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Quantitative Models Characterizing Seed Germination Responses to Abscisic Acid and Osmoticum[1]

Bing-Rui Ni and Kent J. Bradford*

Department of Vegetable Crops, University of California, Davis, California 95616–8631

ABSTRACT

Mathematical models were developed to characterize the physiological bases of the responses of tomato (*Lycopersicon esculentum* Mill. cv T5) seed germination to water potential (ψ) and abscisic acid (ABA). Using probit analysis, three parameters were derived that can describe the germination time courses of a seed population at different ψ or ABA levels. For the response of seed germination to reduced ψ, these parameters are the mean base water potential ($\bar{\psi}_b$, MPa), the standard deviation of the base water potential among seeds in the population (σ_{ψ_b}, MPa), and the "hydrotime constant" (θ_H, MPa·h). For the response to ABA, they are the log of the mean base ABA concentration ($\overline{ABA}_b$, M), the standard deviation of the base ABA concentration among seeds in the population (σ_{ABA_b}, log[M]), and the "ABA-time constant" (θ_{ABA}, log[M]·h). The values of $\bar{\psi}_b$ and $\overline{ABA}_b$ provide quantitative estimates of the mean sensitivity of germination rate to ψ or ABA, whereas $\sigma^{\psi}{}_b$ and σ_{ABA_b} account for the variation in sensitivity among seeds in the population. The time constants, θ_H and θ_{ABA}, indicate the extent to which germination rate will be affected by a given change in ψ or ABA. Using only these parameters, germination time courses can be predicted with reasonable accuracy at any medium ψ according to the equation probit(g) = $[\psi - (\theta_H/t_g) - \bar{\psi}_b]/\sigma_{\psi_b}$, or at any ABA concentration according to the equation probit(g) = $[\log[ABA] - (\theta_{ABA}/t_g) - \log[\overline{ABA}_b]]/\sigma_{ABA_b}$, where t_g is the time to radicle emergence of percentage g, and *ABA* is the ABA concentration (M) in the incubation solution. In the presence of both ABA and reduced ψ, the same parameters can be used to predict seed germination time courses based upon strictly additive effects of ψ and ABA in delaying the time of radicle emergence. Further analysis indicates that ABA and ψ can act both independently and interactively to influence physiological processes preparatory for radicle growth, such as the accumulation of osmotic solutes in the embryo. The models provide quantitative values for the sensitivity of germination to ABA or ψ, allow evaluation of independent and interactive effects of the two factors, and have implications for understanding how ABA and ψ may regulate growth and development.

It has become clear in recent years that ABA plays a role in seed development, dormancy, and germination (17). However, a number of studies have indicated that seed water relations may be even more important than ABA in regulating seed development and maturation and preventing precocious germination (reviewed in refs. 9, 16). Studies on mature seeds have also shown a close relationship between the effects of ABA and of reduced ψ (Table I) on seed germination (18, 21, 22, 28). However, the precise nature of this relationship and its physiological basis are not fully understood.

Trewavas (24) has recently discussed some of the problems inherent in attempting to quantitate the sensitivity of a particular developmental process to hormonal or environmental regulation. He suggests three requirements for unambiguous measurement of growth substance sensitivity: (a) perturbation of growth substance levels near their endogenous concentrations; (b) limited experimental manipulation or excision of tissue; and (c) assessment of the contribution of a growth substance to the control of the process when a number of factors interact. Similar constraints would be presumed to apply to evaluating the role of an environmental factor, such as reduced ψ, in a particular developmental process. In addition, Trewavas (24) has emphasized the possibility that individual tissues or cells within tissues may differ in their sensitivity to a hormonal or environmental factor, resulting in wide dose-response curves and varying responses to a given dosage among cells or tissues. Such a pattern is evident in seed germination, because a population of seeds does not germinate simultaneously, but shows a distribution of germination times that may be differentially affected by hormonal or environmental factors. Seed germination is well suited for sensitivity studies, because the supplied levels of hormonal or environmental stimuli can be controlled in the external medium, and no experimental manipulation or excision of the tissue is required. Quantitative assessments of the sensitivity of germination to ABA and ψ and of their interactions have been made (*e.g.* 21, 22, 28, 29). However, these studies did not develop a general physiologically-based quantitative model of the interactions of ABA and ψ, and did not account for the variation in sensitivity among individual seeds. The present paper describes an analysis of the influence of ABA and ψ on seed germination that achieves these objectives.

For reasons discussed previously (4), the analysis is based on the timing of radicle emergence after imbibition rather than on final germination percentages or growth rates after germination. It has been shown that the time to radicle emergence of a given percentage of seeds imbibed at a particular ψ is fundamentally related to two parameters, the base water potentials of the seeds in the population ($\psi_b(g)$), and the "hydrotime constant" (θ_H) (4, 6, 11). These parameters are defined by the equation

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

where ψ is the water potential of the imbibition medium,

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Table I. *Abbreviations Used in This Paper*

ψ	water potential
$\psi_b(g)$	base water potential of percentage g
t_g	time to radicle emergence of percentage g
θ_H	hydrotime constant
ABA	molar concentration of (±) abscisic acid
$ABA_b(g)$	base *ABA* for percentage g
θ_{ABA}	ABA-time constant
$\overline{ABA}_b$	mean base *ABA* reducing germination to 50%
σ_{ABA_b}	standard deviation of $ABA_b(g)$
$t_g(0)$	time to germination in water
$t_g(ABA)$	time to germination in a particular *ABA*
$d_g(\psi)$	delay in germination due to reduced ψ
$d_g(ABA)$	delay in germination due to increased *ABA*
$t_g(\psi)$	time to germination at a particular ψ
$t_g(\psi, ABA)$	time to germination at a combination of ψ and *ABA* levels
$\overline{\psi}_b$	mean base water potential reducing germination to 50%
GR_g	germination rate (inverse of time to germination) for percentage g
σ_{ψ_b}	standard deviation of $\psi_b(g)$
ψ_π	osmotic potential
RWC	relative water content
$\psi_b(g)^*$	distribution of $\psi_b(g)$ after incubation at low ψ
$\psi_b(g)_{ABA}$	base water potential distribution as a function of *ABA*

$\psi_b(g)$ is the base water potential of percentage g, and t_g is the time to radicle emergence of percentage g. The equation states that if θ_H is constant, the increase in t_g as ψ is reduced is inversely proportional to the difference between ψ and $\psi_b(g)$. It also indicates that the differences in times to germination among seeds in the population are due to variation in $\psi_b(g)$ among individual seeds, which can be quantitated by σ_{ψ_b}, the standard deviation of $\psi_b(g)$. Methods for determining these parameters and examples of the application of this analysis to seed germination data have been published (4, 6).

ABA delays or prevents seed germination, and its inhibitory effect is empirically similar to that of reduced ψ (16, 21, 28). Inhibition of rape (*Brassica napus* L.) and muskmelon (*Cucumis melo* L.) seed germination by increasing *ABA* was very similar to that caused by decreasing ψ, with a linear interaction between ψ and *ABA* such that each could fully replace the other in a quantitatively reciprocal manner (21, 28). Subsequent work on rape seed suggested that the effect of ABA was to increase the yield threshold (minimum turgor) for cell expansion and to decrease the cell wall extensibility (22). For muskmelon seeds, it was proposed that if the germination rate is proportional to the difference between the embryo turgor and the minimum turgor required for radicle emergence (or the embryo "growth potential"), then a lowering of embryo turgor by a decrease in ψ or an increase in the yield threshold due to ABA would have equivalent effects, resulting in the observed linear relationship. Using an ABA-deficient tomato (*Lycopersicon esculentum* Mill.) mutant, Groot (10) proposed that, in addition to reducing embryo growth potential, ABA inhibits germination by preventing GA-induced weakening of the endosperm cap covering the radicle tip. This hypothesis is consistent with the observation that tomato seeds become less sensitive to ABA if the endosperm cap is removed (10, 18).

Because the responses of seed germination to ABA and to ψ are apparently similar and quantitatively additive, the methods used to characterize the water relations of germination (4) might also be applicable to the analysis of the ABA sensitivity of germination. If so, this would allow the quantitation and prediction of germination responses to ABA across a wide range of ABA concentrations, including those near the endogenous levels. Furthermore, the additivity of ψ and ABA effects on germination could be modeled mathematically and tested experimentally. It has been argued that the parameters derived from this analysis have physiological relevance rather than simply being an empirical fit to the data (4, 6). Analysis of the sensitivity of germination to ABA by this approach may reveal aspects of the physiological basis of ABA action. The present work was undertaken to determine whether the water relations analysis of germination could be adapted to describe ABA effects on germination, and if so, to use the analysis to model the interaction of ABA and ψ and to investigate its physiological basis.

THEORY

The water relations analysis of seed germination rates employed here has been described in detail elsewhere (4), and the symbol conventions and definitions described there will be used here. The analysis of ABA responses assumes that there is a base *ABA* that will prevent germination of a specific fraction or percentage g of the seed population [$ABA_b(g)$, M]. The value of $ABA_b(g)$, or sensitivity to ABA, may vary among seeds in the lot. The analysis further assumes that the delay in time to radicle emergence at an external medium *ABA* between 0 and $ABA_b(g)$ is inversely proportional (on a logarithmic concentration scale) to the difference between *ABA* and $ABA_b(g)$. If this is so, then an "ABA-time constant" (θ_{ABA}, log[M]·h) can be defined according to the equation

$$\theta_{ABA} = (\log[ABA] - \log[ABA_b(g)])t_g$$
$$= (\log[ABA/ABA_b(g)]t_g \quad (2)$$

Using the methods described previously (4), germination time courses at a range of *ABA* levels can be used to derive the values of θ_{ABA} and $ABA_b(g)$ by repeated regression analyses of probit(g) as a function of ($\log[ABA] - \theta_{ABA}/t_g$) (which is equivalent to $\log[ABA_b(g)]$ by rearranging Eq. 2). The mean base *ABA* ($\overline{ABA}_b$, or $ABA_b(50)$) is the ABA concentration reducing germination to 50% (where probit(g) = 0), and the distribution of ABA_b among seeds in the population is characterized by σ_{ABA_b}, the standard deviation of $ABA_b(g)$ (given by the inverse of the slope of the probit regression line).

To model the interactions between ψ and ABA in influencing seed germination rates, we can assume that the time required to germinate in either osmotic or ABA solutions can be partitioned into two components, $t_g(0)$ representing the time to germination in water, and an additional time delay

(d_g) due to the reduced ψ ($d_g(\psi)$) or presence of ABA ($d_g(ABA)$). Thus, the total time to germination for a seed at a particular ψ ($t_g(\psi)$) is equal to $t_g(0) + d_g(\psi)$, and at a particular *ABA* ($t_g(ABA)$) is equal to $t_g(0) + d_g(ABA)$. For a strictly additive relationship between ψ and *ABA*, the time to germination in the presence of both *ABA* and reduced ψ ($t_g(\psi, ABA)$) would be equal to $t_g(0) + t_g(\psi) + d_g(ABA)$. From these relationships, it can be shown that

$$t_g(\psi, ABA) = t_g(\psi) + t_g(ABA) - t_g(0) \quad (3)$$

The time to germination of any specific percentage of the seed population at any combination of ψ and *ABA*, therefore, should be equal to the sum of the times predicted for that percentage from Equations 1 and 2 at those ψ or *ABA* levels if they were present alone, minus the time to reach that germination percentage in water.

MATERIALS AND METHODS

Tomato seeds (*Lycopersicon esculentum* cv T5) were placed on two 4.5 cm filter papers in 5 cm Petri dishes moistened with 4.0 mL of water, or of ABA, PEG, or ABA + PEG solutions. The dishes were covered with tightly fitting lids to prevent evaporation, and incubated in a controlled temperature chamber in the dark at 25 ± 1°C. Four replicates of 50 seeds each were tested for each treatment in a randomized complete block design. The PEG solutions were prepared according to Michel (20) using PEG 8000 (Carbowax PEG 8000, Fisher Scientific Co., Fair Lawn, NJ) and the ψ values of the solutions were verified with a vapor pressure osmometer (model 5100C, Wescor Inc. Logan, UT) calibrated against NaCl standards. The ABA solutions were prepared by dissolving (±)*cis-trans*-ABA (Sigma) in a few drops of 1 N KOH and diluting with distilled water. The pH of the stock solution was adjusted to 7.0 with 1 N HCl. Solutions containing both PEG and ABA were made by dissolving PEG in solutions of the desired ABA concentration. Seeds incubated on solutions containing PEG were transferred to fresh solutions after the first 24 h and then weekly thereafter, whereas ABA solutions were changed every 2 weeks. Clearly visible radicle protrusion was used as the criterion for the completion of germination.

Data analyses were conducted using repeated probit analyses as described in detail previously (4). Briefly, germination percentages were transformed to probit values. Values of $\psi_b(g)$ for each observed germination percentage were then calculated according to

$$\psi_b(g) = \psi - \theta_H/t_g \quad (4)$$

for seeds germinated at different ψ, and values of $ABA_b(g)$ were calculated according to

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

for seeds germinated at different *ABA*. Repeated probit analyses of probit(g) as a function of $\psi_b(g)$ or $\log[ABA_b(g)]$ were performed using different values for θ_H or θ_{ABA} until the optimal regression (least residual) was obtained. Probit analyses were conducted using the PROC PROBIT routine of the SAS statistical package (SAS Institute Inc., Cary, NC) which employs a maximum-likelihood weighted regression method. The values of $\overline{\psi}_b$ and $\overline{ABA}_b$ were then obtained from the regression equation at probit(*50*) = 0, and σ_{ψ_b} and σ_{ABA_b} are the inverses of the slopes of the regression lines. Mean germination rates ($GR_{50} = 1/t_{50}$) were determined from separate probit analyses of individual germination time courses for each ψ or *ABA* treatment.

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. The embryos were removed from the seed by excising the endosperm/testa cap surrounding the radicle tip using a razor blade and applying gentle pressure at the opposite end of the seed. Embryo extraction was performed rapidly in a humidified box to reduce evaporative water loss. The embryonic water content of seeds fully imbibed on water was taken as 100% RWC. The ψ_π values determined for embryos imbibed in either PEG or ABA were corrected for water content changes as described previously (4) using a value of 17% RWC for the nonosmotic water volume of the embryo (our unpublished results).

For ABA analysis, seeds were incubated on water for 1 d, on −0.3 MPa PEG 6000 for 2 d, or on −0.6 MPa PEG 6000 for 5 d. These times are approximately half of the time required for 50% germination at each ψ, or near the midpoint of the lag phase of imbibition before germination. After the incubation period, the endosperm cap tissues opposite the radicle were removed and collected on solid CO_2. The embryos were removed from the remaining endosperm with gentle pressure and were collected separately on solid CO_2. Approximately 100 seeds were used per replicate, and three replicate extractions were performed for each tissue type. Tissues were powdered in liquid N_2 and extracted with boiling water (19). The water extracts were evaporated under vacuum, and the residue was dissolved in 50% methanol, loaded on a Sep-Pak C column (Waters Associates, Milford, MA), and eluted with 50% methanol. ABA in the extracts was measured by an indirect ELISA procedure (26) utilizing monoclonal antibody to ABA (Idetek Inc., San Bruno, CA). Values reported are corrected for losses during extraction on the basis of a [^{3}H]ABA internal standard added to each sample before extraction. The average recovery of ABA was 84%.

RESULTS

Germination in Either ABA or Reduced ψ

Cumulative germination time courses at a range of ψ or *ABA* levels are shown on logarithmic time scales to separate the curves for clarity (Fig. 1, A and B). The markers represent the experimental data; the solid curves will be discussed subsequently. Small reductions in medium ψ or increases in medium *ABA* delayed radicle emergence, but had little effect on the final germination percentages. Final germination percentage was reduced only when the medium ψ was <−0.52 MPa or the medium *ABA* was >50 μM. Although the effects of decreasing ψ and increasing *ABA* in delaying and inhibiting germination are obviously similar, they do not appear to be identical when the pattern of germination events is considered across all ψ and *ABA* levels.

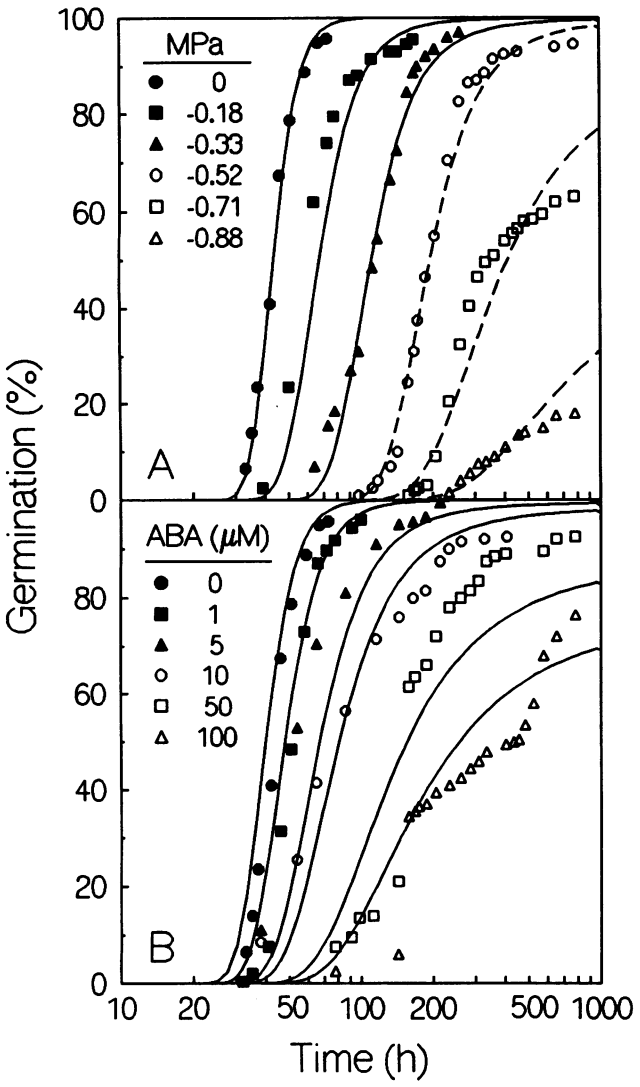

Figure 1. Cumulative germination time courses of tomato seeds incubated at a range of ψ (A) or ABA (B) levels. The symbols represent the actual data, and the curves are the time courses predicted according to the parameters derived from the probit analyses shown in Figure 2. The time axis is plotted logarithmically to separate the curves for clarity.

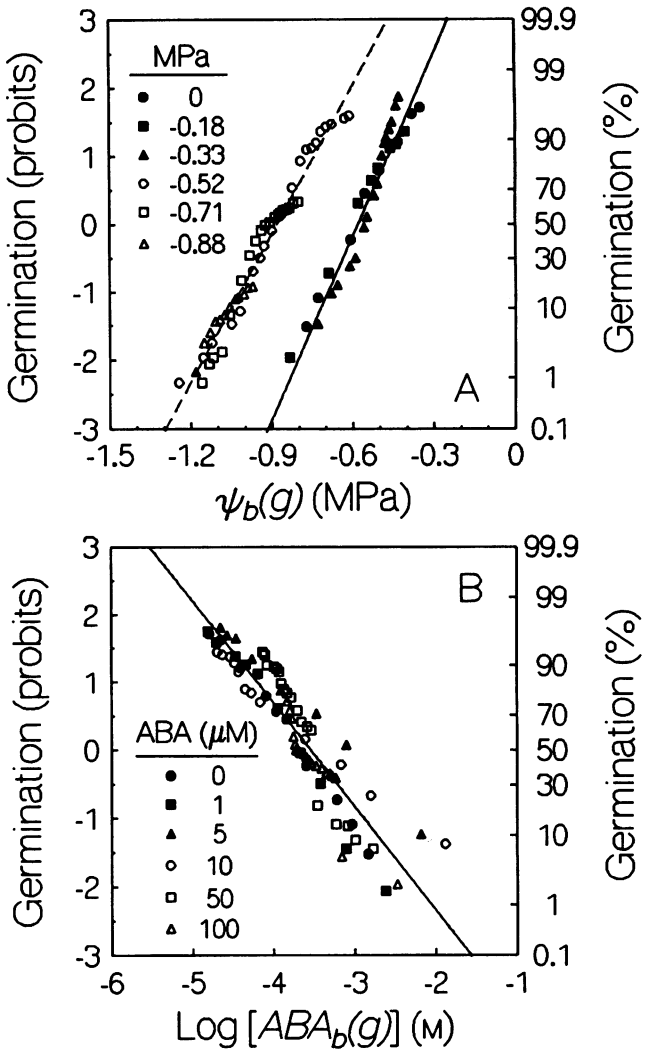

Figure 2. Probit analyses of the germination time course data in Figure 1. A, The probit of germination percentage (compare left and right axes) is plotted as a function of the value of $\psi_b(g)$, calculated according to Equation 4. Two regressions are shown, one for data above −0.5 MPa and a second for data below −0.5 MPa. The values of the parameters derived for the high ψ region (solid symbols and line) were θ_H = 25 MPa·h, $\overline{\psi}_b$ = −0.58 MPa, and σ_{ψ_b} = 0.11 MPa, and those for the low ψ region (open symbols and dashed line) were θ_H = 71 MPah, $\overline{\psi}_b$ = −0.88 MPa, and σ_{ψ_b} = 0.14 MPa. B, The probit of germination percentage is plotted as a function of log[$ABA_b(g)$], calculated according to Equation 5. In this case, all data could be fit to a single relationship, yielding the values of θ_{ABA} = −118 log[M]·h, log[$\overline{ABA}_b$] = −3.54 log[M] (288 μM), and σ_{ABA_b} = −0.66 log[M].

To characterize the germination time courses at different ψ or *ABA* levels, the values of the parameters required to describe the entire population response to ψ and *ABA* can be determined by probit analysis, according to the following equations:

$$\text{probit}(g) = [\psi - (\theta_H/t_g) - \overline{\psi}_b]/\sigma_{\psi_b} \quad (6)$$

for the ψ response and

$$\text{probit}(g) = [\log[ABA] - (\theta_{ABA}/t_g) - \log[\overline{ABA}_b]]/\sigma_{ABA_b} \quad (7)$$

for the *ABA* response. It was not possible to find a single set of constants that fit the data well across all ψ levels. Rather, the data at high ψ (>−0.5 MPa) could be accommodated by one set of constants, whereas data at ψ <−0.5 MPa required

another set of constants (Fig. 2A). For the data from 0 to −0.33 MPa, the values derived from this analysis (Fig. 2A, solid symbols and line) are $\theta_H = 25$ MPa·h, $\overline{\psi}_b = -0.58$ MPa, and $\sigma_{\psi_b} = 0.11$ MPa. For the ψ range from −0.52 to −0.88 MPa (Fig. 2A, open symbols and dashed line), the values are $\theta_H = 71$ MPa·h, $\overline{\psi}_b = -0.88$ MPa, and $\sigma_{\psi_b} = 0.14$ MPa. This indicates that with prolonged incubation at low ψ, the $\overline{\psi}_b$ shifts downward and θ_H increases. Using these values, the predicted germination time courses were plotted using Equation 6 (curves in Fig. 1A).

Applying the same procedure to the data for germination at different *ABA* levels (Fig. 2B), a single regression could be fit to all data, giving values of $\theta_{ABA} = -118$ log[M]·h, $\log[\overline{ABA}_b] = -3.54$ ($\overline{ABA}_b = 288$ μM), and $\sigma_{ABA_b} = -0.66$ log[M]. (The negative value for σ_{ABA_b} is due to the negative slope of the regression.) Because $ABA = 0$ for germination on water, and the analysis is based upon a logarithmic relationship with *ABA*, data for germination in water cannot be used directly in the regression. However, we can determine the maximum *ABA* giving a time course that is not significantly different from that in water, or the threshold *ABA* for a detectable response by the seeds. This value is calculated to be 0.5 μM ABA, and the control (water) data for the *ABA* treatments are plotted at this threshold value in the figures. The values of $ABA_b(g)$ are an indication of the sensitivity of individual seeds to complete inhibition of radicle emergence by ABA. The slowest seeds to germinate are inhibited at the lowest *ABA*, whereas the first seeds to germinate require much higher *ABA* to prevent radicle emergence (Fig. 2B). According to the probit equation, the first 2% of seeds to germinate (two standard deviations or probit units from the mean) would require 6 mM *ABA* to prevent radicle emergence, whereas the slowest 2% of seeds in the population to germinate would be inhibited at only 14 μM, a more than 400-fold difference (2.64 log[M] units) in sensitivity to *ABA* among individual seeds in the population. The same parameters can be used to predict the complete germination time courses at each *ABA* (Fig. 1B, curves).

From Equations 1 and 2, it can be seen that

$$GR_{50} = 1/t_{50} = (\psi - \overline{\psi}_b)/\theta_H = (\log[ABA/\overline{ABA}_b])/\theta_{ABA} \quad (8)$$

which indicates that a given GR_{50} could be achieved by either reducing ψ or increasing *ABA*. Rearranging Equation 8, it can be shown that the ratio of $\log[ABA/\overline{ABA}_b]$ to $(\psi - \overline{\psi}_b)$ is equal to θ_{ABA}/θ_H. For the high ψ range, this ratio has a value of 4.6 log[M]/MPa. This provides a quantitative comparison of the effectiveness of *ABA* in delaying or preventing germination in terms of corresponding MPa units, or vice versa. That is, a reduction in the external ψ of 0.1 MPa (when $\psi > -0.5$ MPa) would have approximately the same effect as a threefold increase in *ABA* (0.46 log[M] units = 2.9-fold).

Germination in Both ABA and Reduced ψ

The germination responses to combinations of reduced ψ and increased *ABA* are shown in Figure 3. The markers are the experimental data, and the solid curves are the time courses predicted on the basis of the parameters derived in Figure 2 for the separate ψ or *ABA* treatments alone, using the additive model of Equation 3 (see "Theory"). By mathe-

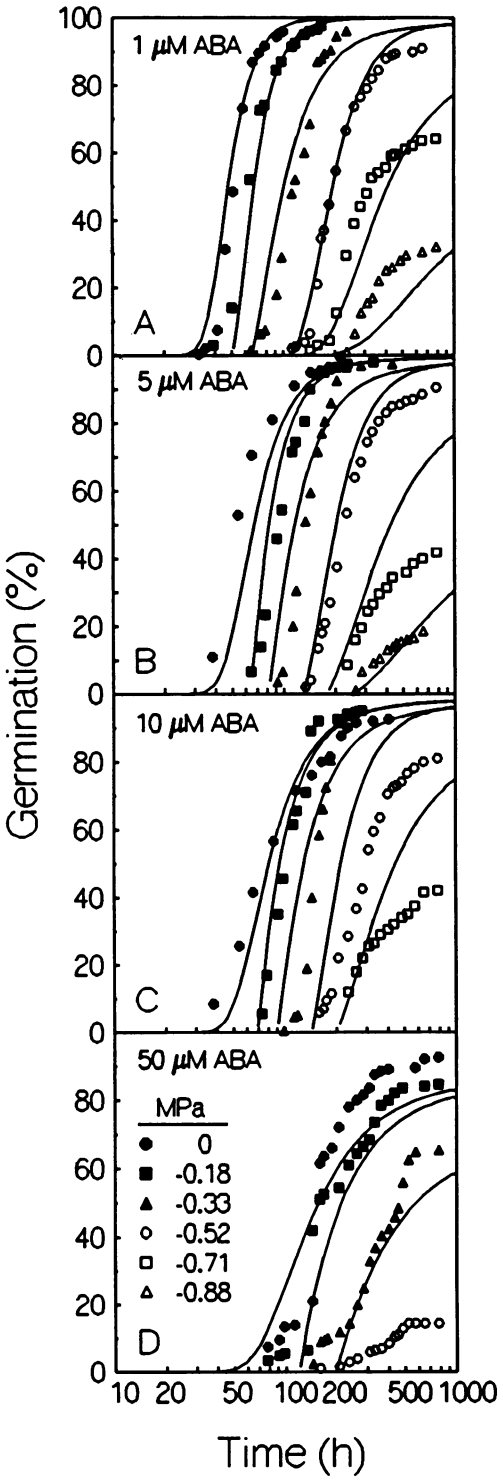

Figure 3. Germination time courses of seeds imbibed in combinations of ABA and reduced ψ. Solutions contained 1 (A), 5 (B), 10 (C), or 50 (D) μM ABA plus PEG to give the ψ indicated by the symbol type. The symbols represent the actual data, and the solid curves are the predicted time courses based upon strictly additive effects of ψ and ABA, according to the parameters derived in Figure 2 and calculated according to Equation 3. In panel D, the value used for $\overline{\psi}_b$ in the predicted curves was −0.42 MPa, based upon the data in Figure 6 showing that $\overline{\psi}_b$ increased linearly at high *ABA*.

matically taking into account the population distributions of germination responses to ψ and *ABA* (embodied in $\overline{\psi}_b$, σ_{ψ_b}, $\overline{ABA}_b$, and σ_{ABA_b}) and their respective time constants (θ_H and θ_{ABA}), the overall correspondence between the curves predicted by this simple model and the actual data is remarkably good, particularly at the higher ψ and lower *ABA* levels (Fig. 3). The poorer correspondence of the model to the actual data at the lowest ψ and highest *ABA* levels can be attributed partly to experimental error in knowing the actual ψ or *ABA* levels present in the germination dishes, as the predicted t_g becomes very sensitive to ψ or *ABA* as those values get closer to their base values (see ref. 4 for discussion of this point). Whereas the curves in Figure 3, A to C were all generated using the same sets of parameters for high ψ, low ψ, and *ABA*, the curves for 50 μM ABA (Fig. 3D) have used a different value for $\overline{\psi}_b$, for reasons that will be described subsequently. It is noteworthy that this model predicts a change in shape of the cumulative germination curves to give very abrupt initial increases in percentage for intermediate ψ and *ABA* combinations, which are also evident in the experimental data (particularly in Fig. 3, B and C).

The mean responses to simultaneous reductions in ψ and increases in *ABA* can be illustrated by the GR_{50} values for each ψ and *ABA* combination (Fig. 4). This graph suggests an additive response to combinations of ψ and *ABA*, as would be expected from the analysis above. Similar relationships between ψ and *ABA* in influencing germination percentages or rates have also been reported for rape and muskmelon seeds (21, 28). That an additive model does account well for the data can be seen by calculating the GR_{50} values predicted by Equation 3 ($1/t_{50}(\psi, ABA)$) and comparing them to the actual values shown in Figure 4. The predicted values corresponded to the actual values over the entire ψ by *ABA* matrix with an r^2 of 0.96 ($P < 0.0001$) and a slope of 0.97 (not significantly different from 1) (Fig. 5).

To further investigate how reduced ψ or increased *ABA* are affecting germination rates, the data of Figure 3 can be reanalyzed to estimate the effects of *ABA* on the ψ responses of the seeds, or the effects of reduced ψ on their ABA sensitivity. For example, if the data at the range of ψ values employed are analyzed separately at each *ABA*, we can estimate the value of $\overline{\psi}_b$ at each *ABA* (Fig. 6A). For the high ψ range, there was a decline in $\overline{\psi}_b$ from −0.58 in water to −0.88 MPa at 10 μM *ABA*, then a linear increase in $\overline{\psi}_b$ at higher *ABA*. For seeds incubated at $\psi < -0.5$ MPa, $\overline{\psi}_b$ remained near −0.9 MPa regardless of the *ABA* level. Thus, incubation at low ψ caused a shift in $\overline{\psi}_b$ to lower values (*cf.* Fig. 2A), which was not further influenced by *ABA*, but at high ψ values, increasing *ABA* up to 10 μM progressively lowered $\overline{\psi}_b$. Above 10 μM *ABA*, $\overline{\psi}_b$ increased markedly, indicating that the seeds were much more sensitive to reductions in ψ in the presence of high *ABA*, a phenomenon observed previously in several species (18, 21, 28, 30). If the linear increase in $\overline{\psi}_b$ with increasing *ABA* above 10 μM is extrapolated to the point where $\overline{\psi}_b = 0$ MPa (*i.e.* where germination would be inhibited by 50% in water), the predicted value is −3.59 log[M] (257 μM), very similar to the value of −3.54 log[M] (288 μM) for $\overline{ABA}_b$ calculated from probit analysis of ABA data alone (Fig. 2B). Thus, low concentrations of ABA (<10 μM) delay germination even though $\overline{\psi}_b$ is decreasing (which would normally increase germination rate). At higher concentrations, ABA causes an increase in $\overline{\psi}_b$ (*i.e.* in the minimum ψ allowing germination, or the ψ threshold) until germination is prevented even at 0 MPa when $ABA = \overline{ABA}_b$. Due to the effect of high ABA on $\overline{\psi}_b$, a value of −0.42 MPa (derived from Fig. 6A) was used in the $t_g(\psi)$ equation to model the combined effects of 50 μM ABA and reduced ψ on germination (Fig. 3D).

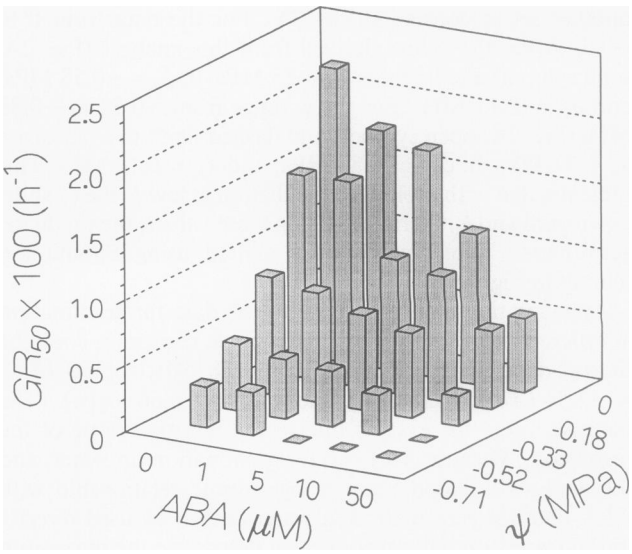

Figure 4. Mean germination rates (GR_{50}) of tomato seeds imbibed in combinations of reduced ψ and *ABA*. The values were determined from the individual time courses in Figure 3. Only treatments in which final germination exceeded 50% are shown.

In contrast to the effects of *ABA* on $\overline{\psi}_b$, decreasing ψ had relatively little effect on $\overline{ABA}_b$, indicating little change in the sensitivity of germination to *ABA* at different ψ values (Fig. 6B).

Solute Accumulation in Embryos Incubated at Reduced ψ or in *ABA*

Incubation of tomato seeds at $\psi < -0.5$ MPa resulted in a lowering of $\overline{\psi}_b$ of about 0.3 MPa, whereas incubation in 100 μM ABA increased $\overline{\psi}_b$ by about the same amount (Fig. 6A). One component of $\overline{\psi}_b$ is the ψ_π of the embryo (4). Therefore, we determined whether extended incubation at low ψ or in the presence of ABA would alter embryonic ψ_π. At a time when germination had just begun in water (32 h), the water content of embryos from ungerminated seeds was 62.9% (dry weight basis; RWC = 100%) and the ψ_π was −1.42 MPa (Table II). Seeds imbibed for 32 h in −1.0 MPa PEG solution had a measured embryo ψ_π of −2.03 MPa, but this was entirely due to the reduced embryo water content, as an ψ_π of −2.04 MPa would be predicted by the RWC of only 74.7% (Table II). After 11 d at −1.0 MPa, however, the embryonic RWC had increased to 91.3% of the control and ψ_π had remained almost constant at −1.97 MPa. In this case, correction for RWC would have predicted an ψ_π of only −1.59 MPa, 0.38 MPa higher than the measured value. When these seeds were transferred from PEG solution to water for 3 h, embryonic

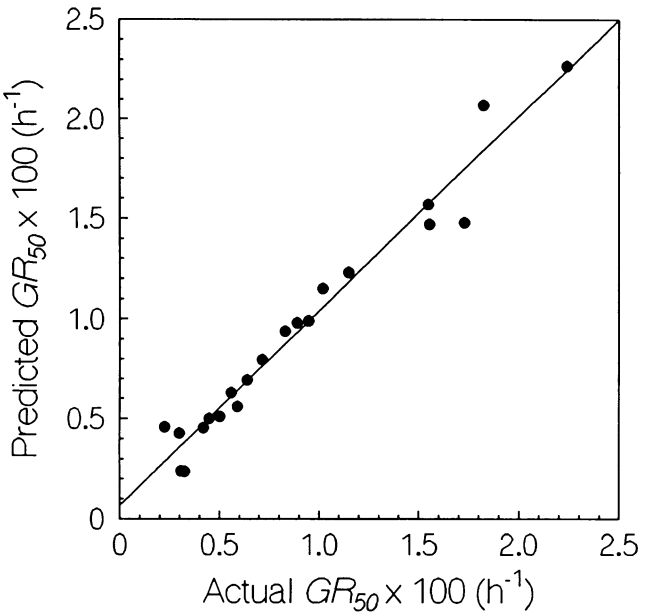

Figure 5. The relationship between the actual GR_{50} values from Figure 4 and the GR_{50} values predicted on the basis of the parameters derived in Figure 2, assuming a simple additive relationship between ψ and *ABA* in delaying germination (Eq. 3). The regression equation is $y = 0.0007 + 0.97\ x$, $r^2 = 0.96$, which does not differ significantly from a 1:1 relationship.

RWC returned to 97.8% of the control value, but ψ_π remained −0.24 MPa lower than the value predicted if no change in solute content had occurred (Table II). These results indicate that 0.24 to 0.38 MPa of solutes had accumulated in the embryos during prolonged incubation at −1.0 MPa, resulting in an increase in embryo water content.

This apparent solute accumulation was confirmed in a time course experiment in which embryonic ψ_π was measured at intervals during incubation in −1.0 MPa PEG solution (Fig. 7A). The ψ_π at RWC = 100% was estimated both from the embryo RWC of seeds incubated at −1.0 MPa, and by transferring such seeds to water for 3 h to allow full imbibition before measuring embryo ψ_π. As in Table II, the values of ψ_π predicted by the latter method were ≈0.1 MPa higher than those predicted by the former method (difference significant at $P < 0.01$). The values presented for ψ_π corrected to RWC = 100% are pooled means from both methods. Whereas the measured ψ_π fell by only 0.1 MPa over the 11-d incubation due to dilution by increasing embryo volume, the total solute accumulation in the embryos was equivalent to 0.31 MPa (Fig. 7).

Because incubation at low ψ results in osmotic accumulation in embryos, we tested whether ABA would also influence embryonic ψ_π or RWC. Seeds imbibed for 1 d in 100 μM ABA had embryonic ψ_π and RWC values not significantly different from those of the water-imbibed control (Table II). After 4 d in ABA solution, embryonic RWC had increased to 120% and ψ_π to −1.19 MPa. However, dilution by the increased water content would have resulted in an ψ_π of −1.15 MPa without any change in solute content (Table II). Thus, at the time that some seeds were beginning to germinate at this *ABA* (Fig. 1B), there was no evidence for changes in solute content in ungerminated seeds, but embryonic water content had increased by 20%. This is in contrast with the results for rape seed, in which ABA also did not affect solute content, but did prevent any increase in embryo volume (21, 22).

Endogenous ABA Contents of Embryos and Endosperm

It could be hypothesized that the additive effects of ψ and ABA on germination rates are due to elevated endogenous ABA levels induced by the reduced ψ. This possibility was tested by measuring the ABA contents of embryos and the endosperm cap directly opposite the radicle tip after incubation at 0, −0.3, and −0.6 MPa. According to analysis of

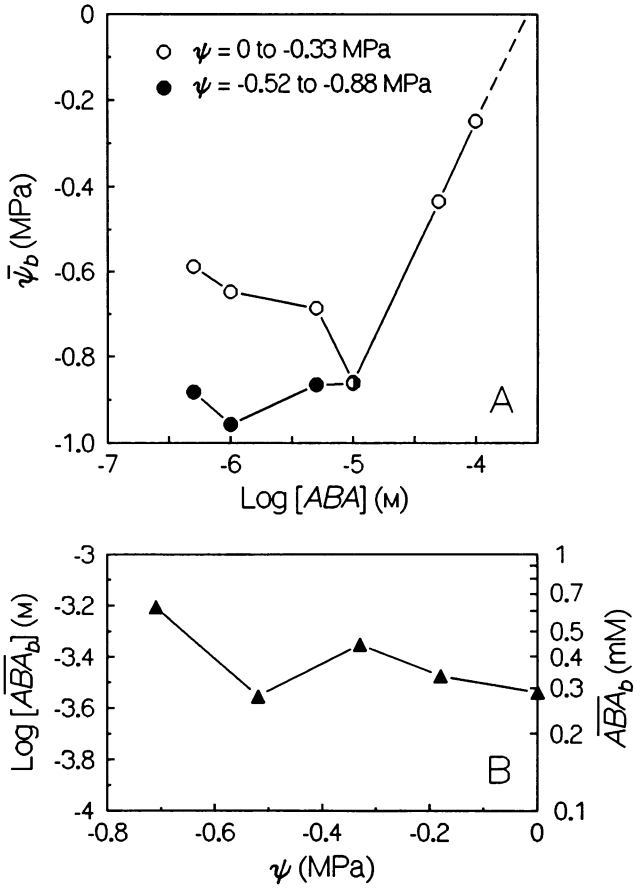

Figure 6. The influence of *ABA* on the value of $\bar{\psi}_b$ (A) and of ψ on the value of $\overline{ABA}_b$ (B). For A, the response to ψ was determined separately for germination time courses at each *ABA*, and the values of $\bar{\psi}_b$ were determined by probit analysis. The ψ response was analyzed separately for the high ψ (0 to −0.33 MPa, closed symbols) and the low ψ (−0.52 to −0.88 MPa; open symbols) regions. At *ABA* > 10 μM, insufficient seeds germinated at low ψ to estimate $\bar{\psi}_b$ (see Fig. 4), but at high ψ, the relationship between log[*ABA*] and $\bar{\psi}_b$ is described by $\bar{\psi}_b = 2.20 + 0.61 \log[ABA]$ ($r^2 = 0.99$). The dashed line shows the extrapolation of this relationship to $\bar{\psi}_b = 0$; the *ABA* at this point should be equivalent to $\overline{ABA}_b$. B, The response to *ABA* was determined by probit analysis at each ψ level to estimate the values of $\overline{ABA}_b$.

Table II. *Water Content, Relative Water Content, and Osmotic Potential of Tomato Embryos after Imbibition of Seeds on Water, −1.0 MPa PEG Solution, or 100 μM ABA*

Seeds were imbibed as indicated before the embryos were extracted for water content (dry weight basis) and ψ_π measurements. The predicted ψ_π values are those that would be expected for the observed embryo RWC assuming no change in solute content relative to the control imbibed on water. Means ± SE are shown (n = 5).

Imbibition Conditions	Water Content		ψ_π	
	Embryo	RWC	Observed	Predicted
	%		MPa	
H_2O (32 h)	62.9 ± 2.9	100	−1.42 ± 0.05	−1.42
−1.0 MPa (32 h)	47.0 ± 0.4	74.7	−2.03 ± 0.09	−2.04
−1.0 MPa (11 d)	57.4 ± 0.9	91.3	−1.97 ± 0.04	−1.59
−1.0 MPa (11 d) → H_2O (3 h)	61.5 ± 2.0	97.8	−1.70 ± 0.08	−1.46
100 μM ABA (1 d)	61.4 ± 2.2	97.6	−1.36 ± 0.09	−1.46
100 μM ABA (4 d)	75.2 ± 4.0	120	−1.19 ± 0.05	−1.15

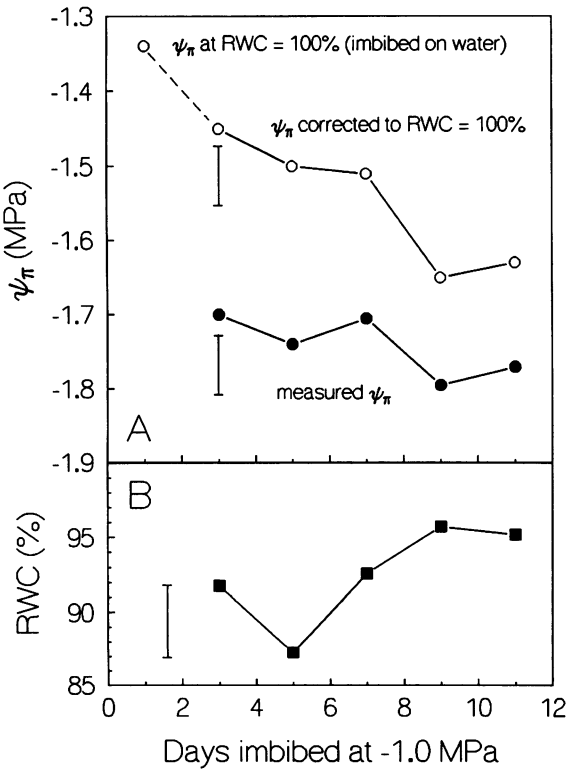

Figure 7. Osmotic potential (A) and RWC (B) of tomato embryos during incubation at −1.0 MPa. The closed circles in A are the actual measured values of ψ_π. The open circles are the ψ_π values corrected to 100% RWC, using the data in panel B and a value of 17% for the nonosmotic RWC. Error bars indicate LSD ($P < 0.05$).

variance, there was a highly significant interaction between ψ and tissue type due to a slight increase in ABA in the embryos and a slight decrease in ABA in the endosperm caps as ψ decreased (Table III). Although statistically significant, these changes amounted to <15% of the ABA content of the same tissues imbibed on water. It is unlikely that these small changes in endogenous ABA levels could be mediating the effects of ψ in delaying germination, as it can be calculated from Equation 8 that a 24-fold increase in *ABA* (1.38 log[M] units) from the apparent threshold of 0.5 μM would be required to mimic the effect of −0.3 MPa on GR_{50}. Even considering the possibility that the internal ABA concentration only attains half of that in the external medium (25), the change in endogenous ABA content of the embryos is at least an order of magnitude too low to account for the delay in germination at reduced ψ. On the other hand, using the average value of 168 ng ABA/g dry weight for the embryo (Table III) and an embryo water content of 63% of the dry weight (Table II), and assuming that the endogenous ABA is uniformly distributed in the embryo water, we can calculate that the endogenous ABA concentration would be ≈1 μM. Although this can be taken only as a rough estimate, it suggests that the threshold exogenous ABA concentration for a germination response (0.5 μM) would change the endogenous ABA content by only 50%. A similar conclusion can be derived from the data of Groot (10).

DISCUSSION

The methods developed here provide a means to quantitatively describe the responses of seed germination to ψ, ABA, or combinations of reduced ψ and ABA. The parameters derived are estimates of the mean sensitivity to inhibition by reduced ψ ($\overline{\psi}_b$) or ABA ($\overline{ABA}_b$) and of the variation among individual seeds in their sensitivities to these factors (σ_{ψ_b}, σ_{ABA_b}). The analysis also indicates that the time required for a seed to germinate is inversely proportional to the difference between the actual ψ or log[*ABA*] of the seed and its own ψ_b or log[ABA_b], with proportionality constants of θ_H and θ_{ABA}, respectively (Eq. 8). Once the parameters of the model are determined for a particular seed lot (Fig. 2), the germination response of the entire seed population to ψ or *ABA*, separately or in combination, can be predicted (Figs. 1, 3, A–C). When both reduced ψ and ABA > 10 μM are present simultaneously, the effect of ABA on $\overline{\psi}_b$ must also be considered (Fig. 3D).

Table III. *Endogenous ABA Levels in Tomato Embryos and Endosperm Caps after Imbibition at a Range of Water Potentials*

Tomato seeds were imbibed at 0, −0.3, or −0.6 MPa for 1, 2, or 5 d, respectively. Embryos and endosperm caps were excised and assayed for ABA using an indirect ELISA procedure. Values are means ± SD (n = 3).

Imbibition ψ	ABA Content	
	Embryo	Endosperm cap
MPa	ng/g dry wt	
0	154 ± 3	224 ± 1
−0.3	175 ± 9	194 ± 11
−0.6	175 ± 13	182 ± 9

These relationships are presented in the form of a flowchart indicating how the various parameters respond to different ψ and ABA levels, and how they combine to determine the rate and extent of germination (Fig. 8). We will now illustrate this model with specific examples of how various ψ and ABA levels result in observed germination behaviors.

The relationships between the distributions of $\psi_b(g)$ and the consequent germination time courses at various ψ levels are shown in Figure 9, A and B, based upon the parameters derived for the tomato seed lot under study. The fraction of the seed population having a particular value of ψ_b is shown as a normal distribution with a mean of −0.58 MPa and a standard deviation (σ_{ψ_b}) of 0.11 MPa (Fig. 9A, solid curve; represented by $\psi_b(g)$ in Fig. 8). After prolonged incubation at $\psi < -0.5$ MPa, a shift in $\psi_b(g)$ occurs such that the mean is now −0.88 MPa and σ_{ψ_b} is 0.14 MPa (Fig. 9A, dashed curve; represented by $\psi_b(g)^*$ in Fig. 8). This response of tomato seeds to incubation at low ψ has been described previously for

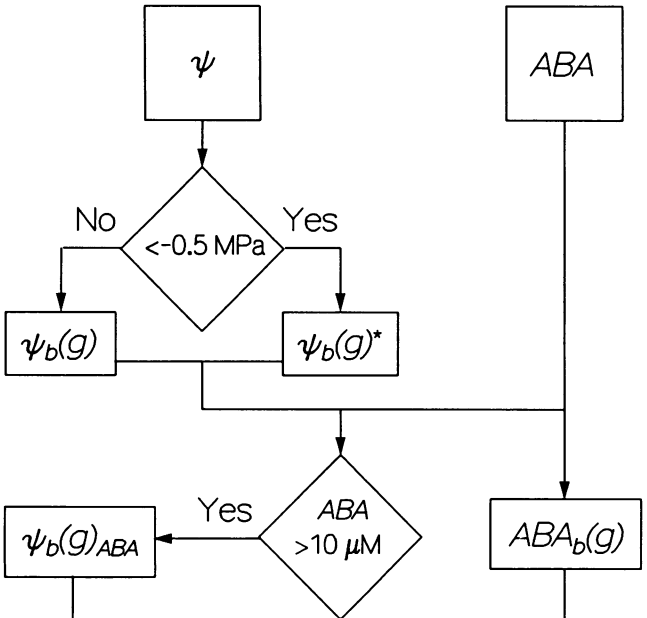

Figure 8. Flow chart describing the influence of ψ and *ABA* on the time to germination of tomato seeds. The values of the symbols shown (± the standard deviations of the distributions around the means) are: $\psi_b(g) = -0.58 \pm 0.11$ MPa; $\psi_b(g)^* = -0.88 \pm 0.14$ MPa; $\psi_b(g)_{ABA} = 2.20 \pm 0.61(\log[ABA]) \pm 0.11$ MPa; $ABA_b(g) = -3.54 \pm 0.66 \log[M]$; $t_g(\psi) = \theta_H/(\psi - \psi_b(g))$; $t_g(ABA) = \theta_{ABA}/\log[ABA/ABA_b(g)]$; $t_g(0) = \theta_H/(0 - \psi_b(g))$; $\theta_H = 25$ MPa·h; $\theta_H^* = 71$ MPa·h; $\theta_{ABA} = -118 \log[M]$·h.

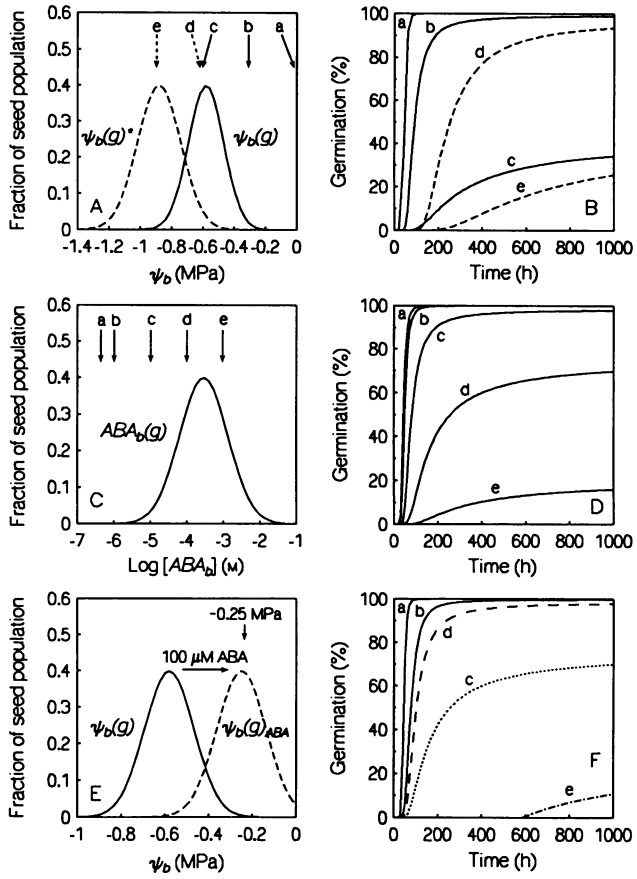

Figure 9. Examples of germination time courses (B, D, F) resulting from the distributions of $\psi_b(g)$ (A, E) or $ABA_b(g)$ (C) under various conditions of ψ and *ABA*. Note that the time scales are linear in B, D, and F, in contrast with the logarithmic scales used in Figures 1 and 3. The letters above the arrows in A and C indicate the ψ or *ABA* level present (read on the *x* axis), and the corresponding germination time courses are labeled with the same letters in B and D. In A and B, the solid curves relate to the distribution of $\psi_b(g)$ at high ψ, and the dashed curves relate to $\psi_b(g)^*$, the distribution after extended incubation at low ψ. In F, curve a is in water with $\overline{\psi}_b = -0.58$ MPa; curve b in −0.25 MPa solution with $\overline{\psi}_b = -0.58$ MPa; curve c in 100 μM ABA; curve d in water with $\overline{\psi}_b = -0.25$ MPa; and curve e in −0.25 MPa + 100 μM ABA ($\overline{\psi}_b = -0.25$ MPa).

seeds with the endosperm cap covering the radicle tip removed (6), and is demonstrated here to occur in intact seeds as well. The time to germination is inversely proportional to the difference between the actual ψ and the $\overline{\psi}_b$ of a particular seed fraction ($t_g(\psi)$ in Fig. 8; *cf.* Eqs. 1 and 8), so that for seeds germinating in water (Fig. 9A, arrow a), a cumulative normal distribution of germination percentages in time is generated (Fig. 9B, curve a). When ψ is reduced to −0.3 MPa (Fig. 9A, arrow b), the times to germination are increased in proportion to the smaller difference between ψ and ψ_b for each seed fraction (Fig. 9B, curve b). If ψ is reduced to −0.6 MPa (Fig. 9A, arrow c), over 50% of the seeds would not germinate if no change had occurred in $\psi_b(g)$ because ψ would be less than their ψ_b, and the germination of the remaining seeds would be considerably delayed (Fig. 9B, curve c). However, incuba-

tion at low ψ caused a shift in the $\psi_b(g)$ distribution to lower values ($\psi_b(g)$*) (Fig. 9A, dashed curve), resulting in the actual germination time course for seeds incubated at −0.6 MPa (Fig. 9B, curve d). After the shift to $\psi_b(g)$*, some of the seeds can germinate at −0.9 MPa (Fig. 9B, curve e), a ψ that would have completely prevented germination of the unadapted seeds (Fig. 9A, arrow e).

The shift in $\psi_b(g)$ detected by the analysis of germination time courses (Fig. 2) was confirmed by the measurement of solute accumulation in the embryo. Solute accumulation could fully account for the 0.3 MPa reduction in $\psi_b(g)$ observed in seeds incubated at low ψ, as a 0.31 MPa more negative ψ would be required to reduce the growth potential (or the ψ_π difference between the embryo and the external solution) of the adjusted embryos to a threshold level as compared with unadjusted embryos (Figs. 2A, 6A). That is, if the data of Figure 2A were plotted as a function of a base turgor rather than of $\psi_b(g)$ (see ref. 4), the regressions for the two ψ ranges would essentially coincide after the osmotic accumulation process had occurred at low ψ. The different θ_H values derived for the high and low ψ regions are largely due to the time required for the adaptation process to occur (Fig. 7), rather than to an inherent change in the rate of progress toward germination per unit difference between ψ and $\psi_b(g)$ (see also ref. 6). Haigh (12) also observed a 0.3 MPa reduction in ψ_π of tomato embryos when they were imbibed on water after a previous imbibition period in osmoticum. Embryo solute accumulation appears to be dependent upon incubation at ψ values equal to or below the initial $\overline{\psi}_b$ of the seeds, as there was no evidence for changes in $\psi_b(g)$ or ψ_π for seeds incubated at $\psi > -0.5$ MPa (Fig. 2A) (13). Solute accumulation in the embryo during prolonged imbibition at reduced ψ could contribute to the increasing germination rate with increasing preimbibition time that has been observed for tomato (14), because the lower $\psi_b(g)$* would result in shorter times to germination when the seeds are reimbibed on water (*cf.* Fig. 9A). In contrast with the osmotic adjustment observed here for tomato embryos, no accumulation of solutes was detected in lettuce (*Lactuca sativa* L.) embryos incubated under similar conditions, even when RWC was taken into account (4, 27).

Weakening of the endosperm tissue directly opposite the radicle tip would also result in an apparent reduction in $\psi_b(g)$ because the endosperm restrains the embryo and limits its water uptake (6, 12). There is good evidence that weakening of the endosperm cap occurs during prolonged incubation in osmotic solutions and is related to subsequently increased germination rates (12, 15). It has been suggested that, whereas endosperm weakening occurs during imbibition in osmotic solutions, a second phase of weakening must occur prior to radicle emergence (12, 15). Values of $\psi_b(g)$ are most likely related to the resistance of the endosperm during the second phase of weakening, because the procedure is based upon the accomplishment of radicle protrusion rather than on the resistance to puncture of excised endosperm caps.

The response of germination to ABA can be described in terms of the sensitivity of particular seeds to ABA, or the distribution of $ABA_b(g)$ (Figs. 8, 9C). In water or up to the threshold level of external *ABA* (Fig. 9C, arrow a), germination rate is maximal for the particular seed lot (Fig. 9D, curve a). As *ABA* increases, the value of $\log[ABA] - \log[ABA_b(g)]$ decreases (Fig. 9C, arrows b–e), resulting in slower germination and eventually inhibition of germination (Fig. 9D, curves b–e). This relationship is indicated by $t_g(ABA)$ in Figure 8 (*cf.* Eqs. 2, 8). At concentrations less than 10 μM, ABA had little effect on $\overline{\psi}_b$ (Fig. 6A), indicating that ABA can delay germination independent of interactions with the water relations parameters of germination. When *ABA* increased above 10 μM, however, there was a linear increase in $\overline{\psi}_b$ that matched almost exactly the relationship predicted from the analysis of germination responses to ABA alone (Fig. 6A; $\psi_b(g)_{ABA}$ in Fig. 8). This increase in $\psi_b(g)$ at high *ABA* is in agreement with the conclusion of Schopfer and Plachy (22) that ABA increased the yield threshold for growth of germinating rape seeds. At $ABA > 10$ μM, there is a delaying effect of ABA that is evident at lower concentrations, and also an increase in $\psi_b(g)$ that affects the response to ψ as well (Fig. 8).

These interactions between ψ and ABA can be illustrated by examining the consequences of changes in $\psi_b(g)$ at different ψ or *ABA* levels (Fig. 9, E and F). Simply reducing ψ to −0.25 MPa will delay germination somewhat (Fig. 9F, curve b) compared to the control germination curve in water (Fig. 9F, curve a). Imbibition in 100 μM ABA alone will also delay germination and inhibit it by about 40% (Fig. 9F, curve c). In addition, the distribution of $\psi_b(g)$ present in seeds imbibed at high ψ or low *ABA* (Fig. 9E, solid curve), shifts to a mean of −0.25 MPa in the presence of 100 μM ABA (Fig. 9E, dashed curve; Fig. 6A). If the sole effect of ABA was to increase $\psi_b(g)$, then germination would be inhibited much less, as shown by the predicted curve for germination in water where the $\overline{\psi}_b$ has been increased from −0.58 to −0.25 (Fig. 9F, curve d). The additional effect of ABA (the difference between curves c and d in Fig. 9F) is due to the delaying effect of ABA superimposed on the change in $\psi_b(g)$. The combined effects of ψ and *ABA* remain strictly additive, but only if the correct $\psi_b(g)$ value based upon the ABA level present is used in the equation (as in Fig. 3D). As a consequence of the increase in $\psi_b(g)$, the effect of the combination of −0.25 MPa and 100 μM ABA is much greater than would be predicted from their independent effects alone (Fig. 9F, curve e). This relationship can explain the apparently synergistic interaction of ABA and osmoticum observed in some cases in which the combined effects of *ABA* and ψ are greater than the sum of the effects of either factor alone (21, 28, 29). It also is in agreement with the proposals that reduced ψ delays or inhibits germination by lowering embryo turgor in excess of a threshold (4, 28) or by reducing "growth potential" (22), whereas ABA (at high concentrations) acts in part by increasing the threshold (22, 28).

As shown in Figure 8, the combined effect of ψ and ABA on the time to germination of a particular fraction of the seed population ($t_g(\psi, ABA)$) is determined by the sum of the predicted germination times for: (a) the given ψ and distribution of $\psi_b(g)$ ($t_g(\psi) = \theta_H/(\psi - \psi_b(g))$, including the effects of solute accumulation and *ABA* on $\psi_b(g)$, $\psi_b(g)$*, and $\psi_b(g)_{ABA}$, when appropriate); (b) the *ABA* level present and the distribution of $ABA_b(g)$ ($t_g(ABA) = \theta_{ABA}/\log[ABA/ABA_b(g)]$); and (c) the time to germination in water ($t_g(0) = \theta_H/(0 - \psi_b(g))$). It is evident that when no exogenous ABA is present, $t_g(ABA) = t_g(0)$, leaving only the ψ response; similarly, if $\psi = 0$ MPa, $t_g(\psi) = t_g(0)$, leaving only the ABA response.

Therefore, this model can fully account for the observed germination time courses at any combination of ψ or *ABA* from threshold levels to those completely inhibitory to germination.

Although the present model is derived from responses to exogenous ABA, available data indicate that endogenous ABA probably acts similarly. A 10-fold reduction in endogenous ABA content of seeds of the ABA-deficient tomato mutant *sit*^{W} compared with wild-type seeds (cv Moneymaker) resulted in a lowering of 0.5 MPa in the mean ψ required to inhibit germination (10). We have recently repeated these experiments and found $\overline{\psi}_b$ values of −0.35 MPa for wild-type seeds compared with −1.2 MPa for *sit*^{W} seeds. Both the wild type and *sit*^{W} seeds were much more sensitive to ABA than were T5 seeds, with $\overline{ABA}_b$ values of 6 and 13 μM, respectively (our unpublished results). Liptay and Schopfer (18) showed that a rapidly germinating genotype of tomato (PI341988) was more tolerant of reduced ψ and increased ABA levels than was a slowly germinating genotype (ST). Reanalyzing their data by the present method, we derive values of $\overline{\psi}_b = -0.67$ MPa and $\overline{ABA}_b = 776$ μM for PI341988, and $\overline{\psi}_b = -0.36$ MPa and $\overline{ABA}_b = 135$ μM for ST. These values for $\overline{\psi}_b$ and $\overline{ABA}_b$ bracket those for the T5 seed lot used here, and demonstrate that rapid germination is associated with low $\overline{\psi}_b$ and high $\overline{ABA}_b$ values, whereas slow germination is associated with the opposite trend.

In tomato, the primary site of action of ABA is in the endosperm cap (18), where it inhibits the expression of cell wall hydrolyzing enzymes apparently responsible for cell separation and weakening (10). If germination occurs when embryo turgor exceeds the restraining force of the endosperm cap, then low levels of ABA may only delay expression of such enzymes or reduce their steady-state levels, whereas higher concentrations would completely inhibit them. Alternatively, ABA could induce germination inhibitors in a concentration-dependent manner. Either case would result in only delayed germination at low *ABA*, but increasing inhibition and higher $\psi_b(g)$ values at higher *ABA*. ABA action on the endosperm cap would be consistent with the increase in embryo RWC without accompanying solute accumulation in the presence of ABA (Table II). Wall relaxation in the embryo (12) and expansion of the endosperm cavity (1) apparently continued in the presence of ABA, allowing uptake of water, but emergence of the radicle was still blocked by absence of weakening in the endosperm cap. It is intriguing that the increase in embryo water content after prolonged incubation in ABA (20%) is equivalent to the amount by which embryo volume is initially constrained by the surrounding endosperm (13). Partial weakening of the endosperm cap occurs during incubation at reduced ψ, although at a slower rate than in water (12, 15), and solute accumulation can occur (Table II; Fig. 7), eventually allowing germination at ψ values that would initially have been inhibitory (Fig. 9B). The effects of reduced ψ are not mediated by elevated endogenous ABA (Table III) or increased sensitivity to ABA (Fig. 6B). Thus, although both ABA and reduced ψ can result in superficially similar germination time courses, the present results indicate that their mechanisms of action are quite distinct. Similar conclusions have been reached concerning the regulation of seed development and gene expression by ABA and osmoticum (2, 3, 8, 30; see 9, 16, for reviews). ABA and osmoticum could influence seed development and germination via distinct, although perhaps overlapping, effects on gene expression.

The mathematical form of the models presented here is directly analogous to that used to describe the rates of biological processes as a function of temperature, *i.e.* thermal time analysis (7, 11). In the case of temperature, thermal time (degree-hours or degree-days) is accumulated as the product of the temperature in excess of a base temperature multiplied by the duration at that temperature. The rates of many biological processes at different temperatures often can be normalized to a single rate on a thermal time scale. A normalization function for seed germination at different ψ values, or a "hydrotime" scale, has also been developed (4, 6). The present experiments suggest that for seed germination, at least, the concept of "ABA-time" (log[M]·h) also has physiological meaning, and that the metabolic processes preparatory to radicle emergence at different ψ or ABA levels may proceed at rates that are equivalent when compared on the appropriate ABA or hydrotime scales. The additive effects of ABA and ψ on germination rates may indicate that both of these factors alter a fundamental mechanism governing the rates of specific physiological processes, although perhaps through separate pathways.

The models presented may not be specific to seed germination rates and could have general applicability to other growth and developmental processes. Two key features of the model, the reciprocal relationship between the level of a controlling factor and the time required for an effect and the variation among cells or tissues in sensitivity to various factors, have been discussed independently by Trewavas (24). At present, we could only speculate on the nature of the receptors and transduction pathways that would allow the rates of specific processes to be quantitatively sensitive to ψ or ABA (the physiological bases of θ_H and θ_{ABA}) (23). On the other hand, it is clear that this type of regulation does occur with respect to gene expression in response to reduced ψ or increased *ABA* (3, 5, 9, 16, 30). Although not providing these mechanistic answers directly, the approach presented here does allow quantitation of sensitivity to various control factors near their endogenous threshold levels, estimation of their contribution to control in the presence of other interacting factors, and incorporation of the population distributions of sensitivity and response into the overall analysis. Therefore, it represents a significant step toward satisfying the criteria proposed for obtaining meaningful information about plant growth regulation (24), and will allow quantitative predictive tests to be made of new hypotheses generated from its further application.

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