grown plants, we obtained similar results in a small field experiment at Davis.

In a recent field study with citrus trees on Troyer citrange rootstock in saline soils of the Coachella Valley, we found a strong positive correlation between the severity of Phytophthora root rot and the level of salinity. Although greenhouse studies indicated that salinity stress could alter rootstock susceptibility to *Phytophthora,* they also revealed that root growth nearly ceased under chronic salinity stress. Thus, rootstocks exposed for long periods to salinity levels of 3 to 4 dS/m (1,900 to 2,500 mg/L) were unable to replace roots decayed by *Phytophthora.*

While all the *Phytophthora* isolates we have worked with so far are fairly tolerant of salinity, an isolate of *P. parasitica* that we recovered from citrus soils of the Coachella Valley had the greatest tolerance. It survived and reproduced in soil at salinity levels equal to or greater than that of full-strength sea water. Thus, we expect that many *Phytophthora* spp. remain active in soils at salinity levels that would severely stress most crop plants. This clearly is the case in the Coachella Valley and probably accounts for the severity of Phytophthora root rot in some citrus groves. These relationships between salinity and root diseases may occur in other crops elsewhere, but they may be overlooked, or the symptoms of chlorosis, wilt, and death of plants simply confused with direct salt injury.

It is still unknown precisely how environmental stresses and root pathogens interact in disease. However, the knowledge that these interactions occur, and information on the levels of stress that can trigger changes in plant susceptibility, should help researchers and growers devise cultural practices to minimize losses, and could help breeders detect and deploy genes providing more stable disease resistance.

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Effects of salt on cell membranes of germinating seeds

Richard D. Bliss **Kathryn A. Platt-Aloia** **William W. Thomson**

Salt stress inhibits growth throughout the plant life cycle, but seed germination is generally the most sensitive stage. Salt stress mimics water stress in many ways, leading to the suggestion that the principal effect of salt is osmotic. However, since some salts are more inhibitory than others, specific toxic effects must also be involved. Although sodium chloride is among the less toxic salts, it is one of the most common and thus one of the most troublesome to agriculture.

The mechanisms by which salt inhibits seed germination are not known, but there is good reason to suspect that cell membranes are the sites for primary or secondary salt effects. Sodium chloride interferes with a wide variety of membrane functions, including permeability, transport of both organic and inorganic solutes, and secretion. Salt sometimes causes structural alterations. Salt stress induces changes in membrane lipid composition in some plants and also causes release of membrane proteins in root cells. These structural modifications may be especially important, since many salt-tolerant species achieve their resistance by isolating salts from sensitive cellular processes through membrane compartmentation.

The interaction of salts with cell membranes during germination is complicated by the dramatic changes occurring in the seed during this time. The transition from a dry to hydrated state as the seed absorbs water during imbibition has a potentially profound effect on seed membranes, since membrane structure depends heavily on the interaction of the membrane molecules with water. (The term "imbibition" is applied to the rapid uptake of water by dry seeds when they are placed in a moist environment.) Some membrane changes probably occur during imbibition, as the amount of water around the cell membranes increases. However, the exact nature of these changes is not certain, since small differences in membrane molecular composition profoundly affect the membrane's response to changes in hydration. The presence of salt during imbibition is quite likely to influence these changes in membrane structure.

In our studies, we have used the electron microscope to examine changes in the seed cell membranes during the early phases of germination. The technique of freeze-fracture electron microscopy was used, because it is especially suitable for study of membrane structure. In this method, frozen tissue is broken open in a vacuum chamber, and an extremely thin layer of platinum and carbon is deposited on the broken surface. The tissue is then dissolved away, leaving the thin platinum-carbon surface replica, which is examined in the microscope. Since the fracturing process frequently breaks open the frozen membranes, the platinum-carbon replica shows the interior structure of these membranes (fig. 1).

Small particles are usually visible on the fractured surface. They are membrane protein molecules that were buried in the membrane interior before it was broken open. The number of these protein particles per unit area is characteristic of the kind of membrane being examined. Changes in either the density or size of these particles reflect developmental changes occurring in the membrane, which may be the result of normal physiological processes or of environmental influences.

We have used this technique to study membranes of primary root (radicle) cells in cowpeas germinated in both water and salt solutions. The membrane appears normal in micrographs of dry seeds, and there is no evidence that it is modified (fig. 2). However, the number of protein particles is unusually high, and some of them appear to be clumped together as multiparticle aggregates.

When seeds are imbibed in water, the appearance of the membrane changes somewhat. In seeds that were imbibed in water for 17 hours, the number of particles per unit area of membrane declined to about half the density in dry membranes, apparently because of membrane expansion during imbibition (fig. 1). In other words, the total number of parti-

cles in the membrane may not have changed, but these particles were spread out over a larger area as the membrane expanded to accommodate the swelling of the cells. The average particle size increased slightly, probably because of protein interaction with water. The particles appeared to be more uniformly distributed and much less clumped than in the dry seed.

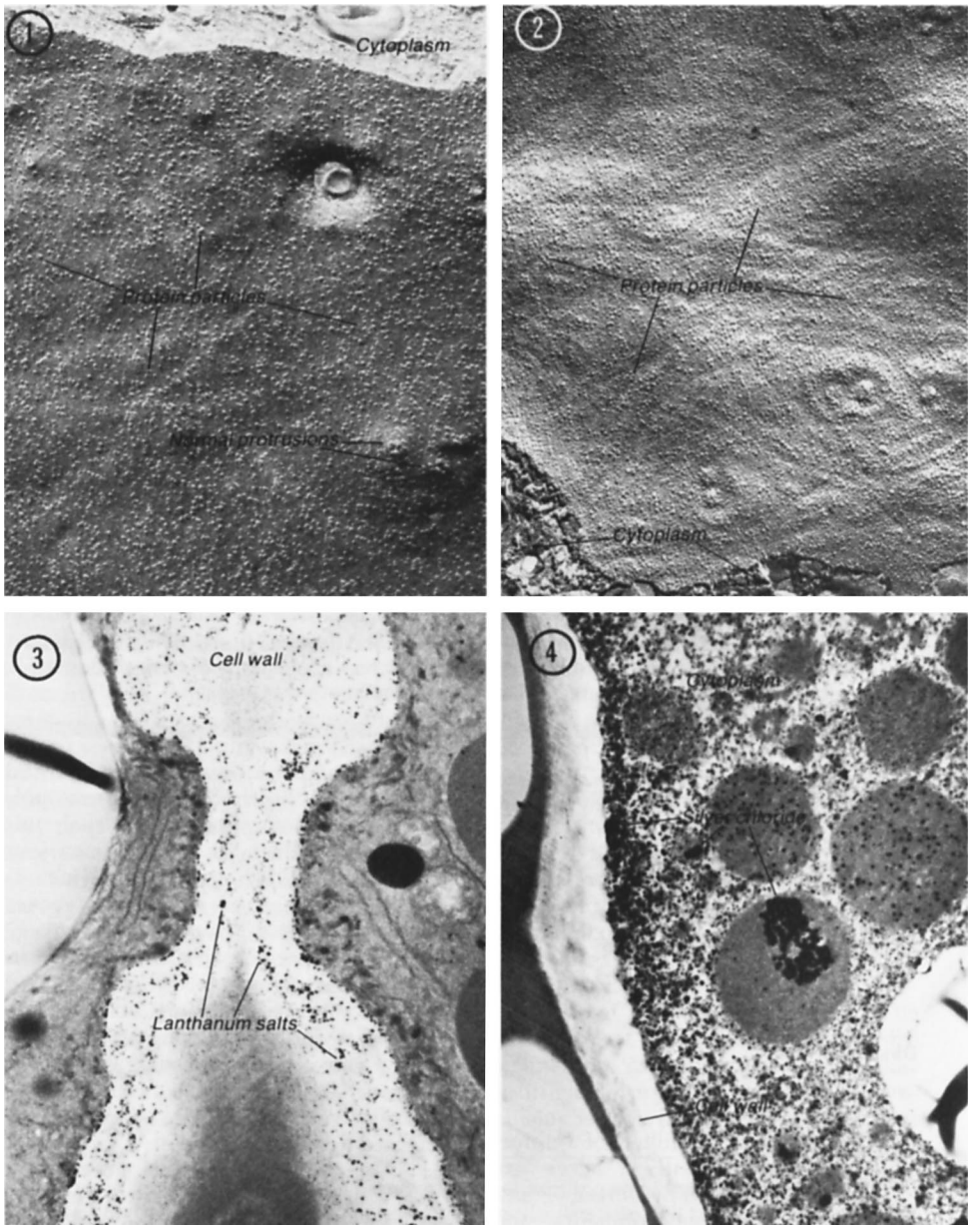
The cell membrane of seeds imbibed in 0.2 M (12,000 mg/L) salt solution expanded about as much as that of water-imbibed seeds. However, there were many more particles per unit area than in the water-imbibed seed and more than would be expected if the particles in the dry membrane were uniformly spread out in the salt-imbibed membrane. Evidently, salt modifies a process that occurs in water-imbibed seeds. The salt appears to have caused the number of membrane protein particles to increase, either by insertion of new particles into the membrane or by disaggregation of existing particles. The average particle size, however, was only about 7 percent smaller than that of particles in the membranes of water-imbibed tissue. The unusually high particle density in the salt-imbibed tissue apparently was not due solely to particle disaggregation, or the particles would have been even smaller.

Another way of examining membrane function is to treat imbibing seeds with various salts and to ascertain, using microscopy methods, whether the salt can permeate into the cytoplasm. Lanthanum nitrate is a useful salt for this purpose, since the deposits can be seen easily in the electron microscope. Our experiments show that lanthanum cannot penetrate the cell membrane, even when it is present at the beginning of imbibition. Lanthanum can accumulate in the cell wall, but not in the cytoplasm (fig. 3). The dry membrane must not be completely porous, or the lanthanum would penetrate to the cytoplasm.

The smaller ions of more common environmental salts may not behave the same as lanthanum at the membrane, however. Usually, these ions are much more difficult to visualize in the microscope. This problem can be overcome in some cases by treating the salt-imbibed tissue with a chemical that reacts with the salt, forming a compound that can be seen and identified in the microscope. For example, silver ions react with the chloride ions of sodium chloride to form insoluble silver chloride crystals, which are readily visualized in the electron microscope.

With this approach, we have demonstrated that chloride can penetrate into the cytoplasm when seeds are soaked in sodium chloride solution. Silver chloride crystals were visible in the cytoplasm of a cotyledon cell (fig. 4). Thus, it is possible that salt could exert some toxic effect within the cell in addition to its effect on membranes during imbibition.

In summary, our experiments demonstrate that salt does affect the cell membrane of imbibing seeds and that some, but not all, salts can penetrate the membrane. Thus, there may be intracellular, as well as membrane, responses to the presence of salt. It seems likely to us that the inhibitory effect of salt on seed germination will ultimately be traceable to the effect of salt on a number of cellular processes.



Freeze-fracture electron micrograph of cell membrane in primary root of seed imbibed in water (1) shows changes in membrane proteins (small bumps). Protein particles in cell of dry seed (2) are smaller and more numerous. (3) Thin-section electron micrograph of cotyledon cells from cowpea seed imbibed in lanthanum nitrate solution. Lanthanum appears as black particles in cell wall (light area) but not in cytoplasm, indicating that lanthanum cannot cross membrane into cell interior. In cell tissue from a seed imbibed in sodium chloride solution and then treated with silver nitrate (4), resultant silver chloride precipitate (black particles) is visible in the cytoplasm, but is almost absent from the cell wall (light area), indicating that salt penetrated to the cell interior.

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