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TEACHING TOOLS IN PLANT BIOLOGY™: LECTURE NOTES

Genetic Improvements in Agriculture

Humans are just one out of millions of species of life on earth, but our impact far outweighs our numbers. We have dramatically changed the planet in countless ways, from carving up mountains and damming rivers to filling our skies with orbiting satellites. We've pushed other species to extinction: some well documented, such as the iconic dodo (*Raphus cucullatus*), but many before they've even been named. Other species we've selected for domestication and manipulated to better suit our needs. In the 15,000 years of our domestication of dog (*Canus lupus*), we've created hundreds of genetically distinct breeds that range in size from the Great Dane (>50 kg) to the Chihuahua (< 3 kg), and in behavior from retrievers to fighters to herders. In 10,000 years of agricultural innovation, we've domesticated plants to produce more food, resist more pests, facilitate harvesting, and provide better nutrition than their wild relatives. An understanding of the history and future of genetic improvements in agriculture is particularly relevant as we look ahead at the challenges brought by increasing population, degrading soils, disappearing water reserves, escalating energy prices, and unpredictable climate changes.

THE DISTANT PAST

Crop Plant Domestication and Beyond

The transition from hunter to farmer marks one of the most significant achievements in human history. Called the Neolithic Revolution, it radically changed human activities and social structures. Freed from the need to constantly forage, individuals began to take on new roles and acquire new skills. Settlement and secure food sources meant that family sizes could increase. At the same time, the use of land for farming rather than hunting started a trend of habitat destruction and deforestation that persists today.

We can only imagine the events that contributed to the transition from gathering to cultivating. Presumably as people returned to favored sheltering sites, they inadvertently increased the abundance of plants whose seeds they had collected. At some point, the connection between seed and plant was made, and intentional genetic selection began to take place. The major crops we rely on as our primary food sources were domesticated as crops between 13,000 and 5000 years ago. The earliest record of agriculture is in modern Iraq, in the valleys of the Tigris and Euphrates Rivers. Here, wheat (*Triticum* spp), barley (*Hordeum vulgare*), pea (*Pisum sativum*), and other beans were cultivated, accompanied by a thriving and complex culture that ultimately produced the Ancient Egyptian, Greek, and Roman civilizations. Sites of early domestication in Asia and the Americas also coincide with sites of great ancient civilizations; maize (*Zea mays*) was domesticated in the Mesoamerica regions, squash (*Cucurbita* spp) and potatoes (*Solanum tuberosum*) in the Andean highlands, and rice (*Oryza sativa* subsp *indica* and *japonica*) in India and China (Diamond, 1997, 2002; Londo et al., 2006).

The morphological and genetic changes that occurred during plant domestication have been revealed through archeological and genomic studies. Archeologists studying early human settlements have collected and dated remains of meals at these sites. In a collection of ears of maize retrieved from a single archeological site, a severalfold increase in cob size over a span of 6500 years has been documented. In fact, over the thousands of generations of cultivation and selection by humans, maize has changed so dramatically from its progenitor that it bears little resemblance to it (Doebley, 2004; Doebley et al., 2006). The closest wild relative of domesticated maize (*Z. mays* ssp *mays*) is teosinte (*Z. mays* ssp *parviglumis*). The seed spike or ear of teosinte consists of up to 12 kernels, each encased in a hard covering. The hard covering protects the teosinte seed from damage while inside an animal's digestive system but makes preparing it as food more difficult. It's thought that a mutation in the *teosinte glume architecture1* gene that reduces this covering was one of the earliest events in maize domestication.

Seed indehiscence or nonshattering is another trait that has been selected for in many grasses. When mature, the seeds of wild relatives break off the ear (they are said to shatter or dehisce), facilitating their dispersal but making the seed very difficult to harvest. In the wild relatives, an abscission layer forms that facilitates the breaking off of the seeds. In the domesticated plants, this layer does not form or is reduced. Archaeological records show an increase in the frequency of seeds with the nonshattering trait over time during the period of domestication. Furthermore, domestication gene variants have been identified that contribute to the nonshattering trait.

Larger-scale genomic changes correlated with domestication are apparent as well. Many of our crops maintain a higher number of chromosomes than their wild progenitors, which is often correlated with increased size. In many cases, this has resulted from the combination of chromosome content from more than one ancestral species. For example, wheat is a hexaploid plant carrying two copies of each chromosome from three progenitors. In the *Brassica* tribe, crops including mustard (*Brassica juncea*), turnip (*Brassica rapa*), broccoli (*Brassica oleracea* ssp *italica*), and oilseed rape (*Brassica napa*) are derived from three genomes combined in various ways. A study looking at the genome of indica rice revealed that its genome has been extensively modified through deletions, insertions, rearrangements, and mutations. Just as selective breeding has radically altered the ancestral dog, the food we eat comes from plants that have no more similarity to their wild relatives than a Chihuahua dog has to a wolf.

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THE RECENT PAST

The Needs of a Growing Population

The century that took us from gas lamps to Google and steamships to space shuttles also brought great advances in genetics and crop breeding. In 1800, the world population was ~1 billion people. In 1900, at the beginning of the 20th century, the population had increased to 1.65 billion people. By 2000, that number had increased to over 6 billion and is predicted to reach 10 billion by 2050. To feed the growing population, new plant varieties and cultivation methods were developed. This article focuses on the genetic improvements that led to high-yielding plants, but the genetic potential of these new varieties was realized by increased application of fertilizers, increased mechanization of planting and irrigation, and increased expansion of cultivated lands.

The Development of Hybrid Seed

Although the insights of Gregor Mendel and Charles Darwin occurred in the 19th century, these ideas didn't come together into the science of genetics until the early 20th century. Alongside the fruit fly (*Drosophila melanogaster*), maize was one of the first organisms in which genetic science was forged and applied, and studies in maize led to important discoveries about chromosomes and mobile DNA, as well as contributing directly to the science of plant breeding.

The discovery of hybrid vigor (also known as heterosis) by maize geneticist George Shull in the first decade of the 20th century made a major contribution to increased crop yields. Shull showed that in some but not all crosses between two purebred individuals, the resulting hybrid is bigger than each parent; geneticists still argue about what causes this effect and why. Shull's finding opened the door to increased yields and changed the way maize and other crops are bred and sold. Producing hybrid seed is labor intensive because one parent has to be detasseled or emasculated to prevent self-fertilization and ensure outcrossing. Companies were formed to provide this service, including Hi-Bred Maize, formed in 1926, which later became Pioneer Hi-Bred. Although purchasing seed every year increased farmers' expenditures, it was more than compensated for by the enhanced yields conferred by hybrid seeds. Between 1935 and 1960, the percentage of hybrid maize seed planted in the US rose from 0 to 99% (Shull, 1909; Fedoroff, 2010; Goff, 2011).

The (First) Green Revolution

In 1944, plant breeder Norman Borlaug took up the challenge of improving wheat yields in Mexico, through a project funded by the Mexican government and the Rockefeller Foundation at what would later become the International Maize and Wheat Improvement Center (CIMMYT). Like any successful agricultural endeavor, the program he developed incorporated modifications to agronomic practices as well as to plant genetics. The dramatically increased yields that followed are attributed to increased use of fertilizers and the development of dwarf wheat varieties that assimilate more of their resources into their seeds. For this work, Norman Borlaug was awarded the 1970 Nobel Peace Prize. When these wheat varieties were adopted in India and Pakistan, crop yields also rose dramatically, turning these countries from grain importers to grain exporters.

Rice breeding efforts, led by Henry Beachell and then Gurdev Khush at the International Rice Research Institute (IRRI), also led to semidwarf, higher yielding rice. Khush describes the impact of these programs, "It took almost 10,000 years for food grain production to reach 1 billion tons, in 1960, and only 40 years to reach 2 billion tons, in 2000. This unprecedented increase, which has been named the 'green revolution,' resulted from the creation of genetically improved crop varieties, combined with the application of improved agronomic practices" (Khush, 2001).

The Green Revolution of the 20th century depended on genetically improved crop plants but also on governments' support and cooperation in providing infrastructure (systems for distribution of water, seeds, and fertilizers) and financial resources (subsidies for training and farm improvements). The Green Revolution bypassed most of Africa, partly because that continent was still struggling out of its colonial history and developing the stable governments and infrastructures needed to support fledgling agriculture. Compounding this, many of the high-yielding modern varieties developed through the CIMMYT and IRRI breeding programs were optimized for growth in other regions and do not thrive in sub-Saharan Africa. Developing crops optimized for the challenges of sub-Saharan Africa is an ongoing process, as described further below (Evenson and Gollin, 2003; Thurow and Kilman, 2009; Ejeta, 2010).

Modern Molecular Plant Breeding

Marker-Assisted Selection

In many cases, resistance to a specific pathogen is conferred by a single resistance gene. Breeders increase the resistance of a plant line by crossing it with a plant carrying the resistance gene. A disease resistance gene is said to be introgressed into an elite line, and ultimately an elite line that incorporates only one gene from the resistant parent is produced. The first cross between an elite line and a resistant line produces progeny with half of their genes from each parent. Rebuilding the elite genome requires repeated backcrossing to the elite line, diluting out the other genome by half with each generation. Until recently, breeders had only the plant's phenotype to go by when breeding. This meant that if they were crossing a disease resistance trait into an elite line, they had to check the progeny for the desired trait by infecting the plant and seeing resistance. Marker-assisted selection (MAS; also known as marker-assisted breeding or molecular breeding) is a tool that accelerates breeding through the use of markers, or DNA sequences, that segregate with or near the desired trait. Thus, an introduced disease resistance gene can be detected in a plant through analysis of a sample of its DNA, rather than by examining its response to a pathogen. MAS allows the identification of multiple traits at a time, facilitating complex breeding schemes (Collard et al., 2005; Collard and Mackill, 2008; Moose and Mumm, 2008).

The development of submergence-tolerant rice provides a nice example of the use of MAS. Rice is grown in flooded fields and can tolerate short periods of total submergence. However, after 2 or 3 d under flood-waters, most rice will literally drown due to a lack of oxygen. Flooding is unpredictable, and severe flooding regularly leads to food shortages and famine. Flash floods and typhoons in Bangladesh and India are estimated to cause yield losses of 4 million tons annually, enough to feed 30 million people. In the 1970s, a rice variety was identified that is capable of surviving prolonged flooding by reducing its metabolic activities (essentially, holding its breath). This trait was identified in a low-yielding rice variety not of interest to growers. The submergence tolerance gene was identified, and once it was cloned, DNA markers were used to track the gene as it was introgressed by MAS into elite varieties, including Swarma, a popular rice grown in India. Since August 2009, IRRI has distributed Swarna-Sub1 seeds to 100,000 farmers in India, and in 2010, it accounted for over one-quarter of the planted rice in India (Xu et al., 2000, 2006; Septiningsih et al., 2009).

Genome-wide association screening is another new breeding method that accelerates the development of improved plant varieties. This method is particularly important in the identification of genes that make a small but real contribution to a complex trait. Using this method, breeders have successfully identified regions of DNA that correlate with enhanced disease resistance, enhanced yields, increased accumulation of β-carotene, and enhanced drought tolerance and used these data to breed plants with enhanced characteristics (Kump et al., 2011; Tian et al., 2011).

The Development of Transgenic Plants

In the 1970s, scientists developed tools that enabled them to recombine DNA from different sources. In the 1980s, these methods were extended to allow genes to be inserted into a plant genome. Plants or other organisms carrying a gene introduced using recombinant DNA methods are often referred to as transgenic organisms or genetically modified organisms (GMOs). (For reviews on the development of recombinant DNA and plant transgenic methods, see Gasser and Fraley [1989], Berg and Mertz [2010] and Teaching Tools in Plant Biology 23 "A Really Useful Pathogen, *Agrobacterium tumefaciens*".)

This new technology accelerated plant breeding projects (Flavell, 2010). For example, rather than crossing an elite plant with a wild relative exhibiting pathogen resistance and then having to rebuild the elite genome, it is now possible to introduce only the gene conferring resistance. Genetic modification (GM) methods are particularly useful for plants like banana (*Musa* spp) that are propagated clonally rather than sexually or plants like cassava (*Manihot esculenta*) whose genetic resources are still being developed.

GM methods are the only method available when the gene of interest is not present in the germplasm of the crop plant. As an example, GM is being used to develop banana plants resistant to the banana wilt disease caused by *Xanthomonas*. Because bananas are relatively easy to grow on small plots of land, they are a food staple for >70% of Ugandans and widely grown by small-scale farmers. Banana *Xanthomonas* wilt was first detected in Uganda in 2001, and cumulative crop losses since then are estimated at more than $200 million. Ugandan scientists have inserted two genes from pepper (*Capsicum annuum*) into banana plants, conferring resistance to the pathogen. Banana is normally propagated vegetatively, and pepper and banana are different species that are not sexually compatible, so a genetic modification approach was necessary to transfer the pepper resistance gene into the banana plants. The resulting banana plants are GMOs and susceptible to the regulatory system established to monitor genetically modified plants. They are also an important resource in the fight to eliminate this disease and retain the banana as a food source (Tripathi et al., 2010).

One of the first applications of GM technology was the development of plants expressing an insecticidal gene derived from the *Bacillus thuringiensis* soil bacterium. The bacteria produce a protein that is selectively toxic to certain insects, and spraying a plant with a solution of the bacteria can confer protection to some insects. Alternatively, the gene encoding the insecticidal protein, commonly referred to as the *Bt* gene, can be introduced into the genome of a plant. The transgenic approach is more effective on some organisms, such as the European maize borer (*Ostrinia nubilalis*) that lives within the internal tissues of the plant and is difficult to control by spraying. *Bt* maize and cotton (*Gossypium hirsutum*) were among the first GMOs produced and have been grown in fields since the 1990s. About a third of GMOs planted carry the *Bt* gene, and many studies have demonstrated that fewer pesticides are used on fields planted with *Bt* crops, benefiting not only the beneficial insects that are not being killed by broad-spectrum sprays but also protecting the health and safety of farm workers and ultimately consumers and, through suppression of pest population levels, growers of non-GM crops (Lemaux, 2009; Hutchison et al., 2010; Ronald, 2011).

Herbicide tolerance is the trait most widely incorporated into GM plants. Fields planted with transgenic plants carrying a gene that breaks down glyphosate herbicides (e.g., Roundup) can be cleared of weeds through the application of this relatively nontoxic herbicide. The benefits of this trait include higher yields (due to less competition from weeds), reduced use of persistant, toxic herbicides in favor of glyphosate, and reduced soil erosion as a consequence of less aggressive weed-controlling tillage (Fernandez-Cornejo and Caswell, 2006; Brookes and Barfoot, 2010).

In 2010, 15 years after their first commercial planting, GM crops were planted on 150 million hectares in 25 countries by 15 million farmers, 14 million of which were small and resource-poor farmers in developing countries. Growth in adoption of these plants is continuing both in terms of land area planted and numbers of countries incorporating them into their agricultural programs (James, 2010). The American Society of Plant Biologists "believes strongly that, with continued responsible regulation and oversight, GM will bring many significant health and environmental benefits to the world and its people."

Emerging Methods for Crop Improvement

Traditional plant breeding and transgenic production via recombinant DNA methods are but two ends of a spectrum of methods available for genetic improvement of plants. Many new approaches have been developed that fall somewhere in between the slow, limited capabilities offered by within-species sexual crosses and the introduction of foreign DNA. Below are a few of the new and emerging methods being used for genetic improvement. Discussions about the regulation of plants produced using them, and how consumers will perceive those plants, are ongoing.

Cisgenics and Intragenics

Recombinant DNA methods provide the ability to introduce genes that are derived from distant relatives. However, these cross-species DNA transfers are also worrying to consumers; one of the symbols of the anti-genetic engineering movement is a fish/tomato hybrid (inspired by an early experiment that introduced a fish-derived antifreeze gene into tomatoes). An alternative use of recombinant DNA methods, known as cisgenics, is to introduce genes from the same or another cross-compatible species. A limitation of this method is that it depends on the availability of a suitable gene. As an example of cisgenics, resistance genes from wild relatives can be introduced into a cultivated variety without extensive backcrossing; this method can be particularly useful in plants such as potato and apple that are vegetatively propagated. Intragenic plants are produced using a gene from a cross-compatible species that has been modified, for example, to change the expression level or pattern or in a form designed to silence the endogenous gene (e.g., antisense constructs). Thus, by eliminating concerns about cross-species gene transfer, cis- or intragenic strategies can make genetically modified plants more acceptable to consumers. Nevertheless, because the chromosomal region into which the DNA is inserted is uncontrolled and variable, some still feel that these methods carry an unacceptable level of uncertainty. These methods also introduce foreign DNA into plants in the form of the T-DNA sequences and borders. Claims of sequences from the potato genome homologous to the T-DNA borders have recently been discredited. The European Parliament has ruled that it perceives no difference between modified plants produced using cis- or intragenic constructs and those produced using transgenic constructs.

Chimeric Grafting or Transgrafting

Chimeric grafting, also known as transgrafting, involves grafting a nontransgenic shoot to a transgenic root (or vice versa). Transgrafting can eliminate issues arising from the presence of transgenes in edible fruit or seed and the potential spread of foreign DNA by pollen transfer. The root system can be engineered with genes that confer resistance to diseases or pests or that confer a greater capacity for nutrient or water uptake. Alternatively, the root system can be engineered to be a source of small interfering RNAs. Gene silencing through antisense, hairpin, or small RNA expression has been used in plant genetic engineering for years, originally to develop virus resistance plants and later to silence endogenous plant genes. Because small RNAs can be mobile in plants and can move from roots to shoots and vice versa, resistance to viruses in grafted wild-type shoots can be conferred by mobile small RNAs produced in genetically engineered roots. Similarly, endogenous genes in wild-type tissues can be silenced or modulated by the action of mobile microRNAs or other small RNAs; as an example, tuber formation in potato was accelerated in wild-type stocks grafted to scions overexpressing the microRNA *miR172*. Root stocks have also been engineered to produce the FT protein, which is phloem transmissible and induces early flowering. This trait is an enormous advantage in breeding and scoring fruit phenotypes of species that take several years to reach maturity. The regulatory status of foods grown from transgrafted tissues is uncertain in some countries, whereas in others they are treated as though they are genetically engineered.

Zinc-Finger Nucleases and Transcription Activator–Like Effector Nucleases: DNA Nucleases with Engineered Specificities

Zinc-finger nucleases (ZFNs) and transcription activator–like effector nucleases (TALENs) are engineered proteins that can introduce double-stranded DNA breaks at specific sites in the genome. The double-strand breaks are imprecisely repaired by the cellular machinery, so short insertions or deletions can be introduced. In some cases, a donor DNA template can be introduced with the nuclease, which results in the repaired breakpoint incorporating a new or different DNA sequence. The elegance of these nucleases is that they can be specifically targeted to any site in the genome, which means that single genes can be modified with great precision.

ZFNs were developed in the mid-1990s and are hybrid proteins that include a specific DNA binding domain attached to a non-specific DNA endonuclease (FokI). The zinc-finger domain folds into a characteristic structure that inserts an α-helix into the major groove of the DNA and recognizes and binds to a specific three-basepair DNA sequence. Mutagenesis and screening has led to a library of zinc-finger protein sequences that correlate with three-basepair recognition sequences. By linking multiple zinc-finger domains together, the DNA recognition site can be extended to 9 or 12 base pairs, which often can be found uniquely within a genome.

TALENs are hybrid nucleases based on transcription-activator like effectors (TALEs) from *Xanthomonas* bacteria, which are DNA-binding effector proteins that activate transcription of host genes. These effectors bind DNA through a series of 33 – 35 amino acid domains, each of which recognizes a single basepair in a sequence-specific manner. Thus, a protein can be engineered to bind a specific DNA sequence by selecting and joining the appropriate amino-acid repeat units. The code that correlates the protein sequence to the DNA recognition site and the huge potential offered by this ability to program a specific DNA-binding domain were recognized in 2009.

The DNA-binding specificity of TALEs and zinc fingers can be exploited further by linking them to other types of proteins besides nucleases, for example transcriptional activators or repressors. Again, their big advantage is that they can confer specific and precise DNA targeting of the nuclease or other functional domain. Their disadvantage is that they can be difficult and/or expensive to produce.

CRISPR/Cas RNA-mediated gene targeting

An RNA-mediated system by which to target a DNA endonuclease to a specific region has been developed, based on a prokaryotic defense system called the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system. This interesting defense mechanism involves acquiring a short DNA sequence from an invading DNA element (virus or plasmid), and incorporating the sequence into a CRISPR array. Transcription and processing of the CRISPR array leads to the production of short CRISPR RNAs (crRNAs). In association with trans-acting crRNAs (tracrRNAs), crRNAs direct a DNA endonuclease such as Cas9 to the original source sequence (i.e., the invading DNA or virus), targeting it for cleavage and elimination.

This system has been modified to for use in genome engineering, using a single-guide RNA (sgRNA), which is a fusion of the crRNA and tracrRNA into a single molecule. When the sgRNA is expressed in a plant cell, along with a plant codon-optimized Cas endonuclease, double-stranded DNA breaks can be targeted to specific regions of the genome. As with the ZFNs and TALENs, these DNA breaks can lead to short insertions or deletions, or the insertion of new DNA sequences derived from an introduced DNA template. Because the mutation and gene-replacement events occur at a relatively high frequency, their presence can be screened through DNA analysis, eliminating the need for antibiotic resistance or other selectable markers, and allowing for very precise genome editing. Furthermore, because the DNA recognition occurs through RNA rather than engineered proteins, this system is less expensive and easier to use than the protein-based targeting systems.

THE FUTURE

Breeding for Improved Human Health

We can safely say that the Neolithic Revolution launched us into the space age. With food production centralized and secure, time was freed for other pursuits, such as developing science and culture and ultimately building rockets. However, human physiological needs have not changed at the same rapid rate as human social development. Over a very long evolutionary period, human bodies were optimized for a diet much richer in fruits and leaves than most people now get in their postagricultural grain- and bean-rich diets. In comparison to the diet of our hunter-gatherer ancestors, this diet is also very high in starch and fat, contributing to chronic diseases like hypertension, cardiac disease, diabetes, and obesity in the developed world, whereas the high cost of food contributes to hunger and malnutrition in the developing world. In either case, most humans consume a diet deficient in the plant pigments, vitamins, and micronutrients essential for human health (O'Keefe and Cordain, 2004; Newell-McGloughlin, 2008).

Carotenoids are yellow and orange plant pigments that are abundant in leaves, sweet potatoes (*Ipomoea batatas*), pumpkins (*Cucurbita* spp), and carrots (*Daucus carota*). β-Carotene is a carotenoid that is converted to vitamin A and retinol, the essential photopigment for vision. Vitamin A deficiency causes blindness and other detrimental health effects. The World Health Organization estimates that 127 million preschool children are affected by vitamin A deficiency, which leads to immune system depression and increased mortality and is the underlying cause of blindness in half of a million children every year (Beyer, 2010). Efforts are underway to breed plants enriched in β-carotene. Several approaches are being used, including GM and non-GM approaches. Seeds of provitamin A–enriched Golden Rice, developed using GM methods, should be available to small farmers soon. High β-carotene maize, cassava, and sweet potato also have been developed using either GM or non-GM methods. The nonprofit organization HarvestPlus focuses on the development of biofortified crops for the developing world, including a provitamin A–enriched sweet potato that is currently being grown by half a million families (HarvestPlus). Many other biofortification projects are underway to increase levels of protein, iron, zinc, antioxidants, and other beneficial components in food (DellaPenna and Pogson, 2006; Pfeiffer and McClafferty, 2007; Butelli et al., 2008; Dennis et al., 2008; White and Broadley, 2009).

Breeding for Drought Tolerance

Water availability limits plant growth throughout many regions of the world, and this is a problem that is going to get worse. Globally, 70% of water usage is for agricultural purposes; it takes ~3000 liters of water to feed one person for one day. Water use has increased dramatically with increased agricultural output, resulting in depletion of waterways and underground aquifers. Riparian ecosystems are diminishing and dying, and aquifers that have been accumulating water for centuries are being depleted at a rate that is unsustainable (Comprehensive Assessment of Water Management in Agriculture, 2007).

Rain-fed agricultural lands are highly vulnerable to devastating crop losses. Africa has experienced three-quarters of the world's severe droughts in the past 10 years and has the lowest proportion of irrigated lands (FAO AQUASTAT Survey, 2012; United Nations Economic Commission for Africa, 2008). Drought isn't limited to Africa, and the past few years have seen major droughts and associated crop losses in Australia, Russia, and China, leading to steep increases in food prices and accompanying social unrest worldwide. This trend is likely to persist as a consequence of altered weather patterns resulting from climate change (Food and Agriculture Organization of the United Nations, 2003; Gornall et al., 2010).

Three major seed companies have been developing maize that is less vulnerable to drought. Syngenta and Pioneer Hi-Bred have used MAS to identify a suite of genes that collectively optimize water use, which they are marketing as Agrisure Artesian and Optimum AQUAmax, respectively. Monsanto's maize expresses a bacterial RNA chaperon that is thought to stabilize drought-responsive mRNAs (Castiglioni et al., 2008). With the support of international aid agencies in a program called Water Efficient Maize for Africa, this drought tolerance trait has been bred into maize varieties optimized for different growing regions, including Africa. Monsanto has waived its royalties so that the seed can be distributed at minimal or no cost to resource-poor farmers (African Agricultural Technology Foundation, Monsanto).

Agricultural Innovation in Africa

Hunger and malnutrion are widespread in Africa. The rate of population growth in sub-Saharan Africa is the highest in the world, but at the same time, agricultural yields are usually lower in Africa than in other regions. The reasons for this are diverse but include a lack of agricultural infrastructure and equipment, including seeds optimized for sub-Saharan African environments and farm practices, insufficient mechanization and training, and a lack of access to and affordability of water, fertilizers, and pesticides (Thurow and Kilman, 2009). Most farmers in Africa cultivate small plots for primarily local consumption; 36% of all African labor is used in subsistence agriculture. Can small-scale farmers retain self-sufficiency but with improved yields, better nutrition, and fewer losses to pest, pathogens, and drought? This question has greater urgency in light of the expectation that the population of Africa will double in the next 40 years, from 1 billion to 2 billion (United Nations Economic and Social Affairs, 2004).

An agricultural revolution in Africa requires strong support from governments. Fortunately, the governments of countries throughout the continent are working closely together to develop shared electricity grids and water management schemes, transport networks, and common markets. The Alliance for a Green Revolution in Africa, chaired by the former Secretary General of the United Nations, Kofi Annan, is working to ensure that African farmers have access to seeds and fertilizers, as well as financing, training, and access to fair markets. Partners supporting these efforts include the Rockefeller Foundation, the Howard G. Buffett Foundation, and the Bill and Melinda Gates Foundation. A major thrust of these efforts is to develop Africa's human capacity through education, innovation, and technology transfer.

In the development of Africa's agricultural potential, low-cost, region-specific innovations can have big impacts. For example, with only 4% of Africa's arable land currently irrigated, there is tremendous opportunity for yield increases simply by bringing water to the growing plants. Water management programs include building systems of reservoirs in which to store rain water and implementation of low-cost, low-waste-drip irrigation systems. The rapid expansion of cell phone networks across the continent has given farmers access to information about weather and farming practices and improved ability to negotiate fair prices for their inputs and products (Juma, 2011).

Breeding programs for cassava, pearl millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), and other drought-tolerant staple crops for Africans are rapidly progressing using conventional, marker-assisted, and GM breeding technologies. Biofortification efforts are underway to improve the nutritional qualities of cassava, on which more than a quarter of a billion people depend, as well as other staple and indigenous crops. Through these programs, the research infrastructure and training of African scientists is expanding rapidly. Through improved infrastructure, training, and plant varieties, some predict that Africa will transition from a net food importer to a net food exporter within the next generation (Juma, 2011).

The Second Green Revolution

One of the criticisms that has been voiced about the Green Revolution is that its yield increases were dependent upon application of synthetic pesticides and fertilizers. It's been claimed that the Green Revolution isn't "green," in that its practice essentially converts oil to food. Agronomists and plant scientists are addressing these concerns by developing lower-input, environmentally friendly agricultural systems and plants (Fedoroff et al., 2010). For example, desirable traits are being combined to produce maize plants with increased drought-tolerance and water use efficiency that are resistant to microbial and insect pests. Experiments are underway to use plants directly to address environmental challenges, for example, through biological desalination, carbon sequestration, and air, soil, and water purification, and efforts to develop algae, perennial grasses, and lignocellulosic biomass as sustainable biofuels are ongoing (Gomez et al., 2008; Carroll and Somerfield, 2009; U.S. DOE and USDA, 2009).

Food shortages lead to price increases, which disproportionally harm the poorest people. Increases in food prices lead to political unrest and violence, and as Bob Marley sang, "A hungry man is an angry man." Impacts on agricultural productivity due to climate change are expected to disproportionally affect tropical regions, a bitter irony considering that many of the resource-poor people living in tropical regions contributed little to the conditions underlying climate change. Those of us with access to resources, including knowledge and training, have a responsibility to strive toward a better future for all. In his 1970 Nobel speech, Norman Borlaug stated, "Man can and must prevent the tragedy of famine in the future instead of merely trying with pious regret to salvage the human wreckage of the famine, as he has so often done in the past. We will be guilty of criminal negligence, without extenuation, if we permit future famines."

The pressures of increased population, dwindling reserves of fresh water and oil, and climate change uncertainty are significant global problems (Pretty et al., 2010). Meeting the hunger challenge requires the development of plants that not only produce more, healthier food, but that do so under more stressful conditions. The most basic goal ahead of us is to produce more food to feed additional mouths. A better scenario would include better nutrition, greener agriculture, and food security in all regions of the world. Using conventional and marker-assisted breeding as well as transgenic technologies, plant breeders are contributing toward these goals. Collectively, the development of plants to meet these needs is often referred to as the Second Green Revolution.

FREQUENTLY ASKED QUESTIONS ABOUT GMOs

As plant biologists, many of us have first-hand knowledge of recombinant DNA technology and transgenic plants, but to others, these ideas can be both foreign and somewhat discomforting. Scientists must contribute to discussions about the risks and benefits of GMOs. We've briefly addressed below five commonly asked questions. For more thorough discussions of these questions, see resources listed below, including Lemaux (2008, 2009), Parrott (2010), and Ronald (2011).

What Risk Assessments Are Performed on GM Crops?

The introduction of genetically modified organisms into the environment is governed by a framework of risk assessment. Typically, assessments are made as to risks to human health (including toxicity and allergenicity) and biological diversity from novel phenotypes, risks of the evolution of resistance in target pathogens or pests, risks to nontarget organisms, and risks of the movement of transgenes into different organisms through gene flow (Craig et al., 2008). Currently, different countries and regions regulate GMOs in different ways, creating conflicts and tensions. Efforts are underway to agree upon globally unified standards and practices, such as the Cartagena Protocol on Biosafety, but the challenges are significant. The voices of scientifically literate individuals are needed in these efforts. Moving toward regulatory harmonization will ultimately lower the costs and times associated with regulatory assessment, ultimately benefitting everyone who is concerned with food and environmental safely (Craig et al., 2008).

Will Genes from GMOs Contaminate Wild Populations?

Angiosperms reproduce by sperm cells contained in pollen being carried by wind or animals to other individuals. What happens to pollen from plants carrying transgenes? Several studies have indicated that pollen drift from GMO plants in the United States do not pose any increased health or environmental risk and that any resulting hybrids are potentially a nuisance but not uncontrollable. Organic farmers have expressed concern that pollen carrying transgenes will move into their fields and contaminate their product. In response to this concern, acceptable levels of transgenic DNA presence in organic products have been suggested. To help avoid unwanted pollen movement, strict guidelines are in place to geographically contain where GMOs are grown, and exclusion zones are required around GM fields. Some crop plants like potato or sugar beet (*Beta vulgaris*) can be harvested before the plant flowers, preventing pollen formation. Other approaches include looking at ways to splice out the transgenic DNA from pollen or make it incompatible with other varieties (Ellstrand, 2003; National Research Council and Institute of Medicine of the National Academies, 2004; Ronald and Adamchak, 2008; McHughen and Wager, 2010; Stokstad, 2011).

Will Anti-Insecticidal or Other Genes Harm Unintended Targets?

The effects of *Bt* on nontarget organisms, including arthropods, nematodes, and soil microorganisms, have been extensively studied. Results of these studies indicate that nontarget invertebrates are better off living on a *Bt* crop field than an herbicide-treated field, but when compared with a nontreated field, some invertebrates are less abundant in the *Bt* field. Further studies have examined the possible effects of predators of parasitoids of herbivores exposed to *Bt* and found no indication of direct effects of *Bt* plants on natural enemies, although adverse effects can be observed when their prey/hosts are *Bt* susceptible, mostly likely due to reduced prey/host quality (Romeis et al., 2006). No significant differences have been detected in soil microorganism populations between *Bt* and non-*Bt* fields. Many concerns about GMO's impact on the environment stem from a study published in 1999 that claimed that Monarch butterfly larvae were harmed by ingestion of pollen carrying the *Bt* gene. The experimental design of these studies was flawed, and the conclusions invalid, but the interpretations of this study persist in the popular media (Gatehouse et al., 2002; Lemaux, 2009). Well-designed, ecologically relevant studies (i.e., good science) are the sole means through which environmental impacts of transgenic crops can be assessed.

Concerns have been raised that antibiotic selectable markers used in transgenic plants might contribute to antibiotic-resistant bacteria, but there is no evidence to indicate that horizontal gene transfer occurs between GMOs and bacteria in the gut or soil. Nevertheless, breeders are moving away from the use of antibiotic resistance markers in the production of GMOs, and alternative technologies are being developed.

GMOs have been in our food chain and environment for a decade or more with no adverse impact (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, 2004; National Research Council and Institute of Medicine of the National Academies, 2004; European Commission Joint Research Centre, 2008; Lemaux, 2009; Ronald, 2011).

Will GMOs Take Away Choice and Exploit Small Farmers?

A common misconception of GM plants is that they are produced by rich corporations that exploit the poor by forcing them to buy seeds they cannot afford to grow crops they do not want. This simply isn't true. During 2010, 15.4 million farmers grew GM crops, of which 14.4 million (>90%) were small resource-poor farmers in developing countries. Nearly all farmers chose to keep planting GM crops after their first experience with them because of the significant benefits they offer (James, 2010). The trend is expected to continue as more plants and traits are made available, including drought-tolerant maize, Golden Rice, and *Bt* rice (Qaim and Zilberman, 2003; Qaim, 2010).

GM is widely recognized as an important tool in our plant breeding toolbox. The British Royal Society recently stated, "Both genetic improvement and better crop management are vital and both should be resourced in parallel." Most humanitarian organizations recognize that the beneficial outcomes stemming from GM technology are important contributors to worldwide food production, particularly the new traits that enhance yields under drought and other adverse conditions. It is important that scientists, philanthropists, and biotechnology companies in the developed world support developing countries as they develop indigenous infrastructure and tools for developing their own GM crops.

Are GM Crops Safe to Eat?

GMO plants are subject to extensive safety testing and regulatory oversight, far more than any plants bred through non-GM means. More than a decade after their initial commercial release, no detrimental health effects have been identified in consumers of these plants, be they human, animal, or human by way of animal. The U.S. National Academies and the British Royal Society have indicated that GM crops are safe to eat. More specifically, it has been repeatedly demonstrated that the process of genetic modification itself has no more risks of unintended consequences to human health and the environment other breeding methods (Lemaux, 2008, and references therein; Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, 2004; European Commission Joint Research Centre, 2008; European Commission Directorate-General for Research and Innovation, 2010).

GMOs in the News

Recently, the discussion about whether GM plants are safe and desirable has been heating up. In May 2012, a group of activists calling themselves “Take the Flour Back” threatened to destroy a field trial of GM wheat at Rothamsted Research, a publically funded research center in England. The wheat was engineered to produce (*E*)-β-farnesene, which is an alarm pheromone produced by aphids. The experiment was designed to determine whether the production of this compound would confer protection against aphid herbivory. Rothamsted scientists and their supporters responded with a public appeal to “Don’t Destroy Research.” Ultimately, the protestors did not harm the plants, and the researchers were able to explain the purpose of their studies to a wider public.

In August 2013, one of the Golden Rice field trials in the Philippines conducted by International Rice Research Institute was vandalized by anti-GMO protestors. The scientific community was quick to respond, with a petition in support of Golden Rice field trials collecting more than 5000 signatures within a month of the vandalism occurring and culminating in a high-profile editorial in the journal *Science*, called “In Defense of GMOs” signed by 11 scientific leaders.

In January 2013, Mark Lynas, a high-profile author and journalist, offered a public apology for his earlier contributions to the anti-GMO movement. As he said, “What happened between 1995 and now, that made me not only change my mind but come here and admit it? Well, the answer is fairly simple: I discovered science.” This articulate lecture has been viewed over 60,000 times since being posted online and prompted countless conversations about the science of GMOs.

In the United States, several ballot initiatives have been put forth that demand that foods containing ingredients from GM plants be labelled. In November 2012, California voters turned down Proposition 37, the “Mandatory Labelling of Genetically Engineered Food Initiative.” A similar initiative, Initiative 522, is going to the voters of Washington State in November 2013, and initiatives in several other states may reach that point soon. Arguments for labelling laws center around a consumer’s “right to know” what is in the food they eat. Arguments against this include the effect on food prices that such a labelling law will have and the lack of any scientific evidence to support such labels. These ballot initiatives have led to very public and heated discussions about the process of genetic engineering and revealed some of the reasons that people worry about GM plants and others are exploiting these fears.

Sowing Seeds of Doubt

Although there is no evidence to support such assertions, headlines regularly declaim that GM food causes cancer, organ failure, loss of fertility, diabetes, and other health problems. Sensationalism sells. Yet, even though the claims are unsupported, these reports impact public perception. The more a technology is associated with risk in the media, the greater the public experiences it as risky. This effect is called the social amplification of risk. The more people associate GM technology with risk, the more difficult it becomes for the benefits to be realized.

The importance of the popular and social media in shaping the public’s perception of science is illustrated by the impact of a flawed study published by Wakefield and others in 1998 that implicated a childhood mumps-measles-rubella vaccine with autism. Although this finding has been thoroughly, repeatedly, and emphatically refuted, the correlation lives on in the minds of many parents and is pervasive on the Internet. After 1998, the number of children who were fully immunized against these diseases dropped significantly, and the number of cases of these preventable diseases increased dramatically. Parents who wanted to protect their children from harm put them at risk by avoiding a vaccine that had been falsely reported as hazardous.

Sowing seeds of doubt is a method used effectively by those who oppose GM plants. In 2012, an article published by Séralini and others was published with an accompanying flurry of media attention. The authors claimed that their study showed an increase in mortality amongst rats fed GM corn and illustrated their report with photos of rats suffering from massive tumors. Immediate responses included a call for the ban of GM food in Kenya, the destruction of a shipment of GM soybeans in France, an inquiry into the article by the European Food Safety Authority, and more than a dozen letters to the editor of the publishing journal expressing concerns about the scientific standards of the article. Specific concerns included sample sizes too small to be statistically significant, bias in the choice of data presented, and an inappropriate test organism for the study’s objectives. Compounding the scientific concerns, the handling of the press release itself was unusual, in that the article was released ahead of time only to reporters who signed a non-disclosure agreement that prevented them from consulting other experts about the research.

A closer look at the Séralini affair

We urge students to look at of the data presented in the Séralini article and the subsequent concerns published in the same journal, but here we provide a brief summary of some of the major concerns about its veracity.

The article's title is "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize". To investigate toxicity, groups of ten male and ten female rats were fed one of ten different regimes. The control rats had 33% of their diet derived from non-GM corn, some rats had 11%, 22% or 33% of their diets derived from GM (herbicide resistant) corn, some rats had 11%, 22% or 33% of their diets derived from GM corn that had been sprayed with Roundup herbicide in the field, and some had their diet supplemented with 33% non-GM corn, and had Roundup added to their water at 0.1 part per billion (ppb), 9×10^5 ppb, or 5×10^6 ppb (the maximum tolerance for Roundup in corn is 1×10^3 ppb). Survival of the rats over time was plotted, along with survival of each group at 600 days (male) or 680 days (female). Although the authors interpreted their results as evidence for increased mortality in rats fed the GM corn and / or Roundup, statistical analyses do not support these interpretations (see for example Ollivier, 2013). For example, the three groups in which 9/10 male rats survived to 600 days were the group fed 22% or 33% GM corn, and the group with 5×10^6 ppb Roundup in their water; more rats survived in these groups than in the control group. The variation between these groups is comparable to the variation in number of heads that would result from ten coin tosses. It is not appropriate to draw conclusions from small, unreplicated samples, just as it would not be valid to try to correlate the number of heads in ten coin tosses with anything else.

One of the most sensationalist aspects of this article was the publication of photographs of rats disfigured by very large tumors, which were reproduced widely in the popular press. Clearly, these images were intended to sway public opinion and introduce an indelible connection between GMOs and massive tumors. However, the numerical data presented revealed that rats in the control groups also developed tumors; photographs of these individuals were not published. As described in greater detail by Sanders et al. (2013) and Barale-Thomas (2013), this variety of rats is useful for short-terms toxicology studies because it is prone to tumors, but, for this same reason, not appropriate for long-term studies (unless your goal is propaganda).

Scientists regularly announce the publication of an article with a press release. Often, these press releases include a copy of the to-be-published article, with an "embargo", a restriction that the content cannot be published in the press prior to the publication date of the article. The embargo period allows journalists time to write their own articles about the new science. In the best case, the journalist uses this time to solicit comments from other experts. The Séralini article was unusual in that the journalists could only obtain a copy of the paper if they signed a non-disclosure agreement, meaning that they could not solicit unbiased comments. This tactic ensured that most of the initial publicity was uncritical; essentially, it resulted in an alarmist media blitz telling the public that GMOs cause cancer.

The debate about the use of GM technology is emotionally charged, and there are many forces that inflame it. It is widely believed that GM can be one of many tools employed to ensure a safe and adequate food supply, particularly in the light of global change and population increases. Public mistrust is one of the greatest obstacles to the realization of the potential of GM methods. Most scientists support the continuing assessment of all new crops, including those developed by GM tools, and support informed public debate about their use. There is no room in these discussions for the kinds of people described by Norman Borlaug as "politically opportunistic pseudoscientists".

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RECOMMENDED READING AND RESOURCES

(This is a representative list of sources intended to help the reader access a huge body of primary literature and governmental reports. We apologize in advance to those whose work is not included. All websites were accessed in September, 2013).

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Breeding for Drought Tolerance

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