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Prospects in Plant Genetic Engineering

Kenneth A. Barton and Winston J. Brill

Agriculture is both the oldest and the largest of the world's industries. Over a period of thousands of years, a broad spectrum of interacting natural and artificial selective pressures has influenced the evolution of crop plants toward those now found under cultivation. Throughout this evolutionary period efforts have been directed toward increasing crop quality and productivity without understanding the contributing molecular features. While the supply of available nutrients for human consumption worldwide has never been in excess, increases in agricultural productivity within the past few decades have been dramatic. A significant reason for the successes of modern agriculture has been an increased reliance on advanced technology, and a successful integration of new technology with the results of intensive plant breeding programs. However, methods of crop improvement in the past few decades have initiated a series of new problems that are now becoming recognized.

Summary. The functional expression of a novel gene in a genetically engineered plant has not yet been reported. One major barrier in movement toward this goal is our limited understanding of the molecular bases of gene expression. Attempts to establish genetic engineering as a practical facet of plant breeding are also complicated by the fact that genes for most important plant characteristics have not yet been identified. However, the benefits to be gained from all aspects of plant improvement are stimulating research into both the development of plant transformation technology and the isolation and characterization of genes responsible for valuable traits. As scientists develop greater knowledge of plant molecular genetics, we can expect to see practical applications in such diverse areas as improvement of plant nutritional quality, decreases in fertilization requirements, and increases in resistance to environmental stresses and pathogens.

Genetically superior plants derived from modern crop improvement programs typically require a high level of crop management. Included in a management regime may be the input of increasingly expensive nitrogen fertilizer as well as the extensive use of pesticides and herbicides, all of which can result in toxic residue accumulation in the environment. In addition, the high degree of inbreeding and the narrowing of the genetic base of widely cultivated crops cause increasing concern about the susceptibility of crops to major disease outbreaks and imply that important genetic traits may be lost as world germplasm is reduced (*1*). With problems such as these it is not surprising that the advent of recombinant DNA technology is generating excitement. A whole range of very specific plant genetic modifications can now be considered, with the use of methods that may someday generate a genetic diversity not naturally present in cultivated plants.

The molecular genetics of prokaryotic organisms is extremely complex and in many respects poorly understood. The flow of stored genetic information in nucleic acids to the appearance of functional gene products elsewhere in the cell requires completion of an intricate sequence of events, with many points where positive or negative control over expression can be exerted. Genetic regulation present in simple eukaryotes, such as yeast, can be more complex, with the added potential for various interactions between organelles, and with an increasing number of both nuclear and cytoplasmic genes. Higher eukaryotes, among them crop plants, provide the still greater problems of cellular differentiation; for example, thousands of active and interacting genes in a leaf cell may be totally quiescent in a root cell of the same organism (*2*). The same natural laws that govern the expression of DNA placed in new genetic environments through classical plant breeding apply to the expression, or lack of expression, of DNA placed in plants by recombinant DNA technology. To be successful in plant genetic engineering, we must begin to develop an understanding of the elements that control gene expression. The significance to gene expression of precise DNA constructs is now beginning to be understood in bacterial, yeast, and

Dr. Barton is a molecular geneticist and Dr. Brill is director of research at Cetus Madison Corporation, Middleton, Wisconsin 53562.

even mammalian systems, in part because of the development of methods for inducing cell transformation. With transformation methods evolving and useful genes being discussed, genetic transformation of plants can now be considered realistically.

Plant Transformation Vectors

Although the transfer of cloned DNA between microorganisms is routinely carried out in many laboratories, the absence of convenient vector systems has inhibited similar experiments with higher plants. However, rapid progress in this area is being made and a variety of vectors are expected to come into practical use in the near future. One limitation to current vector design is the lack of an ideal transformation marker—a gene present on the vector which enables convenient identification of transformed cells. The ability to provide a dominant selection for plant cells deficient in alcohol dehydrogenase activity and to subsequently identify alcohol dehydrogenase-positive revertants (*3*) makes the alcohol dehydrogenase gene an attractive marker for plant host-vector systems. It is also possible that dominant chimeric antibiotic-resistance genes, similar to those now functional in mammalian cells (*4*), can be constructed for expression in plant cells by using genetic control regions from plant DNA spliced to protein-coding sequences from other organisms. As more convenient markers become available, the development of mechanical gene introduction methods [for example, microinjection or polyethylene glycol–mediated uptake of DNA by protoplasts (*5*)] will be greatly facilitated. In the absence of conveniently scored markers, many plant transformation experiments have relied on natural routes of entry into plant cells—the routes of plant pathogens. Although a variety of pathogenic organisms may be modified to serve vectorial functions as more becomes known about their mechanisms of infection and replication, efforts to date have centered on the double-strand DNA plant viruses (Caulimoviruses) and *Agrobacterium*.

Only a small number of Caulimoviruses are known, and all are similar in many respects. The most widely studied, cauliflower mosaic virus (CaMV), has a limited natural host range which has been extended only slightly in vitro (*6*). The transcription and replicative mechanisms of the virus are complex, and the virus is not seed-transmissible. However, the potentially valuable characteristics of CaMV include the capacity of the viruses to infect intact plants, and to then move systemically through the hosts. A vector that would avoid the need for cell culture would be valuable indeed. Unfortunately, experiments directed toward use of CaMV as a gene vector have revealed stringent genome size limitations (*7*), thereby restricting the amount of foreign DNA that can be transported.

Agrobacterium tumefaciens, a soil bacterium that incites crown gall disease in a wide variety of dicotyledonous plants, has provided greater success than CaMV as a plant vector (*8*). Virulence is conferred on the bacterium by genetic information carried on large plasmids, the Ti (tumor-inducing) plasmids (*9*). At the time of infection, a segment of the Ti plasmid, called T-DNA, is inserted into the nuclear DNA of the host plant (*10*). Genes contained within the T-DNA are functional in transformed cells, and T-DNA gene products are responsible for both hormone independence of crown gall cells in tissue culture (*11*) and the synthesis of novel metabolites called opines (*12*). Opines, simple derivatives of amino acids and keto acids, are specifically catabolized by *Agrobacterium* as both carbon and nitrogen sources. The T-DNA insertion into the host plant genome therefore appears as an excellent example of genetic engineering in nature, for it assures a supply of nutrients to the invading bacterium by altering the host plant metabolic pathways.

Tobacco cells containing an intact T-DNA cannot regenerate into normal plants because of hormonal imbalances resulting from the action of T-DNA gene products. However, if the genes responsible for the imbalance are spontaneously deleted from the infected cell, healthy plants containing the remaining T-DNA genes can regenerate (*13*). It is also possible to "disarm" T-DNA in vitro by experimentally deleting one or more genes of the T-DNA (*14*). Tobacco cells transformed with disarmed Ti plasmids are fully capable of regenerating into healthy plants, and it seems likely that other plants that adapt well to tissue culture can be similarly transformed. Eukaryotic DNA placed in the T-DNA of a disarmed Ti plasmid is transported into the plant cell, and the DNA is structurally stable in passage through meiosis, into seeds of the regenerated plants (*14*). The Ti plasmid can therefore be realistically used as a vector for dicotyledenous plants, although refinements over the current experiments can be expected to result in still more convenient Ti-derivative vectors. Ti plasmids can also now be used to facilitate construction and experimental testing of dominant selectable markers, which may soon be available for transformation of both monocots and dicots.

Rapid progress in vector construction has outdistanced two other areas of research which are critical to plant genetic engineering successes. Initial transformation experiments will be carried out at the level of a single cell in culture, but relatively few agronomically significant crops can yet be regenerated routinely from cell culture (*15*). Until this technology develops further, or until alternative vectors become available which avoid the need for tissue culture, many crops cannot be modified by recombinant DNA methods. The second problem is equally significant—what genes can we transfer into plants that will improve a crop species? Much of the development of present cultivars has relied on selection in classical breeding programs for polygenic characteristics such as increased yield or protein content, without an understanding of the molecular basis for such traits. In contrast, success in plant genetic engineering will rely, to a great degree, on a thorough knowledge of the genetics and regulation of the traits to be transferred. A number of systems exist in plants which are being considered for manipulation through genetic engineering, although a few examples demonstrate the magnitude of problems to be encountered.

Seed Proteins

The seeds of legumes and cereal grains provide humans directly with approximately 70 percent of their dietary protein requirement (*16*). Throughout seed development, storage proteins are synthesized and accumulated within the seed, apparently to provide a source of amino acid reserves during early seed germination (*17*). High levels of such protein in seeds provides an enriched amino acid source for both human and animal consumption. However, various deficiencies of seeds in certain essential amino acids do not allow either cereal grains or legumes to provide a balanced diet without supplementation of the limiting amino acids from other sources (*18*). One widely discussed approach for overcoming the nutritional deficiencies of seeds would be to genetically engineer genes encoding the various storage proteins to include new codons for the deficient amino acids, either by inserting additional amino acids into the protein, or substituting existing amino acids with ones

more nutritionally desirable. However, there are a variety of technical problems to resolve before such an engineering project can be successful. Structural conservation of the zein storage proteins of maize (*19, 20*) and apparent structural conservation of messenger RNA in legumes (*21*) provide good examples of such problems.

There are a number of zein proteins in maize, each deficient in the essential amino acids lysine and tryptophan (*18–20*). The zein proteins amount to 50 percent or more of the total corn endosperm protein. Extensive microheterogeneity exists between the different proteins (*19, 20*), although there is apparently a strong conservation of an unusual, highly ordered protein secondary structure (*20*). The basis for the complex folding of zein is the presence of a sequence of 20 amino acids, with alternating hydrophobic and hydrophilic regions, which is repeated nine times in the protein chain. A number of zein genes with minor sequence variation comprise a moderately reiterated multigene family in the maize genome, presumably a result of gene amplification (*19, 20*). Throughout the amplification process and subsequent divergent evolution of the zein genes, there has been a conservation of the secondary folding characteristics of the resulting polypeptides (*20*). Attempts to alter the genomic coding sequence of zein proteins by genetic engineering must take into consideration the possible effect of amino acid changes on protein secondary structure, since stability and accumulation of the zein proteins during embryogenesis may well be due to protein structural features. To complicate matters further, there is now evidence that a conservation of nucleotide sequence in the vicilin genes of the seeds of various legumes may be significant to aspects of messenger RNA structural stability and metabolism (*21*). If this proves correct, the genetic engineer must consider the effect of codon substitution not only on protein stability but also on the folding characteristics of the messenger RNA.

Because the storage protein systems now under scrutiny are encoded in multigene families, engineering of a single gene for higher levels of an amino acid would have a relatively small effect on total seed protein composition unless the engineered gene was transcribed very actively or was amplified in the genome. Alternative approaches toward improvement of seed protein composition, such as introduction of entirely novel proteins that are highly enriched in specific amino acids, can be considered. However, the problems of RNA and protein stability remain, and there is the additional complication of obtaining accurate and high-level developmental expression of the new gene. The transfer of genes encoding known storage proteins to systems now low in protein, or the provision of additional copies of genes to systems already producing storage proteins, are further possibilities for improving seed protein quality or quantity; however, channeling normal amino acid pools into large amounts of a protein not normally present may well create serious metabolic imbalances, not only within the protein-producing cell but within the plant as a whole. Decreases in seed yield or alterations of other important seed characteristics could easily negate improvements in protein content.

Nitrogen Fixation

The growth of agricultural crops is dependent on an enormous supply of usable nitrogen, either mechanically applied as ammonia, urea, or nitrate fertilizer, or naturally produced in the soil through microbial reduction of atmospheric nitrogen. Increasing energy costs have encouraged research into novel approaches to increase the available supply of naturally reduced nitrogen. Although most organisms cannot assimilate atmospheric nitrogen (N_2), a limited number of prokaryotes are able to reduce N_2 directly to ammonia in a process called nitrogen fixation. The complex of enzymes required for nitrogen fixation has been studied in detail in *Klebsiella pneumoniae*, which contains a cluster of 17 contiguous *nif* genes organized in seven operons (*22*). Both the enzymatic machinery and the metabolic controls on nitrogen fixation in *K. pneumoniae* are quite intricate. Information gained from study of this bacterium, which fixes N_2 asymbiotically, is helping to decipher the more complex but agronomically important symbiotic processes of *Rhizobium*. Symbiotic nitrogen fixation occurs within highly differentiated root nodules formed by interactions of *Rhizobium* with plants of the family Leguminosae (soybean, alfalfa, peanut, bean, pea, clover, for example), enabling these plants to grow without addition of nitrogenous fertilizer. The possibilities that increased nitrogen fixation will increase current legume yields or that additional plant varieties could be made capable of carrying out nitrogen fixation are being investigated from several approaches.

The formation of nodules that are effective in nitrogen fixation depends on genetic information present in both the bacterial and host plant cells (*23*). Because *Rhizobium* can be easily manipulated in the laboratory, the potential for generating improvements in the bacterial contribution to symbiotic nitrogen fixation seems high. For example, *Rhizobium*-coded nitrogenase, the enzyme directly responsible for N_2 reduction, has a side reaction that hydrolyzes adenosine triphosphate (ATP) and forms H_2 (*24*). The reaction serves no apparent function and expends considerable energy. Some, but not all, nitrogen-fixing bacteria contain a hydrogenase that regenerates ATP by the oxidation of H_2; the ATP is then available for use in further nitrogen fixation. There is now experimental evidence that strains of bacteria containing the hydrogenase may fix nitrogen more efficiently (*25*). Clearly, introduction of the hydrogenase gene to additional strains of *Rhizobium* has the potential to improve nitrogen fixation and perhaps to increase the yield of legume proteins (*26*).

The potential for improving nitrogen fixation through genetic engineering of host plants is not as well defined. Host proteins are involved in the plant-bacterial symbiosis, but except for leghemoglobin (the protein responsible for protection of the oxygen-sensitive nitrogenase), the functions of host proteins specific for the symbiosis have not yet been resolved (*22*). However, there are examples of variable nitrogen-fixing efficiencies among legume cultivars (*27*). This suggests that exchange or alteration of the "symbiosis genes" in plants might result in enhanced nitrogen fixation efficiency. Until the process is better understood and the genes involved have been identified, we can only speculate on the feasibility of such projects.

Extension of symbiotic or asymbiotic nitrogen fixation to plants which do not now benefit from the process, such as the cereals, would be extremely valuable. Evidence now suggests that free-living nitrogen-fixing bacteria can be encouraged to associate with roots of cereals, enabling the plant host to receive some nitrogen through bacterial nitrogen fixation (*28*). It may be possible to genetically alter the nitrogen-fixing bacteria to bind more tightly to the roots of the cereal and thus create a more beneficial association. However, creation of a new cereal symbiosis which results in nodulation will only be possible when more is known about the host genes that contribute to the nodulation process. The possibility that genes from the bacterial *nif* complex can be moved into cereals by genetic engineering is being explored in

several laboratories. However, overcoming the obstacles preventing proper regulation of prokaryotic gene expression in eukaryotic cells will be difficult. In addition, it is unlikely that the host cellular metabolism can be easily adapted to the stringent metabolic requirements for efficient nitrogen fixation even if *nif* gene expression is obtained.

Pest and Pathogen Resistance

A significant proportion of the total world crop production is lost each year because of pest or pathogen damage (*29*). Crop protection is afforded by strict quarantines of produce and crops from infected areas, crop rotation, more sanitary seed preparation and storage, and the use of chemical pesticides. However, the cheapest, and historically one of the most effective, means of combating both pests and disease is through the use of resistant plant varieties. The cultivation cost of resistant plants is no greater than that for susceptible varieties, and the adverse side effects sometimes resulting from chemical control measures can be avoided. It is likely that molecular biology will eventually play a major role in crop protection (*30*) by (i) increasing our understanding of the mechanisms of pathogenicity, (ii) permitting early detection of infection (*31*), (iii) providing means of direct control of disease symptoms and pests, and (iv) enabling us to engineer resistant crop varieties. The most significant practical applications should follow rapidly behind research into the molecular basis for the disease or pest attack. A few specific examples demonstrate the potential for future successes in this area.

The phenomenon of "induced resistance" in plants has an intriguing similarity to immunization by vaccination in mammals. When plants are either inoculated with pathogens or treated with chemicals that cause chronic but localized cell damage, they frequently exhibit enhanced and broad-spectrum resistance to subsequent infection (*32*). Such resistance has been observed with respect to bacterial, fungal, viral, and nematode infections, and the resistance occurs in many, if not all, plant species. The response has been elicited by application to some hosts of either attenuated pathogenic strains or various fractions of destroyed pathogens (*32*). Although the mechanisms of induced resistance are not yet understood, it is apparent that the capability to manipulate these systems in a practical direction will have strong impact on agricultural productivity. It seems likely that herbicides or other crop additives which induce pest resistance will be developed; but it may also be possible (when the mechanisms are known) to engineer plant varieties to exhibit higher levels of broad-spectrum resistance. This may be as conceptually simple as directing plants to constantly produce low levels of endogenous elicitors, or as complex as altering the genetic pathways of resistance.

Several approaches may be used to genetically engineer crop plants with greater insect resistance. A variety of plant secondary metabolites naturally discourage predators through various mechanisms, such as accumulating metabolites that mimic insect hormones thereby upsetting maturation of insects (*33*). The transfer to crop plants of genetic pathways required to synthesize such metabolites may provide resistances not now found in cultivated crops. Alternatively, various polypeptide insecticidal toxins are now in use as biological control agents, including a range of toxins produced in strains of the bacteria *Bacillus thuringiensis* (*34*). While such toxins have the practical ecological advantage of being specific for certain insect species, they now have to be applied to crops in costly spraying programs. The production of such proteins within the cells of genetically engineered plants might provide pest resistance at both reduced cost and with improved environmental safety over present control measures.

Photosynthesis

The ultimate value of plants is their ability to convert solar energy into stored chemical reserves through the processes of photosynthesis. Complex reactions that convert atmospheric CO_2 into carbohydrates and release O_2 to the environment are all carried out either within the chloroplasts of higher plants or in reactions proceeding coordinately between cytoplasmic and organelle enzymatic pathways. Although the chloroplast is dependent on the cell nucleus for information contributing toward its functioning and survival, a separate chloroplast genome, present as 40 to 60 copies of a large circular chromosome, is found within each of the organelles (*35*). The presence of as many as 50 chloroplasts per cell results in the presence of thousands of copies of each chloroplast gene per cell.

Although little is known about the regulation of chloroplast gene expression, new techniques are becoming available to study these mechanisms. Recent development of transformation methods for both the cyanobacterium *Anacystis nidulans* (*36*) and the photosynthetic eukaryote *Chlamydomonas* (*37*) portend rapid progress in elucidating the functions of photosynthetic machinery through analysis and complementation of mutant genes.

As more becomes known of photosynthetic pathways, many areas of potential improvement may be envisaged. Because many enzymes function coordinately during photosynthesis, it is likely that species variation will be found at critical reactions. Transfer of more efficient Calvin cycle enzymes (the pathway responsible for CO_2 fixation) between plant varieties may well provide for higher rates of carbon fixation. For example, ribulosebisphosphate carboxylase (the major enzyme of the Calvin cycle) has been shown in vitro to vary with respect to kinetic rate constant, depending on the plant source of the enzyme (*38*). This suggests that exchange or modification of genes encoding subunits of the carboxylase might result in an enzyme that provides more efficient CO_2 fixation in the engineered plant. The possibility carries an additional scientific intrigue: one type of the enzyme's two different types of subunits is encoded by chloroplast genes, the other by a small number of nuclear genes (*39*). Exchange or alteration of genetic information for the two types of subunits therefore depends on our gaining an understanding of and developing the technology for both nuclear and plastid transformations.

Additional prospects for improvement of photosynthetic capabilities may be found in the exchange of various photosystem components between different plants to optimize electron transfer. An increased electron flow rate through photosystems I and II might raise the level of light saturation, enabling more efficient light harvesting (*40*). The number and complexities of photosynthetic reactions will make this area extremely attractive for genetic manipulation. Ironically, the complexity of the process will probably delay many genetic engineering successes until a more complete understanding of photosynthetic interactions is gained.

Stress Tolerance

Despite continuing efforts to improve cultivation practices, crop plants will always be subject to a variety of environmental extremes. In even the most productive agricultural regions, drought and

temperature stress can occur throughout the growing season, resulting in injury and reduced plant yield. As more suboptimal lands are brought under cultivation, or as continued use alters the soils of current growing regions of the world, predictable stresses are becoming widespread: heavily irrigated soils are plagued by salt buildup and mineral toxicity; irrigation water supplies are being depleted in some regions that will soon be subjected to chronic drought; continued and expanded use of marginal land results in trace element deficiencies and the need for increased use of remedial fertilization. It is therefore apparent that crop plants that are tolerant of such extremes as drought, high salt, mineral deficiency or toxicity, or radical temperature alteration would be valuable.

When stress resistance characteristics are variable within a crop species, classical breeding programs can be devised to transfer the trait to new cultivars of economic importance (*41*). However, plant species of questionable economic value (such as weeds) frequently exhibit dramatic stress resistance. While such plants are incompatible for breeding with cultivated species, it is tempting to consider transfer of resistance traits by genetic engineering. Extensive research efforts are being directed toward developing a greater understanding of the physiological, biochemical, and genetic bases for responses of plants to the environment.

Many of the adaptations of plants to such stress as water deficit or high temperatures involve highly specialized plant morphology. For example, a reduction in leaf surface area and the presence of fewer stomatal openings promotes greater plant water retention (*42*). Unfortunately, such structural features are likely to result from the interaction of many different genes, the molecular controls of which are not yet accessible. Metabolic responses that are directly induced by stress, such as reductions in cell growth rate (*42*) or the synthesis of new classes of "heat-shock" proteins (*43*), are more easily studied in the laboratory. Further research into these areas may result in the identification of genes involved in stress responses, and eventually may suggest ways to engineer resistance in new plant varieties.

Alternative Applications

The few systems mentioned above have been widely discussed in recent years because of the tremendous potential economic impact of improvement. The complex functions of most of these systems, involving many genes of unknown identity, make them now difficult to exploit. However, the improbability that we will see rapid successes in such complex areas as improving plant protein levels or in the construction of plants that fix their own nitrogen does not mean that plant genetic engineering is far from reality. Transfer of single gene traits is now technically feasible.

It is likely that herbicide-resistant plants will soon be developed through transformation technology. A single new gene may be all that is required for this trait and direct selection for transformed cells in tissue culture is provided by the herbicide resistance. Some pathogen resistances may be only slightly more difficult to transfer, since direct selection may again be possible for the desired resistance, both in tissue culture and in the intact plant. Whether or not the initial plant varieties resulting from such experiments can be easily integrated into practical breeding programs is, for now, an unanswerable question—we cannot predict how such genetic alterations will affect the metabolism of an organism as complex as a higher plant.

A recurring problem in considering any specific application of genetic engineering in plants is the lack of understanding of the molecular genetics involved. Before practical applications can be routinely expected, basic research is required in almost all areas of plant molecular biology. In particular, novel approaches are needed to aid in the identification of the genetic components of plant characteristics. Model systems such as yeast, algae, or bacteria, which are more conveniently manipulated under laboratory conditions than are higher plants, will be useful for some applications and will perhaps aid in isolation of some single or closely linked genes. More complex traits, those which are not expressed in model systems or which are polygenic in character, will need to be explored in other ways. One promising mechanism may involve the use of transposable elements. Plant transposons, analogous to those in prokaryotes, are genetic elements that are able to move to new locations in the plant genome (*44*). Upon moving into a specific genetic locus, a transposon may alter an identifiable gene function. With the use of recombinant DNA technology, it is possible to isolate and characterize DNA surrounding the site of transposon insertion, thus identifying genes responsible for a specific trait (*45*). It may be possible in this way to characterize the major components of some of the more complex plant traits, where genes cannot be identified in other ways.

Conclusions

The potential for improvement of crop plants through genetic engineering seems vast. Although only a few broad areas have been considered in this article, it should be apparent that the present limit on application of the many ideas for crop improvement is basic understanding of the genetic components responsible for plant characteristics. Once genes necessary for valuable plant traits have been identified, there will rapidly be a variety of practical applications. Initially it should be possible to develop convenient germplasm screening methods for the plant breeder, reducing the time required to organize and analyze genetic crosses. Certainly the transfer of genes into new plant species beyond the range of classical breeding will be attempted, and as we delve more into plant biochemistry, molecular biology, and physiology, new applications and new approaches will naturally evolve. Plant molecular biologists can be expected to follow the leadership of scientists working on the better developed animal and bacterial systems; however, recent excitement in plant research is certain to stimulate faster progress in plant genetic engineering. Besides the obvious value to food production, advances in plant biotechnology will contribute to health care (novel pharmaceuticals and more efficient pharmaceutical production), to floriculture (new species of decorative plants), to forestry (acceleration of breeding programs), to the fiber industry (improved and increased fiber production), and to generation of usable energy (production of biomass for conversion to ethanol). The future of plant genetic engineering will be exciting, as much because of applications we cannot yet predict as because of those already expected.

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Isolation of Agronomically Useful Mutants from Plant Cell Cultures

R. S. Chaleff

Summary. Enormous genetic variability is accumulated by plant cells proliferating in culture. Additional variability can be induced in cultured cell populations by exposure to mutagens. This pool of genetic diversity can be examined for agronomically desirable traits at two levels of differentiation. Populations of plants regenerated from callus cultures can be screened by conventional methods. Alternatively, selective culture conditions favoring growth of specific mutant types can be applied at the cellular level. The several characteristics that have been introduced by these methods to date are a harbinger of future contributions to be made by cell culture to the genetic improvement of crops.

It was not until the 1930's that several prior decades of research culminated in the successful propagation of plant organs and tissues in culture. Thereafter, progress in plant tissue culture was rapid. The techniques of culture in vitro were extended to many species and, aided by advances in the knowledge of plant hormones that were made in part through use of tissue culture, regeneration of plants from cultured tissues was achieved in the late 1950's. The first application of these developments was to the clonal multiplication of plants. The ability to regenerate large numbers of plants from masses of disorganized tissue (callus) proliferated in vitro and from cultured organs and axillary buds proved more efficient than conventional methods of asexual plant propagation. The lists in recent reviews (*1*, *2*) of the hundreds of species that have been propagated through tissue culture document the extent to which this application of plant tissue culture technology has been developed and utilized.

In the 1960's, research in plant cell and tissue culture produced a number of achievements that individually represented significant technical advances and refinements. But when considered collectively these contributions effected a qualitative change in the conceptual view of the field. In 1960 Bergmann (*3*) demonstrated that single cultured cells plated in an agar medium would divide and form calluses. That same year Cocking (*4*) introduced an enzymatic procedure for isolating large numbers of protoplasts from higher plant tissues. In 1965 Vasil and Hildebrandt (*5*) demonstrated the totipotency of single plant cells by accomplishing the development of a complete and fertile plant from a single isolated somatic cell. Shortly thereafter Guha and Maheshwari (*6*) obtained haploid plants from immature pollen (microspores) contained within cultured *Datura* anthers. And in 1971 Nagata and Takebe (*7*) regenerated plants from cultured tobacco protoplasts. However, the turning point was in the realization that these discoveries, by making possible (albeit with only a small number of spe-

The author is staff scientist at the Central Research and Development Department, Experimental Station, E. I. Du Pont de Nemours and Company, Wilmington, Delaware 19898.