To be or not to be transgenic

To the Editor:

It is sad and ironic that even though much progress has been made in deciphering the genetic content of food plants and modifying their genomes for the betterment of humankind, many of the principles of modern plant genetics, firmly established decades ago, are now so easily forgotten or ignored. Such is the case with many of the alarmist arguments raised in the News Feature by Emily Waltz[1] in the March issue entitled "Tiptoeing around transgenics." Waltz focuses on the controversy surrounding the regulation of modern (and, in fact, not-so-modern) biotechnological techniques, such as those that alter single base pairs by replacing one nucleotide with another (that is, create single-nucleotide polymorphisms or SNPs). We feel it is important to stress that such genetic changes must be viewed in a historical and biological context to understand why calls for new layers of regulation over technologies that introduce SNPs and other changes are unwarranted.

The most relevant counterargument to the need for regulation is the fact that mutations normally happen. Mutations occur spontaneously in nature, and their rate can be increased by the use of mutagens. On the whole, mutation is a good thing, for without mutation, we would still be biofilm on the bottom of the ocean. Although typical mutation rates are quite low when calculated on a gene or base-pair basis, they are high enough that new mutations are the rule rather than the exception. For example in *Arabidopsis thaliana*, the mutation rate per base pair per generation is estimated to be 7 per billion base pairs[2]. Given that there are 125,000,000 base pairs in the *A. thaliana* genome, 1.75 new SNP mutations are expected per generation per diploid plant. Although SNPs appear to occur at about the same rate in all plants, crop plants have larger genomes, and thus more SNPs. Just one average hectare of 240,000 soybean plants can be expected to contain about 1.8 million novel SNPs.

Not only are SNPs commonplace but techniques that create SNPs have a long history of safe use by breeders. Before the advent of the techniques described by Waltz, the only tool available to breeders to alter DNA sequences was the use of radiation and chemical mutagens. The Food and Agricultural Organization (Rome) and International Atomic Energy Agency maintain a database (http://www-infocris.iaea.org/MVD/) that currently lists 2,543 known plant varieties developed through mutagenesis, including many common or widely grown and consumed crop plants, of which 14% were derived with chemical mutatagens[3]. Chemical mutagens are still used to create the same kind of SNPs[4] that are cited as a cause for concern in the Waltz News Feature. Although the genetic basis and extent of SNPs for the mutant phenotype are usually unknown when mutagenesis is employed, the resulting crops are considered as safe as others and are not subject to premarket regulatory review.

Breeders depend on sheer luck to find an alteration in the gene encoding their trait of interest when they employ mutagenesis, and they must accept random alterations elsewhere in the genome whenever these do not affect crop growth, performance and yield to an unacceptable point. Today, by using *in vitro* techniques, breeders have the ability to target the gene of interest, and not introduce unintended and unwanted mutations elsewhere in the genome. If anything, therefore, modern techniques should decrease concerns for safety, not raise them.

This leads us to the question of whether SNPs alter protein safety. SNPs accumulate in plants and animals. One simple means of quantifying SNP formation is by comparing SNP differences between pairs of genotypes or varieties[5]. For example, 0.05% of bases in soybean coding regions are SNPs, or one SNP per 2,000 bp in coding regions; the frequency in noncoding regions is 0.5%, or 1 per 191 bp[6]. This is similar to the level of SNPs in the human genome. Such crops as maize are much more diverse; in this cereal, SNPs account for as many as 1.3% of base pairs[7]. Tenaillon *et al.*[8] have estimated that any two alleles of a maize gene for a 300–400-amino-acid protein would differ by 3.5 amino acids due to SNP accumulation. Within a diverse population, there are likely to be 15–20-amino-acid differences between proteins from two alleles of a single maize gene. It is therefore not surprising that attempts at protein engineering have not converted enzymes into toxins, as toxic proteins have substantial structural differences from other proteins and need to perform specific physiological roles to act as toxins[9].

SNPs are thus really minor variations compared with the larger-scale changes that have accumulated in crops during domestication and breeding. A case in point are the elongated tomatoes on today's market (which could fall under the category of cisgenics, another technology highlighted by Waltz). However, in the tomato's case, its DNA got copied and moved to another location in the genome through naturally occurring mechanisms[10], most probably after the tomato's arrival in Spain[11].

The Waltz article also discusses the new technology developed by Pioneer HiBred (Des Moines, IA, USA) in which transgenic plants produce nontransgenic, male sterile plants that are used in hybrid production. The argument is made that although these plants are not transgenic *per se*, they should be viewed as such. But if this same 'sins of the fathers' argument were applied elsewhere in agriculture, humans should not consume modern-day tomatoes because their parents contained a toxin. This nonsensical argument is not applied to conventional plant varieties and, therefore, there is no reason why it should be applied to transgenics.

Despite millennia of plant genetic modification, thus far we have not found

a single verified report whereby breeding or radiation and/or chemical mutagenesis resulted in a toxin, allergen or other hazard that was not known to exist before. These facts support the conclusion that DNA insertions and other types of mutations do not pose unreasonable risks to the environment or to human and animal health, regardless of how they came about.

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Broad consent in biobanking

To the Editor:
The Feature in the February issue by Scott *et al.*[1] on the policy challenges of biobanking characterizes broad specimen donor informed consent as "ethically contentious." A survey of public attitudes is cited. This same survey found that a significant percentage of individuals are prepared "to consent broadly to future research use and to forego additional choices as a result"[2].

With our perspectives in patient advocacy or at research centers aimed at bringing new regenerative therapies to patients, we have consistently emphasized the value of research donors' perspectives. In the context of protocols for creating immortalized cell lines for banking and distribution, we have also witnessed support for broad consent. Indeed, enthusiasm is even more pronounced among those touched by disease, and patient donors actually express concern that study-specific consent can be burdensome and impede research.

This experience suggests to us that broad consent is ethically responsible, provided there is comprehensive oversight and a robust informed consent process. With the continued support of donors, we look forward to applying this model in biobanking efforts.

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An accelerated workflow for untargeted metabolomics using the METLIN database

To the Editor:
Metabolites, which are typically recognized as small molecules that are involved in cellular reactions, provide a functional signature of phenotype that is complementary to the upstream biochemical information obtained from genes, transcripts and proteins. The high correlation between metabolites and phenotype has created a surge of interest in the field that is reflected in the number of metabolomic publications growing from just a few articles in 1999 to over 5,000 in 2011. Although relatively new compared with its genomic and proteomic predecessors, research in the field of metabolomics has already led to the discovery of biomarkers for disease, fundamental insights into cellular biochemistry and clues related to disease pathogenesis[1,2].

The success of metabolomics over the past decade has relied largely on advances in mass spectrometry instrumentation, which make it possible to detect thousands of metabolites simultaneously from a biological sample. Coupled with developments in bioinformatic tools such as XCMS Online (https://xcmsonline.scripps.edu/)[3], it has now become relatively routine to comprehensively compare the intensities of thousands of metabolite peaks in one sample group to those in another in an untargeted manner. This approach, called untargeted metabolomics, has the potential to implicate unexpected pathways with a unique phenotype or disease process.

Despite the attractiveness of having a comprehensive and unbiased approach for profiling metabolites that is analogous to those used in the other 'omic' sciences, an overwhelming proportion of the metabolomic community exclusively uses a targeted platform in which only a specified list of metabolites is measured. The benefit of such a targeted platform is speed. Unlike the untargeted platform, after the targeted mass spectrometry methods are established, minimal effort and resources are required to profile these specific metabolites over a large number of samples. In contrast, the major bottleneck of untargeted metabolomics has been the challenge of determining the identities of the peaks found to be dysregulated in the untargeted profiling data.

Traditionally, the untargeted metabolomic platform involves multiple steps (**Fig. 1**). The first step is acquiring global mass spectrometry data for each of the samples. Next, these data are analyzed using bioinformatic software that performs quantitative analyses to find peaks that are significantly different between sample groups. The investigator then typically searches the mass-to-charge (m/z) ratios of the peaks of interest manually in metabolite databases. Searches that return hits within the mass accuracy of the instrument are considered to be putative identifications. To confirm the identifications, tandem mass spectrometry (MS/MS) data from the research sample are then compared to the MS/MS data of a commercial standard. To obtain the MS/MS data, a targeted MS/MS analysis is typically performed on one of