Herb Quick

Date palms were successfully regenerated from cell tissue culture by manipulation of the make-up of the growth medium.

opment phase, the auxin is excluded from the medium.

Aeration is important for embryo or organ development. Anaerobic conditions cause alcohol accumulation and can lead to intoxication of the cells and diminution of their capacity for organized development.

Embryos can be produced in darkness, but shoot and roots are more likely to develop in illuminated cultures. Gro Lux and Cool-White fluorescent lamps that emit high levels of blue and red light have been most effective. Low light intensities, about 100 foot-candles as encountered in household illumination, are preferable during the shoot and root differentiation steps. However, just before transplanting to soil, plants from tissue should be hardened by exposure for a two- to three-week period to a higher light intensity, near 1,000 foot-candles. Plant regeneration of some species is controlled by photoperiodism, like their flowering behavior. Thus, for example, kalanchoe cells regenerate better under short days, and walnuts proliferate shoots more readily under long days.

The temperature in which cells are incubated can also be important to plant regeneration. Differences in day and night optima, or thermoperiodism, must not be ignored when working with plants that differ in origin from tropical to temperate and alpine habitats.

All plant cells probably have totipotential, or the capacity to reproduce entire plants. Nevertheless, the ease of expression of that potential differs among plant species and varieties, as well as among cells within the same plant. Some cells are more responsive than others to manipulations that result in embryo or organ formation. J. G. Torrey has identified the manipulatable cells by the term meristemoid. In appearance, meristemoids resemble very closely cells of apical meristems or of embryos as found in seeds. Indeed, meristemoids are more often isolatable from apical meristems and young embryos. Non-meristemoid cells sometimes differentiate into meristemoids *in vitro*—for example, tobacco stem pith, carrot root phloem, potato leaf mesophyll, and citrus nucellus. Unfortunately, the basis for this differentiation remains unclear.

Meanwhile, the chances of obtaining plants in cell cultures can be increased by observing a few key relationships. Cells of plants that are in the juvenile phase of development, that is, not yet competent to flower, are more regenerative than those of adult plants. Even among juvenile sources, the younger the plant or organ, generally the higher their tendency to contain regenerative cells. Seasonal climatic requirements of the donor plant or organ must be satisfied before cell culture: it must be chilled, grown under appropriate photoperiod, and the like. As a rule, highly regenerative cell lines can be derived from a poorly regenerating tissue by repeatedly selecting for the trait during the course of subcultures.

Molecular biology may someday furnish methods that would enable selective control of gene repression and derepression. Perhaps, then, it will be possible to achieve expression of totipotential of any plant cell, whether meristemoid or nonmeristemoid and regardless of species or variety.

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Somaclonal variation

Thomas J. Orton

Successful application of *in vitro* cell and tissue culture technology to crop improvement hinges on the ability to regenerate plants of known genetic constitution. For example, when using cell or tissue culture as a means of cloning, or amplifying numbers of plants for field or seed production, it is essential that regenerated "copy" plants be genetically similar or identical to the original. Alternatively, when using this approach to develop a new improved variety, a selection scheme would be devised that would theoretically find only cells with altered genotypes at loci whose function bears on a desired character, but which were genetically identical to the original tissue donor in all other respects. However, some of the earliest research papers in this area have documented the existence of spontaneous genetic variability in both cultured cells and corresponding regenerated plants. A useful label, "somaclonal" variation, has recently been advanced for this phenomenon—"soma," occurring in somatic tissues as opposed to sexual progeny, and "clonal," expressed as differences among and within clones.

The cumulative evidence from over 400 scientific papers shows that somaclonal variation in cultured cells is more the rule than the exception. The most common observations are of changes in the number and structure of chromosomes, the subcellular organelles that carry individual genes. Evidence suggests that these chromosomal changes increase with culture age and are antagonistic to the regeneration process. Regenerated plants with altered chromosomal changes often show changes in leaf shape and color, growth rate and habit, and sexual fertility. Such changes are sometimes seen in regenerated plants with apparently normal chromosome constitution, implying that somaclonal variation may extend to the level of individual genes.

Somaclonal variation is obviously highly undesirable in situations where cell or tissue culture is being used to preserve genetic identity. A limited number of specific observations point to its possible use as a means of expanding the pool of desirable genetic variability for crop improvement. Examples include altered plant habit and flower type in chrysanthemum and increased yield and disease resistance in regenerated plants of sugarcane and potato as compared with the original tissue donor. Unfortunately, the phenomenon is ill-understood, and we are presently unable to direct its manifestations in any way.

Research conducted recently at Davis has shed some new light on somaclonal variation. Using celery as a model organism, we have shown that variation occurs at the level of the single gene as well as the chromosome, although the precise nature of the lesions has not yet been pinpointed. Certain of these chromosomal and single gene changes are transmittable to regenerated plants, and they behave sexually in a predictable fashion. Some populations of regenerated plants show chromosomal abnormalities but little or no accompanying visible alteration, perhaps because of observed mixtures of normal with

Samuel W. Woo

Two plants regenerated from same celery callus culture demonstrate somaclonal variation. Plant on left closely resembles the original. Plant on right has smaller, more divided leaves, and its growth is much slower. Genetic studies will investigate whether variation resulted from DNA changes or lingering cultural effects.

aberrant cells within plants. Approximately 70 percent of plants regenerated from cell cultures of a commercial celery variety and grown in three field locations were visibly normal, while the remaining 30 percent showed striking differences in growth rate or habit, leaf shape, color, or flowering behavior. None of the plants exhibited characteristics that could be considered superior to the original type. Experiments to assess the relative physiological and genetic contributions to this variation are in progress.

Further results with celery suggest that genotype of the tissue donor, medium constitution, and culture age are the significant factors mediating somaclonal variation, whereas differentiated state (leaf, stem, and the like) and random effects are not important. The differences observed among genotypes were particularly interesting: some lines showed a rapid, progressive accumulation of variation (and loss of ability to regenerate), whereas others consistently remained stable (and able to regenerate). We therefore speculate that a combination of appropriate genotypes and media may be at least partially effective in controlling somaclonal variation. Perhaps it will be possible to identify genes responsible for inhibition or enhancement of variation and to transfer them sexually into desired backgrounds. Solutions to these problems will eliminate a major block to the application of new molecular and cellular technology in plant breeding and field production.

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Cell mutagens

Gary E. Jones

Realizing the full potential of plant somatic cell genetic techniques will depend on development of methods for isolating a wide variety of cultured cell strains with characteristics different from those of cells in the original cultures. To isolate such variant cell strains, techniques well known in microbial studies will have to be applied to cultured plant cell systems.

One especially important method is the application of mutagens to plant cells to greatly increase the frequency of variant strains in populations of cells so that they may be more easily identified and selected. However, work in several laboratories has shown that many chemicals that are potent mutagens in microbial systems are only marginally or not at all effective on cultured plant cells. The search for chemicals effective on a wide variety of plant cells is therefore an important aspect of plant somatic cell genetics.

Testing whether a compound is mutagenic requires that the expression of an easily seen characteristic be substantially different in parent cells than in the variants derived from them after treatment with the agent. In our laboratory, we have been attempting to develop such a testing system. Resistance to a nucleic acid precursor analog called 6-azauracil appears to be a useful characteristic for such work. Parent cells growing in culture are highly sensitive to this compound, but variants can be found that are resistant to it, and the difference is easily assayed.

We have demonstrated that this difference results from an enzyme deficiency in the variant cells. They lack an enzyme (uracil phosphoribosyltransferase) that converts the analog into the compound that actually kills the cells. Strains of cells from two different species of plants (diploid *Haplopappus gracilis* and haploid *Datura innoxia*) resistant to this analog have been isolated and shown to lack the enzyme. We have studied the effects of several commonly used mutagens on these cells but have not yet found one that effectively increases the frequency of azauracil-resistant cells in treated populations.

Two possible causes for these results must be considered. First, the agents so far tested might not be mutagenic in cells of these species. Or, second, resistance to azauracil might not be the result of real mutations in the gene responsible for the structure of the enzyme but instead might arise from nongenetic causes. In the latter case, mutagenic agents would not be expected to affect the frequency of resistant cells. Although we cannot yet prove that stable azauracil resistance has a genetic cause, several characteristics of the resistant cells indicate that this is the case. Among these traits is the complete lack of uracil phosphoribosyltransferase activity after cells have been cultured for more than two years without exposure to the analog.

One feature of our studies shows that such work must be interpreted with care. In many experiments, we demonstrated that several potentially mutagenic agents appeared at first to increase the frequency of resistant cells, sometimes by as much as 50-fold. However, the vast majority of these resistant populations did not retain the characteristic when subsequently cultured in medium not containing the azauracil. Rapid loss of the resistance strongly indicates that these cells were not genetically altered, although we do not know why they initially appeared to be resistant. In fact, when careful study of the initially resistant strains was carried out, the frequency of stably resistant cells was not very much increased by the treatment with mutagen. Such results demonstrate that detailed investigation of resistance to any compound must be performed before conclusions about the mutagenic effects of any agent can be claimed, even if stable resistance can be shown to be the result of a true genetic change.

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