Plant Genetics: Increasing Crop Yield

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Summary: Cell cultures of crop plants provide new opportunities to recover induced mutations likely to increase crop yield. Approaches include regulating respiration to conserve carbon fixed by photosynthesis, and increasing the nutritive value of seed protein. They depend on devising selecting conditions which only desired mutant cells can survive. Protoplast fusion offers some promise of tapping sources of genetic variation now unavailable because of sterility barriers between species and genera. Difficulties in regenerating cell lines from protoplasts, and plants from cells, still hamper progress but are becoming less severe. Recombinant DNA techniques may allow detection and selection of bacterial cell lines carrying specific DNA sequences. Isolation and amplification of crop plant genes could then lead to ways of transforming plants that will be useful to breeders.

Plant genetics is going through a period of rapid change in concepts and methods. These changes reflect recent developments in molecular biology that can be applied to plant systems, as well as research that exploits features that are unique to plants. In this article I discuss somatic cell genetics, and the implications of work with recombinant DNA. My objective is to show how certain ideas may be applied to plant breeding to increase crop yield.

A variety of factors limit the yield of field crops. These range from the CO_2 concentration in the atmosphere to the density of plants in the stand. Table 1 shows the major limiting factors grouped according to the energy cost of relieving them. Seed costs are relatively small. In 1975 they made up about 3.0 percent of the total farm production costs for the United States (*1*). Expenditure on fertilizer and lime was about three times as great. Genetic improvements that increase yield and reduce energy costs are clearly of great importance and generally require only modest expenditure on research and development. Recent reviews of progress in plant somatic cell genetics have appeared in collections of papers dealing with haploids (*2*), the culture of cells, tissues, and organs (*3, 4*), protoplasts (*5*), and chloroplasts and mitochondria (*6*). Other reviews (*7*) relate work in somatic cell genetics specifically to plant improvement. I shall consider these topics under three general headings: cell culture systems, protoplast fusion, and recombinant DNA.

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Cell Culture Systems and the Regulation of Respiration

Less than 10 years ago, plant cell culture was a small and relatively specialized branch of experimental botany compared with the expanded interest in this field today. The early work established nutrient requirements and optimum concentrations of inorganic salts, carbon sources, and hormones to promote vigorous growth of cell lines as unorganized callus on solid media or cell suspensions in liquid media. It was found that shoot or root formation could be promoted by manipulating hormone concentrations and the intensity and duration of light. The ready availability in recent years of inexpensive laminar flow hoods has helped by providing contamination-free work surfaces. As a result, cell cultures of a great many plants can be grown by following well-established procedures (*8*). The most rapidly growing *Haplopappus* or soybean suspension cultures have doubling times of 22 to 31.5 hours (*9*), a rate of division which is about half that in a whole plant meristem and from 66 to 100 times slower than a bacterium such as *Escherichia coli*. Nevertheless, plant cell cultures can provide large populations in a shorter time and smaller space than either plants or seedlings, and are thus well suited for mutant induction and selection.

Net photosynthesis is equivalent to gross photosynthesis less respiration. Respiratory loss of CO_2 occurs through photorespiration and dark respiration. Because CO_2 is the most important limiting factor in the photosynthesis of field crops, methods of reducing CO_2 losses due to respiration are clearly worth exploring as a means of increasing yield. Unorganized green tissues grown in the light use CO_2 as a carbon source (*10*). Direct selection based on differential rates of growth within a cell line treated with a mutagen might therefore be used to recover desirable mutants with enhanced net photosynthesis. Mutants with reduced rates of respiration, and hence greater net photosynthesis, might also be obtained.

Photorespiration involves the oxidation of glycolic acid, an early product of carbon fixation, to glyoxylic acid. Glyoxylic acid is further converted either to formic acid and CO_2, or to glycine which is further metabolized to serine with release of NH_3 and CO_2 (*11, 12*). Several mechanisms of glycolate biosynthesis are known. One involves the enzyme ribulose diphosphate (RuDP) carboxylase (E.C. 4.1.1.39) which also possesses an oxygenase activity that produces phosphoglycolic acid which is then hydrolyzed to glycolic acid (*13*). Enhancement of RuDP oxygenase activity at the expense of oxygenase activity by increased O_2 levels could thus explain why oxygen inhibits photosynthesis (the Warburg effect). To date, attempts to block the oxygenase without inhibiting carboxylase activity have failed (*13*). However, Zelitch (*12*) found that glycidate, an epoxide inhibitor of glycolate synthesis in tobacco leaf disks, had no effect on the oxygenase activity of the isolated enzyme in air or oxygen. Glycidate also had no effect on RuDP oxygenase activity in tobacco leaf disks under conditions where glycolic acid synthesis was inhibited by at least 50 percent. This suggests that RuDP oxygenase activity accounts for only part of the glycolate synthesized and that another mechanism, that is glycidate sensitive, is responsible for the bulk of synthesis in tissues undergoing rapid photorespiration. Another possibility is that glycidate inhibits the breakdown of some other metabolite, such as glutamate, that inhibits the synthesis of glycolate.

Photosynthesis is limited by photorespiration in many major crop species including wheat, rice, soybean, potato, peanut, barley, sugar beet, cassava, and banana. These are called C_3 plants because their primary photosynthetic products are three-carbon compounds. The net rates of CO_2 fixation in C_3 plants are usually about half those of C_4 plants such as maize, sorghum, millet, and sugarcane (*11*). The more efficient C_4 plants characteristically have two kinds of photosynthetic tissue. The mesophyll cells fix CO_2 by way of phosphoenolpyruvate (PEP) carboxylase (E.C. 4.1.1.31) to form oxaloacetate and thence the four-carbon compounds malate or aspartate. A current hypothesis is that one of these acids, depending on the plant species, is transferred to the cells sheathing the vascular bundles of the leaves where decarboxylation occurs (*14*). The CO_2 released in the bundle sheaths is then refixed by RuDP carboxylase as in the C^3 species. The peculiar anatomy of C^4 species may thus be responsible for some part of their greater yield. For example, locally high CO_2 concentrations in the bundle sheath cells may partially inhibit photorespiration (*15*). However, unorganized callus tissue of a C_4 species was found to lack detectable photorespiration which was clearly shown by callus of a C_3 species (*16*). In the genus *Atriplex*, conventional sexual crosses between C_3 and C_4 species have been made. Among F_2 and F_3 progeny of hybrids between *A. hastata* and *A. rosea*, individuals with the anatomy of the C_4 parent *A. rosea* were present but none had photosynthetic rates that approached it (*17*). These findings suggest that C_4 rates of photosynthesis do not depend on anatomical features and that they may therefore be attained in C_3 plants by genetic modifications that fall short of C_4 anatomy.

Although plants with low rates of photorespiration and high rates of net photosynthesis were observed within a tobacco cultivar, attempts to establish this phenotype in several generations of pedigree selection were unsuccessful (*18*). Several other approaches under test include selection experiments in which mutagen-treated tobacco haploid cell lines are grown photoautotrophically. The cells are first placed in a medium without sucrose and are exposed to 1 percent CO_2 in air. The ambient atmosphere is then changed to 60 percent O_2 with 0.03 percent CO_2 (the balance being N_2) whereupon most cells, like seedlings with normal rates of photorespiration, die after a few weeks. Some oxygen-resistant cell lines selected under these conditions are expected to have slower than normal rates of photorespiration (*19*). Cell lines may also be selected for vigorous autotrophic growth in low concentrations of CO_2 (*19*).

Table 1. The major factors limiting field crop productivity grouped according to the energy cost of relieving them.

Fixed:
CO_2 concentration (0.03 percent)
Length of growing season
Total sunshine
Soil type
Expensive to vary:
Available moisture (irrigation)
Soil fertility (fertilizer)
Pests and diseases (pesticides)
Weeds (cultivation, herbicides)
Storage after harvest
Nutritional value
Marketing
Inexpensive to vary:
Crop density
Planting date
Seed quality (disease-free, high viability, uniform size)
Crop genotype

Another method of controlling photorespiration is to select for cell lines with increased intracellular concentrations of metabolites that can be expected to exert a feedback control on the synthesis of a branch point intermediate. For example, recent work by Oliver and Zelitch (*20*) showed that glycolate synthesis in tobacco leaf tissue is inhibited and that net photosynthesis increases when leaf disks are floated on solutions of glutamate, aspartate, phosphoenolpyruvate, or glyoxylate. Since glycolate oxidation is a key step in photorespiratory CO_2 release, selection for the accumulation of some of these compounds may lead to feedback inhibition of glycolate synthesis and thus reduction of photorespiration. For example, some cell lines resistant to the analog α-methylaspartate (*19*) may have an increased pool of aspartate. Blocking glycolate synthesis and glycolate oxidation with other inhibitors was shown to reduce photorespiration and increase, by more than 50 percent, net CO^2 fixation in tobacco leaf disks (*11*).

An entirely different approach to effecting economies in carbon metabolism is to regulate the alternate respiratory pathway in plant mitochondria (*21*). This pathway is insensitive to cyanide and antimycin A, both of which block cytochrome oxidase (E.C. 1.9.3.1). It has a low energy yield (P/O ratio = 1) compared with the more efficient cytochrome oxidase (P/O ratio = 3). Polacco and Polacco (*22*) set out to select mutant cell lines of tobacco in which the alternate pathway was eliminated. Their premise was that if all dark respiration could be channeled through the more efficient terminal oxidase, plant yield might be increased. From 25 to 75 percent of respiration in rapidly growing intact tobacco cells was cyanide resistant. A first attempt was based on an earlier observation (*23*) that a mutant of *Ustilago maydis*, resistant to the mitochondrial succinic dehydrogenase inhibitor carboxin, lacked the alternate oxidase. A stable carboxin-resistant tobacco mutant recovered from cells treated with ultraviolet light and exposed to 1 m*M* carboxin, was shown to breed true from seed (*24*). However, the alternate oxidase of the mutant was unimpaired and its mitochondrial succinic dehydrogenase (E.C. 1.3.99.1) was as sensitive to carboxin as that of wild type.

A second method for selecting mutants lacking the alternate oxidase is to use their predicted antimycin A sensitivity to rescue them from a negative selection agent, one which preferentially kills growing cells. Sodium arsenate appears to qualify as a negative selection agent in cultured plant cells. For example, cells previously treated with antimycin A and salicylhydroxamic acid (SHAM), an inhibitor of the alternate pathway, survive short exposure to sodium arsenate and grow after they have been washed to remove these agents. In contrast, cells treated in the same way but without SHAM are killed (*24, 25*).

Calculations of the possible benefits of changing P/O ratios in crop plants suggest that the gains in efficiency may be low (*26*). However, several observations indicate that this may be an unwarranted generalization. For example, Heichel (*27*) compared photosynthesis and respiration rates in two maize inbreds. The faster growing inbred had an insignificantly greater rate of photosynthesis but significantly slower respiration rates in leaves and shoots. This inbred grew faster because it was superior in conserving fixed carbon. Grime (*28*) showed that leaves of shade-tolerant species grown in full sunlight have consistently lower rates of respiration than leaves of species from open habitats. Even though shade-intolerant species have higher rates of photosynthesis at low light intensities, these higher rates do not confer shade tolerance because they are, in part, offset by high rates of respiration. In microorganisms also there are examples of differences in the efficiency of converting a hexose carbon source to cell mass. Thus the aerobic yield of *Candida* per gram of hexose was reported to be some 20 percent greater than *Saccharomyces* or *Aerobacter* (*29*).

Cell Culture Systems and Other Selections

The method of exposing mutagen-treated plant cells to inhibitors and other toxic agents to select resistant survivors has broad applications beyond those considered above. The limits to what is possible are set by (i) whether or not one can regenerate plants from surviving cells, (ii) whether the property expressed in undifferentiated cells will be expressed in the mature plant or the part of it that is harvested, and (iii) the investigator's ingenuity in devising selection systems. One obvious application is the selection of disease-resistant plants from cell lines. To date, the method is limited to selections that are based on the use of pathotoxins excreted by fungi or bacteria (*30*) or a pathotoxin analog (*31*). For these it is very efficient. For example, callus of Texas cytoplasmic male-sterile maize, like the mature plant tissue, is sensitive to the toxin of the fungal pathogen *Helminthosporium maydis* race T. A resistant cell line was obtained after exposure of the callus to *H. maydis* toxin (*32*) from which plants that were male fertile were regenerated (*33*). Sugarcane cell lines resistant to *H. saccharum* toxin have also been selected for commercial use. Evidently sensitivity or resistance to these pathotoxins are properties of the cells that, for practical purposes, are unaffected by differentiation. At present, the method is limited because the majority of fungal and bacterial plant pathogens have no pathotoxins that can be isolated from culture filtrates or extracts. Cell selection systems for resistance to these await a better understanding of the mechanisms that determine pathogen specificity (*34*).

Selection methods that seek to improve the nutritive value of seed proteins are more difficult. For example, Polacco (*35*) has set out to increase the amount of urease (E.C. 3.5.1.5) in soybean cell lines. The enzyme is present in soybean seeds and is richer in methionine than other soybean seed proteins. Plants with large amounts of urease might also assimilate urea fertilizer more efficiently. Selections designed to detect mutants that overproduce urease include resistance to hydroxyurea, a potent inhibitor of soybean urease, and growth on urea plus methylammonia. This last compound is a repressor of urease synthesis that is not itself a nitrogen source. Even though soybean cell lines have not yet generated plants, such selection schemes suggest ways of screening cell lines of other crop plants, or of screening seedlings.

There are many other selections that are of interest, such as resistance to heavy metals through failure to accumulate them (*36*), herbicide tolerance (*37*), resistance to chilling at low temperatures (*38*), and overproduction of specific amino acids (*39*).

Protoplast Fusion

Conventional plant breeding makes use of several methods to tap genetic variation that is normally not available because of sterility barriers. For example, failure of endosperm development after fertilization which leads to abortion of hybrid embryos can be overcome by dissecting out immature embryos and culturing them on a synthetic medium (*40*). Incompatibility mechanisms can sometimes be circumvented by adding mentor pollen (*41*) as a source of growth factors. In theory, protoplast fusion should greatly expand the range of hybrids that might be obtained. Indeed, protoplasts from different plant species, genera, and families have been fused (*42*), and plant protoplasts have even been fused with human cells (*43*). For the present the major problem preventing further rapid development is that protoplasts of rather few plant species can be coaxed into regenerating plants.

Protoplast fusion appears to be increased by agents that bring about partial plasmolysis, such as sodium nitrate (*44*) or polyethylene glycol (*45*). Once fusion has occurred, hybrid protoplasts must be selected from the many unfused protoplasts and fusion products of like protoplasts. Carlson and co-workers (*46*), who were the first to produce a somatic hybrid, made use of the fact that cells of the hybrid *Nicotiana glauca* × *N. langsdorfii* do not require auxin in the growth medium, whereas the cells of both parental species do. A more general method with wider applications would be to use either readily available, or easily induced, forcing markers in the cell lines to be fused. Cocking *et al.* (*47*) tested protoplasts of different species for naturally occurring differences in sensitivity to various drugs. Using this approach they produced a tetraploid somatic hybrid of *Petunia parodii* × *P. hybrida* from fused diploid leaf protoplasts (*48*). Hybrid protoplasts, callus, and finally small plants, were selected on a medium that did not support the growth of *P. parodii* protoplasts beyond colonies of about 50 cells in size. The medium also contained actinomycin D (1.0 μg/ml), which had been found to inhibit selectively the growth of *P. hybrida* protoplasts. Some variation in chromosome number from a low of 24 to a high of 28 was observed among the selected plants. Comparison of the plants with 28 chromosomes with colchicine-induced tetraploid ($4N = 28$) forms of both parents and their sexual hybrid confirmed them as somatic hybrids.

In fungi, prototrophic diploid fusion products can be readily selected from mixtures of complementing haploid auxotrophs. Auxotrophic mutants of plants could be similarly useful. For example, in the liverwort *Sphaerocarpos donnellii*, Schieder (*49*) mixed protoplasts of a green, nicotinic acid–requiring female with protoplasts of a pale-green, glucose-requiring male and was able to select a complementing hybrid. Attempts to generate auxotrophic mutants in photosynthetic eukaryotes have produced a very limited range compared with that found in fungi (*50*). For example, Redei (*51*) recovered only thiamin-requiring auxotrophs in extensive mutant hunts in *Arabidopsis*.

Several reasons have been advanced to explain the scarcity of auxotrophic mutants. The method of treating haploid cultured cells requires them to be true haploids so that recessive mutants are not masked by duplicate genes on partially homologous chromosomes. Treating pollen with a mutagen and using it to produce seeds (*52*), or treating seeds (*53*), and then screening for recessive homozygotes in the selfed progeny (M2) of the treated material (M1) may impose a developmental screen that eliminates many auxotrophic mutants (*54*). However, a number of mutants resistant to drugs, antimetabolites, and heavy metals have been produced by these methods (*36, 55*) and could be used to force protoplast fusion.

An alternative forcing method is to use semilethal, recessive chlorophyll-deficient mutants that complement in diploids. Melchers and Labib (*56*) succeeded in recovering somatic hybrids between two light-sensitive chlorophyll mutants of *Nicotiana tabacum* by this means. A similar method was used by Gleba *et al.* (*57*) to recover somatic hybrids of *N. tabacum* except that one parent had a chloroplast mutant and the other a semidominant chromosomal mutant. These authors were thus able to distinguish between regenerated hybrid plants in which nuclear fusion had occurred and hybrids in which the nucleus of one parent was present with the organelles of the other parent, or the organelles of both parents.

It is generally recognized that even when methods for selection and regener-

ation of hybrid protoplasts are developed there will be problems owing to genetical and physiological imbalance affecting development, morphology, and fertility. All of these problems are encountered in sexual hybrids between forms that do not normally interbreed. It may be possible to make use of chromosome elimination under conditions of stringent selection to establish stable hybrids and thus obtain gene transfer between forms that could never be sexually hybridized. For example, chromosome elimination was observed in soybean × *Nicotiana glauca* somatic hybrid cell lines that went through repeated divisions, forming millions of cells, during culture for more than 6 months (*58*). In these lines, apparently random loss of *N. glauca* chromosomes was associated with the loss of alcohol dehydrogenase and aspartate amino transferase isozymes characteristic of *N. glauca* (*59*). Protoplast fusion is most unlikely to produce either bizarre combinations or multipurpose crops, such as solanaceous plants with potato tubers and tomato fruits. Its value is much more likely to be as a means of transferring chromosomes or chromosome fragments.

Protoplast preparation and regeneration offers other opportunities. Plant protoplasts take up a variety of particles in suspension including not only foreign bodies such as virus particles (*60*), latex spheres (*61*), bacteria (*62*), and nitrogen-fixing blue-green algae (*63*), but organelles such as chloroplasts (*64*), and nuclei (*65*). Although mitochondria and chloroplasts contain their own DNA (*66*), they depend on structural genes located in the nucleus for the synthesis of chlorophylls and carotenoids and some of the enzymes involved in photosynthesis and respiration. This interdependence will probably increase the difficulty of recovering stable associations of foreign organelles introduced into plant protoplasts as regenerated plants.

Recombinant DNA

In a number of reports (*67*) it has been claimed that phenotypic changes occurred in plant tissues treated with foreign DNA as a result of the transcription and translation of the introduced DNA. Where genetic tests of such changes were made the introduced marker was not stably integrated within the recipient genome but was transmitted irregularly in sexual progeny. Thus, inositol-independent transformants of the fungus *Neurospora crassa*, obtained by treating an inositol-requiring strain with a DNA preparation from a wild-type strain, were stable during the somatic cell cycle but showed rare, non-Mendelian transmission to sexual progeny (*68*). Conidia from colonies of stable, transformed lines grown on medium containing ethidium bromide or acridine gave rise to up to 7.5 percent of colonies that had reverted to inositol requirement. Both of these agents induce loss of autonomous episomes from bacteria, suggesting that transformation in this case resulted from an episome carrying the information for inositol synthesis (*69*). Although transmission of an episome through seeds of a crop plant may also be irregular, and thus of little direct value for crops that are planted from seed, stable transmission of episomes in vegetatively reproduced crops such as potato and cassava could be very valuable. Unfortunately, the published information on attempts to transform plants is not as experimentally complete as the *Neurospora* example and is also subject to other interpretations (*70*). In several of the experiments the DNA used was unfractionated and extracted from donor cells or tissues, as was the case in the *Neurospora* experiment. However, two groups have used *E. coli* bacteriophages carrying the lactose operon and have interpreted growth of tomato callus (*71*) or sycamore (*Acer pseudoplatanus*) suspension cultures (*72*) in which lactose was supplied as a carbon source, as expression of the *E. coli* β-galactosidase gene. In neither series of experiments were genetic tests possible, since no plants could be regenerated. It has been suggested that plant β-galactosidases were responsible for the growth observed (*70*).

It is now clear that the expression of bacterial DNA in plant cells is likely to meet its most severe test in efforts to transfer genes for nitrogen fixation to nonlegumes (*73*). Two research groups have succeeded in mobilizing the nitrogenase region of the chromosome of the free-living nitrogen-fixing bacterium *Klebsiella pneumoniae*. Dixon *et al.* (*74*) were able to select a P plasmid that carries the nitrogen-fixing (*nif*) genes and it was shown that these genes could be transferred to *E. coli* and to *Azotobacter* (*75*). The genetic engineering in this case made use of cointegration, a mechanism that brings about natural recombination between different plasmids present in the same cell. Ausubel *et al.* (*76*) have used recombinant DNA techniques in vitro to construct a small plasmid that carries *nif* genes of *Klebsiella*. This is a nonmobilizable plasmid but may be used to transform cells of other species. The prospect of using this genetic information to confer nitrogen-fixing ability on nonlegumes such as wheat and maize is alluring but, aside from the genetic problems, which are considerable, it faces another obstacle. Nitrogenase enzymes can only function in anaerobic conditions. *Klebsiella* fixes nitrogen as an anaerobe. Legumes have leghemoglobin in their root nodules which excludes oxygen from the nitrogen-fixing bacterial symbiont *Rhizobium*.

Although it is likely that *nif* genes are not the only bacterial genes a breeder would wish to introduce into crop plants, it is clear that general methods for introducing DNA from other sources, particularly other species and genera of plants, would be of even more use. Transformation experiments that make use of unfractionated total DNA extracts are likely to have a low probability of success. Recombinant DNA techniques that employ λ phage or plasmids of *E. coli* may provide methods for preparing relatively large amounts of specific chromosome fragments from a variety of sources. This fractionated and amplified DNA could then be employed in transformation experiments. Such a technique would be simplified if the genes to be cloned and amplified could be detected by their expression in a bacterial host. To date the only eukaryotic DNA reported to be functionally expressed in *E. coli* is from the fungi yeast and *Neurospora* (*77*). In these examples expression was detected by repair of auxotrophic deficiencies in the *E. coli* host cells. Transcription and translation of DNA from higher eukaryotes is clearly a problem. It may prove necessary to transfer initiation and termination sequences along with the desired segment of chromosome to be cloned. There is also likely to be a need to identify clones that code for proteins for which there are no convenient assays in *E. coli*.

Plant gene expression in a prokaryote would have other useful features, such as offering the opportunity to study the structure and regulation of genes controlling important plant metabolic pathways. However, if expression proves to be too difficult there are other ways of detecting specific foreign eukaryotic DNA in prokaryotic cells. One method involves the use of radioactively labeled RNA or DNA probes to identify colonies of cells that carry a particular DNA sequence (*78*). These labeled probes can be prepared when it is possible to isolate the messenger RNA produced by the eukaryotic DNA sequence. Another method depends on immunoassay for translation products in phage plaques or bacterial colonies (*79*).

Several suggestions have been put forward for developing DNA vectors for transforming higher plant cells. These suggested vectors include small plasmid-like circular DNA's reported in plant mitochondria (*66*), fragments of chloroplast DNA that include the replication function (*80*), the double-stranded DNA caulimoviruses of the Cruciferae (*81*), bacterial plasmid vehicles enclosed in RNA virus protein capsids (*82*), and the crown gall system. This last is perhaps one of the most highly developed vector systems and is especially interesting since it appears to involve a naturally occurring exchange of DNA between a bacterium and a plant.

In nature, part of a large plasmid carried by the crown gall bacterium *Agrobacterium tumefaciens* is capable of transfer to a wide range of dicotyledonous plant hosts. Infection occurs at a wound site and results in a gall, or tumor, which after excision and transplantation is capable of indefinite growth on a fresh host, or on a culture medium, in the absence of *A. tumefaciens*. The tumor induction (TI) is strictly dependent upon the presence of the large plasmid in *A. tumefaciens*. The mechanism of tumor induction in crown gall is still unknown. The presence of TI plasmid DNA sequences in bacteria-free tissue has been detected (*83*), but the nature of its incorporation and its subcellular location are not known. In addition, the tumor may acquire the ability to synthesize one of the arginine derivatives nopaline octopine. It can be inferred that the incorporated plasmid DNA, which is transcribed, determines the ability to synthesize these compounds. It has not been possible to transfer the ability to synthesize these compounds without inducing tumors. However, some teratoma-like galls are able to regenerate normal plants. Since these plants retain the ability to synthesize octopine or nopaline, they may provide a method for recovering plants that express other markers introduced with a teratoma-inducing plasmid (*84*).

The TI plasmid confers on its bacterial host the ability to degrade nopaline or octopine to arginine so that these compounds may serve as nitrogen or carbon sources on a defined medium. Either character can be used as a marker for transferring the TI plasmid to avirulent strains of *Agrobacterium* and to *Rhizobium* in culture (*85*).

It is clearly too early to begin to assess the implications of recombinant DNA techniques for increasing crop plant yield. The redesign and improvement of any complex mechanism depends on understanding how it works. The contributions to studies of genetic structure, regulation, and function in higher plants that are likely to result from work in this field can hardly fail to contribute to the breeding of better crops. Immediate goals are to use the methods we now have to isolate specific crop plant genes and to develop and refine methods for achieving frequencies of transformation high enough to be useful to a breeder. We will then see whether or not the introduction of short foreign DNA sequences has a disruptive effect on the genetic systems of modern crop cultivars.

Guidelines for Plant Recombinant DNA Research

In June 1976 the National Institutes of Health published guidelines regulating the conduct of research with recombinant DNA (*86*). It appears that these guidelines, or a substantially similar form of them, will become law in the United States. Although concern over the safety of recombinant DNA experiments focused on ways to reduce the hazards to man and other hosts of *E. coli*, it was also recognized that some recombinant DNA experiments may pose hazards to plants. The guidelines therefore recommend levels of physical and biological containment which, at the time they were discussed, seemed appropriate. The guidelines now prohibit the release of any organism resulting from recombinant DNA. Release really means a reduction in the level of physical containment. Thus a crop plant that incorporates foreign DNA introduced under P2 physical containment and the equivalent of EK1 biological containment (standards that are required for most plant experiments) cannot be grown in a field-test plot, an ordinary greenhouse, or a laboratory with less than P2 physical containment. As it stands the ban prevents any practical use of recombinant DNA technology in agriculture. It has been widely assumed that requests for reduction in containment or release will be considered by the NIH committee, or some other body, on a case-by-case basis. Understandably, the wisdom of vesting such decisions that directly affect agriculture solely with NIH has been questioned by plant scientists. Requests to subject organisms prepared by recombinant DNA techniques to field tests may be made quite soon. Strains of *Rhizobium* with improved effectiveness, competitiveness in the soil, and tolerance of a wider range of soil conditions are now being sought by breeding by plasmid promoted chromosomal recombination between different species (*87*).

The original rather limited inputs of plant and nonmedical scientists to developing the guidelines (*88*) have now been supplemented by a number of proposed revisions from individual scientists and groups (*89*). These proposals include (i) specifying a mechanism for approving release of organisms that incorporate recombinant DNA, (ii) a better definition of "plant products dangerous to any species" that call for higher containment levels, (iii) a recognition of the high level of biological containment afforded by plant cell protoplasts and undifferentiated plant cells in culture, and (iv) a recognition that containment criteria for research on biological control agents of major agricultural pests should be related to the risks to nontarget organisms.

Conclusions

Probably the biggest problem in applying new concepts to increasing crop plant yield is the technology gap between plant genetics, physiology, and biochemistry on the one hand, and plant breeding on the other. Breeders have to be concerned with total crop performance and can rarely spend much time analyzing single gene effects. They have concentrated instead on quantitative methods for dealing with the effects of many genes using sometimes single plants but more often populations in small plots as their basic units (*90*). In contrast, the work of plant geneticists and physiologists tends to be much more narrowly focused on the biochemistry and control of individual metabolic pathways. Use is made of isolated enzymes, organelles, cells, pieces of excised tissue, and individual leaves or single plants. When the results and methods have practical significance they are often difficult or impossible to apply directly to the greenhouse and breeding plot for the selection of superior individuals from among segregating families. For major innovations in crop improvement plant breeders need methods by which they can follow rates of photorespiration, cyanide-insensitive dark respiration, net photosynthesis, nitrogen fixation, and content of lysine, tryptophan, and methionine, and so on. It is my belief that our increasing facility with cultured cells of crop plants will lead first to methods of producing new germ plasm resources for use in orthodox breeding and eventually to ways of selecting finished varieties in a petri dish or flask.

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88. On 1 to 2 April 1976, the following special consultants were asked to a meeting of the NIH Recombinant DNA Program Advisory Committee to comment on the draft guidelines as they affected plants: P. R. Day, T. Diener, E. Jaworski, L. G. Nickell, N. Panopoulos, A. Vidaver, and M. Zaitlin. This group suggested the following addition to the guidelines; Ecological Safeguards on Release of Plant Material: (i) Those specific traits introduced by recombinant DNA shall not be transmitted by organisms such as arthropods and fungi to other plants. This is to ensure that new properties are not spread among plants indiscriminately. For example, an infectious portion of a recombinant DNA molecule could be purged by heat therapy or seed transmission to select forms which the desired DNA was integrated in the host genome. (ii) Edible portions of the plant shall be no more toxic than the original plant material to animals. (iii) Standard U.S. Department of Agriculture (USDA) plant quarantine procedures should be followed before releasing modified plants to ensure that no unduly competitive or ecologically undesirable forms are released that could be difficult to control.
89. A short meeting was held during the conference on Genetic Engineering for Nitrogen Fixation at Brookhaven, 16 March 1977. A meeting sponsored by the U.S. Department of Agriculture and the National Science Foundation on 12 to 13 April in Warrenton, Virginia, considered the guidelines in relation to recombinant DNA research in agriculture and other nonmedical subjects.
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I thank M. Berlyn, O. Gamborg, J. C. Polacco, R. Sparks, J. Thorne, and I. Zelitch for helpful comments and suggestions.