Plant and Soil **184:** 131–141, 1996.

Water in aerenchyma spaces in roots. A fast diffusion path for solutes

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Received 10 January 1996. Accepted in revised form 8 July 1996

Key words: aerenchyma, cryo-microscopy, intercellular water, maize, root cortex, solute diffusion, *Zea mays*

Abstract

During a study of the diffusivity of sulphorhodamine G in the cortical apoplast of maize roots widely discrepant rates were found between different samples. In roots which had developed large aerenchyma spaces, the diffusion in some regions was very fast, indistinguishable from the rate in water. In other regions the rate was as much as 100 times slower. Examination of frozen intact roots with the cryo-scanning electron microscope showed the presence of liquid filling some of the aerenchyma spaces, while other spaces of the same root contained air. X-ray microanalysis of the liquid (for oxygen) showed that the liquid was water with few detectable ions. Similar liquid was present in small intercellular spaces within the spoke-like radial files of cells between the large spaces, or between remnants of collapsed cell walls at the edges of the large spaces. It is proposed that regions of roots with high diffusivity are those in which some of the aerenchyma spaces are filled with water. In seeking the origin of this liquid, the progress of aerenchyma formation could be followed in the frozen tissues. The first change observed in a group of contiguous cells was a loss of vacuolar solutes and of cell turgor. Next the walls broke apart and collapsed back onto the surrounding turgid cells leaving a volume of ion-poor liquid. The liquid was probably not that found in some aerenchyma spaces of the mature roots, because the final stage of space formation was a loss of the liquid, leaving an air filled cavity surrounded by a composite lining formed from the collapsed walls of the broken cells. It is likely that the liquid in the spaces of mature aerenchyma is exuded from the remaining living cortical cells at times when the root turgor is high. This would be consistent with several recent studies which have shown periodic exudation of water from the surface of turgid roots. The spasmodic occurrence of root cortex tissue with enhanced diffusivity would have important implications for the transport of nutrient ions across the root.

Abbreviations: CSEM – cryo-scanning electron microscope, EDX – energy dispersive X-ray microanalysis, SR-G – sulphorhodamine G.

Introduction

In many plants large intercellular spaces (aerenchyma) develop in the root cortex by cell death and disintegration or by differential division and separation (Esau, 1962). These spaces have long been known to be a major aeration pathway, their development stimulated by low oxygen partial pressure and by flooding (Armstrong, 1979; Justin and Armstrong, 1987; Laan et al., 1989; Smirnoff and Crawford, 1983). Low oxygen enhances aerenchyma formation in maize (Drew et al., 1979, 1981; Konings, 1982; McPherson, 1939). A recent study (Armstrong et al., 1994) has shown, by direct measurements with microelectrodes, that even in non-aerenchymatous roots of maize some oxygen moves from the shoots to the root cortex in the intercellular space system and it is assumed that much more oxygen would move through the large aerenchyma spaces.

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The aerenchyma in maize roots is formed by cell death. Some cells in the cortex lose turgor, appear irregular-concave, lose some contact with neighbouring cells, and finally collapse around the periphery of large spaces (Campbell and Drew, 1983; McPherson, 1939). The resultant final form of the cortex is an array of spoke-like radial files of cells which remain alive, separated by large, gas-filled spaces (Drew et al., 1980; McPherson, 1939). These spokes of cells are still capable of metabolic activity as is shown by their development of chlorophyll when roots are illuminated (Armstrong and Armstrong, 1994). Such aerenchyma in maize has mainly been described in roots grown under anaerobic or waterlogged conditions (see above), or under nutrient stress (Drew et al., 1989; Konings and Verschuren, 1980). It may also be formed in maize roots that are growing in well aerated, non-flooded soil, and especially in dry soils (M McCully and M Canny, unpublished), as has been described for various plants adapted to dry soil conditions (e.g. Beckel, 1956; North and Nobel, 1991).

The formation of large spaces in the cortex eliminates a large part of the cellular pathway into the root and is thus likely to affect nutrient uptake (Drew et al., 1980; Drew and Fourcy, 1986; Drew and Saker, 1986). However, uptake experiments with Rb^+, ^{32}PO and $^{36}Cl^-$ on root segments of maize with and without aerenchyma showed no reduction in uptake due to aerenchyma (Drew et al., 1980; Drew and Saker., 1986). This surprising result raises the question of whether aerenchyma provides a special mechanism which maintains ion movement across the cortex, and this question provides the basis of the present study.

The tracer studies of Drew et al. (1980) and Drew and Fourcy (1986) were by means of electron microprobe analyses, after lyophilization. They observed Rb^+ and Sr^{2+} were in cell wall remnants, and walls and cytoplasm of intact cells, but because the method used permits no proper distinction between symplastic and apoplastic transport, it is not known what pathway is important in nutrient movement across the cortex in aerenchymatous roots.

As Drew and Fourcy (1986) already suggested, if walls lining the aerenchyma spaces contain lipid or suberin-like material (McPherson, 1939) they are probably not very permeable. Furthermore, Canny and Huang (1994) showed, by measuring diffusion rates in the cortex of maize roots with a tracer dye, that the diffusivity of dye in the root cortical cell-wall apoplast is between $^1/_{40}$ to $^1/_{1000}$ of that in water. This suggests that a symplastic pathway across the cortex is much more likely than an apoplastic pathway.

However, as pointed out by Canny (1995), the cell wall pathway is not the only apoplastic pathway. Liquid-filled spaces provide another (rapid diffusion) path. Passioura (1988), reviewing water transport in and to roots, suggested that the permeability of the cortex apoplast would be high if intercellular spaces were lined with water. Moreover, Canny and Huang (1993) have reported liquid-filled small intercellular spaces in maize roots.

The present study began as an extension of the dye diffusion measurements in maize roots of Canny and Huang (1994). Our intention was to speed up the collection of data by using the technique of cutting hand sections of fresh tissue under paraffin oil, developed by O'Dowd and Canny (1993) to preserve the location of the diffusing dye, as has been done for other roots by Bayliss et al., (1996). Though it yields lower resolution images than the freeze-substitution method the paraffin method allows the collection of so much more data that the calculated means have lower variances (Bayliss et al., 1996). To our surprise, and in contrast to the measurements on maize of Canny and Huang (1994), some maize roots showed very fast diffusion of the dye, indistinguishable from the rate in water. This prompted an investigation of the distribution of water in the root tissue in parallel with the measurements of diffusion, and also, in looking for the source of the water, an investigation of the origins of the aerenchyma spaces. The cryo-scanning electron microscope (CSEM) was used to yield images of the water frozen in situ, and to preserve the fragile structures of the dying cortical cells (Huang et al., 1994). In addition the X-ray microanalytical capacity of the CSEM was used to analyse the elements in the root liquids. These analyses enabled us to identify which cells were alive by the high ion (K^+) content of their vacuoles (McCully, 1994), and also to identify material as water, by measuring its oxygen peak with the light-element detector (McCully, 1995a).

Our results suggest that there is water in some lacunae of the aerenchyma, and in some of the normal intercellular spaces in undamaged roots that are not waterlogged. This water may provide a radial path for rapid diffusion of ions across the cortex. Thus aerenchyma in these roots may function directly in plant water/ion relations as well as in their traditional role in aeration of the root tissues.

Material and methods

Plant material

For the study of diffusion into the root apoplast, plants of *Zea mays* L. cv. Seneca Chief were grown in a field plot at Agriculture Canada's Experimental Farm, Ottawa, in sandy loam soil, with natural rainfall during the summer of 1994. Nodal roots, tiers 2 to 4 (terminology of Hoppe et al., 1986) of plants 6 to 8 weeks old were carefully excavated by digging a trench beside the plant and gently scraping away the soil to locate each root. Roots were immediately wrapped in wet paper towels and transported to the laboratory and observed within half an hour.

For the study of the aerenchyma, seeds of the same variety were planted in potting soil in 30–cm pots in the greenhouse (23 °C) with supplemental high intensity light (18 h) and daily watering and weekly fertilizing. Nodal roots up to 60 cm long from plants 6 to 8 weeks old were used and samples were taken at 10 cm intervals, starting 5 cm behind the tip and extending back to 30 cm.

Dye diffusion

At least 25 preparations from 10 separate roots were examined. These did not include any sections that contained branch roots or branch primordia as these were discarded. Pieces of root about 4 cm long were gently washed clean and carefully blotted dry, and the cut ends sealed with petroleum jelly. Each piece was dipped into aqueous sulphorhodamine G (SR-G) (1 m*M*) for 30 s, quickly rinsed in water and blotted dry. Sections were immediately cut with a razor blade on a microscope slide under paraffin oil and mounted in the oil (O'Dowd and Canny, 1993) so the water soluble dye was prevented from spreading all over the place. SR-G was used because it does not penetrate cell membranes except at low pH, it is negatively charged so does not bind to cell walls, and with a molecular weight of 553 it is only a little larger than most organic molecules of the soil solution that can move in the apoplast (Canny and Huang, 1994). Sections were observed with epi-fluorescence optics (UVFL objectives on an Olympus Vanox microscope) with green excitation. The red fluorescence can be detected at low dye concentrations and there is little autofluorescence of the tissue (Canny and Huang, 1994). Photomicrographs were recorded on Kodak T-Max 100 35-mm film.

Aerenchyma

The potted plants were brought to the laboratory. The pots were cut open to allow gentle access to the roots. The selected roots were carefully separated from the soil and quickly hand-sectioned to check for the presence of aerenchyma. If aerenchyma was present root pieces (~ 10 cm long) were immediately frozen in liquid N_2 or in N_2 slush cooled to – 210 °C. A segment (2 mm) was cut from the middle of the root piece under liquid N_2 and quickly mounted on a stub with Tissue-Tek (Miles Inc., Elkhart, IN, USA) and returned to the liquid N_2.

For the following methods see also Huang et al. (1994). Some of the frozen samples were fractured in the pre-stage cryo-chamber (CS 1500, Oxford Instruments, Eynsham, Oxford, UK) cooled to - 180 °C under vacuum. Other frozen samples were first planed with a cryo-microtome (-80 °C) (CR200, Research and Manufacturing, Inc., Tuscon, AZ, USA). Then the planed sample was transferred under liquid N_2 to the cryochamber. Samples were very lightly etched by warming to -90 °C for a few seconds on the stage of the CSEM (JEOL 6400, JEOL Ltd., Tokyo, Japan).

Samples were coated with evaporated aluminium and observed in the microscope at -170 °C with an acceleration voltage of 7–10 kV for observation, and 15 kV for analysis. Micrographs were taken on 120 rollfilm (Kodak T-Max 100).

Energy-dispersive X-Ray (EDX) micro-analyses were made with a Link eXL, LZ-4, (Oxford Instruments, Eynsham, Oxford, UK) on planed samples, with the Be window to measure K^+ concentration in the cortical cells and large xylem elements, to check whether they were alive or dead (McCully, 1994). Analyses were made with the ultra-thin window on material in the spaces, to show that it was water by its large oxygen peak and small or absent carbon peak (Marshall and Patak, 1993; McCully, 1995a), and to check the concentration there of any ions.

Results

Roots

Although the plants were not waterlogged and did not appear to be nutrient deficient, roots of the plants grown in the field, as well as those grown in pots in the greenhouse, started to develop aerenchyma within the first 5

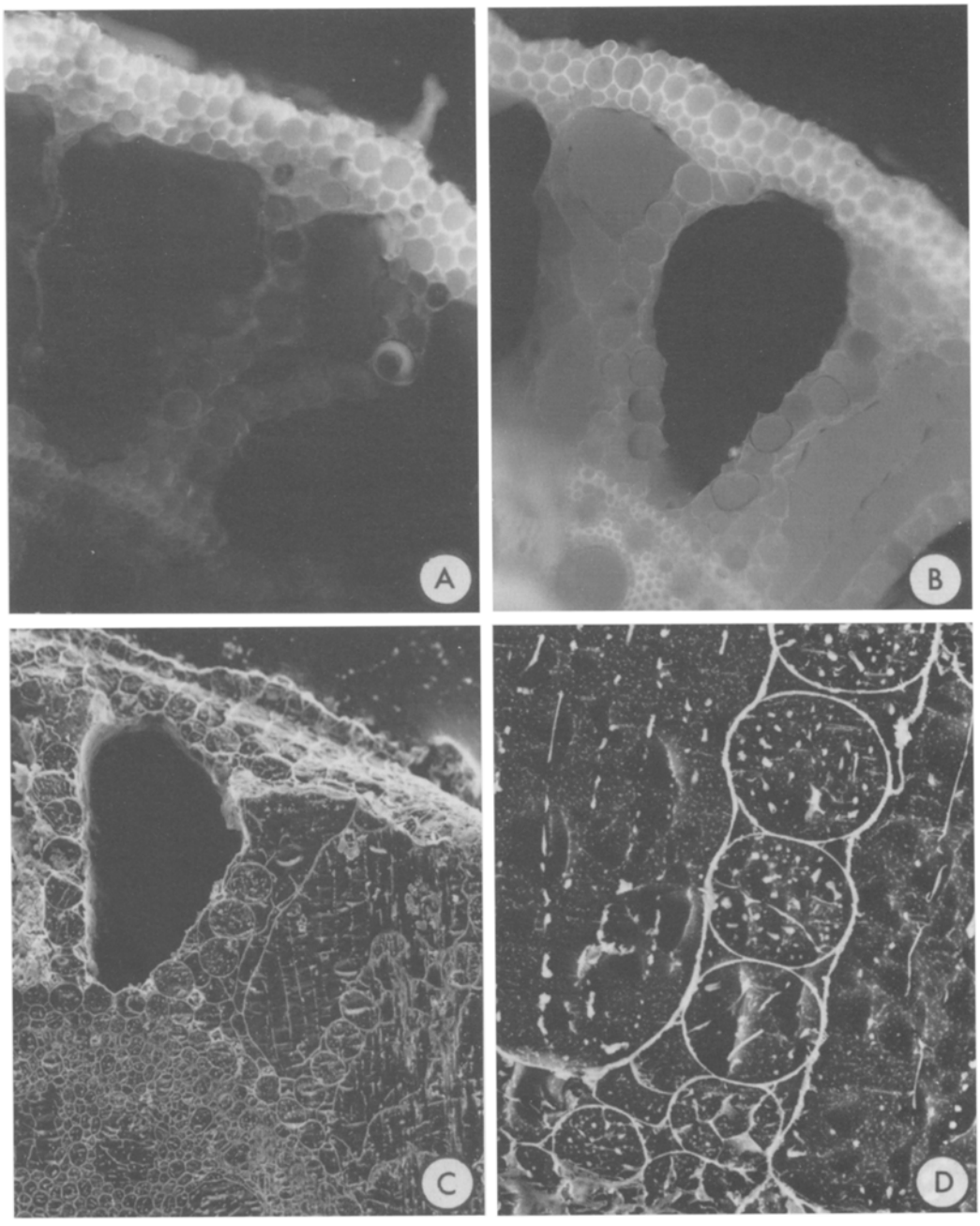

Figure 1. **A** and **B** Hand cut transverse sections of pieces of aerenchymatous maize roots 20–40 cm from the tip which had been dipped in SR–G. The sections were cut under paraffin oil and mounted in the same oil. In some roots the dye moved slowly and mostly did not traverse the aerenchyma (A, after 20 min dye diffusion). In other roots dye movement across the cortex was rapid via liquid-filled spaces in the aerenchyma (B, after 2 min dye diffusion). Fluorescence optics, green excitation. Exposure and development times for these micrographs were similar. × 100. **C** and **D**. Transverse faces of frozen maize roots 20–40 cm from the tip viewed with CSEM. Spokes of living cells traverse the cortex between the large aerenchyma spaces. Some spaces are filled with water while adjacent spaces are empty (C). The aerenchyma spaces are lined with wall–like material. This lining is seen particularly clearly in D where water-filled spaces are separated by living cells. Water also fills the small spaces between this lining and the walls of the living cells. C × 100, D × 330.

to 10 cm from the tip. All roots used looked white and healthy, and appeared to have been growing rapidly.

Dye diffusion

Sections observed under green excitation showed very little autoflorescence. Sections cut immediately after the root pieces had been dipped in dye showed one of two different patterns of dye diffusion. In preparations from some roots the dye advanced very slowly, as had been observed by Canny and Huang (1994) and by Bayliss et al. (1996). Little or no dye penetrated beyond the first few cell layers in the time observed (Figure 1A), and was not, or only faintly visible in the cell walls along the spokes across the aerenchyma spaces. In other roots the diffusion of the dye was very fast. The dye crossed the cortex and stele in a few seconds, at a rate indistinguishable from its diffusion in water (Figure 1B). In roots of this kind, some of the aerenchyma spaces which had not been opened by the sectioning seemed to be liquid-filled and showed bright fluorescence from the dye (Figure 1B). The reality of these liquid-filled spaces was checked by examining aerenchyma of frozen roots in the CSEM.

The extent to which diffusion was delayed by the hypodermis was determined by gently abrading the surface of the root with sandpaper to open the first two layers to the dye solution. This did not affect the results. Some roots showed dye diffusing rapidly throughout the cortex and others did not.

Water in the aerenchyma

In total, 19 roots of 6 plants were examined in the CSEM. In 10 roots some of the large aerenchyma spaces were liquid-filled as in Figure 1C. There were both full and empty large spaces along the root, even at any one level (Figure 1C). No liquid-filled spaces were seen in the other 9 roots. EDX micro-analyses showed that the liquid was water with hardly any detectable ions. Similar liquid was often observed in all the roots in the small intercellular spaces within the spokes of cells, or between remnants of collapsed cell walls (Figures 1C, and D) at the edges of the larger spaces. At the higher resolution of Figure 1D it could be seen that the liquid-filled aerenchyma space was lined by wall material. In seeking the source of the water in the spaces, the CSEM was used to follow the development of the spaces in the younger parts of the roots, and whether collapsed cells left liquid behind.

Aerenchyma development

In the younger regions of the roots, before any aerenchyma had developed, there were groups of cortical cells filled with liquid containing almost no detectable ions, in the midst of cells having a normal ion content in their vacuoles. In planed samples this difference could be seen readily, even without micro-analysis. The contents of ion-poor cells were always low in electron emissivity (appearing black) and the cells were irregularly concave in outline, and had lost turgor, but were still attached in places to their neighbours (Figure 2A). The contents of the ion-rich cells were streaked with lines of sequestered solutes which appeared white due to emitted secondary electrons. The cells were convex in outline, and turgid (Figure 2A). The ion-poor cells were dying and collapsing and were the first visible stage of the formation of an aerenchyma space. A next stage is seen in Figure 2B where the ion-poor cells were losing their water, and their cell walls were collapsed and torn by the pressure of the surrounding, turgid cells. This stage was found 5 to 15 cm from the tip.

The merging process that turns separate cells into composite spaces takes place in the liquid phase. Cell walls of the ion-poor cells (Figure 2A) broke down and the liquid merged into a liquid-filled space (Figures 3C, D and E). But while the early stages of space formation are in the liquid phase a continuum of liquid does not persist, and dry collapsing cells were seen quite early (Figure 2B). A further stage is seen in Figure 2C, where at the edge of a large space, a number of cells have collapsed onto a spoke of turgid cells, forming a compressed many-walled lining. Though the continuum of liquid was lost, the spaces did not become free of water. Water remained in some angles and between sheets of the collapsing mass of cells. In Figures 2D and 3A it can be seen that the composite lining can hold water drops in the intercellular-space apoplast between the lining and the turgid spoke cells. Sometimes this water was seen as crystals formed during the freezing (Figure 3B).

Thus though extracellular water appeared during space formation (Figures 3C–E), and remained even when the large aerenchyma spaces with their linings had become gas-filled (Figures 2C, D), this water which likely originated from the dying cells was probably not the source of the abundant water that sometimes filled the aerenchyma spaces in mature roots (Figures 1B, C and D), and which allowed such rapid diffusion of the dye (Figure 1B). The large spaces shown in Fig-

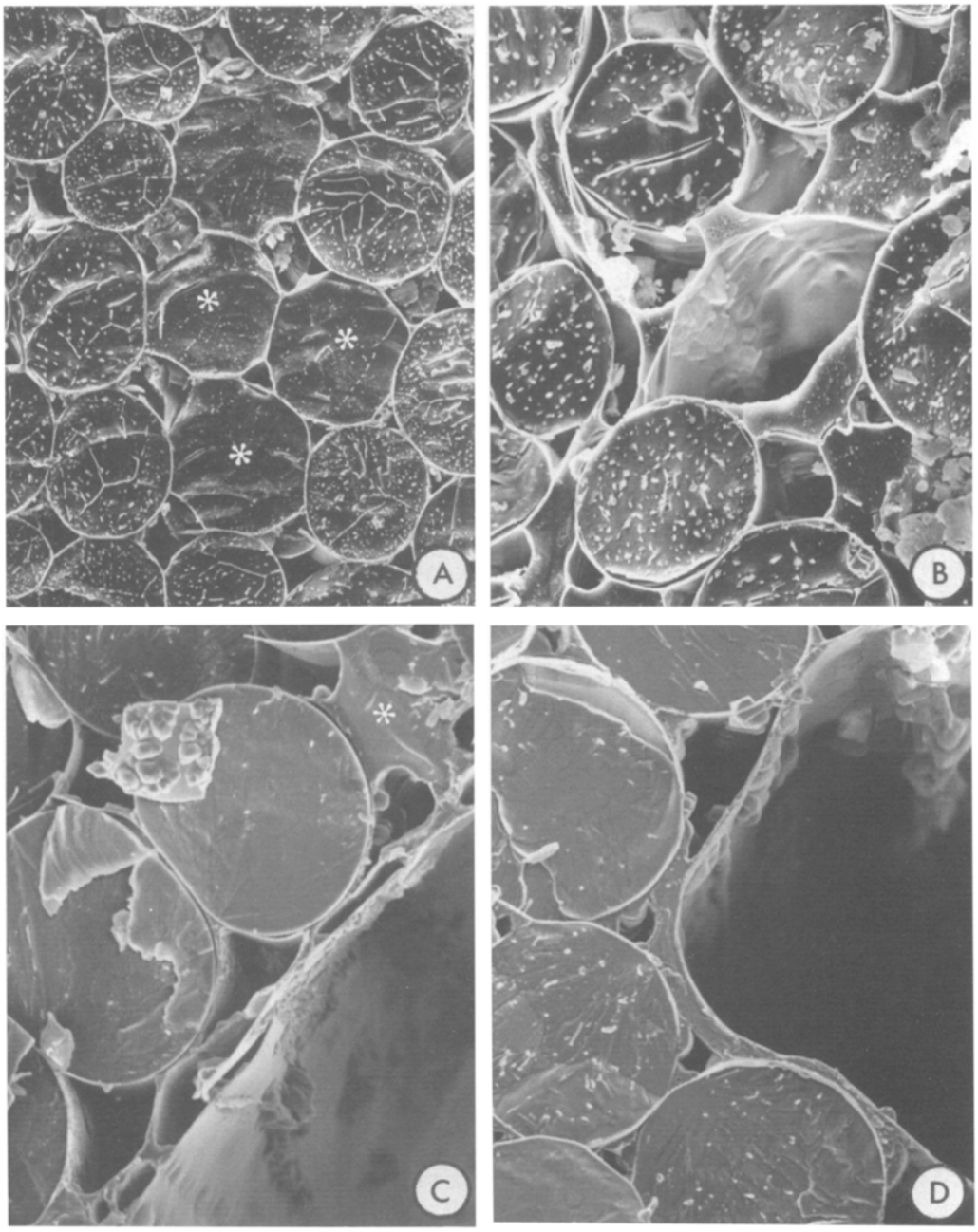

Figure 2. All are preparations as in Figure 1C and 1D; **A** and **B**, 5–15 cm from tip. A: An early stage in aerenchyma development. Cells lacking detectable ions (asterisks) are beginning to collapse and separate from each other forming small intercellular spaces which are mostly water-filled. Walls of the collapsing cells will form the lining around the large aerenchyma space which will form. B; A later stage where several cells have collapsed and lost their contents leaving small, empty or partly empty spaces enclosed by cell walls. Development of a large aerenchyma space has progressed further in Figures **C** and **D**. The walls of the collapsed cells now line the periphery of the large space. A water-filled collapsing cell (asterisk) is still present in C but the rest of the peripheral spaces are dry. A broken fragment of cell wall covers part of one of the cells in C. In D there is a water layer between the aerenchyma lining and the living spoke cells. A × 330; B × 400; C × 500; D × 525.

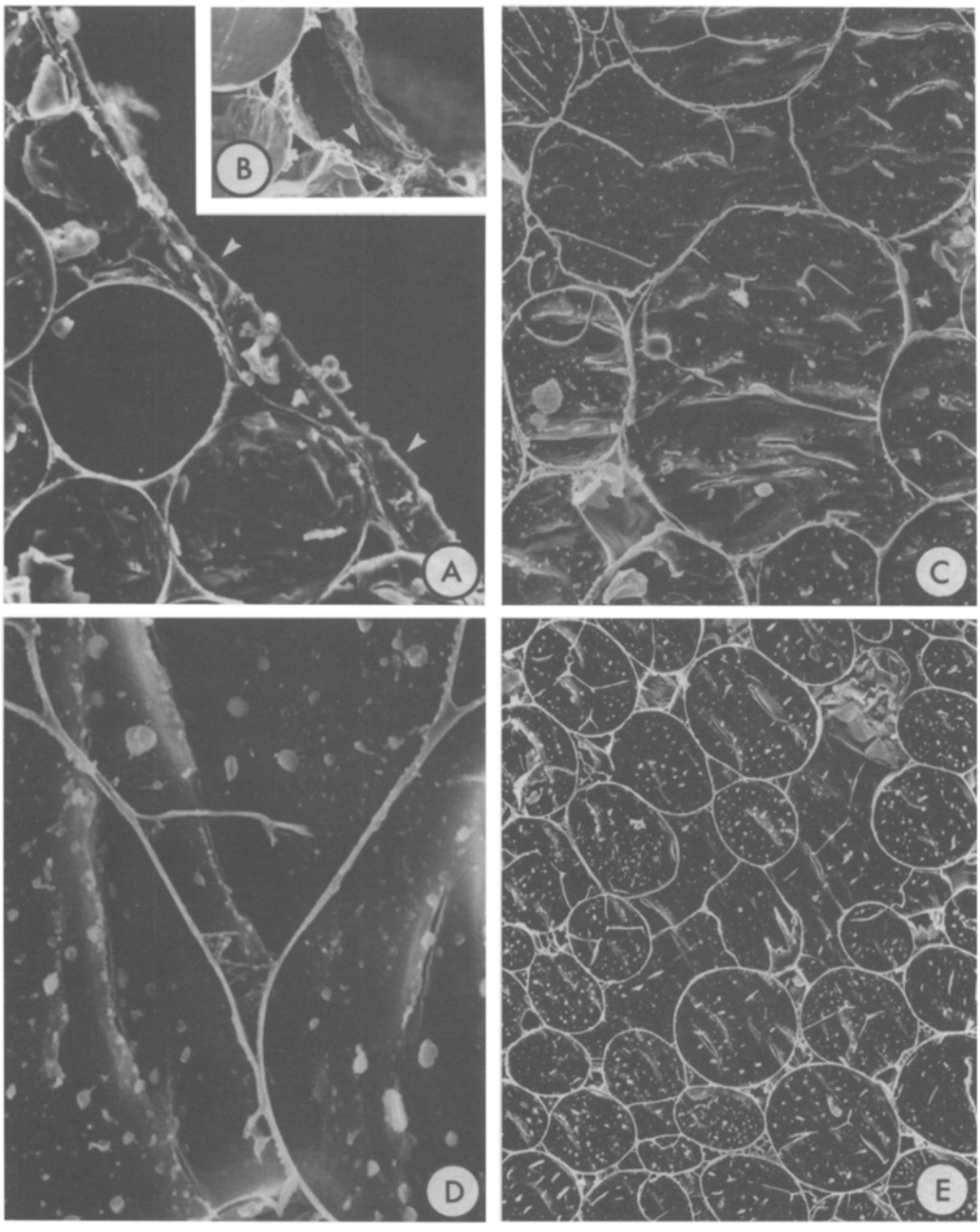

Figure 3. All preparations are as in Figures 1C and D and Figure 2A to 2E; **C, D** and **E**, 5–15 cm from tip. In **A.** a water layer (arrows) lines the large aerenchyma space, in **B** a thin water layer within a small space between cell wall remnants at the periphery of the aerenchyma has formed large crystals during freezing (arrows). Figures C, D and E show roots in which cell walls have broken but have not formed a lining around the large resulting space. The collapsing cells and the surrounding space are water-filled. A, B × 400; C × 350; D × 950; E, × 250.

ures 1A-D, 2C, D and 3A were found 20 to 40 cm from the tip.

Immaturity of the xylem

Analyses with EDX showed that the K^+ concentration in the late metaxylem vessels was 99 ± 45 m*M*, evidence that they were not yet mature and conducting (McCully et al., 1987; McCully, 1995a).

Discussion

Diffusivity in the root cortex apoplast

The apoplast of the roots must be considered as a complex of different apoplastic spaces (Canny, 1995). In these maize-root cortexes the apoplast comprises at least three distinct spaces: the cell-wall apoplast, gas-filled intercellular space and liquid-filled intercellular space. As argued also in that review (Canny, 1995), cell walls are spaces where diffusivity of solutes is low.

If there is no liquid in these intercellular spaces, diffusion of the SR-G across the root cortex would be slow because the dye is restricted to the wall apoplast. But when the spaces contain liquid, or even when the liquid adheres as a layer to the cell walls on the borders of the spaces, the diffusion of the dye would be at the same rate as in water, increasing by a factor of 40 to 1000 times. Thus, the presence or absence of liquid in or around large aerenchyma spaces could explain the slow and fast dye diffusion results (Figures 1A, B) in the present study.

We have been puzzled by the passage of the dye through the hypodermal apoplast. The hypodermis, where it has developed Casparian bands, has been shown in many situations to be a barrier to diffusion in the cell-wall apoplast (see Peterson, 1988). We did not specifically check if the root segments used in the present study had formed Casparian bands but similar soil-grown primary roots of the same maize variety had mature Casparian bands within less mature root regions than most of those we were observing (Watt et al., 1996). These latter authors also found diffusion of SR-G across the hypodermal apoplast. Canny and Huang (1994) found that the apoplast of some regions of maize root hypodermis was no less permeable than other cell walls. If small cracks in the hypodermis in the roots used in the present study did allow passage of the dye (but see below), then it would be seen to spread out from these at regions, rather than moving uniformly into the cortex as was observed (Figure 1B).

Liquid-filled spaces

We do not think that the water-filled spaces were artifacts caused by damage during collection and processing of the roots. Excavations were very gentle and the roots were never exposed to any mechanical stress that would cause them to crack. Furthermore, if cracks were present we would not expect water to have entered and flooded the spaces because the surrounding soil was never waterlogged. Indeed, water could not enter through small holes and replace air in the spaces because a large pressure gradient is required to push a water meniscus through a small hole (see Lewis, 1988).

The best argument against water in the aerenchyma spaces being a preparative artifact is its detection in material prepared by a completely different method (Figures 1B, C), one using unfrozen tissue. Also, large redistributions of water during preparation for the cryo-SEM are unlikely as discussed by Canny and Huang (1993) and Huang et al. (1994). Liquid in the intercellular spaces was seen in maize roots Canny and Huang, 1993), and in barley roots by Stelzer et al. (1988). These authors did not consider the liquid spaces as possible paths for diffusion into the root.

That some intercellular spaces, both large and small, contained water even during the day when roots might be expected to have a negative water potential, is consistent with the discovery that late metaxylem vessels remain immature and non-conducting 20 or 30 cm from the root tip (McCully 1994; McCully and Canny, 1988; St Aubin et al., 1986). Because this part of the root is not connected to the transpiration stream by the low-resistance conduits of the late metaxylem vessels, its relative water content remains high and is not significantly affected by transpiration (McCully, 1995b; Wang et al., 1991). Our EDX measurements of high K in the vessels indicate that the vessel elements were alive and non-conducting.

The source of the water in some of the large spaces of about 50% of the roots examined is unclear, but its presence is in accord with the new view of roots developing from a number of recent reports (see McCully, 1995a). Some roots some of the time are much wetter than had been generally believed. Schwenke and Wagner (1992) presented evidence for an explanation of root pressure that involved cyclic changes in cortical cell permeability, and the excretion of liquid both to the xylem and to the exterior of the root. It would seem inevitable that such excretions of liquid would produce also liquid-filled intercellular spaces. The phenomenon of hydraulic lift, movement of water from deep soil to shallow soil through the root system, has been well documented (Blum and Johnson, 1992; Caldwell and Richards, 1989; Dawson, 1993). The mechanism of this movement is not known, but active secretion by the upper roots cannot be ruled out. Indeed, it has become much more likely since the demonstration of cyclic diurnal exudation of water into the soil (Watt et al.,

1997), and the appearance of water drops under pressure at the root surface (McCully, 1995a). If there is guttation outwards to the soil and upwards to the shoot, internal guttation into cortical spaces seems possible.

Aerenchyma development

In a transmission electron microscopic study of early events in aerenchyma formation of maize roots, collapse of cells was followed by thinning of the cell walls (Campbell and Drew, 1983). In rice, first the middle lamellae degenerated, and then followed collapse of cells, and cell wall disintegration (Webb and Jackson, 1986). The breaking of cell walls found by us (Figure 3C, D and E) might suggest cell wall deterioration, and the parting of cells (Figure 2B) suggest the disintegration of the middle lamellae. Webb and Jackson (1986) propose that the processes of aerenchyma formation differ in rice and maize in the order in which these events happen. From our data the order of the processes cannot be determined, but we have shown that in maize, cell wall and middle lamellae deterioration both occur.

We have seen an earlier stage of the process, which was also recorded by Huang et al. (1994). Aerenchyma formation started with loss of vacuolar solutes (Figure 2A). If the solutes move to neighbouring cells, perhaps these cells are controlling the cavity formation. On the other hand, the dying cells with ion-poor vacuoles were seen in clusters (Figure 2A), and the space enlarged as other cells collapsed (Figure 2C). From this point of view it is possible that the dying cells release a message which initiates the process of cell death in their neighbours. From the regular structures finally produced, it is clear that aerenchyma formation is a controlled process.

The compressed walls of the collapsed cells form a continuous lining of the aerenchyma spaces, so not all the collapsing cells split apart. Cell walls which remain attached may have undergone some alteration, or, their middle lamellae may be altered unilaterally. There are different forms of lysigenous aerenchyma: lysigenous lacunae with diaphragms between them arranged radially and containing mainly collapsed cells (for example in maize and rice), and lysigenous lacunae separated by radially arranged diaphragms of intact cells, with tangentially arranged diaphragms of collapsed cells stretched between them forming a spider-web structure as in some Cyperaceae, e.g. *Eriophorum* spp. (Armstrong, 1979; Smirnoff and Crawford, 1983). The regularity of these patterns excludes the possibility that the spaces are made only by fortuitous cell death.

Implications for the plant of some water-filled aerenchyma spaces

Aerenchyma has long been considered as a pathway for oxygen diffusion into the root, important for survival in waterlogged situations (see Introduction). The presence of water-filled aerenchyma spaces would seem, to contradict this function. The observations that the roots used in our research were not translucent, but white, and that spaces were not always seen to be filled, suggest that water in the aerenchyma is not a continuum, but interrupted by volumes of gas. Also, as with the water secretions observed by McCully (1995a), the water content of the spaces may vary with time. In contrast to our findings, and in agreement with the traditional view, when measurements of porosity with a pyonometer were compared with calculations from microscopic sections (Van Noordwijk and Brouwer, 1989), there was little difference between the volume occupied by air and the volume of aerenchyma space in maize roots. In that experiment, if the roots had water in the aerenchyma, then its total volume would have been a small proportion of the aerenchyma space. However, in a later paper using the same methods they record in a range of plants that the spaces measured by light microscopy of transverse root sections "showed, on average, 60% more intercellular spaces in the root cortex than the measurements of gas-filled porosity, probably because some gaps and spaces were not gas-filled." (Van Noordwijk and Brouwer, 1993).

Aerenchyma is formed also in dry conditions by plants adapted to flooding (Justin and Armstrong, 1987). Furthermore aerenchyma development is enhanced under nitrogen- (Drew et al., 1989; Konings and Verschuren, 1980) and phosphate- (Drew et al., 1989) deficiency. Plants adapted to very dry conditions also develop aerenchyma in their roots (Beckel, 1956; North and Nobel, 1991). A water phase in the root cortex apoplast could provide a fast pathway for solute diffusion into the root, and aerenchyma formation, by extending the possible volume of the liquid phase, could enhance nutrient transport, especially in dry conditions.

The presence of water in the cortical apoplast could explain some of the discrepancies mentioned in the Introduction. As explained there, uptakes of labelled Rb^+, ^{32}P and $^{36}Cl^-$ were the same in non-aerenchymatous and aerenchymatous roots, despite the

absence of much of the pathway in the latter (Drew et al., 1980; Drew and Saker, 1986). This discrepancy was attributed to greater fluxes through the remaining spokes of cells in aerenchymatous roots. But a fast diffusion pathway through water in spaces and water adhering to cell walls, is an alternative explanation. Because the cations were found in cell walls and wall fragments, their movement was thought to be through the cell-wall apoplast (Drew et al., 1980; Drew and Fourcy, 1986). But the lyophilization process used would concentrate and deposit solutes on these walls, mimicking the effect of wall transport. That transport is mainly not in the walls surrounding aerenchymatous spaces was suggested by Drew and Fourcy (1986) on the grounds that they contain lipid or suberin (McPherson, 1939). Canny (1995) argues that solute transport in the plane of all cell walls is by slow diffusion. The wide range of values of diffusivity found in maize roots by Canny and Huang (1994) could well be due to differences in water content of the intercellular-space apoplast. These authors considered only small spaces, not aerenchyma.

Acknowledgements

We thank Hank Datema and his staff for growing the greenhouse plants, Agriculture Canada for the use of the field plot, Cheng Huang for overseeing the cryo-analytical electron microscopy, Wayne England for making the plates, and the Natural Sciences and Engineering Research Council of Canada for operating grants to MEM and MJC.

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Section editor: H Lambers