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GENETIC ENGINEERING IS NOT AN EXTENSION OF CONVENTIONAL PLANT BREEDING; How genetic engineering differs from conventional breeding, hybridization, wide crosses and horizontal gene transfer

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Genetic engineering is not just an extension of conventional breeding. In fact, it differs profoundly. As a general rule, conventional breeding develops new plant varieties by the process of *selection*, and seeks to achieve expression of genetic material which is already present within a species. (There are exceptions, which include species hybridization, wide crosses and horizontal gene transfer, but they are limited, and do not change the overall conclusion, as discussed later.) Conventional breeding employs processes that occur in nature, such as sexual and asexual reproduction. The product of conventional breeding emphasizes certain characteristics. However these characteristics are not new for the species. The characteristics have been present for millenia within the genetic potential of the species.

Genetic engineering works primarily through *insertion* of genetic material, although gene insertion must also be followed up by selection. This insertion process does not occur in nature. A gene "gun", a bacterial "truck" or a chemical or electrical treatment inserts the genetic material into the host plant cell and then, with the help of genetic elements in the construct, this genetic material inserts itself into the chromosomes of the host plant. Engineers must also insert a "promoter" gene from a virus as part of the package, to make the inserted gene express itself. This process alone, involving a gene gun or a comparable technique, and a promoter, is profoundly different from conventional breeding, even if the primary goal is only to insert genetic material from the same species.

But beyond that, the technique permits genetic material to be inserted from unprecedented sources. It is now possible to insert genetic material from species, families and even kingdoms which could not previously be sources of genetic material for a particular species, and even to insert custom-designed genes that do not exist in nature. As a result we can create what can be regarded as synthetic life forms, something which could not be done by conventional breeding.

It is interesting to compare this advance to the advances that led to creation of synthetic organic chemicals earlier in the 1900s. One could argue that synthetic chemicals are just an extension of basic chemistry, and in certain senses they are. Yet

when we began creating new chemicals that had not previously existed on the earth, or which had only been present in small quantities, and began distributing them massively, we discovered that many of these chemicals, even though they were made of the same elements as "natural" chemicals, had unexpected adverse properties for the environment and health. Because we had not co-evolved with them for millenia, many (though by no means all) had negative effects. Among the serious problems were PCBs and vinyl chloride, which were found to be carcinogens, and numerous organochlorine pesticides, which were found to be carcinogens, reproductive toxins, endocrine disruptors, immune suppressors, etc. After several decades of use, these effects caused such concern that we passed the Toxic Substances Control Act which required premarket screening of synthetic organic chemicals by EPA for such effects as carcinogenicity, mutagenicity and impact on wildlife, and changed our pesticide rules similarly. There are many ways in which these two scientific advances are not analogous, but the experience with synthetic organic chemicals underlines the potential for unexpected results when novel substances are introduced into the biosphere.

We will discuss three specific ways in which genetic engineering differs from conventional breeding, and some of the implications for safety, in more detail. The argument is frequently made that genetic engineering is not only an extension of conventional breeding, but is more precise, and therefore safer. We believe that in fact it represents a quantum leap from conventional breeding, is more precise in one way, but more unpredictable in others. We will discuss the following key areas of difference and their implications for unexpected effects: scope of genetic material transferred/unnatural recombination, location of the genetic insertions, and use of vectors designed to move and express genes across species barriers. As a subset of the last category there is the use of foreign promoters (genetic "on" switches) and foreign marker genes (particularly genes coding for antibiotic resistance). Finally we will discuss implications for FDA policy.

Scope of Gene Transfers

As for the scope of genetic material transferred, genetic engineering allows the movement of genetic material from any organism to any other organism. It also offers the ability to create genetic material, and expression products of that material, that have never existed before.

This radically differs from traditional breeding, which merely permits the movement of genetic material between different varieties within species, between closely related species, or closely related genera. Even hybridization and wide crosses cannot move genetic material much beyond these limits. The vast bulk of hybrid crops consist of the mating of two genetically pure lines (i.e. lines that are homozygous for all alleles) of the same crop to create a line which is heterozygous. Thus, hybrid corn is simply the crossing of two pure corn varieties to produce a mixed line. Occasionally, though, in conventional breeding, plant breeders will cross a wild relative of a crop (usually a different species within the same genus) in order to transfer particular traits

from that wild relative (such as resistance to a given disease) to the crop. However, hybrids between two species are also known to occur naturally, although such hybrids are primarily restricted to plants with certain characteristics—such as perennial growth habit—which most crop plants lack (Ellstrand et al., 1996).

Wide crosses, also used by breeders, also occur in nature, but they are rare. When breeders perform wide crosses, they mate two different genera. While the pollen of species A may successfully fertilize the egg of species B, the embryo may not be able to naturally survive and develop into a seedling. The plant breeder, through a technique called embryo rescue, will remove such an embryo from the original hybrid seed and put it into a nutritional environment in the laboratory (one containing various nutrients and plant hormones) and raise it into seedling and adult plant. While such wide crosses are artificial in one sense (the plant wouldn't normally germinate or survive to adulthood), they still represent the mixing of genomes from plants that are fairly closely related and in which fertilization can occur. Wide crosses will happen between plants from two different genera within the same family and often the same sub-family. Wide crosses cannot be achieved with plants from widely different families. Thus, while wide crosses, as breeders perform them, do not occur in nature, they represent only a slight stretching of the boundaries of what can occur in nature. In a sense wide crosses represent a stretching of these boundaries by inches compared to miles with GE. After all, with GE, one can mix genes not only from widely different plant families, one can put genes from any organism on earth, or can create genes which have not existed before and put them, into plants.

The mixing of genes from very different sources is likely to introduce new elements of unpredictability. Because conventional breeding, including hybridization and wide crosses, permits the movement of only an extremely tiny fraction of all the genetic material that is available in nature, and only allows mixing, and recombination, of genetic material between species that share a recent evolutionary history of interacting together, one would expect that the products of conventional breeding would be more stable and predictable. The genome is a complex whole made up in part of genes and genetic elements that interact in complex regulatory pathways to create and maintain the organism. Any new genetic material that enters the genome must fit into this complex regulatory whole or it may end up destabilizing the whole. Think of the genome as a complex computer program or as an ecological community. When one introduces a new subprogram within the larger complex computer program, no computer programmer can reliably predict what will happen. Because of the complexity of such large programs, a small new subprogram can have unpredictable effects and may ultimately cause the whole program to crash. With a complex ecosystem, the introduction of a new species can have a range of effects, from virtually nothing to a catastrophic effect on the ecosystem; most of these changes cannot be reliably predicted knowing just the biology of the introduced species.

The view that genetic engineering may be more prone to unexpected outcomes because it creates profound disruption in the normal interactions of genes is supported

by differences in the success rate in producing viable stable offspring, for genetic engineering versus conventional breeding. In nature, most offspring are viable; the vast majority of seeds germinate and produce organisms that survive and reproduce. In conventional breeding, scientists grow many plants and keep only a few with the most desirable traits; however the ones they discard are still almost always normal examples of the species. This is not true for products of genetic engineering. In the early days of GE, although one could select cells which contained and expressed the desired trait (due to the use of marker genes), it was necessary to attempt to grow the engineered cells into whole plants to determine the overall impacts of the GE. A very large percentage of the transformed cells either were not viable, were grossly deformed, or failed to stably incorporate the desired trait, i.e. failed to produce that trait in the plant in successive generations (Crouch, personal communication). Some of the malformations may be due to difficulties with tissue culture of the transformed cells; however unexpected genetic effects also appear to be a causative factor. In fact, only one in thousands (or tens of thousands or in some cases even millions) of attempts achieves the desired results in terms of a seed that incorporates the desired traits, and expresses them in a useful fashion generation after generation, and doesn't have undesirable side effects. Assertions that genetic engineering is a highly precise process therefore seem misleading.

Location of Gene Insertion

GE can control relatively precisely the trait that is being inserted into a host plant genome. However it cannot yet control the location where the trait is inserted into the genome with any precision, nor guarantee stable expression of the transgene. The process of insertion of foreign genetic material via GE into the host plant genome and the expression of such material is called transformation. Transformation is currently accomplished through several relatively crude methods which are relatively random in where the genes end up. One transformation method frequently used consists of a manipulating a bacteria in the genus *Agrobacterium*. These bacteria are among the few known which can transfer their genetic material to another kingdom/phyla. These bacteria cause a disease in plants (either a tumor-like growth called crown gall disease at the infection site, or uncontrolled sprouting of roots from the infection site) by attaching to the plants, transferring bacterial DNA into the plant and getting that DNA incorporated into the host plant genome. *Agrobacterium*-mediated plant transformation involves engineering the *Agrobacterium* by deleting the disease-inducing genes, retaining the bacterial transfer DNA (T-DNA) and inserting the genetic traits/elements to be transferred. This engineered *Agrobacterium,* sometimes called a bacterial "truck" is then just mixed with the desired plant cells and allowed to transform/infect them. The use of *Agrobacterium*-mediated transformation occurs primarily with dicots (non-grass like plants) and is difficult to do with grains.

The direct gene introduction methods include chemical treatment or electroporation of protoplasts and use of the "gene gun." Chemical treatment or electroporation consists of exposing plants to chemicals or an electrical field that makes

the protoplasts' cell membrane more "porous" facilitating the uptake of DNA from the surrounding medium. The gene gun is used to "shoot" microscopic particles (such as gold) covered with DNA into the plant tissues themselves. In all three cases, once the DNA is in the plant cell it still needs to be incorporated in the host genome. This is done using genetic elements (T-DNA) from *Agrobacterium.* The direct gene introduction methods are often used in transforming cereals which are fairly resistant to *Agrobacterium*-mediated transformation. Furthermore, the direct introduction methods routinely lead to insertion of multiple copies of the genetic construct either at a single site or in multiple locations in the host genome. *Agrobacterium*-mediated transformation, on the other hand, usually leads to insertions at a single site in the plant genome. In either case, however, the site or sites are fairly random. Because the effect of a gene on the whole organisms is significantly governed by its location, the lack of control over location is a significant cause of unexpected effects.

In terms of the location of genetic material in traditional breeding, since it occurs between organisms that share a recent evolutionary background, it involves the shuffling around of different versions (called alleles) of the same gene. Furthermore, these genes are usually fixed in their location on the chromosome by evolution. With GE, the genetic insertion happens in unpredictable places which can lead to unpredictable effects. Thus in this key regard, genetic engineering is more random than conventional breeding.

A clear example of unpredictability was seen in an experiment performed with *Arabidopsis thaliana*, a plant in the mustard family that is frequently used for biological research (Bergelson et al., 1998). The experiment compared several lines, one developed by conventional breeding, and two by genetic engineering, that all exhibited the same trait of herbicide tolerance. Researchers at the University of Chicago induced herbicide (chlorsulphuron) tolerance (HT) into *A. thaliana* via a form of conventional breeding called mutation breeding and via GE. In mutation breeding, the researchers exposed an ecotype (or variety) of *A. thaliana* (ecotype Columbia) to a chemical, ethyl methanesulphonate, that induces mutations in genes. They then isolated individuals that had a mutant version of the allele coding for the enzyme acetolactate synthase (*Csr1-1*) which conferred resistance to chlorsulphuron. They isolated those individuals (by spraying the plants with chlorsulphuron; only the resistant ones survived) and backcrossed them to wild-type *A. thaliana* ecotype Columbia for six generations. For the GE plants, they inserted the *Csr1-1* gene using a certain vector and created two separate GE lines, i.e. two separate transformation events. Each line contained insertions of the *Csr1-1* transgene at a single site.

A. *thaliana* is normally a self-pollinating species with very low rates of cross-pollination. Thus, it was thought that there would be virtually no gene flow to other individual *A. thaliana* plants and thus virtually no risk of transgenes moving from engineered *A. thaliana* to non-engineered neighbors. There were also questions as to whether there were any ecological differences between conventional breeding and GE versions. An experiment was designed to test these issues. The experiment entailed

planting 144 plants—of which 25% were wild type, 25% contained the HT gene via mutation breeding, and 50% contained the HT gene via GE (25% from line 1 and 25% from line 2)—and then collecting about 100,000 seeds from the wild-type plants and looking to see how many carried the HT gene, i.e. to look at the per-plant outcrossing rate.

The results were quite surprising. The per-plant outcrossing rate was 0.30% for mutant fathers (i.e. containing the HT gene from mutation breeding) and 5.98% for transgenic fathers (i.e. containing the HT gene from GE). Thus, transgenic *A. thaliana* were 20-fold more likely, on average, to outcross than ordinary mutants (i.e. those derived from mutation breeding). Further genetic investigation found that the outcrossing rates in the two GE lines were very different—1.2% and 10.8%. Thus, the two GE lines of *A. thaliana* demonstrated 4-fold and 36-fold higher rates of outcrossing compared to traditional breeding. Since the HT gene was the same, the differences between GE and mutation breeding appear to be associated with the overall process of genetic engineering. The differences between the two GE lines appears to be due to the difference in location as to where the insertions happened, as the entire genetic construct was the same. This experiment clearly shows that a difference exists between conventional (including mutation) breeding and genetic engineering. In essence, in the one GE line, the act of genetic engineering had transformed a species that was normally an inbreeder to an out-crosser.

Another example of an unexpected effect that may have been due to the location of the insertion occurred in an experiment with yeast that involved adding not a transgene from another species but inserting multiple copies of a naturally occurring yeast gene. In that experiment, the scientists found that a three-fold increase in an enzyme in the glycolytic pathway, phosphofructokinase, resulted in a 40-fold to 200-fold increase of methyglyoxal (MG), a toxic substance which is known to be mutagenic, depending on the yeast line. As the scientists themselves concluded, "the results presented here indicate that, in genetically engineered yeast cells, the metabolism is significantly disturbed by the introduced genes or their gene products and the disturbance brings about the accumulation of the unwanted toxic compound MG in cells. Such accumulation of highly reactive MG may cause a damage in DNA" (Inose and Murata, 1995). The position of the insertion could potentially explain why the same genetic construct caused from a 40-fold to 200-fold increase in MG concentration, depending on the different yeast transformation events.

Use of vectors designed to move and express genes across species and ecological barriers

A third important way genetic engineering differs from conventional breeding is in its introduction of genes that move and cause expression of desired traits.

Since traditional (or conventional) breeding involves mixing of genetic material from species that are sexually compatible/fertile, there is no need for special genetic elements to facilitate this process; sexually compatible species already contain such

elements. The same is not true with GE, which utilizes non-sexual means of reproduction and usually entails the movement of genetic material between species that could rarely, or never, exchange genetic material in natural conditions. To facilitate this process, GE makes use of vectors specifically designed to move and express genes across species and ecological barriers. These vectors consist of genetic elements and sequences derived from efficient genetic parasites (viruses, plasmids, mobile elements, etc.) and are specifically designed to breach species barriers, i.e. to smuggle genes into cells that would otherwise exclude them and get the genetic material inserted into the genome of the host plant.

In microorganisms, plasmids are known to move readily between bacteria. In crop genetic engineering, plasmids are invariably used and are derived from the tumor-inducing plasmid (the Ti-plasmid) of the bacterium *Agrobacterium,* as discussed earlier.

Genetic elements from *Agrobacterium* are never added to a plant's DNA through conventional breeding, and are only see in the DNA of other species in nature in plants infected with crown gall or hairy root disease, in the infected cells.

Virtually all crop plants derived via GE also contain a powerful promoter (a genetic regulatory or "on"-switch) from the Cauliflower mosaic virus (the CaMV 35S promoter), which in nature causes a disease in plants in the mustard family. Normally, genes in a plant have their own promoters so that the gene will be turned on at the right time in development and will be expressed at the right level, i.e. the right amount of the desired gene product will be produced. A promoter from a plant virus is used because viruses are genetic parasites that have the capability to infect a plant cell and hi-jack its cellular machinery to make multiple copies of itself in a short period of time. The CaMV 35S promoter is used precisely because it is such a powerful promoter, which leads to hyperexpression of the transgenes, having them be expressed at perhaps 2 to 3 orders of magnitude higher than of the organism's own genes. The CaMV 35S promoter effectively puts the transgene(s) outside of virtually any regulatory control by the host genome as the natural plant promoters for each gene allow.

Thus, the use of a foreign promoter is needed in GE and is not found in traditional breeding, including hybridization and wide crosses and so constitutes a difference between GE and conventional breeding. Indeed, for most of the genes that are being transferred (gene for herbicide tolerance, gene for Bt endotoxin), if one used the naturally occurring promoter for that gene, the plant would never be able to recognize the inserted gene and express it. Thus, a promoter that works in plants must be used; hence the reason for the widespread use of a plant viral promoter. Most promoters that work in plants fail to get the gene expressed at a high-enough level to do the work; hence the use of the CaMV 35S promoter (the strongest of the various promoter in the CaMV).

Use of such strong promoters also raise safety concerns. Since the CaMV 35S is so strong, not only can it affect the introduced transgenes, it can also affect genes (either

turn them "on" or turn them "off") thousands of base pairs upstream and downstream from the insertion site on a given chromosome and even affect behavior of genes on other chromosomes. Consequently, depending on the insertion site, a gene that codes for a toxin could be turned "on," leading to production of that toxin.

The potential of CaMV to turn genes "on" is of particular concern because of what we are learning about how plants normally turn many genes "off," through a phenomenon known as gene silencing. Gene silencing appears to be a key defense against intrusion of foreign DNA, particularly from disease-causing organisms, and also regulates normal gene expression. In the last 5-10 years, scientists have come to realize that genetic material can in fact move between organisms that are incapable of mating with each other. Such lateral movement of genetic material is called horizontal gene flow (vertical gene flow is the movement of genes from parent to offspring), and occurs in nature more frequently than has been assumed. Such horizontal gene flow is know to occur in microorganisms; indeed, it is one of the main ways that antibiotic resistance or pathogenicity is passed around among bacteria. Furthermore, numerous viruses can insert themselves into host genomes.

Until recently, such horizontal gene flow was considered rare or non-existent in plants. In 1998, scientists reported evidence that genes from a fungus had invaded 48 out of 335 genera of land plants surveyed and that such movement occurred on some 32 separate occasions (Cho et al., 1998). They extrapolated these genes had invaded higher plants via horizontal transfer over 1,000 times and that such a "massive wave of lateral transfers is of entirely recent occurrence" (Cho et al., 1998: 14244). Just a couple of months ago, a paper in the November 19, 1999 issue of the *Proceedings of the National Academy of Sciences* demonstrated that sequences from a previously unidentified tobacco pararetrovirus had repeatedly integrated themselves into tobacco chromosomes (Jakowitsch et al., 1999). Prior to this study, plant viral sequences were thought to integrate rarely, if at all, into host genomes.

The recognition that genetic material can move laterally between species, along with the recognition in recent years that DNA is not as fragile as previously thought and can persist for extended periods of time in a variety of habitats—aquatic, terrestrial, etc.—has lead to increasing research on the mechanisms that organisms use to prevent the intrusion of foreign DNA. After all, since genomes are complexly regulated wholes, work with other complex systems has shown that intrusion of new elements can lead to destabilization of that whole. Indeed, normal development in a plant requires an exquisite coordination of genes, with the right set being turned on at the proper moment in development. The plant's regulatory system should have a mechanism to ensure that unwanted disruptions of such an elaborately coordinated system are prevented or minimized.

Such defenses have been found to exist, and consist of pre-integration and post-integration (i.e. before and after the foreign DNA has been incorporated into the host chromosome) mechanisms (Traavik, 1998). The pre-integration mechanisms include

preventing the DNA from entering the cell or digesting the foreign DNA (with nucleases) that enters the cell.

The post-integration mechanisms consist of forms of "gene silencing." Gene silencing was initially discovered in transgenic plants and was initially thought to occur only in the case of transgenes. It is a significant impediment to genetic engineering because it leads to instability. A review of this topic in 1994 stated this succinctly: *"While there are some examples of plants which show stable expression of a transgene these may prove to be the exceptions to the rule*. In an informal survey of over 30 companies involved in the commercialization of transgenic crop plants, which we carried out for the purpose of this review, almost all of the respondents indicated that they had observed some level of transgene inactivation. *Many respondents indicated that most cases of transgene inactivation never reach the literature*" italics added (Finnegan and McElroy, 1994: 883).

Gene silencing can occur by preventing either transcription (making a messenger RNA [mRNA] copy of a DNA sequence) or translation (making a protein from the mRNA); the former occurs in the nucleus, the latter in the cytoplasm. Hypermethylation of genetic material is one mechanism associated with preventing transcription (Matzke and Matzke, 1995) while formation of aberrant RNA molecules, with occasional DNA methylation, is the main mechanism for posttranscriptional silencing (Scheid et al, 1998).

Indeed, transgene silencing is becoming a frequently observed phenomenon. Although not completely understood, a number of factors have been shown to affect transgene inactivation including insertion of multiple copies of the transgene, hyperexpression of transgenes (e.g. due to use of CaMV 35S promoter) and environmental factors (Srivastava et al., 1999). High numbers of copies of the transgene and presence of multiple insertion sites lead to a much higher incidence of gene instability. Since these are characteristics of direct gene transfer methods, which are commonly used on cereals, we should expect a higher probability of problems in such crops.

Perhaps the first, and most well studied, example of such unstable transgene silencing was seen in work done in Germany with petunias that were engineered with a single gene from corn to produce a new salmon red flower color (Meyer et al., 1992). After transforming the petunias, the scientists worked with a line that contained a single copy of the inserted gene at a single insertion site (i.e. the most stable situation). Some 30,000 transgenic petunias carrying a single gene conferring the salmon red flower color phenotype were grown outside and observed for differences. Initially, the scientists were looking for mobile elements (so-called "jumping genes" or transposons) that were naturally occurring—such mobile elements would "jump" into the color gene disrupting it and leading to a different color—and thought to occur at frequencies of 1 in a 100 to 1 in 100,000. The unexpected result was the finding that significant numbers of the plants were either weakly colored, white, had variegated colors or different

sectors of the flower being different colors. Since petunias produce up to 50 flowers over the course of the growing season, any changes in individual plants can be easily seen. Furthermore, the number of non-salmon red flowers increased during the season. At the beginning of the season, the percentage of flowers with various color patterns were as follows: salmon red, 91.6%, weakly colored, 7.6%, sectored colored, 0.3%, variegated, 0.2%, and white, 0.3%. By the end of the season the figures were 37.6%, 60.9%, 1.1%, 0.2% and 0.2%, respectively. The change in color was linked both to the age of the plant and to environmental circumstances as well—there was a three-week period near the end of the growing season with particularly hot days and bright sun. Analysis at the molecular level revealed that the bulk of the non-salmon red flowers exhibited methylation of the promoter (which was the CaMV 35S) associated with the transgene.

This result, which was quite unexpected, clearly demonstrates that the transgene are unstable and prone to being switched off or turned down. It also demonstrates the important effect of the environment as the flower color changed over time; by the end of the season over 62% of the flowers no longer exhibited the full salmon red color. Thus, there appears to be greater instability in the transgene expression over time, depending on both the age of the plant and environmental conditions.

Since this study, more work on gene silencing has been done. The newer work has also shown that "gene silencing" is not restricted to transgenes, but is found to occur naturally under certain conditions. The current scientific thinking is that gene silencing evolved for three purposes: to regulate normal gene expression; to inactivate foreign DNA that comes from pathogens; and to prevent genetic events—such as movement of "jumping genes" (transposons)—which may disrupt the normal structure and function of the genome. In an overall sense, gene silencing serves to protect a genome from being disrupted by external or internal forces. Since it serves as a mechanism for detecting and destroying foreign genetic material, it's not surprising that gene silencing happens so frequently in GE. Thus, for GE to work, the genetic engineer must use all sorts of mechanisms to attempt to overcome the plant's natural defenses. That's the reason for the use of powerful genetic elements from viruses (promoters and enhancers), pathogenic bacteria, and mobile elements (e.g. transposons).

Given the inherent unstability of transgenes and the phenomenon of gene silencing, which can be influenced by environmental conditions, we would expect to see problems associated with transgene stability in the field. The unpredictable influence of the environment may explain what went wrong in Missouri and Texas with thousands of acres of Monsanto's glyphosate tolerant cotton and Bt cotton, respectively. In Missouri, in the first year of approval, almost 20,000 acres of this cotton in malfunctioned. In some cases the plants dropped their cotton bolls, in others the tolerance genes were not properly expressed, so that the GE plants were killed by the herbicide (Fox, 1997). Monsanto maintained that the malfunctioning (a result of gene unstability) was due to "extreme climatic conditions." A number of farmers sued and Monsanto ended up paying millions of dollars in out-of-court settlements. In Texas, a

number of farmers had problems with the Bt cotton in the first year of planting. In up to 50% of the acreage, the Bt cotton failed to provide complete control (a so-called "high dose") to the cotton bollworm (*Helicoverpa zea*). In addition, numerous farmers had problems with germination, uneven growth, lower yield and other problems. The problems were widespread enough that the farmers filed a class action against Monsanto. Just a few months ago, Monsanto settled the case out of court, again by paying the farmers a significant sum (Schanks [plaintiffs attorney], personal communication).

Horizontal gene flow is the closest thing to genetic engineering in nature. However it appears that only a limited number of microorganisms can insert DNA into plants, and that plants have evolved defenses against this. Further, each insertion is a one-time event, whereas with GE, rather than a single mutant individual appearing, the environment is flooded with many many transformed plants, containing DNA from sources that bacteria would never naturally carry. Again, GE is a quantum leap from the natural phenomenon. The simultaneous introduction of the CaMV promoter gene, to override silencing, destanbilizes the engineered genome

Another significant differences between conventional breeding and GE is the virtually ubiquitous use of marker genes that code for antibiotic resistance. Such marker genes are needed to facilitate identification of the fairly rare cases where genetic transformation has been successful. The widespread use of genes that code for resistance to antibiotics raise the potential question as to whether such genes be horizontally transferred to bacteria rendering them resistant to the antibiotic in question.

Conclusion

Genetic engineering clearly differs from conventional breeding in several ways. Conventional breeding relies primarily on *selection,* using natural processes of sexual and asexual reproduction. Genetic engineering utilizes a process of *insertion* of genetic material, via a gene gun or other direct gene introduction methods, or by a specially designed bacterial truck, which does not occur in nature. Genetic engineering can insert genetic material from any life form into any other; conventional breeding generally can only work within a species, or at most, within closely related genera, as when they do wide crosses. Conventional breeding relies on mixing characteristics from different populations within a species and then selecting from a plants natural complement of genetic elements. However genetic engineering relies on inserting genetic elements, and they end up in random locations, which can disrupt complex gene interactions. Many of the products exhibit unexpected effects.

Genetically engineered plants almost always contain a viral promoter gene, the "on"switch for the gene inserted; genetic material from *Agrobacterium*, which facilitates

transfer of the genetic construct into a plant's genome; and in most cases a bacterial antibiotic marker gene. These are never deliberately introduced in products of conventional breeding.

There are thus key identifiable scientifically documentable differences between genetic engineering and conventional breeding, both in the process, and in the genetic makeup of the product. Indeed, in any situation in which DNA is recoverable, the presence of engineered DNA can be identified in the product.

Whether these differences are "significant" or "insignificant", however, is a value question and a philosophical question, not a scientific question. We see genetic engineering as a quantum leap from conventional breeding—as different from it as nuclear power generation is from a coal-fired plant. In our view traditional breeding is humankind's attempt to manipulate natural breeding processes for our own benefit. But this attempt, while wildly successful in one sense (the creation of all the major crop plants from weedy wild relatives), has only mildly pushed the barriers of genetic material transfer. GE, on the other hand, does away with all such barriers in the natural world, permitting scientists to manipulate genetic materials in a way that was inconceivable before.

A number of scientists, particularly ecologists, see the differences as we do. However other scientists, particularly molecular biologists maintain it is a continuum. In the end, it is a matter of values, judgment and opinion as to who is correct. While science can inform this debate, this is not a question science can answer. Indeed, it can well be argued that there is no one right answer as to whether this difference is "significant" or not.

In terms of a FDA decision about labeling, this debate about how "significant" the difference is *should be irrelevant*. If there is a documentable difference between two foods, or two processes, and consumers care about the difference, then under the Food Drug and Cosmetic Act FDA has the authority to require labeling and should do so. It does not matter if it is a small difference or a large difference. Nor does it matter whether consumers are "right" or "wrong" to care about this difference.

Labeling would not be a deviation from previous FDA policy. Indeed, the failure to require labeling is a deviation from previous FDA policy. The difference between frozen peas and fresh peas, one could easily argue, is much less than the difference between genetically engineered peas and conventional peas. The frozen and fresh peas can be genetically identical. The frozen peas may even be nutritionally superior, even as consumers choose fresh peas thinking fresh is better. Yet FDA appropriately requires labeling about the difference, and allow consumers to make their own choices about what to buy, even if those choices are a "mistake", leaving it to the marketplace to educate consumers about pluses and minuses of each type of product. It should do the same for any food produced by, or derived from food produced by, a process that

inserts genetic material using a gene gun, a specially-designed vector, or a comparable method.

Additional reasons to label include helping consumers manage food allergies and sensitivities and allowing the public health community to track and identify any unexpected effects. These are discussed in more detail in Consumers Union's comments to FDA on Docket No. 99N-4282.

The science is also clear that this unique and identifiable process of genetic engineering creates a new and unique potential for unexpected effects, due to the unique nature of the material being inserted, from a genome which has not previously interacted with the host genome, due to lack of control over the location at which the gene is inserted, and due to the introduction the CaMV "promoter" gene, which overrides the existing genetic programming. FDA therefore has an obligation to require mandatory reviews of all genetically engineered food before it goes on the market, and develop ways to screen for unexpected effects which could have health consequences.

There are also predictable risks, such as potential risks of toxins, allergens and nutritional changes and antibiotic market genes, which FDA should address. These are discussed further in our comments to the docket.

The details of what that safety review entails should be developed through a further process of notice and comment.

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