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Dormancy and Germination of Abscisic Acid-Deficient Tomato Seeds[1]

Studies with the *sitiens* Mutant

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ABSTRACT

The role of abscisic acid (ABA) in the dormancy induction of tomato (*Lycopersicon esculentum*) seeds was studied by comparison of the germination behavior of the ABA-deficient *sitiens* mutant with that of the isogenic wild-type genotype. Freshly harvested mutant seeds, in contrast to wild-type seeds, always readily germinate and even exhibit viviparous germination in overripe fruits. Crosses between mutant and wild-type and self-pollination of heterozygous plants show that in particular the ABA fraction of embryo and endosperm is decisive for the induction of dormancy. After-ripened wild-type seeds fully germinate in water but are more sensitive toward osmotic inhibition than mutant seeds. Germination of both wild-type and mutant seeds is equally sensitive toward inhibition by exogenous ABA. ABA content of mature wild-type seeds is about 10-fold the level found in mutant seeds. Nevertheless, it is argued that the differences in dormancy between the seeds of both genotypes are not a result of actual ABA levels in the mature seeds or fruits but a result of differences in ABA levels during seed development. It is hypothesized that the high levels of ABA that occur during seed development in wild-type seeds induce an inhibition of cell elongation of the radicle that can still be observed after long periods of dry storage.

Studies with ABA-deficient mutants of *Arabidopsis thaliana* provided clear evidence that the induction of dormancy during the development of seeds on the mother plant depends on an increase of endogenous ABA (11). Recently, Le Page-Degivry *et al.* (14) demonstrated that application of fluridone caused a strong reduction of ABA levels and prevented the development of embryo dormancy in developing seeds of *Helianthus anuus*.

Generally, upon maturation of seeds, ABA levels strongly decrease. For seeds of *A. thaliana*, it was hypothesized that the inhibitory effect of ABA on germination is fixed during seed development in a state of dormancy that does not depend on the actual presence of ABA for its maintenance in the mature seed (12). Bonamy and Dennis (1) showed that in mature peach seeds the ABA level diminished much more rapidly during incubation at 20 than at 5°C, whereas dormancy was only relieved at 5°C.

It was the aim of the present study to test whether the hypothesis formulated for seeds of *A. thaliana* also holds for tomato (*Lycopersicon esculentum*) seeds. The experiments were performed with the *sitiens* (sit^w) mutant of cv Moneymaker, isolated and phenotypically characterized by Koornneef *et al.* (13). It was shown before that developing sit^w seeds contain about 3% of the ABA level in wild-type seeds (8). The strong reduction of ABA levels did not show significant influence on seed development. From our present work, however, we have found that the mutation caused vivipary and lack of dormancy in ripe and mature seeds. An attempt will be made to characterize and localize the ABA-induced dormancy.

Compared to the tiny seeds of *A. thaliana*, the relatively large seeds of tomato are better objects for a study of the localization of hormone action in the different seed parts during development and germination. Previous experiments with the GA^3-deficient *gib-1* mutant of tomato (in those studies still denoted as *ga-1*) have shown that endogenous GA promotes tomato seed germination by induction of cell wall-hydrolyzing enzymes in the endosperm, thereby facilitating a weakening of the mechanical restraint of the endosperm region opposing the radicle tip (6, 7). We will study whether the direct or indirect role of ABA in the induction and maintenance of dormancy is possibly related to the GA-induced processes in the seeds. The availability of a *gib-1* sit^w recombinant line proved to be of advantage in this respect.

Special attention will also be given to a possible role of ABA in the reduction of the cell expansion force of the embryo. Studies with rapeseed have shown that the inhibition of cell expansion by applied ABA interfered with the process of cell-wall loosening (18).

MATERIALS AND METHODS

Seed Material

Wild-type tomato (*Lycopersicon esculentum* Mill. cv Moneymaker) and the isogenic ABA-deficient line sit^w, the isogenic

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[3] Abbreviations: GA, gibberellin; GA_{4+7}, mixture of gibberellin A_4 and gibberellin A_7.

GA-deficient line *gib-1*, and the isogenic recombinant line *gib-1 sit*^{w} were obtained from Professor J.H. van der Veen and Dr. M. Koornneef of the Department of Genetics, Agricultural University, Wageningen, The Netherlands. Plants for seed production were raised at two locations. At location 1, seeds were produced year-round in small scale in a heated greenhouse with minimum temperatures of 21 (day) and 19°C (night). A photoperiod of 16 h was maintained by additional illumination from high-pressure mercury halide lamps (HPI/T, Philips, NL) at 18 $W \cdot m^{-2}$. The plants were cultivated in pot soil (92% peat, 8% sand, and 22.5 g N:P:K = 1:1:1) or on hydroponic culture (19). The *sit*^{w} plants were misted regularly with tap water to prevent wilting, and during summer the greenhouse was whitewashed to protect the plants against direct sunlight.

At location 2, seeds were produced in large scale in a nonheated greenhouse during summer and autumn without additional illumination. The plants were cultivated in soil (sandy loam), the *sit*^{w} plants were misted regularly with tap water to prevent wilting, and the greenhouse was whitewashed to protect the plants against direct sunlight. At this location, the older leaves below the clusters with ripe fruits were removed regularly. The *sit*^{w} and *gib-1 sit*^{w} plants were sprayed once or twice a week with about 2 mL of 10 μM (±)ABA (Fluka, Buchs, Switzerland) to reduce wilting. Of the *gib-1* and *gib-1 sit*^{w} plants, the top and flower bud regions were sprayed once a week with a solution of 10 μM GA_{4+7} (Berelex; ICI, Bracknell, Berkshire, UK) to stimulate shoot growth and development of fertile flowers.

Seeds were isolated from ripe fruits and incubated in 1% HCl for 1 h to remove the remnants of the mucilagenous locular tissue. Thereafter, the seeds were rinsed with tap water, dried at room temperature, and stored in closed plastic containers in a refrigerator at 7°C until use. Comparison between wild type and mutant always occurred with seed lots from the same harvest date.

Germination Conditions

Triplicates of 50 seeds were sown in 5-cm glass Petri dishes on one layer of filter paper (Schleicher and Schüll No. 595) moistened with 1.5 mL of 0.25 $mg \cdot L^{-1}$ thiomersal (BDH, Poole, Dorset, United Kingdom) to prevent fungal growth, with or without growth regulators or osmotic material. ABA and GA_{4+7} were dissolved in 1 N KOH and diluted with distilled water, and the pH of the stock solutions was adjusted to 7.0 with 1 N HCl. The Petri dishes with seeds were placed in closed plastic boxes and incubated at 26 ± 1°C in the dark unless mentioned otherwise. In experiments with intact seeds, visible radicle protrusion was used as the criterion for germination unless mentioned otherwise. When the layers opposing the radicle tip were removed, 2 h after the start of inbibition (detipped seeds), a certain minimum protrusion was taken as the criterion.

The osmotic potential of the PEG (molecular mass 6000 D) (Serva, Heidelberg, Germany) solutions was calculated according to the formula of Michel (15). The PEG solutions were refreshed every 2 d. In some of the experiments, seeds were irradiated intermittently with red light for 10 $min \cdot h^{-1}$ during the first 24 h after the start of imbibition. Red light (620–700 nm, 2.6 $W \cdot m^{-2}$) was obtained by filtering irradiation from six red-fluorescent tubes (TL 20 W/15, Philips, Eindhoven, the Netherlands) by 3-mm Plexiglas (Red 501, Röhm & Haas, Darmstadt, Germany).

ABA Determinations

Quantitative determinations of endogenous ABA were performed according to techniques described by Vermeer *et al.* (21), using a Perkin-Elmer 8320 gas chromatograph equipped with an electron capture detector. Recovery was determined with the use of [^{14}C]ABA that was added to the first extraction medium. It was shown to be at least 95%.

Determination of the Osmotic Potential of the Fruit Juice

Fruit juice was collected from ripe fruits 10 d after they had turned from orange to red. The juice was filtered, and the osmotic potential of the juice was determined with an osmometer (Knauer, Oberursel, Germany), calibrated with mannitol solutions and calculated according to the method of Michel *et al.* (16).

Puncture Force Determinations

The force needed to puncture the endosperm plus testa layers opposing the radicle tip was determined and taken as a measure of the mechanical restraint of these seed layers. The puncture force determinations and statistical calculations of the data were performed according to the previous description (6).

RESULTS

Development of Dormancy

The development of dormancy in wild-type seeds was strongly influenced by the cultivation conditions (Table I). Wild-type plants raised in pots or on hydroponic culture at location 1 produced seeds that germinated directly after harvest only for about 15% (at 26°C in darkness). However, wild-type seeds originating from location 2 germinated for >80% directly after harvest. In contrast, freshly harvested *sit*^{w}/*sit*^{w} seeds from both cultivation conditions germinated at a high percentage, whereas *Sit*/*sit*^{w} seeds that had been formed in a *sit*^{w}/*sit*^{w} fruit germinated at an intermediate frequency. Thus far, additional experiments have failed to explain which conditions at location 1 were essential for the induction of seed dormancy.

Table I. *Germination of Freshly Harvested Seeds Produced under Different Conditions*

Seeds were dried before the germination tests. See "Materials and Methods" for the description of the conditions of cultivation.

Production and Genotype	Germination (mean ± SD)
	%
Location 1	
Sit/*Sit* seeds on *Sit*/*Sit* plants	15 ± 1
Sit/*sit*^{w} seeds on *sit*^{w}/*sit*^{w} plants	57 ± 11
sit^{w}/*sit*^{w} seeds on *sit*^{w}/*sit*^{w} plants	97 ± 1
Location 2	
Sit/*Sit* seeds on *Sit*/*Sit* plants	82 ± 4
sit^{w}/sit^{w} seeds on *sit*^{w}/*sit*^{w} plants	96 ± 0

The germination characteristics of the seeds developing after self-pollination of *Sit/sit*^{w} plants were also studied (Fig. 1). It appeared that all seeds that gave rise to wilting seedlings had germinated within the first 3 d. Of the seeds that formed normal seedlings, 45% also germinated within that time but at a slower rate. The remainder of those seeds required GA_{4+7} to germinate. Thus, *sit*^{w}/*sit*^{w} seeds also showed a lack of dormancy after development in ABA-rich *Sit/sit*^{w} maternal tissues.

Viviparous Germination

Developing seeds of the *sit*^{w} mutant did not germinate prematurely in the fruits, but when ripe fruits remained attached to the plant, viviparous germination of the mutant seeds occurred within the fruits (Fig. 2). Three weeks after the mutant fruits had turned red about 50% of their seeds had germinated (Fig. 3). This phenomenon did not occur in wild-type fruits. In overripe fruits of self-pollinated *Sit/sit*^{w} plants, about one-quarter of the seeds germinated viviparously when the fruits remained attached to the mother plant. A wilting test showed that all the seedlings that developed from these viviparous seeds had the wilty phenotype and, therefore, the *sit*^{w}/*sit*^{w} genotype. The ABA content of the juice of ripe *sit*^{w}/*sit*^{w} fruits was about 10% of the level found in wild-type fruits (Table II). The osmotic potential of the fruit juice was similar for both genotypes.

Alleviation of Dormancy

Germination of dormant wild-type seeds was stimulated by a pretreatment at 2°C, particularly in combination with red light, and by GA_{4+7} (Table III). The level of dormancy was also decreased by several weeks of dry storage.

With wild-type and *sit*^{w} seeds, the force required to puncture the layers opposing the tip of the radicle and the percentage of germination were observed during incubation in water (Fig. 4). The seeds produced at location 1 were tested immediately after harvest or after 8 weeks of dry storage at 7°C. Two hours after the start of incubation, the endosperm resistance of both genotypes was similar, and it was not affected by dry storage. When wild-type seeds were incubated immediately after harvest, only a few seeds germinated, and the median puncture force did not decrease during incubation. Apparently, the dormant state of these seeds prevented endosperm weakening. In wild-type seeds, 8 weeks of dry storage induced the capacity to weaken the endosperm, which was followed by germination. Both processes occurred at the same rate as was observed in the nondormant *sit*^{w} seeds both before and after dry storage.

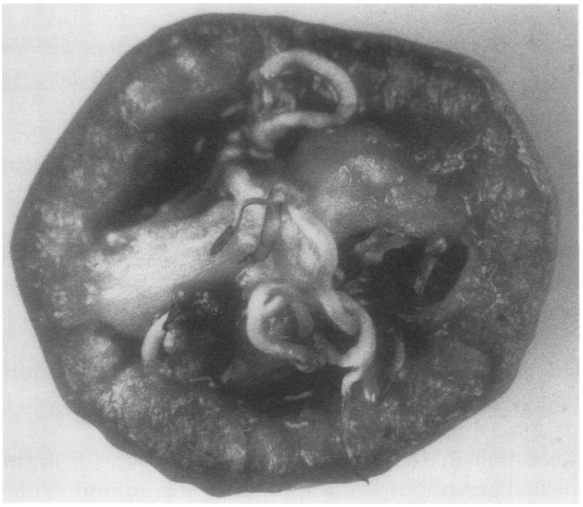

Figure 2. Viviparously germinating mature *sit*^{w}/*sit*^{w} seeds in an overripe *sit*^{w}/*sit*^{w} fruit.

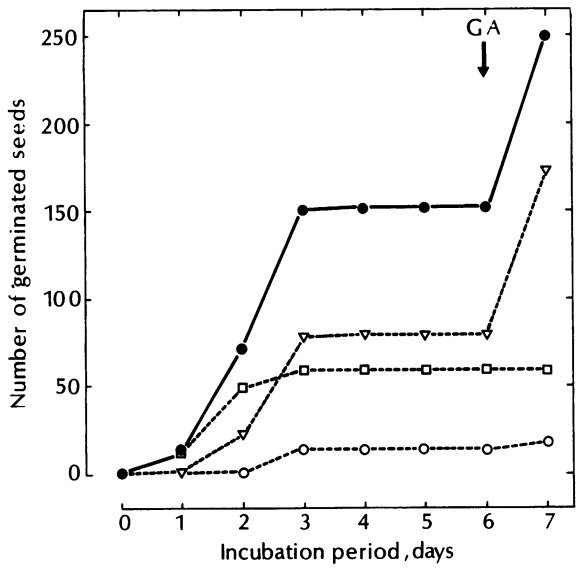

Figure 1. The influence of the genotype of the embryo on the germination of 250 freshly harvested mature seeds from self-pollinated *Sit/sit*^{w} plants. Seeds were collected from ripe fruits, dried overnight, and incubated in water the next day. Germination was recorded each day (●). At each recording day, germinated seeds were removed from the Petri dishes, transplanted in soil, and checked for wilting behavior of seedlings under water stress: □, wilting; ▽, nonwilting. The phenotype of some seedlings (7%) could not be classified (○) because they did not survive the transplantation. All seeds that had not germinated on day 6 were subsequently treated with 10 μM GA_{4+7}.

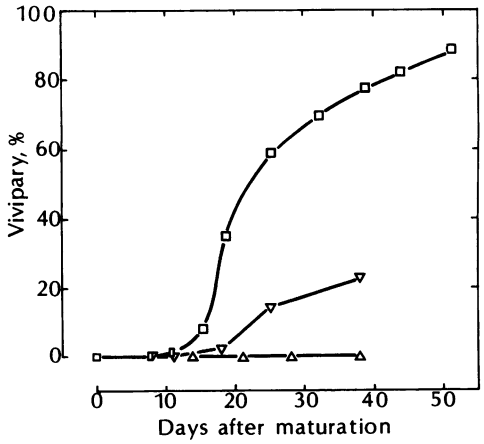

Figure 3. Percentage of seeds germinated within ripe fruits derived from self-pollination of *Sit/Sit* (△), *Sit/sit*^{w} (▽), and *sit*^{w}/*sit*^{w} (□) plants. Maturation is defined as the moment the fruit turns red.

Table II. *ABA Content and Osmotic Potential of the Juice of Ripe Sit/Sit and sit^w/sit^w Fruits*

The data represent the means ± SD of two fruits for each genotype.

	Sit/Sit	*sit^w/sit^w*
ABA content, μM	0.84 ± 0.18	0.08 ± 0.01
Osmotic potential, MPa	−0.85 ± 0.11	−0.93 ± 0.07

ABA Levels in Mature Seeds

The levels of endogenous ABA were determined in mature seeds of both genotypes that had been raised at location 1 (Table IV). Freshly harvested seeds of wild type contained about 10-fold higher ABA levels than fresh *sit^w* seeds. Dry storage reduced the levels by half, and 18 h of imbibition in water caused a further strong reduction of ABA content in the wild-type seeds.

In another experiment, seeds of wild type were raised at both locations. Both germination capacity and ABA content were determined on several batches of freshly harvested seeds collected at different harvest dates (N.J. Pinfield and H.W.M. Hilhorst, unpublished data). The germination of the five batches from location 1 ranged from 9 to 30%, with an average of 16%. The ABA content of those seeds varied between 0.43 and 2.94 ng per seed, with an average of 1.39 ng per seed. The 11 wild-type seed batches produced at location 2 germinated between 58 and 98%, with an average of 85%, whereas their ABA content ranged from 0.08 to 0.24 ng per seed, with an average of 0.14 ng per seed.

Inhibition of Germination by Exogenous ABA

Because the difference in germination of wild-type and *sit^w* seeds in water lasted for only short periods of storage, we tested whether longer lasting effects could be seen under less favorable conditions, such as incubation in ABA or PEG. In these experiments, seeds that were produced in large scale at location 2 were used and stored dry for at least several months. Exogenous ABA inhibited the germination of after-ripened seeds of both genotypes to the same extent (Fig. 5). The same was true for the ABA inhibition of embryo growth in detipped seeds, in which the interfering mechanical restraint of endosperm and testa was removed in the area opposing the radicle. The seeds were detipped 2 h after the start of incubation, and in all detipped seeds the radicle protruded to a limited extent of about 1 mm. ABA did not effect this early protrusion; it only inhibited the further extension of the radicle (Fig. 5). The inhibition of radicle growth required slightly higher ABA concentrations than the prevention of germination, and 50% inhibition occurred in both genotypes at about 2 and 5 μM, respectively.

GA-induced endosperm weakening was also inhibited by exogenous ABA (Table V). Incubation of deembryonated halves of *gib-1* seeds in 0.2 μM GA_{4+7} caused weakening of the endosperm, whereas complete inhibition of the endosperm weakening was achieved by simultaneous incubation in 1 μM ABA.

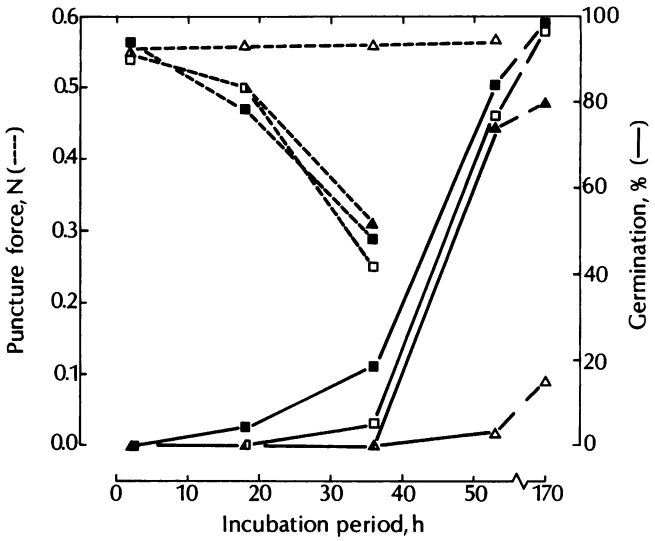

Figure 4. Changes with incubation time of the median force required to puncture the layers opposing the radicle tip (broken lines) and of germination (solid lines) of wild-type (△, ▲) and *sit^w* (□, ■) seeds. Seeds were incubated either 1 d after harvest and drying (open symbols) or after 8 weeks of dry storage at 7°C (closed symbols).

Table III. *Influence of a Pretreatment at 2°C, After-Ripening at Dry Storage, and Different Incubation Conditions on the Germination of Freshly Harvested, Wild-Type Seeds, Produced at Location 1 (See "Materials and Methods" for Description)*

Seeds were incubated at 26°C in water or 10 μM GA_{4+7} and 7 d in darkness (dark) or 1 d under intermittent red followed by 6 d darkness (red). nt, Not tested. Results are means ± SD.

Pretreatment	Incubation Conditions		
	H_2O, dark	H_2O, red	GA_{4+7}, dark
	% *germination*		
None	11 ± 5	35 ± 6	100 ± 0
6 d, 2°C, imbibed	31 ± 3	95 ± 4	nt
7 weeks, 7°C, dry	77 ± 3	nt	100 ± 0

Table IV. *ABA Content of Mature Wild-Type and sit^w Seeds Produced at Location 1*

The seeds were extracted at harvest (nondried), after 1 year dry storage at 7°C or after 1 year storage followed by 18 h imbibition of 50 seeds in 1.5 mL water. Data represent the means ± SD of duplicate samples of 50 seeds.

Line and Extraction	ABA Content
	ng·seed^{-1}
Wild type	
At harvest	1.8 ± 0.2
After 1 year storage	0.8 ± 0.1
After 1 year storage plus 18 h imbibition	0.1 ± 0.0
sit^w/sit^w	
At harvest	0.2 ± 0.0
After 1 year storage	0.1 ± 0.0

Effect of Osmotic Inhibition

Osmotic inhibition of the germination of intact seeds or the radicle extension from detipped seeds was studied in relation to both ABA and GA deficiencies. To prevent interference with the early protrusion that immediately followed detipping, a minimal extension of 1 mm was taken as the criterion for both germination and radicle extension.

In contrast to ABA, PEG also inhibited the early protrusion. The critical osmotic potential of the PEG solution that was required for 50% inhibition of germination was 0.5 MPa lower in *sit*^{w} seeds than in wild-type seeds (Fig. 6A). Intact seeds of the GA-deficient *gib-1* line and of the recombinant *gib-1 sit*^{w} did not germinate in water or PEG (Fig. 6A). Detipping caused a decrease in the critical osmotic potential in the wild-type by 0.5 MPa (Fig. 6). Unfortunately, seed-borne infections prevented the prolonged incubation of detipped *sit*^{w} seeds that was required at strong negative osmotic potentials. After detipping the radicles of GA-deficient seeds also extended, their resistance to osmotic stress was about 0.6 MPa less than in wild type, and the recombinant took an intermediate position (Fig. 6B).

Observations of the rate of germination also showed differences between the genotypes. In water, *sit*^{w} seeds germinated a few hours earlier than wild-type seeds; in −0.5 MPa PEG, the germination of *sit*^{w} seeds was less delayed than wild-type seeds (Fig. 7A). In water, radicles of all genotypes extended at the same high rate from detipped seeds (Fig. 7B). In −0.5 MPa, PEG radicle protrusion from detipped seeds of GA-producing genotypes (*sit*^{w}, wild type) started earlier and occurred at a higher rate than that of GA-deficient genotypes (*gib-1 sit*^{w}, *gib-1*), whereas radicles of ABA-producing genotypes (wild type, *gib-1*) protruded later and at a slower rate than those of ABA-deficient genotypes (*sit*^{w}, *gib-1 sit*^{w}).

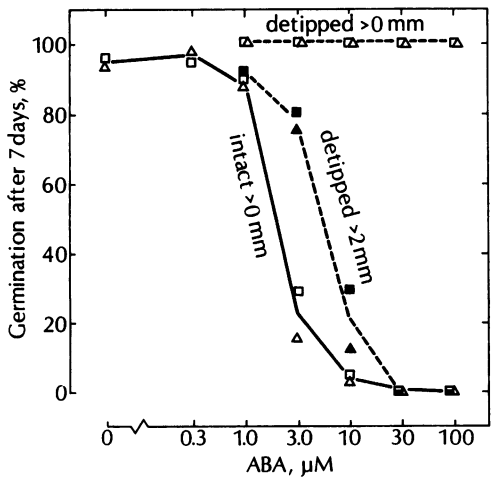

Figure 5. Effect of exogenous (±)ABA on the germination, or growth, of intact (solid line) or detipped (broken lines) seeds of wild type (△, ▲) and *sit*^{w} (□, ■). In detipped seeds, endosperm plus testa layers opposing the radicle tip were removed within 4 h after the start of incubation. The criterion for germination was either visible radicle protrusion (open symbols) or radicle growth surpassing 2 mm (closed symbols).

Table V. *Influence of ABA on the GA_{4+7}-Induced Endosperm Weakening, Tested by Measuring the Force to Puncture Endosperm plus Testa Layers Originally Opposing the Radicle Tip*

The seed was cut and the radicle was removed after 3 h incubation in distilled water. The puncture force was determined either directly or after additional incubation for 21 h in 1.5 mL test solution at 26°C in darkness. The puncture force is expressed as the mean ± SD of two duplicate samples of 10 deembryonated seed parts.

Incubation Period	Incubation Medium		Puncture Force (n = 20)
	GA_{4+7}	ABA	
h	*μM*		*N*
3	0	0	0.57 ± 0.07
24	0	0	0.58 ± 0.06
24	0.2	0	0.40 ± 0.08
24	0.2	0.3	0.53 ± 0.08
24	0.2	1.0	0.57 ± 0.05
24	0.2	3.0	0.57 ± 0.06
24	0	3.0	0.56 ± 0.06

DISCUSSION

Role of ABA in Induction of Dormancy

The present experiments clearly prove that in tomato endogenous ABA plays an important role in the induction of dormancy during seed development. The results are in good agreement with the studies of the role of ABA in dormancy induction in *A. thaliana* (11). As in *Arabidopsis*, the ABA fraction in tomato embryo and endosperm is decisive for induction of dormancy. In our previous study, we showed that during seed development ABA levels in wild-type seeds reached a maximum of 36 ng·seed^{-1} at 30 d after pollination, in contrast to 1 ng·seed^{-1} in *sit*^{w} seeds (8). *Sit/sit*^{w} seeds developing in *sit*^{w}/*sit*^{w} fruits contained maximally 10 ng·seed^{-1} at 40 d after pollination. Germination percentages of freshly harvested mature seeds in water showed a good correlation with the ABA levels during development. Germination of both *Sit/Sit* seeds and *Sit/sit*^{w} seeds from *sit*^{w}/*sit*^{w} fruits was inhibited, whereas *sit*^{w}/*sit*^{w} seeds from both ABA-deficient *sit*^{w}/*sit*^{w} fruits and from ABA-rich *Sit/sit*^{w} fruits germinated directly after harvest (Fig. 1). There is some evidence from the data that maternal ABA may add to the action of zygotic ABA because *Sit/Sit* seeds were more dormant than *Sit/sit*^{w} seeds from *sit*^{w}/*sit*^{w} fruits (Table I). Differences in translocation of maternal ABA from the foliage to the developing seeds might be the reason for this. Whereas wild-type leaves produce large amounts of ABA, particularly during water stress, *sit*^{w} leaves do not (3). Hoad (9) found in lupin that the level of ABA in the phloem sap increased with water stress and, concomitantly, the ABA content of the developing seeds. A small increase of the ABA levels in both testa and cotyledons, in reaction to water stress, was also reported for developing soybean seeds (2).

Maintenance of Dormancy

Freshly harvested seeds of the different genotypes still showed a difference in ABA content (Table IV). It has to be questioned whether ABA present in mature seeds is still

responsible for inhibition of germination. Experiments with after-ripened seeds gave an indirect answer to this question. Seeds of both wild type and *sit*^{w} were equally sensitive to exogenous ABA; 50% inhibition occurred at about 2 μM ABA (Fig. 5). At the end of imbibition, tomato seeds had taken up about 4 μL water·seed^{-1} (our unpublished data). In case of unobstructed diffusion, each seed should have absorbed about 2 ng ABA from the 2 μM solution. However, Velasco and Stoner (20) showed that in tomato seeds diffusion barriers exist that reduce the penetration of ABA to about 50%. Thus, the absorption from a 2 μM ABA solution will be about 1 ng·seed^{-1}. That amount approaches the 0.8 ng·seed^{-1} of endogenous ABA in dry after-ripened seeds of the wild type (Table IV). However, those seeds fully germinated in water at 26°C. The lack of an inhibiting effect of endogenous ABA is most likely due to the considerable reduction of the ABA level during imbibition. It was shown in lettuce seeds that absorbed ABA, at a level comparable to the endogenous amount in dry seeds, was only inhibitory to germination if it was maintained throughout incubation (10). We suppose that in freshly harvested tomato seeds the endogenous ABA level of 1.8 ng·seed^{-1} will also be reduced during imbibition. Therefore, it is unlikely that actual ABA levels in mature seeds are responsible for the observed differences in dormancy between wild-type and *sit*^{w} seeds.

The data in Figure 4 also show that endosperm hydrolysis, a GA-dependent process, does not occur with freshly harvested wild-type seeds. Again, because it is unlikely that this inhibition occurs because of direct ABA action, it will be an extended result of ABA action during seed development, which may interfere with GA production, transport, or sensitivity. Studies with *A. thaliana* (12) and *H. annuus* (14) support the hypothesis that dormancy is related to decreased GA sensitivity. Seed of ABA-deficient lines of *Arabidopsis* were 100-fold more sensitive to GA_{4+7} than ABA-producing lines, and preincubation in water of dormant *Helianthus* embryos resulted in both an increase of the sensitivity to GA and a relief of dormancy.

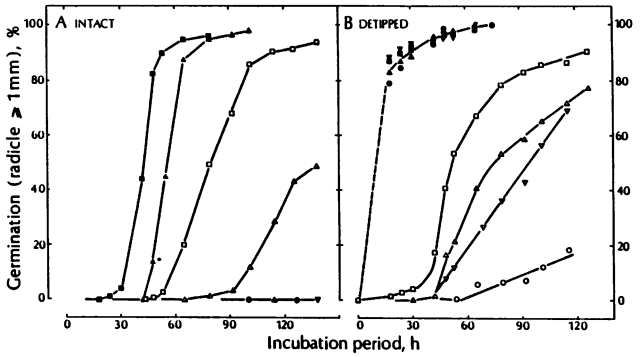

Figure 7. Rate of germination of intact (A) or detipped (B) seeds of wild type (▲, △) and *sit*^{w} (■, □), *gib-1 sit*^{w} (▼, ▽), and *gib-1* (●, ○) in water (closed symbols) or −0.5 MPa PEG (open symbols). In detipped seeds, endosperm plus testa layers opposing the radicle tip were removed within 4 h after the start of incubation. The criterion for germination was radicle growth surpassing 1 mm.

Vivipary

Differences in the actual ABA levels are also an unlikely explanation for the differences in viviparous germination between genotypes as observed in the present study (Fig. 3). Although the juice of ripe wild-type fruits contained 10 times higher ABA levels than that of *sit*^{w} fruits (Table II), a concentration of 0.84 μM ABA was still too low to explain direct inhibition of germination (Fig. 5). The most likely explanation for the different tendency to vivipary is the difference in sensitivity to osmotic stress. After-ripened seeds of *sit*^{w} were inhibited by a 0.5 MPa lower osmotic potential than wild-type seeds (Fig. 6). The osmotic potential of the fruit juice was about −0.9 MPa in both genotypes (Table II). This potential only inhibits the germination of wild-type seeds. Although Table II gives at first view the opposite impression, the primary control of viviparous germination is of an osmotic nature. The conclusions are in accordance with those of

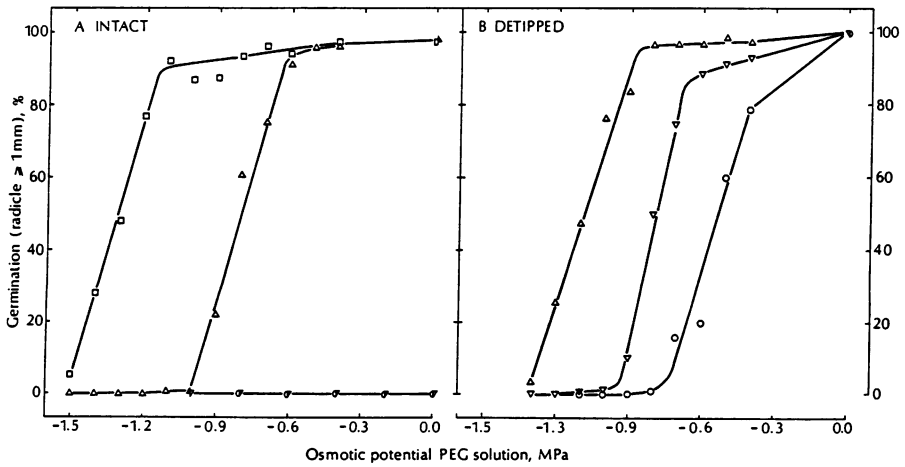

Figure 6. Effect of osmotic potential of the incubation medium on the germination of intact (A) or detipped (B) seeds of wild type (△), *sit*^{w} (□), *gib-1 sit*^{w} (▽), and *gib-1* (○). In detipped seeds, endosperm plus testa layers opposing the radicle tip were removed within 4 h after the start of incubation. The criterion for germination was radicle growth surpassing 1 mm, which was scored after 12 d.

Dörffling (4), who also postulated a secondary role of ABA in the prevention of vivipary in tomato.

Persistence of ABA-Induced Dormancy Phenomena in After-Ripened Seeds

It was mentioned above that after-ripened seeds of *sit*^{w} and wild type showed similar endosperm weakening and germination (Fig. 4). They differed only in the moment that radicle protrusion from intact seeds started (Figs. 4 and 7A). However, the application of osmotic stress revealed that the results of the ABA action during development still persisted in after-ripened seeds. Even after several years of storage, seeds of the ABA-deficient mutant showed much higher osmotic resistance, germination occurred at more negative osmotic potentials (Fig. 6A), and at −0.5 MPa radicle protrusion occurred much earlier than in wild-type seeds (Fig. 7A). Recently, Ni and Bradford (17) showed that the ABA levels in tomato embryos and endosperm were not influenced by imbibition at a range of water potentials up to −0.6 MPa during 5 d. Also, the ABA content of *sit*^{w} seeds was not increased by incubation in osmotic solutions (M.H.M. Cornelussen and H.W.M. Hilhorst, personal communications). It is hypothesized that the high ABA levels that occur during seed development in wild-type seeds induce an inhibition of cell elongation of the radicle that can still be observed after long periods of dry storage when ABA levels have been reduced considerably. In seeds of *Brassica napus*, applied ABA inhibited cell elongation at the site of cell wall loosening without affecting the osmotic potential of the seeds (18). It cannot be decided yet whether endogenous ABA similarly affected tomato seeds.

Separation of the Actions of ABA and GA

The present data support the revision of the hormone balance theory of seed dormancy that was based on studies in *A. thaliana* (12). Also, during seed germination in tomato, endogenous ABA and GA do not act simultaneously. ABA induces dormancy during seed development, whereas endogenous GA does not essentially interfere with seed growth and development (5). However, GA is absolutely required for germination.

The data from our present study show that ABA-induced dormancy resulted in a lower resistance to osmotic stress, which in intact seeds only became visible when seeds could produce GA (Figs. 6A and 7A). In water, radicle growth of GA-deficient genotypes only occurred in detipped seeds. GA deficiency further decreased the resistance to osmotic inhibition (compare *gib-1* to wild type in Figs. 6B and 7B). The difference caused by ABA during development was maintained, however be it at less negative osmotic potentials (compare *sit*^{w} to wild type and *gib-1 sit*^{w} to *gib-1* in Fig. 7B).

Apart from the separation of ABA and GA action in time, their sites of action also differ. Endogenous ABA induces a difference in the growth potential of the embryo, whereas the main effect of endogenous GA is on the weakening of the mechanical restraint of the endosperm.

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