Foreign Gene Transferred into Maize

The genetic engineering of cereal crops moves nearer to reality as researchers introduce a foreign gene into corn plants

RESEARCHERS have achieved a long awaited goal—the transfer of a foreign gene into a major cereal, namely maize. The result marks a new milestone in efforts to apply the techniques of modern biotechnology to the cereals, corn, rice, and wheat, that supply much of the world's food supply. These crops have generally not been amenable to the same gene transfer techniques that have worked for other kinds of plants.

The successful gene transfer into maize, which is described in detail on page 204, means that there is now a direct way for introducing desirable new traits into this cereal, and perhaps into the others as well. For example, researchers have already used gene transfer technology to introduce characteristics such as herbicide, insect, and disease resistance into some plants, including petunia and tobacco, but, until now, have had to rely solely on the standard methods of plant breeding for producing corn strains with novel characteristics.

These methods are often time-consuming and may not be applicable at all if the desired trait is carried by a species that is sexually incompatible with maize. Although the maize plants produced so far by gene transfer have been infertile, this problem is likely to be only a temporary obstacle to the application of the technology to corn.

In the current work, Carol Rhodes, Dorothy Pierce, Irvin Mettler, Desmond Mascarenhas, and Jill Detmer of Sandoz Crop Protection Corporation in Palo Alto, California, transferred a gene encoding resistance to the antibiotic kanamycin into corn plants.* They chose this gene not because anybody wants kanamycin-resistant corn, but because acquisition of the resistance trait provides a ready means of selecting those plants that have acquired the new gene.

A critical step in the transfer procedure was the development of a method for regenerating whole maize plants from the denuded cells called protoplasts. The inability to regenerate cereal protoplasts has been one of the major problems hampering efforts to introduce genes into the major cereals.

The transfers into petunia, tobacco, and other plants that belong to the dicot class do not usually require this step because they are achieved with the help of a DNA-transferring bacterium called *Agrobacterium tumefaciens*. Unfortunately, however, *A. tumefaciens* does not readily infect most monocots, the class of plants to which the cereals belong.

The cereals are becoming amenable to modern biotechnology procedures.

Genes can be introduced into plant cells without the aid of *A. tumefaciens*, provided that the rigid walls covering the cells are first digested away to produce protoplasts. A few years ago, for example, Michael Fromm and his colleagues at Stanford University found that corn protoplasts would take up foreign DNA if they were subjected to an electric field, but at the time whole corn plants could not be obtained from the protoplasts.

The barriers to cereal protoplast regeneration have begun falling, however. Beginning about 2 years ago, several groups achieved rice protoplast regeneration (*Science*, 2 January 1987, p. 31), and Rhodes with Keith Lowe and Karen Ruby, who are now at Advanced Genetic Sciences in Oakland, California, reported in the January issue of *Bio/Technology* that they had regenerated whole maize plants from protoplasts.

To perform the transfer of the antibiotic resistance gene, the Sandoz group first introduced the cloned gene into maize protoplasts by a method similar to that used by Fromm. For regeneration, the protoplasts are then cultured on filters over a layer of maize "feeder cells." The feeder cells apparently supply some as yet unidentified nutrient that stimulates the conversion of the protoplasts into callus tissue, an undifferentiated mass of cells that will eventually develop into the maize plants. "Plating efficiency is greatly increased when you use feeder cells," Rhodes points out.

This is important because only a small percentage—no more than 5% and usually just 1%—of the protoplasts take up the foreign DNA and incorporate it into the nuclear genome. Addition of kanamycin to the growth medium then allowed the selection of calli that had acquired resistance to the antibiotic. Rhodes and her colleagues confirmed that the resistant calli and the plants that eventually developed from them actually carry the antibiotic resistance gene and make the enzyme that it encodes.

Meanwhile, efforts to perform similar transfers of antibiotic resistance genes into rice plants have apparently hit something of a snag. "The bottleneck in many laboratories has been in going from callus to plants," says Edward Cocking of the University of Nottingham, one of the pioneers of the research on rice protoplast regeneration. The kanamycin itself may be inhibiting the formation of whole plants from the rice calli. Rhodes says that her group has not encountered problems with the selection protocol they use with corn calli.

The researchers have encountered one difficulty, however. The corn plants that they have regenerated so far from protoplasts have proved to be infertile—and therefore incapable of yielding corn seeds. Even with successful gene transfer technology, development of new plant strains may well depend on standard breeding methods.

The reason for the current lack of fertility of the regenerated corn plants is unclear, although it is unlikely to be an insurmountable problem. "I wouldn't be too distressed at that," Cocking comments. "It may be just an effect of the culture conditions." Alternatively, the infertility problem might be a property of the particular cell line used for the experiments. Meanwhile, Rhodes says, "Even we if can't get progeny, we can still look at gene expression in the plants themselves."

The newfound ability to regenerate rice and maize protoplasts also means that these cereal plants have become amenable to a number of biotechnology procedures in addition to gene transfer. For example, protoplast fusion is another way of producing hybrids of plant species that may be sexually incompatible. Moreover, whole chromosomes or organelles such as chloroplasts can be introduced into protoplasts as a way of conveying properties that are encoded by many genes or by chloroplast genes. Resistance to some herbicides is an example of the latter. If all this may now be achieved for rice and corn, will wheat be far behind? ■

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*Pierce has since moved to EniChem in Monmouth Junction, New Jersey, and Mascarenhas is now at Biogrowth in Richmond, California.