Stress Interactions—Back to the Future

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Interrelations between stresses have long been known (Levitt, 1980). For instance, an adaptation to one stress, such as freezing temperatures, may carry with it an adaptation to another stress, such as water stress. By “going back” to such interrelations, we have since discovered (Levitt, 1985) a new kind— an interaction between two *adaptations to the same stress*, one adaptation (a stress avoid-

ance) interacting with and controlling the development of another adaptation to the same stress (stress tolerance). Thus, by going back to earlier work, we have found a new path to future research. In this sense, I am proposing going “back to the future”.

Any such suggestions to go back to earlier work might raise strong objections. After all, the “cutting edge” of science is where most of the exciting advances are being made, where research is most heavily funded, and where manuscripts are most likely to be

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accepted for publication. Why then should we go back? The answer is that in our desire to progress as rapidly as possible, we have concentrated our efforts on the forefront of science, where all the new techniques are. To do this, we have left behind unsolved problems that are basic to those currently being investigated. We must now return to these neglected stress problems. The following are two examples of such problems:

1) The locus of the stress-induced injury to the cell. According to current dogma, the locus is the membrane system. This conclusion is logical and is based on both theory and experimental evidence. Nevertheless, there may conceivably be some other loci, at least in some cases. There are four essential components of a living cell (Palade, 1963)—the codes, the ribosomes, the membranes, and a source of energy. Disruption of any one of these four components must injure or kill the cell. By analogy with Koch's principles, any attempt to explain the nature of the stress injury must be preceded by a determination of the locus of injury.

2) A second question, discussed decades ago, is the moment of injury. Does this injury occur only during exposure to the stress, or does it continue or even begin after release from the stress? Recent investigations (see Palta's contribution in this colloquium) have shown that the injury may indeed increase, or, conversely, may be partially or fully repaired after release from the stress.

Thus, a return to earlier results may indeed suggest new paths of research for the future that may become the new cutting edge of research in the field of environmental stress. This return has been the basis of my recent investigations. I must emphasize that I am not advocating going back to the invention of the wheel. The investigator must quickly differentiate between the long-known and the related unknown.

The following is an attempt to go back to the future to achieve a better understanding of the interactions between adaptations to drought-induced water stress. The materials and methods have been described elsewhere (Levitt, 1983, 1985).

INTERACTIONS BETWEEN AVOIDANCE AND TOLERANCE

My first attempt was to return to the old, well-established concept of two kinds of resistance—avoidance and tolerance. The first question raised was: Does the plant have to develop only one or the other or are both required? For instance, it was suggested long ago that the shedding of leaves from plants wilted by drought-induced water stress is purely a stress-and-strain avoidance, due to the conservation of water by the remainder of the plant (and the soil); the plants, therefore, have no need for tolerance.

Newer results (Levitt, 1985) demonstrate that this explanation is too simple. By means of very simple measurements, we were soon able to demonstrate that, just as in the case of low temperature stress, a mild, drought-induced water stress leads to the acclimation of cabbage leaves to drought. Furthermore, this acclimation involved an increase in both avoidance and tolerance. The tolerance increased from a survival by non-acclimated leaves of a dehydration down to a 30% to 40% relative water content (RWC), to survival after acclimation down to 13% RWC, a loss by dehydration of 87% of the leaf's water.

There was nothing really new to this finding, nor to the fact that it resembled the acclimation of the same cabbage leaves to freeze-induced dehydration at low temperatures. However, it was perhaps unrecognized previously that in the case of the drought-induced water stress, acclimation by avoidance was also very pronounced, at least in the case of cabbage. In fact, it was so pronounced, due to increased cutinization and wax deposit on the leaf surface, that no appreciable uptake of water occurred (and, therefore, also no recovery of turgor) when a wilted, excised leaf was submerged in water for 24 hr. In contrast, the full original water content and turgor were regained in minutes or an hour, if the petiole was excised when submerged.

Further investigations revealed a previously unknown interaction between avoidance and tolerance (Gaff, 1981; Levitt, 1985). Avoidance of drought-induced water stress is essential for the development of tolerance, for in the absence of adequate avoidance,

dehydration occurs too rapidly during acclimation for the development of tolerance. Even resurrection plants (e.g., *Borya*, *Xerophyta*, and *Myrothamnus*) lose their ability to "resurrect" if dehydrated too rapidly (Gaff, 1981).

At least some of the acclimation depended on the age, both actual and relative, of the leaf. Thus, a six-leaf seedling, when wilted by withholding water for a long enough period (e.g., 1 to 2 weeks or more), lost its two lowest and oldest leaves by chlorosis, followed by browning, shriveling, and shedding. The leaves above these two retained their normal green color, and even their turgor, for a much longer time. The older leaves appeared to commit suicide to save the younger leaves.

When the turgid, acclimated, upper leaves were excised and allowed to dehydrate on the shelf of the growth chamber, their thickness and area decreased markedly. They may dehydrate to lower than 13% RWC without any loss of their dark green color and without becoming appreciably limp or wrinkled. Of course, the very low water content prevents limpness. Nevertheless, the leaves appear so normal that they suggest a search for a medium in which they might recover full turgor, like resurrection plants. None has so far been found.

TURGUR INTERRELATIONS BETWEEN STRESSES

Another interrelation occurred between stresses. Turgor is a factor in acclimation to freezing temperatures (Cox and Levitt, 1976). In fact, no acclimation occurred if cabbage leaves were kept fully turgid at normally acclimating temperatures. This effect is similar to drought-induced acclimation to water stress, which occurs only if water is withheld long enough for some loss of turgor to occur. Now another adaptation has been found (Levitt, 1986) between turgor and acclimation to drought-induced water stress—turgor adjustment (TA). By this process, the flaccid, excised leaves were able to regain their turgor in the absence of any water uptake, even, in fact, in spite of a small water loss. This adaptation has been explained by a wall adjustment (WA)—an elastic tightening of the cell wall during acclimation, leading to a decrease in the cell volume enclosed by the wall at zero turgor. In practice, zero turgor is achieved at incipient plasmolysis. This finding points to a need for a thorough investigation by electron microscopy of changes in the cell wall during acclimation to drought. Again, this adaptation occurred, not in the older, least-acclimated leaves, but in younger, upper, most-acclimated leaves, though not in the youngest and smallest (only partially expanded) leaf.

Although the above concept of TA has not been rigorously tested, one prediction has been validated. If the TA is due to a "tightening" of the cell wall, leading to a reduced wall-enclosed volume at incipient plasmolysis, the osmotic potential must decrease (an increased negative value) by an amount that can easily be calculated from the above values. In point of fact, the osmotic potential was equal to the predicted (calculated) value (Table 1).

This result means that the change in osmotic potential during TA is due solely to "passive", osmotic loss of water by reverse osmosis and no active (photosynthetic) accumulation of solutes occurred. This relationship is to be expected, of course, since the stomata must have been closed in the wilted leaf, preventing the uptake of CO₂ and, therefore, the accumulation of photosynthate. This reverse osmosis applies only to excised leaves; earlier tests showed that attached leaves do accumulate solutes when acclimated.

Other predictions can also be tested. a) Does, for instance, the

Table 1. Relation of measured to calculated osmotic pressure (OP) after turgor adjustment (TA) of excised and wilted cabbage leaves kept at constant dehydration level [measured by relative water content (RWC)]. Each value an average to three to six leaves. Calculated OP = 100/RWC at TA × original OP.

Plant age (days)	Time for TA (hr)	RWC (%)	OP (MPa)	
			Measured	Calculated
40	73–121	73–81	1.36	1.30
55	73	73–75	1.54	1.54
58–63	72–96	66–81	1.54	1.54

suicidal destruction of the older leaves lead to acclimation of the younger ones by a source and sink relation, the older leaves transporting substances to the younger leaves? b) Are the two turgor effects due to abscisic acid? Thus, we have definitely gone back to the future, and these future paths are already before us.

Although the above interrelations and interactions are theoretical, they point to some possible, practical implications. In the future, for instance, we maybe able to calculate how much water to supply to a wilted crop, and at what rate, to permit adequate acclimation.

INTERACTIONS BETWEEN MEMBRANES AND CYTOPLASM

I stated above that there are four essential components of the living cell. Yet all living plant cells also have a fifth component — the cytoplasmic matrix, in which all the other components, except the external membranes, are embedded, and even these membranes are in direct contact with the matrix. Therefore, when the cell is exposed to environmental stress, interactions between the membranes and the cytoplasm are highly likely.

Half a century ago (see Levitt, 1980), it was shown that acclimation to certain environmental stresses (freezing temperature, drought-induced water stress) produces a pronounced increase in cell permeability to thiourea and urea. This result has been reconfirmed unequivocally (unpublished data). Yet all attempts to find a similar change in permeability to water have failed.

This apparent discrepancy may be explained by an old controversy that was aired 40 years ago at scientific meetings but not published. In this controversy, the Austrian plant physiologist Karl Höfler stood alone against even his own students in maintaining that plant cell permeability is a function, not of the membranes alone, but of the whole cytoplasmic layer, including the outer plasma membrane, the inner tonoplast, and the cytoplasmic matrix between them.

Höfler's concept may be understood if we go back to the old description of cytoplasm as a highly hydrophilic colloidal gel. According to this concept, such a gel would consist of a loosely bonded network of only 5% to 10% colloidal particles, the spaces between them being filled with free aqueous solution. The rate of diffusion of small molecules (both solute and aqueous solvent) through the cytoplasm would not be decreased by more than the above 5% to 10%, in comparison with diffusion through pure water. The limiting

barrier to diffusion would therefore be the largely apolar plasma membranes.

Due to its well-established higher cell sap concentration, the acclimated cell is less-dehydrated osmotically at any one dehydrating stress than is the nonacclimated cell. If, for instance, the nonacclimated cell is 65% dehydrated (to 35% RWC), and the acclimated cell is only 50% dehydrated (to 50% RWC), the diffusion of small molecules would be through a cytoplasm layer that is fully 50% water in the acclimated, but only 35% water in the nonacclimated, cell. This degree of acclimation, therefore, would increase the cell permeability to about 1.5 times the nonacclimated (a ratio of 50:35), *provided that the measurements are made in the stressed (dehydrated) cell*. Höfler did exactly this, since he used a plasmolytic (dehydrating) technique that he had developed for measuring cell permeability.

We must, therefore, conclude with Höfler that the cell permeability of the stressed (dehydrated) cell is controlled, not only by the membranes, but also by the cytoplasmic matrix. Furthermore, we can even postulate the nature of the substance whose accumulation in the cytoplasm gives rise to the increase in cell permeability. The most likely candidates are proline and betaine, since these accumulate in the cytoplasm during acclimation, balancing the sugar accumulation in the vacuole.

This concludes our imaginary trip back; the future is up to you.

Literature Cited

- Cox, W. and J. Levitt. 1976. Interrelations between environmental factors and freezing resistance of cabbage leaves. *Plant Physiol.* 57:553-558.
- Gaff, D.F. 1981. The biology of resurrection plants, p. 114-116. In: J.S. Pate and A.J. McComb (eds.). *The biology of Australian plants*. Univ. of Western Australia Press.
- Levitt, J. 1980. *Responses of plants to environmental stresses*. 2nd ed. Academic, New York.
- Levitt, J. 1983. Plasmolysis shape in relation to freeze hardening of cabbage plants and to the effect of penetrating solutes. *Plant Cell Environ.* 6:465-470.
- Levitt, J. 1985. Relation of dehydration rate to drought avoidance, dehydration tolerance, and dehydration avoidance of cabbage leaves, and to their acclimation during drought-induced water stress. *Plant Cell Environ.* 8:287-295.
- Levitt, J. 1986. Recovery of turgor by wilted, excised cabbage leaves in the absence of water uptake. *Plant Physiol.* 82:147-153.
- Palade, G.E. 1963. Cell structure, p. 57-74. In: M. Fishbein (ed.). *Birth defects*. Lippincott, Philadelphia.