

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

IV. Characteristics of the Perisperm during Seed Development

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ABSTRACT

We previously reported that an apparent water potential disequilibrium is maintained late in muskmelon (*Cucumis melo* L.) seed development between the embryo and the surrounding fruit tissue (mesocarp). To further investigate the basis of this phenomenon, the permeability characteristics of the tissues surrounding muskmelon embryos (the mucilaginous endocarp, the testa, a 2- to 4-cell-layered perisperm and a single cell layer of endosperm) were examined from 20 to 65 days after anthesis (DAA). Water passes readily through the perisperm envelope (endosperm + perisperm), testa, and endocarp at all stages of development. Electrolyte leakage (conductivity of imbibition solutions) of individual intact seeds, decoated seeds (testa removed), and embryos (testa and perisperm envelope removed) was measured during imbibition of freshly harvested seeds. The testa accounted for up to 80% of the total electrolyte leakage. Leakage from decoated seeds fell by 8- to 10-fold between 25 and 45 DAA. Presence of the perisperm envelope prior to 40 DAA had little effect on leakage, while in more mature seeds, it reduced leakage by 2- to 3-fold. In mature seeds, freezing, soaking in methanol, autoclaving, accelerated aging, and other treatments which killed the embryos had little effect on leakage of intact or decoated seeds, but caused osmotic swelling of the perisperm envelope due to the leakage of solutes from the embryo into the space between the embryo and perisperm. The semipermeability of the perisperm envelope of mature seeds did not depend upon cellular viability or lipid membrane integrity. After maximum seed dry weight is attained (35–40 DAA), the perisperm envelope prevents the diffusion of solutes, but not of water, between the embryo and the surrounding testa, endocarp, and mesocarp tissue.

In some species, hydraulic continuity between the maternal plant and the fruit tissues is broken at seed maturity, allowing desiccation of the seed while the plant remains at high Ψ^3 (1). In fleshy-fruited species, however, the seeds mature and re-

main within a relatively moist environment where desiccation is prevented or delayed until after fruit senescence (21). However, we previously reported that the measured Ψ of mature muskmelon seeds was ≈ 0.5 MPa lower than that of the surrounding mesocarp (edible fruit tissue) (20). It is unclear how this Ψ disequilibrium could be maintained between tissues in intimate contact enclosed within the fruit, unless the testa or other tissues became highly impermeable to water. As this did not appear to be the case in muskmelon, the hypothesis was advanced that apoplastic solutes in the embryo, if isolated from the surrounding fruit mesocarp, might contribute to the lower Ψ values measured in muskmelon seeds relative to the mesocarp (20). This requires that the diffusion of solutes from the embryo free space into the surrounding testa and fruit mesocarp be prevented, thus allowing the embryo to control its apoplastic solute environment independent of the surrounding tissues.

Within the mucilaginous endocarp and testa, muskmelon embryos are enclosed in an envelope composed of a 2- to 4-cell layered perisperm and a single cell layer of endosperm (16), but the physiological function and permeability properties of these tissues are unknown. A similar thin endosperm envelope forms a semipermeable membrane surrounding the lettuce (*Lactuca sativa* L.) embryo (8, 17). Klein *et al.* (11) demonstrated that the lettuce endosperm is impermeable to leucine, and Speer and Hsiao (17) showed that it excludes molecules >300 D regardless of whether it is alive or dead. Weges (19) found that K^+ ions leaked from lettuce embryos did not diffuse through the endosperm and were trapped in the fluid layer between the embryo and the endosperm. Similarly, Hill and Taylor (8) reported that solutes released from dead lettuce embryos cannot pass through the endosperm and accumulate in the extra-embryonic fluid. Pesis and Ng (14) found no correlation between muskmelon seed vigor and electrolyte leakage, suggesting that the perisperm envelope of muskmelon may have similar characteristics to the endosperm in lettuce.

In this study, the movement of water and solutes through the muskmelon endocarp, testa, and perisperm envelope was examined during seed development by measuring water loss or electrolyte leakage. Our objective was to determine whether the permeability properties of the endocarp, testa, or perisperm envelope could allow the embryo to maintain a Ψ

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³ Abbreviations used: Ψ , water potential; ψ_s , solute potential; ψ_p , turgor pressure; DAA, days after anthesis; OD, osmotically distended; WC, water content.

independent of the surrounding fruit tissues by acting as a barrier to either water or solute diffusion.

MATERIALS AND METHODS

Plant Material

Muskmelon (*Cucumis melo* L. cv Top Mark, Asgrow Seed Co., Inc., Gonzales, CA) plants were field-grown at the University of California, Davis, as previously described (20). Perfect (hermaphrodite) flowers were tagged at anthesis and fruits were harvested at 5-d intervals from 20 to 65 DAA. Seeds were removed from the fruit and wiped with paper towels to remove the mucilaginous endocarp prior to conductivity measurements. A commercial lot of Top Mark seeds was used in the experiments in which seeds were pretreated prior to conductivity measurements, unless otherwise stated.

Mature muskmelon seeds are nonendospermic with the bulk of the storage reserves contained in the cotyledons. The embryo is bounded by a single cell layer of residual endosperm enclosed within a 2- to 4-cell-layered perisperm which develops from the maternal nucellus (16). The residual endosperm and perisperm (hereafter termed perisperm) are contiguous and form an envelope that completely surrounds the embryo. The testa (actually testa + tegment), derived from the inner and outer integuments, surrounds the perisperm, endosperm, and embryo. The testa consists of two halves (16) that can be easily separated and removed, leaving the decoated seed (embryo with surrounding perisperm envelope intact). The testa is covered by a mucilaginous endocarp composed of small, thin-walled elongated cells.

Desiccation Rates

The kinetics of water loss were measured for 55 DAA seeds to determine the permeability to water of the endocarp, testa, and perisperm envelope. Seeds were isolated from newly harvested fruits with the gelatinous endocarp attached to the outer layer of the testa (intact + endocarp), with the endocarp removed (intact), and with both the endocarp and testa removed (decoated). Water loss was measured at 5 min intervals from groups of 30 seeds placed on a balance inside a desiccator containing activated silica gel maintained at $30 \pm 1^\circ\text{C}$ and 5 to 12% percent RH (measured with a Vaisala Helsinki, Finland HMI 31 humidity sensor). Plots of $\ln \text{WC}$ versus desiccation time were linear down to $\approx 10\%$ WC, indicating that the initial rates of water loss fit exponential decay kinetics. The slopes of semilogarithmic plots provide a relative measure of the rate of water loss and were statistically compared using the 95% confidence intervals of the regression slopes.

Conductivity Measurements

Intact seeds were dissected into testa, decoated seeds, and embryos prior to conductivity testing. Removal of the perisperm envelope from dry seeds was not always possible because of its close association with the embryo. As a result, leakage from embryos was measured after removing the perisperm envelope from imbibed, decoated seeds.

Single seeds or seed tissue samples were placed in individual

wells of a seed tray. Each well was filled with 3.5 mL of deionized water to begin the experiment. The electrical current generated across a potential difference of 2.0 V was measured in μamps using a computerized conductivity analyzer (model ASAC-1000, Neogen Food Tech Corp., Lansing, MI) (9). Each sample was measured periodically over a 24-h period at $21 \pm 1^\circ\text{C}$ unless stated otherwise. Each seed or seed tissue treatment was replicated 20 to 40 times, while each developmental stage was tested 2 to 4 times. Currents were converted to μmho , averaged, and the variability displayed as $\pm \text{SE}$. The K^+ concentration in the leachate was measured by atomic absorption spectroscopy (model 460, Perkin-Elmer Inc., Norwalk, CT).

To investigate the semipermeable properties of the perisperm envelope, decoated seeds were subjected to a number of pretreatments designed to disrupt the tissue. Seeds were pre-soaked in the specified solvents or solutions for 12 h, rinsed in deionized water, and electrolyte leakage was measured for an additional 24 h. Subsamples of 25 pretreated seeds were rinsed in water and incubated on two thicknesses of hydrated germination blotter paper sealed inside plastic boxes at 25°C . Germination percentage was scored as radicle emergence over a 10-d period. Physical treatments were also used: imbibed seeds were autoclaved at 120°C for 20 min, microwaved at maximum power (700 W) for 3 min, frozen at -30°C and thawed at room temperature, and 50 DAA seeds were aged for 4 months at 30°C (15% WC, dry weight basis) prior to germination or conductivity testing and WC determinations. After electrolyte leakage from decoated seeds had been measured, the perisperm envelopes were removed and leakage from the embryos was determined for a second 24-h period.

Solute Potential of the Free Space

Commercial Top Mark seeds were imbibed for 24 h in water at 25°C . Sixty-seven embryos were carefully isolated from intact seeds in a humidified chamber, vacuum infiltrated with water for 240 s, blotted to remove excess moisture, and centrifuged in a Centrex microfilter tube (Schleicher and Schuell, Keene, NH) for 10 min at 480g (5). The osmolality of the 20 to 40 μL of solution recovered in the receiver unit of the microfilter tube was measured in a vapor pressure osmometer (model 5100 B, Wescor Inc., Logan, UT). The osmometer was calibrated in the 0 to 100 $\text{mOs}\cdot\text{kg}^{-1}$ range prior to each measurement. Osmolality was converted to osmotic potential (at 25°C) by multiplying the value in $\text{Os}\cdot\text{kg}^{-1}$ by $2.48 \text{ MPa}\cdot\text{kg}\cdot\text{Os}^{-1}$. Osmometer measurements were replicated two to three times for each sample.

Dead muskmelon seeds often have an elevated water content and are swollen in appearance in comparison with viable seeds. Much of this excess water is held in the free space between the embryo and the perisperm envelope. Commercial Top Mark seeds were imbibed for 24 h, microwaved (boiled) in water for 3 min and incubated in deionized water (changed every 12 h) for an additional 72 h. At this time, greater than 90% of the seeds exhibited the swelling characteristic of nonviable seeds. The fluid in the free space was collected by either withdrawing the free solution trapped between the perisperm envelope and embryo with a hypodermic needle and syringe or by centrifuging intact or cut seeds for 10 min

at 12,000g. The osmolalities of the solutions collected were measured as described above. As there was no significant difference in the ψ_s values obtained by different sampling techniques, the results were pooled and expressed as the average \pm SE ($n = 10$).

Microscopy

Hydrated, decoated seeds were fixed in a mixture of 70% EtOH, 5% formalin, 5% glacial acetic acid, and 20% distilled water prior to dehydration in an EtOH and tert-butyl alcohol series following the procedure described by Jensen (10). Samples were embedded in paraffin (Paraplast, Monoject Scientific, St. Louis, MO) and 5 to 8 μm sections were cut. Sections were stained with either 0.25% toluidine blue O in 4.0% formalin; a triple stain of 3.0% safranin in 50% EtOH, 1.0% aqueous crystal violet, and 1.0% fast green in absolute EtOH; or saturated Sudan IV in 95% EtOH. Sections were observed and photographed using light microscopy.

Seed Water Content

To demonstrate that mature seeds act as osmometers due to the semipermeability of the perisperm envelope, groups of 25 viable and nonviable (aged) seeds were incubated at 20°C in PEG 8000 solutions ranging from 0.0 to -1.6 MPa in 0.4 MPa increments calculated according to Michel (13). The Ψ of each solution was verified with a vapor pressure osmometer. Seeds were imbibed for 24 h, blotted, weighed, and transferred to the next lower Ψ in the series. Following incubation in the -1.6 MPa solution, the seeds were transferred back to water for an additional 24-h period and reweighed. Water content was expressed as a percentage of the dry weight after drying at 130°C for 24 h.

RESULTS

Desiccation Rates

The rates of water loss from 55 DAA intact seeds with the endocarp present, intact seed with endocarp removed, and decoated seeds were examined to determine whether the endocarp, testa or perisperm present a significant resistance to water movement. The dehydration time courses showed exponential initial declines in WC with time (Fig. 1), as plots of \ln WC versus time were linear until seed WC fell below 10% (Fig. 1, inset). The rate of water loss did not differ significantly between intact and decoated seeds, but was $\approx 35\%$ slower when the endocarp was present, with half-times of 30, 28, and 41 min, respectively. These rapid rates of water loss indicate that the tissues covering the embryo are highly permeable to water. Essentially identical values were also obtained for 30-DAA seeds (data not shown), indicating that the permeability to water of the tissues covering the embryo changes little during development.

Electrolyte Leakage during Development

Electrolyte leakage from immature 20 DAA and mature 60 DAA intact and decoated seeds, embryos, and testae were compared to determine whether a barrier to electrolyte dif-

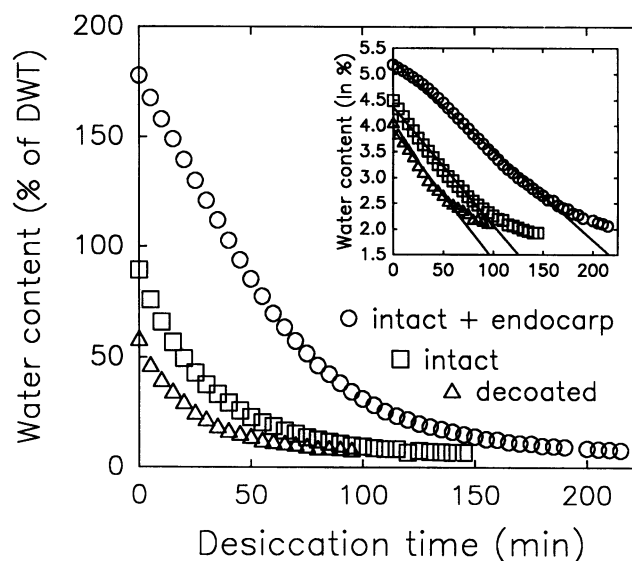


Figure 1. Desiccation rates of intact 55 DAA muskmelon seeds with the endocarp present (intact + endocarp), with the endocarp removed (intact), or with the endocarp and testa removed (decoated). Groups of 30 seeds were placed on a balance inside a desiccator containing activated silica gel ($30 \pm 1^\circ\text{C}$, $\text{RH} < 12\%$) and water loss was recorded at 5 min intervals. Seeds were then oven dried and WC was calculated on a dry weight (DWT) basis. Inset, the same data plotted as \ln WC versus time, with linear regressions through the data above 10% WC. The slopes of the semilogarithmic regression lines ($r^2 \geq 0.99$) were -0.017 , -0.023 , and $-0.025 \ln \text{WC h}^{-1}$ for intact + endocarp, intact, and decoated seeds, respectively.

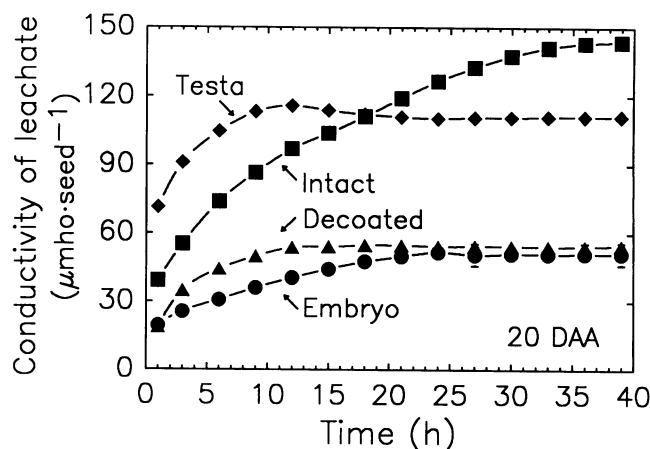


Figure 2. Electrolyte leakage from fresh 20-DAA intact and decoated seeds, testae, and embryos. Error bars represent \pm SE for 30 individual seeds when larger than the data symbols.

fusion forms during development. At 20 DAA, testae lost electrolytes rapidly during the first 5 h of the test and conductivity values reached a plateau thereafter (Fig. 2). Electrolyte leakage from intact seeds was slower than from testae, but final conductivity values for intact seeds were 20% greater (Fig. 2). The conductivity of leachate from decoated seeds increased gradually to a plateau at 12 h and remained constant thereafter (Fig. 2). The conductivity of leachate from embryos increased gradually to a plateau after 20 h (Fig. 2). At 24 h,

conductivities of leachates from decoated seeds and embryos were almost identical at $58 \mu\text{mho}\cdot\text{seed}^{-1}$, roughly half of the final values for intact seeds and testae.

Intact 60 DAA seeds leaked electrolytes rapidly to a plateau of $56 \mu\text{mho}\cdot\text{seed}^{-1}$ after 9 h (Fig. 3A). Conductivity of leachate from the testae reached near maximal values at 30

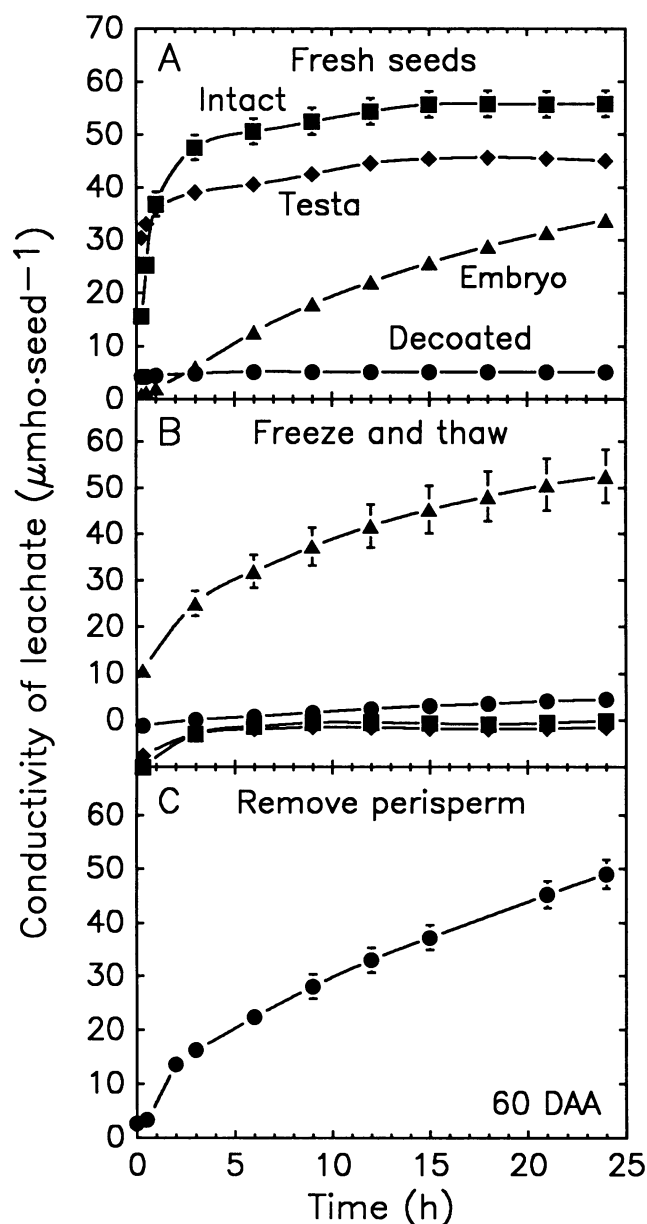


Figure 3. Electrolyte leakage from fresh 60 DAA intact and decoated seeds, testae, and embryos before and after freezing. A, Leakage time courses for fresh seeds and tissues. B, After the measurements in panel A, the samples were frozen and thawed and conductivities were remeasured in the same solutions for an additional 24 h. The final values recorded in panel A have been subtracted to show only the changes in conductivity due to freezing. C, The perisperm envelope was removed from frozen and thawed decoated seeds and embryo leakage was measured for an additional 24 h in fresh solution. Error bars represent \pm SE for 30 individual seeds when larger than the data symbols.

min and increased only slightly thereafter to $45 \mu\text{mho}\cdot\text{seed}^{-1}$ at 24 h. Decoated seeds leaked very little, and the conductivities remained nearly constant at $5 \mu\text{mho}\cdot\text{seed}^{-1}$. Leakage from embryos increased continually to a value of $31 \mu\text{mho}\cdot\text{seed}^{-1}$ at 24 h (Fig. 3A). The K^+ contents of the leachate of decoated seeds and embryos were 0.46 and $5.4 \text{ mg}\cdot\text{mL}^{-1}$, respectively.

The difference in conductivity values and K^+ concentrations between leachates from decoated seeds and embryos indicated that the perisperm blocked diffusion of electrolytes. After the initial conductivity measurements, the 60 DAA seed samples were frozen, thawed, and remeasured to determine whether the barrier formed by the perisperm envelope had been destroyed (Fig. 3B). No additional leakage from intact seeds and testae occurred after freezing, and only a small increase (from 5 to $12 \mu\text{mho}\cdot\text{seed}^{-1}$) was observed for decoated seeds. However, significant additional leakage from embryos occurred following freezing (Fig. 3B). Removing the perisperm envelope from decoated seeds after freezing and thawing increased the rate of leakage to match that of the frozen and thawed embryos (Fig. 3, B and C). The total leakage from frozen isolated embryos and from embryos isolated from decoated seeds after freezing were comparable after 48 h (data not shown).

As the perisperm of mature seeds prevented electrolyte leakage, we investigated when this property developed. Total electrolyte leakage over 24 h for decoated fresh seeds decreased during development and became negligible after 40 DAA (Fig. 4). Since mature embryos do leak electrolytes when imbibed (Fig. 3A), the perisperm envelope apparently becomes impermeable to electrolytes after 40 DAA.

Influence of Chemical or Physical Treatments on Leakage

Various chemical and physical treatments were applied to decoated seeds in an effort to induce leakage through the

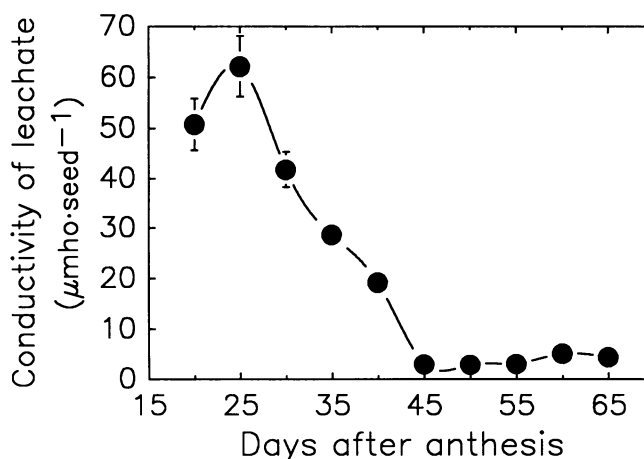


Figure 4. Total electrolyte leakage after 24 h imbibition from decoated seeds throughout development. Data for each developmental stage represent the average of two to four experiments of 20 to 40 seeds each. Error bars represent \pm SE when larger than the data symbols.

perisperm envelope and to determine the nature of the diffusion barrier. Only 5 M NaOH and 95% EtOH significantly increased electrolyte leakage, while autoclaving and 100% MeOH caused slight increases (Table I). The 8 N HCL and 5 M NaOH treatments apparently degraded both the embryo and perisperm envelope, as both were soft and discolored. Since EtOH and MeOH partially increased the permeability of the perisperm envelope, decoated seeds were presoaked in 100, 95, 75, or 50% aqueous alcohol solutions prior to conductivity measurements. Ethanol was more effective than MeOH in inducing leakage, with 75% EtOH resulting in the highest conductivity (Table II).

Embryo leakage was dramatically increased if decoated seeds were given one of the following pretreatments prior to

Table I. Effects of Various Pretreatments on Seed Germination, Swelling, and Electrolyte Leakage

Decoated muskmelon seeds were presoaked in the specified solutions for 12 h or physically treated prior to germination tests or conductivity measurements. Germination (radicle emergence) or osmotic distension (OD) were counted on subsamples of 25 seeds after 10 d incubation on water-saturated blotters at 25°C. OD seed are characterized by large volume increases and fluid accumulation between the embryo and perisperm envelope. WC was determined after 2 to 3 d imbibition, prior to radicle emergence in viable seeds. Conductivity measurements were made on the imbibition solutions of 30 individual decoated seeds after 24 h. The perisperm was then removed from the same samples (embryos) and conductivity was measured after a further 24 h soak in fresh solutions.

Treatment	Germination	OD	WC	Conductivity	
				Decoated seeds	Embryos
		%		$\mu\text{mho} \cdot \text{seed}^{-1} \pm \text{SE}$	
Water	98	0	67	6 ± 1	30 ± 2
100% Hexane	92	0	69	7 ± 1	34 ± 4
10% Triton X-100	82	0	66	8 ± 1	23 ± 3
5 M NaOH	0	0	94	88 ± 10	39 ± 4
8 N HCL	0	0	106	*	*
10 M Butyric acid	0	42	*	*	*
95% EtOH	0	25	98	39 ± 8	97 ± 4
100% MeOH	0	95	119	14 ± 5	92 ± 4
100% Acetone	0	45	*	*	*
Aged	0	94	116	3 ± 1	92 ± 4
Frozen and thawed	0	96	109	5 ± 1	80 ± 6
Autoclaved	0	100	128	20 ± 4	74 ± 5
Microwaved	0	100	124	*	*

* Data not available.

Table II. Effects of Methanol and Ethanol Pretreatments on Leakage from Decoated Muskmelon Seeds

Decoated muskmelon seeds were presoaked in the alcohol solutions for 12 h, then imbibed in water for 24 h prior to conductivity measurements on leachate from 30 individual seeds.

Alcohol	Percentage (by volume)			
	100	95	75	50
	$\text{conductivity } (\mu\text{mho} \cdot \text{seed}^{-1} \pm \text{SE})$			
Methanol	14 ± 5	10 ± 2	16 ± 4	6 ± 3
Ethanol	20 ± 5	38 ± 8	56 ± 4	34 ± 4

removal of the perisperm envelope: MeOH, EtOH, aging, freezing, and thawing, or autoclaving (Table I). These treatments were accompanied by elevated seed WC and loss of viability (Table I). When hydrated, nonviable intact seeds swelled, splitting the testa and exposing the water-soaked embryo enclosed in the perisperm envelope (Fig. 5A). In lettuce seed, this swollen condition has been termed 'osmotically distended' (8, 17). Osmotic distention occurs when embryo leakage is induced, the semipermeability of the perisperm or endosperm envelope is maintained, and water is absorbed osmotically. The WC of decoated seeds exhibiting osmotic distention averaged 115% as compared to 67% for viable decoated seeds (Table I). The WCs of both viable and dead seeds were reduced as the seeds were transferred through a series of osmotic solutions, then returned to the initial WC when transferred back to water (Fig. 6), indicating little loss of solutes even after several days of incubation. Differences in the relationship between Ψ and volume were evident between viable and nonviable seeds. Nonviable OD seeds showed only slight water loss until Ψ was reduced to -1.2 to -1.6 MPa; viable seeds exhibited the opposite pattern of

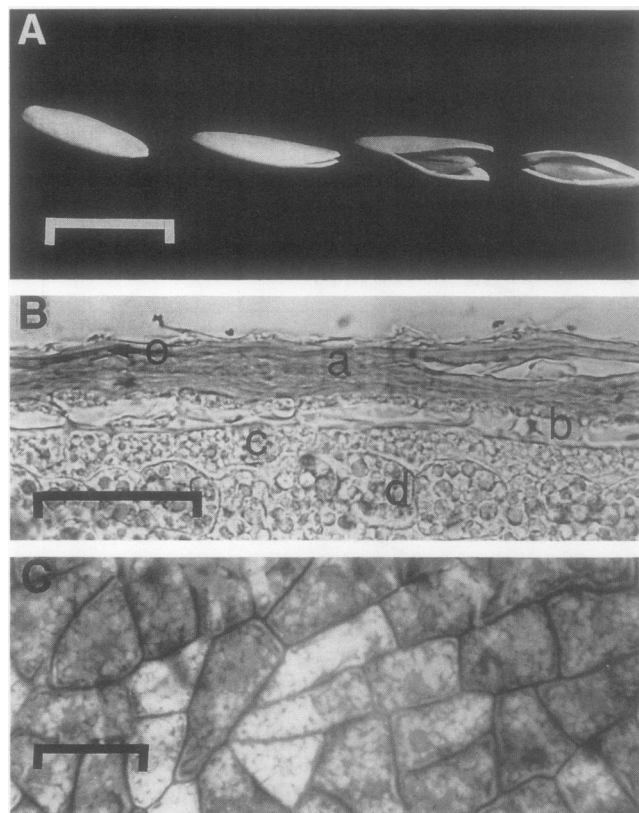


Figure 5. A, Left to right, a fully hydrated viable seed, a partially hydrated OD seed, and two fully hydrated OD dead seeds (scale = 10 mm). The swollen perisperm envelope surrounding the embryo is visible within the split testae of the OD seeds. B, Transverse section of a 55 DAA decoated muskmelon seed stained with Sudan IV: a, perisperm; b, endosperm; c, epidermis of embryo; d, storage parenchyma of cotyledons; e, positive staining for cutin or suberin (stained dark orange in original photo) (scale = 40 μm). C, Outer paradermal view of the perisperm (scale = 20 μm).

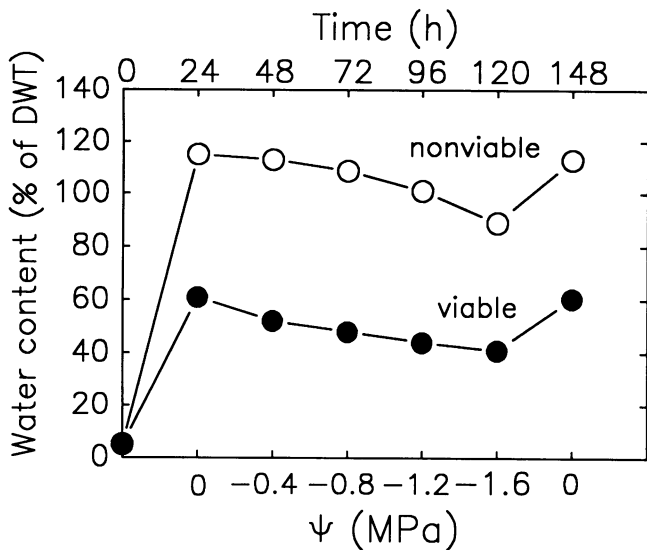


Figure 6. Water contents of viable and nonviable 55 DAA decoated seeds. Groups of 25 seeds were incubated sequentially for 24 h each on PEG solutions of the indicated Ψ , then returned to water.

considerable water loss between 0 and -0.4 MPa, then smaller reductions in WC at lower Ψ s (Fig. 6). If handled carefully, the OD seeds were able to undergo repeated expansion and reduction in volume in response to changes in external Ψ .

Solute Potential of the Free Space

The apoplastic ψ_s was estimated for both viable and nonviable seeds to determine whether the osmotic environment within the perisperm is independent of that of the external solution. The ψ_s of the solution extracted after vacuum infiltration of viable embryos isolated from imbibed mature seeds was -0.08 MPa. Second and third infiltration and centrifugation cycles produced identical values of -0.06 MPa. In contrast to these high ψ_s values for viable seeds, the average ψ_s of the fluid isolated from the free space of OD seeds was -0.66 ± 0.02 MPa.

Anatomy of the Perisperm Envelope

In 20 DAA seeds, the perisperm consists of two to four layers of very fragile, thin-walled, vacuolated cells that were easily damaged during embedding and sectioning. There was no endosperm layer associated with the perisperm and no evidence of suberization in the perisperm cell wall at 20 DAA (data not shown). In 55 DAA seeds, the perisperm, when viewed in transverse sections, consisted of two to four layers of dark-staining dense material with no distinctive cellular structure (Fig. 5B). Underneath the perisperm was a single layer of large rectangular endosperm cells with thick cell walls (Fig. 5B). Adjacent to the endosperm were the epidermis and storage parenchyma cells of the cotyledons (Fig. 5B). In paradermal view from the exterior, the perisperm is composed of irregularly shaped cells that are densely cytoplasmic with prominent nuclei and thick cell walls (Fig. 5C). The endosperm appears much the same in both transverse (Fig. 5B) and paradermal views (not shown). The periclinal walls of the

outer layer of perisperm cells stain dark orange with Sudan IV (a positive reaction for suberin and cutin [4]) in contrast to the pale yellow-orange color of the remaining perisperm (Fig. 5B).

DISCUSSION

We previously reported (20) that an apparent Ψ disequilibrium existed between the Ψ of the muskmelon seed and surrounding mesocarp tissue during the later stages of development. Barriers to water movement between the seed and fruit tissue could allow Ψ gradients to be maintained for extended periods. The outer perisperm gave a positive reaction for cutin and suberin when stained with Sudan IV (Fig. 5B). However, water loss from both mature and immature muskmelon seeds is extremely rapid, indicating that the cuticle in the perisperm (16), the testa, and the endocarp are not effective barriers to water movement (Fig. 1), so the apparent Ψ disequilibrium condition is not due to impermeability to water.

Since no hydraulic barrier was found, experiments were conducted to determine whether the perisperm envelope acts as a barrier to solutes, allowing the embryo to maintain a local Ψ environment independent of the surrounding fruit tissues. The perisperm envelope is not a barrier to electrolyte leakage in immature seeds (Fig. 2), but its permeability decreases with seed development (Fig. 4) until in mature seeds it acts as a semipermeable membrane, allowing the passage of water but not solutes (Fig. 6). The semipermeable properties of the mature perisperm or endosperm envelope explain the poor correlation between electrolyte leakage and seed quality in muskmelon (14) and lettuce (8). The perisperm envelope surrounding mature seeds is semipermeable even when viability is lost and lipid membranes are disrupted, as freezing, autoclaving, and soaking in hexane caused only slight increases in electrolyte leakage from decoated seeds (Table I). Aqueous EtOH solutions increased perisperm envelope leakage, while aqueous MeOH was much less effective (Table II). The cutin and/or suberin present in the outer cell layer of the perisperm envelope (Fig. 5B) could contribute to its semipermeable properties. However, since neither compound is reportedly soluble in aqueous EtOH solutions (12), the effects of the solvent on perisperm permeability must lie elsewhere.

The appearance of dead, fully hydrated seeds is quite different from that of imbibed viable seeds (Fig. 5A). The testae of OD seeds split and their WC's are almost double those of viable seeds (Table I). This occurs because the majority of the solutes in viable seeds are contained in the symplast (ψ_s of the apoplast was >-0.1 MPa), while in dead seeds, solutes are lost from the symplast into the apoplast and are trapped there by the perisperm envelope (ψ_s of fluid within the perisperm was -0.66 MPa). Water is taken up osmotically until hydrostatic pressure develops across the perisperm envelope and Ψ equilibrium is reached with the imbibition solution ($\Psi = 0$ MPa), at which point the hydrostatic pressure across the perisperm envelope would be 0.66 MPa in OD seeds. Osmotically distended seeds behave like dialysis bags, as solutes are retained inside, but water can pass freely through the perisperm envelope to the solution outside. When placed on osmotic solutions, OD seeds can reversibly change in volume

in response to the Ψ of the external solution (Fig. 6; 17). The different relationships between Ψ and WC for viable and OD seeds suggests differences in the elastic moduli of the embryo and the perisperm envelope. The pattern for the perisperm would be characteristic of a stiff wall (high elastic modulus) under tension, as relatively small reductions in WC result in considerable reductions in Ψ . The pattern for the viable seeds is indicative of a low elastic modulus, or of walls not fully stretched, as relatively small initial reductions in Ψ are accompanied by considerable water loss (Fig. 6).

Speer and Hsiao (17) concluded that an enzymic process is involved in the formation of OD lettuce seeds. Our data and those of Hill and Taylor (8) with lettuce indicate that any treatment capable of disrupting embryo membrane integrity, but incapable of breaking the perisperm or endosperm envelope, leads to the formation of OD seeds. In all cases, OD seeds were nonviable (Table I). Triton X-100 and 100% hexane, two treatments designed to disrupt lipid membranes, were ineffective in inducing OD seeds and had little effect on viability or leakage (Table I). This indicates that the semipermeable barrier created by the perisperm envelope does not rely on a lipid membrane and that neither compound could penetrate the perisperm envelope. Speer and Hsiao (17) found that the lettuce endosperm excluded compounds >300 D and that semipermeability did not depend upon a lipid membrane or living cells. As the mol wt of hexane is only 86 D, the muskmelon perisperm apparently has a lower mol wt exclusion limit than does the lettuce endosperm. In muskmelon, the K^+ concentration in the embryo leachate exceeded that in the decoated seed leachate by 12-fold. Similarly, the lettuce endosperm has been shown to block K^+ diffusion (19). Although some of the disparity in the concentration of K^+ in the leachate of decoated seeds and embryos may be explained by ionic binding of K^+ to the perisperm cell walls, it seems unlikely that this alone could account for the magnitude of the concentration differences found. An ion exchange mechanism for retaining K^+ within the perisperm should nonetheless allow slow leakage of the ion with time down the concentration gradient, but leakage from decoated seeds tended to remain negligible for long periods (Figs. 3A and 6). The permeability characteristics of the muskmelon perisperm reported here and of the lettuce endosperm (8, 17, 19) indicate that the pore size for these tissues is considerably smaller than the 1300 to 1600 D limit (≈ 40 Å pore size) previously determined for cell walls of living plant tissues (3). Perisperm and endosperm cells are morphologically dissimilar (Fig. 5B), and it is unclear whether only one or both of these cell layers form the diffusion barrier. *Luffa cylindrica* (Cucurbitaceae) seeds have a perisperm but no endosperm (16) and do not leak electrolytes after freezing (our unpublished results), suggesting that the perisperm may be the primary barrier.

We have demonstrated that the perisperm acts as a barrier to the diffusion of solutes but is highly porous to water. Can the semipermeability of the perisperm contribute to the Ψ disequilibrium measured between the muskmelon seed and fruit tissue (20)? If the apoplastic solutes in the embryo are trapped by the perisperm, or if apoplastic solutes are actively maintained at high concentrations by the embryo, the Ψ measured in decoated seeds would be essentially that of the

apoplast and might account for the lower than expected Ψ values. High apoplastic solute concentrations have been detected in the seeds of some legumes during development (23). However, analysis of the free space of mature muskmelon embryos showed that the ψ_s of the apoplast is >-0.1 MPa. Although the vacuum infiltration method tends to underestimate the ψ_s of the apoplast (5), the concentration was nearly sevenfold less than that required to explain the disequilibrium condition. Furthermore, high apoplastic solute concentrations could not account for the Ψ disequilibrium condition due to the semipermeable characteristics of the perisperm. If a low ψ_s were present in the apoplast of viable embryos, water should move through the perisperm and accumulate in the free space, creating OD seeds, which are not normally observed when fresh muskmelon seeds are imbibed.

Several other factors could account for the low seed Ψ values. As discussed previously, systematic errors in the Ψ measurements could be responsible for the apparent disequilibrium condition (20). We describe elsewhere (22) that excessive pressure while blotting of imbibed intact seeds can remove loosely held water from the testa and lower the apparent Ψ of the seed. As the seeds were blotted to remove the endocarp before Ψ measurement (20), this could contribute to the lower Ψ values detected. However, little of the loosely held water should be present in the testa at the Ψ of mature seeds *in situ* (<-2.0 MPa). We have also found that the testa constrains the embryo as it nears full imbibition, restricting its uptake of water by developing pressure within the testa (22). As the Ψ measurements reported previously were conducted on decoated seeds (20), part of the drop in Ψ values could be due to the release of this pressure when the testae were removed. Yet again, at the Ψ s within the fruit, the embryo would be well below full hydration (21) and its volume should not be constrained by the testa. On the other hand, the Ψ gradient we observed between embryonic and maternal tissues could be real, as Saab and Obendorf (15) reported that the Ψ of soybean (*Glycine max* L.) embryos changed independently of that of the surrounding maternal tissues and of the culture solution during *in vitro* growth. In that case, the Ψ of the culture solution in sealed flasks should determine the Ψ of the entire system, analogous to the situation of the muskmelon seed enclosed within the fruit. It remains an intriguing question whether, or how, the embryo can maintain a total Ψ different from that of its surroundings in the absence of a high resistance to water movement.

The properties of the muskmelon perisperm described here are also intriguing. Mature perisperm cell walls limit solute movement whether alive or dead, but do not form a barrier to water movement. The perisperm is highly elastic and can withstand considerable hydrostatic pressure. No physiological significance has previously been attributed to the perisperm envelope. We may speculate that the perisperm has evolved to isolate the embryo from the harsh chemical environment of ripening and decaying fruits, to protect the seed from soil-borne pathogens by limiting solute leakage, or to control embryo development by restricting access to metabolites after adequate embryo size is attained. In a number of species, endosperm or perisperm envelopes are also involved in regulation of dormancy and germination (2, 6, 7, 18, 19, 22).

Further research is needed to determine the structural basis of the semipermeable and elastic properties of the perisperm and to elucidate its function during seed development and germination.

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