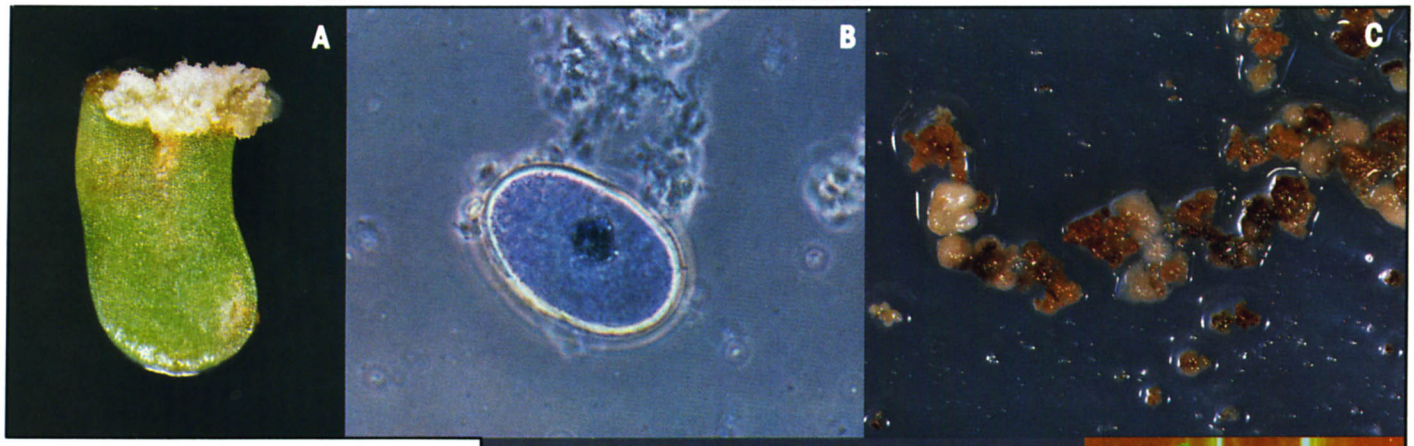
JKC Suzanne Stavarek



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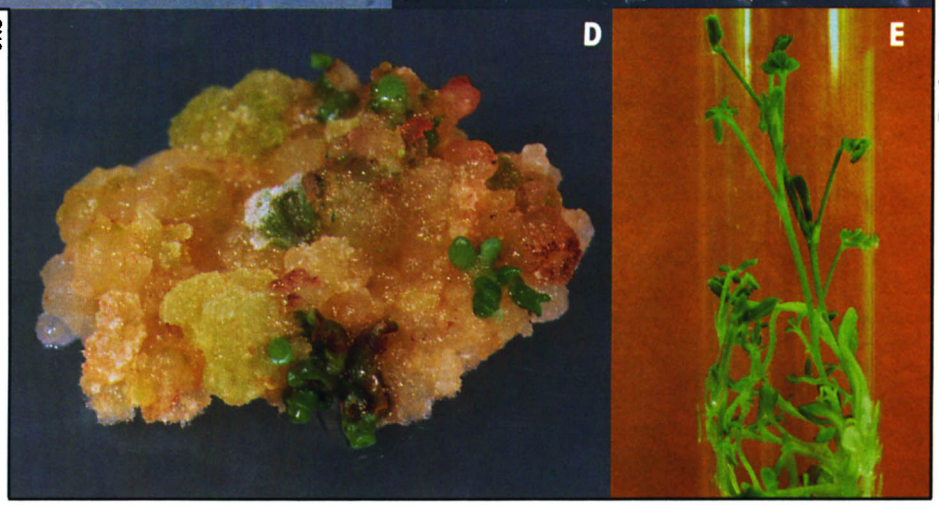

Selecting for salt tolerance: (A) Alfalfa cotyledon produces callus from cut portion. (B) A single alfalfa cell has potential to regenerate into a complete alfalfa plant. (C) Cells are challenged with salt in nutrient medium; light-colored areas are living and presumably salt-resistant; brown and black areas are dead. (D) Resistant cells, on new medium, begin to differentiate into plantlets. (E) Regenerated alfalfa plant, when hardy enough, will be transplanted into soil for further study.

as yield, stress resistance, or nutritional quality, but it may present methods to release previously unavailable variability for such traits. Clearly, MPGE does not stand alone in plant improvement; integration with conventional methods is absolutely necessary.

In considering agricultural applications of genetic engineering, some requirements should be mentioned that are the subjects of current and future research. (1) Cell or tissue culture systems are needed to select for the target characteristic (gene). (2) Systems must be developed to regenerate plants from cell cultures rapidly and in large numbers. (3) Gene expression in cell cultures must also be sustained at the stage of growth in which the plants will be utilized. (4) Good vectors and probes must be developed to transmit and identify the desired genes. These are substantial criteria for utilizing MPGE, but they are certainly not beyond the scope of plant biology research.

Some opportunities for using molecular plant genetic engineering in plant improvement

- ☐ To make transfers of genes from one species to another that would not be possible with nonmolecular methods.
- ☐ To transfer genes at a single step, rather than through repeated crosses or backcrosses.
- ☐ To transfer only the target gene, without detrimental genes linked or otherwise associated with it.
- ☐ To transfer genes rapidly in species with long generation times.
- ☐ To transfer genetic information to plants that will facilitate new end-products.
- ☐ To conserve plant genes in cloned DNA gene banks.
- ☐ To assess genetic variation and genetic relationships among species by molecular methods.
- ☐ To capitalize on "spinoff" technology for use in conventional gene transfer systems — for instance, tissue, cell or protoplast culture methods.

Developing salt tolerance

D. William Rains

Salinity and its potential influence on plant productivity can be managed either by physically manipulating the environment in which the plant grows or by biologically manipulating the plant to reduce the harmful effects of excess salt. The concept of biological manipulation is based on the observation that salt tolerance appears to be genetically controlled and that plants vary widely in their sensitivity to high levels of salt.

Sometimes traditional breeding methods cannot be used to develop salt-tolerant crops. Natural genetic variability in tolerance to salinity may be lacking or not accessible by usual breeding methods. In these cases, it is appropriate to apply new techniques to achieve this goal.

Plant cell culture techniques may be used to obtain salt-tolerant cell lines. This use of cell cultures is based on the application of mutant selection techniques developed in microbial and fungal systems (see articles by Z. R. Sung and G. E. Jones in this issue). The general selective strategy is to produce a cell culture of a crop plant and to challenge these cells with an inhibitory salt concentration by incorporating the salt into the nutrient medium in which the cells are grown. The rare cells that can survive and divide under these conditions are candidates for further study. By inducing the survivors to regenerate whole plants, it is possible to determine whether the salt tolerance observed in the selected cells is stable and transmitted genetically.

In our laboratory we have obtained a line of salt-tolerant alfalfa cells by this means. The salt-selected line grows better than unselected cells at a high level of salt (1 percent sodium chloride), indicating that the selection isolated variant cells with an increased capacity for growth in the presence of high

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D. W. Rains examines a culture plate containing alfalfa cells growing on a high-salt medium in "cell city," where billions of cells are grown and selected for salt tolerance under light- and temperature-controlled conditions.

salt. This selected line additionally displays other characteristics suggesting that the tolerance results from a shift toward a true halophytic nature. Besides tolerating high salts, halophytes actually require some salt, as evidenced by poor growth in its absence and growth stimulation when salt is added.

The capacity of a cell culture to regenerate plants usually diminishes over time. This presents difficulties in cell selection programs, because, by the time the selection and testing stages are completed, the selected cells may fail to respond to standard regeneration conditions. This was indeed the case with our alfalfa cell line. By testing some 200 media modifications, we developed a sequence of media that enabled us to regenerate plants from the salt-tolerant cultures. The regenerated plants could then be tested to determine whether the salt tolerance of the cells in culture was carried through to the whole plant.

Unfortunately, in our case, the regenerated plants were very weak and we were not able to demonstrate any increase in salt tolerance. Our evidence, and that from other laboratories, indicates that our approach is sound, however, and we are confident that continued efforts will produce salt-tolerant crop plants that transmit this valuable trait to their progeny.

D. William Rains, Professor, Agronomy and Range Science, and Director, Plant Growth Laboratory, U.C., Davis.

Genetic disease resistance

David G. Gilchrist
Noel T. Keen

The most widely used plant disease control method has been the incorporation of single, usually dominant, genes for disease resistance into cultivated plants. In some cases, disease control also has been accomplished by withdrawal from plants of certain dominant alleles conferring vulnerability to attack by pathogens that produce specific toxins. By either approach, genetic resistance affords the only practical control strategy in most major crops.

Two current problems with the use of resistance genes are: (1) they are frequently overcome by new forms of the pathogen, which appear to evolve from the original pathogen population by either genetic recombination or mutation; (2) suitable genes conferring disease resistance often are not available in a plant species or genus, or they cannot be incorporated into agronomically useful cultivars because of cross-fertility barriers. Recent development of recombinant DNA techniques opens the possibility of genetically engineering plants by introducing desired disease resistance genes from both intraspecific and intergenic sources. Such techniques would be immediately applicable to these two problems and would also increase understanding of the mechanisms of natural disease resistance and nonhost immunity.

The major limitation to direct implementation of this technology is that no single resistance mechanism has been characterized completely, nor have the interactive gene products been identified. Extensive physiological information indicates that resistance phenomena fall into two general classes, inducible and constitutive.

In the former case, resistant plant genotypes appear to recognize some feature of the invading pathogen, which then initiates an induced defense response. This is typified by hypersensitive necrosis of plant cells at the infection site and is associated with the accumulation of antibiotic chemicals called phytoalexins. Recent evidence from University of California, Riverside, is consistent with the hypothesis that recognition involves resistance gene products in the form of receptor molecules that interact with specific complex cell surface carbohydrates (elicitors) of the invading pathogen. The exact nature of the pathogen genes that may determine this unique carbohydrate structure is unknown,