Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7496–7499, September 1991
Plant Biology

The *aba* mutant of *Arabidopsis thaliana* is impaired in epoxy-carotenoid biosynthesis

(abscisic acid/neoxanthin/violaxanthin/xanthophylls/zeaxanthin)

CHRISTOPHER D. ROCK* AND JAN A. D. ZEEVAART†

Michigan State University—Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

Communicated by N. Edward Tolbert, May 30, 1991 (received for review December 14, 1990)

ABSTRACT **The three mutant alleles of the *ABA* locus of *Arabidopsis thaliana* result in plants that are deficient in the plant growth regulator abscisic acid (ABA). We have used $^{18}O_2$ to label ABA in water-stressed leaves of mutant and wild-type *Arabidopsis*. Analysis by selected ion monitoring and tandem mass spectrometry of [^{18}O]ABA and its catabolites, phaseic acid and ABA-glucose ester (β-D-glucopyranosyl abscisate), indicates that the *aba* genotypes are impaired in ABA biosynthesis and have a small ABA precursor pool of compounds that contain oxygens on the ring, presumably oxygenated carotenoids (xanthophylls). Quantitation of the carotenoids from mutant and wild-type leaves establishes that the *aba* alleles cause a deficiency of the epoxy-carotenoids violaxanthin and neoxanthin and an accumulation of their biosynthetic precursor, zeaxanthin. These results provide evidence that ABA is synthesized by oxidative cleavage of epoxy-carotenoids (the "indirect pathway"). Furthermore the carotenoid mutant we describe undergoes normal greening. Thus the *aba* alleles provide an opportunity to study the physiological roles of epoxy-carotenoids in photosynthesis in a higher plant.**

Abscisic acid (ABA) is a sesquiterpenoid plant growth regulator involved in many physiological and developmental processes such as transpiration, germination, dormancy, and adaptation to environmental stresses (e.g., drought, chilling, and pathogen attack) (ref. 1; for review, see ref. 2). Although the structure of ABA (Fig. 1*D*) has been known for 26 years (3), the biosynthetic pathway in higher plants has not been fully elucidated. The evidence favoring the indirect pathway from xanthophylls, as opposed to the direct pathway from farnesyl pyrophosphate (4), can be summarized as follows. (*i*) The viviparous mutants of maize *vp-2, vp-5, vp-7*, and *vp-9* are blocked in the early stages of carotenoid biosynthesis and are ABA-deficient (5, 6). (*ii*) The carotenoid biosynthesis inhibitors fluridone and norflurazon also inhibit ABA biosynthesis (7, 8). (*iii*) $^{18}O_2$-labeling experiments with water-stressed leaves show ^{18}O incorporation into the side-chain carboxyl group of ABA but little incorporation in the oxygen functions on the ring (9–11), indicating that there is a large ABA precursor pool (presumably xanthophylls) that contains oxygens on the ring (Fig. 1). (*iv*) Xanthoxin, a C_{15} metabolite of epoxy-carotenoids, is found in plants (12) and is readily converted to ABA *in vivo* (13, 14) and *in vitro* (15). (*v*) A 1:1 molar correlation between decreases in *trans*-violaxanthin and 9′-*cis*-neoxanthin (Fig. 1) levels and concomitant increases in ABA and its catabolites has been shown for dark-grown water-stressed bean leaves (16, 17). In contrast to higher plants, phytopathogenic fungi synthesize ABA by a direct pathway from farnesyl pyrophosphate (18, 19).

Koornneef *et al.* (20) isolated three alleles (*aba-1, -3*, and *-4*) of a single locus (*ABA*) from *Arabidopsis thaliana* and showed a correlation between ABA deficiency in seeds of the *aba* genotypes and the phenotypic severity of leaf transpiration rates, reduced growth, and reduced seed dormancy. Application of ABA to the mutant plants restored the normal phenotype. Here we report ^{18}O-labeling studies and quantitation of ABA and carotenoids in leaves of wild-type and the three *aba* genotypes. The results show a correlation between ABA deficiency associated with the *aba* alleles and epoxy-carotenoid deficiency.

MATERIALS AND METHODS

Plant Material. Seeds of *A. thaliana* ecotype Landsberg *erecta* (collection number W20) and the mutant genotypes *aba-3* [isolation mutant G4 (20); collection number W122], *aba-1* (A26; W21), and *aba-4* (A73; W123) were obtained from Maarten Koornneef (Agricultural University, Wageningen, The Netherlands). The genetic nomenclature recommended at the Third International *Arabidopsis* Meeting (East Lansing, MI, April 1987) is used (see ref. 21). The *aba-1* genotype also included the recessive markers *ttg* (transparent testa glabra) and *yi* (yellow inflorescence). Seeds were germinated on 1% agar in Petri dishes for 2 weeks after storage at 4°C for 2 days to break dormancy. Seedlings were transplanted to trays containing a mixture of perlite/vermiculite/peat moss [1:1:1 (vol/vol)]. Plants were grown in a high-humidity growth chamber maintained on a diurnal cycle of 9 hr of light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 23°C and 15 hr of dark at 20°C. When stem elongation started, ≈7 weeks after germination, rosette leaves were harvested and frozen in liquid N_2. For water-stress experiments, detached rosettes were dehydrated with a hair dryer until 14% of the fresh weight was lost. The stressed material was immediately incubated in 20% $^{18}O_2$/80% N_2 (vol/vol) or air at room temperature in the dark for 4 or 8 hr and frozen in liquid N_2. The $^{18}O_2$ (97–98% enrichment) was from Cambridge Isotope Laboratories (Woburn, MA).

ABA, ABA-Glucose Ester (ABA-GE; β-D-Glucopyranosyl Abscisate), and Phaseic Acid (PA) Analysis. Frozen tissue was extracted overnight at 4°C with acetone containing 0.01% 2,6-di-*tert*-butyl-4-methylphenol and 0.25% glacial acetic acid. To each extract was added 20,000 dpm of [^{3}H]ABA, [^{3}H]ABA-GE, and [^{3}H]PA (22) to quantify losses during purification. The samples were homogenized with a Polytron (Brinkmann) and filtered, and 10 ml of 1.0 M potassium phosphate (pH 8.2) was added. The acetone was evaporated at 35°C using a Rotovapor (Brinkmann), and the aqueous solution was passed through a cellulose filter to remove precipitated material. The pH was adjusted to 3.0 with 6 M HCl and ABA, ABA-GE, and PA were partitioned five times

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ABA, abscisic acid; ABA-GE, ABA-glucose ester (β-D-glucopyranosyl abscisate); PA, phaseic acid.

*Present address: Department of Biology, Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280.
†To whom reprint requests should be addressed.

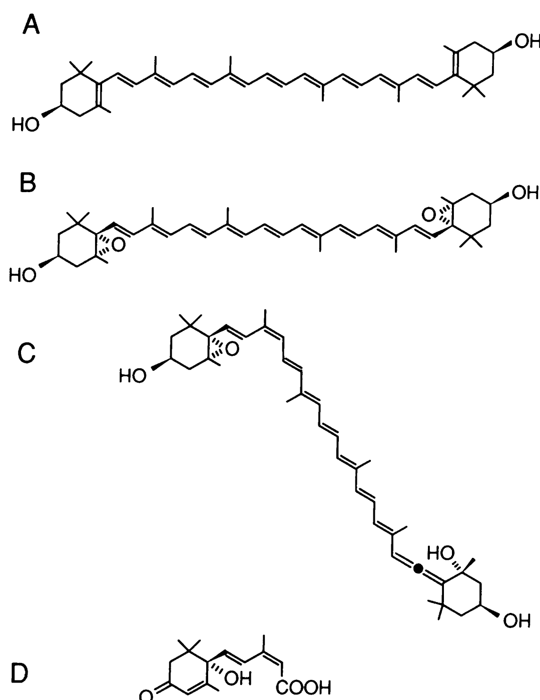

FIG. 1. Structures of zeaxanthin (*A*), *trans*-violaxanthin (*B*), 9′-*cis*-neoxanthin (*C*), and (*S*)-(+)-abscisic acid (ABA) (*D*).

into an equal volume of ethyl acetate. The combined organic fraction was evaporated and the residue was chromatographed by reverse-phase HPLC with a μBondapak C_{18} semipreparative 0.78 × 30 cm column (Waters). A convex gradient (Waters no. 5) was run in 20 min from 0 to 50% ethanol in water containing 1% glacial acetic acid at a flow rate of 2.5 ml/min. The eluant was monitored by UV absorbance at 262 nm. The fractions containing ABA-GE isomers plus PA (17–19 min) and ABA isomers (20.5–24 min) were collected and dried. The ABA-GE was hydrolyzed to the free acid by 2 M NH_4OH for 2 hr at 60°C and dried. Free ABA (from ABA-GE) was then separated from PA with a Nova-Pak C_{18} 0.39 × 15 cm analytical column (Waters) using a 20-min linear gradient from 10% to 60% (vol/vol) methanol in water containing 1% glacial acetic acid at a flow rate of 1.5 ml/min. The retention times of PA and ABA were 17 min and 22 min, respectively. Typical recoveries for ABA and PA were >70% and for ABA-GE was >50%.

The ABA, ABA-GE (free acid), and PA samples were methylated with ethereal diazomethane, and a portion of each sample was quantified by GC-selected ion monitoring with a JEOL model AH-505 double-focusing mass spectrometer equipped with a Hewlett-Packard model 5890A gas chromatograph and a 30-m × 0.259-mm (internal diameter) DB-23 capillary column (J. & W. Scientific, Rancho Cordova, CA) with He as the carrier gas. Flow rate was 1 ml/min. The GC oven temperature was programmed from 80°C to 200°C at 40°C/min, then from 200°C to 250°C at 10°C/min. Standard curves of ABA-methyl ester and PA-methyl ester with ABA-ethyl ester as an internal standard were constructed for quantitation. ABA-methyl ester ions were monitored at m/z = 278, 280, 282, and 284 for ^{18}O-labeled samples and at m/z = 294, 296, 298, 300, and 302 for PA-methyl ester. Corrections were made for the natural abundance of stable isotopes by subtracting the theoretical contribution from the measured ion abundance. Tandem mass spectrometry was performed on a Finnigan model TSQ-70 triple-quadrupole mass spectrometer as described (11).

Carotenoid Determinations. Carotenoids were extracted according to Britton (23) with modifications. Samples were manipulated in dim light to avoid isomerization of carotenoids. Frozen tissue (≈1 g) was extracted overnight at 4°C in 40 ml of methanol containing 1% sodium bicarbonate plus 0.1% 2,6-di-*tert*-butyl-4-methylphenol and homogenized. The extract was filtered, and the chlorophyll a and b concentrations were determined according to Holden (24). The samples were diluted 1:10 with water, NaCl was added to saturation, and the samples were repeatedly partitioned with 40 ml of diethyl ether until no color remained in the aqueous fraction. The ether was evaporated and the residues were saponified with 10% (wt/vol) KOH (in methanol) for 3 hr at room temperature under a stream of N_2. The samples were again diluted 1:10 and partitioned with diethyl ether. The ethereal extracts were stored overnight at −70°C, and ice crystals were removed by filtration. The ether was evaporated, and the residue was resuspended in 90% hexanes/10% ethyl acetate (vol/vol) and chromatographed by normal-phase HPLC with a μPorasil semipreparative 0.78 × 30 cm column (Waters) using a linear gradient from 10% to 100% ethyl acetate (in hexanes) in 65 min at a flow rate of 2.5 ml/min. The major carotenoids were collected and identified by their absorbance maxima, fine structure spectra, and acid shifts of absorbance maxima (23, 25). *cis*-Carotenoid isomers were characterized by rechromatography of iodine-treated *trans*-carotenoid isomers and by spectrophotometric analysis and predicted equilibrium stoichiometries (26, 27). In addition, the identities of violaxanthin, neoxanthin, and antheraxanthin isomers were confirmed by their retention times in reverse-phase HPLC systems (16, 17).

Carotenoids were quantified by integration of the area under the absorbance curve at 450 nm. A standard curve of β-carotene was constructed, and corrections were made for differences in specific extinction coefficients (refs. 23 and 25 and unpublished data).

RESULTS

ABA Biosynthetic Capacity Is Negatively Correlated with the Phenotypic Severity Associated with the *aba* Alleles. The *aba* alleles differ in their phenotypic severity of increased leaf transpiration, reduced growth, and reduced seed dormancy, which is correlated with reduced endogenous levels of ABA (20). Plants homozygous for the *aba-3* allele have close to normal growth rates, whereas *aba-1* and *aba-4* plants are more pronounced in their negative effects on plant size and vigor (ref. 20 and unpublished data). Biosynthesis of ABA in leaves of wild-type Landsberg *erecta* and *aba* genotypes was determined by ^{18}O-labeling and quantitation of *de novo* [^{18}O]ABA and metabolites. The results (Table 1) show that the phenotypic severity of the alleles was correlated with reduced ABA

Table 1. Quantitation of ^{18}O-labeled ABA and catabolites from water-stressed leaves of wild-type and the three *aba* genotypes of *Arabidopsis* after incubation for 4 or 8 hr in $^{18}O_2$

		^{18}O-labeled metabolites, ng/g of fresh weight			
Genotype	$^{18}O_2$, hr	ABA	ABA-GE	PA	Total
Landsberg *erecta*	4	28.1	0.2	104.2	132.5
	8	45.1	0.6	123.4	169.1
aba-3	4	1.6	Trace	1.1	2.7
	8	2.9	0.1	1.5	4.5
aba-1	4	0.7	Trace	0.4	1.1
	8	1.2	0.1	0.8	2.1
aba-4	4	0.3	0.1	NA	—
	8	1.0	0.1	NA	—

Mutants are listed in increasing order of phenotypic severity (20). Samples were quantified as their methyl ester derivatives by GC-selected ion monitoring and data are the average of two experiments. NA, not analyzed.

biosynthesis. Although leaves of plants homozygous for the least severe allele *aba-3* accumulated ABA and catabolites that were ≈50% of wild-type turgid levels (unpublished data), *aba-3* plants synthesized ABA and its catabolites, PA and ABA-GE, at ≈3% of the wild-type level in ^{18}O-labeling experiments (Table 1). Leaves of plants homozygous for the more severe alleles *aba-1* and *aba-4* had correspondingly lower ABA biosynthetic capacities (Table 1).

ABA Precursor Pool Size Is Correlated with ABA Biosynthesis in *aba* Genotypes. Analysis of ^{18}O-labeled ABA by tandem mass spectrometry allows determination of the position and extent of ^{18}O incorporation into the ABA molecule (10, 11). Comparison of ^{18}O incorporation, by wild-type and *aba* plants, into the ring-attached oxygens of ABA (Table 2) indicates a correlation between ABA biosynthesis (Table 1) and turnover of the ABA precursor pool containing oxygens on the ring (presumably xanthophylls). Leaves of plants homozygous for the most severe allele *aba-4* had the highest percentage of ^{18}O incorporation into the ring-attached oxygens of [^{18}O]ABA. After 4 hr of water stress in an atmosphere containing $^{18}O_2$, almost 60% of [^{18}O]ABA from *aba-4* plants was labeled in the ring 1′-hydroxyl group (Table 2), whereas in the wild-type Landsberg tissue only 4% of the [^{18}O]ABA contained ^{18}O at this position. Plants homozygous for the less-severe *aba-1* allele had an intermediate level of ^{18}O ring incorporation (Table 2), which indicates that ABA precursor pool turnover in *aba-4* and *aba-1* plants was correlated with the phenotypic severity of these alleles (20). However, the percentage of total [^{18}O]ABA labeled in the ring-attached oxygens was about the same for the least severe allele *aba-3* and the wild-type plants (Table 2), although the former produced ABA at a rate of only 3% of the latter (Table 1). In the wild-type Landsberg *erecta* plants, incorporation of ^{18}O in the ring-attached oxygens was 6–15 times greater, on a fresh-weight basis, than the incorporation by the *aba* plants. {This calculation is the product of [^{18}O]ABA levels (Table 1) and the respective percentage of [^{18}O]ABA that is ring-labeled (Table 2)}. Thus, the ABA precursor pool containing oxygens on the ring is much smaller in the *aba* plants than in wild-type plants.

Epoxy-Carotenoid Deficiency and Zeaxanthin Accumulation Are Correlated with the Small ABA Precursor Pool in *aba* Genotypes. The results of the $^{18}O_2$ incorporation studies with the *aba* genotypes (Tables 1 and 2) suggested that the xanthophyll levels of *aba* plants were reduced. Fig. 2 shows chromatograms of carotenoids from leaves of wild-type and *aba-4* plants. It is clear that *aba-4* plants had reduced levels of the major epoxy-carotenoids *trans*-violaxanthin and 9′-*cis*-neoxanthin (Fig. 2, peaks 5 and 7) and that this mutant accumulated zeaxanthin (peak 3). Quantitation of carotenoids and chlorophylls from leaves of Landsberg *erecta* and the three *aba* genotypes is presented in Table 3. Zeaxanthin, the biosynthetic precursor to the epoxy-carotenoids antheraxanthin, violaxanthin, and neoxanthin (28) accumulated in leaves of all the *aba* genotypes. β-Carotene, the precursor to zeaxanthin, also accumulated in *aba-4* plants (Table 3). There was a reduction of *trans*-violaxanthin and 9′-*cis*-neoxanthin in all *aba* plants. Lutein, the most abundant xanthophyll in wild-type leaves and a product of the α-carotene branch pathway (28), was significantly reduced in the *aba* genotypes. Furthermore, the quantitative differences in zeaxanthin and β-carotene accumulation (Table 3) are correlated with the phenotypic severity (20) of the different *aba* alleles. From these results we conclude that the ABA precursor pool containing oxygens on the ring is composed of the epoxy-carotenoids violaxanthin and neoxanthin.

Total carotenoids and chlorophylls were not significantly different in wild-type and *aba* plants on a g (fresh weight) basis (Table 3). However, the lower chlorophyll b level in *aba-4* plants is of interest because chlorophyll b and epoxy-carotenoids are associated predominantly with the light-harvesting photosynthetic complexes (29), and epoxy-carotenoids are necessary for assembly of photosystem II light-harvesting complexes *in vitro* (30).

Table 2. ^{18}O incorporation into the ring-attached oxygens of ABA from water-stressed leaves of wild-type and the three *aba* genotypes of *Arabidopsis* after incubation in $^{18}O_2$ for 4 or 8 hr

Genotype	$^{18}O_2$, hr	% of total [^{18}O]ABA
Landsberg *erecta*	4	4.3
	8	8.7
aba-3	4	6.9
	8	8.0
aba-1	4	29.8
	8	27.4
aba-4	4	58.6
	8	60.2

Mutants are listed in order of increasing phenotypic severity (20). Samples were analyzed as their methyl ester derivatives by GC-selected ion monitoring and tandem mass spectrometry (11). The label was always present in the 1′-hydroxyl group (10); in addition, 5–10% of the 1′-hydroxyl-labeled ABA was also labeled in the 4′-keto group. Percent of total [^{18}O]ABA labeled in the ring-attached oxygens is shown.

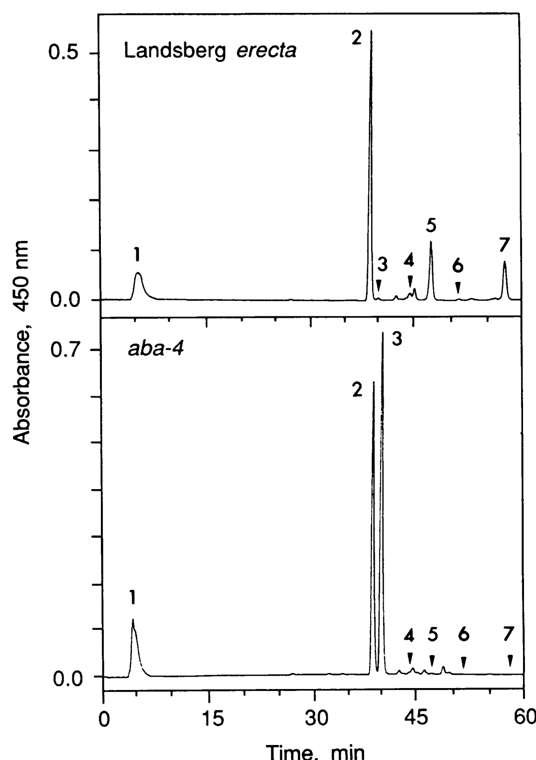

FIG. 2. HPLC chromatograms of carotenoids extracted from leaves of wild-type Landsberg *erecta* and the *aba-4* genotype of *A. thaliana*. Peaks: 1, β-carotene; 2, lutein; 3, zeaxanthin; 4, antheraxanthin; 5, *trans*-violaxanthin; 6, 9-*cis*-violaxanthin; 7, 9′-*cis*-neoxanthin.

DISCUSSION

The data presented in Tables 2 and 3 provide strong correlative evidence for the indirect pathway of ABA biosynthesis from violaxanthin and neoxanthin. If a direct pathway from farnesyl pyrophosphate exists in *Arabidopsis*, it is of negligible physiological importance. From extrapolation of the data in Tables 1 and 3, we predict that a complete loss of

Table 3. Quantitation of carotenoids and chlorophylls from leaves of wild-type and the three *aba* genotypes of *Arabidopsis*

Compound	Concentration, μg/g (fresh weight)			
	Landsberg *erecta*	*aba-3*	*aba-1*	*aba-4*
β-Carotene	23.8 ± 3.54	24.7 ± 3.38	25.1 ± 1.67	33.0 ± 3.72*
Lutein†	58.0 ± 4.31	39.0 ± 2.62‡	38.4 ± 1.28‡	45.1 ± 4.94§
Zeaxanthin†	1.2 ± 0.34	40.0 ± 2.36‡	44.7 ± 2.32‡	52.2 ± 5.46‡
Antheraxanthin	0.9 ± 0.13	1.1 ± 0.12	0.9 ± 0.11	0.6 ± 0.14
trans-Violaxanthin	17.2 ± 0.92	0.4 ± 0.03‡	0.4 ± 0.03‡	0.3 ± 0.06‡
9-*cis*-Violaxanthin	0.6 ± 0.05	0.3 ± 0.04	0.3 ± 0.06	0.2 ± 0.04
trans-Neoxanthin	1.5 ± 0.29	ND‡	ND‡	ND‡
9′-*cis*-Neoxanthin	20.0 ± 2.15	0.2 ± 0.02‡	0.1 ± 0.02‡	0.2 ± 0.07‡
Total carotenoids	122.3 ± 8.68	105.0 ± 8.16	109.4 ± 4.26	133.4 ± 12.68
Chlorophyll	1120 ± 65	1070 ± 55	1150 ± 58	1250 ± 27
Chlorophyll a/b	2.63 ± 0.20	2.40 ± 0.14	2.58 ± 0.24	3.00 ± 0.22

Mutant alleles are listed in increasing order of phenotypic severity (20). Leaves from two to six plants were extracted with methanol and chlorophyll and carotenoids were measured. Data are mean ± SEM of five experiments, two measurements per experiment, except for chlorophyll, which was six experiments. ND, not detected.
*Mutant significantly greater than wild type, $P < 0.08$ (one-sided t test).
†Includes *cis* isomers.
‡Mutant significantly different from wild type, $P < 0.02$ (two-sided t test).
§Mutant significantly different from wild type, $P < 0.12$.

epoxy-carotenoids would result in absence of ABA and would be lethal.

The correlations of phenotypic severity (20), reduced ABA biosynthesis (Tables 1 and 2), and reduced epoxy-carotenoid content (Table 3) suggest that the *ABA* locus affects an enzyme that functions in the epoxidation of xanthophylls. Such an enzyme has been identified in chloroplast envelopes (31) and as a component of the xanthophyll cycle (32), which is involved in the dissipation by zeaxanthin of excess energy in photosynthesis (33, 34). The data presented here imply that the same epoxidase is involved in the xanthophyll cycle and in epoxy-carotenoid biosynthesis. The mutations in the various alleles are presumably leaky, and the residual epoxidase activity determines the rate-limiting step of violaxanthin and neoxanthin biosynthesis, and consequently of ABA biosynthesis, in *aba* plants.

Because *aba* plants have increased ABA precursor pool turnover (Table 2), these genotypes may be useful to study the regulation of epoxy-carotenoid and ABA biosynthesis. The involvement of violaxanthin, neoxanthin, and zeaxanthin in photosynthetic processes makes the *aba* alleles of *Arabidopsis* a promising experimental tool to investigate the function of epoxy-carotenoids and the xanthophyll cycle in plants.

Note Added in Proof. It has come to our attention that Duckham *et al.* (35) simultaneously reported the characterization of the *aba* mutant of *A. thaliana*.

We thank Drs. Tim Heath and Doug Gage of the Michigan State University-National Institutes of Health Mass Spectrometry Facility (Grant DRR00480) for assistance with mass spectrometry, and Dr. Maarten Koornneef for providing seeds of *A. thaliana*. This work was supported by the U.S. Department of Energy under Contract DE-AC02-76ERO-1378, by the National Science Foundation Grant DMB-8703847 to J.A.D.Z., and by a Michigan State University College of Natural Science Doctoral Fellowship to C.D.R.

1. Peña-Cortés, H., Sánchez-Serrano, J. J., Mertens, R., Willmitzer, L. & Prat, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9851–9855.
2. Zeevaart, J. A. D. & Creelman, R. A. (1988) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 439–473.
3. Ohkuma, K., Addicott, F. T., Smith, O. E. & Thiessen, W. E. (1965) *Tetrahedron Lett.* **29**, 2529–2535.
4. Milborrow, B. V. (1974) *Annu. Rev. Plant Physiol.* **25**, 259–307.
5. Moore, R. & Smith, J. D. (1985) *Planta* **164**, 126–128.
6. Neill, S. J., Horgan, R. & Parry, A. D. (1986) *Planta* **169**, 87–96.
7. Moore, R. & Smith, J. D. (1984) *Planta* **162**, 342–344.
8. Gamble, P. E. & Mullet, J. E. (1986) *Eur. J. Biochem.* **160**, 117–121.
9. Creelman, R. A., Gage, D. A., Stults, J. T. & Zeevaart, J. A. D. (1987) *Plant Physiol.* **85**, 726–732.
10. Zeevaart, J. A. D., Heath, T. G. & Gage, D. A. (1989) *Plant Physiol.* **91**, 1594–1601.
11. Rock, C. D. & Zeevaart, J. A. D. (1990) *Plant Physiol.* **93**, 915–923.
12. Parry, A. D., Neill, S. J. & Horgan, R. (1990) *Phytochemistry* **29**, 1033–1039.
13. Taylor, H. F. & Burden, R. S. (1973) *J. Exp. Bot.* **24**, 873–880.
14. Parry, A. D., Neill, S. J. & Horgan, R. (1988) *Planta* **173**, 399–404.
15. Sindhu, R. K., Griffin, D. H. & Walton, D. C. (1990) *Plant Physiol.* **93**, 689–694.
16. Li, Y. & Walton, D. C. (1990) *Plant Physiol.* **92**, 551–559.
17. Parry, A. D., Babiano, M. J. & Horgan, R. (1990) *Planta* **182**, 118–128.
18. Neill, S. J., Horgan, R. & Walton, D. C. (1984) in *the Biosynthesis and Metabolism of Plant Hormones*, eds. Crozier, A. & Hillman, J. R. (Cambridge Univ. Press, Cambridge, U.K.), pp. 43–70.
19. Okamoto, M., Hirai, N. & Koshimizu, K. (1988) *Mem. Coll. Agric. Kyoto Univ.* **132**, 79–115.
20. Koornneef, M., Jorna, M. L., Brinkhorst-van der Swan, D. L. C. & Karssen, C. M. (1982) *Theor. Appl. Genet.* **61**, 385–393.
21. Bleecker, A. B., Estelle, M. A., Somerville, C. & Kende, H. (1988) *Science* **241**, 1086–1089.
22. Cornish, K. & Zeevaart, J. A. D. (1984) *Plant Physiol.* **76**, 1029–1035.
23. Britton, G. (1985) *Methods Enzymol.* **111**, 113–149.
24. Holden, M. (1976) in *Chemistry and Biochemistry of Plant Pigments*, ed. Goodwin, T. W. (Academic, New York), Vol. 2, pp. 1–37.
25. Braumann, T. & Grimme, L. H. (1981) *Biochim. Biophys. Acta* **637**, 8–17.
26. Molnár, P. & Szabolcs, J. (1980) *Phytochemistry* **19**, 623–627.
27. Khachik, F., Beecher, G. R. & Whittaker, N. F. (1986) *J. Agric. Food Chem.* **34**, 603–616.
28. Jones, B. L. & Porter, J. W. (1986) *CRC Crit. Rev. Plant Sci.* **3**, 295–324.
29. Siefermann-Harms, D. (1985) *Biochim. Biophys. Acta* **811**, 325–355.
30. Plumley, F. G. & Schmidt, G. W. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 146–150.
31. Costes, C., Burghoffer, C., Joyard, J., Block, M. & Douce, R. (1979) *FEBS Lett.* **103**, 17–21.
32. Siefermann, D. & Yamamoto, H. Y. (1975) *Arch. Biochem. Biophys.* **171**, 70–77.
33. Demmig, B., Winter, K., Krüger, A. & Czygan, F.-C. (1987) *Plant Physiol.* **84**, 218–224.
34. Demmig-Adams, B., Adams, W. W., III, Heber, U., Neimanis, S., Winter, K., Krüger, A., Czygan, F.-C., Bilger, W. & Björkman, O. (1990) *Plant Physiol.* **92**, 293–301.
35. Duckham, S. C., Linforth, R. S. T. & Taylor, I. B. (1991) *Plant Cell Environ.* **14**, 631–636.