Plant Physiol. (1993) 101: 607–617

Germination and Dormancy of Abscisic Acid- and Gibberellin-Deficient Mutant Tomato (*Lycopersicon esculentum*) Seeds[1]

Sensitivity of Germination to Abscisic Acid, Gibberellin, and Water Potential

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Germination responses of wild-type (*MM*), abscisic acid (ABA)-deficient (*sit*^{w}), and gibberellin (GA)-deficient (*gib-1*) mutant tomato (*Lycopersicon esculentum* Mill. cv Moneymaker) seeds to ABA, GA_{4+7}, reduced water potential (ψ), and their combinations were analyzed using a population-based threshold model (B.R. Ni and K.J. Bradford [1992] Plant Physiol 98: 1057–1068). Among the three genotypes, *sit*^{w} seeds germinated rapidly and completely in water, *MM* seeds germinated more slowly and were partially dormant, and *gib-1* seeds did not germinate without exogenous GA_{4+7}. Times to germination were inversely proportional to the differences between the external osmoticum, ABA, or GA_{4+7} concentrations and the corresponding threshold levels that would either prevent (ψ_b, $\log[ABA_b]$) or promote ($\log[GA_b]$) germination. The sensitivity of germination to ABA, GA_{4+7}, and ψ varied widely among individual seeds in the population, resulting in a distribution of germination times. The rapid germination rate of *sit*^{w} seeds was attributable to their low mean ψ_b (−1.17 MPa). Postharvest dormancy in *MM* seeds was due to a high mean ψ_b (−0.35 MPa) and a distribution of ψ_b among seeds such that some seeds were unable to germinate even on water. GA_{4+7} (100 μM) stimulated germination of *MM* and *gib-1* seeds by lowering the mean ψ_b to −0.75 MPa, whereas ABA inhibited germination of *MM* and *sit*^{w} seeds by increasing the mean ψ_b. The changes in ψ_b were not due to changes in embryo osmotic potential. Rather, hormonal effects on endosperm weakening opposite the radicle tip apparently determine the threshold ψ for germination. The analysis demonstrates that ABA- and GA-dependent changes in seed dormancy and germination rates, whether due to endogenous or exogenous growth regulators, are based primarily upon corresponding shifts in the ψ thresholds for radicle emergence. The ψ thresholds, in turn, determine both the rate and final extent of germination within the seed population.

Seed germination is sensitive to both endogenous plant growth regulators and environmental factors. Both ABA and reduced ψ (Table I) inhibit seed germination, and their interactions have been analyzed for several species (Schopfer and Plachy, 1985; Kermode, 1990; Welbaum et al., 1990; Ni and Bradford, 1992). The inhibitory effect of exogenous ABA on germination is empirically similar to, and quantitatively additive with, that of reduced ψ (Schopfer and Plachy, 1985; Welbaum et al., 1990; Ni and Bradford, 1992). In addition, high ABA concentrations caused an increase in the sensitivity of germination to reduced ψ (Ni and Bradford, 1992). GAs are also involved in the regulation of seed development and germination but with opposite effects to those of ABA. In general, ABA delays or prevents seed germination and determines the depth of dormancy during development, whereas GA breaks dormancy and promotes germination upon imbibition by the mature seeds (Karssen et al., 1983, 1987; Koornneef et al., 1989; Groot and Karssen, 1992). The quantitative amounts of ABA during development result in different depths of dormancy, which in turn require different amounts of GA to stimulate germination. Hence, ABA-deficient mutants produce nondormant seeds that germinate rapidly, whereas GA-deficient mutant seeds will not germinate without additional GA (Karssen et al., 1983, 1987; Groot and Karssen, 1992).

The responses of germination to ABA, osmoticum, and their combination have been quantitatively characterized using a population-based threshold model (Ni and Bradford, 1992). Seed germination rates and percentages can be predicted with reasonable accuracy at any given ABA concentration or ψ on the basis of the derived threshold values for each factor and their respective time constants. The basic assumption of the model is that the rate of approach of a seed toward germination is proportional to the difference between the factor level and the seed's threshold value for the regulatory factor. The response of germination to reduced ψ, for example, can be expressed as

$$\theta_H = [\psi - \psi_b(g)]t_g \quad (1)$$

or

$$GR_g = 1/t_g = [\psi - \psi_b(g)]/\theta_H \quad (2)$$

where θ_H is the "hydrotime constant," ψ is the water potential of the imbibition medium, $\psi_b(g)$ is the base (threshold or minimum) ψ allowing germination of percentage g, t_g is the time required for germination of percentage g, and GR_g is the germination rate of the seeds of fraction or percentage g (Gummerson, 1986; Bradford, 1990). The normal distribution

[1] Supported by National Science Foundation grants DCB-8817758 and IBN-9204782 to K.J.B.

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The abbreviations used in this article are listed in Table I.

Table I. *Abbreviations used in this paper*

ABA_0	the highest ABA concentration having no effect on germination
ABA_{endo}	endogenous ABA
ABA_{exo}	exogenous ABA
$ABA_b(g)$	base ABA concentration inhibiting germination of percentage g
$ABA_b(50)$	mean base ABA concentration reducing germination to 50%
GR_g	germination rate (inverse of time to germination) for percentage g
GA_0	GA concentration stimulating maximum germination rates
GA_{endo}	endogenous GA
GA_{exo}	exogenous GA
$GA_b(g)$	base GA concentration stimulating germination of percentage g
$GA_b(50)$	mean base GA concentration stimulating 50% germination
gib-1	GA-deficient mutant tomato
MM	wild type cv Moneymaker tomato
ψ	water potential
$\psi_b(g)$	base water potential inhibiting germination of percentage g
$\psi_b(50)$	mean base ψ reducing germination to 50%
ψ_π	osmotic potential
RWC	relative water content
σ_{ψ_b}	standard deviation of $\psi_b(g)$
σ_{ABA_b}	standard deviation of $ABA_b(g)$
σ_{GA_b}	standard deviation of $GA_b(g)$
sit^w	ABA-deficient mutant *sitiens* tomato
t_g	time to radicle emergence of percentage g
$t_g(0)$	time to radicle emergence of percentage g in water
$t_g(ABA)$	time to radicle emergence of percentage g in ABA
θ_H	hydrotime constant
θ_{ABA}	ABA time constant
θ_{GA}	GA time constant

of $\psi_b(g)$ values among seeds in the population can be characterized by its mean [$\psi_b(50)$] and SD (σ_{ψ_b}), derived using repeated probit analysis (Bradford, 1990). The resulting probit equation is

$$\text{probit}(g) = [\psi - (\theta_H/t_g) - \psi_b(50)]/\sigma_{\psi_b} \quad (3)$$

which relates g to t_g at any constant ψ. Seed germination time courses can then be predicted at any ψ on the basis of the three basic parameters θ_H, $\psi_b(50)$, and σ_{ψ_b}.

The same formal equations also can be used to quantitate and predict seed germination behavior in the presence of exogenous ABA (Ni and Bradford, 1992). The defining model is

$$\theta_{ABA} = (\log[ABA] - \log[ABA_b(g)])t_g \quad (4)$$

and the probit equation relating germination percentage to ABA concentration and imbibition time is

$$\text{probit}(g) = \{\log[ABA] - (\theta_{ABA}/t_g) - \log[ABA_b(50)]\}/\sigma_{ABA_b} \quad (5)$$

In this case, the relevant parameters are the "ABA time constant" (θ_{ABA}), the log of the exogenous ABA concentration (log[*ABA*]), the normally distributed log threshold or base ABA concentrations inhibiting germination of percentage g (log[$ABA_b(g)$]), the log of the mean base or threshold ABA concentration (log[$ABA_b(50)$]), and the SD of ABA_b values (σ_{ABA_b}). To determine whether endogenous ABA influences germination in a manner consistent with this model, we have examined the sensitivity to both ABA and ψ of sit^w seeds (Karssen et al., 1987; Groot and Karssen, 1992) and its wild-type parent, *MM*.

Seeds from a tomato mutant deficient in GA (*gib-1*) require applied GA to germinate, and germination percentage increases as the GA concentration increases (Groot, 1987; Groot and Karssen, 1987; Groot et al., 1988). We have examined whether the responses of seed germination to GA can be analyzed using the same model used for ABA responses but based upon a threshold concentration of GA permitting, rather than inhibiting, germination. The defining model would be

$$\theta_{GA} = (\log[GA] - \log[GA_b(g)])t_g \quad (6)$$

and the relationship among germination percentage, imbibition time, and GA concentration is

$$\text{probit}(g) = \{\log[GA] - (\theta_{GA}/t_g) - \log[GA_b(50)]\}/\sigma_{GA_b} \quad (7)$$

where θ_{GA} is the "GA time constant," log[*GA*] is the GA concentration of the imbibition medium, log[$GA_b(g)$] is the normally distributed threshold or base GA concentration permitting germination of percentage g, and log[$GA_b(50)$] and σ_{GA_b} are the mean and SD of GA_b values, respectively. The model predicts that, as the GA concentration increases above the threshold level for a given seed, the time to germination will be shortened proportionately, because θ_{GA} is a constant (Eq. 6).

We show here that these models adequately describe the germination characteristics of wild-type, sit^w, and *gib-1* seeds and their responses to applied hormones and reduced ψ. The influence of both endogenous and exogenous growth regulators on germination behavior and water relations can be quantitatively evaluated using this approach. We further demonstrate that the effects of ABA and GA on germination and dormancy are mediated primarily through changes in the threshold ψs permitting radicle emergence.

MATERIALS AND METHODS

Plant Material

Tomato (*Lycopersicon esculentum* Mill.) seeds of the homozygous *gib-1* and sit^w mutants and their wild-type isogenic parent (cv *MM*) were obtained from Dr. C.M. Karssen, Agricultural University, Wageningen, The Netherlands. Plants of all genotypes were grown in potting soil in a greenhouse. The *gib-1* plants were sprayed with 10 μM GA_{4+7} once or twice each week to stimulate shoot growth and flower development (Groot, 1987). Seeds were extracted from mature fruit, fermented in the juice for 1 d, rinsed, dried, and stored at 4°C.

Seed Germination

Four replicates of 50 seeds each were placed on two 4.5-cm filter papers in 5-cm Petri dishes moistened with 4 mL of water, ABA, GA_{4+7}, or PEG solutions. The dishes were covered with tightly fitting lids, placed in a covered polystyrene box lined with water-saturated tissue paper, and incubated in the dark at 25 ± 1°C. The PEG solutions were prepared according to the procedure of Michel (1983) using PEG 8000 (Carbowax PEG 8000; Fisher Scientific Co., Fair Lawn, NJ), and the ψs of the solutions were verified with a vapor pressure osmometer (model 5100C; Wescor Inc., Logan, UT) calibrated with NaCl standards. It has been reported that the exclusion of PEG from the filter paper matrix can cause errors in ψ determinations (Hardegree and Emmerich, 1990). However, a similar solution volume to filter paper weight ratio was present in both the osmometer measurement and in the germination dishes. The ψ measured by the osmometer should, therefore, correspond to that in the incubation dishes. The ABA and GA_{4+7} solutions were prepared by dissolving ABA (Sigma, St. Louis, MO) and GA_{4+7} (Abbott Laboratories, North Chicago, IL) in 1 M KOH and diluting with distilled water. Solutions containing PEG were changed after the first 24 h and then weekly thereafter, and ABA and GA solutions were changed every 2 weeks. Clearly visible radicle protrusion was used as the criterion for the completion of germination.

Measurement of Embryonic RWC and ψ_π

Embryo water contents (dry weight basis) were measured by oven drying at 130°C for 1 h. Embryo ψ_π was determined by thermocouple psychrometry on frozen and thawed tissue using the vapor pressure osmometer. For each genotype, the embryonic water content of seeds that had fully imbibed on water was taken as 100% RWC. The ψ_π values determined for each genotype that had imbibed in PEG, ABA, or GA_{4+7} were corrected for water content changes as described previously (Ni and Bradford, 1992).

Data Analysis

Data analyses to determine the values of the model parameters were conducted using repeated probit regression as described previously (Bradford, 1990; Dahal and Bradford, 1990; Ni and Bradford, 1992).

RESULTS

Germination of *MM* Seeds in ABA, GA, or Reduced ψ

Groot and Karssen (1992) reported that the occurrence of postharvest dormancy in *MM* seeds is sensitive to environmental conditions during seed development. The final germination of our *MM* seed lot was only about 75% in water because of partial dormancy. Although 1 μM ABA had no effect on germination in comparison with the water control, higher concentrations progressively delayed and inhibited germination (Fig. 1A). The threshold model fit the data well (r^2 = 0.93; Table II), and the predicted curves are shown in Figure 1A. The low $ABA_b(50)$ of 6 μM (Table II) indicates the relatively high sensitivity of this seed lot to ABA. The σ_{ABA_b} value (0.84 log[M]) reflects wide variation in ABA sensitivity among individual seeds (Table II).

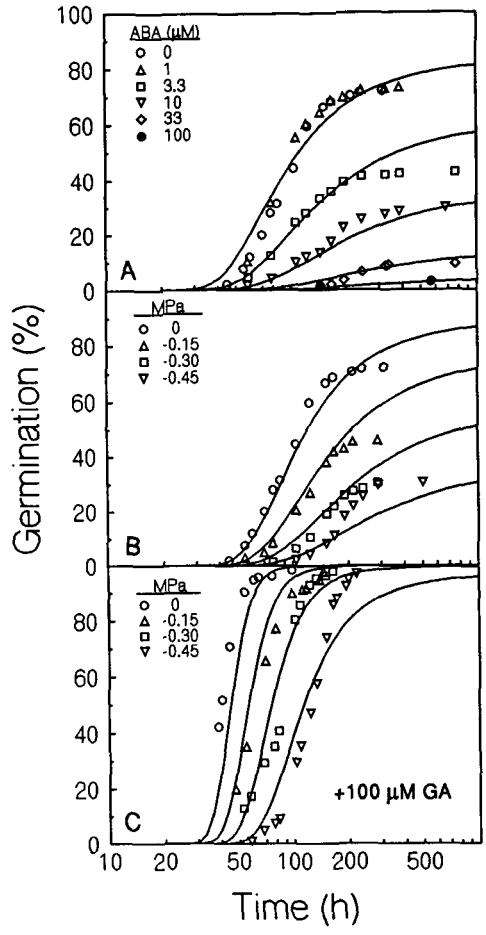

Figure 1. Cumulative germination time courses of *MM* seeds incubated at a range of ABA concentrations (A), ψ (B), and ψ + 100 μM GA_{4+7} (C). The symbols represent the actual data, and the solid curves are predicted by the threshold models (Eqs. 3 and 5) using the parameters in Table II. The time axis is plotted on a logarithmic scale to separate the curves for clarity.

Germination of *MM* seeds was markedly delayed and inhibited by small reductions in ψ (Fig. 1B). This sensitivity to ψ was evident in the high $\psi_b(50)$ value of −0.35 MPa (Table II). With the addition of 100 μM GA_{4+7}, however, germination rate was greatly enhanced, and seed dormancy was completely broken (Fig. 1C). Germination was complete within 100 h on 100 μM GA_{4+7}, a time when <50% germination had occurred on water (Fig. 1, A and B). Germination was also much less sensitive to reduced ψ in the presence of GA_{4+7} (Fig. 1C). This increased tolerance to low ψ was mainly attributable to a GA-induced lowering of $\psi_b(50)$ from −0.35 to −0.75 MPa, although the θ_H was also reduced by 20% (Table II).

The distributions of $\psi_b(g)$ for *MM* seeds in the presence and absence of GA_{4+7} are shown diagrammatically in Figure 2A. The relative frequency of a particular ψ_b value within the

Table II. *Parameters of water relations, ABA, and GA models characterizing seed germination time courses*

The parameters were derived using probit analysis of data from all factor levels. The time constants include θ_H (MPa h), θ_{ABA}, and θ_{GA} (log[M] h). The mean base values are $\psi_b(50)$ (MPa), $ABA_b(50)$ and $GA_b(50)$ (log[M] or μM). The corresponding SD (σ) of each threshold distribution and coefficients of determination (r^2) of the probit regressions are also shown.

Genotype	Factor	Time Constants	Mean Base Values	σ	r^2
MM	ABA	−108 log[M] h	−5.2 (6 μM)	−0.84 log[M]	0.93
MM	ψ	42 MPa h	−0.35 MPa	0.28 MPa	0.95
MM	ψ + 100 μM GA_{4+7}	34 MPa h	−0.75 MPa	0.15 MPa	0.86
sit^w	ABA	−48 log[M] h	−4.9 (13 μM)	−0.54 log[M]	0.91
sit^w	ψ	45 MPa h	−1.17 MPa	0.33 MPa	0.96
sit^w	ψ + 4.5 μM ABA	72 MPa h	−0.90 MPa	0.43 MPa	0.91
gib-1	GA_{4+7}	99 log[M] h	−5.9 (1.3 μM)	1.01 log[M]	0.97
gib-1	ψ + 10 μM GA_{4+7}	40 MPa h	−0.41 MPa	0.40 MPa	0.96
gib-1	ψ + 100 μM GA_{4+7}	41 MPa h	−0.71 MPa	0.23 MPa	0.97

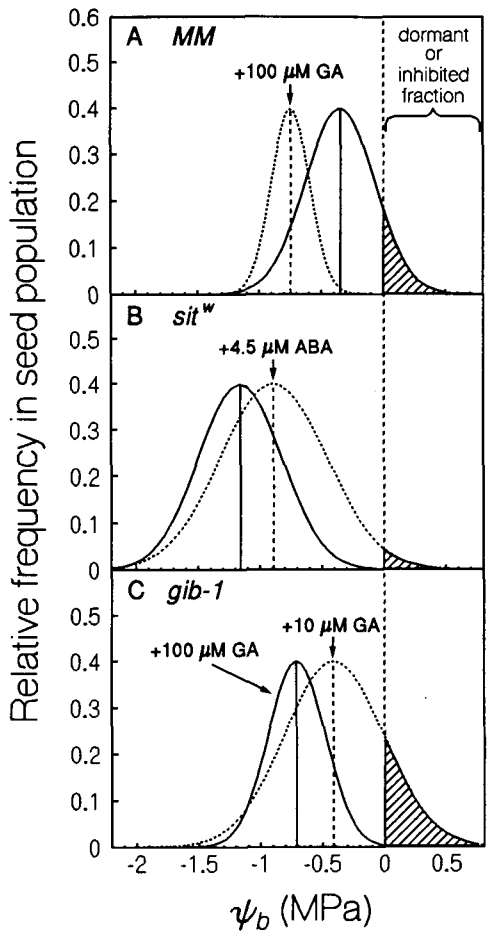

Figure 2. Relative frequency distributions of $\psi_b(g)$ values within seed populations of *MM* (A), *sit*^{w} (B), and *gib-1* (C). The ordinate indicates the relative frequency within the seed population of the possible values of ψ_b indicated on the abscissa. The vertical dotted line at 0 MPa shows the highest possible $\psi_b(g)$ at which germination will occur in water. The shaded areas indicate the fraction of dormant or inhibited seeds in each case.

seed population is illustrated as a normal distribution based upon the $\psi_b(50)$ and σ_{ψ_b} values in Table II. The high $\psi_b(50)$ and relatively large σ_{ψ_b} for *MM* seeds result in a distribution that extends above 0 MPa (shaded region in Fig. 2A). Because ψ cannot exceed 0 MPa, the fraction of the seed population with an estimated $\psi_b(g) > 0$ MPa would be unable to germinate even on water, accounting for the percentage of dormant seeds. In addition, as the external ψ is reduced, the ψ_b of an increasing fraction of the population would exceed the current ψ level and be prevented from germinating. The time to completion of germination of the remaining seeds would also be delayed, because the time to germination is inversely proportional to the difference between the seed ψ and its own ψ_b threshold, generating the ψ response curves shown in Figure 1B. In the presence of 100 μM GA_{4+7}, the $\psi_b(g)$ distribution is shifted to lower values and is narrower because of the smaller σ_{ψ_b} (Fig. 2A). The distribution does not extend above 0 MPa, in agreement with 100% germination on water or breaking of dormancy (Fig. 1C). The greater tolerance to reduced ψ in the presence of GA_{4+7} (Fig. 1C) is also evident from the position of the $\psi_b(g)$ distribution, because ψ would have to be reduced considerably before it would be below the threshold of a significant fraction of seeds in the population. We can conclude, therefore, that both dormancy and its release by GA_{4+7} in *MM* seeds are based upon the values of the $\psi_b(g)$ distributions within the seed populations and that the time to germination, final germination percentage, and sensitivity of germination to reduced ψ are all linked manifestations of this underlying ψ threshold distribution.

Germination of *sit*^{w} Seeds in ABA or Reduced ψ

In contrast to its wild-type parent, all homozygous *sit*^{w} seeds germinated rapidly in water, and higher concentrations of ABA were required to inhibit germination of this genotype in comparison with *MM* seeds (Fig. 3A). The more rapid germination of *sit*^{w} seeds resulted in a θ_{ABA} of −48 log[M] h, as compared to −108 log[M] h for *MM*, and the $ABA_b(50)$ was 13 μM, double that of *MM* seeds (Table II).

Bradford (1990) derived a factor $[1 - \psi/\psi_b(g)]$ that could be used to normalize the times to completion of germination

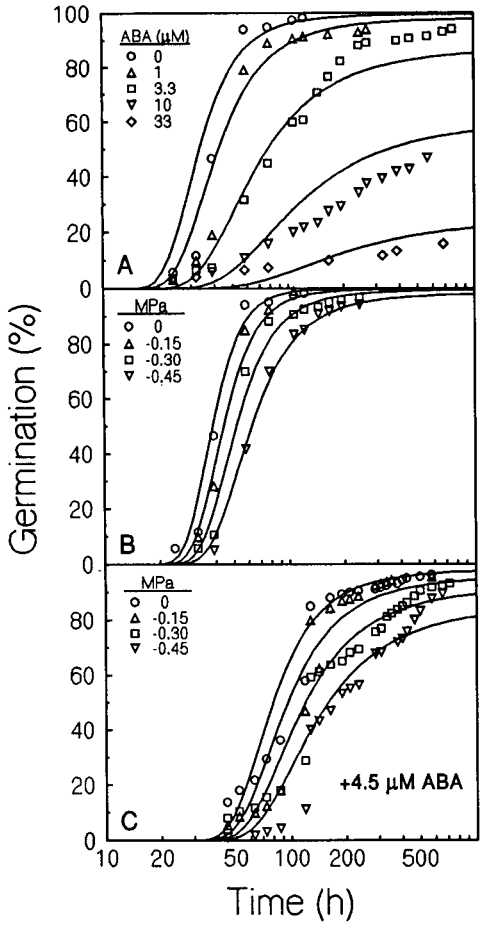

Figure 3. Cumulative germination time courses of *sit*^{w} seeds incubated at a range of ABA concentrations (A), ψ (B), and ψ + 4.5 μM ABA (C). The symbols represent the actual data, and the solid curves are predicted by the threshold models (Eqs. 3 and 5) using the parameters in Table II. The time axis is plotted on a logarithmic scale to separate the curves for clarity.

at different ψ values to a common time course equal to that in water. The same procedure was used here to normalize the germination time courses at different ABA concentrations to a common time course equal to that in water. For the *sit*^{w} seeds, we can calculate from Table II that the highest ABA concentration that would have no effect on germination (ABA_0) would be 0.3 μM (i.e. 99.9% germination = 3 SD units above the mean; $\log[ABA_b(99.9)] = -4.9 - 3(0.54) = -6.5$ log[M], or 0.3 μM). The maximum ABA concentration permitting germination of any individual seed would be $\log[ABA_b(g)]$, so $\log[ABA_0] - \log[ABA_b(g)]$ represents the maximum range of ABA concentrations allowing germination of percentage g. Normalizing the ABA model (Eq. 4) by dividing both sides of the equation by $\log[ABA_0] - \log[ABA_b(g)]$, the resulting equation is

$$t_g(0) = \left(\frac{\log[ABA] - \log[ABA_b(g)]}{\log[ABA_0] - \log[ABA_b(g)]}\right) t_g(ABA) \qquad (8)$$

where $t_g(0)$ is the time to germination at the minimum threshold ABA concentration (equivalent to that in water) and $t_g(ABA)$ is the time to germination at a given higher ABA concentration. Figure 4 shows the germination time courses of *sit*^{w} seeds at various ABA concentrations (from Fig. 3A) normalized to a single curve approximating the time course in water (closed symbols and solid line). The ability of this normalization equation to collapse the array of curves in Figure 3A to a single curve in Figure 4 is an indication of the degree to which parameters derived from the model describe the actual germination responses.

The rapid germination of *sit*^{w} seeds was particularly evident at reduced ψ. Germination of *sit*^{w} seeds was complete within 200 h at −0.45 MPa (Fig. 3B), whereas only 30% of *MM* seeds were able to germinate at this ψ (Fig. 1B). The rapid germination observed in this genotype resulted entirely from its low $\psi_b(50)$ (−1.17 MPa), because its θ_H value was essentially the same as that of *MM* seeds (Table II). Using the ABA model, we can calculate that the *sit*^{w} seeds on 4.5 μM ABA should germinate at approximately the same rate as *MM* seeds on water. We tested this prediction by germinating *sit*^{w} seeds in water or osmotic solutions containing 4.5 μM ABA (Fig. 3C). The addition of ABA did delay germination to approximate that of the *MM* seeds in water, but a higher concentration of ABA was apparently required to completely reproduce the wild-type behavior at all ψ values (compare with Fig. 1B). The $\psi_b(50)$ value of *sit*^{w} seeds increased from −1.17 to −0.90 MPa in the presence of 4.5 μM ABA, which is still much lower than the $\psi_b(50)$ of −0.35 MPa of *MM* seeds (Table II). This change in the $\psi_b(g)$ distribution of *sit*^{w} seeds due to ABA is shown in Figure 2B, which illustrates that,

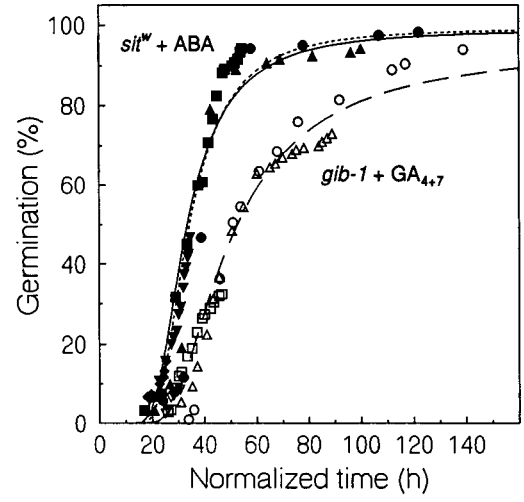

Figure 4. Cumulative germination time courses for *sit*^{w} (from Fig. 3A; closed symbols) and for *gib-1* seeds (from Fig. 5A; open symbols) at a range of ABA and GA_{4+7} concentrations, respectively, plotted on a normalized time scale. The data for *sit*^{w} seeds are normalized to the predicted time in water (solid curve) using Equation 8 and the values in Table II, and those for *gib-1* seeds are normalized to the predicted time course occurring in 100 μM GA_{4+7} (dashed curve) using Equation 8 (with GA substituted for ABA) and the values in Table II. The dotted line represents the theoretical germination time course predicted by the model for *gib-1* seeds germinating in 1 mM GA_{4+7}.

although ABA increased the mean ψ_b value of the population, the majority of seeds still had thresholds considerably below those of *MM* seeds (Fig. 2A). Previous work with a different genotype showed that the mean ψ_b value would increase further at higher ABA concentrations (Ni and Bradford, 1992). The θ_H also increased from 45 to 72 MPa h in the presence of 4.5 μM ABA (Table II). Thus, ABA delayed germination by a dual effect on the θ_H and on the $\psi_b(g)$ distribution.

Germination of *gib-1* Seeds in GA or Reduced ψ

Seeds of the *gib-1* mutant showed a complete and quantitative dependence of germination on GA_{4+7}. No germination was observed without applied GA_{4+7}, and the germination percentage and rate progressively increased with increasing GA_{4+7} concentrations (Fig. 5A). The models of Equations 6 and 7 fit the GA response of *gib-1* seeds very well ($r^2 = 0.97$; Table II; predicted curves in Fig. 5A). The very low value of $GA_b(50)$ (1.3 μM) indicates the high sensitivity of germination

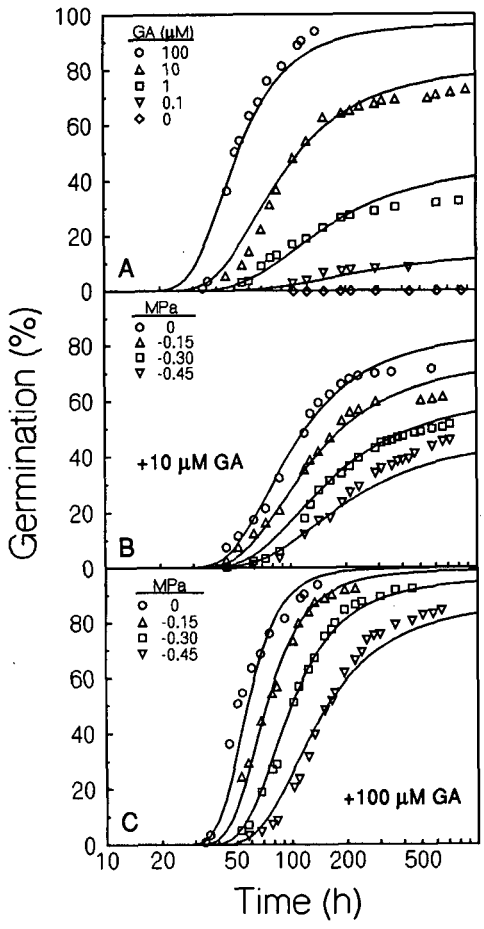

Figure 5. Cumulative germination time courses of *gib-1* seeds incubated at a range of GA_{4+7} concentrations (A), ψ + 10 μM GA_{4+7} (B), and ψ + 100 μM GA_{4+7} (C). The symbols represent the actual data, and the solid curves are predicted by the threshold models (Eqs. 3 and 7) using the parameters in Table II. The time axis is plotted on a logarithmic scale to separate the curves for clarity.

to GA in these GA-deficient mutant seeds. As for ABA, a function can be developed to normalize germination time courses at any GA concentration to that predicted for a saturating dose of GA. The function will be the same as Equation 8 with GA values substituted for ABA, except that GA_0 will be the saturating dose of GA_{4+7} (100 μM) rather than the minimum threshold dose. This function worked well to collapse the array of time courses in Figure 5A to a single normalized time course (Fig. 4, open symbols and dashed line).

When incubated in osmoticum containing 10 μM GA_{4+7}, the germination time courses and water relations parameters of *gib-1* seeds were similar to those of *MM* seeds in the absence of GA (compare Figs. 1B and 5B; Table II). When both genotypes were supplied with 100 μM GA_{4+7}, the germination behavior and water relations parameters of *gib-1* and *MM* seeds were again very similar (compare Figs. 1C and 5C; Table II). GA_{4+7} could, therefore, restore both the germination rate and water potential sensitivity of *gib-1* seeds to equal that of its wild-type parent. This is evident in the $\psi_b(g)$ distributions of *gib-1* seeds in either 10 or 100 μM GA_{4+7} (Fig. 2C), which correspond closely to those of *MM* seeds in water or 100 μM GA_{4+7} (Fig. 2A). Projecting this trend to lower GA_{4+7} concentrations, we can suggest that the threshold distribution would continue to increase, resulting in the progressive delay and reduction in germination evident in Figure 5A. On the other hand, when GA_{4+7} stimulates germination of dormant *gib-1* seeds, the entire population distribution of $\psi_b(g)$ is shifted to lower values, accounting for both the increased rate and final percentage of germination.

Even in 100 μM GA_{4+7}, germination of *gib-1* or *MM* seeds was not as rapid as that of sit^w seeds (Figs. 1C, 3A, 4, and 5C). It is interesting that inserting a value of 1 mM GA_{4+7} into the GA model (Eq. 7) using the parameters for the GA response in Table II resulted in a predicted time course for *gib-1* seeds that was essentially identical with that of sit^w seeds in water (dotted curve in Fig. 4). We tested this prediction experimentally by germinating *gib-1* and *MM* seeds in 1 mM GA_{4+7}, but the time courses were not significantly more rapid than those in 100 μM GA_{4+7} (data not shown). Although experiments at these very high hormone concentrations must be interpreted with caution, this result suggests that, although the model predicts that sit^w seeds germinate as if they were in the presence of 1 mM GA_{4+7}, germination of *gib-1* or *MM* seeds cannot be accelerated to an equivalent degree by supraoptimal GA. Low concentrations of ABA during development may precondition the seeds for a subsequent germination rate that cannot be duplicated by excess GA applied to seeds that had developed with normal ABA concentrations.

Effect of Reduced ψ, ABA, and GA on Embryo ψ_π

The changes in $\psi_b(g)$ values due to genotype, ABA, and GA described above could result from accumulation or loss of solutes in the embryos, because embryo ψ_π determines the maximum turgor force that can be generated by the embryo to penetrate through the enclosing endosperm tissues. To test this possibility, embryo ψ_π was measured in embryos of seeds from which the radicle had not protruded at a time when some seeds had just completed germination under each im-

bibition condition (Table III). Among the three genotypes, *sit*^{w} embryos had the lowest ψ_π (−1.41 MPa), 0.14 and 0.19 MPa lower than that of *MM* and *gib-1* embryos, respectively (Table III). This pattern is consistent with the lower $\psi_b(50)$ of *sit*^{w} seeds, but the differences in ψ_π are far too small to account for the differences in $\psi_b(50)$ (Table II). Similar results were also found by H.W.M. Hilhorst at Wageningen Agricultural University (personal communication). The presence of ABA caused a small increase in embryonic RWC but did not cause any change in ψ_π that could not be accounted for by the increased water content (compare observed and predicted values in Table III). When seeds were incubated on 100 μM GA_{4+7} solutions, however, the predicted embryo ψ_π values for both *MM* and *gib-1* seeds were about 0.1 MPa lower than the corresponding measured values, indicating a net accumulation of solutes equivalent to about 0.1 MPa (Table III). Overall, the changes in embryonic ψ_π due to genotype or hormones were small compared to the corresponding differences in $\psi_b(g)$.

DISCUSSION

Population-Based Threshold Model

The population-based threshold model worked well to describe germination of *MM*, *sit*^{w}, and *gib-1* seeds in response to reduced ψ and a range of exogenous ABA and GA_{4+7} concentrations, with >86% of the variation in germination times accounted for by the model in all cases (Table II). A good match between the actual data and the curves predicted by the models is evident for all three genotypes across all ψ and hormone levels (Figs. 1, 3, and 5). It should be emphasized that the parameters of the models and the predicted curves are not empirical fits to each individual time course. Rather, as discussed previously (Ni and Bradford, 1992), the parameters derived from simultaneous analysis of data at all factor levels are estimators of the mean sensitivity of a seed population to ψ [$\psi_b(50)$], ABA [$ABA_b(50)$], or GA [$GA_b(50)$], the corresponding SD values of the threshold distributions (σ_{ψ_b}, σ_{ABA_b}, and σ_{GA_b}, respectively), and the proportionality or time constants that relate the difference between the external level and the threshold value of a factor to a corresponding time to completion of germination (θ_H, θ_{ABA}, and θ_{GA}). When the three basic parameters for each model are determined, germination time courses of the entire population in response to ψ, ABA, or GA can be predicted by changing only the factor level in Equations 3, 5, or 7. In addition to the factors described here, the same basic model is likely to be applicable to other agents that affect germination, including ethylene, light, nitrate, or aging (Abeles, 1986; Hilhorst and Karssen, 1989; Tarquis et al., 1992).

A novel feature of this model is its ability to simultaneously account for both the timing and the final extent of germination at different factor levels. This is a result of the distribution of different factor threshold values among individual seeds and the constant proportionality between the germination response rate and the difference between the current factor level and its threshold. Other approaches to quantitating the sensitivity of germination to environmental or physiological factors from germination response curves, such as those derived from the Michaelis-Menten enzyme kinetic equation (Weyers et al., 1987; Hilhorst and Karssen, 1989) or from fitted monomolecular, logistic, or Gompertz functions (Tipton, 1984), require the estimation of separate values for maximum response rate and other "constants" for each individual curve and do not explicitly incorporate the population aspects of germination behavior into the analysis. The models developed here characterize the physiological sensitivity and variation in sensitivity of the seed population to a given factor and then use this distribution to generate the predicted response at any factor level.

We have also demonstrated previously how the apparently additive or synergistic action of multiple factors (e.g. ABA and ψ) can be predicted by mathematically combining the separate response functions for each factor (Ni and Bradford, 1992). The model also predicts that equivalent effects on

Table III. *Water content, RWC, and ψ_π of embryos after imbibition by tomato seeds of water, 33 μM ABA, or 100 μM GA_{4+7}*

Seeds imbibed for the period indicated (just before radicle emergence), the embryos were excised, and the embryonic water contents (dry weight basis) and ψ_π were measured. The water content of the embryos of each genotype that had imbibed water was taken as 100% RWC, and the RWC of the embryos that had imbibed ABA or GA are calculated as a percentage of this value. The predicted values of ψ_π corrected for changes in embryo RWC were calculated by dividing the measured ψ_π of the water-imbibing embryos of each genotype by the factor [1 − (100 − RWC)/83] using a constant nonosmotic volume of 17% (Ni and Bradford, 1992). Means ± SE are shown (n = 4).

Genotype	Imbibition Condition	Embryonic Water Content		ψ_π	
		Percentage	RWC	Observed	Predicted
				MPa	
MM	H_2O (45 h)	49.9 ± 1.3	100	−1.27 ± 0.03	−1.27
MM	33 μM ABA (7 d)	52.6 ± 2.2	105	−1.22 ± 0.04	−1.20
MM	100 μM GA_{4+7} (32 h)	49.4 ± 1.6	99	−1.38 ± 0.02	−1.29
sit^{w}	H_2O (24 h)	50.3 ± 3.2	100	−1.41 ± 0.03	−1.41
sit^{w}	33 μM ABA (36 h)	53.8 ± 2.4	107	−1.32 ± 0.06	−1.30
gib-1	H_2O (45 h)	56.5 ± 2.4	100	−1.22 ± 0.06	−1.22
gib-1	100 μM GA_{4+7} (36 h)	59.8 ± 1.7	106	−1.22 ± 0.02	−1.14

germination would result from a given change in either the factor level or in the threshold value, because the response is proportional only to the difference between them (Bradford et al., 1992). The model, therefore, can be used to characterize responses due to changes in either factor levels or tissue sensitivity and can discriminate between the two possibilities.

Application of a population-based model is not limited to seed germination, because it is general in plants that individual tissues or cells within the same tissue may differ in their hormonal sensitivity, resulting in wide dose-response curves and variable responses to a given dosage among cells or tissues (Trewavas, 1991). In fact, the concept of target tissues or cells that are particularly responsive to a given factor is virtually axiomatic in our understanding of hormonal regulation of growth and development. Although this variation in sensitivity has been recognized, it has seldom been quantified as a significant physiological parameter in its own right.

Seed germination is perhaps only a more obvious example of this fundamental physiological feature because a seed population is a collection of individual seeds that do not germinate simultaneously but, rather, show a distribution of germination events over time. This distribution in time is mainly attributable to the differences in sensitivity to environmental or hormonal factors among individual seeds in the population. We can calculate, for example, that the differences in the sensitivity to ABA [$(ABA_b(g)$ values] between the first 10% and the last 10% of germinating seeds ($\pm 1.28\ \sigma_{ABA_b}$ around the mean) were as large as 140-fold for *MM* seeds and 25-fold for *sit*^{w} seeds. Differences were even greater among *gib-1* seeds in response to GA_{4+7} (390-fold). The analytical methods developed here using seeds as a model system should be applicable to quantifying and understanding the variations in sensitivity that occur in other systems as well, such as, for example, nonuniform stomatal closure in response to ABA (Terashima et al., 1988) and mild water stress (Sharkey and Seemann, 1989; Ni and Pallardy, 1992), variable changes in cytosolic calcium concentration among epidermal guard cells in response to a single ABA concentration (McAinsh et al., 1992), tissue-specific gene expression patterns in response to hormones or water stress (Cohen et al., 1991; Li et al., 1991; van Loon and Bruinsma, 1992), and differences in α-amylase production among individual aleurone cells in response to GA (Jacobsen and Knox, 1973).

A final implication of the present analysis is that, in analogy to thermal time, the concepts of "hydrotime," "ABA time," and "GA time" also have physiological meaning for seed germination. The application of thermal time, or a heat-units or degree-days analysis, to normalize the rates of biological processes at different temperatures is well established in modeling of crop growth or insect development (Fry, 1983). Germination behavior at different ψ, ABA, or GA levels can be normalized using hydrotime, ABA time, and GA time in analogy to the use of thermal time to normalize germination rates at different temperatures (Fig. 4; Bradford, 1990; Dahal and Bradford, 1990; Dahal et al., 1990). That is, progress toward germination is proportional to the prevailing level of ψ, ABA, or GA in much the same way that biological developmental rates are proportional to temperature. An alternative way to view this is to consider that, as ψ is reduced or ABA concentration is increased, biological time "stretches" or slows down so that more clock time is required to reach the same physiological state (i.e. radicle emergence in this case).

In contrast, as GA concentration or ψ is increased, biological time "contracts" so that more development is achieved per unit of clock time, as would occur if the temperature were increased. There is also evidence that biological or metabolic time can accumulate under conditions in which radicle emergence is prevented by low ψ or ABA, shortening subsequent times to germination when the inhibitor is removed (Finch-Savage and McQuistan, 1991; Dahal et al., 1992). This is not to say that the underlying mechanisms of thermal time, hydrotime, ABA time, and GA time are necessarily identical. These rather novel concepts of biological time are, however, a well-supported consequence of our analysis of seed germination, and this alternative viewpoint may have more general implications for understanding the regulation of plant development.

Regulation of Germination and Dormancy in Tomato Seeds

It has been well documented in studies using ABA- and GA-deficient mutants of *Arabidopsis thaliana* and tomato that ABA determines the depth of dormancy during development and the amount of GA required to break dormancy during imbibition (Karssen et al., 1983, 1987; Groot, 1987; Koornneef et al., 1989; Groot and Karssen, 1992). Our results are in excellent qualitative agreement with those recently reported by Groot and Karssen (1992) using the same tomato genotypes. Our work extends their analysis to provide quantitative measures of ψ, ABA, and GA sensitivity for each genotype and develops a comprehensive model explaining the underlying linkage among germination rates, sensitivity to ψ, and dormancy.

In our analysis, the influence of both endogenous and exogenous ABA and GA on dormancy and germination can be explained largely on the basis of their effects on the $\psi_b(g)$ distributions within the seed population. When GA was absent, as in the *gib-1* mutant, the $\psi_b(g)$ distribution was entirely above 0 MPa so that no germination occurred even on water. As the exogenous GA_{4+7} concentration increased, the $\psi_b(g)$ distribution shifted to lower values (Fig. 2C), allowing a larger percentage of seeds to germinate and reducing the time to germination (Fig. 5A).

In contrast, low ABA levels during development of *sit*^{w} seeds resulted in very low $\psi_b(g)$ values, absence of dormancy, and rapid germination, and exogenous ABA delayed and inhibited germination by causing an increase in both $\psi_b(g)$ and θ_H (Fig. 2B; Table II; Ni and Bradford, 1992). Partial dormancy in *MM* seeds was due to a high $\psi_b(g)$ distribution that extended above 0 MPa; that fraction of the seed population for which $\psi_b > 0$ MPa was unable to germinate even on water or was dormant (Fig. 2A).

If the distribution of $\psi_b(g)$ determines when and if a given seed will germinate, what determines the values of $\psi_b(g)$? A scheme summarizing our current understanding of the factors controlling tomato seed germination is shown in Figure 6. Whether radicle emergence occurs or not under a given condition is determined by the balance of forces between the growth potential or turgor of the embryo (essentially the difference in ψ_π between the embryo and the external me-

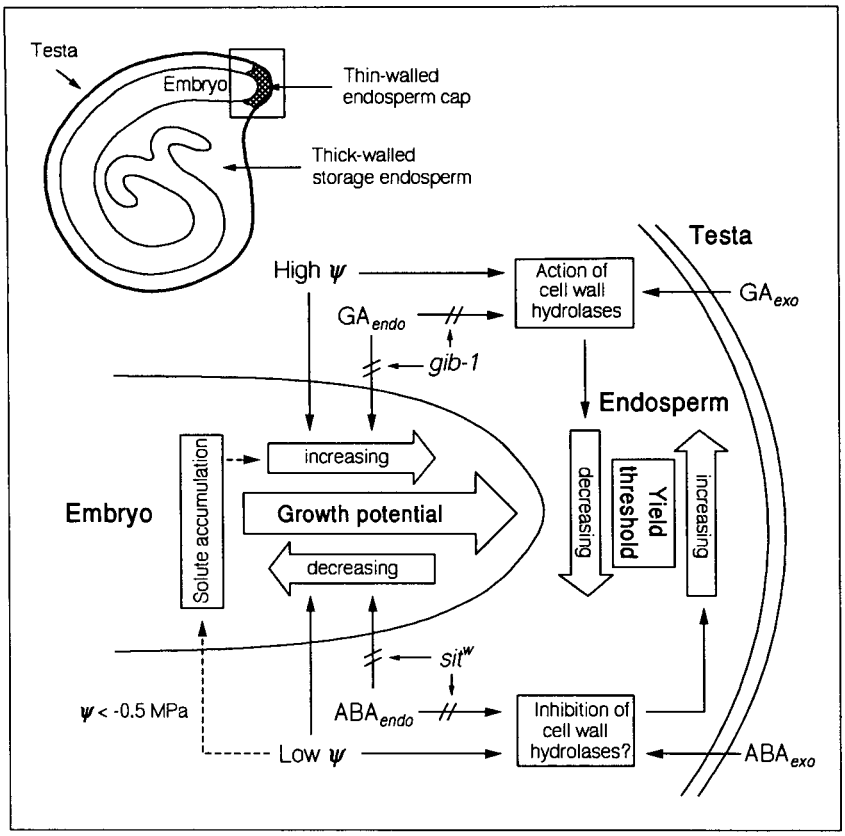

Figure 6. A diagram summarizing the water and hormonal relations in the embryo and the endosperm cap that influence radicle emergence of tomato seeds. The inset illustrates the enclosure of the embryo by the endosperm and the thin-walled endosperm cap region opposite the radicle tip that weakens to allow radicle emergence (Haigh, 1988; modified from Groot, 1987).

dium) and the yield threshold or mechanical resistance of the endosperm cap. The value of $\psi_b(g)$ is the net result of these opposing forces. The primary control of germination timing seems to reside in the endosperm cap, whereas secondary effects on cell wall loosening of the embryo are expressed after the endosperm has weakened sufficiently to allow embryo expansion (Haigh, 1988; Karssen et al., 1989). High ψ increases the growth potential of the embryo and allows the weakening process in the endosperm to proceed. Low ψ decreases embryo growth potential directly by lowering turgor and also delays or inhibits endosperm weakening (Dahal and Bradford, 1990).

On the other hand, prolonged incubation at low ψ (≤ -0.5 MPa) results in solute accumulation in the embryo, increasing the embryo growth potential, decreasing $\psi_b(g)$ by as much as 0.3 MPa, and allowing germination at ψ values that were initially inhibitory (Ni and Bradford, 1992). However, the small differences in embryonic ψ_π detected here among genotypes or hormone treatments in water (Table III) were far too small to account for the observed differences in $\psi_b(g)$ (Table II; Fig. 2). For example, ψ_π of *MM* and *sit*^{w} embryos differed by only 0.14 MPa, and the difference in $\psi_b(50)$ was 0.82 MPa. Imbibition in ABA or GA_{4+7} caused large changes in $\psi_b(g)$, but ABA had no effect on ψ_π and GA_{4+7} lowered ψ_π by only 0.1 MPa. In addition, there was no significant difference in ψ_π between *MM* and *gib-1* embryos when both seeds imbibed on water (Table III), indicating that the inability of *gib-1* seeds to germinate was not due to insufficient embryo turgor. It can be concluded that the differences in $\psi_b(g)$ among the three genotypes and the shifts in $\psi_b(50)$ caused by addition of GA_{4+7} or ABA are not due to large changes in embryonic ψ_π.

Alternatively, the changes in $\psi_b(g)$ could be dependent on the rate or extent of the weakening of the endosperm tissue directly opposite the radicle tip, because the endosperm completely encloses the embryo and limits its water uptake (Fig. 6; Groot, 1987; Haigh, 1988; Karssen et al., 1989; Dahal and Bradford, 1990). It has been suggested that values of $\psi_b(g)$ are related to the resistance or yield threshold of the endosperm during a final weakening stage just before radicle emergence (Karssen et al., 1989; Dahal and Bradford, 1990). Linear relationships have been found between germination rate and endosperm puncture force (Karssen et al., 1989) and between germination rate and $\psi_b(g)$ (Dahal and Bradford, 1990; Ni and Bradford, 1992). Hence, it is very likely that $\psi_b(g)$ and endosperm resistance to penetration are closely correlated.

There is good evidence that ABA inhibits and GA promotes tomato seed germination by affecting the rate and extent of endosperm weakening, with lesser effects on embryo growth capacity (Groot and Karssen, 1987, 1992; Groot et al., 1988). Tomato endosperm cell walls are composed primarily of a mannan polymer with smaller quantities of Glc and Gal (Groot et al., 1988). GA_{4+7} induced endo-β-mannanase and increased mannohydrolase activities in the endosperm of *gib-1* seeds, and the puncture force required to penetrate the endosperm cap was significantly reduced coincident with the initiation of radicle emergence (Groot and Karssen, 1987; Groot et al., 1988).

When ABA was added to the incubation solution, however,

the GA-induced endosperm weakening was completely inhibited and the puncture force remained high (Groot and Karssen, 1992). The *sit*^{w} mutation that lowers endogenous ABA contents during seed development prevents the induction of dormancy, speeds germination, and dramatically lowers the $\psi_b(g)$ for germination (Figs. 2 and 3; Table II; Groot and Karssen, 1992). To our knowledge, it has not been tested experimentally in tomato seeds whether ABA and/or low ψ act by inhibiting the synthesis or activity of the same set of cell wall hydrolases that are stimulated by GA, although it is a likely hypothesis. In isolated lettuce endosperms, ABA does inhibit the appearance of mannanase activity, and it has been argued that endogenous ABA is an in vivo regulator of the synthesis of this enzyme (Dulson et al., 1988). However, the effects of low ψ on tomato seed germination are not mediated through increases in endogenous ABA (Ni and Bradford, 1992). The changes in endosperm strength due to ABA and GA correspond well with the effects of these hormones on $\psi_b(g)$ (Fig. 2), indicating that $\psi_b(g)$ is determined mainly by modulation of cell wall hydrolase activity leading to endosperm weakening (Fig. 6).

Received August 24, 1992; accepted October 29, 1992.

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