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Effects of abscisic acid on reserve deposition in developing *Arabidopsis* seeds

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SUMMARY

Arabidopsis thaliana mutants that were either ABA-deficient (*aba1-1*) or ABA-insensitive (*abi3-1*) and their recombinant (*aba1-1, abi3-1*) were used to determine whether ABA plays a role in the regulation of deposition of reserve material during seed development. The total net import of assimilates into seeds of these genotypes was unaffected compared to wild-type seeds, but the distribution of these assimilates over the various types of storage material depended on the genotype. All mutants were to the same extent impaired in the synthesis of long-chain fatty acids: their seeds contained three times less eicosenoic acid (20:1) in the triacylglycerol fraction compared to wild-type seeds. Moreover, recombinant (*aba1-1, abi3-1*) seeds accumulated considerably less neutral lipids than wild-type and single-mutant seeds, and simultaneously the amounts of soluble carbohydrates and starch were increased. Absence of and insensitivity to ABA apparently cause inhibition of acyl-chain elongation and of lipid accumulation, and as a result a higher proportion of the imported assimilates is stored as carbohydrates.

Key-words: ABA-deficient (*aba1-1*) mutant, ABA-insensitive (*abi3-1*), abscisic acid, *Arabidopsis thaliana*, carbohydrates, lipids, seed development, starch, storage product accumulation.

INTRODUCTION

In most plant tissues photosynthates are stored as carbohydrates. However, seeds of many species store mainly triacylglycerols, as a way to concentrate energy in a small volume (Slack & Browse 1984). Although assimilates usually arrive as sucrose, and oil-rich seeds are also capable of storing starch (Norton & Harris 1975; Hara *et al.* 1985), in general carbohydrates are only temporarily present during seed development. Apparently the cells in these oil-rich seeds differ from vegetative cells with respect to the mechanism that determines the deposition of storage material. Relatively little attention has been paid to the hormonal regulation of the distribution of assimilates among different types of storage material. Although the effect of abscisic acid (ABA) on the accumulation of storage proteins in developing seeds is well-documented (Black 1991;

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Abbreviations: x:n, fatty acid containing x carbons and n double bonds.

Thomas *et al.* 1991), only few studies describe the influence of ABA on the conversion of carbohydrates to fatty acids. Ackerson (1984) found an effect of exogenous ABA on the sucrose partitioning in soybean embryos cultured *in vitro*: embryos cultured in the presence of ABA accumulated more protein, sugars and starch, at the expense of lipid accumulation. Finkelstein & Somerville (1990) studied the accumulation of both storage protein and triacylglycerols in seeds of a series of ABA-insensitive mutants of *Arabidopsis thaliana*. Seeds of one of these mutants (*abi3-1*) had a threefold reduced content of 20:1 fatty acids. This result is in close agreement with a promoting effect of applied ABA on the accumulation of long-chain fatty acids in cultured embryos of *Brassica napus* (Finkelstein & Somerville 1989). Holbrook *et al.* (1992) and Zou *et al.* (1995) reported that applied ABA and its metabolites are able to stimulate both the total fatty acid accumulation and the elongation of 18:1 and 20:1 fatty acids in microspore-derived embryoids of *Brassica napus*.

These latter studies, however, have two drawbacks: the use of embryos cultured *in vitro*, and the use of applied ABA. It is still an open question whether the effects of applied ABA on embryos cultured *in vitro* simulate the effects of endogenous ABA during *in planta* development of seeds. *In vitro*, ABA may act primarily as a germination suppressor, resulting in continued development of the embryoids. An important difference between the two systems is that the fatty acid composition of *in vitro* cultured zygotic embryos may deviate considerably from that of *in planta* developed embryos (Finkelstein & Somerville 1989; Dutta & Appelqvist 1991; Kim & Janick 1991), depending on the culture conditions (Möllers *et al.* 1994). However, Taylor *et al.* (1990) achieved almost similar fatty acid profiles in embryoids as compared to mature seed. In general, the use of applied ABA has also several disadvantages (Trewavas & Jones 1991): uptake, transport and metabolism of ABA have to be checked, and the tissue of the control treatments may have endogenous ABA above the threshold required for the response being studied. The use of ABA mutants offers the possibility of studying both the role of endogenous ABA in *in planta* development of embryos, and the effects of lower ABA levels or insensitivity to ABA.

The main objective of the present study was to aquire insight into the influence of ABA on the deposition of reserve material in seeds during development. For that purpose, the fatty acid and carbohydrate composition of seeds of several *Arabidopsis* hormone mutants during development was compared with that of wild-type seeds. Both an ABA-deficient (*aba1-1*) and an ABA-insensitive (*abi3-1*) mutant and their recombinant (*aba1-1, abi3-1*) were included.

MATERIALS AND METHODS

Plant material

All *Arabidopsis thaliana* (L.) Heynh. mutants were either derived from or back-crossed with the pure line Landsberg *erecta* (wild type). The isolation and characterization of the ABA-deficient (*aba1-1*, isolation number A26) and the ABA-insensitive (*abi3-1*, isolation number CIV) mutant were described by Koornneef *et al.* (1982, 1984). The recombinant *aba1-1, abi3-1* was originally isolated from a population segregating for the *aba1-1* gene (Koornneef *et al.* 1989) and recognized by the dark green colour of the mature seeds. All plants were self-fertilized.

Culture conditions

Dry stored seeds of wild-type, *aba1-1* and *abi3-1* plants were sown in 9-cm diameter Petri dishes on moist filter paper (Schleicher & Schüll nr. 595; Dassel, Germany). Since *aba1-1, abi3-1* plants yielded no viable mature seeds due to lack of desiccation tolerance, this mutant was maintained by transferring near-mature seeds from the siliques directly to moist filter paper. The seeds were allowed to germinate in a climate room at 24°C, under continuous fluorescent light. After 3 or 4 days, the seedlings were planted out in 5·5 cm pots with a mixture of sand and humus. The plants were grown in a greenhouse at day temperatures of 17–22°C and night temperatures of 15–17°C, under natural light, supplemented with artificial illumination (Philips, TLD 58W/84) to give a 16-h day length. Because the ABA-deficient mutants were very sensitive to wilting, a mist bench was created within the greenhouse to maintain a very high relative humidity (85–100%).

Harvest of seeds

Due to practical limitations, the seeds used in this study originated from different series. In the first series of experiments (March 1991), both weight and carbohydrate content of wild-type and *aba1-1, abi3-1* seeds were determined. In the second series (December 1991), seeds of wild-type and the single mutants (*aba1-1* and *abi3-1*) were used for determination of both carbohydrate and lipid composition. In a third series (April 1992), the lipid composition of wild-type and *aba1-1, abi3-1* seeds was investigated.

Individual flowers were tagged at the day of anthesis and siliques were harvested at various stages after flowering, ranging from the youngest seeds that could be collected (6 days after flowering (DAF)) to mature seeds (17–22 DAF, depending on the season). Collection of the seeds in each series was stopped when siliques had dried.

Prior to opening, the siliques were transferred to an enclosed box with a high relative humidity, since young seeds readily lose water. Seeds were carefully removed from the siliques, counted and immediately transferred to vials containing either a mixture of chloroform and methanol (1:1, v/v) (for lipid determinations) or 80% (v/v) methanol (for carbohydrate determinations). These vials were stored at − 70°C until analysis. In all series of experiments, 50–80 seeds were harvested of each genotype, in triplicate.

Weight determinations

Individual seeds of both wild-type and *aba1-1, abi3-1* plants were harvested at various stages ranging from 7 DAF to 22 DAF, as described above, but after removal from the siliques each seed was immediately enclosed in a small, preweighed tin container (approx. 7 mg). The weight of these containers was determined on a high-precision balance (Mettler UM3). After drying for 16 h at 105°C the containers were cooled over silica and weighed again, to determine the dry weight of the seeds. Four siliques were harvested per genotype and per stage, and four representative seeds were chosen from each silique.

Neutral lipid determinations

The contents of vials containing 50–80 seeds and 0·3 mg triheptadecanoin (as an internal standard) were transferred to a hand-operated 1 mL-Potter tube and homogenized. The lipid content of the seeds was analysed essentially as described by Hoekstra & Van Roekel (1988). The homogenate was washed and dried, polar lipids were removed by passage over a SEP-PAK silica cartridge, and neutral lipids were trans-methylated in

0·3 N KOH in methanol at 70°C for 15 min. Methylated fatty acids were collected by phase separation in hexane and injected in a GC (Shimadzu GC-8A, equipped with a J&W Megabore column, DB225 [J&W Scientific, Folsom, CA], 30 m, operated at 210°C; flame ionization detector). *Arabidopsis* seed lipids consist mainly of triacylglycerols (± 90%, Kunst *et al.* 1992).

Carbohydrate analysis

Before homogenizing the seeds, 25 μg melezitose was added to the 80% methanol as an internal standard. The homogenate was heated for 15 min at 75°C and centrifuged (5 min, 10 000 *g*). The pellet was stored for analysis of polysaccharides; the supernatant was vacuum-evaporated and its residue was taken up in 0·5 mL purified water (Milli-Q purification system, Millipore, Molsheim, France) and injected into a Dionex HPLC system (Dionex Corporation, Sunnyvale, CA). The HPLC was equipped with a CarboPac PA100 (4 × 250 mm) column with appropriate guard column and a pulsed electrochemical detector (PED) with an Au working electrode and an Ag/AgCl reference electrode. Usually, mono-, di- and trisaccharides were separated by isocratic elution in 0·1 N NaOH for 30 min, at a flow rate of 1 mL min^{-1}, at ambient temperature. Peaks were identified by comparing their retention times with the retention times of a mixture of standard sugars; both samples and standards were run at various NaOH concentrations in the eluent, to confirm this identification.

Polysaccharides (the carbohydrate fraction that was not extractable in 80% methanol, as described above) were hydrolysed according to Fry (1988). Pellets were resuspended in 50 μL of 58% (v/v; *c.* 11 M) H_2SO_4 and incubated at room temperature for 1 h. Subsequently, an anti-bumping granule and 0·5 mL water were added and the sample was stirred and heated at 120°C for 1 h. After cooling, 0·5 mL water was added and the sample was injected directly into the HPLC (see above) without further dilution. No substantial breakdown of monosaccharides was observed when storage of samples before injection was limited to 2 days. The monosaccharides were separated by a modified elution programme: the column was pre-equilibrated with 0·1 N NaOH and 1·1 M sodium acetate for 5 min and with 0·1 N NaOH for 15 min. The sample was injected at the end of a 10 min gradient from 0·1 N NaOH to 0·01 N NaOH and eluted isocratically at 0·01 N NaOH for 35 min. The column equilibration procedure was repeated before every analysis.

RESULTS

Fresh and dry weight of seeds during development

In a preliminary experiment it was investigated whether ABA influenced the import of assimilates into the seeds, by comparing the gain in fresh and dry weight of seeds of wild-type plants and of *aba1-1, abi3-1* plants. The latter mutant was chosen since it could be expected that ABA effects are more pronounced in this recombinant compared to the *aba1-1* or *abi3-1* single mutants (Koornneef *et al.* 1989). Moreover, analysis of the weights of individual developing seeds would hopefully provide a 'calibration curve' to be used in further experiments, thus avoiding the laborious and time-consuming work of weighing such small seeds.

It was evident that *aba1-1, abi3-1* seeds were somewhat retarded in their development with respect to the accumulation of both fresh and dry weight, compared to wild-type

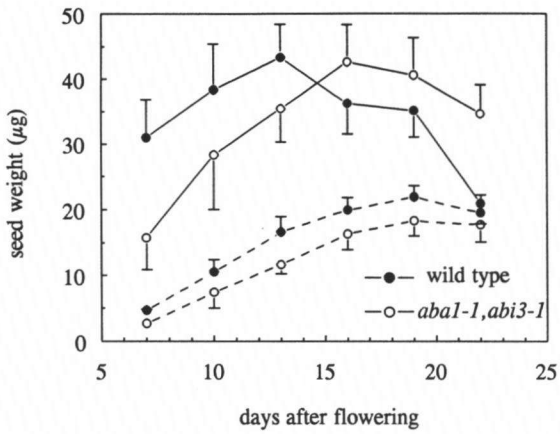

Fig. 1. Fresh (solid lines) and dry (dotted lines) weight of wild-type and *aba1-1*, *abi3-1 Arabidopsis* seeds during development. Data are the means ± SD for 16 seeds from four siliques.

seeds (Fig. 1). Nevertheless, the maximum fresh and dry weights of both lines were not significantly different. At day 22, siliques of *aba1-1, abi3-1* plants contained both viviparously germinated seeds (since these seeds lack ABA and have no dormancy, Koornneef *et al.* 1989) and dried, dead seeds (since these seeds have no desiccation tolerance, Koornneef *et al.* 1989). The latter seeds were omitted from the weight determinations.

In further experiments, it turned out that the rate of seed development varied considerably between different series (cf. Parcy *et al.* 1994). Therefore, the seed weight determined in one series cannot be transferred to another series for calculations of assimilate deposition on a dry weight basis.

Neutral lipid content

No differences were observed in neutral lipid contents during development, comparing single mutants and wild-type seeds, whereas seeds of the *aba1-1, abi3-1* recombinant were strongly inhibited in their lipid accumulation (Fig. 2). Another evident feature is the difference between the series of December 1991 and April 1992: seeds of plants grown during the winter season (Fig. 2A) started the accumulation of lipids later than seeds produced during the spring (Fig. 2B).

Another obvious difference among the genotypes lies in the fatty acid composition of the neutral lipids. The seeds of all three mutants contained about three times less 20:1 than wild-type seeds (Fig. 3, Table 1). The proportion of 20:1 in *aba1-1* seeds during development displayed a peak at 11–13 DAF but decreased later during development (Fig. 3). However, this peak was not significant when the data were expressed as absolute amounts of 20:1 fatty acid per seed (data not shown).

The lower proportions of 20:1 in the single mutants were counterbalanced by an increase in 16:0, 18:1 and 18:2. However, in the double mutant *aba1-1, abi3-1* the proportion of 18:1 was not elevated whereas the proportions of 16:0 and 18:2 were higher than in the single mutants and in the wild-type (Table 1; De Bruijn *et al.* 1993). These differences were not only evident in mature seeds, but also throughout

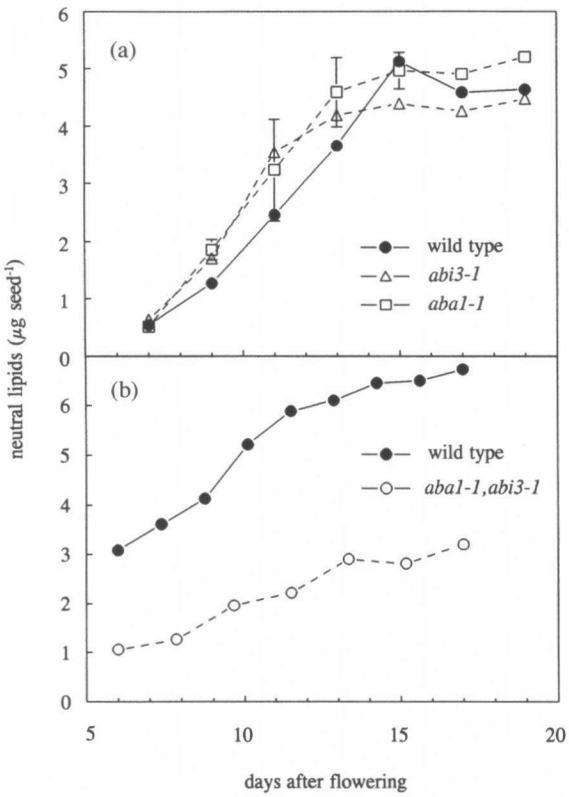

Fig. 2. Accumulation of neutral lipids during seed development in seeds of wild-type, *abi3-1* and *aba1-1* (A) or wild-type and *aba1-1*, *abi3-1* (B) *Arabidopsis* plants. Data for the *aba1-1* seeds are the means ± SD of triplicate samples.

development (data not shown). However, the absolute amounts of 16:0 and 18:1 in the double mutant are more or less comparable to those in the single mutants, since the double mutant seeds have a considerably reduced lipid content.

Carbohydrate content

Soluble carbohydrates. Figure 4 summarizes the results for fructose, glucose and sucrose (*c.* 80–85% of total soluble carbohydrates), determined during seed development. In general, fructose and glucose decreased initially, peaked later during development and declined again at maturity. In contrast, sucrose gradually increased during development. The amounts of glucose and fructose in seeds of *aba1-1* and *abi3-1* plants were similar to those in the wild-type seeds. In the seeds of the recombinant *aba1-1, abi3-1*, glucose and fructose remained relatively high throughout development. As a result, the amounts of glucose and fructose at maturity were eight times higher than those in mature wild-type seeds.

The amounts of sucrose in *aba1-1* and *abi3-1* seeds were up to two times higher than in wild-type seeds. The increase in sucrose near maturity was even more pronounced in the recombinant *aba1-1, abi3-1*, showing a fourfold increase compared to wild-type seeds.

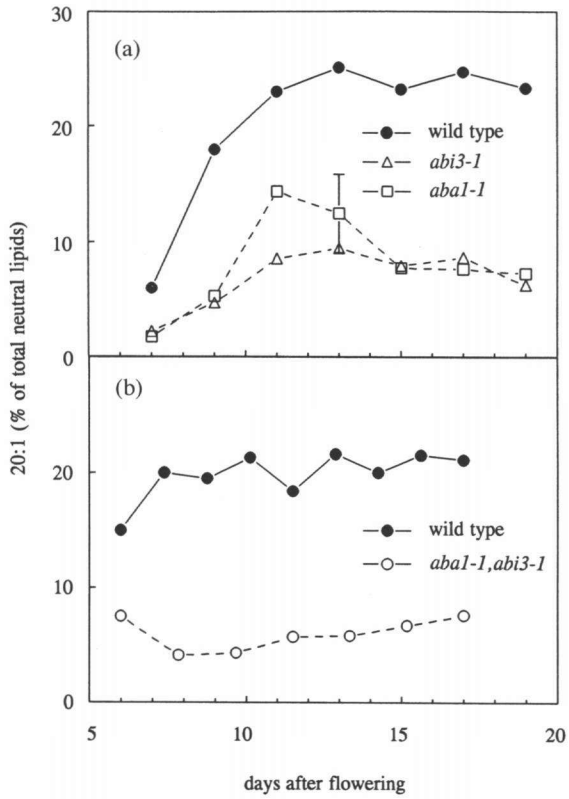

Fig. 3. Accumulation of 20:1 fatty acid during seed development in seeds of wild-type, *abi3-1* and *aba1-1* (A) or wild-type and *aba1-1*, *abi3-1* (B) *Arabidopsis* plants, relative to the total neutral lipid content. Data for the *aba1-1* seeds are the means ± SD of triplicate samples; SD is indicated when it exceeded the size of the symbols.

Insoluble carbohydrates. The non-soluble fractions that remained after extraction with methanol were hydrolysed with sulphuric acid under conditions that yielded monosaccharides from all types of polysaccharides (including cellulose and other cell wall polymers). A variety of monosaccharides was found, the most prominent ones being glucose, arabinose, galactose, xylose, rhamnose and ribose (Fig. 5). Fructose was hardly detectable. The accumulation pattern of arabinose, galactose, rhamnose and xylose was similar in all genotypes: the amounts of these sugars in the polysaccharide fraction gradually increased during development. Contrary to this, glucose and ribose generally declined towards maturation. In wild-type seeds ribose decreased almost to zero; in *abi3-1* and *aba1-1* (data not shown) and in *aba1-1, abi3-1* seeds (Fig. 5) minor levels were still left at the end of development.

It is very likely that the glucose found after acid-catalysed hydrolysis predominantly originated from starch, since data on starch content of seeds from the same series of experiments hydrolysed by amyloglucosidase (De Bruijn *et al.* 1993) matched these graphs. In Fig. 6, starch contents of the seeds of all genotypes are displayed: apparently the single mutants initially had slightly higher starch levels compared to wild-type seeds; at the end of development starch had declined in all genotypes, except in the *aba1-1, abi3-1* mutant where a considerable amount of starch remained. The differences in

Table 1. Fatty acid composition of *Arabidopsis* seeds of several genotypes. Total neutral lipids, extracted from mature seeds, were transmethylated and the acyl composition was determined by gas-chromatography of the methylesters. Data for the *aba1-1* seeds are means of triplicate samples

	Fatty acid (% of total)								
Genotype	16:0[a]	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:1
Wild type	6.1	2.7	17.3	29.3	17.8	1.8	23.3	1.4	0.3
abi3-1	9.2	3.2	25.1	37.0	18.4	0.5	6.3	0.5	ND[b]
aba1-1	8.5	3.3	25.0	35.4	19.2	0.7	7.3	0.5	0.3
Wild type	6.5	3.3	22.2	27.4	16.7	1.7	21.1	1.0	0.1
aba1-1, abi3-1	11.6	3.0	18.6	41.6	16.7	1.0	7.6	ND	ND

[a]x:n: fatty acid containing x carbons and n double bonds. [b]ND: not detectable.

starch in Fig. 6B are even more pronounced when expressed on a dry weight basis: at 10 DAF 15.1% of the dry weight of *aba1-1, abi3-1* seeds consists of starch vs. 6·7% in wild-type seeds, at 19 DAF the ratio was 2·9% vs. 0·4%.

DISCUSSION

The first question that must be addressed is whether the observed differences in storage material accumulation between wild-type seeds and seeds of mutants that have either a lower ABA content or a reduced responsiveness to ABA are actually caused by ABA. Both mutations that were used in this study were leaky and it can be argued that this complicates the interpretation of the observed phenomena.

It is well accepted that the *aba1-1* mutant used in the current study is impaired early in the ABA biosynthetic pathway (Duckham *et al.* 1991) and that the observed phenotype of this mutant can be fully reverted by the application of ABA (Koorneef *et al.* 1982; Karssen *et al.* 1983). The nature of the mutations at the *ABI3* locus is more complex. Parcy *et al.* (1994) have shown that *abi3* mutants display changes in gene expression during the early stages of seed development and that the *ABI3* gene product is already visible at 4 DAF. Our results also indicate that effects of both mutations were already present at day 6 (Fig. 3), so the role of *ABI3* is probably not confined to the switch between a maturation or a germination programme (as proposed by Nambara *et al.* 1995) but it is active at multiple developmental stages. Although it cannot be excluded that the *ABI3* protein is also involved in ABA-independent regulatory pathways (Parcy *et al.* 1994), it is generally accepted that the *ABI3* gene, similar to the *VP1* gene in maize, plays a key role in the ABA signal transduction chain (Finkelstein 1994; McCarty 1995).

With respect to triacylglycerol accumulation, at least three effects were discerned: (1) the ABA-deficient (*aba1-1*) or ABA-responsiveness (*abi3-1*) mutants and the recombinant of these mutants (*aba1-1, abi3-1*) accumulated considerably less long-chain fatty acids in their seeds, (2) *aba1-1, abi3-1* seeds contained only half the amount of total triacylglycerols of the wild type and the single mutants and (3) *aba1-1, abi3-1* seeds had relatively less 18:1.

Both ABA-deficiency and ABA-insensitivity resulted in lower levels of 20:1 in the seeds and a concomitant increase in 16:0, 18:1 and 18:2 (Fig. 3, Table 1). These data

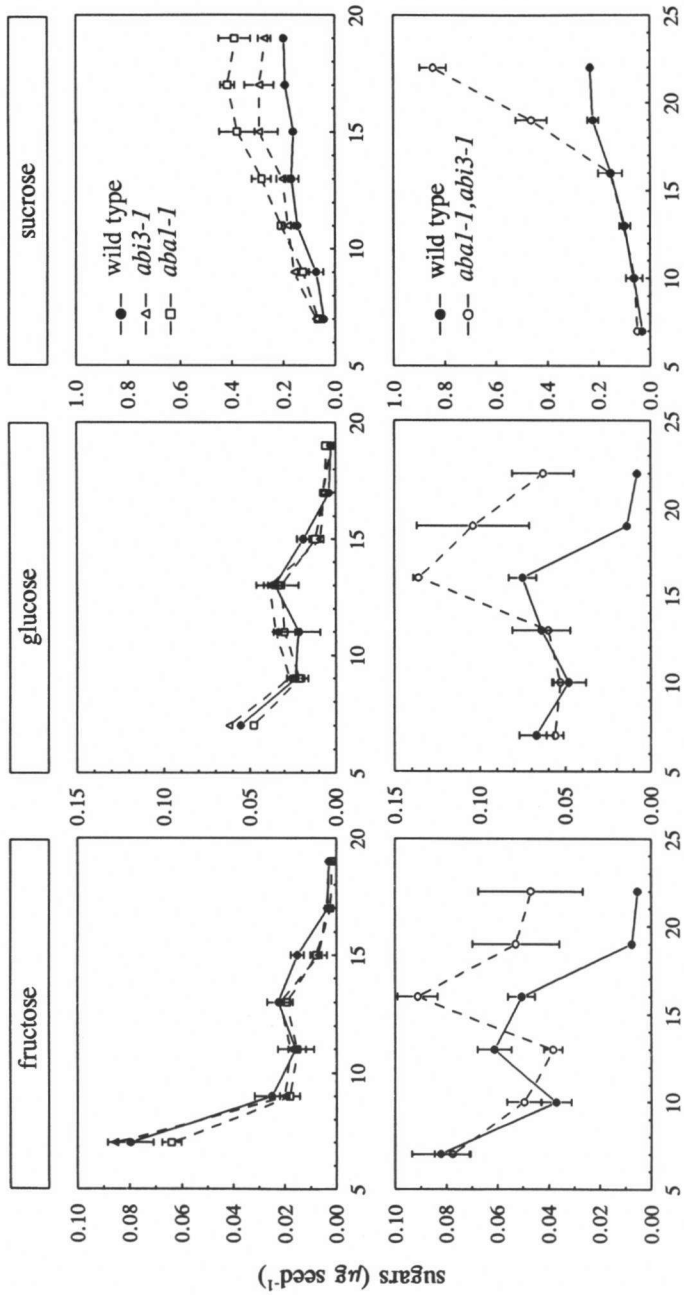

Fig. 4. Levels of fructose, glucose and sucrose during seed development in seeds of wild-type, *abi3-1* and *aba1-1* (upper panels) or wild-type and *aba1-1*, *abi3-1* (lower panels) *Arabidopsis* plants. Data are mean ± SD of triplicate samples.

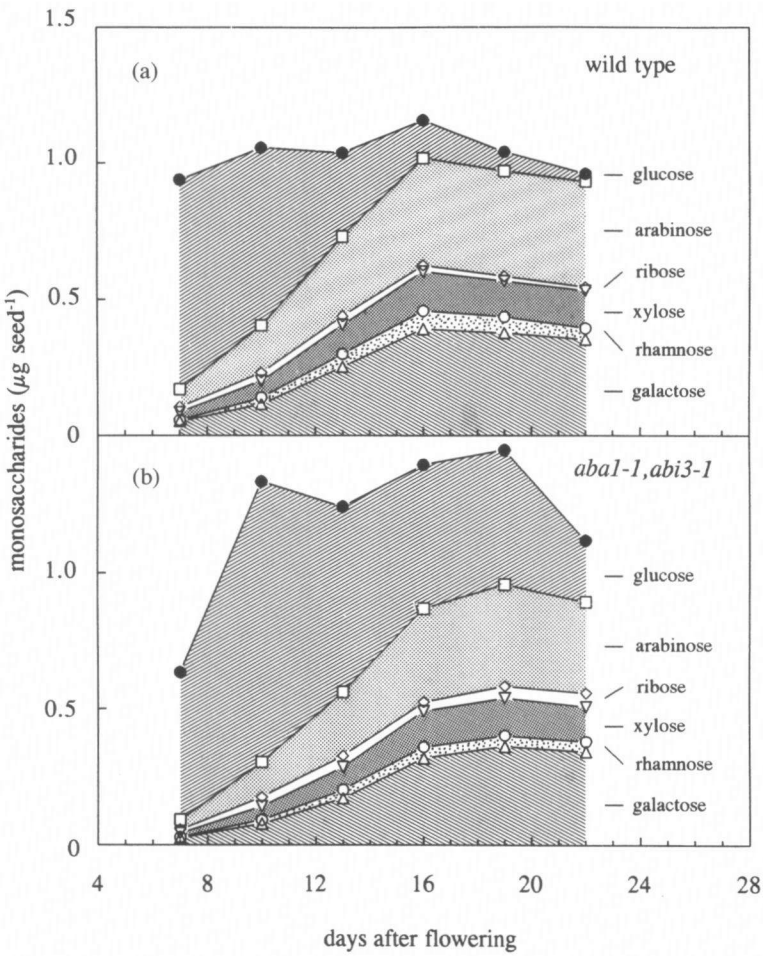

Fig. 5. Cumulative levels of monosaccharides after total hydrolysis of a methanol-insoluble residue from homogenates of seeds of wild-type (A) and *aba1-1*, *abi3-1* (B) *Arabidopsis* plants. Data are means of triplicate samples.

confirm the observations of Finkelstein & Somerville (1989), Holbrook *et al.* (1992), Möllers *et al.* (1994) and Zou *et al.* (1995), who observed an increase in the fraction of long-chain fatty acids (20:1 and 22:1) in *Brassica* embryos cultured *in vitro* after incubation with ABA. Moreover, Holbrook *et al.* (1992) and Zou *et al.* (1995) found increased incorporation of precursors in long-chain fatty acids in cell-free homogenates of *Brassica* embryos after preincubation with ABA or analogues. From their work it is clear that ABA directly interferes with the activity or presence of the elongase enzyme(s), and not with the presence of cofactors or malonyl-CoA or the transport of 18:1 from the plastids to the site of elongation. Since the incubation times with ABA in their studies were rather long (3–7 days), it is not possible to decide whether ABA directly inhibited the enzyme(s) or acted on the level of gene expression.

The present result that both ABA-deficiency and ABA-insensitivity caused decreased amounts of 20:1 conflict with the data of Finkelstein & Somerville (1990), who found wild-type 20:1 amounts for the *aba1-1* mutant. A possible explanation is that the

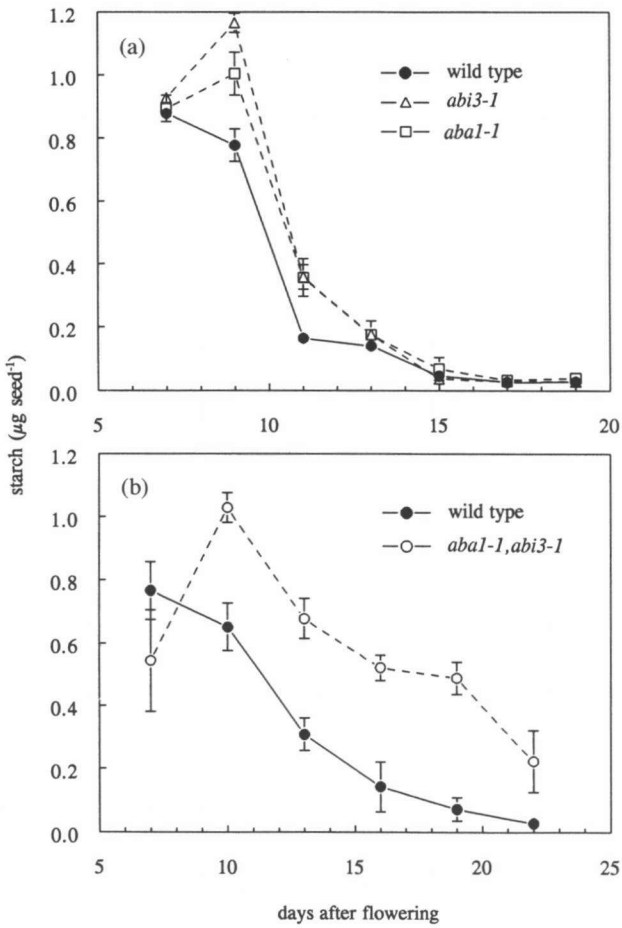

Fig. 6. Levels of starch during seed development in seeds of wild-type, *abi3-1* and *aba1-1* (A) or wild-type and *aba1-1*, *abi3-1* (B) *Arabidopsis* plants. Starch levels were calculated from amounts of glucose determined after hydrolysis of methanol-insoluble material. Data are mean ± SD of triplicate samples.

threshold level of ABA needed for this response is around the endogenous ABA level in *aba1-1* mutants (at least 20 times lower than in wild-type seeds, Karssen *et al.* 1983). The culture conditions of the plants may slightly have influenced the endogenous seed ABA level. We experienced a large variation between batches of plants, not only with respect to seed weight or accumulation of lipids, but also to the overall development and appearance of the plants; the plants used for the present experiments were of relatively good quality, had high fruit yield and good seed set, and usually seeds of the first initiated siliques were used. Perhaps these conditions contributed to low endogenous seed ABA levels, at least lower than the threshold level for an effect on elongation of fatty acids. The finding that similar effects of ABA on elongation were observed in *Brassica* microspore-derived embryoids which were apparently not ABA-deficient (Holbrook *et al.* 1992; Zou *et al.* 1995) can be explained by the observation that ABA levels in *in vitro* cultured embryos are generally much lower than in embryos that develop *in planta* (Finkelstein & Crouch 1986).

The double mutant *aba1-1, abi3-1* differed from the single mutants with respect to the total amount of storage lipids formed (Fig. 2). Also in other systems an effect of ABA on total neutral lipid accumulation was observed (Finkelstein & Somerville 1989; Dutta & Appelqvist 1989; Kim & Janick 1991; Holbrook *et al.* 1992). However, these reports all describe *in vitro* experiments in which ABA is routinely added to the cultures at the end of the culture period to prevent precocious germination, and the presence or absence of ABA may result in totally different developmental stages at the time of harvest for lipid determination. It is still difficult to conclude whether the changes observed in *in vitro* embryos are directly caused by ABA or probably due to differences in developmental stage.

Our results show that the effect of ABA on lipid accumulation also occurs during *in planta* development of seeds. One might argue that seeds of the *aba1-1, abi3-1* mutant also differ from wild-type and single-mutant seeds with respect to lack of desiccation tolerance and that those seeds will not accumulate storage material because they come into a premature vivipary-like germination mode, as concluded by Meurs *et al.* (1992). However, a large effect on lipid accumulation in the *aba1-1, abi3-1* mutant was already observed at 6 DAF (Fig. 2). Nambara *et al.* (1995) reported that the promoter of one of the genes related to germination (a chlorophyll a/b binding protein) was expressed at a higher level in the *abi3* mutant than in the wild type. However, it was not earlier than in the wild type and it did not appear before 8 DAF in seeds that were mature at 16 DAF. The genotypes used in the current study/our experiments germinate at 10–12 DAF when they are removed from the siliques (Koornneef *et al.* 1989). We conclude from this that it is unlikely that the observed early differences in lipid accumulation are due to a precociously entered germinative state. The results indicate that ABA either stimulates the synthesis or inhibits the breakdown of triacylglycerols.

The third difference between wild-type and mutant seeds was the relative amount of 18:1 (Table 1). The increase of 18:1 in the single mutants *abi3-1* and *aba1-1*, combined with the slight increase of 18:2, 18:3 and 16:0, most probably reflect a compensation for the diminished levels of 20:1. Analogous to the situation with other mutants deficient in the elongation of 18:1 (James & Dooner 1990; Lemieux *et al.* 1990; Kunst *et al.* 1992), the increase in 18:1 is the highest, whereas the desaturation pathway seems less favourable (Appleby *et al.* 1974). However, seeds of the double mutant *aba1-1, abi3-1* showed a markedly decreasing proportion of 18:1 towards the end of development (Table 1; De Bruijn *et al.* 1993), and more elevated proportions of 18:2 and 16:0 than in single-mutant seeds. One explanation for this phenomenon is that the fatty acid synthetase activity in this recombinant is decreased while the desaturase activity is not affected. This would explain both the reduced amount of neutral lipids and the relative large proportion of 18:2 compared to 18:1.

Arabidopsis mutants with reduced ABA levels or sensitivity are not only obstructed in the accumulation of long-chain fatty acids, but are also impaired in the synthesis of storage proteins (Finkelstein & Somerville 1990; Meurs *et al.* 1992).

The overall pattern of dry matter distribution in single-mutant seeds was not very different from that in wild-type seeds: amounts of total storage lipids (Fig. 2A), starch (Fig. 6A) and protein (Meurs *et al.* 1992) show only minor differences. However, double-mutant seeds contained only half the amount of lipid (Fig. 2B) and protein (Meurs *et al.* 1992) compared to wild-type seeds. The interesting question is whether those seeds have an alternative for storage of their reserves, since it is clear from Fig. 1 that seeds of both genotypes achieved the same maximum dry weight. Indeed, the lower

lipids levels, observed in the double-mutant seeds were accompanied by higher amounts of soluble carbohydrates (Fig. 4B) and starch (Fig. 6B), compared to wild-type seeds, and the decrease in starch during development was not only retarded but a considerable amount of starch was left in 'mature' seeds. Nevertheless, a rough calculation indicates that the lower lipid and protein content in the double mutant is not fully balanced by the increase in carbohydrates.

The transient peak in starch content of wild-type and single-mutant seeds agrees well with previous findings on other cruciferous seeds (Norton & Harris 1975; Romano *et al.* 1984; Fischer *et al.* 1988). In general, starch functions in those seeds as a temporary buffer of carbon, and in mature seeds hardly any starch is present (Siddiqui & Wood 1977; Thibault *et al.* 1989).

At present, no data are available on starch or carbohydrate levels in mutants that are disturbed in their fatty acid composition. However, studies on *Papaver* somatic embryos have revealed that exposure to abundant carbon leads to starch accumulation, whereas *Papaver* seeds are oil-seeds and normally do not contain starch (Hara *et al.* 1985).

Comparison of Figs 2, 4 and 6 gives some insight into the timing of the processes that are affected by ABA. Early during development, the accumulation of lipids in seeds of the *aba1-1, abi3-1* mutant was inhibited. The surplus of assimilates or metabolites is channelled to starch and this led to a peak in starch content at 10 DAF; during the following days starch breakdown was retarded and hexoses increased. At the end of development, a fraction of the *aba1-1, abi3-1* seeds precociously germinated; this caused a further degradation of starch and a sharp increase in sucrose.

In conclusion, the deficiency of ABA and the lack of responsiveness to ABA in seeds of the *aba1-1, abi3-1* mutant reduces the accumulation of storage lipids in favour of carbohydrates. Although substantial evidence has arisen that ABA does not affect the long-distance transport of assimilates to seeds (Fig.1; Schroeder 1984; Quarrie *et al.* 1988; De Bruijn and Vreugdenhil 1992, 1993), ABA may play a role in the distribution of these assimilates among the various types of reserve material within the seed.

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