

REVIEW & INTERPRETATION

A Comparison between Crop Domestication, Classical Plant Breeding, and Genetic Engineering

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ABSTRACT

Several claims have been made about genetic engineering (GE) in comparison with crop domestication and classical plant breeding, including the similarity of genetic changes between those taking place during domestication and by GE, the increased speed and accuracy of GE over classical plant breeding, and the higher level of knowledge about the actual genes being transferred by GE compared with classical breeding. In reviewing evidence pertaining to these claims, I suggest that (i) it is unlikely that changes introduced by GE will make crops weedier, although exceptions have been noted, (ii) changes brought about by GE currently often involve gain-of-function mutations, whereas changes selected during domestication generally involve loss-of-function mutations, (iii) adoption of GE cultivars has been much faster than any previous introduction and spread of agriculture that occurred earlier but has occurred at about the same rate as the spread of cultivars obtained by plant breeding, (iv) introduction of agriculture reduced the health of agriculturists compared with that of hunter-gatherers, suggesting that introduction of innovations do not automatically improve well being, (v) although GE is not a substitute for plant breeding, it can significantly contribute to plant breeding by generating additional genetic diversity, (vi) uncertainties associated with the site of insertion of transgenes in the genome and the expression of transgenes following insertion, makes GE less rapid and precise than originally claimed, and (vii) a potential advantage of GE over classical breeding is the knowledge of the actual gene(s) being inserted, although few cases of unwanted gene introductions through classical plant breeding have been documented. Further advances in GE will increase the precision of the technique, its relevance to consumers, and its environmental friendliness. What is most needed are even-handed, case-by-case assessments of the benefits and potential pitfalls of GE in comparison with other crop improvement techniques. Classical plant breeding may, in the end, also be regulated in the same way as GE.

THE LAST 20 YEARS have seen the development of genetically engineered plants whereby genes are introduced into a genome by means other than by the usual sexual means, i.e., after fertilization of an ovule in a female parent by a sperm cell contained in pollen grain produced by a male parent. These means include gene transfer by *Agrobacterium tumefaciens*, a pathogenic bacterium that naturally transfers DNA to plants during the disease process (Gelvin, 2000). (It should be noted here that the strains of *A. tumefaciens* that are used in GE have been rendered nonpathogenic.) Other means of gene transfer for GE include biolistics (where

genes are literally shot into plant cells) and a variant, particle discharge (Birch, 1997).

GE has now reached the commercial stage. New cultivars of maize (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], cotton (*Gossypium* spp.), papaya (*Carica papaya* L.), etc. have been developed that carry additional genes conditioning traits as diverse as resistance to insects, viruses, and herbicides. Some of these cultivars have been readily adopted by farmers because they greatly facilitate the management of crops (e.g., Delannay et al., 1995: herbicide resistance), provide a more effective alternative to current control methods [e.g., Bolin et al., 1996: resistance to European corn borer, *Ostrinia nubilalis* (Hbner), in maize], or offer the only viable possibility of disease control in the absence of any natural resistance within the crop gene pool [e.g., Lius et al., 1997: *Papaya ringspotvirus* (PRSV) resistance in papaya].

In the current controversy affecting the release of GE crops, proponents of the genetic engineering technology (e.g., Prakash, 2001) cite the fact that existing crops result from genetic modifications that have taken place for a period of approximately 10 000 yr in several places in the world (Harlan, 1992; Smith, 1995). These modifications include the process of domestication, a selection process conducted under human influence to increase adaptation to cultivated conditions and usefulness to consumers of the harvested products such as grains, fruits, and fibers. They also include selection for adaptation to new environments, as crops were dispersed from their original centers of domestication to other regions or continents (Gepts, 1999, 2001; Gepts et al., 1999).

Furthermore, the last century has seen very active scientific breeding programs that have led to spectacular increases in both the quality and quantity of the crops produced, resulting among other things in the production of sufficient food to feed the current population of the entire planet (even though poverty, warfare, and the lack of infrastructure have so far prevented the eradication of hunger). In light of these successive genetic modifications of crops, the use of the term *genetically modified organism* (GMO) to designate GE crops is a definite misnomer since it implies that before GE there were no genetic modifications. Clearly, nothing could be further from the truth! *Genetically engineered*

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Table 1. Domestication syndrome traits (after Harlan, 1992).

Selection stage	Trait selected		Crop examples
	General	Specific trait	
Seedling Reproductive system	Increase in seedling vigor Increased selfing rate Adoption of vegetative propagation	Loss of seed dormancy	Many crops Tomato Cassava Maize
Harvest or after harvest	Increase in seed yield Consumer appeal	Loss of seed dispersal More compact growth habit Increase in the number or size of inflorescences Increase in the number of seeds per inflorescence Change in photoperiod sensitivity Color, size, taste, texture Reduction in toxic substance	Legumes, maize Wheat, barley, maize Maize, amaranth Legumes, rice Many crops Cassava, lima bean

or *transgenic* would be more appropriate terms.¹ According to the proponents of GE, GE involves basically the same modifications that have been taking place for 10 000 yr and these are thought to be part of a long-term trend of genetic modifications about which few people have shown concerns. This view is presented in scientific journals (e.g., Borlaug, 2000; Prakash, 2001) as well as in news media (e.g., McGloughlin, 2000). Their argument further states that these genetic changes occurred without regulations or oversight and that current regulation of GE is more than adequate. Furthermore, genetic modification is nothing new and the latest round of genetic modifications, brought about by genetic engineering, should therefore evoke no concern, or so the argument goes (e.g., Borlaug, 2000; Prakash, 2001).

Is GE a continuation of the same type of genetic modifications that humans have imposed on crop plants for some 10 000 yr? Is GE merely an improved (i.e., faster and more accurate) version of classical plant breeding as asserted by some [National Research Council (U.S.). Committee on Genetically Modified Pest-Protected Plants, 2000; Royal Society, 1998]. In this interpretive review, I bring up a number of observations. Each observation is immediately followed by a more circumstantiated discussion of that observation.

GENETIC MODIFICATIONS BROUGHT ABOUT BY DOMESTICATION

What Types of Traits?

Observation 1. Most Transgenic Crops Are Not Anticipated to Survive in Nature without Human Intervention; However, Exceptions to this Pattern Can Be Expected on Theoretical Grounds and Have Actually Been Observed

Domestication is a selection process conducted by humans to adapt plants and animals to the needs of humans, whether as farmers or consumers. Interestingly, this process of domestication has been conducted for some 10 000 yr, following the last ice age, in several

regions independently. At least six regions of domestication have been identified, including Mesoamerica, the southern Andes (including the eastern piedmonts), the Near East, Africa (probably the Sahel and the Ethiopian highlands), Southeast Asia, and China. Agriculture is thus one of the few inventions that can be traced back to several locations. From these foci, agriculture was progressively disseminated to other regions, including, for example, Europe and North America.

In spite of the geographically diverse distribution of the domestication centers, a remarkably similar set of traits can be identified that have been selected in widely different crops. These traits jointly make up the domestication syndrome (Hammer, 1984). They result from selection of spontaneous mutations that occurred in wild populations and were selected at various stages of growth of these wild plants (or animals), as well as after harvest (Table 1) (Harlan, 1992). Many traits selected under domestication, because they fit the needs or fancy of humans, are actually deleterious in the wild. As a consequence, fully domesticated crops may not survive in the wild without human intervention in planting and harvesting. Addition of transgenes to highly domesticated crops that cannot survive without human intervention is unlikely to remove the dependency of these crops on humans (Crawley et al., 2001). This is not surprising because this dependency is based on multiple traits (see Table 1) that are unrelated to the traits coded by the transgenes (e.g., disease or pest resistance, nutritional improvement). Likewise, most humans cannot currently survive without crop plants because the plants supply a major portion of human food. Thus, there is a mutualism between humans and their crops enhancing their respective survival.

Partially domesticated crops may constitute an exception to the dependency on humans. Partially domesticated crops are those that display some but not most or all of the domestication syndrome traits. Among these are fruit trees and forage species, for which it is sometimes difficult to distinguish between truly wild and feral populations. The latter escaped cultivation and survive as wild-growing populations without human intervention (e.g., Ladizinsky, 1999). Addition of genes such as herbicide resistance to forage crops may increase their weediness by reducing opportunities to control feral populations. An additional exception is oilseed rape (*Brassica napus* L.), which is prone to form feral populations because of silique shattering (Liljegren et

¹ Genetic engineering is but one of the tools commonly grouped under the term biotechnology, which can be defined as the use of recombinant or non-recombinant DNA technology to study, categorize, and manipulate genetic materials (Duvick 2001). Not all manipulation of DNA involves genetic engineering. For example, indirect selection using DNA markers is usually included under biotechnology but does not involve transformation of plants by GE.

al., 2000), secondary dormancy of the seed (Hails et al., 1997), and accidental discharge from trucks (Crawley and Brown 1995). Pessel et al. (2001) observed that feral populations of oilseed rape tended to persist for at least 8 yr without intervening human actions. Thus, while it can be generally stated that fully domesticated plants are unlikely to survive on their own in the wild, there are exceptions to this pattern. The feral tendency of crops have to be studied on a case-by-case basis.

What Type of Inheritance?

Observation 2. Genetic Modifications Brought about by Domestication Are, in Many Cases, Loss-of-Function Changes, Whereas Those Currently Brought about by Genetic Engineering Often Represent a Constitutive Gain of Function; Expression of Transgenes in the Future Will Likely Be Finely Regulated According to Timing, Tissue, and Amount

The inheritance of domestication traits has been investigated numerous times since the rediscovery of Mendel's laws at the beginning of the 20th Century (for reviews, see Gottlieb, 1984; Hilu, 1983; Knight, 1948; Ladizinsky, 1985). Initially, the traits were analyzed as Mendelian traits because many of them display qualitative variation and discrete phenotypic segregation classes. More recently, these same traits have been analyzed by quantitative trait locus (QTL) approaches, which are more powerful because they allow a genome-wide analysis of influence on several traits at the same time (Lee, 1995; Tanksley, 1993). Such approaches have been applied to a limited number of crops, including maize (Doebley et al., 1990), common bean (*Phaseolus vulgaris* L., Koinange et al., 1996), tomato (*Lycopersicon* spp., Grandillo and Tanksley, 1996), rice (*Oryza sativa* L., Xiong et al., 1999), and pearl millet [*Pennisetum glaucum* (L.) R. Br., Poncet et al., 1998, 2000].

The results of these genetic studies can be summarized as follows. When considering individual traits, the domesticated state is often controlled by recessive alleles at one or, at most, two or three loci (Ladizinsky, 1985). The importance of these major genes was further confirmed by QTL analyses. So far, these analyses paint a remarkably similar picture of the inheritance of the domestication syndrome (Table 2). The consensus genetic control involves, for many traits, a limited number of genes, several of which have a major effect (high R^2). Furthermore, the joint involvement of these genes accounts for most of the phenotypic variation, suggesting a high heritability. Finally, many of the genes are located in a limited number of linkage groups, and, on these linkage groups, are sometimes closely, although

not tightly, linked. This type of inheritance probably reflects conditions during domestication. Major genes controlling highly heritable traits would have facilitated progress from selection during the domestication process.

For the sake of this discussion, it is important to point out that many domestication genes represent a loss rather than a gain of function, as indicated by their recessiveness. There are, of course, many more mutations that convert a functional enzyme or structural protein into an inactive one, than there are mutations that give an enzyme or structural protein an entirely new function. There are some exceptions to this pattern, notably the *Tb-1* domestication gene in maize, which has recently been characterized at the molecular level (Doebley et al., 1997). The *Tb-1* allele of domesticated maize is dominant over the *tb-1* allele of teosinte, the wild progenitor of maize, because of overexpression of the gene in maize in comparison with teosinte. Dominant mutations may have been easier to select in an outcrossing species such as maize.

Many traits controlled by transgenes are gain-of-function traits, such as resistance to insects and tolerance to herbicides. This should not be surprising as it often is this type of trait that provides the added value expected from improved cultivars. These transgenes are currently driven by strong promoters controlling constitutive expression, such as the Cauliflower mosaic virus 35S promoter. Other genes of similar inheritance to be expected in future GE applications code for nutritional (Napier and Michaelson, 2001), medical (Parmenter et al., 1995; Giddings et al., 2000; Walmsley and Arntzen, 2000), pharmaceutical (Giddings et al., 2000), or industrial products (e.g., Poirier, 1999; Lapierre et al., 2001). A few of these applications involving novel traits in the crop species will likely be of concern from the standpoint of human health and the environment.

Some GE applications will, however, involve loss-of-function mutations. GE could play a role, for example, by inactivating certain genes controlling the production of toxic compounds through dominant mutations acting by gene silencing. The associated agronomic changes (e.g., possible increases in susceptibility to diseases and pests) would have to be carefully evaluated. On the whole, one can therefore state that the genetic changes currently brought about by GE are generally different from the ones effected during domestication. In the future, the expression of transgenes is likely to be modified by regulatory sequences to limit the expression to certain tissues, growth stages, genetic backgrounds, and environmental conditions. This approach may help alleviate some of the potential environmental and human concerns associated with GE.

Table 2. Comparison of the inheritance of domestication syndromes in several crops.

Crop	Mating system	Average no. of QTLs or genes/trait	Average R^2 (%)	Total R^2	No. of linkage groups	Source
Maize ($2n = 20$)	Outcrossing	5.3	12 (4–42)	50 (34–61)	5	Doebley et al. 1990
Pearl millet ($2n = 14$)	Outcrossing	2.2	29 (13–64)	57 (25–77)	4	Poncet et al. 1998, 2000
Common bean ($2n = 22$)	Selfing	2.2	23 (12–53)	45 (18–69)	3	Koinange et al. 1996
Rice ($2n = 24$)	Selfing	3.7	14 (7–60)	41 (16–72)	5	Xiong et al. 1999

What Were the Time Line and the Geographic Scope of Domestication and the Spread of Crops?

Observation 3. The Spread of GE Has Been Extremely Rapid and Far More So Than the Development and Spread of Agriculture Itself; Nevertheless, the Speed of Spread of Ge Has Been Comparable to That of Plant Breeding Technologies Such as the Use of Heterosis in Maize and Dwarfing Genes in Cereals

The relative simplicity of the genetic control of the domestication syndrome suggest that domestication could have taken place fairly quickly. The actual duration, defined as the period required to achieve a domesticated phenotype starting from a wild phenotype, is generally not known. There are very few archaeological sequences that encompass the full range of remains, from wild progenitor to domesticated descendant. Such a sequence could shed light on the time it took the first farmers to select a domesticated phenotype. Most of the archaeological sequences include remains of either wild progenitors or domesticated descendants but not both, including in the Near Eastern center of domestication, which has the most abundant archaeological record (Willcox, 1998, Table 1, p. 27–28). On the basis of available data from that and other regions, it appears that the transition from wild to domesticated grains, as measured by morphological changes, took at least a millennium (Willcox, 1998; Long et al., 1989; Piperno and Flannery 2001; Pope et al., 2001; F. Hole, B. Smith, and G. Willcox, pers. comm.). However, this does not mean that selection took place each and every year of those 1000 yr. Plants may have been cultivated intermittently as the need arose. Likewise, there is uncertainty as to the type and intensity of selection pressures applied during this period. Stronger selection pressures would have accelerated the domestication process, whereas increased levels of outcrossing and migration and higher population sizes would have slowed down the process (see next).

Because of the paucity of direct, archaeobotanical data, indirect approaches have been applied to the question of domestication speed. From a genetic or breeding standpoint, the limiting factors to progress during a selection process are the phenotypic variation, the number and magnitude of gene effects, the heritability, the selection intensity (Falconer, 1989), the frequency of mutations (Drake et al., 1998), the level of outcrossing, the effective population size, and the degree of recombination–linkage among genes of the domestication syndrome (Hillman and Davies, 1999; Le Thierry D’Ennequin et al., 1999). Four types of data are relevant to this discussion: the mapping experiments by several groups on crops as diverse as maize, common bean, rice, pearl millet, and tomato (Doebley et al., 1990; Grandillo and Tanksley, 1996; Koinange et al., 1996; Poncet et al., 1998, 2000; Xiong et al., 1999), field experiments and simulations on rachis brittleness of Hillman and Davies (1999), the simulations of selection for multiple genes of Le Thierry D’Ennequin et al. (1999), and the analyses of molecular evolution of several genes in maize (Eyre-

Walker et al., 1998; Hilton and Gaut, 1998). These results can be summarized as follows.

Mapping experiments using quantitative trait loci analysis show that the inheritance of the domestication syndrome is relatively simple, as pointed out earlier (Table 2). Selection acting on phenotypic traits that are highly heritable and controlled at least in part by genes with a major effect is bound to see a rapid response. Modeling of this response to selection, in the case of selection for a nonbrittle rachis coded by a single gene in einkorn wheat (*Triticum monococcum* L.), shows that domestication, as defined by the time span necessary to achieve a gene frequency above 90 or 95%, could have taken place within a time span of 200 yr. A higher level of inbreeding and a stronger selection pressure obviously reduced the time to domestication. These results were further confirmed by a modeling experiment simulating selection of a domestication syndrome involving four traits controlled by one of multiple recessive genes under complete epistasis. Only under high outcrossing rates (approaching complete outcrossing) and migration rates (up to 50% migration) was domestication not achieved. Molecular analysis of several genes in maize suggest that the genetic bottleneck maize underwent during its domestication could have been of short duration, as short as 10 generations, although alternative scenarios involving bottlenecks of longer duration cannot be excluded.

The archaeological and genetic data on the length of the domestication process can be reconciled by positing that the genetic data provide a minimum duration on the basis of genetically limiting parameters, such as the number of genes and their respective effects and the selection intensity. Archaeological data, on the other hand, provide a reality check, especially with regard to selection intensity. The limited data available suggest a domestication process extending over several decades, if not centuries. Thus, the transition from hunting–gathering has taken place over a considerable amount of time, at least from our current perspective.

The spatial scale of development of agricultural societies is also relevant to this discussion. How quickly did agriculture spread from its various centers of origin? The most reliable data come from the Near Eastern center of agricultural origins and the dispersal of agricultural societies into Europe (Ammerman and Cavalli-Sforza, 1984; Price et al., 1995). Agriculture appears to have spread at speeds averaging 1 to 5 km yr⁻¹ having started around 9000 to 8000 BP (before present) in southeastern Europe and being completed around 5000 BP in northwestern Europe.

One could also analyze the introduction of New World crops into Europe on the basis of written and other records. How quickly were these crops adopted after their introduction into Europe following the voyages of Christopher Columbus (1492 and later)? There is no doubt that the so-called “Columbian Exchange” was characterized by an unprecedented exchange of crops leading to profound changes in the agricultural, culinary, and nutritional habits of humans around the globe. For example, it is hard to imagine Italian cuisine



Fig. 1. Common bean representation in the bronze portals—dated to the late 16th century—of the cathedral of Pisa by the Flemish sculptor Giambologna (photo of P. Gepts).

without tomatoes! Information on the speed of adoption of New World crops in Europe, for example, is often buried in difficult-to-obtain sources of information, such as herbals and art history records. The tomato was initially thought to be at best an aphrodisiac or ornamental or at worst a toxic crop, unfit for human consumption. It was only in the 17th Century that tomato were consumed in abundance in Italy and Spain and even later in northern Europe, in spite of having been introduced into Europe before 1550 (Sauer, 1993). In contrast, the first detailed description of common bean, accompanied by a strikingly accurate drawing of a bean plant, was published in 1543 in the Herbal of Fuchs (Fuchs, 1543), suggesting that common bean was well known in Europe, at least by the scientists of the time, less than 50 yr after the voyages of Columbus to the Americas. The bronze portals—dated to 1595—of the cathedral of Pisa, include realistic representations of plants and animals, include Old World legumes such as field bean (*Vicia faba* L.) and lupin (*Lupinus* spp.) and common bean, a New World legume (Fig. 1). The fact that common bean

was represented in a religious site suggests that it was part of every day life at the end of the 16th Century, at least in Tuscany.

These limited data therefore suggest that, at the very least, dissemination of New World crops into Europe took place over one to two centuries, a pace much slower than that observed currently for GE crops. USDA data in 2000 (Fernandez-Cornejo, 2001) show that transgenic field crops occupied a substantial part of the total field crop area in the USA. For example, from 1996 to 2000 planting of herbicide-tolerant soybean surface planted went from 1 million to 43 million acres (1 acre = 0.4 ha) or 54% of the U.S. soybean acreage. Likewise, herbicide-tolerant cotton expanded from 10% of surveyed acreage in 1997 to 46% in 2000. The use of insect-resistant (Bt) maize grew from about 1% of planted corn acreage in 1996 to a peak at about 26% in 1999, before falling to 19% in 2000. GE soybean, cotton, and maize are now planted on approximately 30 million hectares (more than 75 million acres) in every state of the USA except Alaska, Hawaii, Nevada, and Rhode Island.

Had cultivation and the genetic modifications brought about by domestication and subsequent spread of agriculture entailed a serious human health or ecological risk, the slow pace of the changes would have allowed societies to take a step back and adopt changes to their food procurement. Alternatively, the slow pace may have prevented detection of mildly deleterious effects. Deleterious effects of agriculture on the environment are not new. For example, O'Hara et al. (1993) provided evidence of soil erosion in prehispanic times.

One could object that the previous two examples are not quite relevant to the introduction of GE as the first example involves a transition to a completely novel type of society and the second the introduction of new crops within established agricultural societies. The introduction of GE represents, in its narrowest sense (i.e., disregarding socioeconomic, cultural, or environmental issues such as the ownership of the agricultural and food chain), a technical advance that increases the efficiency of plant breeding. A change similar to GE is the introduction of hybrid vigor in maize. Duvick (2001) showed that hybrid maize cultivars spread quickly in the USA once the initial technical difficulties had been overcome. After approximately 5 and 10 yr, 50% of the maize area was planted to hybrid cultivars in Iowa and the USA, respectively (Duvick, 2001, Fig. 2). A similarly rapid spread was observed for wheat varieties incorporating dwarfness genes. In California, for example, the entire wheat growing area was converted to short-statured varieties in 2 yr in the 1960s (C. Qualset, pers. comm.). This rapid spread is not limited to the USA. High-yielding rice varieties were grown over 50% of the Philippine rice-growing area 5 yr after their introduction (Hargrove et al., 1979). Thus, GE cultivars have spread at rates that are comparable to those of cultivars obtained by classical breeding, in the last 100 yr.

Was Agriculture the “Hip” New Thing 10 000 Years Ago?

Observation 4. The Introduction of Agriculture, like That of Any New Technology, Was Accompanied by Desirable as Well as Deleterious Effects

By production and acreage yardsticks, agriculture has been very successful. It has been able to feed sharply increasing human populations and is widely distributed over the entire world. However, the change from hunting–gathering to agriculture has not been an unconditional blessing. Aside from environmental considerations, it is generally thought that hunter–gatherers were better fed and healthier than the farmers who replaced them. The sedentism and urbanization accompanying the advent of agricultural societies led to an increase in infectious diseases (Armstrong et al., 1991; Tishkoff et al., 2001; Volkman et al., 2001) and decreased well being mainly because of a less-diverse nutrition (Kates, 1994). On the other hand, agriculture—by generating a surplus of food—made labor specialization possible and eventually led to the development of civilizations (e.g., Maisels, 1993; Solis et al., 2001). Humanity would eventually recover from the introduction of agriculture and other societal changes, but only in the 17th Century (Current Era) (Kates 1994).

Unlike 10 000 yr ago, when there were probably no widely held environmental concerns and, hence, most certainly no regulatory framework, contemporary societies have established more stringent criteria for technology adaption, resulting in more widespread regulations and we are thus in a better position to study the possible outcomes of the introduction of new technologies. GE has been subjected to more stringent regulatory scrutiny than classical plant breeding. For example, one of the potential concerns raised about GE is escape of transgenes through pollen-mediated gene flow. Yet, gene flow is a feature of many crop plants, even nontransgenic ones (Ellstrand et al., 1999). This raises a general concern about the effects of gene flow from crops—transgenic or not—to their wild relatives, for example on the genetic diversity of wild relatives. Whereas these effects have rarely been studied systematically, this situation raises the question whether cultivars developed by classical breeding should be subject to more stringent regulation, similar to that used for GE cultivars. Whether or not applications of GE (and classical breeding) are a matter of concern can only be decided on a case-by-case basis as some of the examples in the next paragraphs will show.

CLASSICAL PLANT BREEDING

General Steps in the Development of New Crop Cultivars

Observation 5. GE Can Play a Role Primarily in the Introduction of New Genetic Diversity in the Gene Pools Used by Plant Breeders for the Development of New Cultivars

It is often claimed that GE is a faster and more accurate way of developing new crop cultivars than classical

plant breeding [e.g., National Research Council (U.S.) Committee on Genetically Modified Pest-Protected Plants, 2000; Prakash, 2001]. A closer examination of this claim and the underlying facts indicates that this may not always be accurate. The development of new cultivars is the result of a cyclical process, each cycle consisting of several overlapping phases (Allard, 1960; Bos and Caligari, 1995; Brown et al., 1990; Charrier et al., 2001; Comstock, 1996; Hill et al., 1998; Mayo, 1987; Poehlman and Sleper, 1995; Simmonds, 1979; Stalker and Murphy, 1992). The phases include (i) assembling and generating new diversity; (ii) selection and testing to identify superior recombinants; and (iii) release, distribution, and commercialization of new cultivars. The first phase includes such activities as assembling sources of genetic diversity for the major breeding activities from adapted or exotic sources and recombining these sources of genetic diversity to create new gene combinations, either by sexual crosses or GE. In the selection phase, breeders deal with such issues as the timing of the selection (e.g., early vs. late generation), the selection environment (e.g., favorable vs. stress), and the number of years and locations of testing. Finally, the release and distribution of new cultivars should not be overlooked. Having a new, superior genotype is in itself not a guarantee of widespread success of an improved cultivar.

The major contribution of modern plant breeding from a genetic standpoint, as practiced over the last century, has been the maximization of the expression of traits of agronomic interest, principally the tolerance to biological and environmental stresses (Duvick, 1992; Duvick and Cassman, 1999; Fehr, 1984). This has been achieved primarily through the application of powerful tools in quantitative genetics and statistics (Comstock, 1996; Hallauer and Miranda, 1988). While QTL analyses show that major genes have played a role in several cases, the contribution of minor genes and their interactions (see below) has also been very important to maximize trait expression.

The major contribution of GE is the generation of additional genetic diversity. In spite of intensive searches, certain traits cannot be found in the domesticated gene pools or in gene pools of sexually compatible relatives (Harlan and de Wet 1971). The example of high provitamin A “golden rice” comes to mind (Ye et al., 2000). Deficiencies in dietary vitamin A are one of the leading causes of child blindness in the developing world. Golden rice was engineered to contain provitamin A based in the introduction of two genes from daffodil and one from the bacterium *Erwinia uredovora*. On one hand, non-GE rice contains no provitamin A. Thus, the ability to introduce these genes to create a new biochemical pathway in rice is an exciting development. On the other hand, the development of this GE rice genotype is only the first step towards solving the vitamin A nutritional problem by genetic means. Among the steps on the long and arduous road towards this goal are the development of local high-yielding rice cultivars (including transfer from the current temperate *japonica* background to the *indica* background more prevalent in the tropics), clinical trials to demonstrate the effectiveness of these rice

varieties in raising levels of this lipid-soluble vitamin in the human body (many children go blind even though they consume sufficient provitamin A because dietary lipid is in notoriously short supply in many Third World diets), and consumer acceptability tests of the altered color of the grain. These additional activities are not trivial, will take several years and have an uncertain outcome. It should also be kept in mind that there are potential nongenetic alternatives to the genetic means to alleviating vitamin A deficiency. For example, mango and palm oil (Manorama et al., 1996; National Institutes of Health, 2001) are rich sources of provitamin A and can be obtained from tropically adapted plants, grown in small-scale community or school vegetable and fruit gardens (International Life Sciences Institute and Food and Agricultural Organization, 1997).

In summary, GE complements classical breeding because it represents an additional, although powerful, way of generating additional genetic diversity, which is the starting point of any breeding program.

Introduction of Genes into New Genetic Backgrounds and Duration of the Development of New Cultivars by Classical Plant Breeding and GE

Observation 6. GE—at Least in Its Current Incarnation—Carries with It Its Own Set of Pitfalls That, to a Large Extent, Nullify the Initial Time Advantage That May Have Been Conferred by Direct (Nonsexual) Gene Transfer Compared with (Sexual) Backcrossing

As mentioned earlier, one of the major capabilities of GE is to introduce genes from sexually incompatible backgrounds. A gene is therefore placed into a new genetic background to which—during evolution—it has not been exposed, nor has the genetic background been exposed to this gene. Is this a serious concern?

The reductionist approach to molecular biology posits that genes can be studied in isolation and that they have discrete and unambiguous effects on the phenotype. This is reflected to a large extent in the current strategy in genomics research where a limited number of (in some cases, one) genotypes are studied. Disruptions of genes either by physical, chemical, and insertional mutagenesis are used to make inferences about the function of the gene. This approach assumes that genes will be expressed regardless of the genetic background and the environment. Likewise, expression analyses by such technologies as microarrays are conducted with limited reference to quantitative approaches. In reality, some genes may only be expressed in certain genetic backgrounds and environments. This lack of consistent expression results in phenotypic plasticity, whereby the same genotype produces multiple phenotypes depending on the environmental conditions (Pigliucci et al., 1999; Sultan, 2000).

Conversely, genetic heterogeneity refers to a situation where different genotypes produce the same phenotype. Although these are widespread phenomena, the molec-

ular, biochemical, and physiological bases are poorly understood. Much of the available information comes from studies on experimental organisms such as yeast, *Drosophila*, and *Caenorhabditis elegans*. For example, double mutants have been identified that lead to synthetic lethality, which defines a relationship where the presence of one gene allows the organism to tolerate genetic variation in another gene that would be lethal in the absence of the first gene. For example, some 170 genes have been identified in the protein secretion pathway of yeast into the vacuoles. These and other genes are involved in some 240 synthetic lethal interactions (Hartman et al., 2001). In comprehensive synthetic lethal screens, individual genes interacted with between three and eight genes. These epistatic interactions are by no means unique to yeast, but are general among living organisms and, from a methodological standpoint, can be used to actually determine the order of genes in biochemical and developmental cascades (Avery and Wasserman, 1993). In plants, genes involved in pathogen recognition and response pathways may provide a similar example of genetic buffering through redundancies (Dangl and Jones, 2001).

The available data therefore show that far from acting in isolation, genes act in concert with others and are influenced by the environment. The response of transgenes to environmental influences and the genetic background in which they operate has to be taken into account. It should be pointed out here that these concerns are not limited to transgenes but any genes that are introgressed into crops within the same species or from other species, whether by classical breeding or GE. Examples of these phenomena, observed after transfer by sexual crosses from close relatives, include reversal of dominance, epistasis, and differential sensitivity to environmental conditions of the *tb1* gene in maize (Doebley et al., 1995; Lukens and Doebley, 1999) and the effect of epistasis on some traits distinguishing annual species of sunflower (*Helianthus* spp., Kim and Rieseberg, 2001) and on seed yield in wide crosses of rice (Li et al., 1997; Yu et al., 1997) and common bean (Johnson and Gepts, 2002). These observations immediately raise the question whether introduction of genes from more distant genetic backgrounds, such as might be the case with GE, will be faced with a larger or smaller number of interactions. No answer is available to this question, which may well be faced with difficult experimental difficulties related to linkage drag in sexual crosses. From a practical standpoint, however, testing that normally takes place during the breeding process should eliminate deleterious mutations or underperforming genotypes resulting from these interactions, whether they arise from the introduction of genes via GE or classical breeding.

A corollary of Observation 6 is that the purported speed of introduction of genes by GE compared with classical breeding is overstated. There are three main reasons why the duration of the introduction of new genes is more similar between classical breeding and GE than generally stated. First, the length of the process in classical backcross breeding is not as long as usually presented, other things being equal, especially if it is

guided by molecular markers. Crops produced in this way are not considered transgenic because the transfer of genes occurs solely through the usual sexual processes. Classical breeding typically would introduce a single gene by backcrossing. Without molecular markers, at least six backcrosses would be needed. For the average annual crop, this would represent around two calendar years. The use of molecular markers to select for the gene(s) and against the genetic background of the donor parent shortens this introduction by two or three generations so that introduction by classical means can be achieved in somewhat more than 1 yr.

Second, the introduction of genes by GE is faced with uncertainties related to the transgenic nature of the gene introduction (Zhong, 2001). These uncertainties are generally not observed with standard backcrossing. Genetic engineering lacks precision in that the integration point is uncertain. Ideally, the transgene(s) should be integrated in a location of the genome where (i) stable expression and predictable Mendelian transmission is assured and (ii) the expression of endogenous genes is not disrupted or silenced (Iyer et al., 2000; Kunz et al., 2001; Meyer, 2000; Morel et al., 2000). Precise integration of transgenes by homologous recombination, although possible in yeast, is still elusive in higher plants. Additional issues associated with transgenic transfer are stability of gene expression as a function of the environment and the point of integration into the genome ("position effect"). Thus, the initial introduction of transgenes have to be followed by extensive progeny testing to identify those integration events that are consistent with a high level of stable expression, which further reduces the supposed time advantage of GE. An example of the intensive testing to be conducted following transformation is provided by the case of glyphosate-tolerant line 40-3-2 of soybean (Padgett et al., 1995) for which seven generations were needed to verify stability and level of expression by classical breeding procedures. These pitfalls in transgenic methodology and potential solutions are discussed in greater details in a recent review (Zhong, 2001). Potential solutions to these problems have been proposed (reviewed in Allen et al., 2000; Chandler and Jorgensen, 2000; De Wilde et al., 2000; Hohn and Puchta, 1999; Kumar and Fladung, 2001).

Thirdly, current genetic engineering methods are generally genotype dependent. Often the genotypes used for GE are not elite genotypes used in the development of new cultivars. Therefore, successful transformation has to be followed by a classical backcross program to introduce the transgenes into the desired genetic background. These additional backcrosses, even when aided by marker-assisted selection, represent an additional time cost.

A good example of the need to breed the transgene (i.e., getting it into improved genetic background) is currently happening with herbicide resistant soybean developed through GE. The first wave of herbicide resistant soybean varieties proved to be very susceptible to *Sclerotinia stem rot* [cause by *Sclerotinia sclerotiorum* (Lib.) de Bary]. Through necessity, the genetic back-

ground of the germplasm chosen for transformation was very narrow and proved to be highly susceptible to *S. sclerotiorum*. As a result the GE herbicide resistant varieties showed increased susceptibility to *Sclerotinia stem rot* because of their genetic background but not as a result of the introduction of the transgene or any sensitivity to the herbicide (Lee et al., 2000).

In summary, selection for the transformation events with the highest and most stable expression will require multilocation, multiyear testing, making GE more akin to classical breeding. In addition, testing for the possible disruption of existing developmental or biochemical pathways also takes time, especially if the introduced genes come from a widely different genetic background. It is likely that technical advances will advance the precision of genome insertion and the stability of expression of transgenes. In turn, these advances will increase the speed of GE in generating new genotypes.

Linkage Drag Associated with Gene Introgression Using Classical Methods

Observation 7. The Knowledge about the Actual Genes Being Introduced Is an Advantage of GE over Classical Plant Breeding; Although this Knowledge Reduces the Likelihood of the Inadvertent Introduction of Genes Deleterious to Humans Per Se, Few Cases of Such Introduction by Classical Plant Breeding Have Been Reported

When a gene is introduced by backcrossing and selection, it is not only the gene that is introduced but also the flanking regions. This natural phenomenon has been called linkage drag. The flanking regions can carry additional genes most of which are unknown because with the exception of *Arabidopsis thaliana* Heynh. (Bevan et al., 2001; Kaul et al., 2000) and soon rice, no plants have had their complete genomes sequenced (assuming that the raw sequence data will provide all information necessary to understand the function of each gene). Among these genes carried through linkage drag could be some that control the synthesis of potentially harmful compounds. Alternatively, these genes could code for agronomically undesirable traits, such as low yield or disease susceptibility. Depending on the linkage distances, the size of the flanking regions can be decreased by additional backcrossing (Young et al., 1988) although breeders do not have control over the size of the region or the recombination breakpoints. On the other hand, crosses with wild progenitors can potentially reintroduce toxic compounds that were selected against during and after domestication or against which humans have developed suitable processing methods. As sessile organisms, most plants contain nefarious substances that are part of their arsenal against diseases and predators. Examples of these compounds are cyanogenic glucosides in cassava (*Manihot esculenta* Crantz) and lima bean (*Phaseolus lunatus* L.), gossypol in cotton, steroidal alkaloids in potato (*Solanum tuberosum* L.), hemagglutinin and trypsin inhibitor in grain legumes, cucurbitacins in cucurbits, and sulfur-containing compounds in the cabbage

family (Simmonds, 1979). During domestication, selection against these toxic compounds took place and many crops have either lost these compounds or contain significantly reduced amounts. Clearly, testing for nutritional quality has taken place during domestication and has been effective. However, testing was not of the do-no-harm, informed consent type used today! Classical plant breeding has actually introduced very few deleterious genes from wild relatives into crops.

CONCLUSIONS

An analysis of existing data shows that genetic changes induced by domestication are generally not comparable with those currently brought about by GE in that they represent a loss of function, in contrast with the gain of function associated with genes introduced by GE (at least for most current applications). In addition, GE is not necessarily a faster and more precise alternative to classical plant breeding. It is principally a powerful and useful way of generating additional genetic diversity that can then be incorporated into improved crop cultivars by the proven methods and techniques of plant breeding. The potential benefits of GE should be investigated on a case-by-case basis, taking into account the advantages or disadvantages of alternative technologies. The existing regulations in place for GE cultivars may have to be extended to cultivars obtained by classical plant breeding. Where uncertainties exist, experiments specially designed to investigate these uncertainties should be conducted rather than relying on existing data that are sometimes only peripherally relevant. Who should conduct these experiments (private or public sector) and the current U.S. regulatory framework divided over three agencies (EPA, USDA, and FDA) remain bones of contention. Finally, it is also clear that GE is—at this stage—a relatively unsophisticated technology, severely limited by the paucity of economically useful genes available for transformation. Further advances will increase the precision of the technique, its relevance to consumers, and its environmental friendliness. It is likely that technological advances will increase the precision of insertion in the genome and expression of transgenes. Potential solutions to some of these problems are being developed (e.g., Daniell et al., 1998; Scott and Wilkinson, 1999, but see also Cummins, 1998; Stewart and Prakash, 1998). The public deserves an impartial, thoughtful, and mutually respectful discussion of the issues surrounding genetic engineering.

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