PLANT GENETICS, ECOLOGICALLY BASED FARMING AND THE FUTURE OF FOOD

PAMELA C. RONALD

> Many worthy people objected to the production of hybrids on the ground that it was an impious interference with the laws of Nature.—Maxwell Masters, 1899

For 10,000 years, we have altered the genetic makeup of our crops. Conventional approaches are often quite crude, resulting in new varieties through a combination of trial and error, and without knowledge of the precise function of the genes that are being transferred. Such methods include grafting or forced pollinations between different species, as well as radiation or chemical treatments that induce random mutations in the seed. Today, virtually everything we eat is produced from seeds that have been genetically altered in one way or another.

Here I provide examples of crops derived from three modern genetic approaches to plant breeding: genetic engineering, which allows the introduction of genes from one species into another; marker-assisted breeding, which facilitates precision breeding using molecular techniques; and genome editing, which allows for targeted insertions, deletions, or replacement of DNA sequences. Over the last twenty years, scientists and breeders have used these approaches to create crop varieties that thrive in extreme environments or can withstand attacks by pests and disease.

Although seed is just one component of a sustainable agricultural system, it is an important one. The seed carries the traits that farmers and consumers value: flavor, nutrition, tolerance to pests, diseases, environmental stress, and the like). Because planting a new seed variety does not require extra maintenance or additional farming skills, it is scale-neutral technology. This means that farmers of both small and large acreage, including farmers in developing countries, can benefit if the trait is appropriate to their particular geography and farming challenges. In the developed world, most farmers, including organic farmers, buy their seed from for-profit seed companies. In less developed countries, the seed is typically developed and distributed by nonprofit institutions.

To advance the discussions around sustainable agriculture, in this essay, I use specific terminology to describe three modern genetic approaches. I avoid using the term "GMO" (genetically modified organism) because generalizations about "GMOs" are not informative and the term is scientifically meaningless.

Dr Pamela C. Ronald is a Distinguished Professor, Plant Pathology and the Genome Center Director, The UC Davis Institute for Food and Agricultural Literacy University of California, Davis 95616; [pcronald@ucdavis.edu].

Geographical Review 1–8
DOI: 10.1111/gere.12256

Each crop is distinct, each trait is different, each geographic region has unique attributes, and most foods do not contain entire organism. Because there is an abundance of confusion regarding the meaning of the acronym, the FDA does not employ this term. The term "non-GMO" is also poorly defined. Emerging evidenced indicates that food labeled "non-GMO" is more expensive and grown with older, often more toxic, pesticides (a general term that encompasses both herbicides that kill weeds and insecticides that kill insects).

Genetic Engineering

The process of genetic engineering has been used for more than forty years to create life saving drugs (for example, insulin), enzymes for cheeses (approximately 90 percent of U.S. cheeses are made with genetically engineered enzymes), and crops resistant to disease. After decades of careful study and rigorous peer review by thousands of independent scientists, every major scientific organization in the world has concluded that the genetically engineered crops currently on the market are safe to eat and that the process of genetic engineering is no more risky than older methods of genetic alteration (National Academies of Sciences, Engineering, and Medicine 2016). These are precisely the same organizations that most of us trust when it comes to other important scientific issues such as global climate change and the safety of vaccines.

VIRUS-RESISTANT PAPAYA

An important example of the application of genetic engineering is the development of papaya that is resistant to viral infection. In the 1950s, papaya production on the Hawaiian island of Oahu was decimated by papaya ringspot virus. By 1995 the disease was widespread, creating a crisis for Hawaiian papaya farmers.

In 1978, Dennis Gonsalves, a local Hawaiian, initiated research to develop strategies to control the disease. Funded by a grant from the USDA, Gonsalves and his coworkers spliced a small snippet of DNA from a mild strain of the virus into the papaya genome. Conceptually similar (but mechanistically different) to human vaccinations against polio or smallpox, this treatment immunized the papaya plant against infection. The immunized plants yield twenty times more than the conventional varieties and were distributed freely to local growers. The story of Hawaiian papayas is an example where genetic engineering was the most appropriate technology to address a specific agricultural problem. In the 1990s, there was no other technology or farming practice available to protect the papaya from this devastating disease, nor is there today. The genetically engineered papaya carries trace amounts of the viral protein. Organic or conventional papayas infected with the virus carry tenfold more viral protein.

INSECT-RESISTANT EGGPLANT, MAIZE, AND COTTON

Genetic engineering has also been used to introduce the Bacillus thuringiensis "Bt" gene into crops such as eggplant, maize, and cotton. The Bt insecticide is highly specific to caterpillar pests, but is nontoxic to birds, fish, and humans. It is less toxic than table salt. For these reasons, Bt is a popular insecticide in the organic industry. Organic farmers apply it in a spray formulation. However, in some countries and for some crops, the sprays are expensive, hard to find, and don't prevent the insect from getting inside the plant.

For example, in Bangladesh, the fruit- and shoot-borer caterpillar can destroy a farmer's entire eggplant crop if it is not controlled. For this reason, farmers spray chemical insecticides several times a week. But many of these chemicals are harmful to human health, especially when farmers and their families don't have access to proper safety gear. To reduce chemical sprays on eggplant, scientists at the Bangladesh Agricultural Research Institute and Cornell University applied a genetic approach that builds on the organic farming approach.

Scientists cut the gene for Bt out of the bacteria and, using genetic engineering, inserted the gene directly into the eggplant genome. In 2015, Bangladesh eggplant farmers reported that they were able to reduce their chemical sprays by a huge amount—often down to zero. They could also save their seeds and replant them next year.

Maize farmers in the United States have also benefited from the Bt trait to control the European maize borer. The USDA reports a tenfold reduction in chemical insecticide sprays over the last fifteen years due to planting of Bt corn (Fernanadez-Cornejo and others 2014). Because the National Organic Program standards prohibit the planting of genetically engineered crops, organic farmers do not directly benefit financially from planting BT corn. However, organic farmers benefit from reduced application of chemical insecticides by their neighbors, which results in less chemical drift onto the organic farms. Organic farmers also benefit economically, because when the insects deposit eggs in neighboring Bt cornfields, the larvae die (Hutchison and others 2010). Thus, Bt cornfields become an effective "dead end" trap crop for the European maize borer resulting in fewer European maize-borer infestations in the region. Cumulative benefits for Bt maize growers in Illinois, Minnesota, and Wisconsin over fourteen years were estimated at \$3.2 billion. More than \$2.4 billion of this total accrued to non-Bt maize growers (Hutchison and others 2010).

Cotton farmers around the world have also benefited from the Bt trait. A team of Chinese and French scientists reported in the journal *Nature* that widespread planting of Bt cotton in China drastically reduced the spraying of harmful chemicals, increased the abundance of beneficial organisms on farms, and decreased populations of crop-damaging insects (Lu and others 2012). Planting of Bt cotton also reduced insecticide poisonings of farmers and their families (Pray and others 2002). In Arizona, farmers who plant Bt cotton spray half as much insecticide as do neighbors growing conventional cotton. The Bt farms also have greater biodiversity. In India, farmers growing Bt cotton increased their yields by 24 percent, their profits by 50 percent, and raised their living standards by 18 percent, according to one common standard that measures household expenditures (Kathage and Qaim 2012).

INSECT-RESISTANT CROPS AND INTEGRATED PEST MANAGEMENT

It is important to note that seed alone will not solve all pest problems; farming practices are also important. One drawback of using any insecticide, whether it is organic, synthetic or genetically engineered is that pests can evolve resistance to it. For example, one crop pest, the diamondback moth (*Plutella xylostella*), a global pest of vegetables, evolved resistance to Bt in response to repeated sprays of Bt in fields of conventional (nongenetically engineered) vegetable crops.

Based on this case, laboratory studies, and computer modeling, the U.S. Environmental Protection Agency (EPA) mandated an integrated pest management strategy to reduce the evolution of pests on Bt crops. Farmers were required to plant part of their maize crop as non-Bt corn. This “refuge strategy”, creating refuges of crop plants that do not make Bt toxins, is an important element of long-term insect resistance management because it promotes survival of susceptible insects.

HERBICIDE-TOLERANT CROPS

Genetic engineering has also been used to introduce herbicide tolerance into crops, an application that remains controversial because the crops are used in conjunction with chemical herbicides such as glyphosate. Glyphosate blocks the chloroplast enzyme EPSPS (5-enolpyruvoyl-shikimate-3-phosphate synthetase) that is required for plant growth. When sprayed on leaves, these herbicides kill the entire plant in two weeks. Farmers and home gardeners have used glyphosate-based herbicides, such as Roundup, since the 1970s to control weeds.

Planting of herbicide-tolerant crops is correlated with an increase of low-till and no-till agriculture, which leaves the fertile topsoil intact and protects it from being removed by wind or rain (National Academies of Sciences, Engineering, and Medicine 2016). Because tractor tilling is minimized, less fuel is consumed and greenhouse gas emissions are reduced.

The popularity of herbicide-tolerant crops and glyphosate has spurred the evolution of herbicide-resistant weeds. These studies highlight the fact that application of glyphosate (or other herbicides) should not be relied on solely to the exclusion of other weed control measures. Rather than applying a single herbicide repetitively over large areas, agronomists and weed-control specialists advocate an integrated pest-management strategy to mitigate rate of development of resistance to a single herbicide.

The case of herbicide-tolerant crops and weed control highlights the confusion with the term “GMOs.” Even before the advent of genetic engineering, most farmers were using some type of chemical herbicides—except organic farmers, who use alternative approaches such as tilling, which has other environmental costs. Furthermore, conventional breeding strategies have also been used to generate herbicide-tolerant crops that are paired with older and more toxic herbicides (the so-called “non-GMO” approach).

BIOFORTIFIED RICE

According to the World Health Organization, Vitamin A deficiency (VAD) is the main cause of preventable blindness in children. To address this serious vitamin deficiency, the World Health Organization has supported distribution of Vitamin A pills and gardening programs to promote growing of nutrient rich vegetables (Paine and others 2005). Despite these efforts over more than fifty years, an estimated 250 million preschool children remain vitamin A deficient and an estimated 250,000 to 500,000 vitamin A-deficient children become blind every year, half of them dying within twelve months of losing their sight. As a complementary approach to supplementation programs, which often do not reach the rural poor, the nonprofit Rockefeller Foundations supported the development of rice varieties enriched with beta carotene—the nutrient found in carrots and other foods that the human body converts to vitamin A—through genetic engineering, known as golden rice. Models suggest that widespread consumption of golden rice would save thousands of lives. The positive effects of golden rice are predicted to be most pronounced in the lowest income groups, and at a fraction of the cost of current supplementation programs golden rice is expected to be released in 2018 in Bangladesh, where one in every five preschool children is vitamin A-deficient. Because this is a publicly funded project, there are no restrictions on seed use. Farmers can share and replant the seeds (Stein and others 2006).

Marker-Assisted Breeding

Modern genetic analysis also facilitates a process called marker-assisted breeding. In conventional breeding, large populations of plants, usually over multiple generations (seven to ten years), are screened for the desired trait. This is a labor-intense and time-consuming process. In marker-assisted breeding, DNA of the individuals of a population are examined to identified the desired genetic compositions. Only lines with desired combinations are followed. This saves tremendous amount of labor and can speed up the pace of breeding. Researchers do not need to grow a plant to maturity to find out what characteristics the plant will express. A quick look at the genome of the seed will be informative.

An exciting example of how marker-assisted breeding has been applied is the story of Submergence tolerance 1 (*Sub1*) rice. Rice grows well in standing water, but most varieties will die if they're submerged for more than three days. In South and Southeast Asia, where many farmers and their families live on less than US$2 a day, 4 million tons of rice—enough to feed 30 million people—is lost every year to flooding. The International Panel on Climate Change predicts that flooding will increase as the climate changes.

My colleagues and I used a combination of sequencing, genetic engineering, and marker-assisted breeding to develop rice varieties that are tolerant of 18 days of flooding (Xu and others 2006). At the University of California, Davis, we isolated the *Sub1* gene and demonstrated its function through genetic engineering. Researchers at the International Rice Research Institute used marker-assisted breeding to introduce *Sub1* into rice varieties that are adapted to farms in South and Southeast Asia. The *Sub1* plants can withstand fourteen days of submergence, and they yield and taste the same as the older conventional variety (Xu and others 2006). In each of the six years from 2008 to 2015, farmers in Bangladesh and India were able to harvest three to fivefold more grain from the *Sub1* varieties as compared to the conventional varieties under flooded conditions. This is important as floods are predicted to become more frequent as the climate changes.

In 2015, 4.9 million farmers grew *Sub1* rice, setting a record for the most rapidly adopted rice variety in the history of modern rice farming. The *Sub1* varieties, distributed through government breeding stations, with support from the Bill and Melinda Gates Foundation, have enhanced the productivity of poor farm families in India, benefiting disadvantaged farmers that historically cultivated plots that are flood prone (Dar and others 2013).

Genome Editing

In the last few years, genome editing has emerged as another important tool for plant breeders. This approach can be used to create mutations in specific genes, to delete genes, and to insert genes. It also can be used to tune the activity of genes over a 1,000-fold range. According to Dr. Jennifer Doudna, professor of chemistry and of molecular and cell biology at the University of California, Berkeley, and one of the scientists who first showed the application of this system, genome editing can be used "in much the way that you would use your word processing program to change a typo in a document" (Doudna 2016).

Genome editing is based on a DNA targeting and editing system discovered in bacteria. To create a mutation, scientists synthesize a single-stranded molecule—called a guide RNA—that will target specific regions of the double-stranded DNA. When the guide RNA binds to the target region on the DNA, it recruits a bacterial enzyme to cut the DNA to generate a break. Organisms have evolved cellular mechanisms to repair the damaged DNA by connecting the two broken ends. During the repair process, errors often occur, introducing mutations near the original break point. Scientists make use of the endogenous error-prone repair mechanism in the cells to introduce mutations.

After the mutations are created, the guide RNA and bacterial DNA-cutting enzyme can be removed from the plant, so that the only modification left is the targeted mutation. The engineered organisms contain no foreign DNA. This is a major difference compared with genetic engineering. But it is also a limitation. For example, as of today, genome-editing technology cannot be used to engineer papaya for resistance to viral infection or eggplant for resistance to caterpillars. Thus, although powerful and exciting, genome editing does not replace other genetic approaches such as genetic engineering.

The genome-editing technique has already been used to generate a mushroom with reduced browning, maize plants with enhanced starch content, and dairy cows without horns. Regulation of crops developed through genome-editing system is not yet fixed. The U.S. Department of Agriculture has proposed that crops modified by genome editing be exempt from regulation if similar mutations could also be achieved through chemical or radiation mutagenesis. Some organic farmers have called for National Organic Program Standards to permit the use of crops developed through genome editing in organic production. For example, Urs Niggli, the director of the Research Institute of Organic Agriculture, stated, “It would be unfortunate, if the conventional farmer could use a potato variety which does not need pesticides and organic farmer must continue to use a potato variety, which he must spray with copper” (Maurin 2016).

Conclusion

The hybridization described by Masters in 1899, the genetic engineering of crops launched in 1996 and the genome editing of tomorrow are examples of a continuum of new technologies aimed at helping farmers produce food in a productive and ecologically-based manner. I believe it is important to frame discussions about agriculture in the context of the environmental, economic, and social impacts of agriculture—the three pillars of sustainable agriculture. Rather than focusing on how a seed variety was developed, we must ask what most enhances local food security and can provide safe, abundant and nutritious food to consumers. We must ask if rural communities can thrive and if farmers can make a profit. We must be sure that consumers can afford the food. And finally we must minimize environmental degradation. This includes conserving land and water, enhancing farm biodiversity and soil fertility, reducing erosion and minimizing harmful inputs.

References

Dar, M. H., A. de Janvry, K. Emerick, D. Raitzer, and E. Sadoulel. 2013. Flood-Tolerant Rice Reduces Yield Variability and Raises Expected Yield, Differentially Benefitting Socially Disadvantaged Groups. *Scientific Reports* 3 (3315).

Doudna, J. 2016. Consensus and Controversy in Science: Genes, GMOs and Climate. *The Morton L. Mandel Public Lecture*. Berkeley, Calif.: American Academy of Arts and Sciences.

Fernanadez-Cornejo, J., S. Wechsler, M. Livingston, and L. Mitchell. 2014. *Genetically Engineered Crops in the United States, ERR-162*. Washington, D.C.: U.S. Department of Agriculture.

Hutchison, W. D., E. C. Burkness, P. D. Mitchell, R. D. Moon, T. W. Leslie, S. J. Fleischer, M. Abrahamson, K. L. Hamilton, K. L. Steffey, M. E. Gray, R. L. Hellmich, L. V. Kaster, T. E. Hunt, R. J. Wright, K. Pecinovsky, T. L. Rabaey, B. R. Flood, and E. S. Raun. 2010. Areawide Suppression of European Maize Borer with Bt Maize Reaps Savings to Non-Bt Maize Growers. *Science* 330 (6001): 222–225.

Kathage, J., and M. Qaim. 2012. Economic Impacts and Impact Dynamics of Bt (Bacillus thuringiensis) Cotton in India. Proceedings of the National Academy of Sciences 109 (29): 11652–11656.

Lu, Y., K. Wu, Y. Jiang, Y. Guo, and N. Desneux. 2012. Widespread Adoption of Bt Cotton and Insecticide Decrease Promotes Biocontrol Services. *Nature* 487: 362–365.

Maurin, J. 2016. Eco Researchers Using New Genetic Engineering Method, in Taz.de. Germany. [http://www.ask-force.org/web/Organotransgenic/Maurin-Niglli-CRISPR-Great-Potential-TAZ-20160406.pdf].

National Academies of Sciences, Engineering, and Medicine. 2016. *Genetically Engineered Crops: Experiences and Prospects*. Washington, D.C.: The National Academies Press.

Paine, J. A., C. A. Shipton, S. Chaggar, R. M. Howells, M. J. Kennedy, G. Vernon, S. Y. Wright, E. Hinchliffe, J. L. Adams, A. L. Silverstone, and R. Drake. 2005. Improving the Nutritional Value of Golden Rice Through Increased Pro-Vitamin A Content. *Nature Biotechnology* 23: 482–487.

Pray, C. E., J. Huang, R. Hu, and S. Rozelle. 2002. Five Years of Bt Cotton in China—The Benefits Continue. *The Plant Journal* 31: 423–430.

Stein, A. J., H. P. S. Sachdev, and M. Qaim. 2006. Potential Impact and Cost-Effectiveness of Golden Rice. *Nature Biotechnology* 24: 1200–1201.

Xu, K., X. Xu, T. Fukao, P. Canlas, R. Maghirang-Rodriguez, S. Heuer, A. M. Ismail, J. Bailey-Serres, P. C. Ronald, and D. J. Mackill. 2006. Sub1A Is an Ethylene-Response-Factor-Like Gene that Confers Submergence Tolerance to Rice. *Nature* 442 (7103): 705–708.