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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