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

V. Water Relations of Imbibition and Germination

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ABSTRACT

The initiation of radicle growth during seed germination may be driven by solute accumulation and increased turgor pressure, by cell wall relaxation, or by weakening of tissues surrounding the embryo. To investigate these possibilities, imbibition kinetics, water contents, and water (Ψ) and solute (ψᵣ) potentials of intact muskmelon (Cucumis melo L.) seeds, decoted seeds (testa removed, but a thin perisperm/endosperm envelope remains around the embryo), and isolated cotyledons and embryonic axes were measured. Cotyledons and embryonic axes excised and imbibed as isolated tissues attained water contents 25 and 50% greater, respectively, than the same tissues hydrated within intact seeds. The effect of the testa and perisperm on embryo water content was due to mechanical restriction of embryo swelling and not to impermeability to water. The Ψ and ψᵣ of embryo tissues were measured by psychrometry after excision from imbibed intact seeds. For intact or decoted seeds and excised cotyledons, Ψ values were >-0.2 MPa just prior to radicle emergence. The Ψ of excised embryonic axes, however, averaged only -0.8 MPa over the same period. The embryonic axis apparently is mechanically constrained within the testa/perisperm, increasing its total pressure potential until axis Ψ is in equilibrium with cotyledon Ψ, but reducing its water content and resulting in a low Ψ when the constraint is removed. There was no evidence of decreasing ψᵣ or increasing turgor pressure (Ψ-ψᵣ) prior to radicle growth for either intact seeds or excised tissues. Given the low relative water content of the axes within intact seeds, cell wall relaxation would be ineffective in creating a Ψ gradient for water uptake. Rather, axis growth may be initiated by weakening of the perisperm, thus releasing the external pressure and creating a Ψ gradient for water uptake into the axis. The perisperm envelope contains a cap of small, thin-walled endosperm cells adjacent to the radicle tip. We hypothesize that weakening or separation of cells in this region could initiate radicle expansion.

Seed germination culminates in embryo growth and radicle protrusion through the tissues surrounding the embryo. Processes both in the embryo (solute accumulation or cell wall loosening) and in the enclosing tissues (tissue weakening or cell separation) may be involved in regulating the initiation of germination. In some species, such as rape (Brassica napus), the testa does not represent a significant barrier to radicle growth because it is thin, fragile, and cracks during imbibition. Schopfer and Plachy (21) have shown that germination of rape seeds occurs when cell wall extensibility increases and the turgor yield threshold decreases. They detected no significant solute accumulation or changes in hydraulic conductivity during germination.

In species where the radicle must penetrate an enclosing tissue such as the endosperm or perisperm, such as lettuce (Lactuca sativa), tomato (Lycopersicon esculentum), or muskmelon (Cucumis melo), the embryonic axis might generate additional pressure by accumulating osmotic solutes (2, 3, 22). Factors stimulating seed germination, such as light and growth regulators, can increase the ability of seeds to germinate at lower Ψ (4, 14, 23). However, Weges (25) found no evidence of solute accumulation in lettuce embryos prior to radicle emergence. In lettuce and watermelon (Citrullus lanatus), direct measurements of ψᵣ in excised axes failed to completely account for the increases in growth potential observed (3, 24), indicating that decreased yield threshold or increased cell wall extensibility may be partially responsible for radicle growth during germination. Liptay and Schopfer (16) concluded that differences in the extensive force exerted by the radicle, not in the opposing force of the endosperm, were responsible for genotypic variations in tomato seed germination capacity. Groot and coworkers (10, 11), on the other hand, have emphasized the role of the endosperm in controlling radicle emergence in tomato. Haigh and Barlow (13) found no lowering of embryo ψᵣ or increase in turgor prior to radicle emergence in tomato, but showed that the endosperm enclosing the embryo acts as mechanical barrier to embryo expansion, limiting embryo WC by 20% during the plateau phase of imbibition. Weakening of the endosperm layers opposite the radicle tip is necessary for radicle growth to occur (10-12).

The muskmelon embryo is enclosed within a testa and a thin perisperm/endosperm envelope that can be removed without damaging the embryo (29). The testa and perisperm/endosperm envelope (hereafter termed perisperm) are perme-

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Abbreviations: Ψ, water potential; ψᵣ, solute potential; ψᵣₑ, turgor pressure; ψᵣₑₓₑ, extracellular pressure; DAA, days after anthesis; WC, water content.
able to water movement (29), but their relationship to embryo expansion during imbibition or germination has not been investigated. In this study, the water relations of intact and excised muskmelon seed tissues were examined during imbibition and germination. Our objectives were to determine whether the testa and perisperm limit embryo expansion, inhibiting or delaying germination, and whether radicle growth is accompanied by solute accumulation and increased turgor pressure, by changes in embryo cell wall properties, or by weakening of tissues surrounding the embryo.

**MATERIALS AND METHODS**

**Plant Material**

Muskmelon (*Cucumis melo* L. cv Top Mark, Asgrow Inc, Gonzales, CA) plants were field-grown at the University of California, Davis, as previously described (26). Perfect flowers were tagged at anthesis and fruits were harvested at 60 DAA. Seeds were removed from the fruits and vigorously washed in tap water for 30 min. The cleaned seeds were forced-air dried at room temperature for 6 h, then transferred to a desiccator containing activated silica gel for an additional 42 h at 30°C. Seeds were stored in hermetically sealed plastic bottles at 10°C and <5.0% WC for 10 months prior to the start of the study. Samples were weighed, heated to 130°C for 24 h, cooled in a desiccator, and reweighed to determine fresh weight, dry weight, and WC (expressed on a dry weight basis).

**Imbibition**

Intact seeds, decoated seeds (testa removed but with the perisperm envelope intact around the embryo), cotyledons, and embryonic axes were partially submerged in beakers containing deionized water at 20°C. At 1-h intervals for 12 h and at 2-h intervals thereafter, each sample was lightly blotted with tissue paper to remove excess moisture, weighed, and returned to water. Intact seeds also were imbibed in vigorously aerated water or on water-saturated germination blotter paper...
Water Potential Measurements

The $\psi$ and $\psi_1$ of intact seeds, decoated seeds, embryonic axes, and cotyledons were measured using a thermocouple psychrometer (model SC-10, Decagon Devices, Pullman, WA) using procedures previously described (26). The psychrometer was calibrated daily with NaCl standards that were verified by vapor pressure osmometry (model 5100C, Wescor Inc., Logan, UT). Samples were equilibrated for 2 to 3 h and two to four readings were taken on each sample to ensure that equilibrium had been attained. The volume occupied by the embryonic axes was $\sim$0.2 cm$^3$ (approximately one-eighth the volume of other samples), and equilibration time and variability were reduced by decreasing the volume of the psychrometer cups to approximately 1 cm$^3$ using fluxless solder. Water potential measurements were replicated four to eight times for each reported imbibition time. Following $\psi$ measurements, samples were tightly capped, frozen at $-70^\circ$C for 12 h, thawed for 3 h at room temperature, and remeasured to determine $\psi_1$, $\psi_1$ was estimated as the difference between $\psi$ and $\psi_1$. The $\psi$ and $\psi_1$ of embryonic axes also were measured with the vapor pressure osmometer immediately following measurement by psychometry. No significant differences were detected between the two methods.

Estimation of Testa and Perisperm Restraint to Radicle Emergence

Groups of 25 intact seeds, decoated seeds (only testa removed), and embryos (perisperm and testa removed) were incubated inside scaled plastic boxes on blotters saturated with PEG solutions to estimate the restraint to germination imposed by the testa and perisperm (23). Solutions of either $-0.2$ or $-0.4$ MPa were prepared using the equation of Michel (18). Filter paper discs were placed on the blotters at the start of each experiment and were removed periodically for $\psi$ measurement by vapor pressure osmometry. The reported $\psi$s were determined from an average of three or four measurements taken from 4 to 7 d after planting. Germination was scored when the radicle emerged from the testa or perisperm, or when a positive geotropic response of the radicle was evident in isolated embryos.

Microscopy

Hydrated, decoated 60 DAA seed were fixed in a mixture of 70% ethanol, 5% formalin, 5% glacial acetic acid, and 20% distilled water prior to dehydration in an ethanol and tert-butyl alcohol series following the procedure described by Jensen (15). Decoated seeds were embedded in paraffin (Paraplast, Monorex Scientific, St. Louis, MO) and 5 to 8 $\mu$m transverse, serial sections were prepared from the tip of the embryonic axis using a microtome (model 820, American Optical Company, Buffalo, NY) equipped with stainless steel knives. Sections were stained with saturated Sudan IV in 95% ethanol (5) and photographed through a light microscope.

RESULTS AND DISCUSSION

Imbibition

The WC of intact seeds increased rapidly (phase I) and reached a plateau (phase II) at 85% after approximately 15 h.
Seed WC then remained constant until just prior to radicle emergence at 78 h (Fig. 1A). Imbibition of decoated seeds (testa removed before imbibition) lagged behind that of intact seeds, and the WC did not reach a plateau level but continued to increase gradually until radicle emergence occurred at 66 h and 78% WC (Fig. 1A). The WC values of decoated seeds were lower than for intact seeds due to the high WC of the testa (Fig. 1A). The testa accounted for 42% of the total seed dry weight and 46% of the imbibed fresh weight. During phase II, the WC of decoated seeds isolated from the testa after imbibition was ≈12% less than that of seeds decoated before imbibition, or the equivalent of approximately a 24 h delay in the attainment of a given WC (Fig. 1A; percentages cited refer to the absolute WC values, not to relative differences). The delay in imbibition of embryos when the testa is present indicates that the testa restricts embryo water uptake. However, both the rapid initial absorption of water and the attainment of a plateau or equilibrium WC in intact seeds show that this is not due to water permeability barriers in either the testa or the perisperm. The kinetics of water loss from imbibed seeds also indicated that the perisperm and testa are not barriers to water movement (29).

The imbibition pattern of isolated cotyledons showed an initial rapid water uptake followed by a relatively constant WC during phase II (Fig. 1B). The plateau WC of 85% was attained within 24 h, but if the perisperm was present, an additional 40 h was required to reach the same WC in decoated seeds (cf. with Fig. 1A). The cotyledons account for 84% of the decoated seed fresh weight, so their final WC should be similar to that for decoated seeds. The perisperm delays expansion of the cotyledons, apparently by presenting a resistance to embryo expansion but being capable of gradually stretching under tension. The perisperm can stretch to approximately twice the embryo volume and can withstand a considerable pressure from within (29). The WC of cotyledons isolated after imbibition within the testa was only 60% (Fig. 1B). The testa appears to be relatively inelastic and prevents the cotyledons from achieving full hydration.

Isolated embryonic axes displayed an abbreviated phase I, as the axes exceeded 115% WC in just 3 h (Fig. 1C). The WC then increased steadily until radicle growth began at 45 h and 140% WC. Axes imbibed within the perisperm and testa had a slower rate of water uptake and a plateau WC of ≈90% (Fig. 1C). The reduction in tissue water content by the enclosing tissues was therefore even greater for the axis (≈50%) than for the cotyledons (≈25%). These results are similar to those of Haigh and Barlow (13), who showed that water uptake by tomato embryos is mechanically restricted by the surrounding endosperm. Removal of the embryo from the endosperm in tomato allowed an additional 20% increase in embryo water content. Observations on the water relations of seed germination in *Avena fatua* (17) and *Eucalyptus sieberi* (9) also indicate that physical constraints on embryo expansion by tissues surrounding the embryo may limit hydration in morphologically diverse seeds.

**Testa and Perisperm Restraints to Germination**

As shown above, the presence of the testa delays the initiation of radicle growth by about 12 h, and isolated axes begin to grow approximately 30 h earlier than is evident in intact seeds (Fig. 1). We tested whether this delay in germination time could be related to mechanical restraint by the testa and perisperm by germinating intact and decoated seeds and isolated embryos in a series of osmotic solutions. The change in germination capacity at different Ψs when the embryo coverings are removed gives a quantitative estimate of the strength of the enclosing tissues (23). Freshly harvested 35 DAA intact seeds, decoated seeds, and embryos were used because at this stage it is still possible to remove the perisperm from the embryo with minimal damage. Seeds of this age have just attained maximum dry weight and germination capacity (26), and the perisperm has also just developed its mature properties (29).

Intact seeds germinated poorly in water, and germination was actually improved in the presence of osmoticum (Fig. 2A). An inhibition of germination at high Ψ that is alleviated by removing the testa, elevated O2 partial pressure, or slight reductions in Ψ has been described for muskmelon seeds (7; our unpublished results). This prevented the precise estimation of restraints to germination due to the testa. However, at slightly lower Ψ (~0.35 or ~0.29 MPa) the germination time courses of intact and decoated seeds were quite similar (Fig. 2, A and B). Thus, once the high-Ψ inhibition is alleviated, the testa does not appear to present a significant mechanical restraint to radicle emergence. This is reasonable, as the halves
of the testa often split open slightly at the tip during imbibition, and should not directly restrict radicle growth.

After removal of the testa, germination occurred in water and was inhibited by reduced $\psi$ (Fig. 2B). When the perisperm was also removed, the rate of germination was advanced such that the embryos in $-0.25$ MPa solution exhibited a time course similar to that of decoated seeds in water (Fig. 2C). The mean time to germination of decoated seeds in water was 247 h, while that for embryos imbibed on $-0.25$ MPa solution was 261 h. This indicates that the perisperm exerts a restraint on radicle emergence equivalent to an osmotic potential of about $-0.25$ MPa.

### Water Potentials Prior to Germination

The $\Psi$ and $\psi$, of intact and decoated seeds, cotyledons, and embryonic axes isolated from imbibed intact seeds were measured to determine whether the initiation of axis growth is accompanied by changes in turgor. During initial studies with intact seeds, $\Psi$ values as low as $-0.5$ MPa were measured during phase II of imbibition, when water content was not changing (Fig. 1A). Since water content has attained equilibrium during this period, one would expect that the seed $\Psi$ should approach that of the solution, near 0 MPa. Extensive trials with various methods of imbibition and blotting of the seeds indicated that the $\Psi$ measured for intact seeds was highly sensitive to the pressure applied during the blotting of excess water from the seeds (data not shown). This is apparently due to the large amount of water held at relatively low tension in the testa (e.g. Fig. 1A). Even relatively slight pressure during blotting removes some of this water and reequilibration between the testa and the embryo during the measurement period results in lower and variable $\Psi$ values. The experiments reported were therefore conducted with only gentle wiping of the free water from the seed surface before $\Psi$ measurements or dissection. All manipulations were conducted in a humidified box to minimize water loss from exposed tissues prior to sealing them in the psychrometer chamber. Water potential values $>-0.2$ MPa could then be consistently measured, within the upper limit of sensitivity of the system.

Psychrometric measurements were made during the final stages of phase II (48–68 h of imbibition), just prior to radicle emergence. The $\Psi$ of intact seeds approached 0 MPa, $\psi$, increased from $-0.8$ to $-0.3$ MPa, and turgor ($\psi_p = \Psi - \psi$) decreased from 0.5 to 0.1 MPa during this period (Fig. 3A). Water potentials of decoated seeds and cotyledons isolated after imbibition approached 0 MPa by 65 h, $\psi$, increased from about $-1.2$ to $-0.9$ MPa, while $\psi_p$ values were 0.6 to 0.9 MPa (Fig. 3, B and C). The considerably higher $\psi$ and lower $\psi_p$ values for intact seed is apparently due to dilution of solutes after freezing and thawing by water absorbed from the testa. After freezing, the embryo membranes will leak solutes into the space bounded by the perisperm, which is semipermeable, allowing water uptake but preventing leakage of solutes (28). The loss of turgor in the frozen embryos will lower their $\Psi$, creating a gradient for water to move from the testa through the perisperm. Since the testa holds a quantity of water roughly equivalent to that in the imbibed embryo, considerable dilution and increase in $\psi$, could be expected, as was observed (Fig. 3 A).

The $\psi$, values measured here for 60 DAA decoated seeds are considerably higher than the calculated values based upon the measured $\psi$, of freshly harvested seeds (26) and the dilution due to the increase in seed water content upon imbibition (27). Many seeds accumulate large quantities of soluble sugars late in development which are rapidly metabolized following imbibition (19). Thus, the low $\psi$, observed in fresh seeds may increase during phase II of imbibition to the levels we recorded prior to radicle emergence. More detailed measurements during the early stages of imbibition are required to answer this question.

The $\Psi$ and $\psi$, values measured for embryonic axes during phase II were lower than those of the other tissue samples. The $\Psi$ was $-0.4$ to $-0.6$ MPa, $\psi$, was $-1.6$ to $-1.2$ MPa, and $\psi_p$ was 0.6 to 0.9 MPa (Fig. 3D). It seems unlikely that the embryonic axis and the cotyledons would fail to be in $\Psi$ equilibrium within the intact seed at a time prior to axis growth. There are at least two possible explanations for this discrepancy. Cell wall relaxation during the equilibration period prior to psychrometric measurements would lower $\Psi$ and $\psi$, (1). However, cell wall relaxation should not affect $\psi_p$, which was also lower in axes than in cotyledons. Furthermore, the axes were capable of 50% greater water uptake if removed from the testa and perisperm, indicating that the tension on the axis cell walls due to turgor pressure would have been relatively low compared to when the cells are fully imbibed. Alternatively, if the axis is mechanically restrained from expanding by the perisperm and testa, the pressure exerted by these enclosing tissues (i.e. $\psi_{testa}$ or an extracellular pressure component) would be additive with turgor to increase the total pressure potential ($\psi_p + \psi_{testa}$). Inside the testa, this additional pressure would bring the axis into total $\Psi$ equilibrium with the remaining seedle tissues before the tissues had achieved maximum hydration. The situation would be analogous to that of tissues contained within a pressure-block apparatus, where the total tissue pressure potential remains constant as an external pressure is applied, but turgor is reduced and growth is inhibited (6). Excision from the seed, or penetration through the perisperm/testa, would immediately reduce $\psi_{testa}$ to zero, resulting in the lower $\Psi$ values recorded for excised axes (Fig. 3D) and in a gradient for water uptake to drive initial axis expansion. A very similar situation was recorded for tomato seeds, where the embryo $\Psi$ measured upon isolation from the surrounding endosperm was $-1.5$ MPa, even though the intact seed had equilibrated at near 0 MPa (13). Constraint of embryo expansion by the endosperm and testa apparently created pressure which limited the embryo volume by 20% but increased its $\Psi$ to near 0 MPa in situ. An even greater discrepancy between the $\Psi$ of intact seeds and isolated embryos was reported for E. seiberi, where imbibed intact seeds equilibrated at $-0.6$ MPa, but embryos excised from the seeds had $\Psi$ values of $-4.5$ MPa (9). This effect was still evident in the cotyledons remaining within the inner integument ($\Psi = -4.5$ MPa) even after hypocotyl tissues had emerged from the seed and begun growth ($\Psi > -1.0$ MPa). These authors also concluded that mechanical restraint, rather than hydraulic impermeability, limited water growth.
uptake into the tissues enclosed within the inner integument (9).

The maintenance of the embryonic axis at relatively low WC within the imbibed seed also implies that cell wall relaxation in the embryo is not the factor controlling the initiation of growth. In rape seeds, for example, the testa splits upon imbibition, allowing full hydration of the embryo (21). Under this condition, cell wall relaxation will lower turgor and create a gradient for water uptake. The cell walls of muskmelon axes, however, are already relaxed in the sense that the axes are capable of increasing in volume by 50% when isolated from the testa/perisperm (Fig. 1C). Additional wall relaxation in the presence of an external restraint to expansion would have little effect on \( \psi \), and could not create a gradient for the water uptake required for growth.

Alternative mechanisms to initiate radicle growth would be a decrease in axis \( \psi \), or a weakening of the external restraint (reduction in \( \psi_{\text{ext}} \)) to create a gradient for water uptake. We found no evidence of solute accumulation or a corresponding increase in turgor preceding radicle growth, as \( \psi \) remained constant or decreased slightly with imbibition time (Fig. 3). These results agree with recent studies with rape (21), lettuce (25), tomato (13), and \( E. \) seiberi (9) seeds, where no evidence of solute accumulation prior to radicle emergence was found, and are in contrast to the conclusions of Takeba (22) and Bradford (2) with lettuce. In the latter studies, however, indirect methods were used to infer the occurrence of osmotic adjustment, and other interpretations of the data are possible.

Anatomy of the Perisperm

Since the perisperm exerts a mechanical restraint to radicle emergence (Fig. 2B), and turgor does not increase prior to radicle emergence (Fig. 3D), weakening of the perisperm may be involved in the initiation of axis growth. There is good evidence in several species that endosperm weakening is a key event in germination (10, 11, 13, 20, 25). Haigh (12) identified a region of smaller, thin-walled cells in the tomato endosperm immediately opposite the radicle tip which appeared to separate coincident with radicle growth. Specific anatomical changes also occur in a region of the lettuce endosperm opposite the radicle tip just prior to or coincident with germination (8). We examined the perisperm region adjacent to the radicle tip to determine whether any distinctive anatomical features might be identified. Cells from both the residual endosperm (Fig. 4A) and the perisperm (Fig. 4B) were evident in transverse sections obtained from tissues adjacent to the embryonic axis. A region of endosperm with thickened cell walls appears to form a circle around the tip of the envelope (Fig. 4C). Inside this circular region, the endosperm cells are smaller in comparison to the larger rectangular cells outside. We have no experimental data as yet on cellular or mechanical changes that might occur in this region of the perisperm envelope coincident with germination, but its structure is reminiscent of similar tissues in tomato (12), which are induced by gibberellin to weaken or separate to initiate radicle growth (10, 11). Furthermore, abscisic acid will prevent endosperm weakening and inhibit germination in tomato (10), and the effect of ABA on germination is eliminated if the endosperm cap adjacent to the radicle is removed (16). We have shown that germination of muskmelon seeds is sensitive to inhibition by ABA, and have proposed that it acts by increasing the effective turgor yield threshold for growth (28), which would include the restraint due to the perisperm envelope. It would be of interest to determine the influence of ABA and gibberellins on the region of the muskmelon perisperm envelope identified in Figure 4.

CONCLUSIONS

The embryos, and particularly the axes, of muskmelon seeds are prevented from attaining full hydration by the surrounding perisperm and testa. The enclosing tissues apparently exert pressure on the embryo opposing the uptake of water. When the perisperm and testa are removed, the external pressure is relieved and \( \psi \) is reduced, allowing axis water content to increase by 50%. No evidence was found of osmotic accumulation or turgor increase prior to the initiation of radicle growth. Weakening of the perisperm opposite the radicle tip is apparently required for radicle growth to occur. A distinct anatomical region was identified in the perisperm/endosperm envelope adjacent to the radicle tip. We hypothesize that growth of the embryonic axis may be initiated by weakening or separation of cells in this region.

LITERATURE CITED

priming. Ph.D. Dissertation, Macquarie University, Sydney, Australia.


