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Water Relations of Seed Development and Germination in Muskmelon (*Cucumis melo* L.)[1]

III. Sensitivity of Germination to Water Potential and Abscisic Acid during Development

Gregory E. Welbaum[2], Tahar Tissaoui[3], and Kent J. Bradford*

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

ABSTRACT

Muskmelon (*Cucumis melo* L.) seeds are germinable 15 to 20 days before fruit maturity and are held at relatively high water content within the fruit, yet little precocious germination is observed. To investigate two possible factors preventing precocious germination, the inhibitory effects of abscisic acid and osmoticum on muskmelon seed germination were determined throughout development. Seeds were harvested at 5-day intervals from 30 to 65 days after anthesis (DAA) and incubated either fresh or after drying on factorial combinations of 0, 1, 3.3, 10, or 33 micromolar abscisic acid (ABA) and 0, −0.2, −0.4, −0.6, or −0.8 megapascals polyethylene glycol 8000 solutions at 30°C. Radicle emergence was scored at 12-hour intervals for 10 days. In the absence of ABA, the water potential (Ψ) required to inhibit fresh seed germination by 50% decreased from −0.3 to −0.8 megapascals between 30 and 60 DAA. The Ψ inside developing fruits was from 0.4 to 1.4 megapascals lower than that required for germination at all stages of development, indicating that the fruit Ψ is sufficiently low to prevent precocious germination. At 0 megapascal, the ABA concentration required to inhibit germination by 50% was approximately 10 micromolar up to 50 DAA and increased to >33 micromolar thereafter. Dehydration improved subsequent germination of immature seeds in ABA or low Ψ. There was a linear additive interaction between ABA and Ψ such that 10 micromolar ABA or −0.5 megapascal osmotic potential resulted in equivalent, and additive, reductions in germination rate and percentage of mature seeds. Abscisic acid had no effect on embryo solute potential or water content, but increased the apparent minimum turgor required for germination. ABA and osmoticum appear to influence germination rates and percentages by reducing the embryo growth potential (turgor in excess of a minimum threshold turgor) but via different mechanisms. Abscisic acid apparently increases the minimum turgor threshold, while low Ψ reduces turgor by reducing seed water content.

Abscisic acid and low Ψ[4] prevent precocious germination and extend seed development in many species (1, 6, 7, 9, 13, 16, 17, 24). Immature seeds from ABA-deficient mutant lines of *Arabidopsis thaliana* will germinate precociously when isolated from the silique and incubated under moist conditions (11). This indicates that in the absence of ABA, limited access to water is the primary factor arresting development in these seeds (11, 23). In dry-seeded crops such as wheat, rape, and beans, the fruit tissue surrounding the seed normally desiccates at the same time as the seed, preventing precocious germination (2). However, in fleshy-fruited species such as tomatoes or melons, germinable seeds are held at relatively high water content for extended periods with little precocious germination. Viviparous germination of seeds in overripe tomato fruits was observed, however, in an ABA-deficient mutant (9). The Ψ and ψ_s values of muskmelon fruit tissue and seeds decline during the later stages of seed development after maximum dry weight and full germinability are attained (25). The hypothesis was advanced (25) that the osmotic environment inside mature fruit could prevent precocious seed germination regardless of ABA levels, which characteristically fall during the final stages of seed development (13). To test this hypothesis, the minimum Ψs required for germination of muskmelon seeds at various developmental stages were compared with the Ψ values of seeds developing inside the fruit.

Osmotic solutions and ABA can have similar effects in preventing precocious germination (7, 17, 18). The ABA-deficient tomato mutant *sit*^{w}/*sit*^{w} had a minimum Ψ for germination 0.5 MPa lower than that of the wild type, leading to the proposal that ABA inhibits germination by preventing GA-induced endosperm weakening and by reducing the ability of the embryo to grow at low Ψ (9). In agreement with this, ABA increased the sensitivity of germination to inhibition by water stress in mature rape and tomato seeds (14, 20). The effect of increasing ABA concentrations on germination of rape seed was very similar to that of decreasing Ψ, resulting in a linear interaction between inhibition of germination by ABA and by reduced Ψ (20). Subsequent work on rape seed showed that the effect of ABA was to increase the minimum ψ_p for growth and decrease the proportionality constant between growth rate and ψ_p (extensibility coefficient) (21).

Since ABA is commonly found in developing seeds (13), changes in ABA levels or in responsiveness to ABA during development could be involved in determining the sensitivity of germination to Ψ. Additionally, dehydration of developing

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[2] Current address: Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0327.

[3] Current address: Institut National Agronomique de Tunisie, 43 Avenue Charles Nicolle, 1002 Tunis, Tunisia.

[4] Abbreviations: Ψ, water potential; DAA, days after anthesis; ψ_s, solute or osmotic potential; ψ_p, turgor pressure; σ, standard deviation; Y, yield threshold; WC, water content.

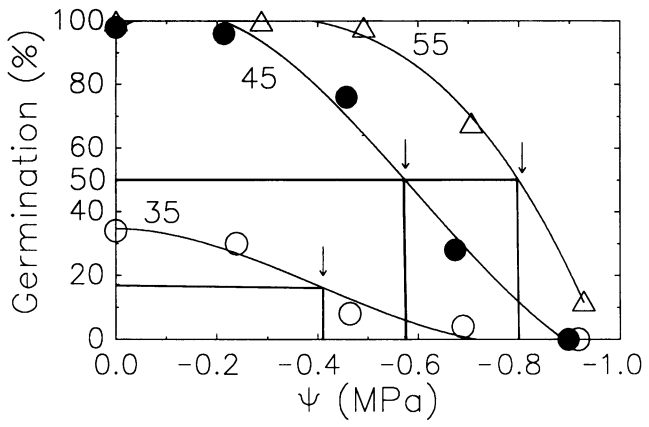

Figure 1. Estimation of Ψs required to inhibit maximum germination by 50% (Ψ_{50}) from curves fitted to plots of final germination percentage *versus* Ψ. The arrows indicate Ψ_{50} values of −0.41, −0.57, and −0.80 MPa for 35-, 45-, and 55-DAA fresh seeds, respectively.

castor bean seeds can alter their responsiveness to ABA (12). If the germination responses of developing muskmelon seeds to ABA parallel their responses to reductions in Ψ, it would support the hypothesis that ABA inhibits germination by altering cellular water relations parameters. The sensitivities of germination to ABA and Ψ were examined during muskmelon seed development, both with and without desiccation, to determine whether the low Ψ of muskmelon fruit tissue prevents precocious germination, whether ABA and Ψ interact to inhibit seed germination, and whether there is evidence that ABA and Ψ act to inhibit germination in a coordinated manner by affecting the same water relations parameters.

MATERIALS AND METHODS

Plant Material

Muskmelon (*Cucumis melo* L. cv Top Mark, Asgrow Inc, Gonzales, CA) plants were field-grown using cultural practices previously described (25). Flowers were tagged at anthesis and fruits were harvested at 5-d intervals from 10 to 50 DAA in 1985 and from 30 to 65 DAA in 1986.

Germination Tests

Seeds were removed from the fruit and wiped with dry paper towels to remove the mucilaginous endocarp. After blotting, the seeds were held for less than 1 h at high RH until the start of the germination test (fresh treatment) or prior to drying. Seeds were forced-air dried at room temperature for 6 h, then transferred to a desiccator containing activated silica gel for an additional 48 h at 30°C. For the 1985 study, 50 seeds were rinsed in tap water, dried to a WC of 6.0%, and placed on rolled germination paper towels saturated with either deionized water or 0.1, 1, 10, or 100 μM (±) 2 *cis*-4 *trans*-ABA (Sigma, St. Louis, MO.) dissolved in a basic aqueous solution and adjusted to pH 7.0. The rolled towels were placed inside plastic bags in a dark incubator at 20°C/30°C alternating temperature (16/8 h, respectively). After 10 d, seedlings were scored in accordance with Association of Official Seed Analysts standards (27) to determine the percentage of normal seedlings.

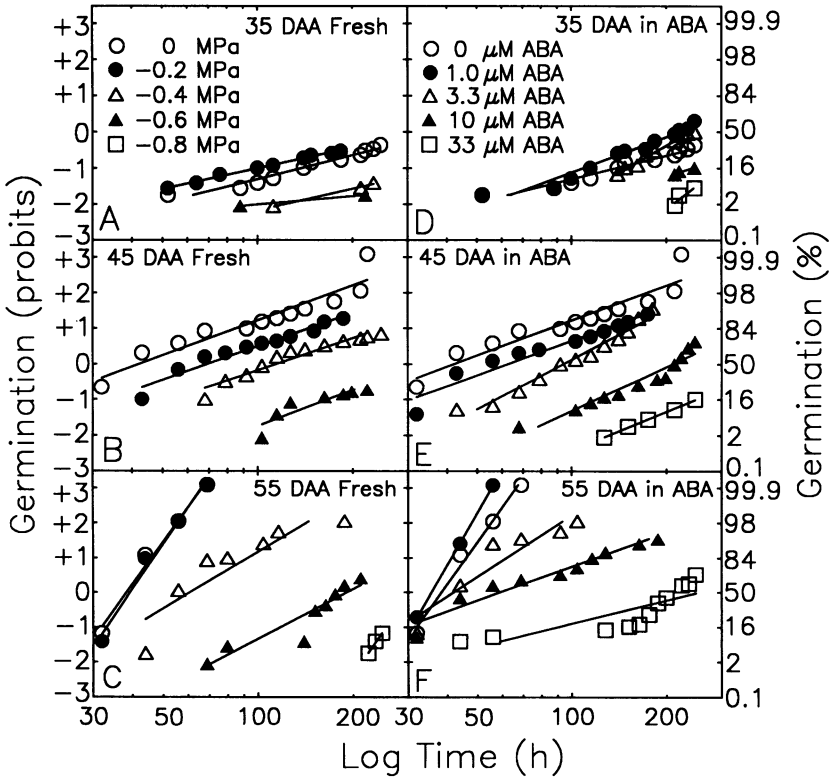

Figure 2. Cumulative germination percentages (on a probit scale) for fresh muskmelon seeds at decreasing Ψs (A, B, C) or increasing ABA concentrations (D, E, F) *versus* log time for 35 (A, D), 45 (B, E), and 55 (C, F) DAA seeds. The final germination percentages, log $\bar{t}$, and σ values are summarized and compared with the corresponding values for dried seeds in Tables I and II.

Table I. *Influence of Ψ and Drying on Germination Percentage, Rate, and Uniformity of Muskmelon Seeds Throughout Development*

Germination percentages, mean times to radicle emergence (log $\bar{t}$), and standard deviations of germination times (σ) are compared for fresh or dried muskmelon seeds harvested at 5-d intervals after anthesis, sealed in plastic boxes on blotter paper saturated with 0 to −0.8 MPa PEG solutions, and scored for radicle emergence at 12-h intervals for 10 d. ANOVA for germination percentages and log $\bar{t}$ revealed highly significant differences among fresh and dried seeds, developmental stages, and Ψ treatments. ANOVA for σ showed no significant differences between fresh and dried seeds, highly significant differences among developmental stages, and significant differences among Ψ treatments.

DAA	Fresh or Dried	Ψ MPa												
		Germination[a]					log $\bar{t}$				σ			
		0	−0.2	−0.4	−0.6	−0.8	0	−0.2	−0.4	−0.6	0	−0.2	−0.4	−0.6
		%					*log h*							
30	F	24	12	0	0	0	2.27				0.07			
	D	66	90	70	10	0	2.10	1.99	2.24		0.17	0.13	0.09	
35	F	34	30	8	4	0	1.97	1.96			0.22	0.22		
	D	52	84	48	20	0	2.07	2.01	2.13	2.26	0.28	0.15	0.16	0.07
40	F	58	54	26	8	0	2.04	1.99	2.15		0.21	0.21	0.15	
	D	98	96	56	10	0	1.93	1.84	2.08		0.21	0.20	0.15	
45	F	98	92	80	24	0	1.67	1.81	1.98	2.12	0.23	0.20	0.17	0.11
	D	96	98	80	22	0	1.62	1.69	1.90	2.10	0.10	0.22	0.24	0.10
50	F	100	98	74	20	6	1.62	1.66	1.80	2.10	0.19	0.20	0.23	0.19
	D	100	98	70	40	8	1.63	1.80	1.79	1.86	0.20	0.21	0.21	0.17
55	F	100	100	98	68	12	1.57	1.58	1.77	2.18	0.07	0.07	0.13	0.12
	D	100	100	100	54	2	1.56	1.58	1.73	2.20	0.09	0.08	0.16	0.15
60	F	98	98	86	34	2	1.53	1.61	1.63	2.03	0.10	0.13	0.11	0.22
	D	100	100	98	80	10	1.54	1.56	1.69	2.20	0.09	0.03	0.19	0.15
65	F	84	94	86	44	12	1.68	1.70	1.74	1.87	0.16	0.21	0.18	0.23
	D	92	94	84	64	8	1.55	1.63	1.73	2.15	0.12	0.22	0.15	0.15

[a] LSD ($P < 0.05$): germination, 19; log $\bar{t}$, 0.36; σ, 0.11.

In 1986, seeds were placed in 11×11×3 cm transparent covered plastic boxes on two germination blotter papers (Filtration Sciences, Mount Holly Springs, PA). The blotters were saturated with 17 mL of solutions containing ABA and PEG 8000 (Union Carbide, Danbury, CT). The ABA concentrations were 0, 1, 3.3, 10, and 33 μM, and the initial water potentials of the PEG solutions were 0, −0.2, −0.4, −0.6, and −0.8 MPa. A complete factorial (5×5) combination of all ABA and PEG concentrations was employed, with two replications of 25 seeds each for each stage of development. The seeds were incubated in the dark at 30 ± 1°C. Seeds were scored for radicle emergence at 12-h intervals for 10 d, and germinated seeds were removed from the boxes. Box lids were wrapped with plastic film to reduce evaporation.

Water Potential and Water Content

The PEG solutions were prepared according to Michel (15), and the actual Ψs of the blotters in each treatment were verified using a vapor pressure osmometer (model 5100C, Wescor Inc, Logan, UT). Daily measurements indicated that blotter Ψ declined by 0.1 to 0.3 MPa during the first 48 h of the germination test due to the concentrating effect of imbibition and then remained essentially constant. The actual Ψ values were determined from an average of two or three measurements taken from 4 to 7 d after planting. For convenience, the Ψ treatments in the figures are labeled with the initial Ψ values. The measured Ψs were used in the calculation of Ψ_{50}. The Ψ and ψ_s of seeds imbibed in water or 100 μM ABA were measured with a thermocouple psychrometer (model SC-10, Decagon Devices, Pullman, WA) using the procedures previously described (25). Briefly, the seed coats were removed from 8 to 12 seeds after 48 h imbibition and the embryos were placed in the psychrometer cup. After equilibration (1–2 h), Ψ was determined by wet-bulb depression based upon calibration curves prepared using standard salt solutions. Following measurement of Ψ, the sample cups were sealed and immediately frozen at −20°C for at least 8 h, thawed, and placed in the psychrometer for ψ_s determination. Seed or embryo WCs were determined by weight loss after drying for 24 h at 130°C.

Ψ_{50} and ABA_{50}

The Ψ and ABA concentration required to inhibit the maximum germination of each treatment by 50% (Ψ_{50} and ABA_{50}, respectively) were determined graphically from a curve fitted to a plot of Ψ or ABA concentration *versus* fresh seed germination percentage (Fig. 1). These parameters represent the population mean values for germination responses to Ψ and ABA. The ranges of Ψs or ABA concentrations required to reduce germination from 90 to 10% ($\Psi_{10\text{-}90}$ and $ABA_{10\text{-}90}$) were determined to assess the variability associated with Ψ and ABA inhibition. At 55 and 60 DAA, germination percentages exceeded 50% at the highest ABA concentration employed, so the ABA_{50} values were extrapolated from a

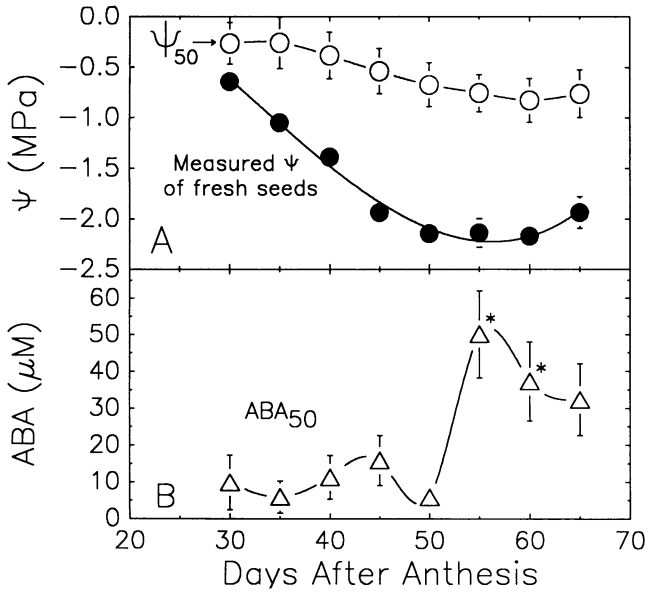

Figure 3. A, Ψ_{50} values for fresh seeds compared to the psychometrically determined Ψs of fresh decoated seeds during development. Measured decoated seed Ψ values are ± SE when the error bars exceed the size of the symbols (from ref. 25). The error bars on the Ψ_{50} values indicate the range of Ψs allowing 10 to 90% germination. B, ABA_{50} values during muskmelon seed development. Asterisks indicate values extrapolated from a polynomial equation, as the highest ABA concentration tested did not reduce germination to 50%. The error bars indicate the ABA concentrations allowing 10 and 90% germination, when the values exceed the dimensions of the symbols.

polynomial equation fitted to the plot of ABA concentrations versus germination percentages.

Statistical Analyses

The 1986 experiment was a completely randomized factorial design with eight developmental stages, five levels of ABA, and five Ψ levels for both fresh and dried seeds. Germination percentages were arcsin $\sqrt{\%}$ transformed to normalize the variances of binomial data before analysis by ANOVA (MSTAT, Michigan State University). The mean log time to germination (log $\bar{t}$) was calculated according to $\log \bar{t} = \sum n_i \log t_i / \sum n_i$, where n_i is the number of newly germinated seeds at time t_i. The time values were $\log_{10}$ transformed to produce log-normal distributions from the positively skewed cumulative germination time courses (22). When germination percentages were plotted on a probit scale versus log t, straight lines of approximately equal slope were produced for different Ψ or ABA treatments at most developmental stages (Fig. 2), indicating a normal distribution of germination events with log time. Since the slope of a probit plot is equal to $1/\sigma$ (inverse of the standard deviation) (8), equal slopes indicate homogeneous variances. Thus, the values for log $\bar{t}$ can be compared by ANOVA. Log $\bar{t}$ is the log time corresponding to a probit value of 0, or 50% germination. The germination rate was expressed as 1/log $\bar{t}$ for treatments in which greater than 16% of the seeds germinated. The significance of the main effects of ABA, drying, and Ψ, and their interactions, on germination percentage, rate, and uniformly (σ) were determined by F-tests. When appropriate, single-degree-of-freedom orthogonal comparisons were conducted to test specific interactions against a linear model.

RESULTS

Germination Responses to Ψ during Development

Cumulative germination time courses at decreasing Ψs are shown on a probit scale *versus* log time for 35-, 45-, and 55-DAA fresh seeds (Fig. 2, A, B, C), and are representative of the 30- and 35-, 40- to 50-, and 55- and 60-DAA age groups, respectively. At 30 and 35 DAA, germination percentages and log $\bar{t}$ values of fresh seeds were very sensitive to reductions in Ψ (Table I). Desiccation increased the germination percentages of 30- to 40-DAA seeds at all Ψs, but had little effect after 40 DAA (Table I). However, the germination percentages of dried 30 and 35 DAA seeds were significantly greater in −0.2 MPa solutions than in water (Table I). Forty- to 50-DAA seeds germinated best in water, and successively lower Ψs increased log $\bar{t}$ and reduced germination percentages, but had little effect on σ (Fig. 2B; Table I). Fifty- and 55-DAA seeds germinated equally well in water and at −0.2 MPa, and further reductions in Ψ reduced germination percentages and increased log $\bar{t}$ and σ (Fig. 2C; Table I). Overall, 50- and 55-DAA seeds were more tolerant of water stress, germinated faster and to a greater final germination percentage when compared to less mature seeds (Fig. 2; Table I).

The Ψ_{50} values of the populations of germinable seeds were determined graphically from plots of germination percentage versus Ψ (Fig. 1). For example, a −0.41 MPa PEG solution reduced the germination percentage of fresh intact 35-DAA seeds by half, from 34% in water to 17% (Fig. 1). The Ψ_{50} decreased gradually throughout seed development from −0.31 MPa at 30 DAA to −0.78 MPa at 64 DAA (Fig. 3A). The psychrometrically measured Ψ of decoated seeds immediately after removal from developing fruits (25) was at least 0.4 MPa below the Ψ needed for fresh seed germination at all stages of development (Fig. 3A).

Germination Responses to ABA during Development

The cumulative germination time courses for 35-, 45-, and 55-DAA seeds imbibed in ABA solutions were also plotted as probits *versus* log time (Fig. 2, D, E, F), and the results are summarized for both fresh and dried seeds in Table II. Responses to ABA during development were remarkably similar to those described above for Ψ. At 30, 35, and 40 DAA, fresh seeds germinated to a significantly higher percentage in 1 μM ABA than in water (Table II). In the 1985 study, 20- to 40-DAA seeds incubated on paper towels saturated with 0.1 μM ABA yielded germination percentages from 4 to 12% greater than in water (data not shown). Over the same developmental period, 1 μM ABA inhibited germination by 4 to 18% compared to water, and there was no germination at 10 or 100 μM ABA. The 1985 study further showed that from 40 to 50 DAA, both 0.1 and 1 μM ABA reduced germination by 8 to 19%, while 10 and 100 μM ABA completely inhibited germination (data not shown). In 1986, maximal germination of 45-DAA seeds was attained in pure water, and adding ABA increased log $\bar{t}$ and decreased germination percentages, but

Table II. *Influence of ABA and Drying on Germination Percentage, Rate, and Uniformity of Muskmelon Seeds Throughout Development*

Germination percentages, mean times to radicle emergence (log $\bar{t}$), and standard deviations (σ) are compared for fresh or dried muskmelon seeds harvested at 5-d intervals after anthesis, sealed in plastic boxes on blotter paper saturated with 0, 1, 3.3, 10, or 33 μM ABA solutions, and scored for radicle emergence at 12-h intervals for 10 d. ANOVA for germination percentages revealed highly significant differences among fresh and dried seeds, developmental stages, and ABA concentrations. ANOVA for log $\bar{t}$ revealed no significant differences between fresh and dried seeds and highly significant differences among developmental stages and ABA concentrations. ANOVA for σ showed no significant differences between fresh and dried seeds, highly significant differences among developmental stages, and significant differences among ABA concentration.

DAA	Fresh or Dried	ABA (μM)														
		Germination[a]					log $\bar{t}$					σ				
		0	1	3.3	10	33	0	1	3.3	10	33	0	1	3.3	10	33
		%					*log h*									
30	F	24	46	16	12	4	2.27	2.24	2.30			0.07	0.11	0.07		
	D	66	74	78	32	10	2.10	2.11	2.20	2.25		0.17	0.15	0.14	0.15	
35	F	34	62	50	14	6	1.97	2.15	2.13			0.22	0.19	0.12		
	D	52	60	40	28	8	2.07	2.10	2.14	2.31		0.28	0.27	0.13	0.15	
40	F	58	80	90	46	24	2.04	1.96	2.11	2.30	4.25	0.21	0.19	0.16	0.07	0.08
	D	98	94	82	54	6	1.93	2.03	2.07	2.27		0.21	0.14	0.21	0.10	
45	F	98	92	94	68	16	1.67	1.76	1.95	2.22	2.28	0.23	0.24	0.19	0.15	0.12
	D	96	94	98	80	18	1.62	1.66	1.76	1.97	2.22	0.10	0.18	0.25	0.29	0.07
50	F	100	98	92	30	0	1.62	1.63	2.06	2.23		0.19	0.20	0.28	0.24	
	D	100	98	56	2	0	1.63	1.65	1.75			0.20	0.21	0.35		
55	F	100	100	98	90	66	1.57	1.55	1.62	1.73	2.18	0.07	0.07	0.12	0.23	0.26
	D	100	100	100	98	90	1.56	1.57	1.588	1.62	1.82	0.09	0.11	0.12	0.15	0.31
60	F	98	100	94	98	58	1.53	1.50	1.50	1.72	2.09	0.10	0.13	0.14	0.24	0.18
	D	100	100	98	100	90	1.54	1.56	1.52	1.66	1.70	0.09	0.13	0.09	0.18	0.28
65	F	84	96	96	88	30	1.68	1.71	1.69	1.67	2.18	0.16	0.17	0.18	0.15	0.16
	D	92	92	92	88	40	1.55	1.55	1.56	1.60	1.84	0.12	0.14	0.16	0.16	0.28

[a] LSD ($P < 0.05$): germination, 19; log $\bar{t}$, 0.11; σ, 0.09.

did not affect σ (Fig. 2E; Table II). Germination percentages and log $\bar{t}$s were similar in water and in 1 μM ABA at 55 DAA, and higher ABA concentrations increased log $\bar{t}$ and σ and decreased germination percentages (Fig. 2F; Table II). The germination percentages of fresh and dried seeds at 45- and 55-DAA did not differ at any ABA concentration (Table II). The ABA_{50} values were <15 μM prior to 50 DAA, then increased markedly at 55, 60, and 65 DAA to values of 50, 37, and 32 μM, respectively (Fig. 3B).

ABA and Ψ Interactions

When ABA and reduced Ψ were combined in the same treatment, interactive effects on germination rate and percentage were observed (Figs. 4 and 5). These effects were tested by subjecting the germination percentages and rates to ANOVA. A highly significant 4-way interaction was detected among ABA concentrations, Ψ levels, fresh versus dried seeds, and stages of development for both germination percentages and rates. The different responses among developmental stages were largely responsible for the 4-way interaction. To isolate the 2-way interaction between ABA and Ψ, fresh and dried seeds were considered separately, and developmental stages were subdivided into three groups based on common germination performance within either fresh or dry treatments. Developmental stages were combined as 30 and 35 DAA, 40 to 50 DAA, and 55 and 60 DAA and are termed groups 1, 2, and 3, respectively, within fresh and dried seed categories, and each group was reanalyzed by ANOVA. The 65-DAA seeds were excluded, because their declining viability did not correspond with the characteristics of other groups.

Both germination percentages and rates differed significantly between fresh and dried group 1 seeds when compared by *F*-test. There were no significant differences between fresh and dry seeds in groups 2 or 3 for either germination percentages or rates. The ANOVA for each developmental group yielded no significant 3-way (fresh/dried, ABA, Ψ) interactions except for the group 2 germination rate. However, all groups had highly significant 2-way interactions between ABA and Ψ. The *F*-test of orthogonal coefficients comparing the ABA and Ψ interaction against a linear model for germination percentages was highly significant for groups 1 and 2, while that for group 3 was not significant (Figs. 4 and 5). The linear interaction for germination rates was not significant for group 1, while for groups 2 and 3 it was highly significant.

The interaction between ABA and Ψ is illustrated in series of three-dimensional graphs showing differences in germination percentages and rates in the presence of various combinations of ABA and Ψ during development (Figs. 4 and 5). Fresh, immature seeds were very sensitive to both ABA and reduced Ψ (Fig. 4, A and C). Germination percentages for most combinations of ABA and Ψ more than doubled after

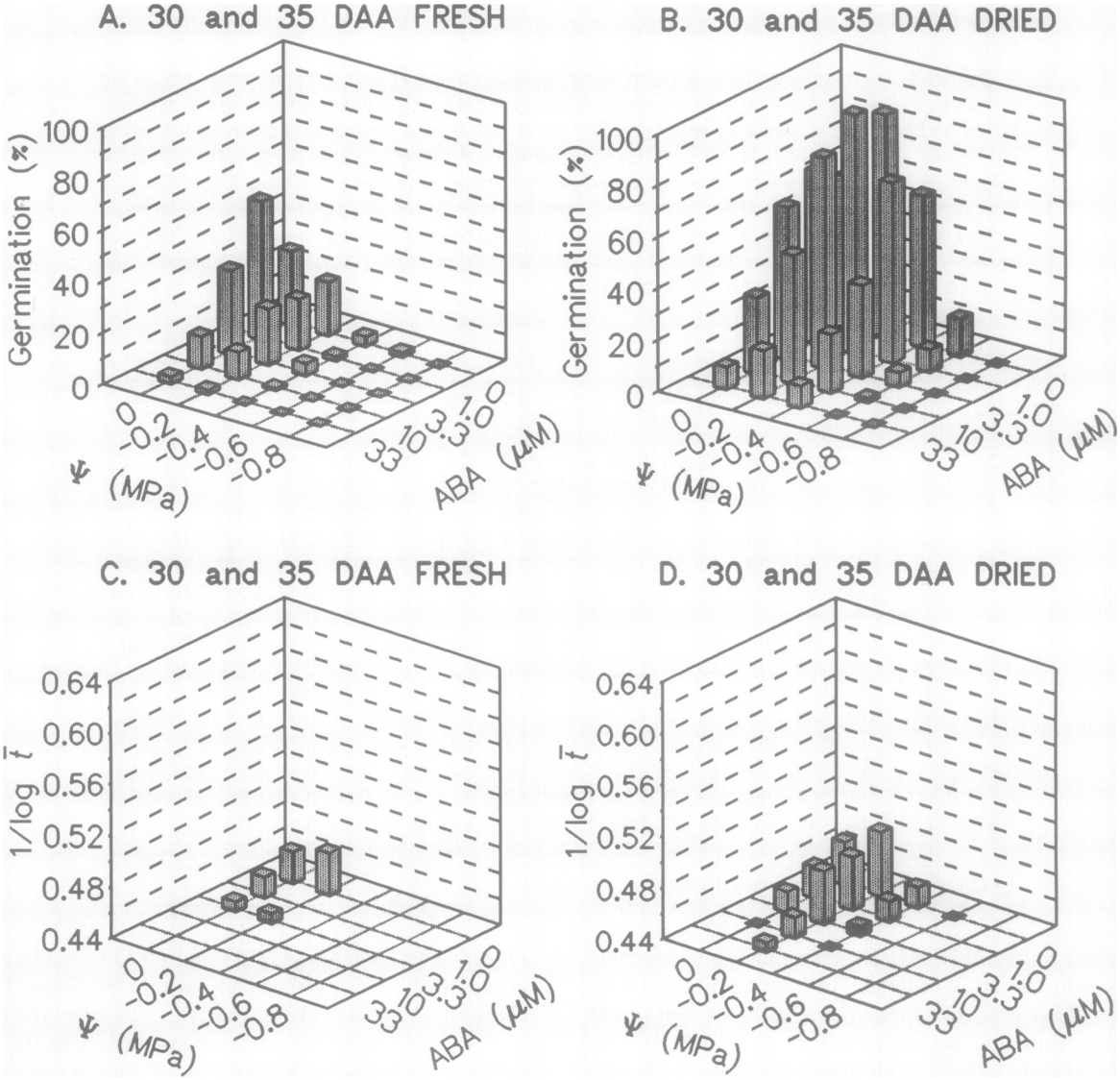

Figure 4. Germination percentages (A, B) and rates (1/log $\bar{t}$) (C, D) for fresh (A, C) and dried (B, D) immature (30- and 35-DAA) muskmelon seeds at 30°C as influenced by Ψ and ABA. $LSD_{(0.05)}$ = 7 (A), 9 (B), 0.04 (C), and 0.03 (D).

drying (Fig. 4, A and B), and drying also increased germination rates significantly over those of fresh seeds (Fig. 4, C and D). The effect of drying was equivalent to advancing fresh seeds in maturity by approximately 5 to 10 d (*cf.* with Fig. 5). Even though final germination percentages of dried immature seeds exceeded 80% in some cases, the rates of germination were still slow compared to more mature seeds with similar germination percentages.

Forty- to 50-DAA seeds exhibited a highly significant linear interaction between ABA and Ψ for germination percentage (Fig. 5A). Approximately 10 μM ABA or −0.5 MPa Ψ acted interchangeably to produce equivalent inhibitory effects on germination, and combinations of ABA and reduced Ψ had additive effects (Fig. 5A). At 55 and 60 DAA, the ABA and Ψ interaction was not significant, because the sensitivity of germination to both ABA and Ψ had decreased (Fig. 5B). An interaction would likely have been evident had higher concentrations of ABA been used. The ABA and Ψ interaction was additive with respect to germination rates for the period from 40 to 60 DAA (Fig. 5, C and D). Germination rates declined at combinations of ABA and Ψ where final germination percentage was unaffected (Fig. 5, B and D), indicating that germination rate was more sensitive to ABA and Ψ than was final germination percentage.

To determine whether ABA affected either water uptake or solute accumulation, 60-DAA decoated seeds were imbibed in 100 μM ABA. Abscisic acid had no significant effect on water uptake rates or final plateau WC levels (data not shown). Water potential and ψ_s were also unaffected by ABA, as after 48 h imbibition, seeds imbibed in ABA had a Ψ of −0.25 ± 0.02 and a ψ_s of −1.67 ± 0.18 MPa, while seeds imbibed without ABA had a Ψ of −0.22 ± 0.02 and a ψ_s of −1.75 ± 0.21 MPa. The ψ_s values for fresh 30- to 65-DAA seeds (25) and the WCs of fresh and imbibed seeds were used to estimate

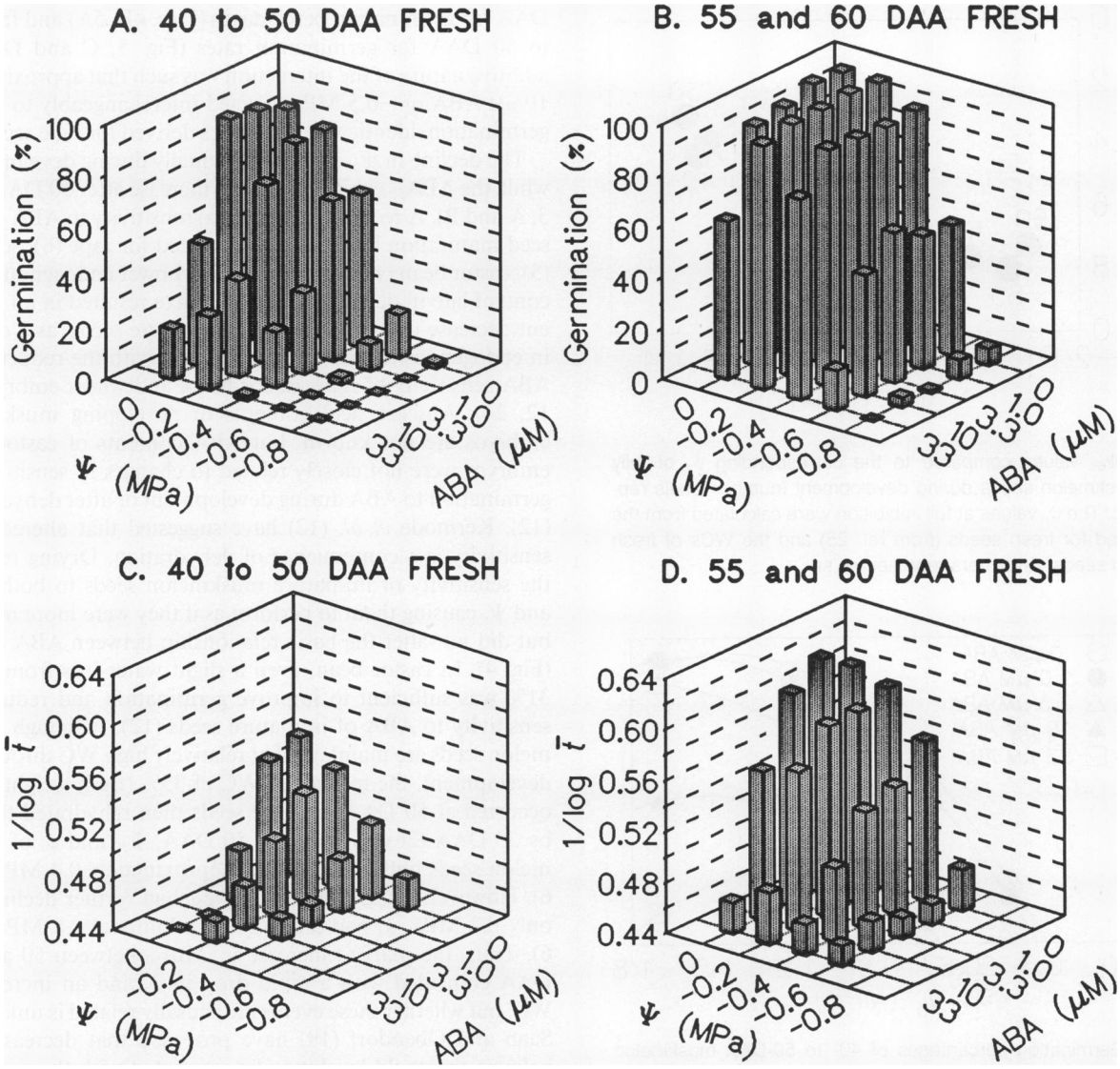

Figure 5. Germination percentages (A, B) and rates (1/log $\bar{t}$) (C, D) for 40 to 50 DAA (A, C) and 55 and 60 DAA (B, D) fresh muskmelon seeds at 30°C as influenced by Ψ and ABA. $LSD_{(0.05)}$ = 9 (A), 7 (B), 0.04 (C), and 0.04 (D).

embryo ψ_s at full imbibition by making corrections for changes in seed relative water content. The ψ_s values calculated for fully imbibed seeds declined from −1.44 to −2.46 MPa between 30 and 65 DAA, while Ψ_{50} fell from −0.31 to −0.78 MPa over the same period (Fig. 6). The ψ_p (Ψ-ψ_s) at various external Ψ values can also be calculated by correcting the ψ_s values for the different embryo WCs attained during the imbibition plateau prior to radicle growth. With increasing ABA concentrations, the calculated ψ_p required to achieve a given germination percentage increased for both fresh and dried 40- to 50-DAA seeds (Fig. 7), indicating that the minimum ψ_p for germination was increased by ABA.

DISCUSSION

Fresh seeds were highly sensitive to reduced Ψ early in development, but tolerance of low Ψ developed gradually as the seeds matured (Figs. 2, 3A). When compared with the psychometrically determined Ψ of fresh decoated seeds (25), the measured Ψ was initially 0.4 MPa below the Ψ_{50} at 30 DAA, and this difference increased to a maximum of 1.4 MPa at 60 DAA (Fig. 3A). This indicates that the seed Ψ is sufficiently low within the fruit at all stages of development to prevent precocious germination. Dos Santos and Yamaguchi (3) demonstrated that low fruit ψ_s is involved in preventing precocious germination in tomato, and Groot (9) showed that the Ψ of tomato fruit tissue was sufficiently low to prevent precocious germination of normal genotypes, but seeds of ABA-deficient mutants germinated in overripe fruit because their sensitivity to osmotic stress was reduced.

Previous studies with either cultured immature seeds (7, 18) or mature seeds (9, 17, 20) have shown that additions of either ABA or osmoticum can produce similar responses. In this study, the inhibitory effects of ABA and Ψ on muskmelon seed germination were remarkably similar at all stages of

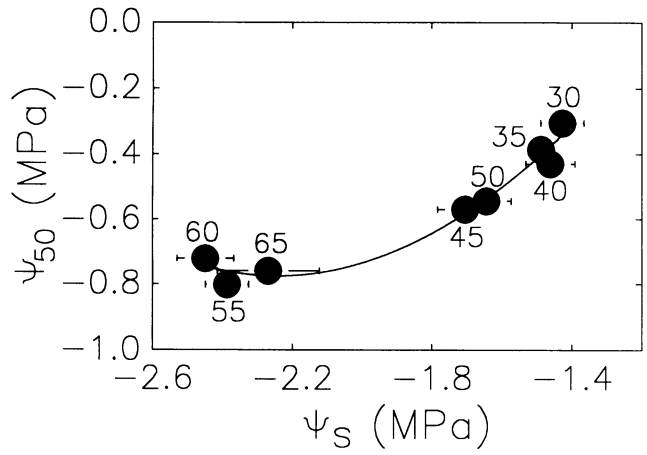

Figure 6. Ψ_{50} values compared to the corresponding ψ_s of fully imbibed muskmelon seeds during development (number labels represent DAA). The ψ_s values at full imbibition were calculated from the ψ_ss measured for fresh seeds (from ref. 25) and the WCs of fresh and imbibed seeds. Error bars represent ± SE.

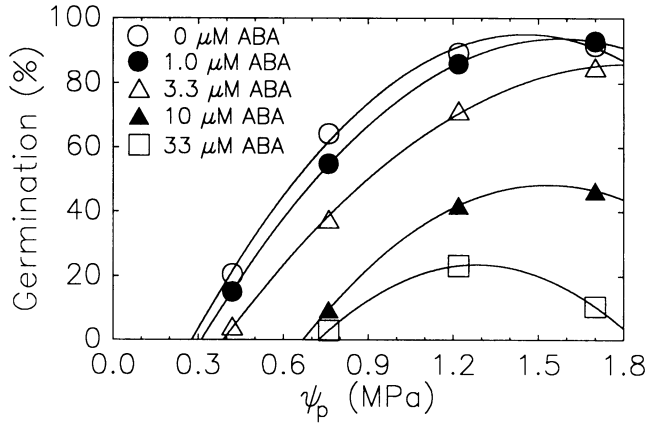

Figure 7. Germination percentages of 40- to 50-DAA muskmelon seeds as functions of the calculated embryo ψ_p at different Ψs and ABA concentrations. Data for both fresh and dried seeds were pooled, and each point is the average of 12 replicates. Turgor values were calculated based upon the ψ_s of fully imbibed embryos, corrected for the lower relative water contents of embryos at $\Psi < 0$ MPa.

development. In immature seeds, both −0.2 MPa and low ABA concentrations did not inhibit germination; rather, significant increases in germination percentages occurred in some cases compared to seeds germinated in water (Fig. 2, A and D; Tables I and II). The inhibition of muskmelon seed germination in water is a recognized but poorly understood phenomena that has been attributed to reduced O_2 availability (4, 10). However, it is unclear how imbibition in either PEG solutions or ABA could increase O_2 availability. At 45 DAA, the uniformity of germination times within the seed population was essentially constant for all Ψ and ABA treatments, but at 55 DAA, σ increased progressively with both decreasing Ψ and increasing ABA (Fig. 2; Tables I and II). The rate of germination was inhibited similarly by additions of ABA or reductions in Ψ, as reported for tomato (14). In muskmelon, the interaction was additive and symmetrical from 30 to 50 DAA for germination percentages (Figs. 4B, 5A) and from 40 to 60 DAA for germination rates (Fig. 5, C and D). The additive nature of the interaction was such that approximately 10 μM ABA or −0.5 MPa Ψ acted interchangeably to inhibit germination, identical to the values derived for rape seed (20).

The decline in Ψ_{50} occurred gradually during development, while the ABA_{50} exhibited a sharp increase after 50 DAA (Fig. 3, A and B). A reduction in embryo sensitivity to ABA during seed maturation has also been reported for rape (6), soybean (5), castor bean (12), and wheat (24). Lower endogenous ABA content late in development could have resulted in an apparent decrease in ABA sensitivity in mature seeds, as a decline in endogenous ABA content coincided with the reduction in ABA sensitivity of rape, castor bean, and wheat embryos (6, 12, 24). Abscisic acid contents of developing muskmelon embryos are not known, but ABA contents of castor bean embryos were not closely related to changes in sensitivity of germination to ABA during development or after dehydration (12). Kermode *et al.* (12) have suggested that altered ABA sensitivity is a consequence of dehydration. Drying reduced the sensitivity of immature muskmelon seeds to both ABA and Ψ, causing them to perform as if they were more mature, but did not alter the basic relationship between ABA and Ψ (Fig. 4). In castor bean, even a slight water loss from 36 to 31% was sufficient to improve germination and reduce the sensitivity to ABA of immature seeds (12). Although muskmelon seeds are maintained at relatively high WC throughout development, the minimum WC of 35% (fresh weight basis) occurred at 50 DAA, and the seeds then rehydrated to 40% by 55 DAA (26). From 30 to 50 DAA, Ψ_{50} and ψ_s of muskmelon seeds both decreased by approximately 0.4 MPa (Fig. 6). However, by 55 DAA, when Ψ_{50} had further declined by only 0.2 MPa, ψ_s had fallen by an additional 0.8 MPa (Fig. 6). Thus, the marked increase in ABA_{50} between 50 and 55 DAA coincided with a rapid drop in ψ_s and an increase in WC, but whether these events are causally related is unknown. Saab and Obendorf (19) have proposed that decreasing ψ_s below a threshold level may be associated with the cessation of embryo growth. During muskmelon seed development, the abrupt changes in ABA_{50}, ψ_s, and WC occur 10 to 15 d after maximum seed dry weight and germinability and coincide more closely with the attainment of maximum seed vigor (25, 26).

The linear additive interaction of ABA and Ψ on muskmelon seed germination (Figs. 4 and 5) and the parallel changes in ABA and Ψ responses during development (Fig. 2) reinforce the idea that a common control point exists for both ABA and Ψ in regulating embryo growth. Schopfer and Plachy (21) have shown that ABA does not affect the hydraulic conductivity or solute accumulation of rape embryos, but inhibits water uptake by preventing cell wall loosening, while low Ψ reduces ψ_p. Our results with muskmelon seeds also show that embryo ψ_s and rates of water uptake were not affected by ABA. Rather, the effect of ABA was primarily to increase the apparent minimum ψ_p required for germination, which can be operationally defined as the yield threshold for radicle emergence (Y) (Fig. 7; 21). From his work on ABA-deficient tomato mutants, Groot (9) concluded that ABA acts in two ways, by preventing GA-induced endosperm weaken-

ing and by reducing the embryo growth potential (ψ_p in excess of the minimum ψ_p required for radicle emergence, or ψ_p − Y). In seeds such as muskmelon (our unpublished results) and tomato (9), the strength of the endosperm or other tissues covering the embryo will contribute to the total Y that must be exceeded for radicle emergence to occur. If the initiation of radicle growth is determined by the effective ψ_p (*i.e.* by ψ_p − Y, the extent by which ψ_p exceeds the total yield threshold), an increase in Y (by ABA) or a decrease in ψ_p (by reduction in Ψ) will have equivalent effects, explaining the strictly additive interaction between ABA and Ψ in the inhibition of seed germination (Fig. 5; 20). Thus, we conclude that both the rate of initiation of germination and the final germination percentage depend upon the extent by which embryo ψ_p exceeds a minimum threshold required to overcome the restraint of any external tissues as well as that of the embryo cell walls. Abscisic acid apparently reduces the effective ψ_p by raising the ψ_p threshold (increasing Y), while lowered Ψ reduces ψ_p by reducing seed water content.

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