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The Isolation of Abscisic Acid (ABA) Deficient Mutants by Selection of Induced Revertants in Non-germinating Gibberellin Sensitive Lines of *Arabidopsis thaliana* (L.) Heynh.

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Summary. By selecting for germinating seeds in the progeny of mutagen-treated non-germinating gibberellin responsive dwarf mutants of the *ga-1* locus in *Arabidopsis thaliana*, germinating lines (revertants) could be isolated. About half of the revertants were homozygous recessive for a gene (*aba*), which probably regulates the presence of abscisic acid (ABA). Arguments for the function of this gene were obtained from lines homozygous recessive for this locus only, obtained by selection from the F_2 progeny of revertant × wild-type crosses. These lines are characterized by a reduced seed dormancy, symptoms of withering, increased transpiration and a lowered ABA content in developing and ripe seeds and leaves.

Key words: *Arabidopsis thaliana* – Abscisic acid – Gibberellin – Physiological mutants – Seeds – Dormancy – Water relations

Abbreviations

ABA	Abscisic acid
GA_{4+7}	Mixture of gibberellin A_4 and A_7
EMS	Ethylmethanesulfonate
NG	Non-germinating
G	Germinating

Introduction

The role of endogenous regulating compounds in the physiology of plants has been studied mainly by correlative studies. The use of genotypes in which the level of one of these compounds is drastically changed adds an important additional tool to plant physiology. This genetic approach ideally requires the use of monogenic mutants or isogenic lines, for only with such single-gene contrasts is it clear that the observed physiological and biochemical differences are causally related.

The hormone abscisic acid (ABA) plays an important regulatory role in a number of physiological processes e.g. the dormancy and germination of seeds, the regulation of water stress, root geotropism and dormancy of buds (Walton 1980). Monogenic mutants with a disturbed ABA metabolism have been studied in tomato by Tal and coworkers (Tal and Nevo 1973). These mutants at the loci *sit*, *not* and *flc*, isolated by Stubbe (1957, 1958, 1959) are characterized by an excessive wilting tendency due to abnormal stomatal behaviour (Tal 1966), which could be reversed by the application of ABA (Imber and Tal 1970). A relation between disturbed ABA metabolism and germination was described in maize by McDaniel et al. (1977) and Smith et al. (1978). They found that precocious germination of viviparous (*vp*) mutants (Robertson 1955) could be attributed to the absence of ABA or to the incapacity to respond to ABA.

In *Arabidopsis* monogenic recessive mutants have been described by the present authors in which germination was reduced or absent under conditions that were suitable for germination of wild-type seeds (Koornneef et al. 1977; Koornneef 1978; Koornneef and van der Veen 1980).

One group of non-germinating mutants, which could be brought to 100% germination by the application of gibberellins (GA_{4+7}, GA_3 and GA_9 were effective), subsequently developed into dwarfs. These dwarfs could be reverted fully or almost fully, to wild-type by GA sprays (Koornneef and van der Veen 1980). These mutants, found in *Arabidopsis* at three loci (*ga-1, ga-2* and *ga-3*) probably lack the capacity to synthesize gibberellins. Strictly comparable mutants have been found in tomato, at only two loci so far (Koornneef et al. 1981; van der Veen and Bosma pers. comm.).

Application of gibberellins stimulates the germination of seeds of many plant species (Jones and Stoddart 1977). Seed germination requires in most cases de novo synthesis or activation of these substances. Besides such promoters, also inhibitors like abscisic acid (ABA) may affect the state of dormancy of a seed.

This concept led to the idea that the germination capacity of non-germinating dwarfs might be restored when the level of inhibitors would be reduced by mutating the genes responsible for their production. Selection

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for this type of revertants is easy since they are self-detecting: germinating seeds among non-germinating seeds. Apart from mutations in genes regulating the production of inhibitors, also other types of external suppressor mutations may be found as well as intragenic reversions. This paper describes the isolation of ABA deficient mutants in *Arabidopsis* by using this revertant technique. For preliminary reports on this subject see Karssen et al. (1980) and Koornneef et al. (1980).

Material and Methods

Plant Material

Arabidopsis thaliana (L.) Heynh. (2 n = 10) is a small fast growing, self-fertilizing crucifer. Seed stocks used in the present experiments were derived from the pure line Landsberg *erecta* (Redei 1962), which will be referred to as the wild-type. For revertant induction we used the non-germinating, gibberellin-sensitive mutant lines NG5 (EMS induced) and 6.59 (fast-neutron induced). Both lines are mutants induced in the wild-type at the *ga-1* locus on chromosome 4 (Koornneef and van der Veen 1980). To check for seed admixture and unwanted cross-fertilization, both lines were also homozygous recessive for *gl-1* (hairless; chrom. 3), except in the first revertant-induction experiment (see later).

Conditions of Culture

The seeds were sown equally spaced in numbers of 25, 30, or 36 in 9 cm petri dishes on perlite saturated with a standard mineral solution, as described by Oostindiër-Braaksma and Feenstra (1973). The seeds were incubated at 4–6 °C for 4–6 days to break dormancy, and subsequently allowed to germinate at a temperature of approx. 24 °C under continuous light (Philips TL 57) at an intensity of 8 W · m². After 8 days at 24 °C, the seedlings were transplanted into soil and cultivated in an air-conditioned greenhouse, where additional white fluorescent light (Philips TL 57) was given during 24 h per day in the winter (October to April).

Germination Tests

Germination tests were performed in plastic petri dishes (Ø 8.5 cm) on two layers of filter paper (Ederol no. 261) saturated with 2 ml of sterile distilled water. To avoid rapid evaporation, each dish was wrapped in a small polythene bag. Temperature and light conditions were as described above for seeds sown on perlite. Germination was scored 7 days after the start of incubation at 24 °C.

The Induction and Isolation of Revertants

To induce revertants, seeds of NG5 and 6.59 were first redried after dormancy breaking on moist filter paper and then treated with 10 mM ethylmethanesulfonate for 24 hrs at 24 °C in dark (Koornneef et al. 1982). After rinsing with tap water, the seeds were immediately sown in petri dishes containing 10 μM GA_{4+7} in the standard mineral medium and transferred to light as described above. The resulting M_1 seedlings developed into the usual NG5 and 6.59 dark-green, bushy dwarfs. At 4 and 5 weeks after EMS treatment the dwarfs were sprayed with 100 μM GA_{4+7} to stimulate anther development and to provide sufficient seed set from selfing. M_1 plants were individually harvested and the M_2 progenies were sown separately at standard conditions. Screening for germinating seeds (the presumed revertants) was done 8 days after the start of incubation. The seedlings obtained were transplanted into soil. The ultimate selection for revertants was based on the germination behaviour of the M_3 lines.

Genetic Characterization

For genetic analysis the revertants were crossed with wild-type and the parental *ga-1* mutant. Revertant types were scored on their capacity to germinate without GA and on the leaf colour which is slightly different from the darker green colour of non-germinating GA responsive *ga-1* mutants. Presumed ABA-types (recombinant, single recessive to wild-type) were scored on several morphological features (described in detail later in the paper). Allelism versus non-allelism was tested on the basis of non-complementation versus complementation to parental *ga-1* mutant type in F_1's of revertant × revertant or to wild-type in revertant × ABA-type crosses.

Localization on chromosomes was done by trisomic analysis (Koornneef and van der Veen 1978). The position of the *aba* locus on the chromosome was determined by linkage analysis of F_2 populations. The recombination fractions were estimated by the Product Ratio Method using the tables of Stevens (1939).

Measurement of Water Loss in Intact and Cut-off Plants

To determine water loss plants were grown for 2 weeks in plastic pots in the greenhouse and were then transferred to a climate chamber (temperature 22 °C; relative humidity approx. 85%, 12 h fluorescent light at approx. 13 W · m², 12 h dark). From the day of transfer onwards, half of the plants were sprayed with 10 μM ABA every second day, the other half with water. After 8 days in the climate chamber the plastic pots, including the soil surface, were wrapped in aluminium foil to prevent evaporation from the soil surface. Water loss during the third light and dark period after wrapping the pots, was determined by weighing the plants with the pots at the change of dark and light. The leaf surface was measured with an area meter. To measure water loss in cut-off aerial parts, well-watered plants were transferred from the climate room to a laboratory room and kept at 22 °C in white fluorescent light (0.6 W · m²). After an acclimatization period of 1½ h the aerial parts were cut off from the roots and stored in 400 ml glass beakers. Fresh weight was determined every hour.

Determination of ABA Content

Quantitative determinations of endogenous ABA were performed according to techniques described by Knegt et al. (1981) with a few additions. During purification of the extracts of seeds and siliquae the extraction into K_2HPO_4 with subsequent acidification to a final volume of 10 ml diethyl ether was performed three times instead of once. After standard purification the extracts of the leaves were purified additionally by using high pressure liquid chromatography with a re-

verse phase RP8 column operated with a linear gradient from 25% to 75% methanol in water. The solvents were 0.1 M towards acetic acid. ABA and trans-ABA are partially separated in this system, but collected in one fraction. The gas chromatograph was operated with He as carrier gas and 5% CH_4 with Ar as make up gas.

Results

A) Isolation of Revertants and Genetic Characterization

The Isolation of Revertants

From the individual M_2 progenies of 2122 EMS-treated seeds of *ga-1* mutants NG5 and 6.59 (M_2 lines), 31 lines could be isolated that showed 50% (up to 100%) germination under standard germination conditions (Table 1). It appeared that 15 of these lines were germinating, GA-responsive extreme dwarfs with a somewhat weaker appearance and a slightly yellow-brown colour on the leaves compared to the parental *ga-1* mutants. For reasons that will be described in the next sections, this type will be called ABA revertant.

Other isolated revertants were mostly slightly taller and/or had a paler green colour than NG5 and 6.59. The two revertants dominant to the parental lines reached a length of respectively 75% and 50% of the wild-type. These two groups will not be further considered here.

The selection of revertants in NG5 was hampered by the partial germination of this mutant as has been described before (cf. Koornneef 1979; Koornneef and van der Veen 1980), probably due to leakiness of this allele. This led to the decision to transplant only the most conspicuous plants from M_2 lines in which germination occurred as these could be expected to be revertants. Further only the progeny of M_2 plants, which showed some deviating features compared to the parental line, were tested as M_3 lines. Only in the first experiment were M_3 lines from each M_2 line with germinating seeds tested. However, it appeared that all lines ultimately selected as revertants on the basis of a high germination percentage in M_3 had a somewhat deviating morphology.

Genetic Characterization of the Revertants

In crosses between the revertants and the parental *ga-1* mutants, the capacity of the revertant to germinate was found to be monogenic recessive to the inability to germinate of the *ga-1* mutants.

Complementation tests with 14 ABA-revertants revealed that 13 of these independently isolated revertants were allelic. The presence of a second locus needs further confirmation.

In F_2's from revertant × wild-type crosses one expects on the basis of two unlinked loci for germination the ratio 13 germ.: 3 non-germ., and when also taking plant phenotype into account, the ratio 9 wild-type: 3 non-germinating GA dwarf: 3 new (recombinant) phenotype: 1 germinating GA dwarf (revertant). Indeed, a deviating non-dwarf phenotype was observed in all F_2's, which when compared to the wild-type had a reduced vitality (smaller, weaker plant), a slightly yellow-brownish colour, and symptoms of withering, mainly in the influorescence (Fig. 1). The withering symptoms were more pronounced in winter than in summer. These symptoms point to ABA deficiency (see also below). Therefore the new recombinant phenotype was called ABA-type and the mutant allele *aba*. By selfing ABA-type F_2 plants, F_3 lines could be established not segregating for revertant types (expected one among three F_3 lines). Lines homozygous for ABA-type were crossed with a parental *ga-1* mutant to give F_2 and F_3. The segregation of these populations was analysed together with those derived from revertant × wild-type crosses. It should be noted that aba^1 was induced in $ga\text{-}1^1$ (line NG5) and aba^2 in $ga\text{-}1^2$ (line 6.59) background. In the *ga-1* mutant × ABA-type crosses the allelic combinations were interchanged, so all combinations were represented. Since χ^2-test of heterogeneity between the four crosses did not reveal significant dif-

Table 1. Frequencies of independently induced revertants

Experiment	Parental mutant	Number of M_2 progenies tested	Number of revertant lines			Total
			ABA-revertants	Dominant	Others	
I	NG5 ($ga\text{-}1^1/ga\text{-}1^1$)	199	4	0	0	4
II	NG5 ($ga\text{-}1^1/ga\text{-}1^1$)	382	4	1	3	8
II	6.59 ($ga\text{-}1^2/ga\text{-}1^2$)	246	1	0	1	2
III	NG5 ($ga\text{-}1^1/ga\text{-}1^1$)	771	2	0	8	10
III	6.59 ($ga\text{-}1^2/ga\text{-}1^2$)	524	4	1	2	7
		2122	15	2	14	31

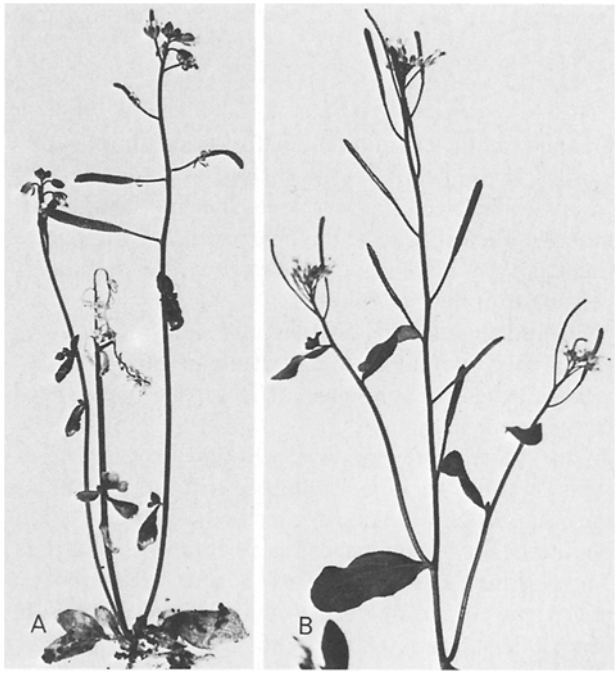

Fig. 1 A and B. A BA-type A26 (**A**) showing a withered main stem and withered siliquae compared with wild-type (**B**)

ferences (except one case, see below), the segregation data of the four crosses listed below could be pooled (Table 2).

Crosses in association; (revertant × wild-type):

A) *ga-1¹/ga-1¹, aba¹/aba¹* × +/+, +/+

B) *ga-1²/ga-1², aba²/aba²* × +/+, +/+

Crosses in dispersion; (ga-1 mutant × ABA-type):

C) *ga-1¹/ga-1¹*, +/+ × +/+, *aba²/aba²*

D) *ga-1²/ga-1²*, +/+ × +/+, *aba¹/aba¹*

These pooled data (Table 2) consistently confirm the segregation at two unlinked loci (*ga-1* and *aba*), in particular the fact that germinating GA dwarfs (revertants) only segregated in F_3 lines when expected.

In F_3 lines from wild-type F_2 plants a significant recessive deficit at the *ga-1* locus was found. With induced mutants a recessive deficit of this magnitude is by no means uncommon and can be ascribed to certation (cf. Koornneef and van der Veen 1980; Koornneef et al. 1982). Since the degree of certation is variable, the single case of significant heterogeneity between crosses can be explained in this way. In addition, the recessive deficit at the *ga-1* locus may also be generated by a reduced survival of non-germinating genotypes when these are induced to germinate by 10 μM GA_{4+7} after 8 days of incubation at 24 °C. This may be partly due to secondary dormancy induced during that period.

With regard to the phenotypes described in the top lines of Table 2 two different aspects have to be considered: 1. Plant phenotype: *aba/aba* leads with a wild-type genetic background (with respect to the *ga-1* locus) to ABA-type plants (slightly yellow-brown colour, withering), whereas with a *ga-1* background (GA dwarf) it leads to GA-responsive dwarfs with a more yellow-brown colour than normal GA dwarfs. 2. Germination: in the presence of *ga-1/ga-1*, which as such leads to a lack of germination, *aba/aba* restores the germination

Table 2. Segregation ratio's in F_2 and F_3 progenies from pooled crosses A, B, C and D (for explanation see text)

		Wild-type G	GA dwarf NG	ABA-type G	GA dwarf (revertant type) G+NG		χ^2_1 (3:1) *ga–1* locus	χ^2_1 (3:1) *aba* locus	χ^2_1 linkage ("2×2 Table")
		+/. +/.	*ga–1/ga–1* +/.	+/. *aba/aba*	*ga–1/ga–1 aba/aba*				
F_2 generation		555	149	179	56	6[a]	3.6	0.1	2.2
F_3 generation									
F_2 plant wild-type:									
Segregating-digenic	(52)[b]	920	203	284	60		39.1**	1.9	0.1
Segregating NG GA dwarf	(22)	507	122	–	–		10.5**	–	–
Segregating ABA-types	(27)	589	–	167	–		–	3.4	–
Non-segregating	(13)	357	–	–	–	–		–	–
F_2 plant NG GA dwarf:									
Segregating "revertants"	(29)	–	554[c]		175[c]		–	0.4	–
Non-segregating	(10)	–	253[c]		–		–	–	–
F_2 plant ABA type:									
Segregating "revertants"	(29)	–	–	509	133	33[a]	0.2	–	–
Non-segregating	(9)	–	–	213	–		–	–	–
F_2 plant reverant type	(18)	–	–	–	240	59[a]	–	–	–

[a] NG GA dwarfs of the revertant type occurred almost exclusively in the progeny of cross B (see text)

[b] In brackets: Numbers of F_2 plants selfed. These F_2 plants were randomly sampled within each of the four phenotypic classes, but not between classes. Note the good fit within classes (4:2:2:1 and 2:1)

[c] Tested by germination behaviour only

** $P < 0.01$

capacity. This seems to imply that GA is only required for germination if ABA is present.

In a few cases seeds that did not germinate on water, gave, upon addition of GA_{4+7}, dwarfs which showed the leaf colour characteristics of the revertant type. Therefore it is concluded that in these cases the germination capacity in *ga-1/ ga-1, aba/aba* was only partly restored. Such incomplete restoration occurred almost exclusively in cross B (alleles *ga-1*^{2} and *aba*^{2}) and was absent in cross C (alleles *ga-1*^{1} and *aba*^{2}) and in crosses A and D (both *aba*^{1} allele). As a check both G and NG revertant types from cross B were selfed. The seeds obtained gave equal germination percentages, viz. $32.0 \pm 5.4\%$ for the G-parents and $31.1 \pm 3.8\%$ for the NG-parents.

A plausible explanation can be found on the basis of the concept of GA-ABA balance in germination. The *ga-1*^{2} mutant never germinates – this is in contrast to the *ga-1*^{1} mutant which sometimes germinates to some extent. To make the "deeply GA-deficient" *ga-1*^{2} mutant germinate a "strong" (i.e. deeply ABA-deficient) *aba* mutation is necessary. Here *aba*^{2} is not strong enough, whilst *aba*^{1} is.

As mentioned before, the mutant alleles *aba*^{1} and *aba*^{2} were initially induced in *ga-1*^{1}/*ga-1*^{1} (line NG5) and *ga-1*^{2}/*ga-1*^{2} (line 6.59) background respectively. From the crosses C (alleles *ga-1*^{1} and aba^2)and D (alleles *ga-1*^{2} and *aba*^{1}) it follows that a in qualitative sense the germination-restoring effect of *aba* mutants does not depend on the allele at the *ga-1* locus: no allele specificity. Nor does the effect of *aba* alleles seem to be locus-specific with respect to the *ga* loci. This follows from a cross, revertant × *ga-2* mutant:
(*ga-1/ga-1*, +/+, *aba/aba* × +/+, *ga-2/ga-2*, +/+), where germinating dwarfs (*aba/aba*) could be selected which were homozygous recessive at the *ga-2* locus (and wild-type at the *ga-1* locus), as determined by means of test crosses.

In general it appears that *aba* alleles improve the germination of genotypes with a reduced germination.

The *aba* gene could be located on chromosome 5 by trisomic analysis. As said before, *ga-1* was located on chromosome 4, which confirms that the two loci are unlinked. Linkage of *aba* with specific chromosome-5 markers was studied in F_2 populations. The markers involved were *ms* (male-sterile), *tt-2* (transparent testa), *tt-3* (transparent testa, anthocyaninless) and *tz* (thiazole-requiring). The results are summarized in Fig. 2 and show that *aba* is located at the end of chromosome 5, as far as this chromosome has been mapped.

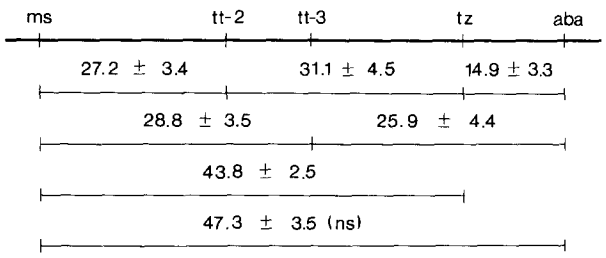

Fig. 2. Provisional linkage map of chromosome 5 and the estimates of recombination percentage between some markers including *aba*. n.s. no significant linkage

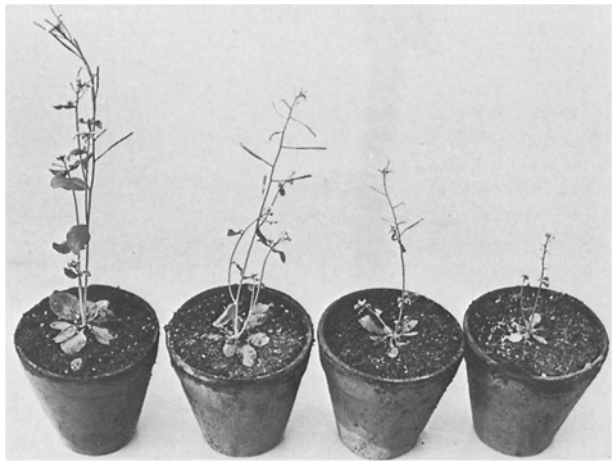

Fig. 3. Adult plants of wild-type and the independently arisen mutants G4 (*aba*^{3}), A26 (*aba*^{1}) and A73 (*aba*^{4})

B) Physiological Characterization

It has been shown in the previous sections that a specific gene (*aba*) with a specific phenotypic expression is able to remove the lack of germination in *ga-1* mutants.

Some phenotypic features of a number of lines the ABA-type (+/+, *aba/aba*) are presented in Table 3 and Fig. 3. All the characteristics show an increasing tendency to deviate from the wild-type in the allelic order *aba*^{3}/*aba*^{3} (G4), *aba*^{1}/*aba*^{1} (A26), *aba*^{4}/*aba*^{4} (A73). The perfect rank correlation between all five parameters implies multiple pleiotropism with specific degrees of expression of the different alleles.

The physiological characterization of the ABA-type was focussed on: 1. The germination behaviour of seeds, because the selection of this genotype was based on this property. 2. water relations of the plant in view of the observation of withering. 3. ABA content, since both previous aspects are related to this compound (Walton 1980).

Germination Behaviour

A comparison of the germination behaviour of seeds from ABA-types (*aba/aba*) with seeds from wild-type

Table 3. Some characteristics of lines of ABA-type as compared with wild-type

	+ / + (wild-type)	*aba*^{3}/*aba*^{3} (G4)	*aba*^{1}/*aba*^{1} (A26)	*aba*^{4}/*aba*^{4} (A73)
Percentage survival after planting	99 ±1[a]	97 ±2[a]	69 ±5[b]	82 ±5[b]
Percentage plants with withered main stem	0	11 ±4[b]	56 ±7[c]	69 ±6[c]
Total plant length (cm)	20.8±0.3[a]	14.8±0.2[b]	8.2±0.3[c]	6.4±0.3[d]
Length of largest rosette leaf (cm)	2.6±0.1[a]	2.1±0.1[b]	1.4±0.1[c]	1.2±0.1[d]
Number of side shoots	4.9±0.1[a]	3.2±0.1[b]	2.7±0.2[c]	2.0±0.1[d]

A different letter indicates a significant difference ($P < 0.05$)

(+ / +), harvested the same day and identically stored, showed that seeds of the ABA-type are characterized by a strong reduction of seed dormancy, as judged in line A26 (*aba*^{1}/*aba*^{1}) from a reduced requirement for light and cold treatment (Fig. 4). This has been found as well for other *aba* alleles.

The germination of both ABA and wild-type could be completely inhibited by exogenous applied ABA (Fig. 5). The response of the ABA-type is only slightly less than that of the wild-type.

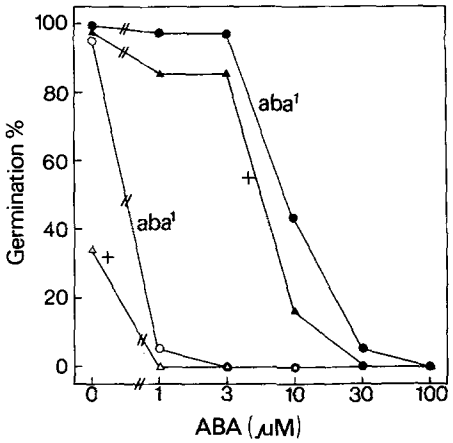

Fig. 5. Germination in white light of wild-type (+) and A26 (*aba*^{1}) at different concentrations ABA, scored 3 days (open symbols) and 7 days (closed symbols) after incubation

Water Relations

When the plants in the greenhouse were either enclosed in plastic bags, which maintained a high humidity, or were sprayed twice a week with an ABA solution (Table 4), the development of symptoms of withering on ABA-type plants was highly reduced. Plants of ABA-type (*aba*^{3}/*aba*^{3}) grown in a climate room showed an enhanced water loss, which could be considerably reduced by ABA sprays (Table 5).

The enhanced rate of water loss in isolated aerial parts of ABA-types (Fig. 6) can be interpretated as a reduced rate of stomata closure upon water stress caused by the absence of water supply from the roots. This may be caused by a reduced availability of ABA.

Endogenous ABA Content

The level of endogenous ABA was determined in dry, ripe seeds and in siliquae with seeds during de-

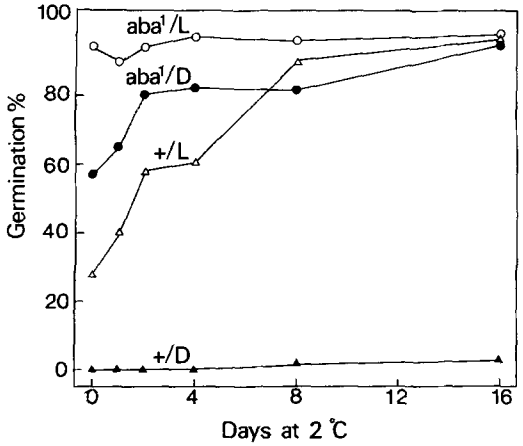

Fig. 4. Germination percentage of ABA type and wild-type in white light (L) and darkness (D) preceeded by different periods of dark-incubation at 2 °C. The seeds were used 4 weeks after harvest

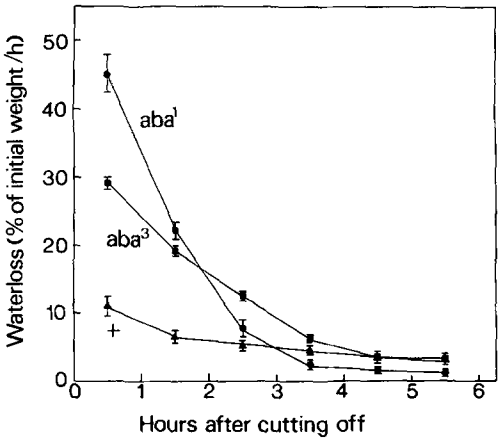

Fig. 6. Water loss by the aerial parts of ABA types and wild-type expressed per hour as percentage of fresh weight at the time of cutting

Table 4. The effect of spraying with ABA and of enclosing the plants in plastic on the percentage of plants with symptoms of withering

	Treatment	Normal	Withered tips of siliquae	Withered siliquae	Withered main stem	
+/+ (wild-type)	Sprayed H_2O	100 (48)	–	–	–	
	1 μM ABA	100 (46)	–	–	–	
	10 μM ABA	100 (48)	–	–	–	
	Enclosed in plastic	100 (25)	–	–	–	
aba^3/*aba*^{3} (G4)	Sprayed H_2O	5 (2)	21 (9)	65 (28)	9 (4)	a
	1 μM ABA	16 (7)	11 (5)	71 (31)	2 (1)	a
	10 μM ABA	83 (38)	11 (5)	7 (3)	–	b
	Enclosed in plastic	83 (35)	–	13 (7)	–	b
aba^1/*aba*^{1} (A26)	Sprayed H_2O	–	8 (3)	22 (8)	70 (26)	a
	1 μM ABA	22 (8)	19 (7)	17 (6)	45 (15)	b
	10 μM ABA	64 (28)	14 (6)	11 (5)	11 (5)	c
	Enclosed in plastic	65 (20)	3 (1)	26 (8)	6 (2)	c

In brackets: the number of plants observed
Within genotypes: a different letter (a, b, c) indicates a significant difference between treatments when testing (χ^2) the normals versus non-normals (withered tips of siliquae, siliquae, and main stem)

Table 5. The effect of the *aba* gene, of ABA sprays and of light on water loss (kg/m^2/h) of *Arabidopsis* plants grown in a climate chamber (plants were cultivated in a 12 h photoperiod or in darkness and sprayed twice a week with water or 10 μM ABA)

	Light period		Darkness	
	H_2O	10 μM ABA	H_2O	10 μM ABA
+/+ (wild-type)	2.5±0.1	1.9±0.1	1.2±0.1	1.0±0.1
aba^3/*aba*^{3} (G4)	6.0±0.5	2.6±0.2	3.2±0.3	0.9±0.1

A 2 × 2 × 2 factorial analysis of variance showed that the main effects, genotype, light and ABA, were highly significant ($P < 0.01$). The three interactions were also significant

velopment (Table 6) and in rosette leaves (Table 7). The ABA content in seeds, in particular during seed development, was found to be much lower in G4 (*aba*^{3}/*aba*^{3}) and reduced below the level of detection in ripe seeds of A26 (*aba*^{1}/*aba*^{1}) and A73 (*aba*^{4}/*aba*^{4}). Rosette leaves contained very low amounts of ABA (Table 7), nevertheless the same rank order wild-type > G4 > A26 appears. In the same order the phenotype of the different alleles was found to deviate from wild-type for a number of characteristics (Table 3).

It appears that ABA-types are deficient in ABA content during various stages of their development, which very probably explains the symptoms of ABA deficiency observed.

Table 6. Endogenous ABA content of ripe seeds and of developing siliquae with seeds. The ripe seeds were extracted within 1 month of harvest, the developing seeds were extracted 10 days after pollination

	Ripe seeds harvested in:				Siliquae with developing seeds harvested in:	
	April 1979		October 1979		January 1980	
	ng/g fresh weight	pg/seed[a]	ng/g fresh weight	pg/seed[a]	ng/g fresh weight	pg/seed[b]
+/+ (wild-type)	71	1.42	27	0.54	117	10.5
aba^3/*aba*^{3} (G4)	8	0.16	7	0.14	12	1.0
aba^1/*aba*^{1} (A26)	nt	nt	< 1	< 0.02	nt	nt
aba^4/*aba*^{4} (A73)	nt	nt	< 1	< 0.02	nt	nt

nt = not tested
[a] seed weight 20 μg/seed
[b] 93% or more of total ABA in siliquae is present in the seeds (Karssen et al., in preparation)

Table 7. Endogenous ABA content of greenhouse grown rosette leaves of four-week-old wild-type and ABA-type

	ABA content (ng/g)
+/+ (wild-type)	6 (8; 3)
aba^3/*aba*^{3} (G4)	2 (2; 2)
aba^1/*aba*^{1} (A26)	1 (2; 0)

In brackets: values of the two replications

Discussion

By means of selection for revertants of non-germinating mutants, interesting physiological mutants with a disturbed ABA metabolism could be isolated. The isolation procedure for ABA-deficient mutants via revertants has the disadvantage of indirectness and inefficiency as it may take three extra generations. However, the short life cycle of *Arabidopsis*, enabled us to remove the parental *ga-1* allele relatively quickly. An alternative would be direct selection for the ABA-type in segregating M_2 populations derived from mutagen-treated wild-type. Here the rather inconspicuous phenotype will be a problem, especially in summer, when the symptoms of withering are almost absent in the greenhouse. Moreover, plants with a slightly deviating colour and a weaker growth occur frequently in M_2 populations of *Arabidopsis*. Selection for non-dormant seeds by sowing these M_2 seeds immediately after harvest may be a more attractive direct selection method. However, the rapid loss of dormancy when the seeds are stored after harvest, also occurring in wild-type, and the large environmental and maternal effects on dormancy may complicate this procedure.

Selection of ABA resistance, which is an attractive procedure especially in cell cultures (Wong and Sussex 1980), probably will not be very efficient either, as the differences between the dose-response curves of mutants and wild-type are relatively small.

The rather unaffected sensitivity of the ABA-type to exogenously applied ABA (Fig. 5) seems to exclude that the ABA-receptor sites are affected in the mutant. Very probably the *aba* gene regulates the biosynthesis of endogenous ABA at all stages of the development of the *Arabidopsis* plant. It cannot be entirely excluded however that the *aba* gene, when homozygous recessive, enhances ABA degradation.

The *aba* mutant may have a similar biochemical background as the *flc, not* and *sit* mutants in tomato (Tal and Nevo 1973; Nevo and Tal 1973), and as the background of some of the *vp* loci in maize (Smith et al. 1978). The ABA mutants in *Arabidopsis* seem unique as they combine the characteristics of ABA-deficient mutants in both the tomato (enhanced transpiration) and the maize (reduced seed dormancy). However, it may very well be possible that no attention has been paid to seed germination in tomato nor to the water relations in maize. An indication for a reduced seed dormancy of the tomato mutants comes from the observation of precocious germination in ripe fruits of the *sit* mutant (Koornneef unpublished).

Compared to maize, where probably five loci (Smith et al. 1978), and tomato where three loci, are known to affect the level of endogenous ABA, in *Arabidopsis* only one and perhaps two loci have been identified so far among 14 independently induced mutants. A simple explanation might, apart from the limited scale of the present mutation-induction experiments, be the fact that our *aba* locus has a relatively high mutation frequency (cf. Koornneef et al. 1981a). Finally, it has to be realized that many ABA-deficient mutants may be lethal because they might as well be deficient in carotenoïds as has been found in maize (Robertson 1955; Smith et al. 1978). All these substances have mevalonic acid as a common precursor. It may be significant that a viviparous mutant of sunflower was also characterized by a reduced pigment content (Wallace and Habermann 1958). It is quite possible that this mutant and other viviparous mutants described in other plant species. e.g. barley (Gustafsson et al. 1969), prove to be deficient in or in sensitive to ABA.

In addition to studies with mutants as cited above, differences in ABA content have also be observed between varieties of cultivated species. Largué-Saavedra and Wain (1974, 1976) found that ABA content was higher in both wilted and non-wilted leaves of drought-resistant cultivars of maize and sorghum as compared to less resistant ones. A correlation between reduced ABA content and short dormancy and low sprouting resistance was found for two barley varieties (Goldbach and Michael 1976). Compared to the drastic effects observed in deficient mutants, the minor and more specific physiological differences between varieties may reflect rather a specific genetic regulation of ABA metabolism than an effect on biosynthesis as is probably the case in mutants. Likewise it is not clear whether relatively small differences in ABA content between different genotypes, as found by Lee and Looney (1977) and Yadava and Lockard (1977) between compact and normal apple types, are a primary effect of mutations in genes directly involved in the regulation of ABA biosynthesis.

The fact that the absence of seed dormancy of ABA-deficient mutants segregates as a single recessive gene in the progeny of heterozygous plants (Table 2), shows that dormancy in ripe seeds related to ABA metabolism is mainly determined by the genotype of the embryo and not by the genotype of the mother plant including the seed coat. The genetic control and the role of ABA in relation to seed dormancy will be described in detail in a later paper.

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