

SOMACLONAL VARIATION

G THE TRANSITION TO APPLIED TECHNOLOGY

AMSTERDAM, Neth.-Baconian induction is alive and well and thriving in plant cell and tissue phenomenology. Or, at least, so it would seem from a perusal of the lecture theatres and poster halls at the VII International Congress of the International Association for Plant Tissue Culture (IAPTC) held here recently. This area of biotechnology, clearly biological, is only passingly technological; it is intriguing but hardly analytical. And the main consequence of this is that a fine, but as yet unclassified, body of phytophenomena ranging from the encouraging to the baffling has been accumulating over the past several years.

Callus formation, vitrification, embryogenesis, organogenesis, and regeneration are central processes in the propagation of plants via cell and tissue culture, and yet all remain poorly understood. Driven by the need for a pragmatic approach to crop improvement, considerable effort is being expended in extending the range of plants in which these phenomena occur. While it is perhaps reassuring to learn from the posters

at the IAPTC meeting that plants as diverse as bamboo, cardamom, garlic, mango, melon, grapevine, begonia, tea, and asparagus are every bit as curious as the model crops in which the phenomena were first observed, the knowledge does little to supplement understanding. And without understanding, one is reduced to empiricism in order to extend the phenomena to species and strains-like the cereals-where the impact of improvement could be greatest. Only lately, and still only rarely, have the powerful tools of molecular biology been used to describe the phenomena and then dissect them into analyzable components.

One example of this "new approach" was given by Ron Phillips of the University of Minnesota (St. Paul) who, in his keynote lecture, asked "Do we understand somaclonal variation?" The answer, despite decades of endeavouring to exploit the wealth of variability in the generation of agriculturally significant qualities, is still "No." Now, however, the phenomenon is yielding at least some of its secrets. Phillips and his colleagues,



working with maize, observed that, during generation of somaclonal variants, transposable elements such as Ac and Spm were activated in three percent of the embryo cultured lines. These elements were not seen in native plants. How could transposable element activation induce the wide variation in quantitative traits seen in the progeny of regenerated plants? The observed heritability of the variant phenotype over several subsequent generations necessitates an explanation that involves stable genetic changes. Phillips argued that, since somaclones vary in many traits, the mechanism could not be gene-specific. Instead he proposed that changes in DNA methylation of the transposable elements-specifically hypomethylation-might mediate the apparently global genetic change.

The extent of variation between somaclones was illustrated by restriction analysis: restriction with *HpaII* of DNA from plants regenerated from a single embryo culture distinguished at least 12 different families of regenerants. Analysis with *MspI* (which has the same site-specificity as *HpaII* but cleaves both methylated and unmethylated sites), however, indicated that there were no base sequence differences between the families of regenerants, only changes in methylation.

On the basis of these experiments, Phillips' current hypothesis on the mechanism of somaclonal variation is that, in the tissue culture environment, a change in cell physiology (possibly affecting the nucleotide pool) induces hypomethylation. Hypomethylation may in itself cause relatively rare base changes; however, to account for the breadth of somaclonal variation, Phillips theorizes that it is hypomethylation that activates the transposable elements. This activation, in turn, could lead to such gross genetic changes as insertion, duplication, deletion, and chromosome exchange. In essence, he sees somaclonal variation, in part at least, as being an epigenetic phenomenon.

Phillips cautions that a more precise definition of the molecular mechanism of somaclonal variation will require sequence and methylation analysis of the somaclones. Furthermore, the changes in chromatin structure associated with somaclonal variation need closer analysis. Nevertheless, in one area at least, plant biology is making the transition from phenomenology to technology.

-John Hodgson