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CELL WALL WRINKLING AND SOLUTE LEAKAGE IN IMBIBING SQUASH AND CARROT SEEDS

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Abstract

Dry seeds placed in an aqueous solution take up water, swell, and leak potassium and a variety of other materials into the solution. It is likely that much of the potassium is from the cell walls. Neutron activation analysis was used to measure the concentration of potassium leaked from both squash embryos and carrot mericarps that had been soaked in solutions of different water content. The cell walls in dry seed tissues are often wrinkled, whereas imbibed tissues have smooth cell walls. Cryogenic preparation for scanning electron microscopy was used to study the degree of cell wall wrinkling in the seed tissues at different hydration levels. The aim of this study was to test the hypothesis that the degree of cell wall wrinkling was related to the leakage of potassium. It was found that the amount of potassium leaked into the soaking solutions was not directly related to the degree of wrinkling of the cell walls.

KEY WORDS: Scanning electron microscopy, seeds, embryos, solute leakage, imbibition, cryogenic preparation, neutron activation analysis, cell walls, squash, carrot, Cucurbita , Daucus .

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Introduction

Seeds, which are of vital importance to mankind, generally have low moisture contents as they reach maturity and thus can be stored for long periods of time. The resumption of growth of the embryo in a seed, which is called germination, may depend upon a variety of factors but an important component is the uptake of adequate water. This imbibition of water causes most dry seeds to swell markedly and in many cases permits a resumption of active metabolic activity leading to growth of the seedling plant. Two phenomena that were studied in this research are associated with the imbibition process in seeds. One phenomenon deals with the structural changes in cell walls that take place as a result of swelling during imbibition. The other phenomenon relating to imbibition deals with the pronounced leakage of various materials, a significant portion of which may come from the cell walls. The leakage of materials at different hydration levels, as measured by the loss of ions, will be compared to major changes in cell wall structure that were studied by observing cryogenically prepared seeds in a scanning electron microscope (SEM).

Imbibition of water by dry seeds is known to be accompanied by the leakage of a variety of substances including electrolytes, sugars, amino acids, organic acids and proteins (Simon and Raja Harun, 1972; Duke and Kakefuda, 1981; Duke et al. 1983; Powell and Matthews, 1981). Most studies of imbibing seeds have indicated that leakage of electrolytes was by passive diffusion (Duke and Kakefuda, 1981), although the results of Marbach and Mayer (1985) suggest that a component of the leakage is not passive. Observations that imbibing dead pea embryos leak in a pattern similar to that found in imbibing live pea embryos (Powell and Matthews,1981), when coupled with observations that dead seed coats also leak K, indicates that the cell walls may be a major source of leachable K.

The loss of water from seed tissues as they dry out during maturation leads to shrinkage of the cells. As a result, cell walls of dry seed tissues often are extensively wrinkled or folded (Buttrose, 1973; Lott, 1974; Webb and Arnott, 1982). The swelling of the seed tissues during

imbibition results in an unwrinkling of the previously wrinkled cell walls (Lott and Kerr, 1986; Webb and Arnott, 1980,1982). Although dry seed tissues would seem to be relatively easy to process anhydrously for transmission electron microscopy (TEM) due to their initially low water content, it is actually very difficult to infiltrate resins into dry seed tissues. The work of Yatsu (1983) developed the idea that the reason dry seed tissues are so difficult to prepare anhydrously is that pores in the cell walls are too small in the dry state to permit passage of epoxy resin molecules. Only when the tissue has swollen, after imbibition of water, can embedding resins adequately penetrate the cell walls. These findings of Yatsu are borne out by the findings of Lott et al. (1984), in which a wide variety of solvents failed to give nominal penetration of epoxy resins into dry pea cotyledon tissue while somewhat better penetration of resins was achieved after some swelling of the tissue was fostered by soaking the tissue in 70% or 80% alcohol. Given the observations that much of the K that leaks from imbibing seeds may come from the cell walls, that cell walls undergo major folding and unfolding during seed maturation and subsequent imbibition, and that epoxy resins do not easily penetrate cell walls in the dry state but do so when imbibed, led us to investigate the possibility that loss of K during imbibiton might be related to the degree of cell wall wrinkling.

In this study the leakage of elements was measured by neutron activation analysis (NAA). This technique has advantages not found in other analytical techniques with comparable sensitivity (Corliss, 1964; Norgowalla and Przyloylowicz, 1973). NAA permits simultaneous and quantitative multi-element analysis, can measure elements in both liquid and solid samples, is quick because no elaborate sample preparation is required, and is relatively free from matrix effects. Low-temperature SEM was used to study the structural changes of the tissues. Previous studies have documented a number of advantages of cryogenically preparing biological samples for study on a cryo-stage in a SEM (Beckett et al., 1984; Beckett and Read, 1986; Echlin et al., 1980, 1982; Lott et al., 1985). One advantage that has not received much attention is the capability of such preparations to reveal differences in the structure of tissues at different moisture contents. An earlier paper by Lott and Kerr (1986), which mainly dealt with dry-to-wet transitions in a variety of samples including seeds, mentioned the possibility of using cryogenic preparation to study samples with different moisture contents.

The studies reported here will investigate, for the first time, if there is any relationship between the degree of cell wall wrinkling and the leakage of elements from seeds soaked in solutions with different water contents. Originally we hypothesized that the leakage of elements from seed tissue during hydration would occur to a substantial degree once a certain concentration of water was attained. We also hypothesized that the concentration of water at which K leakage would occur to a considerable extent would be at a point where the cells had expanded markedly.

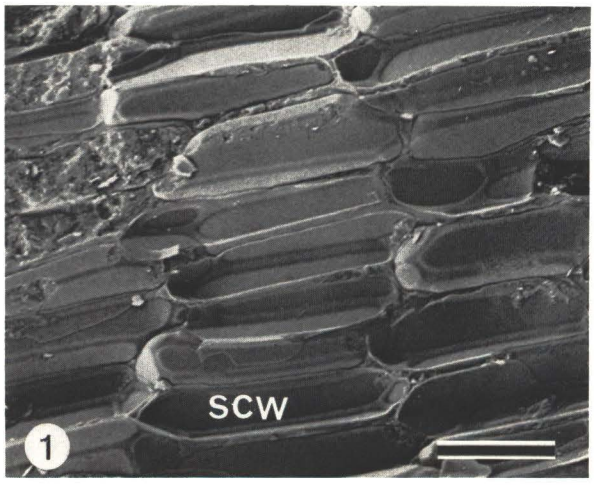
Materials and Methods

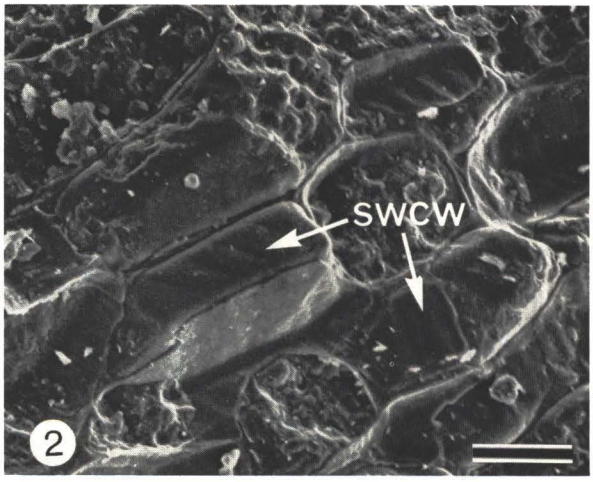
Carrot (Daucus carota L. cv. Imperator 408) mericarps were purchased from Tregunno Seeds Limited, Hamilton, Ontario. A mericarp is one half of a dry dehiscent fruit called a schizocarp. Squash seeds (Cucurbita maxima Duch. cv. Warted Hubbard) were obtained from Stokes Seeds Limited, St. Catherines, Ontario. Prior to use all squash seeds had the outer seed coat removed with a razor blade. The inner seed coat was also removed after brief soaking in water using the procedure of Ockenden and Lott (1986). Five g of squash embryos and 5 g of carrot mericarps were soaked in 20 ml of double distilled water, or absolute ethanol or mixtures of both (30%, 50%, 70%, 80% ethanol). Soaking was for 6 h at room temperature. Once the soaking was complete the seed tissue was carefully separated from the solution. The tissue was then studied by SEM while the elements in the soaking solution were determined by NAA.

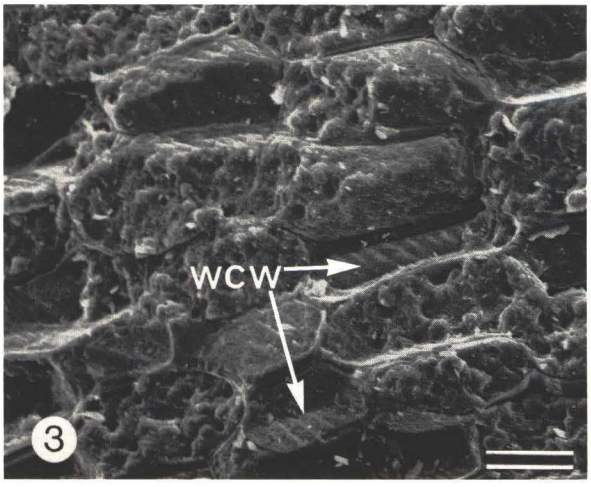
Epidermis-to-epidermis portions from the center of squash cotyledons and entire carrot mericarps were frozen, fractured under vacuum and sputter coated with gold in an EMscope SP2000 Cryo-sputter unit (EMscope Laboratories Ltd., Ashford, England). All samples were then viewed in a frozen state in an ISI DS-130 scanning electron microscope. Accelerating voltages of 12 to 15 kV were used.

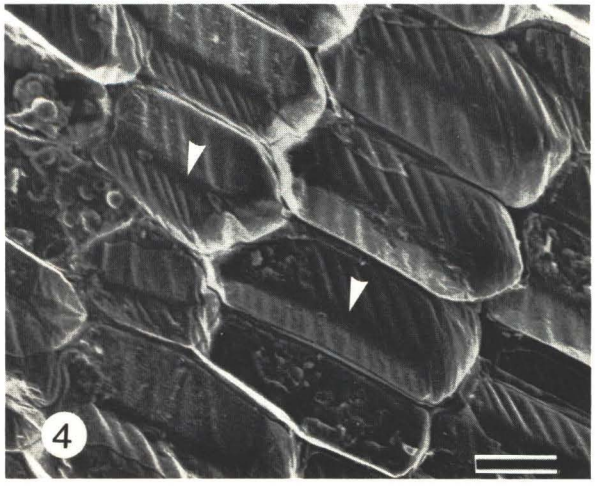
The McMaster University Nuclear Reactor was used to activate standards and samples. Standard reference materials used in this study were: SRM 1643b trace elements in water from the National Bureau of Standards, Washington, DC.; Riverine water, bottle 181 from the National Research Council of Canada, Ottawa; and potassium standard from the Aldrich Chemical Company Milwaukee, Wisconsin.

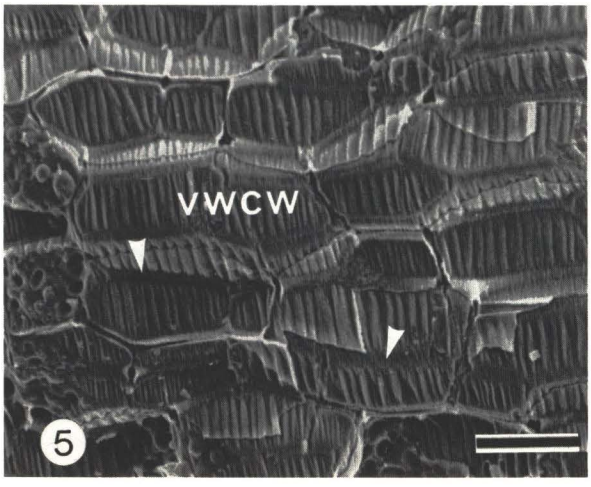
Each 6 ml volume of imbibition solution or standard was acidified prior to activation by the addition of 50 µl of ultra-pure nitric acid (Ultrex, J.T. Baker Chemical Co.). This acidification was necessary to prevent adsorption of elements onto the sides of the polyethylene vials in which all samples were activated. Effective irradiation times were determined for each series of solutions. Once activated, standards and samples were counted using a 28% efficient germanium detector. Delay and count times were maintained at approximately 100 and 600 s, respectively. An APTEC, series 90 multi-channel analyzer (Canberra Industries Inc.) was used in conjunction with this detector to generate spectra. The resolution obtained with this system was 1.8 keV at the 1332 keV cobalt peak. From each spectrum generated, the following peaks were isolated, Mg, Na, K, Cl, Mn, Ca. Quantitative determinations of these elements, for each sample, were made by











Figs. 1-5. Bars represent 50μm. Cucurbita maxima (squash) embryos were soaked in various solutions for 6 h before central cotyledon tissue was prepared cryogenically for scanning electron microscopy. Spongy mesophyll cells located near the lower epidermis were observed in all cases.

Fig.1 Embryo soaked in double distilled water. Cell walls were smooth (SCW).

Fig.2 Embryo soaked in 30% ethanol. Cell walls were slightly wrinkled (SWCW) in most places.

Fig.3 Embryo soaked in 50% ethanol. Cell walls were more wrinkled (WCW) than those in Fig.2.

Fig.4 Embryo soaked in 80% ethanol. Wrinkles in cell walls appeared more pronounced than those observed in cells of embryos soaked in lower concentrations of ethanol. Note that the angular portions of the cell walls which abut inter-cellular spaces were relatively smooth (arrows).

Fig.5 Embryo soaked in 100% ethanol. Cell walls were very wrinkled (VWCW). Unlike samples soaked in 80% ethanol, the angular portions of the cell walls had numerous small wrinkles (arrows).

comparison to the standards. These values were corrected for any differences in irradiation, delay or count times as well as for differences in weight between standards and samples.

Results

For ease of comparison, all micrographs of squash cotyledon tissue have been grouped together (Figs. 1-5) as have micrographs of carrot endosperm tissue (Figs. 6-10). Due to the great size difference between carrot mericarps and squash seeds, the leakage of potassium from each is presented in a μg of K leaked in 6 h of soaking per g of tissue (Fig.11).

Neutron activation analysis of the solutions in which squash embryos and carrot mericarps were soaked for 6 h revealed that K was the main element leaked into water. Elements such as Ca, Mg, Na and others were also leaked but at very much lower concentrations. As a result we used K as the indicator of solute leakage (Fig.11). Far greater amounts of electrolytes were leaked per gram of carrot mericarps than per gram of squash embryos. Seed tissues soaked in absolute ethanol leaked only small amounts of electrolytes. Seeds soaked in different concentrations of ethanol released different amounts of K, as shown in Fig.11. Clearly squash embryos, which were large and without seed coats, responded differently from the small carrot mericarps which contained mainly endosperm tissue and were studied with the mericarp wall layers intact. While carrot mericarps leaked slightly more K into 30% ethanol than into water, the squash embryos leaked more than twice the amount of K into 30% ethanol than was leaked into water.

The structure of cells at different hydration levels was studied using cryogenically prepared seeds. Both squash spongy mesophyll cells (Fig.1) and carrot endosperm cells (Fig.6) appeared turgid and had mainly smooth cell walls following a 6 h immersion in water. At the other end of the hydration scale, both seed tissues soaked in 100% ethanol showed evidence of reduced cell volumes and wrinkling in the cell walls. Spongy mesophyll cells that had been soaked in 100% ethanol had extensively wrinkled cell walls. There were fewer large wrinkles on the approximately flat portions of the cell walls and many more smaller wrinkles in the angular portions of the cells which abut inter-cellular spaces (Fig.5). Carrot endosperm cells soaked in 100% ethanol had some large and randomly arranged indentations and numerous small wrinkles (Figs. 9,10). Perhaps because the cell walls in carrot endosperm are much thicker than those of squash, the degree of shrinkage upon seed dehydration is much less than that observed in squash and a great proportion of the shrinkage occurs as a few large folds.

From this study it is obvious that structural changes can occur gradually as dry tissue becomes partially hydrated. Seed tissues soaked in 30% ethanol (Fig.2) and 50% ethanol (Figs. 3,7) showed that some wrinkling of the cell walls remained. Tissues soaked in 80% ethanol (Figs. 4,8) had very wrinkled cell walls, but the degree of such wrinkling was less than that seen in the dry seed tissues. In both test systems it was found that tissue soaked in 30% ethanol (and 50% with the carrot) fractured in a way that major expanses of cell wall were not exposed. These findings were reproducible but the reason for the difference is not known.

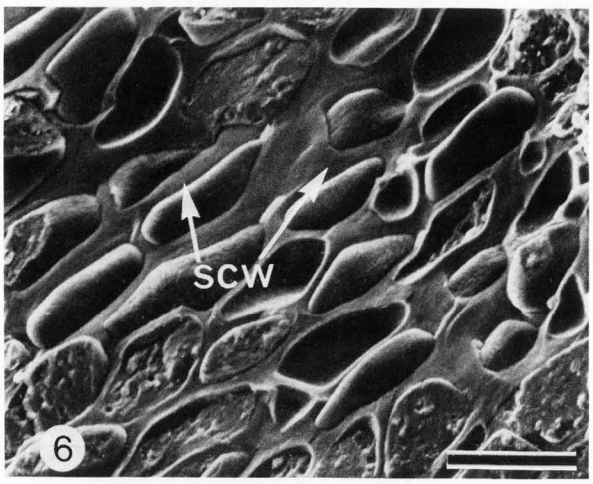
Clearly the results presented here do not support our original hypotheses since there was neither a specific level of hydration at which leakage of K to the external solution markedly increased nor was there a notable correlation between leakage of K and cell wall wrinkling. In both squash and carrot the amount of K leaked into 30% ethanol was equal to or greater than the amount leaked into water yet the cell walls of tissue soaked in 30% ethanol were still somewhat wrinkled. While tissue soaked in 80% ethanol was somewhat less wrinkled than that of dry tissue, the degree of wall wrinkling was nevertheless extensive. The amount of K being leaked into 80% ethanol was approximately 30% of the amount of K leaked into water in the case of squash and over 40% in the case of carrot.

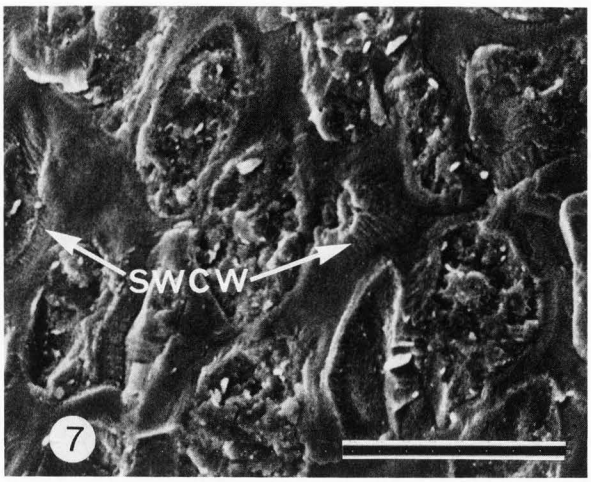
Discussion

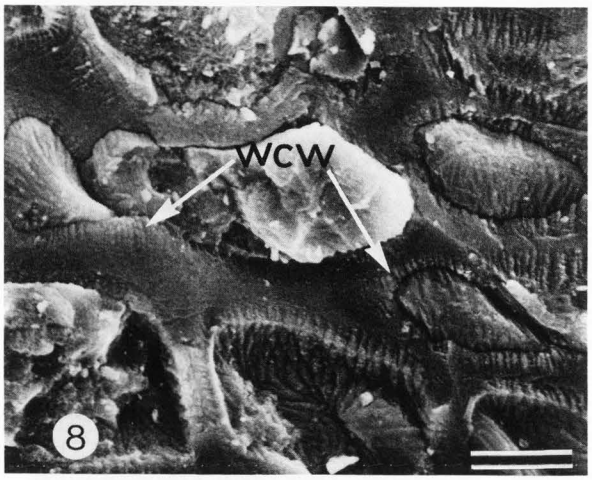
The results presented here have confirmed the usefulness of low-temperature procedures for SEM studies of samples that differ in their hydration levels. The reasons why various room temperature procedures are unsuitable for such studies have been discussed previously by Lott and Kerr (1986) so they will not be considered further in this paper.

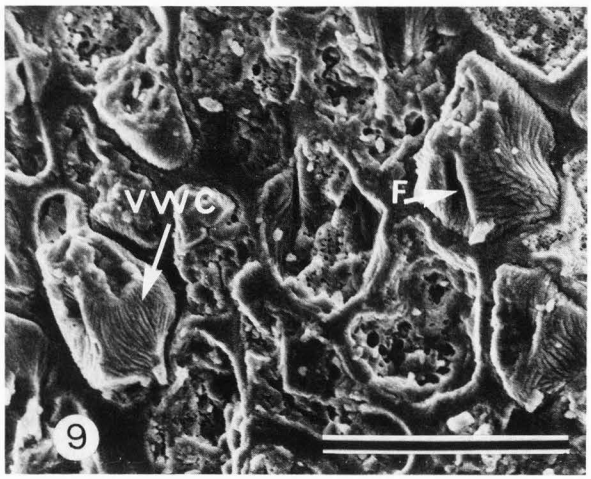
Past studies from several laboratories have shown that the dry seed tissues from certain species have wrinkled cell walls and that following imbibition these tissues have smooth cell walls (Buttrose,1973; Webb and Arnott, 1982; Lott and Kerr, 1986). Thus while the wet and dry extremes have received attention, it was uncertain whether or not the loss of wrinkling during imbibition occurs over a narrow or a wide range of hydration levels. Some studies with processed peanut tissues have indicated that an abrupt swelling occurs at a certain hydration level (Yatsu, personal comm.). It is possible that dry seed tissue would swell gradually if water became available slowly. Alternately it is possible that a certain level of hydration is needed before a substantial change would occur. The latter scenario would be similar to blowing up a balloon where a great deal of pressure may be needed to bring about the first major enlargement. The results presented here demonstrate that the loss of cell wall wrinkling during imbibition can occur gradually, as the tissue gains moisture. In both of the tissues studied some changes in structure had clearly begun with 20% hydration (in 80% ethanol) and were still continuing at 70% hydration (in 30% ethanol).

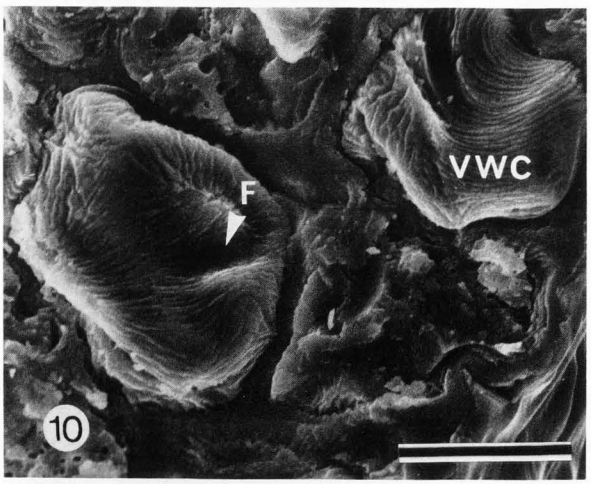
Cell wall wrinkling in dry cucurbit seeds, such as squash and zucchini, have previously been studied (Lott and Kerr, 1986; Webb and Arnott, 1980,1982). To our knowledge there are no similar studies of the endosperm of











Figs. 6-10. Bars on Figs. 6-9 represent 50μm and the bar on fig 10 represents 10μm. Daucus carota L. (carrot) mericarps were soaked in various solutions for 6 h. Mericarps were frozen, cross fractured through the middle, gold coated and studied in an SEM equipped with a cryo-stage. Endosperm cells near the outer portion of the endosperm were studied. Carrot endosperms have thick primary cell walls with very few inter-cellular spaces.

Fig.6 Mericarp soaked in double distilled water. Endosperm cell walls were thick and inter-cellular spaces were not evident. The cell walls were relatively smooth (SCW).

Fig.7 Mericarp soaked in 50% ethanol. Cell walls were slightly wrinkled (SWCW).

Fig.8 Mericarps soaked in 80% ethanol. Cell walls were wrinkled (WCW). This wrinkling was particularly pronounced immediately adjacent to the cell cytoplasm.

Figs.9 and 10. Mericarps soaked in 100% ethanol. Cells were very wrinkled (VWC) and large folds (F) were evident.

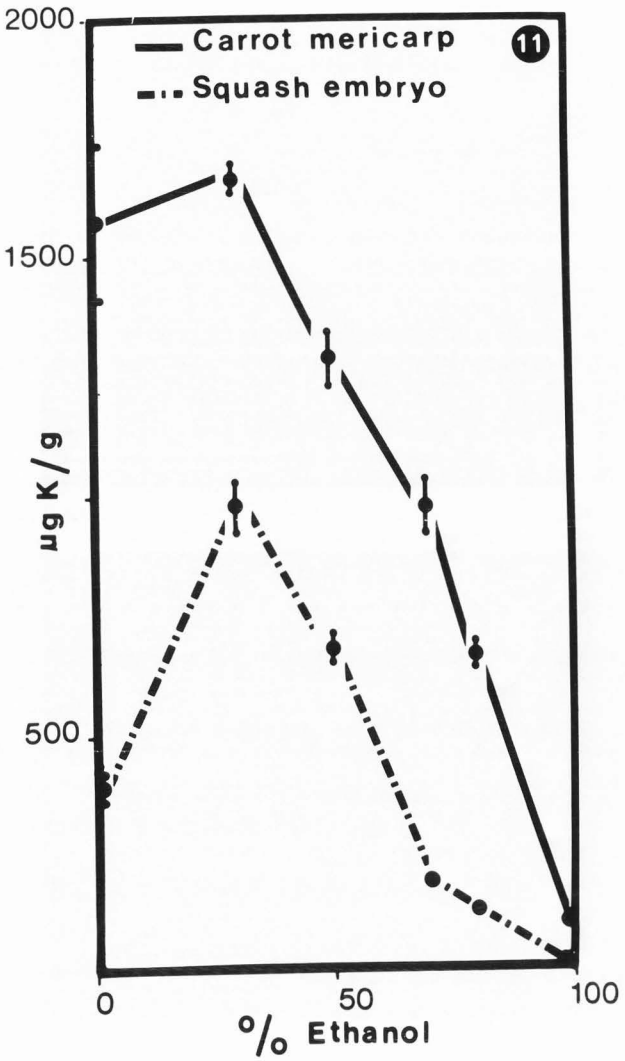


Fig.11 Leakage of K from carrot mericarps and squash embryos into water and 30%, 50%, 70%, 80%, 100% ethanol solutions. Each 5 g sample of tissue was soaked in 20 ml of solution. NAA was used to measure the amount of K in the solutions (expressed in µg / g of tissue soaked).

Umbelliferous seeds such as carrot. While the squash cells shrink mainly along the longest axis of the cells, carrot endosperm cells do not have such a pronounced axis of shrinkage. In carrot endosperm cells, a few large and randomly oriented folds appear to permit most of the shrinkage. Carrot endosperm also differs from squash cotyledon tissue in having thicker primary cell walls and fewer inter-cellular spaces.

While small amounts of elements such as Ca, Cl, Mg, Na and Mn leaked from the squash and carrot tissues, it was found that K was released in the greatest amounts. The results presented here demonstrated conclusively that leakage of K occurred over a range of hydration levels. Leakage did not begin abruptly once a particular water content had been reached. The leakage of K from tissue soaked in solutions with relatively low water content was small compared to the amount leaked when solutions with a higher concentration of water were used. The two test samples also showed distinct differences in the manner in which K was leaked at different hydration levels. For example, in carrot similar amounts of K leakage occurred in samples soaked in water and in 30% ethanol whereas in squash the 30% and the 50% ethanol samples leaked much more K than did the water control. The reasons for this difference are not known but could be due to several factors. The carrots were subject to the constraints of the mericarp wall while the seed coats were removed from the squash embryos prior to the start of the experiment. Preliminary studies indicate that the amount of K leaked from intact squash seeds is different than the results reported here for squash embryos. Carrot mericarps are small compared to squash embryos so that the ratio of tissue surface area to volume of solution would be different for the two systems. Also the squash cotyledon tissue has a much more pronounced system of inter-cellular spaces than does the carrot endosperm, a difference that could influence the ease with which soluble ions could be removed from the tissue. Ethanol soaking is an abnormal system and we have some evidence from growth experiments indicating that ethanol/water mixtures damage cells. This damage may allow leakage to occur at greater rates than would be the case when only water was present. In living cells, initial leakage is greater and then decreases over time as the cells resume active metabolic functioning (Simon and Raja Harun, 1972).

The results presented here showed that leakage of potassium from seed tissue did not depend upon the cells being turgid. Major wrinkling of the cell walls was still present in cases where considerable leakage of potassium had taken place. In carrot a small amount of K was even released into absolute ethanol. The opening up of very small pores in the cell walls to allow passage of large molecules, as suggested by Yatsu (1983), clearly does not require loss of the large wrinkles in the cell walls.

The exact origin of the K that was leaked from squash embryos and carrot mericarp was not determined in this study. The proportion originating from the cell walls and the proportion leaking across the plasmalemmas from the cell contents remains uninvestigated. Cell walls in dry pea embryos contain significant amounts of K (Lott et al., 1984) so it is reasonable to propose that some of the K leaked into the soaking solutions came from the cell walls. Since sodium phytate is virtually insoluble in alcohol concentrations above 70% (Lott et al., 1984), it seems likely that potassium phytate would also be insoluble in such alcohol solutions. The loss of a significant amount of K into 70% and 80% ethanol

suggests that much of the K being leaked was not associated with the phytate mineral reserves inside the protein bodies.

Acknowledgements

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Discussion with Reviewers

H.J. Arnott: The thick cell walls in some seeds (eg. Yucca) are reabsorbed during germination. Does that happen in carrot? If so, perhaps some of the differences you see in comparing squash and carrot may be due to the nature of the cell wall material in the individual cases.

Authors: Although the fate of the endosperm cell walls was not studied as part of this project, we do know that the endosperm of carrot is extensively degraded during seedling growth. Several days after radicle emergence the residue of the endosperm is very soft and contains

numerous calcium oxalate crystals (Lott et al., 1982, Can.J. Bot. 60 , 1404-1408). It is therefore most probable that the cell walls in carrot endosperm are degraded for use by the seedling plant. As you suggest, this may account for differences between cell walls in squash and carrot.

A. Beckett: Do the authors feel that a significant amount of K leaked from the seeds, comes from the cell walls? Is it not more likely that solute leakage is a reflection of the structural integrity of the plasma membranes of the cells? In this case, ethanol treatment would surely do more damage as a solvent than any proposed changes resulting from different states of hydration!

A. Beckett: Why were ethanol, rather than mannitol solutions used to control hydration?

G.H. Haggis: If ethanol at high concentrations dissolves lipids from cell membranes and denatures membrane proteins may this not alter membrane permeability sufficiently to make the study of this paper rather meaningless in relation to K leakage during normal seed imbibition? Having raised this point the authors have to justify the validity of their work, or the reader is left with the impression that their K leakage measurements for the higher alcohol concentrations are of little value.

Authors: We believe that a significant amount of the K that leaks from a seed when it is soaked does indeed come from the cell walls. For example the outer seed coats of squash, which mainly consist of cell walls, leaked 2157 µg of K / g of tissue soaked in water. This is almost 6 times greater than that leaked from an equivalent weight of squash embryos. By comparison, the outer seed coats of squash leaked only 178 µg of K / g of seed coat soaked in absolute ethanol. Clearly then, the cell walls may contain a lot of leachable K but the extraction of that K depends greatly on the hydration of the cell walls. Powell and Matthews (1981), observed that the pattern of leakage from dead pea embryos was similar to that found with living embryos, and argued that leakage during imbibition was a physical diffusion phenomenon. Although ethanol can damage membranes and could extract materials that are not readily soluble in water, the evidence just presented lead us to believe that the cell walls were the logical place for us to concentrate our efforts. The influence of ethanol on the ability of seeds to germinate is a complex one. Many seeds will survive soaking in absolute ethanol or very dilute concentrations of ethanol but will not germinate following soaking in 30-95% ethanol.

For soaking studies no solution is perfect. There are reasons to choose polar or non-polar solutions, large or small molecules, solutions that penetrate or those that do not. We chose ethanol for the following reasons but are aware that the choice can be criticised. (1) Glycerol was not suitable for NAA because heat generated in the reactor damaged the sample containers. We are not certain if mannitol would present similar difficulties. As mannitol is a powder that must be dissolved in water, it is not possible to get solutions with very low water contents. (2) Ethanol is widely used in microscopy and we had experience with it in attempts to embed seed tissue using low-water-content procedures (Lott, Goodchild, and Craig 1984, text reference). (3) Most of the mineral storage in a seed is in the form of phytate and we know that the readily water soluble K phytate is virtually insoluble in ethanol concentrations above 70%. Since most phytate in squash and carrot cells are in less soluble mixed salts it is unlikely that the major ion store inside the cells contributes to leakage in the higher alcohol concentrations. (4) We also required a solution that would penetrate the tissue so that complex interactions between an external osmoticum, water and dry tissue could be avoided.

B.G. Swanson: Do intact squash seeds leak less K+ than squash embryos and if so, how much less?

Authors: Whole squash seeds leaked almost twice as much K into water than did the isolated embryos under the same conditions (373 µg / g of embryo tissue soaked versus 640 µg / g of whole seeds soaked). As noted in reply to the previous question, this result reinforces our interest in the cell walls as a source of leachable ions since the seed coat of squash is mainly dead tissue devoid of cell contents. For details of the structure of squash seed coats see Lott, 1973, Can. J. Bot. 51 , 1711-1714.

B.G. Swanson: Do you have a hypothesis for why the thick cell walled carrot seeds with seed coat attached leaked more K+ than the thin cell walled squash seeds with seed coats removed?

Authors: This question is a complex one since there could be differences due to species, and/or the presence of the testa and/or the thickness of the cell walls. We have no way of separating out the various possibilities with the data we have. Carrot mericarps are small so it would be a laborious process indeed to try and dissect off the mericarp walls without causing major damage to the endosperm. We thus cannot determine the influence of the mericarp wall on the leakage process in carrots. The information for squash is presented in our answer to the preceding question. The thickness of the cell wall may have an influence on the amount of K present in the cell walls but we believe that species-to-species differences may be more significant. Many of the studies of electrolyte loss during seed imbibition have used legumes like peas and soybeans. Seeds of these species have markedly higher K concentration than seeds of many other species. It is possible that some species-to species difference occurred in this study. Our microscopy studies concentrated on two tissues, namely the spongy mesophyll of

squash cotyledons and the outer part of the endosperm of carrot. However other cell types are present in carrot mericarps and squash embryos so we did not make any direct comparison of cell wall thickness in one of the tissues present and total leakage of K in the entire structure. The total amount of K that was leaked into the imbibition solution would depend upon a number of factors. As discussed in the text there is a substantial difference in the size of the two test samples. Regardless of the differences in cell wall width in certain tissues, the small carrot mericarps would have had a greater total surface area in contact with the soaking solution than would the large squash embryos and this could explain why such a difference was found. Also the presence of the pericarp and testa regions in the carrot mericarp would be a probable additional source of K which was not present in the squash samples.

B.G. Swanson: Do you have any idea what the smooth dome-like structures in Fig. 6 are?

Authors: These smooth dome-like structures are likely to be cell contents covered by plasmalemma.

B.G. Swanson: Did you check solubility of K phytate in ethanol solutions? Why not?

Authors: While the number of salts of phytic acid that are available in pure form from chemical companies have been increasing, the sodium salt is readily available and the potassium salt is not. The solubilities of the Na and K salts are similar in any case.

A. Beckett: Was there any evidence of a variation in cell wall wrinkling within seeds as the hydration front moved from the outside to the inside?

A. Beckett: Have the authors studied structural changes in the cell walls of these tissues when soaked for different time periods in water alone? If so, do the changes correspond to those illustrated here where samples have been imbibed in graded solvent solutions which would be expected to cause extraction and chemical change?

Authors: We have previously published (Lott and Kerr, 1986), illustrations of dry-to-wet transitions in a variety of specimens including squash cotyledons. Such transitions were found to occur relatively rapidly so that there were very few cells between a wet area that had smooth cell walls and a dry area that had highly wrinkled cell walls.In the area of transition varying degrees of cell wall wrinkling were observed. The big difficulty with this approach is that the water content of a cell in the transition area cannot be measured, so it was not possible to determine the degree of hydration needed to bring about a certain structural change. Increasing the time of soaking in water will produce a dry-to-wet transition progressively further into the tissue but will not drastically alter the rate at which that transition occurs.