Gene Transfer in Crop Improvement

Robert M. Goodman; Holly Hauptli; Anne Crossway; Vic C. Knauf

Science, New Series, Vol. 236, No. 4797 (Apr. 3, 1987), 48-54.

Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819870403%293%3A236%3A4797%3C48%3AGTICI%3E2.0.CO%3B2-7

Science is currently published by American Association for the Advancement of Science.

49. D. L. Anderson and M. N. Toksöz, *ibid.* **68**, 3483 (1963); L. R. Johnson, *ibid.* **72**, 6309 (1967); P. G. Richards, *J. Geophys.* **38**, 517 (1972); A. C. Lees, M. S. T. Bukowinski, R. Jeanloz, *J. Geophys. Res.* **88**, 8145 (1983).
50. Abstracts from a session on "Deep slab penetration" at the 1986 spring meeting of the American Geophysical Union [*Eos* **67**, 379 and 380 (1986)].
51. B. R. Julian and M. K. Sengupta, *Nature (London)* **242**, 443 (1973).
52. M. K. Sengupta, R. E. Hassel, R. W. Ward, *J. Geophys. Res.* **86**, 3913 (1981).
53. O. L. Anderson, E. Schreiber, R. C. Lieberman, M. Soga, *Rev. Geophys. Space Phys.* **6**, 491 (1968).
54. D. L. Anderson, *Phys. Earth Planet. Inter.*, in press.
55. D. Stevenson, *Geophys. J. R. Astron. Soc.* **88**, 311 (1987).
56. V. F. Cormier and G. L. Choy, *Geophys. Res. Lett.* **13**, 1553 (1986).
57. M. A. Richards and B. H. Hager, *J. Geophys. Res.* **89**, 5987 (1984).
58. J. Bloxham and D. Gubbins, *Nature (London)* **317**, 777 (1985).
59. ———, *ibid.*, **325**, 511 (1987).
60. D. Gubbins and J. Bloxham, *ibid.*, p. 509.
61. *Science Plan for New Global Seismographic Network* (Incorporated Research Institutions for Seismology, Washington, DC, 1984); S. W. Smith, *Eos* **67**, 213 (1986).
62. G. Nolet, B. Romanowicz, R. Kind, E. Wielandt, *ORFEUS Science Plan* (Reidel, Dordrecht, Netherlands, 1986).
63. B. Romanowicz, M. Cara, J. F. Fels, D. Rouland, *Eos* **65**, 753 (1984).
64. B. Romanowicz and A. M. Dziewonski, *ibid.* **67**, 541 (1986).
65. We thank J. Bloxham, D. Giardini, X.-D. Li, and A. Morelli for discussions and D. L. Anderson for suggestions on the manuscript. Some results and figures are from unpublished research in which D. Giardini, X.-D. Li, and A. Morelli were our coworkers. We thank D. Stevenson (*55*) for a preprint. Research described here was made possible by the bulletin data compiled by the International Seismological Centre and digital recordings from two networks: Global Digital Seismographic Network operated by the U.S. Geological Survey and the International Deployment of Accelerometers operated by the University of California, San Diego. Supported by the National Science Foundation under grants EAR81-20944, EAR82-13330, EAR83-17594, EAR84-18332, and EAR85-11400.

Gene Transfer in Crop Improvement

ROBERT M. GOODMAN, HOLLY HAUPTLI, ANNE CROSSWAY, VIC C. KNAUF

Transfer of genes between plant species has played an important role in crop improvement for many decades. Useful traits such as resistance to disease, insects, and stress have been transferred to crop varieties from noncultivated plants. Recombinant DNA methods greatly extend (even outside the plant kingdom) the sources from which genetic information can be obtained for crop improvement. Gene transfer systems based on recombinant DNA are available for several crop species and are under development for others. The concerted use of traditional and more recent methods for plant genetic manipulation will contribute to crop improvement.

RAPID PROGRESS IS BEING MADE IN DEVELOPING THE tools for manipulating genetic information in plants by recombinant DNA methods. Plant genes are being cloned, genetic regulatory signals deciphered, and genes transferred from entirely unrelated organisms (notably bacteria and a virus) to confer new agriculturally useful traits on crop plants. Recombinant DNA methods significantly increase the gene pool accessible for crop improvement.

In this review we summarize and illustrate with selected examples the long history of gene transfer by plant breeders between plant species and even between plants from different genera. We describe the use of recombinant DNA–based methods for gene transfer to plants and indicate with examples how these may contribute to the future of crop improvement. Our analysis highlights the important role continuing development of technology (Fig. 1) has played in expanding the range of organisms from which genetic information can be mobilized to plants. We conclude with some views on issues related to the use of technology in crop improvement and the future strength of agriculture.

R. M. Goodman is executive vice president, research and development; H. Hauptli is principal scientist; A. Crossway is managing scientist; and V. C. Knauf is managing scientist, at Calgene, Inc., Davis, CA 95616.

Gene Transfer Through Hybridization

Plant breeding and intraspecific gene transfer. Plant breeding as a science began in the 19th century with discoveries of how plant traits are inherited (*1*). The early years saw transfer and reassortment of large numbers of genes in heterogeneous cultivated populations (landraces). Breeders steadily expanded their search for new genetic variation to the entire crop species, including noncultivated populations. These were gene transfers within the species. It is from such exchanges that our modern cultivated varieties originated (*2*). Often, however, the crop species does not contain sufficient genetic diversity to allow the desired improvements. The search for added diversity has been a stimulus for plant breeders to adopt new technology.

In simple terms, plant breeding is the selection of plants with desired traits after the sexual exchange of genes by cross-fertilization between two parents. When one parent is a cultivated variety and the other a wild relative, an improved variety is formed by backcrossing to the cultivated parent and selecting for the desired combinations of characteristics. Plant breeding has developed into a sophisticated science, aided in part by the application of statistical tools. The alliance of genetics with probability theory has allowed plant geneticists to arrive at more efficient models for the combination and selection of genes in populations and breeding lines. Statistical methods are now indispensable in the design of field experiments and in the prediction and analysis of results (*1*).

The definition of a plant species rests on the concept of genetic isolation. Nevertheless, sexual exchange of genes between species can and does occur in nature without human intervention. One of the better documented cases of such transfer is that between maize (*Zea mays*) and teosinte (*Z. mexicana*) (*3*). Use by plant breeders of sexual exchanges between species as sources of genetic variability to improve crops has been made possible during the past 80 years by the discovery of efficient ways to circumvent the natural barriers to genetic exchange by sexual mechanisms.

Interspecific gene transfer. For certain crops, plant breeders in the 20th century have increasingly used interspecific hybridization for the transfer of genes from a noncultivated plant species to a crop variety in a related species (Table 1). The exploitation of interspecif-

ic hybridization for crop improvement is illustrated by the advances made in wheat breeding during this century. Gene transfer from related species into cultivated wheat began in 1930. McFadden (*4*) transferred resistances to stem rust and loose smut diseases from tetraploid emmer (*Triticum tauschii*) to hexaploid bread wheat (*T. aestivum*). The resulting bread wheat variety, 'Hope', was widely grown in the United States and was responsible for one of the longest rust-free periods in the history of U.S. wheat cultivation. Other genes for resistances to races of stem rust and powdery mildew and to Hessian fly have since been incorporated from *T. timopheevi*, *T. monococcum*, and *T. turgidum* into a number of bread wheat varieties (*4*).

Another early example of gene transfer to a cultivated crop species by interspecific hybridization is in tomato. In 1936, Tucker and Bohn transferred a gene conferring resistance to race 1 of the fusarium wilt fungus from weedy *Lycopersicon pimpinellifolium* to the cultivated tomato (*L. esculentum*) (*5*). Because occurrence of this pathogen is global, resistance to race 1 conferred by the *L. pimpinellifolium* gene is considered essential in commercial tomato production throughout the world (*5*). More recent applications of interspecific gene transfer include successful wide hybridization between the cultivated soybean (*Glycine max*) and its wild perennial relatives (*6*).

Intergeneric gene transfer. Successful interspecific transfer of traits from wild species to domesticated relatives in the same genus was a precedent for attempts at even wider crosses, including those between members of different genera. A growing understanding of the origins of our crop species was also a factor. There is evidence that some of our modern crop species, such as rapeseed (*Brassica napus*), tobacco (*Nicotiana tabacum*), and wheat, originated in nature by hybridization between different species or genera. The available evidence indicates, for example, that the ancestor of *B. napus* was a hybrid between *B. oleracea* and *B. campestris*. The creation of new plant species has been mimicked in the modern era by the intentional hybridization of species from the genera *Secale* (rye) and *Triticum* (wheat) to create a new cereal crop, *Triticosecale* (triticale) (*7*).

Intentional crosses between species in different genera have also successfully transferred specific traits into crop species (Table 1). Here also some of the better documented examples come from the annals of wheat breeding. Hybridization between cultivated wheat and species of wild grasses from the genera *Aegilops*, *Agropyron*, and *Secale* has been used to transfer various traits, including salt tolerance and disease resistance, into the crop (*8*). Advances in intergeneric gene transfer continue today. For example, the transfer of traits of cold tolerance, insect tolerance, and disease resistance from *Solanum lycopersicoides* to cultivated tomato by intergeneric hybridization may now be possible because Rick and his co-workers have succeeded in obtaining sesquidiploid hybrids between *S. lycopersicoides* and cultivated tomato (*9*).

Methods for production of hybrids. Natural barriers to interspecific and intergeneric hybridization make creating such hybrids difficult. Successful gene transfers by these methods begin with pollination of the flowers of one of the two species with pollen from the other. The gametes of the two species unite, and successive cell divisions produce an embryo. Development of the embryo and the endosperm associated with it gives rise to a mature seed, which upon germination produces a hybrid plant. The resulting complement of chromosomes must be stable so that the hybrid is fertile. Death or sterility can occur because of failure at any of the many steps in the process leading to a hybrid plant.

Even if an interspecific cross produces a viable zygote, incompatible genic interactions can prevent normal embryo or endosperm development. In such situations, the embryo may not survive. Organ culture techniques pioneered in the 1930s have been used to culture isolated embryos. The conditions used are designed to supply the life support for the hybrid embryo that is normally supplied by maternal tissue and the endosperm in early stages of embryo development and by the cotyledons (or in cereals the endosperm) during germination (*10*). Rescue of embryos in culture was a key tool used in obtaining the sesquidiploid of tomato and *S. lycopersicoides* (*9*).

The youngest immature embryos that can be cultured in vitro

Table 1. Examples of agriculturally important genes and traits transferred to crop plants by interspecific or intergenic hybridization. Though selective, the examples given are representative of the plant families in which such transfers have been most successful. The two families dominating the list are the Gramineae (wheats, oat, rice, and maize), and the nightshade family, Solanaceae (tomato, potato, and tobacco). TMV, tobacco mosaic virus.

Crop species	Donor species	Trait
Avena sativa (oat)	*A. sterilis*	Increase yield 25–30%
Beta vulgaris (sugarbeet)	*B. procumbens*	Sugarbeet nematode resistance
Brassica napus (swede turnip)	*B. campestris*	Clubroot resistance
Cucurbita pepo (pumpkin)	*C. lundelliana*	Mildew resistance
Gossypium hirsutum (cotton)	*G. tomentosum*	Nectariless (decreased incidence of boll rot)
Gossypium hirsutum	*G. raimondii*	Rust resistance
Lycopersicon esculentum (tomato)	*L. hirsutum*	Bacterial canker resistance
Lycopersicon esculentum	*L. peruvianum*	Nematode resistance
Lycopersicon esculentum	*L. peruvianum*	Jointless (facilitates clean fruit harvest without stems)
Lycopersicon esculentum	*L. peruvianum*	TMV resistance
Lycopersicon esculentum	*L. pimpinellifolium*	Fusarium wilt race 1 resistance
Nicotiana tabacum (tobacco)	*N. glutinosa*	TMV resistance
Nicotiana tabacum	*N. longiflora*	Blackfire resistance
Oryza sativa (rice)	*O. nivora*	Grassy stunt virus resistance
Ribes nigrum (black currant)	*R. sanguineum*	Mildew resistance
Ribes nigrum	*R. grossularium*	Gall mite resistance
Solanum tuberosum (potato)	*S. acaule*	Potato virus X resistance
Solanum tuberosum	*S. demissum*	Late blight resistance, leaf roll resistance, potato virus Y resistance
Solanum tuberosum	*S. stoloniferum*	Late blight field resistance, potato virus A resistance, potato virus Y resistance
Triticum aestivum (bread wheat)	*Aegilops comosa*	Stripe rust resistance
Triticum aestivum	*Aegilops ovata*	High kernel protein
Triticum aestivum	*Aegilops speltoides*	Stem rust resistance
Triticum aestivum	*Aegilops squarrosa*	Leaf rust resistance
Triticum aestivum	*Aegilops umbellulata*	Leaf rust resistance
Triticum aestivum	*Agropyron elongatum*	Leaf rust resistance, drought tolerance
Triticum aestivum	*Secale cereale*	Yellow rust resistance, powdery mildew resistance, winter hardiness, leaf rust resistance, stem rust resistance
Triticum aestivum	*T. monococcum*	Stem rust resistance
Triticum aestivum	*T. timopheevi*	Stem rust resistance
Triticum durum (durum wheat)	*T. monococcum*	Stem rust resistance
Zea mays (maize)	*Tripsacum dactyloides*	Northern corn leaf blight resistance

generally are those that show observable signs of differentiation. Treatment of the ovule or seed with plant hormones may allow development of the embryo within the incompatible ovule until a stage is reached at which the embryo can be cultured in vitro. For example, gibberellic acid treatment of an immature wheat kernel bearing a wheat-barley hybrid embryo will keep the embryo alive until it is approximately 10 days old, at which time it can be removed and cultured (*11*). When the cultured embryo has fully differentiated, it can then be transferred to a suitable medium for growth and development.

After a hybrid plant has been successfully recovered, differences in the number or compatibility of parental chromosomes may cause sterility. Cytogenetic manipulations have been instrumental in obtaining stable gene transfers. Sterility may result from incomplete or unstable pairing of chromosomes during cell division. It is sometimes possible to facilitate the cross by doubling the chromosome number of one or both of the parents, usually with the use of the mitotic inhibitor colchicine. Advanced generations are then backcrossed to the cultivated parent and monitored cytogenetically to select progeny with chromosomes from the donor species. Further manipulation may result in stable lines with a chromosome pair from the noncrop parent, either added to or substituted for a pair of the crop's chromosomes [(*12*) and Fig. 2].

For a desired gene from the donor to be incorporated into a chromosome of the crop variety, recombination must take place. If the two species are closely related, natural pairing and recombination may occur. Treatments such as irradiation can be used to induce translocation of a chromosome fragment from the donor to a crop chromosome, thus stabilizing the desired gene carried on the donor fragment (*13*).

Development of useful crop varieties. Even when successful, interspecific and intergeneric gene transfers made by sexual methods are laborious and time-consuming; moreover, other problems must be solved before a genotype useful for crop production is obtained. Even after six backcross generations in an intraspecific gene transfer, the natural process of recombination frequently will not separate tightly linked genes (*14*). This means that undesired traits that affect crop quality, yield, or adaptation may be carried along with the desired gene. The difficulty of separating linked deleterious genes has commonly limited the commercial potential of hybridization-derived varieties (*15*). Even if a desired gene is successfully separated from linked deleterious genes, its inheritance and expression may be unpredictably altered in the new genetic background.

Gene Transfer by Nonsexual Methods

Development of nonsexual methods for gene transfer in plants has been possible because plant cells, organs, and tissues can be cultured in vitro. Embryo rescue, which has been central to the success of wide crosses made with sexual methods, is one of many examples of such manipulations. Many of the methods for nonsexual gene transfer depend on our ability to produce in certain plant species (through a process called regeneration) fully differentiated plants from nonsexual tissues or organs. The starting material for regeneration can be pieces of leaves or stems or even various undifferentiated clumps of cells in culture. In some species, regeneration is possible starting even with a single somatic cell.

Concerted work to develop and exploit nonsexual methods for gene transfer to crops is a relatively recent development. Methods based on cell fusion have been studied for about 20 years. Approaches that use recombinant DNA, from which some agriculturally useful crop varieties may soon be obtained, became possible in 1983. There are few examples in which traits of any conceivable use in agriculture have been successfully transferred by nonsexual methods to crops. There are no examples of which we are aware in which crop varieties so derived are being used in commercial agriculture. The first field tests of crop plants modified by gene transfer with recombinant DNA were conducted in 1986. Thus, the introduction of these methods to crop improvement is in its infancy. Neverthe-

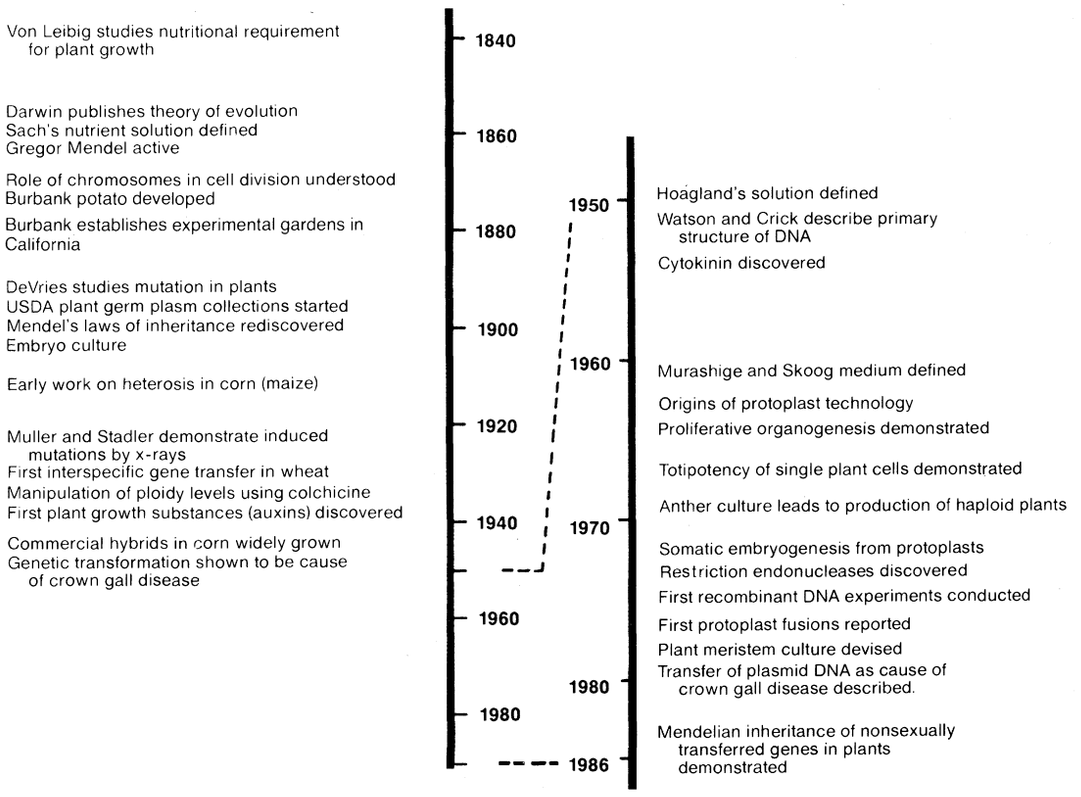

Fig. 1. Scientific and technological developments in several areas have steadily enhanced our ability to manipulate the traits of crop plants. The time line identifies some of the major advances that provide the essential scientific background for crop science at present and in the future.

less, some of these methods have the potential to influence profoundly the future of crop improvement.

Cell fusion. In the 1960s, methods were developed that allowed preparation of large numbers of single plant cells stripped of their cell walls (protoplasts). Protoplasts from different species can be induced to fuse by exposure to certain chemicals or electric current. The resulting somatic hybrid may be grown in vitro to produce callus tissue from which in certain species a whole plant can be regenerated. The objective may be to combine the chromosomes of species that are sexually incompatible or, as a shortcut, to combine the nuclear genome of one species with the cytoplasm (that is, the organellar genomes) of another. Much work has been devoted over the past two decades to attempts to exploit cell fusion as a method to effect gene transfers between different species, but many problems have arisen and little of commercial use in agriculture has resulted (*16*).

The greatest potential for the use of cell fusion methods may be in creating new crop varieties containing the nuclear genome of one species in the cytoplasmic background of another (nuclear transfer) or in a mixed cytoplasm with organelles from both species (cybrids). The plant mitochondrion and chloroplast each possess DNA, which encodes some of the proteins that make up the structure and metabolic machinery of the organelle. (The majority of proteins found in organelles are imported, however, and are encoded by genes in the nucleus.) Although complex and not completely understood, certain agriculturally important traits are the result of interaction between the nuclear and cytoplasmic genomes. For example, a form of male sterility that is useful in commercial production of hybrid seeds results from nuclear-mitochondrial interactions. In species where cytoplasmic male sterility is not naturally found, making artificial combinations between nuclear and cytoplasmic genomes can result in such sterility. Well-studied cases include species of tobacco (*Nicotiana* spp.) and combinations of rapeseed (*B. napus*) nuclear genomes with cytoplasms from radish (*Raphanus sativus*) (*17*).

Much has been written about the potential for recombining genetic traits by cell fusion methods, and considerable work has been done to develop systems that will allow exploitation of these methods. It seems clear as a result of this activity, however, that many difficult questions remain to be answered. As with sexual hybridization, for example, incompatibility between parental species may result in hybrid instability (*18*). As explained above, there are several levels at which incompatibility can operate; somatic cell fusion bypasses some but not all the possible problems. Fusion may also severely compromise the ability of the resulting cell to undergo regeneration. The difficulties unique to cell fusion methods themselves notwithstanding, these methods share with plant breeding the drawbacks of imprecision. Deleterious genes will be transferred, and may be linked to, those encoding desirable traits. In cases where the target is a specific trait encoded by the nuclear genome, selection for the desired trait is made more difficult because a fusion-derived hybrid may have a mixture of cytoplasmic genes in addition to nuclear genes. Gene transfer methods based on cell fusion, like sexual hybridization, result in transfer of many genes. From the resulting hybrids, backcrossing or other schemes that use sexual methods are still needed to obtain plants with the desired combination of parental traits.

Gene transfer by manipulating DNA directly. Methods for transferring DNA directly from one organism to another originated in experiments of the 1940s that established DNA as the chemical basis of genetic inheritance. Transfer of genes or chromosome segments in bacteria by sex factors (plasmids), viruses (transducing phage), or uptake of purified DNA (transformation) were well understood before in vitro gene splicing by recombinant DNA was demonstrated in 1973. Virally mediated gene transfer, direct DNA uptake, and microinjection have been successfully applied to animal cells. All of these approaches are also being applied to plants, but the approach that is most advanced is a bacterially mediated DNA transfer system unique to higher plants.

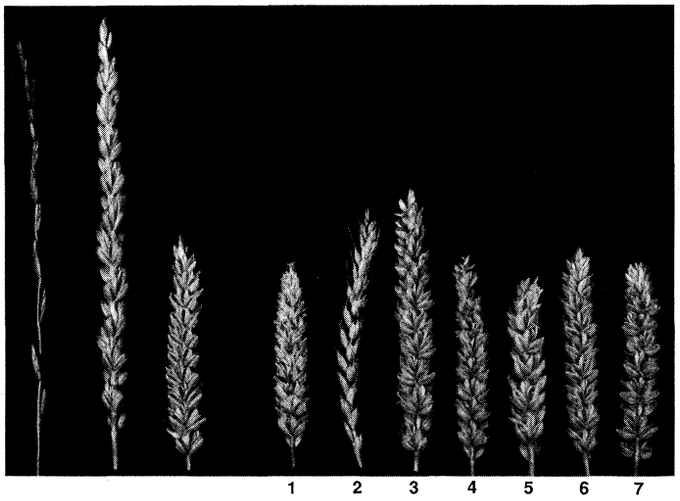

Fig. 2. Spikes of the parental species, intergeneric hybrid, and wheat addition lines developed by cytogenetic manipulation of the intergeneric hybrid. Individual addition lines have a single pair of *Elytrigia* chromosomes in addition to the 21 chromosome pairs normally found in wheat. Left to right: *Elytrigia elongata*, intergeneric hybrid between *E. elongata* and wheat, *Triticum aestivum* cultivar Chinese Spring (common wheat), 1 to 7 label wheat addition lines that have a single *Elytrigia* chromosome pair 1 to 7 added to the full chromosome complement of wheat (*12*). (Photograph courtesy of J. Dvorak, Department of Agronomy and Range Science, University of California, Davis, 95616.)

Nonsexual DNA transfer techniques make possible manipulations that are outside the repertoire of breeding or cell fusion techniques. Genes can be accessed from exotic sources—plant, animal, bacterial, even viral—and introduced into a crop. Because the DNA elements that control gene expression can, and often must, be modified for proper function in the new host, it is possible to control timing, tissue specificity, and expression level of transferred genes. Endogenous plant genes may even be reprogrammed through the reintroduction of an engineered gene. Thus, nonsexual DNA transfer methods expand the sources of variability available for crop improvement to include all living things, and also allow manipulation to achieve quantitative control over gene expression. With methods available for chemically synthesizing DNA or causing specific mutations in naturally occurring genes, entirely novel genes can be used. All of these effects can in principle be achieved with great precision.

Agrobacterium-*mediated gene transfer. Agrobacterium*-mediated gene transfer exploits the natural ability of *Agrobacterium tumefaciens* to transfer DNA into plant chromosomes (*19*). *A. tumefaciens* is a plant pathogen that transfers a set of genes encoded in a region called T-DNA of the Ti plasmid into plant cells at wound sites. The pathogen has a wide range of hosts among higher plants, including many dicotyledonous (broad-leaved) crop plants. The result of gene transfer is generally a tumorous growth called a crown gall in which the T-DNA is stably integrated into a host chromosome. The site of integration appears to be random. The tumor phenotype, which can be maintained indefinitely in tissue culture, results from the expression of genes on the T-DNA that alters the normal balance of growth substances (phytohormones) in transformed cells. The ability to cause crown gall disease can be removed by deletion of genes in the T-DNA without loss of DNA transfer and integration

functions; an *Agrobacterium* strain that does not cause disease is said to be disarmed. In a disarmed strain, the DNA to be transferred is attached to border sequences that define the end points of an integrated T-DNA. To be active in T-DNA transfer, the *Agrobacterium* strain must also express a complex set of virulence genes also encoded on the Ti plasmid.

In the laboratory, disarmed *Agrobacterium* strains can be used to transfer genes to protoplasts with partially regenerated cell walls, suspension cell cultures, leaf pieces, and stem segments. The critical step is recovering a whole plant from cells that have acquired integrated T-DNA. Selectable markers are used to identify and favor the growth of transformed cells. For example, the gene to be transferred is linked within the T-DNA to a gene conferring resistance to an antibiotic, such as kanamycin, which prevents plant growth. Plant cells that survive and can divide and undergo development in the presence of kanamycin are generally only those containing the engineered T-DNA. Because all the genes between the T-DNA borders are transferred, cells expressing the kanamycin-resistance gene are expected to contain any other genes engineered into the T-DNA region (*19*).

Gene transfer by means of engineered *Agrobacterium* strains has become routine in many laboratories for plants in the nightshade family such as tobacco, tomato, and petunia (*19*). Extension to other, more important crops has been difficult but progress is being made, particularly with species in other dicotyledonous families. In some cases (for example, soybean) gene transfer has been demonstrated in cultured cells (*20*), but the ability to regenerate a complete plant from cultured cells containing T-DNA has not yet been reported. Although data have been cited that *Agrobacterium* can transfer T-DNA to monocotyledonous hosts (*21*), clear evidence of T-DNA integration exists only for asparagus, and, even in that case, no transformed plants have been described. Because *A. tumefaciens* does not induce crown galls on monocotyledonous plants, such as rice, corn, and wheat, other methods of gene transfer are being developed for these important crops.

Applications of Agrobacterium-*mediated gene transfer to agriculture.* In the 4 years since this method has been available, exciting progress has been made in applying it to the transfer of agriculturally useful genes. These include genes for insect and disease resistance and tolerance to safer herbicides. This is the only method of nonsexual gene transfer for which there are now practical and useful examples that are being tested and that are serious candidates for use in agriculture.

An early goal in the use of recombinant DNA for crop improvement has been to engineer bacterial or plant genes encoding enzymes that make crop plants tolerant to broad-spectrum, environmentally safer herbicides. One successful strategy has been to transfer a gene for an enzyme that complements the plant enzyme whose action is blocked by the herbicide. This has been done by engineering a bacterial gene so that its enzyme product is insensitive to the herbicide and then transferring it to the plant (*22*). Alternatively, the plant gene itself can be engineered so that the plant produces a larger amount of its own enzyme, making plants that can survive in the presence of the herbicide (*23*). Another strategy is to engineer plants to express genes for enzymes that chemically detoxify the herbicide.

Bacterial genes for insecticidal proteins obtained from *Bacillus thuringiensis* have also been engineered into plants with exciting results. When certain insects feed on these plants, the bacterial toxin within the plant tissues kills them (*24*). The insecticidal protein is considered very safe; it has been widely tested and has been in use for a number of years in a crude powder form made from *B. thuringiensis* cultures. The toxicity of the protein is very specific. It is not toxic to mammals, plants, or even many kinds of insects.

Perhaps the most intriguing and unexpected result of agricultural interest has come from engineering plants with the gene encoding the coat protein from tobacco mosaic virus. Expression of this gene in tobacco and tomato plants resulted in resistance to infection by the virus (*25*). The mechanism of resistance is not understood, but, if it is a general result, we will have at hand a novel genetic approach to the control of virus diseases in plants. The use of insecticides for control of insects that spread certain plant viruses may also be avoided. To date, the search for chemical treatments to prevent or cure infections by plant viruses has been unsuccessful.

A key observation in these early tests of nonsexual gene transfer mediated by *Agrobacterium* is that the genes transferred are inherited predictably. Also, the recipient plant varieties are apparently unchanged except for the acquisition of new traits encoded by the engineered genes. Thus it appears likely that the engineering itself will not compromise plant performance. This does not mean that undesirable results cannot occur. Occasional insertion of engineered DNA into genes that are required for proper functioning of the plant must occur at some frequency. Indeed, such insertions, if not lethal, could be an important scientific tool. The strong selection pressures applied in the gene transfer and regeneration processes, however, undoubtedly favor the recovery of normal plants. In summary, experience suggests that, when a desired trait has been introduced through *Agrobacterium* vectors, the resulting engineered plants are predictable, genetically stable, and useful.

Direct DNA transfer. Purified DNA can be used directly for plant transformation either by direct DNA uptake or by microinjection. Direct DNA uptake involves physicochemical reactions that result in DNA transfer to protoplasts. Microinjection is the mechanical introduction of DNA into cellular compartments with microscopic pipettes. Unlike methods based on *Agrobacterium* systems, direct gene transfer methods are not subject to host range restrictions, but practically are limited by the need to recover a whole plant from the target cells or tissue.

Plant protoplasts can take up nucleic acids directly from the culture medium, a phenomenon first demonstrated with viral RNAs. Integration into plant chromosomes of foreign DNA introduced by direct uptake is a relatively rare event. Treatments, such as polyethylene glycol (PEG) and the application of electrical pulses (electroporation), which increase the permeability of membranes, can result in transformation frequencies of one transformant per thousand protoplasts (*26*). The combination of several uptake-enhancing treatments has increased transformation frequencies into the range of one in a hundred in at least one case (*27*).

With transformation frequencies near 1%, direct DNA uptake becomes an attractive method for gene transfer. In particular, plant species that either are not susceptible to *Agrobacterium* infection or are inefficiently transformed by it might be good candidates for direct DNA uptake if they can be regenerated from protoplasts. Although direct DNA uptake in other plant cells and tissues has been attempted, it has so far been successful only with protoplasts. Thus, application of direct DNA uptake to the cereals may be limited because regeneration of whole plants from protoplasts has not yet been achieved for many cereal species. However, there has been a recent report of regeneration of plants from rice protoplasts and a separate report showing expression of a foreign gene delivered by electroporation to rice protoplasts (*28*). There are reports of stable transformation of maize cell lines by direct DNA uptake (*26*). Thus, prospects for methods to produce engineered plants by direct DNA uptake in the cereal crops are more encouraging.

Microinjection. Microinjection, the most recent addition to the repertoire of plant transformation methods, involves the introduction of DNA solutions under pressure into plant protoplasts by means of micropipettes. The key to successful transformation has

been the development of methods for the immobilization of cells during injection and methods for their subsequent culture (*29*). In one study (*30*), cell lines cultured from microinjected tobacco protoplasts were shown to have integrated the foreign DNA sequences into the nuclear DNA; the average transformation frequency depended on whether the injections were intranuclear (14%) or cytoplasmic (6%). In another study (*31*), transformed cell lines cultured from intranuclear injections of alfalfa protoplasts were identified by screening for enzyme activity encoded by the foreign DNA; transformation frequencies ranged from 15 to 26% depending on the DNA injected. To date, there have been no reports to our knowledge of transformed plants regenerated from cell lines obtained by protoplast microinjection.

Transformation of plant cells by microinjection has only been demonstrated with protoplasts. However, because microinjection is a physical means of introducing DNA, it should be capable of delivering genes into targets other than protoplasts. In this regard, it is important that intact cells have been shown to survive microinjection (*32*). As with direct DNA uptake, microinjection can in principle be used with any crop species from which whole plants can be obtained from single transformed cells.

Virally mediated gene transfer. Viral-based gene expression systems for animals have been developed, both for experimental and therapeutic uses. Some parallel effort has been made to develop vectors based on plant viruses for gene transfer into plants (*33*). In plants, viral-based vectors are not likely to stably transform plant cells because integration of viral genes into plant chromosomes has not been detected. The concept is that the engineered viruses would spread throughout a plant while expressing genes that confer some new trait. Results with the double-stranded DNA of cauliflower mosaic virus in which the strict requirements for gene expression and viral particle packaging have been taken into account in the design of the vector and a recent result from the use of *Agrobacterium* to deliver an infectious maize virus to plants show that virally mediated gene transfer in plants is possible (*34*). The contribution virally mediated gene transfer in plants will make to agriculture, however, is far from clear.

Summary and Conclusions

We believe that outside the agricultural research community few people appreciate how powerful a tool for crop improvement conventional plant breeding has been. The past use of gene transfers between species and even between genera is less appreciated. The history of scientific crop improvement shows how important technological innovation in the past has been in the enhancement of agricultural productivity. Future advances in crop improvement and the solution of the many problems facing agriculture today will depend on the wise use of all resources, including new technology, to advance fundamental knowledge about plants and apply this knowledge in the field.

The advent of recombinant DNA technology has focused scientific and public policy attention on gene transfers between species (*35*). As we have described, however, in plants, and particularly in crop improvement over the past century, interspecific and even intergeneric gene transfer is not new. Gene transfer by recombinant DNA is just the latest in a long history of increasingly more powerful methods available for crop improvement (Fig. 1).

Crop improvement has been a cornerstone of advances in agriculture and in the economic strength of the United States. Technological innovation and continued advances in fundamental science have driven efforts toward crop improvement. A return to prosperity in agriculture and a continuation of the primary role of U.S. agriculture in the economic strength of the nation depend on the availability and wise use of new technology. Research funding and other public policy decisions will profoundly affect the way new technology will be used, in both the public and private sectors of the agricultural research community, for crop improvement.

The research and public policy agenda. The new genetic engineering technologies have the potential to add precisely characterized genes to the preexisting germplasm with which a breeder has to work for a specific crop. Although the result may have considerable economic impact, each addition requires an extensive effort and must be considered as incremental to the 100,000 or so genes it takes to run a crop plant. Many desirable traits or phenotypes are conferred by the coordinated expression of a number of genes. To alter a plant trait in a desirable way, the correct genes to be manipulated must be chosen. This choice depends on an understanding of the biochemical bases of the processes underlying the trait. Our knowledge of the biochemistry of plant traits important in agriculture is, generally speaking, very weak and a major area for future research.

Mechanisms for research funding are needed that bring the practice of scientific disciplines together and that focus on the advances in fundamental knowledge needed to ensure continuing advances in technology (*36*). Much of the attention within the agricultural research community in recent years has been given to the competing demands of plant breeding and molecular biology for scarce resources.

There are already numerous examples of ways in which recombinant DNA research has depended on past achievements in plant breeding and genetics. Proven superior crop varieties developed by plant breeders provide the genetic background for introduction of new genes developed in the laboratory. Isogenic lines (that is, plants differing in a single trait) are being used by molecular biologists to isolate genes related to specific traits such as disease resistance. Mobile genetic elements, first studied by genetic and cytogenetic analysis in the 1940s, have been used to isolate a specific gene from genetic variants in maize (*37*).

There is great potential for new methods to enhance the plant breeding process. Many genetic loci can now only be evaluated in a mature plant. If a transformation event can be identified in which a trait detectable in the embryo has become tightly linked to other traits, the size of experimental plots might be reduced by allowing selection at the embryo stage, or a breeding program may be accelerated by not having to complete a growing season to evaluate results. Either cloning a gene from a crop plant or inserting some unique piece of DNA can establish a specific chromosome position marker that along with restriction fragment length polymorphisms will lead to superior genetic maps for major crops (*38*). These maps will not only serve the breeder as a source of new markers to evaluate crosses but may also permit isolation of potentially important genes, which the plant geneticist can link to known markers.

Improved genetic maps together with traditional cytogenetic techniques may allow for gene isolation from microdissected chromosomes. Such research may lead to new technology for isolating poorly characterized genes, such as those involved in disease resistance, and may expand our knowledge of chromosome architecture and its effects on gene expression. Technological innovations motivated by the use of recombinant DNA in gene transfer may likewise advance cytogenetics. Specific chromosome transfers via microinjection might allow the transfer of complex multigenic traits. Organelle or nuclear transplants could allow traits, such as male sterilities, to be transferred or created. Such transfers, if successful, could be viewed as a more efficient way of making wide crosses between species. As a result, the range of species from which such transfers can be made will be extended.

Research funding should also address the major environmental

issues that face agriculture. In particular, attention should be given to the ways in which the uses of genetic manipulation may reduce dependence on hazardous chemicals in agricultural production systems and promote long-term sustainability of highly productive agriculture (*39*). The possibilities are well illustrated by the recent reports of successful expression of genes conferring tolerance to safer herbicides, resistance to virus infection, and toxicity to insects.

Concerns over the regulation of the uses of recombinant DNA technology have been widely discussed. The unusual power of the technology, uncertainty over the behavior to be expected from organisms modified in novel ways, and the past 40 years of experience with chemicals in the environment make it reasonable and indeed desirable that genetically modified organisms be introduced cautiously. The regulatory framework should, however, be based on a thorough understanding of the scientific issues and should be properly calibrated to the likely risks and rewards (*40*). As we have shown here, there is a wealth of past experience with interspecific gene transfer in crop plants that is relevant to this question and is in general reassuring.

Finally, consideration of the technology used in gene transfer highlights the critical importance of collecting, preserving, and characterizing the world's germplasm for plants and microorganisms. Many of the advances that will enhance agriculture in the future will probably be made as the result of entirely unforeseeable ideas and manipulations by future generations of scientists. We must preserve the raw material from which our successors will work.

REFERENCES AND NOTES

1. R. W. Allard, *Principles of Plant Breeding* (Wiley, New York, 1960); F. N. Briggs and P. F. Knowles, *Introduction to Plant Breeding* (Reinhold, New York, 1967); N. W. Simmonds, *Principles of Crop Improvement* (Longman, New York, 1979); J. Whitson, R. John, H. S. Williams, *Luther Burbank: His Methods and Their Practical Application* (Burbank, New York, 1914–15), vol. 12.
2. E. J. Wellhausen, L. M. Roberts, E. Hernandez X., *Races of Maize in Mexico* (Bussey Institute, Harvard University, Cambridge, MA, 1951).
3. H. G. Wilkes, *Econ. Bot.* **31**, 254 (1977). For a review of this general topic, see C. B. Heisser, Jr., *Bot. Rev.* **39**, 347 (1973).
4. E. S. McFadden, *J. Am. Soc. Agron.* **22**, 1050 (1930); H. C. Sharma and B. S. Gill, *Euphytica* **32**, 17 (1983).
5. C. M. Tucker and G. W. Bohn, *Mo. Agric. Exp. Stn. Bull. 387* (1937), p. 31; G. W. Bohn and C. M. Tucker, *Science* **89**, 603 (1939); J. C. Walker, *Am. Phytopathol. Soc. Monogr. 6* (1971).
6. C. A. Newell and R. Hymowitz, *Crop Sci.* **22**, 1062 (1982).
7. N. W. Simmonds, Ed., *Evolution of Crop Plants* (Longman, New York, 1976).
8. G. Fedak, *Euphytica* **34**, 673 (1984).
9. C. M. Rick, J. W. DeVerna, R. T. Chetelat, M. A. Stevens, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3580 (1986); J. W. DeVerna, R. T. Chetelat, C. M. Rick, M. A. Stevens, in Tomato Biotechnology Symposium, Davis, CA, 20 to 22 August 1986 (Liss, New York, in press).
10. For a review, see G. B. Collins, N. L. Taylor, J. W. DeVerna, in *Gene Manipulation in Plant Improvement*, J. P. Gustafson, Ed., *Stadler Genet. Symp. No. 16*, 323 (1984).
11. A. K. M. R. Islam, K. W. Shepherd, D. H. B. Sparrow, *Proceedings of the Fifth International Wheat Genetics Symposium*, New Delhi, India, 23 to 28 February 1978 (Indian Agricultural Research Institute, New Delhi, 1979), p. 356; G. E. Hart, A. K. M. R. Islam, K. W. Shepherd, *Genet. Res.* **36**, 311 (1980).
12. J. Dvorak and K. C. Chen, *Can. J. Genet. Cytol.* **26**, 128 (1983); G. R. Hart and N. A. Tuleen, *Genet. Res.* **41**, 181 (1983); J. Dvorak, *Can. J. Genet. Cytol.* **22**, 237 (1980).
13. E. R. Sears, *Stadler Genet. Symp. No. 4*, 23 (1972).
14. The length of a chromosome is measured in genetic terms in units, called centimorgans (cM), that are dependent on recombination frequencies along the length of the chromosome. After six backcross generations (which is the generally accepted minimum for breeding new genetic information into a crop variety) the length of a donor chromosome segment bearing the gene of interest was 24 cM on a recipient chromosome 50 cM in length (48%), 32 cM on a recipient chromosome 100 cM in length (32%), and 38 cM on a recipient chromosome 200 cM in length (19%). These theoretical estimates were made on the assumption that donor and recipient were of the same species. If they are not of the same species, as in an interspecific hybrid, recombination is less likely to occur within the chromosome segment, which increases the probable length of the transferred donor chromosome segment after six backcrosses. P. Stam and A. C. Zeven, *Euphytica* **30**, 227 (1981).
15. D. R. Knott and J. Dvorak, *Annu. Rev. Phytopathol.* **14**, 211 (1976); H. T. Stalker, *Adv. Agron.* **23**, 111 (1980).
16. D. A. Evans, W. R. Sharp, J. E. Bravo, in *Crop Species*, vol. 2 of *Handbook of Plant Cell Culture*, W. R. Sharp, D. A. Evans, P. V. Ammirato, Y. Yamada, Eds. (Macmillan, New York, 1984), pp. 47–68.
17. E. Galun and D. Aviv, in *Techniques for Propagation and Breeding*, vol. 1 of *Handbook of Plant Cell Culture*, D. A. Evans, W. R. Sharp, D. V. Ammirato, Y. Yamada, Eds. (Macmillan, New York, 1983), pp. 358–392; G. Pelletier *et al.*, *Mol. Gen. Genet.* **191**, 244 (1983).
18. C. T. Harms, *Q. Rev. Biol.* **58**, 325 (1983).
19. R. T. Fraley *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4803 (1983); G. An, B. D. Watson, S. Stachel, M. P. Gordon, E. W. Nester, *EMBO J.* **4**, 277 (1985); R. B. Horsch *et al.*, *Science* **227**, 1229 (1985).
20. D. Facciotti, J. K. O'Neal, S. Lee, C. K. Shewmaker, *Bio-Technology (New York)* **3**, 241 (1985).
21. S.-P. Hernalsteens, L. Thiu-Toong, J. Schell, M. Van Montagu, *EMBO J.* **3**, 3039 (1984).
22. L. Comai *et al.*, *Nature (London)* **317**, 741 (1984).
23. D. M. Shah *et al.*, *Science* **233**, 478 (1986).
24. M. J. Adang *et al.*, in *Sixth International Congress on Plant Tissue Cell Culture*, University of Minnesota, Minneapolis, 3 to 8 August 1986 (University of Minnesota, Minneapolis, 1986), p. 404.
25. P. P. Abel *et al.*, *Science* **232**, 738 (1986).
26. F. A. Krens and R. A. Schilperoot, in *Laboratory Procedures and Their Application*, vol. 1 of *Cell Culture and Somatic Cell Genetics of Plants*, I. K. Vasil, Ed. (Academic Press, New York, 1984), pp. 522–534; M. E. Fromm, L. P. Taylor, V. Walbot, *Nature (London)* **319**, 791 (1986).
27. R. D. Shillito, M. W. Saul, J. Pazskowski, M. Muller, I. Potrykus, *Bio-Technology (New York)* **3**, 1099 (1985).
28. M. Y. Coulibaly and Y. Demarly, *Z. Pflanzenzuecht.* **96**, 79 (1986); Y. Yamada, Z.-Q. Yang, D.-T. Tang, *Plant Cell Rep.* **5**, 85 (1986).
29. For review see A. Crossway *et al.*, *BioTechniques* **4**, 320 (1986).
30. A. Crossway *et al.*, *Mol. Gen. Genet.* **202**, 179 (1986).
31. T. J. Reich, V. N. Iyer, B. L. Miki, *Bio-Technology (New York)* **4**, 1001 (1986).
32. K. Nomura and A. Komamine, *Plant Sci.* **44**, 53 (1986).
33. J. W. Davies *et al.*, in *Molecular Form and Function of the Plant Genome*, NATO Workshop (Plenum, New York, 1984), pp. 371–385; A. Siegel, *Plant Mol. Biol.* **4**, 327 (1985); L. van Vloten-Doting, J. F. Bol, B. Cornelissen, *ibid.*, p. 323; P. Ahlquist, R. French, M. Janda, L. S. Loesch-Fries, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7066 (1984); R. Hull and J. W. Davies, *Adv. Virus Res.* **28**, 1 (1983).
34. N. Brisson *et al.*, *Nature (London)* **310**, 511 (1984); N. Grimsley, T. Hohn, J. W. Davies, B. Hohn, *ibid.* **325**, 177 (1987).
35. U.S. Department of Agriculture Animal and Plant Inspection Service, *Fed. Regist.* **51**, 23352 (1986).
36. F. Buttel, *Proceedings of the Institute for Alternative Agriculture*, Third Scientific Symposium, Washington, DC, March 1986 (Institute for Alternative Agriculture, Greenbelt, MD, 1986), p. 4.
37. B. McClintock, *Science* **226**, 792 (1984); N. V. Federoff, D. B. Furtek, O. E. Nelson, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3825 (1984).
38. M. Soller and J. S. Beckman, *Theor. Appl. Genet.* **67**, 25 (1984); J. S. Beckman and M. Soller, *ibid.*, p. 35; J. E. Bishop, *Wall Street Journal*, 27 May 1986, p. 2.
39. H. Hauptli and R. M. Goodman, *Proceedings of the Institute for Alternative Agriculture*, Third Scientific Symposium, Washington, DC, March 1986 (Institute for Alternative Agriculture, Greenbelt, MD, 1986), p. 26; C. M. Benbrook and P. B. Moses, *Technol. Rev.* **89**, 55 (1986).
40. H. Hauptli, R. M. Goodman, N. Newell, *Bio-Technology (New York)* **3**, 437 (1985); W. J. Brill, *Science* **227**, 381 (1985).

41. We thank G. Bruening, V. Walbot, B. Stefansson, C. Houck, and C. Shewmaker for reviewing an earlier draft, L. Schlosser for assistance with figures, and C. Facciotti for assistance in preparing the manuscript.