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Arabidopsis Mutants with a Reduced Seed Dormancy[1]

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The development of seed dormancy is an aspect of seed maturation, the last stage of seed development. To isolate mutants of *Arabidopsis thaliana* that are affected in this process, we selected directly for the absence of dormancy among freshly harvested M_2 seeds. The screen yielded two mutants exhibiting a reduced dormancy, *rdo1* and *rdo2*, that are specifically affected in dormancy determined by the embryo. The *rdo1* and *rdo2* mutants show normal levels of abscisic acid and the same sensitivity to abscisic acid, ethylene, auxin, and cytokinin as the wild type. The *rdo2* mutant but not the *rdo1* mutant has a reduced sensitivity to the gibberellin biosynthesis inhibitor tetcyclacis. Double-mutant analysis suggested that the *RDO1* and *RDO2* genes are involved in separate pathways leading to the development of dormancy. We assume that the *RDO2* gene controls a step in the induction of dormancy that is most likely induced by abscisic acid and is expressed as an increase of the gibberellin requirement for germination.

Many plant species produce seeds that are dormant when they are released from the plant. Seed dormancy has been defined as a temporary failure of a viable seed to germinate in conditions that favor germination when the restrictive state (i.e. dormancy) has come to an end (Simpson, 1990). Dormancy is relieved by a cold treatment of seeds allowed to imbibe and during dry storage of the seeds, often referred to as after-ripening. It can be reinduced (secondary dormancy) when the conditions do not meet all requirements for germination (Karssen, 1982). The cycling of dormancy, leading to seedling emergence in specific periods of the year, is thought to reflect the seeds' responsiveness to the environmental factors temperature, light, and nitrate (Hilhorst et al., 1995).

The small crucifer *Arabidopsis thaliana* exhibits seed dormancy, allowing the seeds in the natural situation to survive the dry summer period and germinate in autumn (Baskin and Baskin, 1972). The many environmental and genetic factors that determine seed dormancy in Arabidopsis have been reviewed by Koornneef and Karssen (1994). Dormancy differences between seed lots can be "measured" by comparing the germination percentage of these seed lots after a specified period of dry storage.

The conditions during seed development and the duration and conditions of dry storage after harvest influence the degree of dormancy, which is expressed in the germination behavior of the seeds. Therefore, these parameters and the test conditions have to be identical when genotypes are compared. Despite these precautions, seeds from different plants of a homozygous genotype may show larger differences than statistically expected.

The nature and variability of seed dormancy determine that this trait behaves genetically more as a quantitative trait than as a qualitative trait. However, several Arabidopsis mutants have been described that have a reduced dormancy. One group of these mutants is affected in the testa structure or testa color. It is hypothesized that these mutations allow an easier penetration of the radicle through the testa because this structure is a mechanical barrier (Léon-Kloosterziel et al., 1994). In addition, a number of mutants have been described in which reduced dormancy is due to the embryo genotype. Among these are the mutants at the *ABI3* locus (Koornneef et al., 1984; Nambara et al., 1992; Ooms et al., 1993), the *LEC1* locus (Meinke, 1992; Meinke et al., 1994), and the *FUS3* locus (Bäumlein et al., 1994; Keith et al., 1994). Mutants at these loci affect many aspects of the seed maturation process, during which the seed prepares for survival by the induction of dormancy, the accumulation of storage material, and the acquisition of desiccation tolerance.

Mutants deficient in the hormone ABA are among the most nondormant mutants in Arabidopsis and in other plant species as well (Koornneef, 1986). ABA-deficient mutants of Arabidopsis were selected on the basis of their lack of a GA requirement for seed germination, by the isolation of germinating revertants of nongerminating GA-deficient mutants (Koornneef et al., 1982). Mutants that do not need GA for germination have also been selected on the basis of their resistance to GA biosynthesis inhibitors such as paclobutrazol (Jacobsen and Olszewski, 1993; K.M. Léon-Kloosterziel, unpublished data) and unicazol (Nambara et al., 1991). This resulted in the isolation of additional *aba* and *abi3* mutants and of the *spy* mutant, which is hypersensitive to GAs. However, since the seed maturation mutant *fus3*, in contrast to *abi3*, is not resistant to such GA inhibitors (Keith et al., 1994), a lack of seed dormancy cannot be equated with resistance to GA biosynthesis inhibitors.

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Abbreviations: *Ler*, Landsberg *erecta*; NAA, naphthalene acetic acid.

A direct screen for reduced seed dormancy mutants was applied to identify mutants at other loci that play a role in this process. In this paper, we present the genetic and physiological analysis of two new mutants. Since plant hormones have been reported to be involved in seed dormancy and germination (Karssen et al., 1989), attention has been paid to the effects of hormones.

MATERIALS AND METHODS

Mutant Isolation

M_1 seeds of *Arabidopsis thaliana* (L.) Heyhn, ecotype L*er* were soaked in tubes containing 0.2% agar for 20 h at 4°C, mutagenized with 200 or 300 Gy of γ-irradiation (^{60}Co source), and dispersed on soil in an air-conditioned greenhouse (18–23°C) with additional light during winter (16-h photoperiod; HP1-T/400 W, Philips, Eindhoven, The Netherlands). M_2 seeds were harvested from groups of approximately 500 M_1 plants and sown on water-saturated filter paper (Schleicher & Schuell No. 595) in Petri dishes on the day of harvest. The dishes were incubated in a growth room (25°C, 16-h photoperiod, Philips TL57). Seeds that had germinated within 3 d after imbibition were selected as putative mutants. Freshly harvested wild-type seeds did not germinate under these conditions (less than 1%). The mutant phenotype was confirmed by retesting the germination of the seeds harvested from plants grown from these selected seedlings.

Genetic Analysis

The mutant plants were crossed with the wild type. Nondormant F_3 lines derived from this cross were used for reciprocal crosses and physiological analyses. The nondormant mutants were also crossed among themselves and with *abi2–1*, *abi3–1* (Koornneef et al., 1984), *abi3–5* (Ooms et al., 1993), and *aba-1* (Koornneef et al., 1982) mutants to perform complementation tests and to construct double mutants. Putative double mutants were selected on the basis of ABA insensitivity (*abi*), green seeds (*abi3–5*), and wilty plants (*aba*). Subsequently, double mutants were selected by reciprocal test crossing with each of the nondormant parents, followed by determination of the F_1 seed germination.

Germination Assays

All genotypes were grown together, and mature seeds were harvested at the same time from dehydrated siliques. Batches of 50 to 80 seeds were sown in triplicate in Petri dishes containing water-soaked filter paper (Schleicher & Schuell No. 595) and incubated in a growth room (25°C, 16-h photoperiod). Seeds sown on solutions of ABA (Sigma) or tetcyclacis (BASF, Limburgerhof, Germany) in water were incubated for 3 d at 4°C prior to transfer to the growth room. Germination was scored after 7 d of incubation at 25°C.

Determination of Sensitivity to Ethylene, Auxin, and Cytokinin

Sensitivity to ethylene was determined by sowing seeds on 0, 30, 100, 300, and 1000 μM ACC (Sigma) and 10^{-4} M GA_{4+7} (ICI, Bracknell, UK) on filter paper in sealed Petri dishes. Both a mutant and the wild type were sown in each dish and incubated in darkness at 22°C after 1 d of cold treatment (4°C). Hypocotyl length was determined after 6 d of incubation at 22°C. Sensitivity to the auxin NAA (Sigma) and to the cytokinin BA (Sigma) was determined by a root growth assay in vertical Petri dishes. Seedlings were grown in a vertical position on 4.59 g/L Murashige and Skoog minerals and micronutrients, 2% (w/v) Suc, and 0.7% (w/v) agar with 10^{-9}, 10^{-8}, 10^{-7}, or 10^{-5} M NAA or BA. Root length was determined after 10 d of incubation in a growth room (25°C, 16-h photoperiod) and after 3 d of cold treatment (4°C).

ABA Determinations

Plants were grown under the same conditions as previously described (Rock and Zeevaart, 1991) and used for ABA determinations when still in the rosette stage. In all experiments, ABA content of turgid and water-stressed plants was compared. Turgid material was harvested and frozen immediately in liquid N_2. In the case of water stress, detached rosettes were dehydrated in dry air at room temperature until 15% of the fresh weight was lost. The stressed material was kept in polyethylene bags at 22°C for 5 to 6 h and then frozen in liquid N_2. The frozen material was lyophilized and the dry weight was determined.

The lyophilized material was extracted as described before (Cornish and Zeevaart, 1984). To each extract was added 20,000 dpm of [^{3}H]ABA to quantify losses during purification. The dried, crude extracts were first purified by semipreparative reverse-phase HPLC with a μBondapak C_{18} column (30 × 0.78 cm; Waters) (Rock and Zeevaart, 1991). The fractions containing ABA were collected, dried, and methylated with ethereal diazomethane. Methyl-ABA in these fractions was further purified by normal-phase HPLC with a μPorasil column (30 × 0.4 cm; Waters) (Zeevaart et al., 1989). Quantification of methyl-ABA was performed by GLC with electron capture detector (Cornish and Zeevaart, 1984).

Dry seeds (approximately 1 g of wild type and of each mutant, from seed batches grown and harvested at the same time and stored for 14 months) were extracted overnight. The samples were homogenized with a Polytron (Brinkmann), and the extracts were filtered. The acetone was evaporated, and the oil extracted from the seeds was removed by partitioning the aqueous concentrate with hexane. The water phase was frozen and lyophilized. ABA in the residue was purified by HPLC and measured as described above for plant material.

RESULTS

Mutant Isolation

After γ-ray mutagenesis, 43 M_2 seedlings were selected out of approximately 5000 M_1 plants in the reduced-dor-

mancy screen. Progeny testing showed that eight stable mutants were recovered. Some of these mutants were allelic to ABA-insensitive mutants: three mutants failed to complement *abi3* and one mutant failed to complement *abi2*. Two other mutants were affected in the pigmentation of the testa, resulting in yellow and pale brown seeds. Two mutants with wild-type brown seeds exhibited reduced dormancy. These are described in this paper.

Genetics

Allelism with *abi3* was tested because leaky *abi3* mutants are also nondormant without strong pleiotropic effects. This led to the conclusion that both mutants were not allelic to *abi3* (data not shown). The reduced-dormancy mutants were crossed reciprocally to the wild type and intercrossed. F_1 seeds from these crosses were dormant (Table I), showing that the mutant phenotypes were caused by recessive mutations at two separate loci. These observations also indicate that the mutant phenotype, i.e. the reduced dormancy, is embryo determined, since no maternal effects are present. We designated these reduced-dormancy mutants *rdo1* and *rdo2*. F_2 plants from the cross of *rdo1* and *rdo2* with wild type were harvested to obtain individual F_3 lines. To study the inheritance of the mutations, the germination percentage of the F_3 lines and the parents was determined by sowing them 1 week after harvest. The frequency distribution of the germination percentages is shown in Figure 1. If the frequency distribution is assumed to reflect a monogenic, recessive, embryo-determined inheritance of the mutant phenotype, one-fourth of the lines should fall in the mutant class and approximately one-half of the lines (the heterozygotes) should have a germination percentage slightly higher than the wild type. To show that the distribution in Figure 1 represents heritable variation, the progenies of F_3 lines with the lowest and the highest germination percentages were tested for germination. In Figure 2 the germination percentage of the F_3 is plotted against the germination percentage of the F_4. This shows that the reduced-dormancy phenotype of *rdo2* breeds true in the F_4 and that the extreme classes of the frequency distribution represent the homozygous wild types and homozygous mutants. The F_3 data (where a peak between 20 and 50% may represent the heterozygotes) together with the F_4 data are in agreement with the segregation of a single, recessive gene. Nondormant F_3 lines from the crosses wild type × *rdo1* and wild type × *rdo2* were used for subsequent physiological analyses. They reproducibly showed a higher germination than the wild type, although they were grown in different sea-

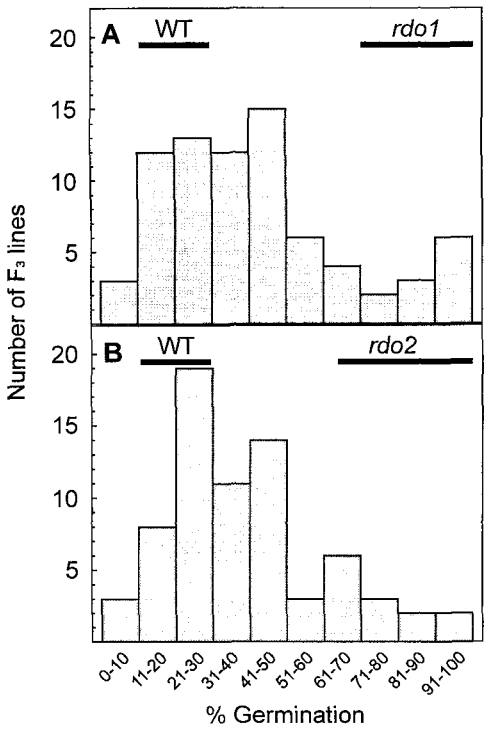

Figure 1. Segregation of reduced-dormancy mutants. Frequency distribution of germination percentages of individual F_3 seed batches from the crosses wild type (WT) × *rdo1* (A) and WT × *rdo2* (B). The seeds were sown 1 week after harvest and germination was scored 1 week after sowing. The bars indicate the range of germination percentages of three WT and four *rdo1* or *rdo2* lines.

Table I. *Germination percentages of F_1 seeds of reciprocal crosses between the mutants rdo1, rdo2, and the wild type (WT)*

The seeds were sown 3 d and 10 d after harvest, and germination was scored 7 d after sowing. Percentages are means of three to seven replicates.

Male	Female					
	rdo1		*rdo2*		WT	
	3 d	10 d	3 d	10 d	3 d	10 d
rdo1	14	97	0	36	0	51
rdo2	1	40	57	97	0	61
WT	0	n.d.[a]	0	42	0	44

[a] n.d., Not determined.

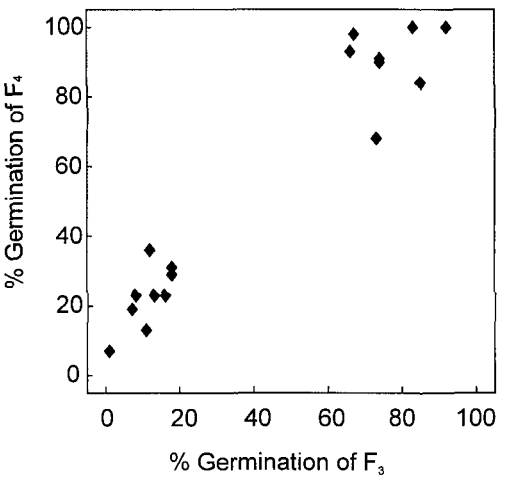

Figure 2. Germination percentage of a number of F_3 lines derived from the cross wild type (WT) × *rdo2* plotted against the average germination percentage of five F_4 lines derived from each F_3 line. Each line represents the progeny of a single plant.

Table II. *Plant parameters of the wild type (WT) and the rdo1 mutant*

The length of the main stem after termination of flowering was determined, together with the length of the sixth silique and the number of seeds, embryo lethals, and nonfertilized ovules in this silique. Values are means of 15 determinations ± SE.

Genotype	Length of Main Stem	Silique Length	Sum of No. of Seeds, Embryo-Lethals, and Ovules	Fertility
	cm	*mm*	%	
WT	26.5 ± 5.3	12.2 ± 0.3	56 ± 2	95 ± 2
rdo1	17.3 ± 4.3	10.6 ± 0.4	51 ± 2	94 ± 2

sons. This is an additional argument for the heritability of the reduced-dormancy trait.

Mutant *rdo1* plants were smaller and had shorter siliques because of fewer seeds in the siliques than the wild type (Table II). The flower buds opened prematurely. The reduced dormancy and the aberrant plant phenotype cosegregated in 140 F_3 and F_4 lines derived from the cross wild type × *rdo1*, indicating that these are pleiotropic effects of the *rdo1* mutation and that the reduced dormancy of this mutant is a heritable trait. The phenotype of *rdo2* plants was normal except for a slightly darker green rosette than the wild type and a slight retardation of flowering (1–2 d), with a normal leaf number.

Seed Dormancy Characteristics of the *rdo1* and *rdo2* Mutants

The ability to germinate as freshly harvested seeds indicates a lack of an after-ripening requirement that is induced during seed development (Karssen et al., 1983). To follow the course of dormancy induction and after-ripening, germination of excised developing seeds and mature seeds in water was determined using the wild type as a dormant and the ABA-deficient mutant (*aba-1*) as a nondormant control (Fig. 3). Wild-type seeds older than 13 DAF did not germinate, reflecting their dormant state. *rdo1* seeds were slowly released from dormancy from 13 DAF onward (Fig. 3A) and this release continued during dry storage of the seeds (Fig. 3B). Dormancy was not induced in *rdo2* seeds. Seeds of the *aba* mutant could germinate at all stages of development. Wild-type seeds were released from dormancy during several weeks of dry storage (Fig. 3B). The degree of dormancy varied between seed batches of different harvest dates, reflected in differences in levels and slopes of curves such as in Figure 3B. The relative germination percentages were always *aba* > *rdo2* > *rdo1* > wild type.

The degree of dormancy can also be described by the ability to germinate in unfavorable conditions, such as darkness and suboptimal or supraoptimal temperatures. Table III shows that *rdo1* and *rdo2* are very similar to *aba* in having a higher capacity to germinate in darkness. At 30°C, germination percentages of *rdo1* and *rdo2* decreased to lower values than those of *aba* but not as low as those of the wild type. This confirms that the new mutants have a reduced degree of dormancy compared to the wild type but are not as nondormant as the *aba* mutant.

Hormone Levels and Sensitivity

Since ABA-related mutants have a reduced dormancy, we investigated whether *rdo1* and *rdo2* were ABA insensitive or ABA deficient. Figure 4 shows that ABA sensitivity of germination of *rdo1* and *rdo2* was slightly lower than the wild type. Mutant plants were not wilty, suggesting that they are not ABA deficient. ABA determinations confirmed that the *rdo1* and *rdo2* mutants had near wild-type levels of endogenous ABA (Table IV). Seeds of both mutants contained at least as much ABA as wild-type seeds, indicating that reduced dormancy of the mutants is not due to reduced ABA specifically in the seeds.

Ethylene can replace GA for Arabidopsis seed germination (Karssen et al., 1989). Ethylene-insensitive mutants of Arabidopsis show poor germination in relation to the wild type (Bleecker et al., 1988). "Easily" germinating seeds, like those of *rdo1* and *rdo2*, might be hypersensitive to ethylene. We investigated the ethylene sensitivity by germinating seeds in the dark in the presence of GA_{4+7} and ACC. Hypocotyl length was inhibited to the same extent in wild type, *rdo1*, and *rdo2* seedlings (Table V); therefore, *rdo1* and *rdo2* cannot be classified as ethylene-response mutants.

The sensitivity to inhibitory effects of the auxin NAA and the cytokinin BA was tested with a root growth assay. This did not reveal a different sensitivity of the mutants compared to the wild type, except for a slight insensitivity with respect to root growth of *rdo1* to NAA (Table V).

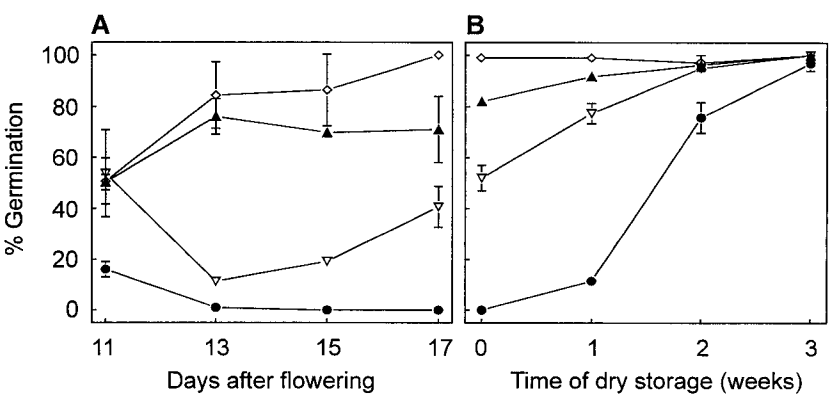

Figure 3. Induction and loss of dormancy. Germination in water of *rdo1* (▽), *rdo2* (▲), *aba-1* (◇), and wild-type (●) seeds that were removed from the siliques at different stages during development (A) and of seed batches sown after different periods of storage of the seeds (B). Percentages are means of triplicates ± SE.

Table III. *Dark germination of reduced-dormancy mutants*

Final germination percentages reached after 16 d (5°C), 5 d (15°C), and 3 d (25, 30°C) of imbibition in darkness of the wild type (WT), dormancy mutants (*rdo1*, *rdo2*), and ABA-deficient mutant (*aba-1*) at different temperatures are given. Percentages are means of numbers indicated (*n*) ± SE.

Temperature	WT	*rdo1*	*rdo2*	*aba*	*n*
°C					
5	71 ± 3	89 ± 1	100 ± 0	98 ± 1	12
15	50 ± 3	86 ± 1	100 ± 0	99 ± 0	21
25	64 ± 4	98 ± 1	95 ± 1	99 ± 1	21
30	3 ± 1	57 ± 2	27 ± 2	87 ± 1	18

Since GAs are required for seed germination (Karssen et al., 1989), we determined the sensitivity to the GA biosynthesis inhibitor tetcyclacis (Fig. 5). Sensitivity to tetcyclacis is reduced in *rdo2*, but the curve of *rdo1* corresponds with the wild-type. The differences between seed batches of different harvest dates with respect to tetcyclacis sensitivity are also shown. However, a similar relative insensitivity of *rdo2*, but not of *rdo1*, was found in four independent experiments using different seed harvest batches. The tetcyclacis sensitivity of a given seed batch does not change with time (data not shown); therefore, the differences in tetcyclacis sensitivity are due to different growing conditions and not to the storage duration of the seeds.

The tetcyclacis insensitivity was a true pleiotropic effect of the *rdo2* mutation as was tested with F_4 lines derived from the cross wild type × *rdo2*. Figure 6 shows that all lines with a low degree of dormancy, expressed as a high germination percentage, germinate on 100 μM tetcyclacis, whereas lines with a low germination percentage hardly germinate in the presence of tetcyclacis.

Interaction with Other Seed Dormancy Mutants

Double mutants were constructed between *rdo1*, *rdo2*, and the nondormant mutants *aba*, *abi2*, *abi3–1*, and *abi3–5*. The germination rates of the different mutants and their double mutants were analyzed. The germination rates of the ABA-related mutants *aba*, *abi2*, and *abi3–5* were higher than those of *rdo1* and *rdo2*, but the double mutants' germination rates were not at all or only slightly higher than those of the single mutants (data not shown). The germination rate of the *rdo1,rdo2* double mutant is given in Figure 7A. The germination rate of the *rdo1,abi3* double mutant was higher than the sum of the single mutants' germination rates, indicating an amplifying effect of these two mutations (Fig. 7B). In contrast to this, the *rdo2,abi3* germination rate was not higher than the sum of the germination rates of *rdo2* and *abi3* (Fig. 7C). The additive effect of *rdo1* to *abi3* was also visible in the loss of viability of *abi3* seeds. The extreme mutant allele *abi3–5* has a reduced desiccation tolerance (Ooms et al., 1993). The time after which one-half of the seeds have lost their viability was 2 times shorter for *rdo1,abi3–5* mutant seeds than for *abi3–5* and *rdo2,abi3–5* seeds.

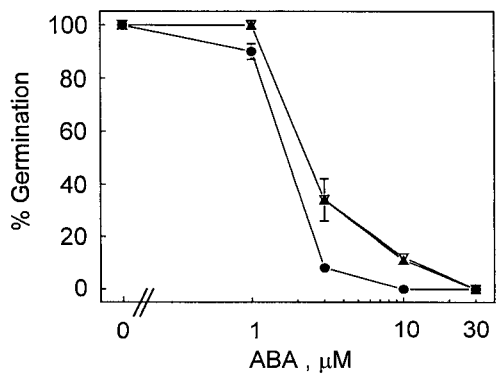

Figure 4. ABA sensitivity of reduced-dormancy mutants. Germination of *rdo1* (▽), *rdo2* (▲), and wild type (●) in different ABA concentrations. Percentages are means of triplicates ± SE.

Table IV. *Endogenous ABA content of turgid and partly dehydrated rosettes and seeds*

Amounts in rosettes are the averages of measurements of two samples. Amounts of ABA in seeds are measurements of one sample for each genotype.

Genotype	Turgid Rosettes	Stressed Rosettes	Mature Dry Seeds
		μg/g dry wt	
L*er* (WT)	0.10	1.11	0.14
rdo1	0.12	0.85	0.15
rdo2	0.12	0.89	0.36

DISCUSSION

In this paper, two Arabidopsis mutants that are specifically affected in the degree of dormancy are described. They originated from a screen for mutant seeds that were able to germinate immediately after harvest. Such a screen can be expected to yield ABA-related mutants since these lack dormancy. The ABA-insensitive mutants *abi2* and *abi3* were found, but no additional ABA-deficient mutants emerged. Two mutants appeared to be seed color mutants in the M_3. A reduced dormancy is common for seed color mutants such as the *ttg* and *tt* (*transparent testa*) mutants (Koornneef, 1981; Léon-Kloosterziel et al., 1994; Debeaujon et al., 1995); therefore, it was concluded that the reduced dormancy of the color mutants found in our screen was most likely caused by a mutation affecting the testa pigmentation. However, the testa is maternally inherited and *tt* mutants are recessive. An explanation for the finding of these mutants in the M_2 can be that a partial dominance,

Table V. *Sensitivity to ethylene, NAA, and BA*

The inhibition of hypocotyl elongation on 300 μM ACC[a] and root growth on 10^{-7} M NAA or BA[a] is expressed as a percentage of the control hypocotyl and roots ± SD.

Genotype	ACC	NAA	BA
L*er* (WT)	38 ± 8	43 ± 7	35 ± 7
rdo1	42 ± 8	66 ± 13	41 ± 10
rdo2	39 ± 8	40 ± 9	35 ± 11

[a] Only the value of one concentration is given, which was representative for all concentrations tested.

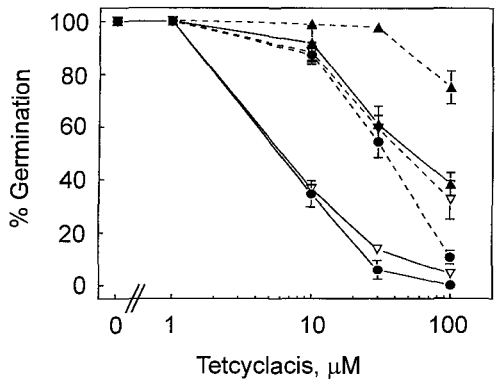

Figure 5. Tetcyclacis sensitivity of reduced-dormancy mutants. Seeds of *rdo1* (▽), *rdo2* (▲), and the wild-type (●) harvested in June 1994 (solid lines) and October 1994 (dotted lines) were tested for their ability to germinate on different concentrations of tetcyclacis. Percentages are means of triplicates ± SE.

not detectable on the basis of seed color, might influence dormancy. However, it cannot be excluded that originally these mutants have been "false positives," of which the next generation revealed the seed color mutation.

The degree of dormancy of a seed lot is reflected in the germination percentage relatively soon after harvest of the seeds, since the release from dormancy of Arabidopsis ecotype L*er* occurs within several weeks of dry storage of the seeds (K.M. Léon-Kloosterziel, M. Koornneef, unpublished observations). Arabidopsis is known to show a large variability in light and GA requirement for seed germination, because of differences in growing conditions (Derkx and Karssen, 1993). We found that the degree of dormancy and concurrently the rate of release from dormancy of the mutants was always higher than that of the wild type but highly variable between seed batches of different harvest dates. To perform a physiological analysis of these mutants, comparisons had to be made between seed lots

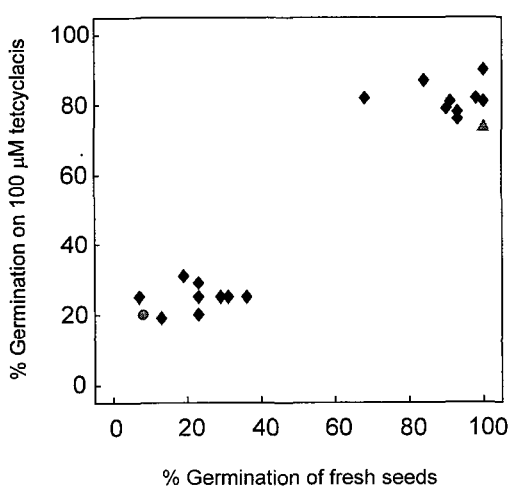

Figure 6. The correlation between reduced dormancy and tetcyclacis insensitivity. The germination percentage of freshly harvested seeds of F_4 lines derived from the cross wild type × *rdo2* (◆), the wild type (●), and the *rdo2* parent (▲) is plotted against the germination percentage on 100 μM tetcyclacis.

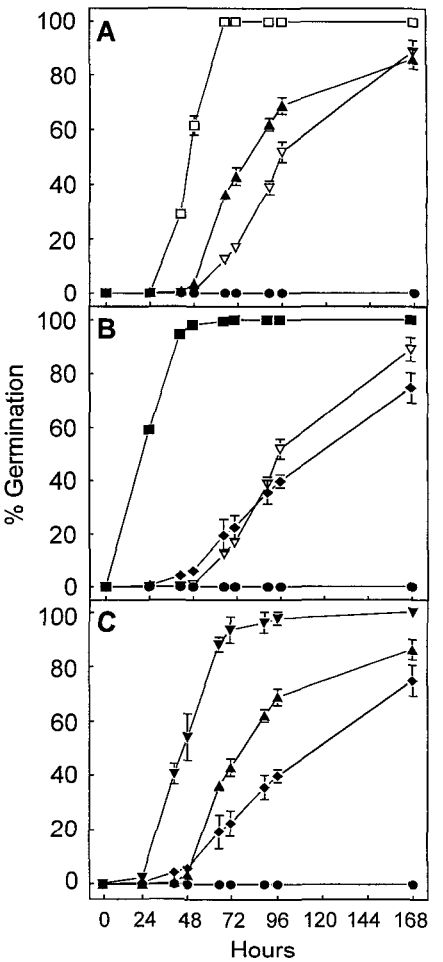

Figure 7. Germination rates of freshly harvested seeds. A, Germination rates of the wild type (●), the *rdo1* (▽), and *rdo2* (▲) mutants and the *rdo1,rdo2* double mutant (□). B, Germination rates of the wild type (●), the *rdo1* (▽), and *abi3–1* (◆) mutants and the *rdo1,abi3–1* (■) double mutant. C, Germination rate of the wild type (●), the *rdo2* (▲), and *abi3–1* (◆) mutants and the *rdo2,abi3–1* (▼) double mutant. Percentages are means of triplicates ± SE.

grown at the same time and in the same conditions. We also repeated most experiments three times to ascertain consistent results. An example of the variability with respect to tetcyclacis sensitivity is shown in Figure 5.

The reduced-dormancy trait of the mutants was caused by a single, recessive mutation at two distinct loci, *rdo1* and *rdo2*, and was embryo determined (Fig. 1; Table I). We did not attempt to locate these mutations on the genetic map of Arabidopsis, because dormancy can vary between ecotypes and can be influenced by several mutations present in marker lines. The difficulty in performing genetics on variable traits like reduced dormancy for which no clear phenotypic classes can be distinguished is also illustrated in Figure 1.

Lack of dormancy can have a hormonal cause as described for ABA-deficient (*aba*) or ABA-insensitive (*abi*) mutants (Koornneef et al., 1982, 1984). The *rdo1* and *rdo2* mutants cannot be classified as ABA-related mutants, since

their ABA content was normal (Table IV) and *rdo1* and *rdo2* seeds were not ABA-insensitive at germination (Fig. 4). The curves of *rdo1* and *rdo2* differed slightly from that of the wild type, but this does not indicate significant ABA insensitivity, since ABA-insensitive mutants are at least 10 times more insensitive to ABA than the wild type (Koornneef et al., 1984; Finkelstein, 1994). The possibility that the reduced dormancy originates in an altered sensitivity to ethylene, auxins, or cytokinins was ruled out by the observation that the mutants and the wild type exhibited the same hypocotyl inhibition by the ethylene precursor ACC and the same root growth inhibition by NAA and BA. One exception to this was the slight insensitivity of *rdo1* to NAA. Nevertheless, this insensitivity is far less extreme than in auxin-resistant mutants of Arabidopsis (Maher and Martindale, 1980; Estelle and Somerville, 1987).

The *rdo* mutants were not ABA deficient in either vegetative parts or in mature seeds. In mature seeds, embryonic ABA, known to be responsible for dormancy induction, prevails (Karssen et al., 1983). Apparently, the *rdo* mutants do not represent the class of maize mutants that are specifically ABA deficient in the seeds (McCarty, 1995). However, the *rdo* mutants had physiological characteristics similar to those of the *aba* mutant. Similar to the *aba* mutant, the reduced dormancy of *rdo1* and *rdo2* resulted in a broader germination window (Table III). Furthermore, seeds of the *aba* and *rdo2* mutants, but not the *rdo1* mutant, were more resistant to the GA biosynthesis inhibitor tetcyclacis (Fig. 5). This indicates that less GA is required for germination than in the wild type. Since the plant height of *rdo2* was normal, it is not likely that the GA response is altered in this mutant. The *spy* mutant of Arabidopsis, which is nondormant and hypersensitive to GA, shows a phenotype comparable with GA-treated plants (Jacobsen and Olszewski, 1993).

The *rdo2* mutants have a lower GA requirement specific for seed germination in common with ABA-deficient (Koornneef et al., 1982; Léon-Kloosterziel et al., 1995) and ABA-insensitive mutants (Nambara et al., 1991). However, a lack of ABA or ABA action does not seem to be the cause of this lack of dormancy accompanied by a reduced GA requirement for germination. It is likely that the *RDO2* locus controls a step between the initial occurrence of ABA and ABA-induced dormancy. Apparently, this step is specific only for the ABA effects on seed dormancy, since no pleiotropic effects on water relations, like those observed in *aba* and *abi* mutants, were present in *rdo2*.

A reduced seed dormancy is not always associated with a reduced GA requirement for germination, since the *rdo1* mutant was as sensitive to GA biosynthesis inhibitors as the wild type. The same has been reported for the *fus3* mutant, which is extremely nondormant and is strongly disturbed in seed maturation (Bäumlein et al., 1994; Keith et al., 1994). Double-mutant analysis led to the conclusion that *FUS3* and *ABI3* control parallel pathways that regulate seed maturation. The reduced dormancy in the *fus3* mutant does not abolish the GA requirement for germination and *RDO1* may control a step in the same dormancy mechanism as the one that is affected in the *fus3* mutant. This can be explained by posing the hypothesis that dormancy has two aspects controlled by different mechanisms in the embryo tissue. Dormancy is induced in a pathway in which the *FUS3* and *RDO1* genes are involved. This is one aspect of the seed maturation process and leads to a developmental arrest. A separate pathway in which the *ABA*, *ABI3*, and *RDO2* genes are involved induces a requirement for de novo GA biosynthesis in addition to this developmental arrest.

This model predicts that the *rdo1,rdo2* and the *rdo1,abi3* double mutants show an additive effect and that the *rdo2,abi3* double-mutant phenotype is like the single mutants' phenotypes, except when the mutants are leaky. The *abi3–1* allele is leaky because most other alleles described have a much more extreme phenotype (Koornneef et al., 1984; Nambara et al., 1992; Ooms et al., 1993). Since we are not certain that the *rdo1* and *rdo2* alleles we isolated are null alleles, the double-mutant analysis may not be completely conclusive. However, the *rdo1,rdo2* and the *rdo1,abi3* double mutants showed an additive germination rate and the *rdo1,abi3* germination rate was higher than the *rdo2,abi3* germination rate, confirming the model of parallel pathways (Fig. 7). Desiccation tolerance, which is low in *abi3–5* mutants, was even more reduced in *rdo1,abi3–5* double mutants. This also confirms the hypothesis that *RDO1* and *ABI3* act in different pathways and may mean that, although the *rdo1* mutant is desiccation tolerant, the *RDO1* gene is involved in this other aspect of seed maturation as is the *FUS3* gene.

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