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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

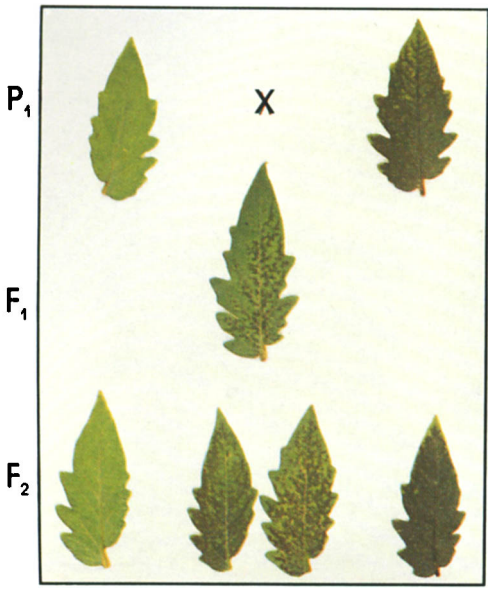
David G. Gilchrist
Noel T. Keen

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,



Inheritance of a single-gene-determined sensitivity to host-selective toxins produced by *Alternaria alternata* f. sp. *lycopersici*, a fungal pathogen specific to tomato. Segregation in F_2 generation fits ratio of 1 resistant: 2 intermediate: 1 susceptible plant from the original cross of homozygous-resistant by homozygous-susceptible parental (P_1) plants.

Lesions on tomato leaves and fruit caused by disease-determining toxins produced by the pathogen *A. alternata* following infection of plant. This disease was important to the fresh-market tomato industry in San Diego and Ventura counties from the early 1960s to the late 1970s. It is now controlled in these areas by genetic resistance in what may have been the first cloning of a gene for disease resistance.

but specific glycosyl transferase enzymes are known to be involved in cell surface carbohydrate synthesis. Attempts to molecularly clone the primary disease-determining genes of certain plant pathogens, especially bacteria, are in progress in laboratories at U.C., Berkeley, Davis, and Riverside. If such genes can be cloned and their gene products more readily isolated, it will be possible to perform experiments to elucidate the molecular function of such genes and to isolate the predicted resistance gene-coded receptors produced by the host plant.

Other bases of resistance appear to be constitutive and, depending on the form of gene expression, may involve the presence or absence of host-plant sensitivity to chemical disease determinants produced by the pathogen. In several diseases, alleles at a single genetic locus in the host control both susceptibility to the pathogen and sensitivity to host-selective toxins produced by the pathogen. Such toxins are useful as direct chemical probes of the genetic interaction. Recent work at U.C., Davis, with such a host-parasite interaction (see photos) indicates that the host-selective toxin inhibits a key enzyme in the pyrimidine biosynthetic pathway, aspartate transcarbamylase; the enzyme from the resistant genotype is relatively less sensitive to inhibition by the toxin than that from the susceptible genotype. Other host-selective toxins have been hypothesized to interfere with membrane integrity, although the exact mechanism is unresolved. Clearly, cloning of specific alleles lacking sensitivity to toxins from the same or unrelated species and introducing the cloned DNA into the cultivated plant is a goal, along with establishing a valuable information base on resistance gene products.

As assay systems for resistance genes have become available at the level of gene action, more laboratories are attempting to identify and clone disease resistance genes and are searching for suitable vehicles to introduce this DNA into desired plants. Although foreign DNA has been experimentally introduced into plant cells using the crown gall Ti plasmid, we are not aware of the successful introduction of defined plant genes into other plant cells by recombinant DNA technology. We predict, however, that this will happen within the next few years. Disease resistance genes are indeed likely to be among the first genes introduced into new plants by genetic engineering, not only because of their considerable economic importance, but because they represent some of the few examples in plants of naturally occurring and defined single genes with definitive phenotypes. Thus, their transfer can be detected easily and rapidly.

David G. Gilchrist, Associate Professor, Plant Pathology, U.C., Davis, and Noel T. Keen, Professor, Plant Pathology, U.C., Riverside.

Enhancing nitrogen fixation

Donald A. Phillips
Larry E. Williams

Production of ammonia from atmospheric nitrogen by the *Rhizobium*-legume symbiosis offers opportunities for genetic improvement of both *Rhizobium* bacteria and host legume. Root nodules formed by rhizobia are the organs responsible for nitrogen fixation. California crops that might benefit most directly from such improvements are alfalfa, clover, common beans, lima beans, garbanzos, and blackeye peas. Additional nitrogen fixed, but not used, by those plants would be bound in an organic form that could carry over to benefit subsequent crops.

Recent genetic information indicates that leguminous plants vary in their capacity to use soil nitrogen and to fix atmospheric nitrogen with *Rhizobium*. Our understanding of how efficiently nitrogen is fixed by common varieties of legumes grown in California is limited, but significant work in this area is being done by L. R. Teuber and K. W. Foster at Davis. Genotypic variation for protein concentration in alfalfa grown sequentially on atmospheric nitrogen and ammonium nitrate has been measured, and over 700 genotypes of large-seeded grain legumes are being assessed in the field for their capacity to use atmospheric and soil nitrogen. Such studies will provide the information required to produce the next generation of nitrogen-efficient legumes for California.

More immediate benefits will be reaped from genetic studies of the bacterial partner, *Rhizobium*. Laboratories at Davis are investigating hydrogen uptake, a process affecting efficiency of nitrogen fixation (see article by R. C. Valentine in this issue), and D. N. Munns at Davis is examining *Rhizobium* strains for natural variation in tolerance to acid and aluminum stress. Collaborative work between the John Innes Institute in Norwich, U.K. and our group in Davis has used conjugal plasmid transfer to produce *Rhizobium* strains significantly superior to either parent. The key plasmid, pIJ1008, carries determinants for hydrogen uptake, which are not found in *Rhizobium* strains that nodulate alfalfa and clover. Although pIJ1008 is expressed most completely in pea rhizobia, it has been transferred to closely related clover and alfalfa bacteria, and those