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PERSPECTIVE

Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics

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The costs of meeting regulatory requirements and market restrictions guided by regulatory criteria are substantial impediments to the commercialization of transgenic crops. Although a cautious approach may have been prudent initially, we argue that some regulatory requirements can now be modified to reduce costs and uncertainty without compromising safety. Long-accepted plant breeding methods for incorporating new diversity into crop varieties, experience from two decades of research on and commercialization of transgenic crops, and expanding knowledge of plant genome structure and dynamics all indicate that if a gene or trait is safe, the genetic engineering process itself presents little potential for unexpected consequences that would not be identified or eliminated in the variety development process before commercialization. We propose that as in conventional breeding, regulatory emphasis should be on phenotypic rather than genomic characteristics once a gene or trait has been shown to be safe.

Although plantings of a few transgenic crops developed through the use of recombinant DNA techniques continue to increase in area globally[1], the costs and uncertainties that result from the rapidly proliferating national and international regulations covering transgenic crops significantly impede further development of additional crops and traits[2,3]. Transgenic crops face a daunting array of pre-commercialization regulatory requirements and post-commercialization market restrictions that traditionally bred crops do not[4,5], even though similar phenotypic traits may be involved in both cases[6]. The cost of meeting regulatory requirements for major globally traded crops (recently estimated at $20–30 million per product[7]) limits commercialization of transgenic crops to a few multinational corporations and to traits that have a large economic payback. High regulatory costs effectively block academic and government research institutions and small businesses from commercializing transgenic crops[5] and discourage the establishment of new biotechnology firms and the flow of venture capital that finances them[7]. Regulatory costs, along with intellectual property acquisition, have contributed to the consolidation of multinational agricultural biotechnology companies[8].

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Regulatory costs also play a role in the growing disparity between the expanding global adoption of the large-market transgenic maize, soybean, cotton and canola crops[1] and the so-called 'small-market' or 'specialty' crops, for which field trials and commercial releases of transgenic food crops have all but stopped[3]. In 2003, fruits, vegetables, landscape plants and ornamental crops accounted for more than $50 billion in value in the United States, representing 47% of the total US farm crop income[9]. Of this, the only transgenic commodities currently marketed are small amounts of virus-resistant papayas and squash, insect-resistant sweet corn, and blue carnations, even though numerous examples of useful transgenic traits have been researched and developed[10,11]. Although market acceptance and intellectual property issues are also serious limitations[12,13], regulatory hurdles clearly present significant challenges that are delaying or preventing commercial release of transgenic specialty crops[3,14].

Comprehensive discussion of regulatory requirements for transgenic crops at the national and international levels is a broader topic than can be covered here, and recent studies have addressed them in detail[4,15]. Sensible proposals for regulatory modifications based on potential for ecological spread and impact were made years ago[16]. Specific recommendations were recently made for how regulations could be streamlined considering biological novelty and likely effect on fitness of specific genes, and the growing familiarity of a number of transgenic tools[17–19]. Here, we propose some specific changes in regulatory approaches based on extensive experience with conventionally bred crops, the first generations of transgenic crops and the growing knowledge of the complexity of genome structure and dynamics[20]. Our goal is to rationalize regulatory requirements so that they are congruent with science-based risk factors, focus scrutiny in safety assessments where it is most important and allow the commercialization of safe transgenic varieties that can provide health and/or economic benefits to consumers or farmers in developed and developing countries. We believe that certain regulatory requirements that were prudent for the initial phases of commercial development of biotech-derived crops actually are not necessary today to ensure a safe food supply. Instead, we propose stratifying various kinds of genetic constructions and experiments into risk classes that will be subject to different, and more proportionate, regulatory requirements.

Deregulate the transgenic process

It seems obvious that the phenotypes of transgenic plants and their safety and behavior in the environment, not the method used to produce them, should be the main focus of regulatory concern. Environmental and toxicological issues will be influenced by the expressed traits rather than the genes *per se*, particularly as DNA and

most encoded enzymes themselves do not appear to pose threats. Thus, the product not the process should be evaluated. Although this rational 'product' not 'process' principle has been repeatedly supported in US National Research Council reports[21–23], and is official US government policy[24], it has not been translated into regulatory practice by the US Department of Agriculture (USDA) and Environmental Protection Agency (EPA), nor by other international biosafety protocols[15]. Instead, transgenic plants are subjected to an array of additional requirements before release into the environment, even though similar traits developed through 'conventional' breeding (e.g., mutation-derived herbicide resistance[6]) are exempt from these requirements. The complex genomic manipulations used in conventional breeding (e.g., wide crosses between species, mutagenesis, protoplast fusion, somaclonal variation, ploidy manipulation) are seldom characterized at the molecular level before variety release. The long history of safe and beneficial use of this array of methods for generating genetic variation argues that the method of modifying genomes *per se* should not drive the regulatory process. Instead, the traits and the phenotypes that they produce, whether developed through traditional or transgenic breeding, should be the focus of risk analyses.

Rationalize the basis for transgenic regulation

The legal authority in the United States for the USDA Animal and Plant Health Inspection Service (APHIS) to regulate transgenic crops derives from its mandate to protect the agricultural environment against pests and diseases. Since some components of transgenic plants often contain DNA from pathogens, such as *Agrobacterium tumefaciens* or cauliflower mosaic virus, APHIS has construed this to create a new category of "regulated article" for plants containing such DNA, even though the components used (e.g., vector or promoter DNA) are unable to cause disease[15]. This is a tenuous platform on which to base the regulatory process, and extensive study and experience indicate that at least the following two types of DNA sequences should be exempt:

***Agrobacterium* DNA**. *Agrobacterium* DNA transfers naturally to plant genomes and some is known to be stably integrated into plant genomes. For example, the tobacco genome contains genes from *Agrobacterium rhizogenes*[25].

Plant viral DNA. DNA from plant viruses used as promoters/terminators or other functional elements, or when used in nonfunctional form to suppress viral genes (and thus impart disease resistance) should be exempt. Viral DNA sequences by themselves do not appear to pose a hazard, and many have become incorporated into the genomes of plants. For example, plantain bananas contain the genome of the banana streak virus, rice contains sequences of the rice tungro bacilliform virus and tomato has sequences from tobacco vein-clearing virus[25]. In addition, viruses are ubiquitous in plant foods. It has been estimated that about 14–25% of oilseed rape in the field is infected with cauliflower mosaic virus in the United Kingdom[26]; similar numbers have been estimated for cauliflower and cabbage. Historically, humans have been consuming cauliflower mosaic virus and its 35S promoter at much higher levels than those in uninfected transgenic plants. Unsupported claims that the 35S promoter is unstable, prone to transfer and insertion into the DNA of other cells, thereby causing cancer in humans[27], have been extensively rebutted by the scientific community and are without merit[28]. Given the extensive exposure of humans to plant viruses and their DNA in most foods, there is no justification for using the presence of small segments of viral DNA resulting from genetic engineering as the basis for calling all transgenic plants containing them "regulated articles."

Exempt selected transgenes and classes of transgenic modification from regulation

In addition to the above, several kinds of transgenes and methods of modification have been widely used in genetic engineering of many crop species. These have been intensively studied, and in some cases transgenic crops incorporating them are in extensive commercial use. Because of their familiarity and known safety, regulatory burdens should be reduced or eliminated when these genes and methods are used. Some examples include:

General gene suppression methods such as antisense, sense suppression or RNAi (RNA interference). The effects of gene suppression are similar to the diverse forms of reduced function alleles that are common in wild populations, and to the natural processes of microRNA inhibition of gene expression during development[29]. These mechanisms are useful for inducing viral and bacterial pathogen resistance, and similar processes of viral resistance are known to occur in wild species.

Nontoxic proteins that are commonly used to modify development. For example, expression of barnase and barstar under tissue-specific promoters is deregulated for inducing or restoring male sterility. Similar uses of these transgenes for other purposes should have a low regulatory burden.

Selected, well known marker genes that impart antibiotic resistance. The product of the *npt*II gene (providing resistance to kanamycin and related antibiotics) was classified as Generally Recognized as Safe (GRAS) during deregulation of the Flavr Savr tomato[30,31]. A working group of the British Society for Antimicrobial Chemotherapy recently made a strong general argument for the safety of virtually all antibiotic resistance genes in plants[32]: "The Working Party finds that there are no objective scientific grounds to believe that bacterial AR [antibiotic resistance] genes will migrate from GM [genetically modified] plants to bacteria to create new clinical problems…. Use of these genes in GM plant development cannot be seen as a serious or credible threat to human or animal health or to the environment." This view largely echoes that of Flavell *et al.*[33] and the US Food and Drug Administration in their "Guidance for Industry" issued in 1998 (ref. 34).

Selected marker genes that impart reporter phenotypes. Strong arguments have been made for the safety of the β-glucuronidase reporter gene[35], which was present in commercially released transgenic papaya[36]. The same is true of green fluorescent protein[37], which seems to be an ecologically neutral marker[38].

Create regulatory classes in proportion to potential risk

Consistent with previous risk-based stratification proposals[16], we seek regulations that treat classes of transgenic organisms differently based on the true risk associated with the traits and gene functions, rather than on the method of introduction of the trait. The establishment of classes based on scientific criteria[16,17] would promote efficiency by enabling companies, public institutions and regulators to focus on important issues associated with new traits, not on the method of genetic change or unimportant linked genes or sequences. We recommend three risk classes, as previously suggested[18]:

Low risk. Exemptions or reduced regulatory oversight of low-risk transgenic organisms are warranted during field testing and commercial use where the imparted traits are functionally equivalent to those manipulated in conventional breeding, and where no novel biochemical or enzymatic functions are imparted; in short, where genetic engineering brings about directed changes in expression of functionally homologous genes to achieve a commercially useful trait (what one of us has termed "genomics-guided transgenes"[38]). Where scientific considerations suggest that the modified traits are likely to be "domesticating" and thus retard spread into wild populations (e.g., sterility,

dwarfism, seed retention, modified lignin), we believe that exemptions are warranted at the field-testing stage, and in most cases at the commercialization stage (assuming domestication genes do not directly impact endangered or threatened species). The recent US National Research Council report on bioconfinement[39] suggested that many transgenic traits will require no confinement; we believe that transgenes for domestication traits in plants are good examples of those where regulation is unwarranted for most species and geographies. For cases where there is ambiguity, exemptions granted at the field-testing stage could be re-reviewed before commercial deregulation.

Moderate risk. Plant-made pharmaceutical/industrial proteins (PMP/PMIP), plants with novel products that have very low human and environmental toxicity, or that are grown in nonfood crops and have low nontarget ecological effects (including, we expect, most plants used for phytoremediation), are candidates for less stringent regulation. In general, the moderate category should not be viewed as a permanent status, and transgenic varieties in this moderate risk class should be transferred to the low or high risk categories after ecological and/or toxicological studies have been conducted. Continued oversight may be appropriate for plants with novel pest management traits such as herbicide tolerance and pest resistance where monitoring of potential development of weed or pest resistance to the management traits is needed.

High risk. Careful regulation of high-risk plants producing PMP/PMIP is appropriate during field tests and commercial production where their transgene products have a documented likelihood to cause significant harm to humans or the environment. Plants with the ability to accumulate high levels of heavy metals or other environmental toxins might also be placed in this category, if their release could present a hazard for herbivores or their prey.

Eliminate the event-specific basis of transgenic regulation

Regulation of transgenic crops is currently based on specific 'events' (that is, specific transgenic insertions into the host genome). Each time a transgene is inserted into a genome, a separate regulatory data package must be submitted for that event. The rationale for event-specific regulation is that the insertion sites for transgenes cannot currently be targeted and therefore can occur randomly in the genome. Some insertions might inactivate or alter the expression of endogenous genes or interact with different genetic backgrounds[40], thereby resulting in unexpected consequences. In addition, different insertion events often vary in transgene expression levels, patterns or stability[41]. The regulatory premise is that these uncertainties significantly exceed those encountered with conventional breeding methods such as introgression or mutagenesis and thus constitute a safety concern that is not otherwise addressed during normal variety development.

Transgenic 'events' are analogous to other genetic modifications. Extensive experience with mutation breeding, in which random genetic changes are induced throughout the genome, does not support undue concern over unexpected consequences of transgene insertions. Over 2,200 crop varieties have been commercialized that had an irradiation-induced mutation step in their pedigrees, and other methods of inducing random mutations have also been used extensively[42]. In these cases, subsequent selection has been almost entirely made on the basis of phenotypic characteristics, generally without any knowledge of the underlying genomic changes causing the phenotype. Multiple mutations with diverse pleiotropic (that is, collateral) effects can be induced by irradiation or chemical mutagenesis, providing ample opportunity for unexpected consequences to occur[43]. However, instances of increases in toxins or other harmful constituents in released varieties due to either introgression or mutation are extremely rare[44,45]. Even in the few cases where potential toxins were present at unexpectedly high levels in conventionally bred cultivars, they were toxins known to be present in those species (e.g., solanine in potato or psoralens in celery), rather than entirely novel compounds, and would be detected using standard phenotypic screens.

Other intensive breeding methods that are routinely used, such as intervarietal hybrids, wide interspecies crosses, inbreeding, ploidy modification and tissue culture, produce abundant pleiotropic effects on gene structure and trait expression in plants[46]. The dwarfing genes that provided the foundation of the 'green revolution' varieties in wheat and rice had multiple pleiotropic effects[47]. These effects are routinely sorted through during conventional breeding. Loss-of-function alleles that may be generated by the transgenic process are common in breeding populations, and events such as transposon and retroviral movement caused by the transformation process are also common, and can induce changes in gene expression at distal sites in the genome. As in conventional breeding, we believe that developers of transgenic varieties should be encouraged to utilize, rather than avoid, both the random and the expected effects produced during genetic engineering to accelerate overall rates of crop improvement.

In a commercial transgenic variety development program, hundreds of individual transformants are screened phenotypically to identify the few that have the most desirable expression of a transgenic trait. This process parallels the breeding of cultivars by introgression of genes from related wild species through sexual crosses. In fact, conventional breeding programs generally evaluate populations with much wider ranges of phenotypic variation than is observed in transgenic programs, and genetic traits can be expressed in the progeny that are not evident in the parents from which they are derived[48]. It is now possible to determine the actual genetic regions that have been transferred through crossing and introgression. For example, the introgression of traits from wild species of tomato into cultivated varieties through sexual crosses resulted in chromosomal segments of variable sizes (encoding dozens to hundreds of unknown genes) being transferred to different varieties[49,50]. However, despite variation in the specific molecular environments in which the introgressed genes were present, the commercial varieties all exhibited the desired phenotype. These findings likely apply to virtually all sexually introgressed genes, since introgression relies upon random recombination to exchange the introduced DNA for that of the recurrent parent.

A given gene inserted into a specific genotype could have different interactions in other genetic backgrounds, possibly resulting in unexpected consequences. Yet, such variable trait expression within a population, technically referred to as 'penetrance,' is routinely observed during recurrent selection for desired traits in conventional plant breeding programs, a practice with over 100 years of safe application. Currently, the cost of meeting regulatory requirements ensures that only one or very few specific transgenic events that achieve deregulation will be backcrossed into other varieties of the same species. The genetic recombination involved in this process guarantees that the original insertion event will end up in different genetic contexts and backgrounds. Nonetheless, the cumulative experience of crossing specific herbicide-tolerance and insect-resistance transgenes into hundreds of soybean, maize, cotton and canola varieties planted on tens of millions of hectares annually indicates that such background effects are not a hazard when combined with standard genotypic and phenotypic selection protocols used in plant breeding.

Although not explicitly required in the United States[51], site-specific sequence data for the entire inserted DNA, along with adjacent genomic sequences near the insertion site, have generally been submitted to regulatory agencies. Such information is required for event-specific tracking purposes as part of the European Union's traceability and labeling requirements for post-marketing surveillance. Some have recently called

for expanding this to require sequencing of a "large stretch of flanking DNA" up to several thousands of bases long and have argued for regulatory rejection if even a single base pair is changed relative to the same sequence in the recipient variety[52]. However, characterization of sequences adjacent to insertion sites is of little value for predicting trait expression or product safety. Even if adjacent sequences predict insertion into a protein coding region, without further study it would not be known whether this is an actively expressed gene or a pseudogene, whether it is a member of a redundant gene family, or whether it actually encodes a protein. Even if an insertion changed the expression of a native protein, its developmental, toxicological and environmental significance would generally be impossible to predict from sequence data alone. The only sure guide, as for introgressed genes, is the phenotype of the plant.

Genomic science does not support event-specific regulation. Recent genome mapping and sequencing results support the contention that site-specific characterization has little value in a regulatory context. Such studies have revealed that genomes are highly dynamic and phenotypically robust to changes at genic and genomic scales. Total DNA content, the number of genes, and gene order can vary considerably even among varieties of the same species[53–55]. For example, different varieties of maize, chili pepper and soybean can differ by as much as 42%, 25% and 12%, respectively, in their DNA contents[56–58]. For soybean, this means that different varieties vary by over 100 million base pairs of DNA, dwarfing the few thousand base pairs that transgenes add to genomes. In maize, significant differences in sequence collinearity occur among varieties while retaining phenotypic function[54,59]. Closely related crops, such as maize, sorghum and rice, have genomic regions with differing arrangements of essentially the same sets of genes[60]. Small insertions and deletions in maize occur on average every 85 base pairs in noncoding regions, and the frequency of point mutations (single nucleotide polymorphisms) in maize breeding germ plasm is as high as 1 every 5 to 200 base pairs[61]. As a result of a large number of deletions that affect gene families in maize, even different individual plants do not have the same number of genes[54,55]. Transposable elements move into and out of genes, where they can alter gene expression or serve as sites of chromosome breakage or rearrangement[62]. Retrotransposons continuously insert themselves between genes[63] and are likely to have resulted in improvements in plant adaptation through both evolution and breeding[64,65]. Even different individuals of the same species differ in the number of transposons and retrotransposons they contain[54]. Such differences underscore the futility of attempting to define a standard genome for a species or even a variety against which to compare changes due to transgene insertion. It is even more unlikely that genomic sequence analyses could usefully predict the ecological consequences of transgenic plants in agronomic or natural environments[66].

Event-based regulation has adverse consequences. Event-based regulation promotes the use of as few insertions as possible followed by backcrossing to transfer the trait into other varieties. This is theoretically feasible in many seed-propagated crops, but it can be commercially and practically daunting. Although backcrossing has become efficient for major crops such as maize or soybean, the comprehensive DNA-based marker systems necessary for efficient backcrossing of many other crops simply do not exist. As a consequence, most varieties developed in backcrossing programs inevitably lag behind the improved varieties that use forward breeding approaches. The lifespan of many crop varieties has also decreased significantly over the past decade, resulting in rapid turnover of the top varieties. Therefore, by the time a single transgenic event is deregulated, enters a backcrossing program and the transgenic version of the desired variety is recreated, the variety may no longer be commercially viable. A tragic example is the delay in release of Golden Rice, which produces β-carotene to help alleviate vitamin A deficiency[67]. Release of Golden Rice awaits deregulation of a single event and backcrossing into locally adapted varieties, rather than simultaneously transforming the required genes into a range of varieties. Thus, restrictive event-specific regulatory policies act to reduce biological diversity by forcing backcrossing of single events rather than use of diverse genetic backgrounds.

In vegetatively propagated trees and vines, including fruits and nuts that employ highly heterozygous varieties and long generation times, backcrossing to transfer an engineered trait is effectively impossible. Existing varieties adapted to local climatic conditions and market preferences will each need to be transformed. Similarly, multiple accessions of forest trees adapted to different ecological zones would each need to be transformed to provide varieties that are adapted to the diverse environments they will occupy for many years. The requirement for complete deregulation data packages for each new event-variety-provenance combination, even after the trait itself has been shown to be safe for a given species, discourages biological diversity and creates financial and practical hurdles.

Regardless of these consequences of event-specific regulation for the variety development and commercialization process, marketing of GM products has been the biggest casualty of this regulatory approach. Individual events must be evaluated and approved or deregulated in each national or international jurisdiction, which can make one variety legal and a second one a 'contaminant' simply by virtue of where the same transgene has incorporated into the genome. This, in turn, has engendered a burgeoning bureaucratic infrastructure of product channeling, identity preservation, commodity testing and auditing based upon individual transgenic events that bears no relationship to true risk or hazard. As this approach to regulation becomes entrenched into international agreements such as the Cartagena Protocol, marketing of GM products will continue to be confronted with market barriers that have no foundation in science or safety.

Eliminate event-specific regulation. We recommend a regulatory approach that would require an initial evaluation of the specific protein/trait/phenotype that results from the transgene in a given species, but a much reduced regulatory package or simply notification for additional events using the same protein/trait/phenotype in that or related species. The US EPA has established general clearances for some plant-incorporated protection genes and proteins, though it still requires event-specific registration and evaluation of each new transgenic variety. Limited molecular genetic characterization of specific events would routinely be done by a developer to uniquely identify a transgenic allele for use in quality assurance or stewardship programs. This is analogous to traditional breeding, where molecular knowledge of the genetic composition of a variety is not required before release, but genetic fingerprinting may be useful for other purposes. Different events will have some variation in intensity and cell/tissue specificity of transgene expression[68], However, the variance seen among transformants during initial screening is greatly reduced by subsequent selection for a specific trait. Nonetheless, in cases where such variations in expression could have nontarget ecological or toxicological effects of consequence, such as where novel pest resistance toxins or high risk PMP/PMIPs (as defined above) are expressed, characterization of the transgene for initial deregulation or registration should include data spanning the range of expression anticipated among multiple commercially relevant events. Since unknown mutations and chromosomal translocations can occur during the transformation and regeneration process[52,69], it is prudent to expect that transgenic varieties will be grown and evaluated for at least three generations before commercial release, as is routinely done for conventional varieties. For vegetatively propagated species,

this might mean two or three cycles of propagation and evaluation rather than sexual generations, as appropriate to the species. However, we see no reason to regulate the exact nature or number of generations, propagation cycles or field trials for additional transgenic events at the national or international level, except as such requirements exist for traditionally bred crops, as for inclusion on approved national variety lists. Applying the distinctness, uniformity and stability criteria for new varieties is best left to regional or national variety evaluation boards, breeding companies and local regulatory agencies based on field experience for specific crops.

Conclusion

We have discussed a number of reasons to substantially modify regulatory data requirements for transgenic crops. Our intent is to give specific advice to regulatory agencies on approaches that are highly discriminating based on product rather than process, as has been urged by several high-level scientific panels. We believe that regulation of transgenic crops should be comparable to and compatible with traditional breeding when similar traits and uncertainties are involved, be updated to reflect experience from nearly two decades of research and commercial experience with transgenic crops and be brought in line with the rapid advances in knowledge of plant genomes. We believe that such changes would reduce costs, open transgenic-based innovations to a broader array of private and public entrepreneurs and thus facilitate the production of improved crops based on the genomics revolution in biology.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details)

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