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Safety Concerns and Genetic Engineering in Agriculture

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Federal agencies are considering various regulations to protect the public from environmental and health problems that might arise from the release of genetically engineered organisms. Concern has been expressed because several agricultural practices, such as the widespread use of DDT in past decades (*1*), have caused serious problems that were unintended and unexpected. Also, movement of weeds and insect pests into new environments has created problems that have become difficult to control. Examples include kudzu, hydrilla, the gypsy moth, and the Japanese beetle. Because of these experiences, it is necessary to consider the potential effects of releasing organisms containing genes from related and unrelated genera. This article will focus on the safety issues involved in using genetically engineered plants and microorganisms (bacteria and fungi) to benefit agriculture. Other applications to which the same principles should hold with respect to safety issues include the use of genetically engineered organisms for mining, waste treatment, and detoxifying chemical spills.

The economic and environmental benefits expected to accrue from agricultural use of recombinant organisms are great (*2*) and should be considered in relation to the potential risks. By splicing foreign genes into plant chromosomes it may be possible to create plants resistant to a wide array of pests. The hope and expectation is that they will lead to decreased use of chemical fungicides and insecticides, many of which are toxic to man. Recombinant DNA techniques may be used to develop plants that utilize fertilizers more efficiently, thereby minimizing fertilizer runoff into streams and lakes. In many crop species a relatively narrow base of germplasm is being used to develop varieties. There is concern that this has created genetic vulnerability to disease (*3*). Genetic engineering can be used to introduce new genes and thereby increase genetic variability for the future. The time it takes to develop new plant varieties should be greatly decreased by this new technology.

Genetically engineered bacteria and fungi also have potential value. For example, *Rhizobium* strains isolated from many locations around the world are being applied to soils in large numbers so that legumes can produce high yields without needing expensive nitrogenous fertilizers. Several approaches are being considered to increase legume yields with genetically engineered *Rhizobium* (*4*). Other microbes, such as mycorrhizae, *Pseudomonas*, and *Frankia* (*5*), are also promising candidates for use in agriculture, and there is a good chance that the value of these organisms can be increased through recombinant DNA technology as well as traditional mutation and recombination techniques. As in traditional agriculture, the value of the new plants and microbes can be assessed only after they have been tested under a variety of field conditions. This article will discuss ways to predict the safety level of an organism that has received several foreign genes.

Of particular concern in the introduction of new organisms is the potential to self-perpetuate and spread. For the purpose of this discussion, however, a problem plant that gets no farther than the next field is not defined as a serious problem. Nor is a microbe that unexpectedly kills plants that it was sprayed on but does not damage plants in a neighboring field.

Plants

Plants have been crossed (traditional "genetic engineering") by man for centuries. New variants resulting from such breeding have not caused serious problems. Most of our high-yielding crops,

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Summary. Predictions about the safety of a recombinant plant or microorganism for agricultural use should be based on our vast experience with traditional practices, such as plant breeding and the use of microbial inoculants. An introduced plant, bacterium, or fungus containing foreign genes should be no greater environmental threat than such organisms without recombinant genes. Problems caused by introduction of organisms such as kudzu and the gypsy moth into a foreign environment do not imply problems for an organism, currently considered safe in its habitat, with characterized recombinant genes added to its genome.

productive forest trees, popular ornamentals, and garden plants have been derived through breeding programs. Some crosses include those that would not occur without man's intervention, such as crosses between high-yielding Midwestern corn and its putative wild ancestor, teosinte (*6*). Species that do not readily cross-pollinate have been crossed, without recombinant DNA technology, by many scientists around the world. As an example, cultivated oats have been crossed with wild species to increase the protein concentration of seeds and to introduce resistance to diseases (*7*). Protoplast fusion between cells of plants that normally are unable to cross have yielded new variants (*8*). Also, plants obtained by mutation have frequently been grown in experimental fields with the hope of detecting useful new phenotypes. These experiments produce novel plants and, with the exception of mutated plants, the progeny are the result of uncontrolled recombination of tens of thousands of genes. The exact properties of progeny from most of these crosses are impossible to predict. Breeders have never taken and do not now take special precautions in testing these plants in the field because they know from experience that these extensive mixings have not produced serious problems. If we compare plants derived from breeding programs with those derived through genetic engineering, it is clear that, in the latter case, the addition of a few characterized genes to the plant results in properties that are relatively easy to predict.

One ecological concern is the inadvertent release of a new weed that will be difficult to control. However, the long and diverse experience of breeders and plant geneticists indicates that genetic crosses among nonweedy plants will not result in a serious problem (*9*). From our growing understanding of the genetic and biochemical basis of competition by weeds, it is obvious that many genes must interact appropriately for the plant to display the undesirable properties of a weed (efficient seed dispersal, long seed viability, rapid growth in an environment not normally favorable to other plants) (*10*). It is possible that hundreds or thousands of specific interacting genes are necessary for a plant to be a weed that will cause problems that approach the magnitude of those of kudzu. Thus the chance is exceedingly small that a cross between nonweeds will yield a weed. Most commercial field tests with genetically engineered plants will involve cultivated crops that have been specifically bred for high yield under intensive agricultural practices. As crops are bred for characteristics favorable to agriculture, competitive properties are weakened. Such crops, if left unattended, are not capable of competing well with other plants (*11*). Addition of characterized foreign genes to these crops should not produce an undesirable weed. Obviously, if weedy species are to be purposely genetically engineered, both the weed and the recombinant derivative need to be considered in light of potential environmental damage. Precautions currently used by scientists who purposely plant problem weeds (or crops that readily cross with problem weeds) should be sufficient for those who would plant weeds with incorporated foreign genes (*12*).

Genetic changes in weeds through man's activity but not involving genetic engineering occurred prior to application of recombinant DNA technology. In recent decades the use of chemical herbicides has caused uncharacterized genetic changes by which weeds have become herbicide-resistant (*13*). Problems can be overcome merely by using a herbicide to which the weed is not resistant, thus removing the environmental pressure for maintaining the resistance genes. A similar situation is found with insecticide application to plants, whereby insecticide-resistant insects may arise (*14*). Therefore, uncharacterized genetic changes causing problems with undesirable organisms have already been generated through traditional practices (*15*). Some of these changes probably are due to a single-gene modification. It would be extremely difficult (if not impossible) to purposely mutate a plant now considered safe to become a serious problem weed. It should be even more difficult to derive such a weed through acquisition of characterized foreign genes.

It can be anticipated that problems encountered in traditional breeding programs will occur in plant genetic engineering. For instance, certain popular corn hybrids were especially susceptible to the fungus *Helminthosporium* (*16*). This resulted in the corn leaf blight that destroyed a large portion of the U.S. corn crop in 1970. Breeders prepare for this type of situation, however. They were ready to quickly replace the susceptible variety with ones resistant to corn leaf blight. Field tests, therefore, are necessary to assess the threat of pathogens and to check for undesirable characteristics of new varieties, whether they are products of traditional breeding or genetic engineering.

There is a very small chance that plants resulting from genetic engineering with uncharacterized genes may produce a toxic secondary metabolite or protein toxin. For this reason, animal feeding experiments might be desirable before an edible crop is introduced commercially. Even through traditional breeding, however, toxin production can be a concern, especially when exotic plants are used in the breeding program. Several plants currently marketed, including rhubarb, cotton, and castor bean, contain toxins and therefore need to be carefully processed. Another example is a cultivar of potato that was removed from market shelves because, under certain stress conditions, it produced potentially hazardous levels of glycoalkaloids (*17*). Plant toxins are natural products, whether polypeptides or secondary metabolites, and should be rapidly degraded and not accumulate in the soil or water supply.

One reason critics urge caution over the release of genetically engineered plants is experience with problem plant species such as kudzu. This plant has been extremely difficult to control since its introduction, from the Orient, to the southern United States (*18*). The problems were not caused by changes in the genetic makeup of the plant, however, but rather by its introduction into a new environment. Each plant species evolved over eons to be competitive, which is why it exists naturally in at least one environment. In that natural environment a variety of factors, such as other plants, pests, and weather, keeps the population in check. Most U.S.–

grown crops were introduced from other countries (*19*), and the Department of Agriculture maintains large collections of wild members of our cultivated species to improve our crops (*20*). These collections are not normally maintained under strict quarantine.

Microorganisms

From the early years of this century certain microbes were grown in large volumes and, in many cases, became the foundation of new industries. Examples include the production of antibiotics, solvents, vitamins, amino acids, *Rhizobium*, *Azotobacter*, *Bacillus*, and yeast. In a number of industries mutated organisms have been utilized (*21*). These organisms have not caused nor will they create environmental or health problems that are difficult to control. This is not surprising considering microbial behavior in the environment. Every time rotted food is discarded in the woods, streams, or fields a culture of millions or billions of uncharacterized microbes is added to the environment. A rotted tree stump contains billions of lignin-degrading fungal cells, which are readily transported by people, animals, and insects that come in contact with the stump. No one is concerned that such uncharacterized organisms will cause difficult-to-control problems. During this century many cultures of bacteria and fungi (inoculants) have been added to soils or plants in the environment in the hope of finding useful applications, as in oil and chemical waste removal (*22*), plant residue decomposition (*23*), plant pest protection (*24*), and plant growth stimulation (*25*). No substantiated damage of any significance has been caused through such practices with fungi and bacteria not considered dangerous to our health or the environment.

Known pathogens have been used in field studies. For example, microorganisms that are weed pathogens have been used in experiments to control weeds (*26*). Precautions are required in these types of experiments; however, it is extremely unlikely that addition of characterized genes to such pathogens would increase their potential to cause serious problems.

There is no reason to think that a bacterium or fungus that is not known to damage the environment will cause environmental problems after it has obtained several characterized foreign genes. It is also extremely unlikely that a dangerous organism in the soil (for example, *Clostridium tetani*) will become more of a problem after acquiring these new genes from the introduced organism. Certainly, microorganisms intentionally and unintentionally added to the environment have naturally exchanged genes with other microorganisms. Such organisms have moved through wind and water and with man to distant places (*27*). Without man's intervention microbes are continually mutating, sharing, and rearranging genes through such agents as transposons, viruses, and plasmids.

Randomly introduced microorganisms generally are unable to predominate in new habitats because preexisting organisms already have evolved to successfully compete for those niches. In most cases a microbe in nature grows far more slowly than it does in laboratory cultures; thus the newly introduced organism will probably have a difficult time surviving and an even more difficult time significantly increasing and maintaining its population, whether it is genetically engineered or not. The extra burden to the organism carrying new genes should decrease its ability to compete and persist.

What is the chance that a harmless microorganism can become a pathogen after it has been genetically engineered to be agriculturally useful? Studies with pathogens have demonstrated that many specific genes with interacting activities (usually not all genetically linked to each other) are required for a microbe to cause disease, persist outside the host, and be transferred to subsequent hosts (*28*). Most of these studies involved animal pathogens, but it is becoming apparent that the same is true for plant pathogens (*29*). The chance that one could accidentally convert a microbe that normally is nonpathogenic to a problem pathogen through introduction of characterized foreign genes seems very small. That an organism has obtained genes involved in pathogenesis does not necessarily mean that the recipient will become a problem pathogen even if it damages a host in the laboratory. To become a serious problem (as defined earlier), it has to maintain the genes, be able to spread from host to host, and retain the genes during times when no host is available. Appreciation of this should minimize concern over natural dynamic exchange of genes among uncharacterized microbes in the field. There are many plant pathogens that can naturally exchange genes with *Escherichia coli*, but we do not see *E. coli* strains becoming pathogenic to plants.

One can argue that special problems may arise because of the very high concentration of recombinant organisms applied to crops. As mentioned earlier, high concentrations of microbes have been purposely added to crops for decades. In many cases such microbes can naturally exchange genes with pathogens. Certainly these inoculants have come in contact with pathogens, but no problems have been reported.

Examples are known from current practices not involving genetic engineering in which acquisition of a single gene or a mutation in a microbe causes ecological and health problems. Applications of certain herbicides or pesticides to soils enrich the soil for microbes (*30*) that degrade the chemical, resulting in the need to apply more of the chemical in subsequent years. Another example is acquisition of antibiotic resistance genes that have caused major medical problems. These problems arose not by man's ability to genetically manipulate organisms but rather by introducing new chemicals to the environment. The problems can be reversed by eliminating application of such chemicals. In fact, many current genetic engineering experiments are focused on projects expected to decrease the use of some industrially produced chemicals (*31*).

Need for Field Tests

Experience has shown that it is important to test the degree of toxicity of each newly synthesized chemical before it is used internally or added to large areas of land. Even if a new chemical is only a slightly modified analog of a known safe chemical, the degree of safety cannot be extrapolated from that safe chemical. In fact, analogs of normal metabolites can be most dangerous. By comparison, minor modifications obtained by breeding safe plants or mutating safe microbes do not yield progeny that become serious problems. Minor modifications are expected from genetic engineering; agronomic problems that may arise can be assessed only by field testing. To allay concerns about the safety of a recombinant organism, it would be useful to follow testing protocols before the organism is generally released. However, the task of designing relevant tests for most situations seems to be enormous, if achievable at all (*32*). How will a greenhouse test show that a corn line resulting from a standard genetic cross will not become a problem weed? If a bacterium increases corn yield in the greenhouse, how will researchers know, without field testing, that it will not harm the following season's crop? Tests aimed toward predicting the level of microbe persistence in a field could be very difficult and not relevant (*33*). Because different soils, soil treatments, and weather conditions can dramatically alter the growth rate, population, and persistence of a microbe, greenhouse or growth chamber experiments have little relevance to field results. It is easy to imagine that one type of pathogen can damage a plant at a density of ten cells per gram of soil whereas another pathogen may initiate plant disease only at a million cells per gram. Current field testing practices seem to be the best guide to predicting safety.

Certain microorganisms and plants have been introduced in the environment without need for regulation. Such organisms containing recombinant DNA should not be of concern unless the organisms or introduced genes have obvious potential problems (for example, *Clostridium botulinum* and the botulinus toxin gene) that require special precautions. It is unlikely, however, that such experiments would be proposed for field testing. Because of the complex interaction of genes required for an organism to cause a serious disease problem or major environmental disruption, it would be extremely difficult to purposely engineer an organism now considered safe to an organism that would be a significant problem. A program that aims to utilize, in agriculture, a plant, bacterium, or fungus considered to be safe but with several foreign genes will have essentially no chance of accidentally producing an organism that would create an out-of-control problem. The chance of a problem resulting from genetic engineering should be viewed in perspective and compared to known problems caused by currently accepted genetic and chemical practices, such as breeding and the use of chemical pesticides. It may be valuable for one or more laboratories to test, under appropriate containment, several worst-case scenarios, as was done with *E. coli* (*34*). Such a test might utilize an *E. coli* strain genetically engineered to contain pectolytic enzymes required for soft-rot disease of plants (*35*). Regulations governing release of genetically engineered organisms should be based on scientific experience and informed debate of the issues.

To summarize, traditional agricultural practices continually improve useful crops and microbes by taking advantage of new genetic modifications. In almost all cases the exact nature of these modifications is unknown. There has not been any special concern about the new variants. By comparison, genetic engineering will make well-characterized and specific modifications. Thus there does not seem to be any reason to expect greater problems arising from recombinant organisms in agriculture than from organisms produced through traditional practices.

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