutation changing the sequence of codon 264 from AGT to GGT, thus changing the predicted amino acid from 264Ser into 264Gly (Botterman and Leemans, 1988; Gronwald, 1994; Sibony and Rubin, 2003).

The occurrence of atrazine-resistance in *Arabidopsis*, not a usual target species for herbicide applications, can be explained by the fact that this resistant accession was collected at a railway station in Ely (UK) in the mid-eighties (P Williams, personal communication). At that time herbicide applications were common to maintain weed-free railways and atrazine was commonly used until its ban in 1993.

Although, in general, little variation in ChlF was observed between the accessions tested, there is additional nuclear-encoded genetic variation as concluded from the small but significant differences in phenotype between Ely and its progeny. This small-scale variation can still be very amenable for quantitative trait locus (QTL) analysis. It has been observed more frequently that the trait values of segregating populations extend beyond the values of the parents (transgression), implying that more variation is present than can be detected by simply surveying accessions. This will especially be the case with traits that are under selection and for which the optimal phenotype can be obtained by different genetic make-up. A similar situation was observed for the length of the circadian period length (Swarup et al., 1999) and is also suggested by the detection of a ChlF QTL in the Ler×Sha RIL population (El-Lithy et al., 2004), despite the fact that the parents did not differ.

However, it is also possible that more variation will be found between accessions when experiments are performed under less optimal conditions. Furthermore, screening for more complex photosynthetic parameters such as qP, NPQ (Niyogi et al., 1998), in addition to the photosynthetic yield (Walters et al., 2003; Varotto et al., 2000), might result in more variation between accessions than observed by only determining yield.

This study demonstrates how screening for natural variation has led to the identification of intraspecific variations in photosynthetic traits in *Arabidopsis* populations. The variation in photosynthetic traits was linked to growth parameters, revealing the resulting fitness penalty.
Supplementary data

A supplementary table is provided at JXB online listing names and stock numbers of all Arabidopsis accessions used in this study. The averaged data ± SE of chlorophyll fluorescence measured as yield (three measurements, one measurement/plant) for the 127 accession are also given in this table.

Acknowledgements

We thank Professor Paul Williams (University of Wisconsin, Madison, USA) for providing detailed information on the location where the Ely accession had originally been found. M El-Lithy is supported by a grant from the Ministry of Higher Education, Egyptian Government.

References


