Content*Select*

John Bryant takes a closer look at some of this month's Original Articles

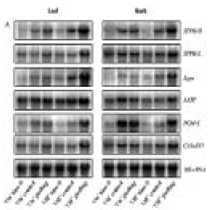
New technique — new insights, inside and out

How often in science do we see that the development of a new technique opens up new areas of research? Indeed, some new techniques, such as recombinant DNA technology and PCR have had very major effects. That is not to say that all technical innovations have such a dramatic an impact on science, but nevertheless new techniques nearly always add to our investigative ability. Thus, **Donald *et al.* (Cambridge and Norwich, pp. 73–77)** describe the application of environmental scanning electron microscopy (ESEM) to the study of living plant tissues. In conventional SEM the sample chamber is evacuated; under high vacuum some specimens become distorted. In ESEM, the chamber is not under vacuum: it contains gas that can be, as described by the authors, water vapour. Thus, in plant tissue, turgor may be controlled. Further, the ionization of the gas by the electrons removes the need to coat the sample with a layer of conductive material. The authors then show how the ESEM may be used to study cellular and tissue responses to stresses applied by a tensometer. Cell and tissue breakage are important in normal developmental processes such as abscission and seed shedding but may also occur because of environmentally derived damage or stress. In the 'model' system described here, the onion epidermis was treated with a chelating agent (thereby weakening the middle lamella); stressing the tissue caused the cells to separate without losing their turgor (implying that broken plasmodesmata along the fracture surface were sealed). However, when a notch was cut in the tissue, individual cells were ruptured along the line of a tear that extended from the point of the notch when the tissue was stressed. These observations could not be made in real time on living tissue with the conventional SEM, thus showing the potential of this new technique.

Babes in the wood: from embryo to plantlet

We often speak of plants' developmental plasticity and their ability to regenerate from excised pieces. However, gardeners and horticulturalists know that, actually, plants are extremely variable in this respect. Some plant species can regenerate from seemingly unpromisingly small fragments (as I know from moving an acanthus in my garden). At the other end of the scale are plants that we cannot get to regenerate from any sort of cutting. The same range of interspecific variation is seen in the propagation of plants from *in vitro* culture, and it is this topic that has been studied by **Corredoira *et al.* (Santiago de Compostela, pp. 129–136)**. They note that chestnut (*Castanea sativa*) is suitable for improvement by GM technology, especially in respect of resistance to fungal diseases. However, this approach has so far failed because of failure to regenerate plants from culture. So, where does one start? There are obvious general principles and there are also procedures known to have worked with similar species, but in the end it has to be a thorough, painstaking empirical approach, evaluating all possible factors. This is clearly seen in the work of these authors and furthermore, the approach has been successful. Good embryogenic cultures have been established and plants have been regenerated from them. The original embryogenic cultures were obtained from leaf explants and the somatic embryos were multiplied either directly or via callus. The 'germination' potential of these somatic embryos was strongly affected by the carbohydrate source and by temperature. Maltose was the ideal carbohydrate and the temperature regime needed to include 2 months at 4 °C. Somatic embryos matured in this way showed a 39 % conversion rate to plantlets, which were then multiplied by micro-propagation. We are still a long way from the first GM chestnut, but the authors' careful work has given them an excellent start.

Continued overleaf



Girdling — ringing the genetic changes

Growers of several types of fruit tree, including peach and various citrus species, traditionally girdle the trees prior to flowering. This means removing a ring of bark from the main trunk so that phloem transport to the lower trunk and to the roots is prevented, increasing the relative sink strength of the fruit (which is already very high) and, as mentioned by **Li *et al.* (Hebrew University of Jerusalem, pp. 137–143)** often causing severe starvation of the roots. Nevertheless, the desired effect is seen in more successful fruit set and greater fruit size, presumably because of the diversion of nutrients to the upper parts of the tree, including the reproductive organs. However, as we have noted previously in these pages, some plants respond to plenty in unexpected ways. What then is the situation with girdled citrus trees? The authors used a variety that fruits every other year and were thus able to study the accumulation of soluble carbohydrate and starch in the leaves, bark and roots of girdled trees in both 'on' and 'off' years. Here we focus on leaves and bark. Girdling certainly leads to an abundant carbohydrate supply above the ring but in 'on' years the fruit act as such a strong sink that there is no accumulation in stems or leaves. In 'off' years when the soluble carbohydrate is not mopped up by the fruit, it is converted to starch in both leaves and bark. Further, this starch accumulation is associated with increases in the levels of mRNAs that encode several enzymes involved in starch biosynthesis. These increases in gene transcription do not occur in 'on' years when little or no soluble carbohydrate is available for starch biosynthesis. Thus gene expression is sensitive to carbohydrate status, adding further weight to the view that sugars may act as signalling molecules.

Island hopping with a flow cytometer

Angiosperms as a group have a very large range in genomic DNA content (i.e. their C-values), amounting to a 1000-fold difference between the largest and smallest genomes. Measurement of C-values can give insights into the evolution and ecology of plant groups. In general, genomes have tended to increase in size during evolution so large genomes are regarded as more 'advanced' than smaller genomes; we note here that monocots generally have larger genomes than dicots. In plant development, increasing amounts of DNA are correlated with larger nuclei and longer cell division cycles. In any one taxon, the lowest C-values are often found in the species with the shortest life-cycle, such as ruderals. There are also correlations between larger genome size and lower temperature so that within any one group there are latitudinal or/and altitudinal gradients in C-value. This brings us to the paper by **Suda *et al.* (Prague, pp. 153–164).** They have measured C-values of angiosperms (in fact, mainly dicots) in Macronesia, those Atlantic islands, including the Canaries, the Azores and Madeira that lie between 15 and 40°N. The islands are very rich floristically, with a high proportion of shrubs and other woody forms. Nuclear DNA contents were measured in 104 species in all, revealing, very surprisingly, that C-values are in general very low, falling into the lowest third of the known range and, for nearly all species, lower than the C-values found in members of the same families growing in mainland Europe or Africa. It is an interesting picture: large shrubs with genomes as small as those of some cool-temperate ruderals. Overall, there was no obvious correlation between C-values and environmental factors, although some correlations were observed within particular groups, e.g with altitude. However the general picture that Macronesian plants have small genomes remains a puzzle and surely shows the way for further research.

Professor J. A. Bryant
University of Exeter, UK
E-mail j.a.bryant@exeter.ac.uk