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Personal View:

The plant response: stress in the daily environment

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STRESS IS NORMAL

Like animals, plants have evolved to survive in almost every climatic and environmental niche available. They have, however, evolved more sophisticated and varied methods to enable them to survive environmental changes in light, temperature, atmosphere composition, water and nutrients and salinity. This, in part, is necessary because of the sessile nature of plants; they do not have the ability to move to more favourable environments. Stress conditions that plants encounter are not always as rare or unusual as we might at first think. The most common environmental variables, necessary for growth, can impose significant stresses on the plant. But should we think of these as unusual and extreme or just part of the normal diurnal responses experienced by the plant?

One example is light. While requiring light for photosynthesis and developmental processes, many plants are damaged by excess light, and mechanisms have evolved to dissipate light energy, for example, using metabolites such as flavonoids, which divert electron flow and avoid harmful build up of free radicals. This sensitivity to excess light, sometimes manifest as photobleaching, is not uncommon in plants which have evolved to live in more shaded environments such as the forest understorey, but which have been developed as crop plants in new more open environments. Even plants that continue to live in such shaded conditions have to adapt to sudden high light; sun flecks which occur as upper canopy trees move in the wind can be damaging to plants which are adapted to low light conditions. Again, pigments, such as anthocyanins, a common feature of understorey leaves, are used to deflect damaging energy. The important issue to understand here is that these conditions are not what we might have thought of as extreme, but rather those which occur regularly in any climate.

Another good example is high temperature. Leaves generally do not attain temperatures much above 35 ºC. They are able to control this by the fact that they can control internal water and air flow, and are not bulky tissues. However, organs such as fruit do not have this same control capacity, and even in temperate climates can attain high temperatures. For instance, an apple growing in an air temperature of not more than 25 ºC, as experienced in temperate climates, can reach temperatures of 40 ºC−50 ºC in the flesh when in direct sunlight (Ferguson *et al*., 1998). These are temperatures that would be sufficient to inactivate many enzymes *in vitro*. The flesh temperature of the same fruit may drop to 10 ºC −15 ºC overnight, representing a possible 30 ºC change in temperature in less than 12 h. The process is cyclic, rising and falling each day under normal, temperate, growing conditions. We think of these high temperatures as extreme and the response in terms of stress physiology. However, we should recognize that experiencing and reacting to such conditions is normal, often happens in diurnal cycles, and is part of regular cellular homeostasis.

THE ANIMAL PARADIGM

It is natural therefore to expect that plants have developed unique mechanisms to respond to environmental conditions, both in terms of growth and development, and tolerance mechanisms. For instance, the growth regulators ethylene and abscisic acid are particularly involved in stress responses. Abscisic acid controls stomatal closure and thus water and gas transfer from leaf to air, and ethylene induces the development of aerenchyma in roots under water logging (low oxygen) conditions, thus allowing greater air distribution to root cells. However, there are a number of mechanisms which are common to stress response mechanisms in animals. Animal systems have often provided the model or paradigm for plant researchers to start unravelling plant response mechanisms. This has been important, but we should not assume that exactly the same mechanisms occur in all details.

One of the most conserved mechanisms is that of ubiquitin-dependent protein degradation. The gene sequence for ubiquitin is one of the most conserved that we know across the plant and animal kingdoms; plants have the proteasome assembly and the enzymic mechanisms for ubiquitination. Although there have been few protein targets identified in plants, it appears that ubiquitin-dependent breakdown is a common feature in response to stress and developmental changes in plants and animals.

Two other response and signalling mechanisms share common features, but also differ in many ways. Our knowledge of calcium signalling, and of programmed cell death in plants, has originated from research in animal systems.

Calcium was long recognized in plants as having restricted transport through living cells. This came mostly from observations that calcium was phloem immobile; once transported into organs such as leaves with the xylem-based water transport system, recycling was not observed, and analysis of phloem exudate supported the concept of calcium being excluded from the living phloem system. Later research on root uptake showed that uptake into cells was generally passive, following an inward chemical and electrical gradient, but with an outward active pumping of calcium. This suggested that intracellular levels of calcium might be low and well controlled. As technology developed, intracellular measurements with calcium selective electrodes confirmed these concepts and showed that cytoplasmic calcium was in the expected sub-micromolar region, and the vacuolar concentrations closer to millimolar. Most of the calcium in the plant appeared to be extracellular, associated with the cell wall and the free space solution. At the about the time these findings were appearing, animal physiologists were showing that calcium played a role as a secondary messenger. Under instruction from extracellular signals transduced at the plasma membrane through transducers such as G proteins and the phosphoinositide system, calcium was shown to be released from intracellular pools such as the endoplasmic and sarcoplasmic reticulae, and transient spikes of calcium were signals for induction of biochemical responses, often through specific calcium-binding proteins such as calmodulin.

This general scheme of low intracellular calcium and transient increases as part of signalling pathways holds for plants, but only in limited situations, as far as current knowledge goes. Low temperature induces calcium transients in most cells, and calcium has been implicated as part of the induction pathway for programmed cell death (O'Brien *et al*., 1998). Inositol tris-phosphate was first shown to release calcium from plant microsomes, implicating the endoplasmic reticulum as a releasable calcium pool (Drøbak and Ferguson, 1985), and although more recent suggestions for such a pool have centered on the vacuole, this organelle has neither a high affinity calcium uptake system, nor the concentration and spatial characteristics necessary for rapid release. Calcium is implicated in stomatal guard cell control, amongst other responses, but the exact nature of the regulation of the calcium signalling system is incompletely known. In summary, there are similarities with animal cells, but the specific attributes of plants in calcium signalling are still not fully understood.

The second plant system which has been investigated based on an animal model is programmed cell death (PCD). The specific requirements for apoptosis in animals, dissolution of cells and re-

moval of contents, while applying in some cases in plants, does not of course involve an immune system and systematic cell disposal. However, a number of apoptotic features exist in plants, including DNA fragmentation by endonucleases, shown as laddering and positive TUNEL responses, and chromatin and cytoplasmic condensation (Jones, 2000). The involvement of caspases is problematic, with no functional homologues of animal caspases yet identified in plants (Woltering *et al*., 2002). However, mitochondrial cytochrome *c* release occurs with heat-induced PCD (Balk *et al.*, 1999), and the mitochondrion has been suggested as the one common controlling agent of PCD in both plants and animals (Jones, 2000). Thus the animal paradigm has proven useful, but should be interpreted with care in plants.

PCD occurs in three main response areas. One is in development, where the best example is tracheid formation in new xylem tissues. Tracheids are formed from living cells, and the process of cell death bears many characteristics of PCD (Kuriyama and Fukuda, 2002). A second is the hypersensitive response, which is a process where cell death is induced in response to pathogenic attack, thus restricting the spread of the pathogen in the plant. This process involves reactive oxygen species and nitric oxide (Delledonne *et al*., 2001), both inducers of PCD or part of the pathway in animals. The third is abiotic stress response, and the best example is aerenchyma development under low oxygen conditions, where cell root cortical cells are induced to die to form larger airspaces, enabling greater diffusion of air from the above parts of the plant (Drew *et al*., 2000). PCD has also been characterised in response to plants to high temperature (McCabe and Leaver, 2000).

One other response system is very similar in plants and animals. Heat shock proteins (hsps), which are induced or up-regulated in response to high temperature and some other abiotic stresses, are universal, and plants have members of the common hsp 60 and 70 chaperone families, higher molecular weight proteins at about 90 and 101 kD, and a group of low molecular weight hsps which are expressed much more frequently and widely than in animals. The functions of the hsps are similar in all organisms, although the exact functions of the low molecular weight group in plants are still not fully characterised (Vierling, 1991).

CROSS TALK

One of the most interesting of the responses is what we are increasingly referring to as cross-talk. This is not only where similar genetic and molecular responses occur with different stress, but more elegantly, where induced tolerance to one stress confers tolerance to another. The best example is with temperature stress. Plants can achieve tolerance to lethal high temperatures by first experiencing high, but permissive temperatures. For instance, many fruit will be damaged at 45 ºC−50 ºC, temperatures necessary for disinfestation methods. However, exposure of the fruit for about 1 h at 38 ºC will prevent that damage. Such induced tolerance has been associated with hsp enhancement (Lurie, 1998). Similarly, damage at low temperatures such as 0 ºC in fruit such as avocados can be reduced if fruit are first held at slightly higher temperatures such as 6 ºC for up to 3 days (Woolf *et al*., 2003). In this case again, it is likely that COR (cold regulated) genes and some hsps are up-regulated.

However, we also find that the induced high temperature tolerance will provide some protection against low temperature damage. Avocado fruit held at 38 ºC for 1 h undergo less chilling injury when transferred to 0 ºC (Woolf *et al*., 1995). This suggests that some of the mechanisms, whether they be stress proteins, or the induction of protective antioxidant enzymes, provide common protection. This is indicative of a more co-ordinated response to stress in plants.

CONCLUSION

Plants have to tolerate a greater range of diurnal environmental changes than animals, Even so, some mechanisms are common, and animal models can give plant researchers a lead into understanding plant processes. It is possible that one of the ways in which plants can tolerate continual changes in their

environment on a daily basis is to combine stress response mechanisms or co-ordinate them. Often one stress is closely associated with another, such as high temperature and drought and high light. It is efficient to use similar mechanisms, triggered by different environmental impacts to ensure the maximum control over environmental change. These stresses should be seen not as extreme and infrequent, as our concept of stress tends to suggest, but rather as a normal part of plant homeostasis; a normal, co-ordinated, continually changing response to ensure continued growth and development.

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