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BRIEF 56

Breaking Barriers with Breeding: *A Primer on New Breeding Innovations for Food Security*

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PREFACE

Advancements in science and technology are enabling humanity to survive the challenges of the times: hunger, malnutrition, climate change, and dwindling natural resources on top of the COVID-19 pandemic.

One of the most game changing innovations in food and agriculture is the advent of genome (gene-) editing. Genome editing is a new breeding technique that allows scientists to improve the characteristics of living organisms, including plants, animals, and microbes. The technologies used for genome editing work like molecular scissors, cutting the DNA in a specific location, then remove, add, or replace known DNA sequences where the cut was made. The most used technologies in genome editing are clustered regularly interspaced short palindromic repeats – CRISPR-associated protein 9 (CRISPR-Cas9), transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and homing endonucleases or meganucleases.

The overwhelming interest and the present information gap in genome editing motivated ISAAA to monitor the advances in genome editing and their implications and contribution in food and agriculture towards greater food security. Articles based on peer-reviewed journals are published every week in the Crop Biotech Update since August 2016. Regulatory updates and other relevant news about genome editing obtained from credible sources are also included. Since July 2020, ISAAA featured the Genome Editing Resource page (https://www.isaaa.org/resources/genomeediting/default.asp) at the ISAAA website, and has since then attracted more than 6,000 unique pageviews. Interest in genome editing in crops, livestock, aquaculture, and health was also evident in the large number of attendees during the ISAAA webinar series on genome editing with an estimated reach of 18,000 from 70 countries in almost two years.

This inspired ISAAA to develop and publish this primer on new breeding innovations for food security. Since ISAAA has been regarded as a credible source of publications on biotechnology for more than two decades, this Primer is expected to raise public awareness and appreciation of new breeding innovations, its products, regulation, prospects, and contribution to food security.

We gratefully acknowledge the following international experts for their dedication to write sections on their fields of expertise in relation to new breeding innovations: Dr. Diana Horvath for crops (The 2Blades Foundation), Dr. Diane Wray-Cahen and Dr. Justin Bredlau for livestock (USDA Washington), Prof. Martin Lema on science-based policy considerations/regulation (University of Quilmes, Argentina), Dr. Gabriel O. Romero for the Asian prospects (Philippine Seed Industry Council), Dr. Margaret Karembu and Godfrey Ngure Mutero for prospects in Africa (ISAAA *Afri*Center), Dr. Mahaletchumy Arujanan for communication (ISAAA and Malaysian Biotechnology Information Center), and Dr. Paul S. Teng for contribution to food security (Chair, ISAAA Board of Trustees).

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Finally, the ISAAA technical and editorial team including Panfilo G. De Guzman, Kristine Grace N. Tome, Clement Dionglay, and Zabrina J. Bugnosen appreciate working with the esteemed experts and the financial donors. We do hope that all our efforts in preparing and publishing this primer will contribute to the global effort of raising the understanding and acceptance of genome editing and its products.

Rhodora O. Romero-Aldemita
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"How will we produce enough food for the expected 2 billion additional people who will join us by 2050? And how can we do this as climate change multiplies both biotic and abiotic threats to our food crops?

From Molecule to Market: Using the Innovative TALENs Plant Breeding Tool to Help Build Global Food Security

By DIANA HORVATH, PhD

Introduction

For vegetables and grains to reach your dinner table, they face a gauntlet of challenges before they even leave their fields. Our food crops are constantly exposed to stresses that affect their growth and productivity—both biotic (living things, like fungi or insects) and abiotic (changing temperature or moisture).

Millions of years of evolution have endowed plants with comprehensive defenses against these threats. For over a century, breeders have used their knowledge of plant genetics to selectively breed food crops with resistance to disease in order to make plants even more resilient. Better genetics, led by scientists, have been a key part of producing enough food to keep pace with the world's population growth, from 1 billion people in the year 1800 to nearly 8 billion today.

Now the human species faces one of its most daunting challenges: how will we produce enough food for the expected 2 billion additional people who will join us by 2050? And how can we do this as climate change multiplies both biotic and abiotic threats to our food crops (Lawal, 2021)?

Rising temperatures expand the range of many disease-causing (pathogenic) fungi, as well as bacteria, oomycetes, and viruses (Bebber et al., 2013). And even though we can breed plants with new defenses, the more rapid life cycles of pathogens means they breed and evolve faster.

Nature has been brutally efficient in evolving threats that attack our food supply, evidenced by over a dozen plant disease pandemics in the past 100 years in our top food crops—wheat, corn, rice, and potatoes—and other important staples, fruits, and vegetables (Ristaino et al., 2020). These attacks and associated food losses imperil the health and nutrition of millions, and cost farmers and consumers billions of dollars each year (FAO, 2019).

Molecular tools offer hope for crop breeding challenges

Scientists are helping farmers to meet these threats with new breeding technologies that provide robust defenses to plant pathogens. Thus, scientists and their ideas are the seeds for success in our quest for global food security (2Blades Foundation, 2021).

Because of climate change and rapid population growth, we need tools to accelerate breeding to grow the food we need, since conventional breeding methods can take decades to develop new varieties that are resistant to plant threats. We must use effective and safe methods to speed up the work and progress toward food security.

Scientists are continuously developing better tools to improve plant breeding. New precision breeding innovations include both new digital tools—devices like sensors, detectors, and robotics—that have been combined with management technologies for precise and more efficient production system control, and genetic tools like new molecular breeding techniques (ISAAA, 2021a) for gene editing, such as CRISPR or TALENs (ISAAA, 2021b).

The development of genome-editing techniques has progressed over the past 20 years, gradually at first and then more rapidly, leading to a Nobel

Prize in 2020 for the CRISPR system (The Nobel Prize, 2020). Implementation of these tools for agriculture has revolutionized efforts to improve crops, allowing scientists to remove or otherwise alter a cell's DNA sequence to modify the function of its individual genes.

The process involves making targeted single- or double-stranded breaks in the DNA at a place of the researcher's choosing, and then letting the cells' natural mechanisms repair the break, making alterations precisely in a target gene to alter specific desired (or undesired) traits. It is a directed and accelerated means of generating sequence diversity. Natural processes also cause sequence variation in nature, and that variation is fundamental for evolution and life.

Such changes are typically small, on the scale of a few base pairs, but precision breeding tools can also allow scientists to make targeted deletions (knock outs) or insertions of new and useful genes ("trans" genes – originating elsewhere) making crops transgenic (a type of genetically modified organism, or GMO), another essential tool in the toolbox for global food security. Transgenic approaches have been uniquely effective at achieving the goal of long-lasting resistance to crop disease, and the crops are widely recognized as safe (National Academies of Science, Engineering, and Medicines, 2016).

Targeted genome modifications in plants can optimize any important trait, including yield, nutritional characteristics, quality (protein or oil types, for example), as well as to improve our crops' tolerance to environmental conditions such as drought or salinity.

What are TALENs?

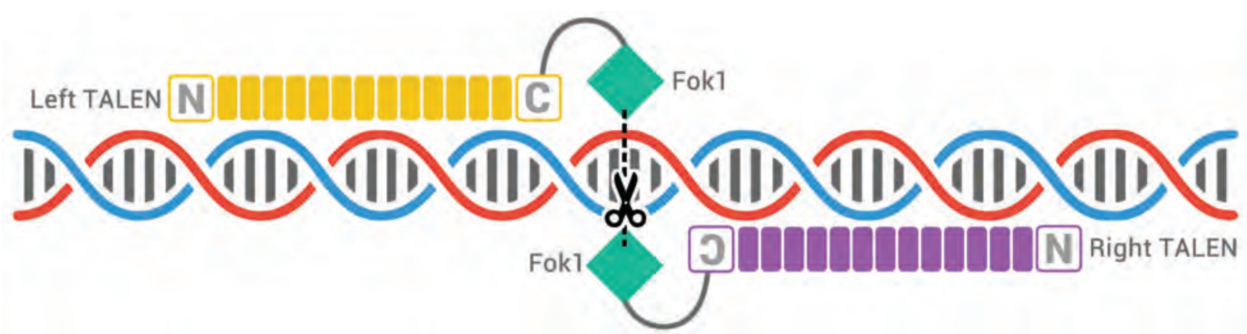
While the best-known gene editing technology is CRISPR-Cas9, the 2Blades Foundation was instrumental in helping to develop an earlier and effective gene-editing tool called TALENs, which has practical applications in plant science and other biosciences. It has been used to improve traits in rice (Li et al., 2012), wheat (Wang et al., 2014), and other crops.

TALENs were developed after researchers at Martin Luther University in Germany made a remarkable discovery in 2007 (Kay, 2007) while studying bacterial spot disease, which attacks pepper and tomato. Plant pathogenic bacteria insert bacterial proteins—TALEs (Transcription Activator-Like Effectors)— into their plant hosts

What are TALENs?

TALENs are tools for precise gene editing made up of protein combinations composed of two parts:

- **a TAL Effector (TALE) DNA binding domain that targets the protein to a specific DNA sequence**
- **a nuclease (N) that cuts DNA. Fok1 is a nuclease that is commonly used in TALENs.**



where they alter the expression of plant genes. By doing so, the bacteria essentially trick their plant host into making conditions more favorable for the bacteria to establish themselves and spread throughout the plant.

In studying the TALEs, the scientists found that these bacterial proteins have a novel repeating structure that binds to specific DNA sequences with exquisite precision, and, most amazingly, the repeats use a simple cipher or "code" to interact with each DNA base.

Not only could researchers identify the genes the bacteria were targeting for manipulation in pepper, rice, citrus, or other crops, but now scientists could create designer TALEs to target any DNA sequence of their choice. This new ease of design and ability to contact any DNA sequence was a huge step forward for precise manipulations of genomes.

In 2009, the discovery of the TALEs and their DNA interaction code was first published in *Science*, a leading peer-reviewed science journal, by Ulla Bonas, Jens Boch, Thomas Lahaye, and Sebastian Schornack, who were then researchers at the Martin Luther University in Germany.

Appreciating the potential of this technology to aid plant science, 2Blades helped the scientists to protect and commercialize uses of their discovery by taking on the patent protection and licensing for research and commercial applications.

The new knowledge of the bacterial protein code allowed researchers to design proteins on demand that hone in on virtually any DNA sequence with high efficiency and accuracy. Scientists soon showed they could direct TALE proteins to not only turn on desired genes, but also to turn off and edit genes with great specificity by fusing TALE DNA-binding domains with other protein functions such as repressors and nucleases.

Nucleases are proteins that cut DNA and, when harnessed for gene-editing, cause the small insertions, deletions, or whole gene insertions that make these "molecular scissors" an incredibly precise means to fine-tune traits in a targeted, predictable fashion anywhere in the genome.

Using the TAL interaction code, researchers could make custom gene editors based on TALEs, combining the highly precise (TALE) DNA binding domain with a nuclease (N) that cuts DNA to create a "TALEN". Fok1 is a nuclease that is commonly used in TALENs.

For crops, TALENs can effect small targeted changes in plants (in a manner that is distinct from genetic engineering tools developed in the 1980s to add genes), and which is largely identical to widely accepted methods of mutagenesis and natural sequence variation (ISAAA, 2021a).

The demonstration that TALENs can be used to select and modify specific DNA sequences was a major breakthrough, greatly simplifying the design and increasing the accuracy of gene editing, thereby reducing the costs and time to develop new crop varieties. The result is that growers can now produce crops with improved traits such as resistance to disease, which is 2Blades' central mission.

Accordingly, the TAL Code was recognized by the Agrow Awards as a Best Novel Agricultural Biotechnology in 2012 and a finalist as a Best New Crop Production Product or Trait in 2017 (2Blades Foundation, 2012a). In addition, TALENs were recognized as Method of the Year in 2011 by the journal *Nature Methods* (*Nature Methods*, 2011).

Comparison of CRISPR and TALENs

CRISPR has become the most popular and well known genome-editing tool because of its simple design and ease of use. Yet, TALENs are extremely precise and have capabilities beyond CRISPR.

They can: target any DNA sequence with fewer errors; discriminate between DNA modifications like methylation that affect a gene's expression; and modify DNA in mitochondria and chloroplasts (organelles that contribute to cell function).

Both TALENs and CRISPR are highly specific, produce few off-target editing events (thus preventing undesired DNA mutations), and can be fused to other functional protein modules for versatility in gene manipulation.

How TALENs aid crop breeding

Precision in gene editing is key to achieving desired crop characteristics. To mitigate biotic threats, TALENs can be used to edit genes to make plants less susceptible to pathogens, confer new specificity to existing resistance genes to match evolving pathogens, and to directly target and alter pathogen DNA. Additionally, researchers can use TALENs to introduce novel resistance genes and to create "multi-gene stacks" that combine multiple genes at a single location in the genome, ensuring that they don't get separated and lost during further breeding for other traits (Luo et al., 2021). This is a critical feature needed for creating long-lasting resistance to disease.

By comparison, today's most successful high-yielding crops were bred by conventional means over centuries to achieve their current selected combinations of genes, yet this method has limitations. Conventional breeding—crossing two varieties—mixes every gene in the plant's genome, including both helpful characteristics (greater yield, disease resistance) and also undesirable characteristics (poor flavor or quality, shorter shelf life, or even toxic compounds).

For example, crop breeders could try to modify a modern corn variety by adding a gene from a wild corn relative that confers resistance to a destructive fungus (Mammadov et al., 2018). Yet, if breeders use conventional means to cross the two species then the new variety will acquire not just the desired trait (fungal resistance) but also other undesired traits (such as long lateral branches) (Hufford and Doebley, n.d.).

Researchers can avoid adding undesired traits by using technologies like TALENs to insert only the crop relative's disease-resistant gene directly into the modern corn variety. This saves many seasons of tedious work crossing and back-crossing the varieties, and can result in new crop varieties which have only the desired set of characteristics.

TALENs can be used to extend the diversity of traits and allows breeders to selectively change the qualities they want, such as: improved yields;

Crops and traits modified using TALENs

Oil quality; reduced polyunsaturated fats | **Bacterial blight resistance, aroma** | **Reduced acrylamide, cholesterols** | **Visible gene marker** | **Powdery mildew resistance**

better taste; resilience to climate change, drought, or salinity; resistance to disease and pests; and improved quality of products derived from crops.

Specific examples of TALEN-derived improvements include: high-oleic soybean oil that is low in polyunsaturated fats; rice that is resistant to bacterial blight; potatoes with less browning, bitter taste, or potentially carcinogenic acrylamides; and wheat that is resistant to powdery mildew (ISAAA, 2021a).

TALENs can help us make our crops more resilient to climate change effects, such as the expansion of host ranges for pests and diseases that attack our crops, more frequent droughts, or increased salinity. TALENs have also been used in industrial applications, such as engineering biofuels from sugarcane and algae to help reduce dependence on fossil fuels.

How to gain access to the TALEN technology

The TALEN technology segment reached approximately $650 million in 2019. With increasing government support for genome editing in the United Kingdom and other nations, market growth is projected to exceed $10 billion by 2026 (Market Watch, 2021).

Despite the popularity of CRISPR, simple IP and licensing remain a distinguishing advantage of TALENs. Importantly, TALENs offer a simpler path to commercialization—without intellectual property disputes that have burdened CRISPR in recent years, including lawsuits, patent disputes, and even patent cancellations (Cohen, 2017; Hiltzik, 2019; Collins, 2020). 2Blades has always been committed to scaled, unencumbered access to TALEN and TAL Code rights for broad use.

The 2Blades Foundation holds exclusive global rights for uses of the TAL Code and TALENs in plants (2Blades Foundation, 2020), including for commercial uses of the technology in plants (Businesswire, 2014). 2Blades has worked extensively to create simple, broad access to this versatile platform to improve the efficiency and precision of plant breeding.

Distinct from strategies for other gene editing tools like meganucleases, zinc-finger nucleases, and CRISPR, 2Blades' strategy for broad licensing has helped to change the way technology licensing is carried out and reflects its mission to help achieve global food security. A portion of the TALENs rights is held by Calyxt, and 2Blades has made cross-licenses and sought joint licensing to make the technology accessible.

Together, the Martin-Luther scientists and 2Blades sought to achieve the greatest uptake of the technology applications for biomedical and research reagent uses by partnering with a large life sciences company. The rights for these applications were exclusively licensed to (ThermoFisher (previously Life Technologies), and 2Blades undertook licensing uses of the TAL Code in agriculture.

Consistent with its two-pronged mission, 2Blades had the goal of making this powerful technology an industry standard and so facilitated broad use of the TAL Code technology, licensing it on a tiered, non-exclusive basis to a wide range of users from large commercial seed companies (e.g., Bayer, Syngenta) to small biotech companies (e.g., Simplot, Epicrop) to enable users to benefit from the new efficiency and precision in the crop improvement process (2Blades Foundation, 2016, 2012b, 2012c, and 2018).

The 2Blades Foundation's commercial license agreements for TALENs give 2Blades access to any improvements to the technology that can be used for the benefit of smallholder farmers.

The 2Blades Foundation also gives no-cost TALEN rights to non-profit and multilateral entities, such as the International Rice Research Institute, the world's premier rice research organization, based in the Philippines and with offices across Asia and Africa (IRRI, 2016). IRRI is using TALENs to facilitate the improvement of rice varieties and support innovation to benefit food security.

To achieve global food security, we must use all the tools in our toolbox

In a single generation we must grow more food, using less land, water, and chemicals. If we don't innovate, millions more could go hungry, economies could fail (Eschen et al., 2021), and the very fabric of our societies could be torn apart.

Failure is not an option. We must use all the tools in our toolbox to avoid future plant pandemics, so that agriculture can help build a world that is more prosperous, productive, and secure—for all people.

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Why are breeders interested in using genome editing, if the traits that they introduce can be done via conventional breeding? Genome editing allows breeders to target only genes of interest, helping to preserve genetic diversity, which is sometimes lost when selecting for traits of interest.

From Labs to Farmers: New Breeding Choices for Better Livestock

By DIANE WRAY-CAHEN, PhD and JUSTIN BREDLAU, PhD

Introduction

We begin the discussion of new breeding innovations in animals by placing genome editing in context with other agricultural breeding techniques that modify DNA. People have practiced the selection, breeding, and modification of livestock since the early times of livestock domestication. Whether we're talking about mass selection (which has been around for centuries), pedigree or progeny selection, marker-assisted selection, genetic engineering (insertion of an rDNA construct) or genome editing, the goal of livestock breeding is to change genetic make-ups to produce new variations of animals that have improvements on traits that are valued by farmers and consumers. Genome editing is just the latest method in this continuum of genetic modification. Since the early days of animal breeding, farmers and breeders have been refining and creating new selection methods to modify animals to increase the precision or accuracy of their ability to select for desired traits, such as temperament (e.g., to allow for domestication), milk or meat yield, heartiness and resistance to disease, size and strength (e.g., for pulling plows), and others.

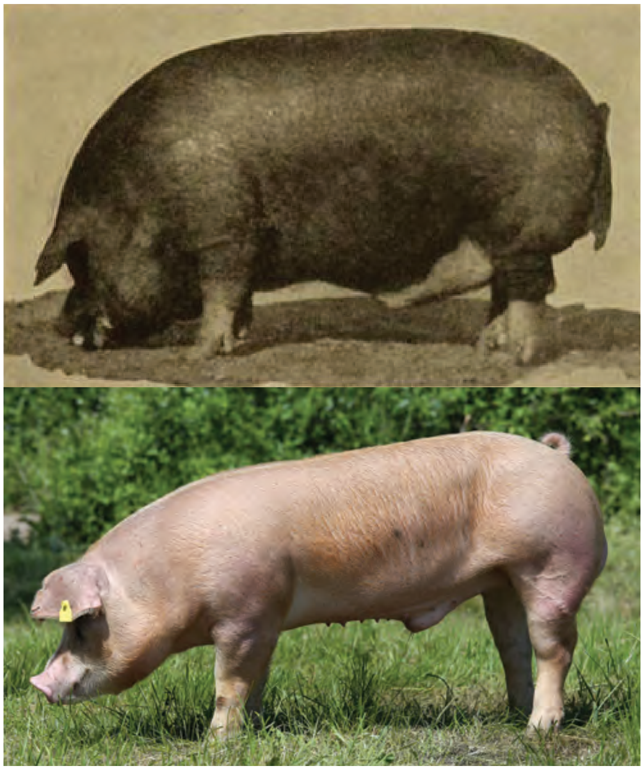

Figure 1. Duroc boars from 1910s (above) and now (below).

Duroc Boars: (top) In Plumb, C (1912). Beginnings in animal husbandry. Webb Publishing Company, St. Paul MN. https://www.flickr.com/photos/internetarchivebookimages/20353356232/
(bottom) https://www.istockphoto.com/photo/duroc-pig-grazing-on-the-meadow-gm1146912528-309197644.

These breeding goals have changed over the years, but the desire and need to modify animals to achieve the particular goals or desired traits for livestock production remain the same. Different generations in different regions have placed value on different traits in livestock. For example, the ideal pig from a hundred years ago looks quite different from the pigs that we raise today (Figure 1), which have a greater emphasis on producing lean meat than fat, as we now place a higher value on high quality protein, than on fat. As farms have become more mechanized, use of cattle for transport has decreased and the breeding goals have been adjusted accordingly.

Using biotechnology to improve livestock is not a new idea. The first genetically engineered animal, a mouse with a growth hormone gene inserted (Palmiter et al., 1982), was produced before the first genetically engineered (GE) plant (Bevan et al., 1983), and the creation of this first GE mouse was shortly followed by the creation of GE fish (Maclean and Talwar, 1984; Zhu et al., 1985) and GE livestock with similar growth hormone genes inserted. The first GE livestock was a rapidly growing pig created at a U.S. Department of Agriculture research center (Hammer et al., 1985). Other GE livestock that were developed include a dairy cow that was resistant to mastitis (Wall et al., 2005) and a pig with a reduced environmental footprint (Golovan et al., 2001). While many types of livestock and fish were created via genetic engineering, only a very limited number of non-laboratory GE animals have been commercialized (Fahrenkrug et al., 2010; Van Eenennaam, 2017). Disease resistance and reduced environmental impact are among traits that are still of interest today.

After the creation of the first GE livestock in the 1980s, three developments have had the biggest impact for innovative breeding in animal agriculture. The first was the development of cloning techniques, first achieved with Dolly the sheep at the Roslin Research Institute in Scotland (Wilmut et al., 1997). Cloning greatly enhanced the ability of scientists to create GE animals and allowed for great advances in genetic engineering. The second was the sequencing of livestock genomes (e.g., chicken: 2004, cattle: 2009, swine: 2012), which greatly increased scientists' understanding of livestock biology and evolution, as well as helped to identify specific DNA sequences that code for certain traits in livestock. Sequencing has also facilitated the acceleration of genetic improvement of livestock and helped to expand the type of traits being selected for breeding programs, including traits of high value and interest in developing countries (Mrode et al., 2019). The third was the development of genome editing—the ability to make targeted changes at specific locations within the genome.

There are currently three types of genome editing nucleases used in livestock: Zinc Finger Nucleases (ZFN), TALEN, and CRISPR (Perisse et al., 2021). As with plants, TALEN and CRISPR applications are more common than ZFN. In recent years, use of CRISPR-Cas9 has become dominant. Advances in CRISPR technologies have greatly simplified the creation of genome-edited animals, allowing efficient production of genome-edited livestock without the use of cloning. Although the mechanisms of these nucleases are somewhat different, all result in DNA breaks and repairs, and use the cell's own DNA repair mechanisms to either delete, silence, change, or insert DNA sequences, as cells do with natural mutations.

Sometimes the process of genome editing is described as being similar to a word processor as it is changing the base pairs or letters in DNA. However, it may be easier to visualize DNA as a blueprint and genome editing as being analogous to a builder making small changes to the blueprint, like modifications to correct an error or defect in the blueprint, or perhaps to add a feature to improve the house (Figure 2). These are small, targeted changes relative to the whole house, perhaps adding a door between the kitchen and dining room to improve functionality or adding a sliding door for more natural lighting and an entrance to a patio to facilitate entertaining outdoors. Likewise, scientists can make small, targeted changes in DNA. Like the builder with the blueprint, animal breeders are not trying to create new animals or breeds from scratch, but rather are starting with already superior breeding animals and adding additional desirable traits, like disease resistance or heat tolerance, to make the animal more resilient and of higher value.

Similar to genetic engineering, breeders are able to use genome editing to introduce traits not available via conventional breeding. However, most breeders who are using genome editing techniques in animals are introducing traits that could have been introduced via conventional breeding or other modification methods that do not involve biotechnology, such as genomic selection.

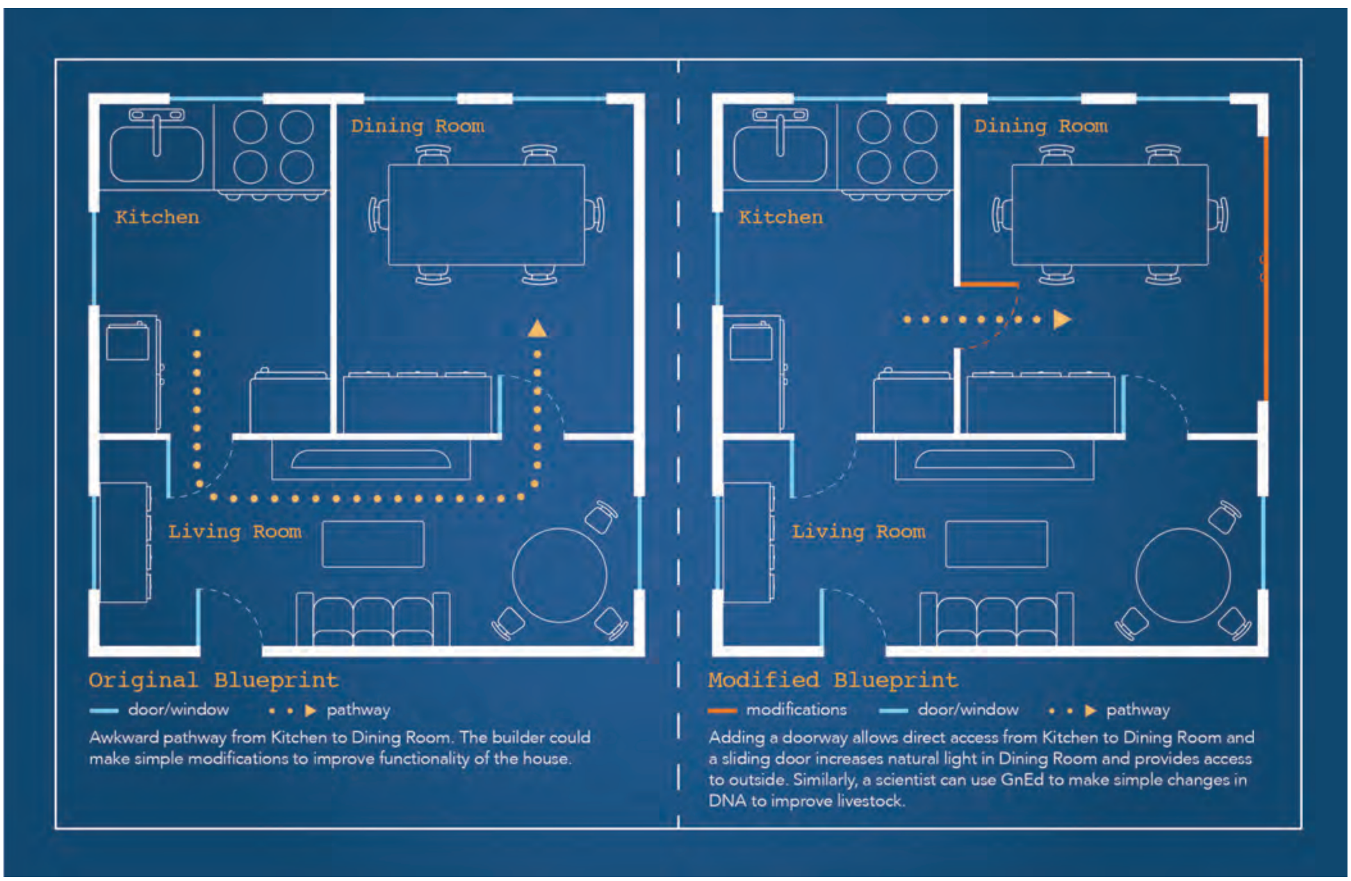

Figure 2. Process of genome editing described using a modified house blueprint.

Blueprints: Ganjofarid Anvarzod, USDA Office of the Chief Scientist

One might reasonably ask, “Why are breeders interested in using genome editing, if the traits that they introduce can be done via conventional breeding?” There are many reasons that breeders want to use genome editing. Genome editing can allow for the introduction of traits that are possible to acquire via conventional breeding, but may be quite difficult to select for, such as those with low heritability. Genome editing allows breeders to target only genes of interest, helping to preserve genetic diversity, which is sometimes lost when selecting for traits of interest. Furthermore, genome editing allows for more rapid genetic gain or progress when introducing or selecting multiple genes at the same time. This is all done with increased precision and efficiency (especially compared to conventional breeding, which produces random combinations of genes).

One very important advantage to using genome editing over other breeding methods is how it reduces the time necessary to improve an animal breed. This is especially important in animals with longer generation times, such as cattle. For example, the Brangus breed (Figure 3) was developed by USDA beginning in the early 1910s. The goal was to create cattle with the meat characteristics of an Angus and the heat and humidity tolerance of a Brahman.

Figure 3. Brangus, a breed resulting from selective breeding and backcrossing of Brahman and Angus.

Photo courtesy of Diane Wray-Cahen

After much breeding and backcrossing, a new breed was established with genetics that are 3/8 Brahman and 5/8 Angus. These high-quality beef cattle could thrive in hot, humid regions and were also selected for having a good temperament. While the breeders were successful in creating a breed well adapted to hot, humid conditions, it took many decades (Go Brangus, 2014). The environment on our farms is changing more rapidly than ever and solutions are needed more quickly than in the past. Using genome editing in combination with conventional breeding methods, such as genomic selection, and assisted reproductive techniques, such as artificial insemination and embryo transfer, will enable much faster genetic gains than is possible with conventional methods alone.

An additional advantage of genome editing techniques as part of livestock breeding programs, is the ability to introduce new traits while preserving genetic diversity of different livestock breeds. Conventional methods of livestock selection and breeding, as used for the development of the Brangus breed, inevitably result in a loss of genetic diversity in the process of selecting for a desired trait. Backcrossing and inbreeding strategies used in selection processes can increase the likelihood of unfavorable recessive traits. Genome editing allows for the introduction of a specific trait of interest, such as resistance to a disease, into a genetic background of an animal that is otherwise well-adapted to a climate or region.

A diversity of traits have been introduced into animals via genome editing, and many more are under development.

The Promise of Genome Editing in Livestock:

The discovery of genome editors, especially CRISPR (Doudna and Charpentier, 2014), with its ease of use, has opened many new options for livestock breeding. The promises and opportunities for food and agricultural applications of genome editing are many. Traits have been created to control diseases and pests, improve animal welfare, create healthier or safer food, improve animal production or yields, improve the quality of animal products (milk, meat, or fiber), and to reduce the impact on the environment or an animal's tolerance to changing climate conditions. Animal biotechnologies are also being used for biomedical uses targeting human health, but these will not be discussed here.

Protection from disease. The focus of much genome editing research in livestock has been on reducing the impact of disease and controlling its spread, including with the control of insects that serve as disease vectors. Diseases result in financial losses to farmers, potential loss of genetic diversity, reduced food security, and also contribute to animal suffering. The goal is not only to reduce the impact and spread of disease, but also to reduce the need for antibiotics and insecticides.

Diseases that researchers and breeders are focusing on include: African swine fever (Figure 4), porcine reproductive and respiratory syndrome (PRRS), avian influenza, trypanosomiasis (sleeping sickness), bovine spongiform encephalopathy (mad cow disease), foot-and-mouth disease, mastitis, and tuberculosis (TB). Much progress has been made on developing animals that are resistant to these diseases. For example, PRRS is a viral disease that affects the respiratory and reproductive systems of swine and results in large economic losses for the pork production industry worldwide (Neumann et al., 2005). Scientists at the University of Missouri developed pigs resistant to infection by the PRRS virus by deleting the CD163 gene (Whitworth et al., 2014; Burkard et al., 2018). Genome editing has been used to produce pigs resistant to foot-and-mouth disease (Hu et al., 2015). Researchers in Kenya are working to develop cattle that are resistant to the trypanosomes carried by Tsetse flies. Genome editing can be applied to enhance resilience to important diseases of poultry, such as avian leukosis (Kučerová et al., 2013) and avian influenza (Lee et al., 2017). CRISPR-Cas9 editing is being applied to improve disease and pest resistance in fish (Zhu and Ge, 2018; Gratacap et al., 2019).

Researchers are developing biotechnology methods to control disease vectors and insect pests of livestock, such as screwworms (Figure 5), as well as insects that damage crops, such as fall armyworms, fruit flies, and diamondback moths (Alphey and Bonsall, 2018). For example, insects have been genetically modified to be conditionally sterile when they lack a dietary supplement (tetracycline) provided during rearing. Upon release, these sterile males mate with wild females and the resulting offspring fail to develop, thereby reducing the target population in the release area without

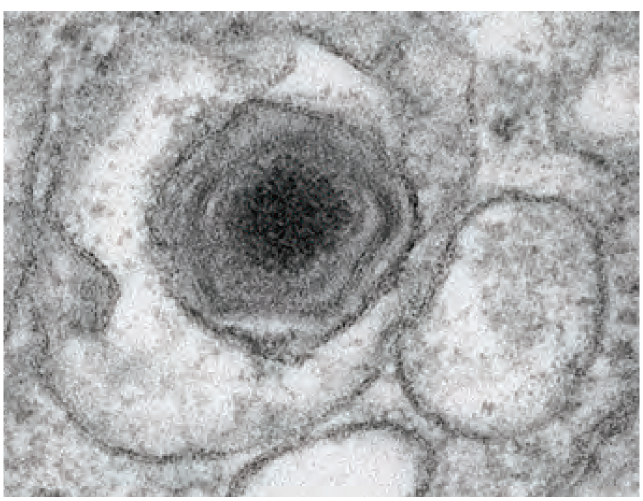

Figure 4. Transmission electron microscope (TEM) view of African swine fever virus.

Photo from Ben Clark, Plum Island Animal Disease Center, U.S. Department of Homeland Security

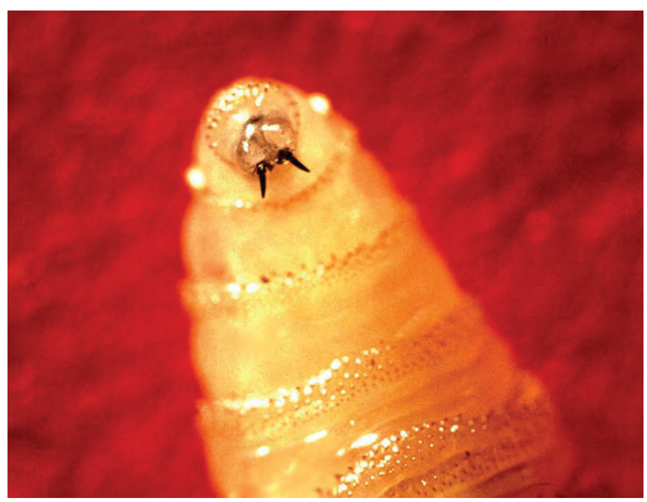

Figure 5. Screwworm larva.

Photo from USDA APHIS (https://www.aphis.usda.gov/aphis/ourfocus/internationalservices/Sterile_Fly_Release_Programs/Screwworm)

Figure 6. A typical horned dairy cow (left) and a genome-edited cow without horns (right) that contains a DNA sequence found in hornless cattle.

Photo courtesy of Alison L. Van Eenennaam, Department of Animal Science at University of California, Davis.

harming any other insects. This method can be more cost-effective and work in more species than classic sterile insect technique that uses radiation to sterilize the insects. More recently, genome editing with CRISPR is being used for sterilizing males and eliminating females prior to a release in some pest insects (Kandul et al., 2019); similar to other methods, the released males mate with wild females which fail to produce offspring, thereby reducing the population over time. Furthermore, genome editing may also one day be used to help protect pollinators from diseases.

Environmental resiliency and adapting to climate change. Researchers and livestock breeders are also working on introducing traits that reduce the environmental footprint of animal agriculture and on creating animals that are more resilient and tolerant to hotter temperatures.

Certain traits could help mitigate the effects of climate change. For example, traits can be introduced to help improved breeds of cattle to be more acclimated to warmer temperatures, which could improve livestock productivity in the tropics and also reduce the carbon footprint of animal agriculture (Karavolias et al., 2021). Heat stress compromises not only animal welfare, but also productivity. This is most evident in European cattle breeds during the hot season or in tropical and subtropical regions. Intense sunlight, heat, and humidity can reduce productivity and reproduction in these livestock. Genome editing has been used to alter coat color in Holstein cattle, which are usually black and white, to create cattle with grey and white coats (Laible et al., 2020) and to make the dominant color in angus to be red, not black. Genome editing is also being used to produce animals with genetics found in certain breeds of cattle that result in slick, short hair (SLICK) that increases heat tolerance in heat sensitive breeds (Hansen, 2020), such as Angus and Holstein.

Improving animal welfare. Genome editing can be used to introduce traits that are focused on addressing farm animal welfare issues. These include traits that eliminate the need for certain farm management practices such as castration and dehorning, as well as allowing sex selection in eggs prior to hatching in laying hen production.

Cattle horns present risks to both farmers and other animals. Therefore, farmers frequently remove horn buds from young calves using chemicals or a hot iron. One company has introduced a gene from hornless cattle into dairy cattle breeds that usually have horns (Carlson et al., 2016), removing the need for disbudding (Figure 6). For pork, several genes are being targeted to reduce or eliminate boar taint (Telugu, 2020), which could eliminate the need to castrate male pigs. Male pigs raised for meat production are castrated shortly after birth, a process that can be painful for animals. This is done because pork from uncastrated male pigs has a strong noxious smell and taste, rendering the meat virtually inedible. Also, sows could be created that contain protective factors in their milk that would help reduce piglet mortality (Han et al., 2020). In poultry, a key industry challenge is the culling of day-old male chicks in the egg layer industry (as the males cannot lay eggs), for which a biotechnology solution is being developed (Doran et al., 2016a). Disease resistance traits, such as those described earlier, also improve animal welfare and in addition reduce potential for zoonotic spread of diseases to humans.

New animal products for the consumer. Other traits are focused on creating healthier and safer food products for the consumer. Genome editing can be used to introduce genetic alterations to improve food quality, create foods with different nutrient profiles, or even reduce the allergenicity of food animal products. For example, CRISPR-Cas9 editing is being used in fish to have higher levels of the healthier omega-3 fatty acids (Zhu and Ge, 2018; Gratacap et al., 2019) and cows have been edited so that they produce these healthier fatty acids in their milk (Liu et al., 2017). In the case of allergen reduction, cows have been developed that produce milk that does not contain beta-lactoglobulin, a protein that is an allergen in cow milk (Wei et al., 2018). In eggs, genome editing could improve food safety by the deletion of allergen coding sequences (Doran et al., 2016a, 2016b; Oishi et al., 2016); this could also be valuable for vaccine production. There is even a trait for chicken eggs that has been created to improve the efficiency of vaccine production (ISAAA, 2021).

Enhancing animal performance and agricultural productivity. Genome editing can also be used to improve animal productivity and traits such as meat production and milk yield or improved fiber production. For example, cattle, pigs, goats, and sheep (Van Eenennaam, 2017) that produce more meat have been created by deletion of the myostatin gene, a mutation that occurs naturally in a number of breeds. Greater muscling and filet size has also been achieved for cultivated fish by this same deletion using genome editing of common carp (Zhong et al., 2016), red sea bream (Kishimoto et al., 2018), olive flounder (Kim et al., 2019), and yellow catfish (Zhang et al., 2020).

For fiber production, wool and cashmere yield can also be improved via genome editing (Perisse, 2021) and biotechnology has also been used in silkworm production for fiber qualities (Zhu et al., 2016, Zhang et al., 2019) and disease resilience (Dong et al., 2020).

Getting traits from labs to farmers:

Work on genome-edited animals has moved beyond proof of principle toward practical applications with the first commercially available genome-edited animal for food, the sea bream mentioned above, expected to enter the market in Japan by the end of 2021 and is to be joined by a genome-edited tiger pufferfish created by the same developers. Commercialization of genome-edited animals could improve livestock resiliency for disease and environmental changes, provide economic benefits to farmers, promote sustainability, and improve animal well-being. Two-thirds of the global cattle population is held by 300 million small holders. Bringing traits to these farmers will require partnerships among universities, government research institutes, charitable funding organizations, as well as the private breeding and production sectors.

Such partnerships have begun moving forward. For example, U.S. companies (Acceligen and TransOva Genetics) are partnering with an Argentine company (Kheiron Biotech) with support from the Bill and Melinda Gates Foundation to combine valued traits from the Gir (a Brazilian breed tolerant of tropical conditions) and Holstein (a breed with high milk production) cattle. This group aims to generate dairy animals that will bring about significant and sustainable production gains through immediate access to improved heat-tolerant and disease-resistant dairy cattle, particularly for African dairy production systems. In Kenya, another project is focused on improving cattle production in Africa. The Mzima cow project of the Centre for Tropical Livestock Genetics and Health, with support from UKAid and the Bill and Melinda Gates Foundation, is aimed at producing genome-edited cattle that are resistant to trypanosomes, the parasite responsible for African Sleeping Sickness (Kemp, 2020).

Conclusion

Genome editing provides the opportunity for traits to be targeted and introduced into local breeds to meet regional needs. In some cases, greater accessibility to assisted reproductive technologies, such as artificial insemination, and associated infrastructure may be necessary to fully capitalize on traits introduced by genome editing. Livestock farmers face many challenges now and in the near future, including climate change, diseases, and shifts in consumer demand. Access to the best selection of tools in the breeding toolbox is crucial to address future challenges, meet sustainability goals, and increase productivity. The next generation of farmers will require more options, not fewer, to meet our growing agricultural needs with a smaller environmental impact.

For Further Reading

Genome sequencing: Swine genome sequencing: https://comparativegenomics.illinois.edu/swine-genome-project; https://www.nature.com/articles/nature11622 (Groenen, M, A Archibald, H Uenishi et al. Analyses of Pig Genomes Provide Insight Into Porcine Demography and Evolution. 2012. Nature 491, 393–398. https://doi.org/10.1038/nature11622)

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These techniques do not lead to different results from those that earlier techniques could obtain; they just allow to reach the same results easily, faster, and with increased knowledge/control of the outcome.

Regulation of Breeding Innovations in Agriculture

By MARTIN LEMA, MSc

The regulation of new genetic variants

One of the main drivers of agriculture since its inception is genetic improvement. New plant varieties, new animal breeds (and, more recently, new microbial strains) represent an opportunity for increased food production, improved food quality, and even completely new traits that can increase product quality.

However, the same power of genetic change can also lead to food and environmental safety concerns. Therefore, in modern times most countries have systems in place that enable governmental oversight of genetic innovations.

The specifics of sanitary regulation may differ between plants, animals, and microorganisms. This is briefly summarized next[1]:

New plant varieties are usually subjected to a *variety registration* process, which routinely serves governments to administer developer's intellectual property rights. However, variety registration may also entail activating a risk assessment process if the trait is new and a clear risk hypothesis is associated with it.

[1] Assuming that reference is made to new genetic variants of species that are already being employed in agriculture. In addition, many countries also count nowadays with a separate "Novel food" regulation that, inter allia, covers the introduction of products from "new" (at least at the national level) species into the food supply. Such regulation may eventually become also relevant to this discussion, since there is at least one example where gene editing has been used to turn a wild relative of tomato into an allegedly edible new tomato-like species (Zsögön et al., 2018).

The cornerstone of animal sanitary regulation, in contrast, is rarely the genetic novelty of an animal breed. Historically, most of the sanitary risks have been associated with diverse zoonosis, while the genetic change of new breeds has hardly ever affected food safety by itself. Therefore, regulation is focused on the surveillance of health parameters for each individual.

Finally, the regulation of new microbial strains for food processing is relatively strict. It is usually based on a detailed compositional and genetic comparison with other strains considered safe for food use. Conversely, new microbial strains for agricultural production (e.g., biofertilizers) are less strictly regulated, except in cases where the microbial species entails known and specific human exposure (occupational) hazards.

These regulations, although quite different according to the biological kingdom, used to be a single one and the same for all known breeding methods, including (a) the selection of new varieties/breeds/strains from wild populations, or from spontaneous mutants in domesticated populations, (b) the hybridization with wild relatives or between separate domesticated lineages, or (c) the creation of new genetic diversity, typically in plants and microorganisms, using somaclonal variation or chemical/radiological mutagenesis.

However, by the end of the 20th century, a new breeding method was introduced worldwide. Genetic engineering added the possibility of moving genes between species, thus introducing new traits that were not within reach of pre-existing methods. For instance, moving a gene from bacteria to crops to confer strong protection against insect pests.

The emerging possibilities of creating new genes by combining pieces of DNA from different species and reintroducing them into food-producing organisms led to the development of a new specific regulation for "recombinant-DNA" technology.

That specific regulation, in most cases, was enacted in addition (not replacing) any pre- existing regulation for new varieties, breeds, or strains. In fact, the new regulation has a different subject: the so-called "transformation events" (shortened in the regulatory slang to "events"). In the field of genetic engineering, a transformation event means the insertion of a piece of foreign DNA into the genome of an organism.

Over the years, countries have been making efforts to harmonize their regulatory approach for recombinant-DNA technology. Such efforts are coordinated in multilateral fora such as the Codex Alimentarius Commission, the World Trade Organization, and the Organisation for Economic Co-operation & Development (OECD). However, the most prominent forum in this regard has always been the Cartagena Protocol on Biosafety under the Convention on Biological Diversity.

The new breeding methods

With the advent of the 21st century, gene editing and other breakthroughs in molecular biology led to new techniques for easing genetic improvement. The term "easing" here is important: these techniques do not lead to different results from those that earlier techniques could obtain; they just allow to reach the same results easily, faster, and with increased knowledge/control of the outcome.

Since these innovative breeding methods do not generate different results, there is no need for a completely new regulation. However, they pose an apparent dilemma for regulatory classification. Let's consider an example (Figure 1).

Now, how should this example be regulated? This technique generates the same outcome of earlier mutagenesis-based breeding techniques such as selecting spontaneous mutants from nature or provoking mutations using somaclonal variation and chemical/radiological substances. These earlier techniques also rely on DNA breakage that is repaired incorrectly to generate new genetic diversity. From that perspective, the result is just another new mutant variety obtained by mutagenesis; and this equality is valid both for the scope of potential benefits as well as regarding any safety considerations.

On the other hand, recombinant-DNA was somehow used in the process. However, that recombinant DNA was inserted in a bacterium

In one embodiment of gene editing (known in regulatory slang as SDN1), a piece of recombinant-DNA is introduced in bacteria to produce a site-directed-nuclease. This nuclease is then purified and introduced into plant cells, where it will seek for a very specific genomic sequence, which is not random but chosen by design.

Then, the nuclease will introduce a break in the DNA molecules having the target sequence, and that is the end of human intervention. Immediately after, the plant cells will repair the broken DNA, in most cases restoring the original sequence without consequences. However, in a few cells the repair mechanism will fail, and a short sequence change will occur, resulting in a loss-of-function mutation (in a predetermined gene).

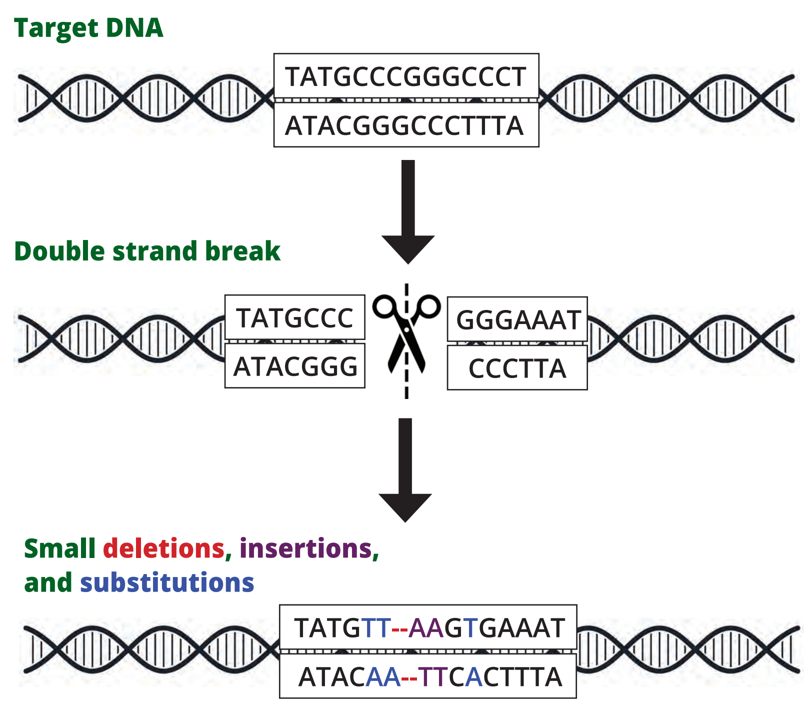

Figure 1. SDN1 (see text for details).

that never left a lab; it is not present in the plant genome to be used commercially. Therefore, there is no transformation event and no transgenesis in the new variety.

Should this product be regulated just as any other new mutant variety? Or should it also be under the regulatory framework for the so-called "modern biotechnology" enacted in the nineties?

This decision is not trivial. Although both regulatory systems would suffice to guard the safety of people and the environment, the regulatory system for "modern biotechnology" is expensive and time-consuming. Besides, it is highly politicized and thus uncertain, especially for newcomers and radical innovations. The burden of this regulation has hindered the use of many potentially useful transgenic organisms developed by public researchers and SMEs, which are left virtually out of the game.

Moreover, the mistrust of many people regarding transgenic organisms (not due to any actual safety issue but raised by decades of misinformation) would also stain these other biotechnology products in those people's eyes if governments classify them altogether.

Overall policy considerations

An ideal sanitary (safety) regulation for agrifood and other products should meet the following criteria:

Fit for purpose: Sanitary regulations are enacted to decide if a product can be safely allowed to enter the market. It is not about what politicians or other influential people feel or prefer about novel products. Therefore, all relevant considerations to assess safety must be included, but none other aimed at influencing trade or consumer choice.

Science-based: In connection with the fit-for-purpose element, regulations should rely only on the most updated scientific and technical analysis tools. Besides, the utility of such tools should be judged against a specific endpoint: deciding if there is enough evidence to conclude that a product can be safely allowed to enter the market.

Risk-proportionate: The objective of sanitary regulation should be avoiding concrete risks. Therefore, the risk level of a product should determine the number of safety studies warranted. Potential risks, in turn, result from the product's characteristics or traits. Therefore, the same burden of proof (and regulatory burden) or, in other terms, the same "level of protection" should be required for products having the same or similar traits.

Separate products from process: In connection with the risk-proportionate principle, better (and more knowledgeable) regulations are triggered by and based on the characteristics of the final product, instead of the process used to obtain it.

International harmonization: The triggers and requirements of regulations for a specific type of product should be equivalent across countries. Whenever gross and unjustified differences in the regulatory burden or the information required arise between governments, a science-based dialogue (and subsequent regulatory updates) should take place to try equalizing those differences.

Country approaches worldwide

During the past decade, countries of different world regions began establishing regulatory criteria for new breeding innovations. This is briefly reviewed next (Figure 2):

North America

The United States and Canada were among the first countries to take concrete regulatory decisions upon the regulatory status of several new breeding innovations. This did not require a modification of their preexisting laws and regulations. These countries have broad concepts like "plant pest" or "novel trait", which trigger a special regulatory oversight that is usually applied to transgenic organisms.

Therefore, the same triggers were applied to decide the status of several organisms derived from these innovative techniques, and many of them were found not to require special oversight.

More recently, there have been updates on the regulation of agrifood biotech in both countries. Learning from a decade of experience and facing the need of expediting consultation processes (due to an increasing pipeline of products), new regulations now include a more explicit exclusion of many innovative breeding techniques.

Latin America

Eight countries in Latin America have already established criteria to determine the regulatory standing of new breeding innovations. This includes Brazil, Chile, Colombia, Ecuador, Guatemala, Honduras, Paraguay, and Argentina. Their approaches are remarkably similar, all of them inspired up to a certain extent by the pioneer regulation that Argentina enacted in 2015. Half of them have already taken regulatory decisions on specific products, determining that the GMO regulation does not apply to them.

The "Argentine approach" to the regulation of these products became very influential in Latin America and overseas. It relies on the Cartagena Protocol definitions to decide if products are considered GMOs or not, on a case-by-case basis. This facilitates harmonization since most countries worldwide are parties to the Protocol and have enshrined its definitions in their national legislation.

Europe

The national GMO regulatory bodies in six countries of the European Union (Finland, Germany, Ireland, Spain, Sweden and the United Kingdom) received early consultations and determined that a certain crop variety mutated by gene editing is not a GMO. This occurred with regards to applications for field trials, which are under national administration.

However, for years, the European Commission did not take any stance regarding the criteria for commercial approvals, which are administered at the supra-state level. In the meantime, official scientific advisors concluded that some NBI products should not be considered GMOs.

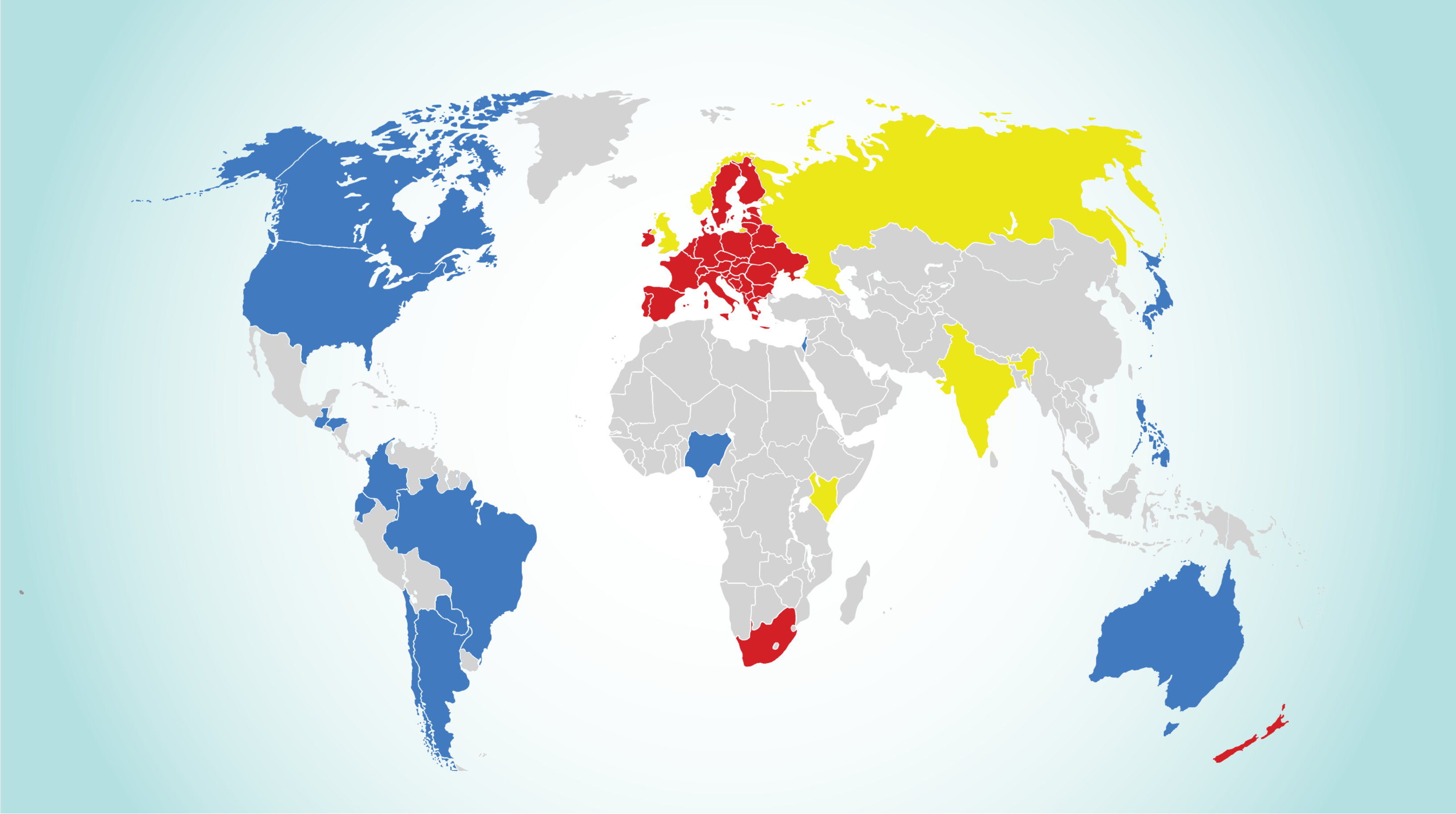

Figure 2. Global regulatory landscape for SDN1 gene-edited products.

Countries in blue are those where such products are likely to be regulated as conventional new varieties after recent regulatory policy updates. In contrast, countries in red represent those where these products should be treated as GMO according to court interpretations based on old regulations. Finally, countries in yellow are those where there are noticeable policymaking discussions over proposals to treat SDN1 as conventional new varieties. See text for details.

Technology detractors forced a clarification by taking the issue to the Justice in France, and the case escalated to the EU Court of Justice (ECJ). Although the Attorney General of the ECJ agreed that the result of some innovative breeding techniques should not be considered GMOs, in the end, the EU Court of Justice itself ruled the opposite. However, it is very important to note that the ruling is based on the GMO definition included in the EU regulations, which is entirely different from that in the Cartagena Protocol.

Finally, several current or former high-level authorities of the EU and its member states have declared that gene editing and other innovative techniques should not be regulated as GMOs or that the EU GMO regulation unnecessarily blocks innovation.

In summary, there is a state of legal, technical, and political contradictions in the EU, and many agree that this can only be solved by updating its legislation on these matters.

Nonetheless, Europe is more than the EU. Other countries in the region are considering the adoption of regulatory policies that have more resemblance to those of the American continent. This includes Norway, the post-Brexit UK, Switzerland, and Russia.

Africa

Nigeria amended its Biosafety Act in 2019 to address gene editing. In Kenya, regulators have developed a Draft Guideline for the same purpose. In South Africa, the Department of Science and Innovation has issued recommendations to the Department of

Agriculture, Land Reform, and Rural Development (GMO regulator). In all cases, the proposed approach is similar to the one described for Latin America.

Besides, the issue is being analyzed in several biosafety offices across the continent. Therefore, it has been included among the priorities of the African Biosafety Network of Expertise (ABNE). ABNE is a program under the African Union Development Agency (AUDA-NEPAD), which generates resources and networking among African regulators to advance continental harmonization and regulatory clarity.

Asia and the Pacific
New Zealand had a very early experience resembling the EU, firstly with regulators judging that a certain mutant gene-edited crop was not GMO. Then the decision was challenged in court and ended up being nullified.

In recent years, Japan and Australia have been issuing and refining their implementing regulations and taking the first decisions on the status of a few products. Preliminarily, it is clear that at least SDN1-type products would be excluded from the regulation usually applied to transgenic organisms.

Israel reportedly has adopted a criterion that excludes products not having recombinant-DNA insertions in the final product from the GMO regulation.

Besides, other regulatory bodies in these regions have begun discussing how to regulate organisms improved with innovative breeding techniques, including China and Korea. In the Philippines and India, there are advanced drafts of new regulatory texts already being circulated.

Lessons from recent regulatory experiences

The importance of legal definitions
There has been an enormous quantity of debates and publications discussing if organisms resulting from innovative breeding techniques should be regulated as GMOs or not.

It has been pointed out, for instance, that some mutations obtained from these techniques cannot be distinguished from those happening spontaneously in nature. Besides, it has been suggested that genetic changes up to 20 nucleotides should not be specially regulated. There have been comparative discussions in the level of safety *vis a vis* "traditional" or transgenic breeding techniques.

However, by the end of the day, the scope of regulation is dictated by its definition of what constitutes a regulated article.

Therefore, the language of current (or future) legal definition is the primary aspect to consider. In most countries, their triggering definition is the same or similar to those of the Cartagena Protocol. Hopefully, that would greatly facilitate harmonization.[2]

Unintended changes
New breeding innovations are much more predictable, controlled, and understood than earlier breeding techniques, as illustrated in Table 1.

Debates on the regulation of New Breeding Innovations, especially regarding genome editing, usually refer to the possibility of unintended changes. These include the so-called "off-target effects" and unintended DNA insertions. Albeit with low frequency, such changes may happen, and this cannot be denied or ignored. However, it should not be disregarded that current capabilities of finding and filtering them out are much higher than for "conventional" breeding, where they can happen, too. Whole-genome sequencing (WGS) studies are powerful tools in this regard.

Regulators asking for thorough checking of unintended changes is not always well received by some developers that use innovative breeding techniques. Developers may expect that their

[2] Having different definitions does not necessarily imply reaching different conclusions. For instance, most of the decisions made by North American regulators are coincident with those of Latin American countries. Conversely, having exactly the same definition does not guarantee coincident decisions in every case. For instance, it is emerging that SDN2 may be considered a non-GMO in some countries, but a GMO in others. This seems to relate more to different molecular models of template-driven DNA repair, and not diverging GMO definitions.

Table 1. Comparison of new breeding innovations and earlier breeding techniques.

Breeding method:	Creating new genetic diversity by mutagenesis	
Principle:	Breaking or distorting DNA and wait for its repair mechanism to commit mistakes	
Technique:	**Ionizing radiation or chemical mutagens**	**Site-directed nucleases (e.g., CRISPR-Cas9)**
Control over target sequences:	None	Tight. Breakage directed to specific sequences of interest
Knowledge on the trait-associated mutation:	Initially none, rarely investigated; regulators usually don't ask.	Known from the start, part of the breeding strategy and the regulatory analysis.
Possibility for off-target mutations having hidden phenotypes (unintended changes):	Enormous, but regulators usually don't ask.	Small, and regulators should ask.
Possibility of finding those off-target mutations:	Very limited. Not required by regulators.	Very easy, indirectly required to reduce the amount of regulatory burden.

products are treated exactly the same as those obtained with earlier methods, e.g., not being required to spend in WGS studies.

However, biotech regulators should check for the absence of unintended changes that may be risky on a case-by-case basis. This would imply that a new trait released to the market will have a higher margin of safety if obtained by New Breeding Innovations compared to the earlier methods.

Moreover, given the early instar of these technologies, a single case of an undetected change (or an "unintended GMO") that is overlooked may badly stain the reputation of these technologies in general. Fortunately, the cost of a WGS study is dropping fast and becoming insignificant compared with the commercial benefit of a novel trait for agriculture.

Minding the gap

In most countries, GMOs for food and agriculture first pass a safety assessment by the "biotech" regulator, and the (transformation) event is approved. Then, the corresponding varieties, strain, products, etc., need to be registered with the regulator of conventional products. This "conventional" regulator may have the power to require safety assessments also, but in most cases, this becomes practically unnecessary due to the earlier intervention of the "biotech" regulator. Both regulators typically don't need to interact much.

Now, innovative breeding techniques have generated another scenario where the biotech regulator intervenes first, but, in many cases, it will be only to determine that the final product is not a GMO, for instance, because there is no transformation event. In such cases, the "conventional" regulator may still need to exert its safety assessment prerogatives in those cases where the trait leads to a credible risk hypothesis.

This difference must be duly accounted for in the regulatory procedures. The "biotech" regulator may envision a risk hypothesis, even if not entitled to assess it (because the product is not a GMO). However, that risk hypothesis must be noted down and notified to both the developer and the "conventional" regulator for its assessment (Figure 3).

Regulation and innovation economy

Research studies on innovation economy recognize the importance of regulation on the rate of technological development, especially for activities that are simultaneously highly innovative and highly regulated.

Therefore, it is quite predictable that products from innovative breeding techniques will have a

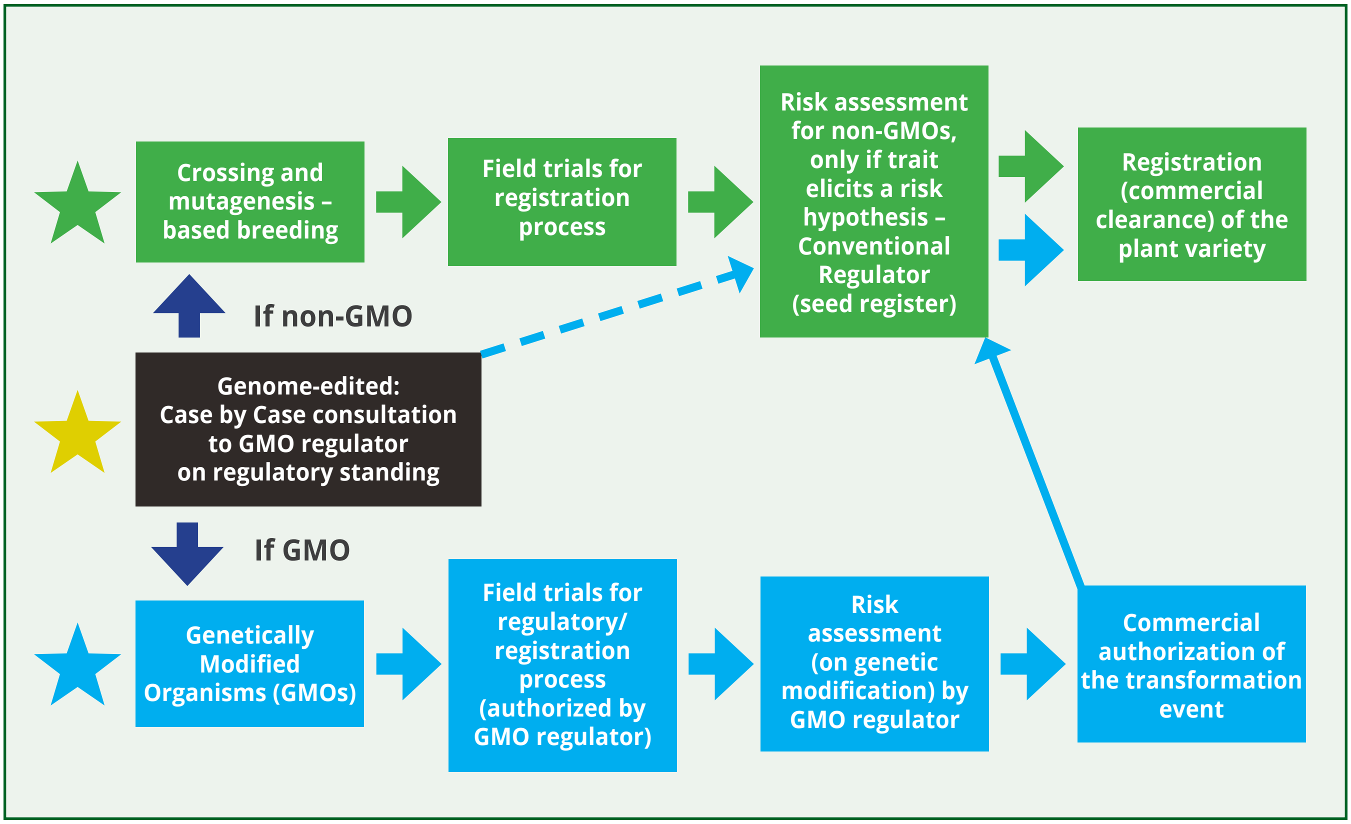

Figure 3. Regulatory process flow for genome-edited plants. This is a simplified representation of the usual regulatory path for registering non-GM new plant varieties (beginning in the green star), and the registration of plant varieties containing biotech events (beginning in the blue star). The possibility of handling gene-edited varieties within these preexisting systems is represented also (beginning in the yellow star).

very different adoption rate and socio-economic impact if regulated as GMOs or as conventional products.

This is beginning to be supported by empirical evidence from North and Latin America as well as Japan, where the regulatory approach described has led to a boom of new applications for very diverse agrifood uses. The menu of innovative traits includes, among others, those related to consumer interests, resilience to climate change, and increased sustainability.

Most applicants are public research institutions and SMEs, which can attract a significant amount of public and private external investment for these projects. However, the Achilles heel of such developers seems to be the predictability of regulatory outcomes. The "boom" is more noticeable in countries like the USA and Argentina, where developers can consult regulators at an early development stage. In these countries, applicants can request a preliminary but formal answer on the regulatory treatment that their products will receive. Conversely, countries that only admit a definitive consultation once the product is fully developed are receiving much fewer applications.

Therefore, a seemingly secondary aspect like enabling a preliminary consultation can significantly impact the quantity of future players. More players mean more creativity, niche exploration, and market competition. In turn, this would translate into more solutions available for different agricultural chains at more competitive costs.

Conclusions

Most innovative breeding techniques are based on scientific knowledge that has been around for many years. Besides, regulators have been preparing to deal with its products for a decade now. Early examples show that the regulation of this kind of biotech products can be made accessible to more developers without compromising safety.

The scientific and regulatory state of the art allows assessing these products with a high level of confidence. They do not defy current regulations for the safe use of new genetic variants and can be handled within existing frameworks introducing minor adjustments. Nevertheless, in some cases, those regulations that depart too much from international consensus, particularly on their GMO definition, may need significant changes to reach harmonization.

Fortunately, many countries now seem to be transiting, at various stages, the same avenue towards effective regulatory criteria that still reassures consumer and environmental protection with the right amount of regulatory burden. Their sensible policies will be rewarded with increased agrifood quality and productivity, as well as many derived socio-economic and environmental benefits for their people and all humankind.

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With the proven precision of genome editing without necessarily introducing a foreign gene, the opponents of GM crops now have less reason to stand in the way of this innovation.

Prospects of New Breeding Innovations in South and Southeast Asia

By GABRIEL O. ROMERO, PhD

The great potential of genome editing, the current toast of new breeding innovations, in developing superior crops has created much excitement around the world, the South and South East Asian region included. While there is general consensus among scientists about the safety and potential benefits of biotechnology including GM crops, political and consumer pressures have derailed research, commercialization, and cultivation of biotech products in most of the region. Now comes genome editing in which plant traits can be improved through simple genetic changes quite similar to those induced by conventional mutagenesis except that precise and direct DNA manipulation is performed. With the proven precision of genome editing without necessarily introducing a foreign gene, the opponents of GM crops now have less reason to stand in the way of this innovation.

To be sure, most if not all countries in the region are GM crop users for direct use as food, feed, and processing. The main differentiation is on the issue of cultivating GM crops, with only a handful allowing it, such as India, Pakistan, Philippines, Vietnam, Myanmar, and Indonesia. Their positive experience with the GM crops attested by government, farmers, and consumers augurs well for the introduction of genome editing technology and products. A few of them have local capability in GM crop development on which they can leverage research activities on the relatively less costly genome editing. In fact, in-country research, some with international collaborations, have started in earnest as early as 2016 in India.

The main issue facing these countries is how to regulate the genome-edited products as their current GM regulations appear to be too overbearing for these products that, except for the use of recombinant DNA, appear to be very much like conventional varieties. As such, the looming question is whether a biosafety assessment is necessary. With the recent ardent campaigns of academics, technology developers, and trade stakeholders, and matched with the openness and willingness of regulatory agencies, an alignment is developing to distinguish genome editing products from GM crops and thus accord them a less stringent assessment nearly as straightforward as varietal registration much like for conventional crops (Turnbill et al., 2021).

Each country in the region presents a unique case of GM use and regulatory history, initial work on genome editing R&D and possible direction of regulatory guidelines for genome editing.

India

India has adopted biotechnology and is involved in R&D of emerging applications like genome editing. India has grown GM cotton since 2002 and is now considered the top cotton exporter in the world. More than 96% of the 11.2 Mha of cotton area in India is planted to GM cotton. More than 85 crop species are currently under various stages of biotech R&D in India. Bt brinjal (eggplant) had been subjected to a moratorium due to an adverse public reaction in the middle of its approval process, and genetically engineered mustard was awaiting approval (Friedrichs et al., 2019).

India has taken steps toward establishing a suitable, science-based guideline for genome-edited products. The genome editing draft guideline recommends that risk assessment should be commensurate with the nature of genetic changes with increasing rigor along with edit complexity. Risk assessment in Group I, with deletions caused by site-directed nucleases type 1 (SDN-1), and Group II, with a few base edits made with site-directed nucleases type 2 (SDN-2), requires: a) evidence for the targeted edit, b) proof of absence of biologically significant off-targets, c) testing for trait efficacy, and d) demonstration of equivalence to reference varieties except the edited trait. For herbicide tolerance or weediness related traits arising from single base pair changes, additional biosafety studies are required. Group III, with large foreign/synthetic DNA replacements induced by site-directed nucleases type 3 (SDN-3), will be considered GMOs and thus subjected to the same stringent risk assessment as for classic transgenic plants. Basic information on the delivery method, molecular basis of edits, molecular characterization, phenotype and biosafety among others is necessary for a thorough assessment of the edited product (Bhattacharya et al., 2021; Menz et al., 2020).

While the genome editing guidelines are still under development, government agencies and public universities have successfully used this new tool in improving rice, banana, and groundnut. Nagaraj et al. (2019) of Tamil Nadu Agricultural University conferred thermo-sensitive genic male sterility in rice using CRISPR-Cas9 editing system. The trait is important in the seed production of hybrid rice, which now accounts for around 7% of the total rice area in India. Farhat et al. (2019) at the National Institute of Plant Genome Research (NIPGR) and Kumar et al. (2020) at The Indian Agricultural Research Institute (ARI) in New Delhi produced drought and salt-tolerance in rice through the CRISPR-Cas9 system. Also at the NIPGR, Kaur et al. (2020) improved the β-carotene biosynthesis in banana fruit through CRISPR-Cas9 directed editing. Rajyaguru et al. (2020) at the Junagadh Agricultural University developed high oleic, low linoleic acid in groundnut through genome editing. The cholesterol-free groundnut is suitable to weight watchers and those with high cholesterol.

Pakistan

Pakistan has adopted a wide range of applications and approved GM crops for cultivation or allowed field trials with GM crops, and local research on

genome editing has started. Pakistan began GM cotton cultivation in 2002 and approved (though immediately recalled) GM corn cultivation in 2014.

The government agencies tasked to oversee GM crops in Pakistan, unfortunately, do not agree on the cultivation of more GM crops other than GM cotton. The Ministry of Climate Change of Pakistan restricted commercialization of GM crops while the Pakistan Environment Protection Agency (Pak-EPA) and the National Biosafety Committee supported it. GM corn is approved in Pakistan but the conflicting positions in government have prevented its cultivation in farmers' fields (Babar et al., 2020).

Another instrumental agency, the Ministry of National Food Security and Research, while supportive of GM crops, invokes genetic purity concerns and the outstanding performance of current non-GM corn. It is concerned that GM corn may contaminate local maize varieties and does not recognize the advantage of GM maize when the conventional maize varieties have good yield and pests are manageable (Shaikh, 2010).

Despite the unpredictability of the regulatory environment in Pakistan around GM crops, and lack of genome editing policy, a local research agency, the National Institute for Biotechnology and Genetic Engineering (NIBGE), began applying the new editing tool to improve rice. Zafar et al. (2020) of NIBGE used CRISPR-Cas9 to create broad-spectrum resistance to bacterial blight in the Super Basmati rice by deactivating its susceptibility genes.

Philippines

The Philippines is arguably a crop biotechnology leader in Asia in cultivation and direct use of GM crops, and local research in genome editing is gaining momentum. The Philippines started growing GM corn in 2003, which now accounts for over 85% of the 1.0 M ha yellow corn in the country. The GM corn traits and hybrids are produced by multinational companies and sold through their local branches and licensees.

The Philippines has enjoyed the fruits of modern plant breeding for more than six decades, and nearly two decades of biotechnology including GM. Locally developed GM crops are soon getting the green light for commercial propagation. A local public university, University of the Philippines (UPLB), has developed transgenic delayed-ripening papaya. UPLB also collaborated with Mahyco in creating Bt eggplant with Mahyco's Bt gene constructs. Public research agencies partner with international entities such as the Philippine Rice Research Institute (PhilRice) with the International Rice Research Institute (IRRI) for the transgenic Golden Rice, and Philippine Fiber Industries and Development Administration (PhilFIDA) with an Indian company (Global Transgenes) and a Chinese company (Biocentury Transgenes), for the transgenic Bt cotton. The Institute of Plant Breeding at UPLB and PhilRice have added the more precise genome editing techniques in their toolbox.

The Philippines is currently implementing the Department of Science and Technology (DOST)-Department of Agriculture (DA)-Department of Environment and Natural Resources-Department of Health-Department of Interior and Local Government Joint Department Circular (JDC) No. 1 "Rules and Regulations for the Research and Development, Handling and Use, Transboundary Movement, Release into the Environment, and Management of Genetically-Modified Plant and Plant Products Derived from the Use of Modern Biotechnology which was issued in 2016. This superseded the DA Administrative Order No. 08 "Rules and Regulations for the Importation and Release into the Environment of Plants and Plant Products Derived from the Use of Modern Biotechnology" issued in 2002.

Towards building capacities of the country for genome editing R&D and regulation of upcoming genome editing products, the National Committee on Biosafety of the Philippines (NCBP) issued a resolution defining a product-based approach for NBTs. The resolution contends that products of NBT/ PBI can be (a) GMO if they contain a novel combination of genetic materials obtained through the use of modern biotechnology and not possible through conventional breeding"; or (b) non-GMOs or conventional products, if they do not contain a novel combination of genetic materials. Only PBI-derived GM plants and plant products would be regulated under the JDC1. Consequently, PBI-derived non-GM plants and plant products would not be regulated under the said Circular. DA shall issue guidelines and take the lead in evaluating and monitoring plant and plant products derived from the use of modern biotechnology, including Plant Breeding Innovations (NCBP 2020). Based on the NCBP resolution, products from SDN-1, SDN-2, and SDN-3 with inserts from the same species would not be regulated as GMOs (Entine et al., 2021).

Meanwhile, as the regulatory guidelines are under development, the DA and the DOST fully support crop biotechnology and genome editing research. The DA-Biotechnology Program Office (BPO) is now evaluating genome editing project proposals. The DOST through its council the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) provides funding support to biotechnology projects and recently to genome-editing projects.

Biotech laboratories in the Philippines have turned to genome editing tools in conferring novel traits that are not achievable with conventional breeding. The International Rice Research Institute (IRRI) and the Institute of Plant Breeding (IPB), which produced a number of GM products, are well capacitated for genome editing projects. Using CRISPR-Cas9, the laboratory of Dr. Inez Slamet-Loedin of IRRI has developed broad-spectrum resistance to bacterial leaf blight (BLB) by removing rice genes that allow BLB infection. Using CRISPR-Cas9, they also increased rice resistance to tungro virus by deleting a rice gene that aids viral multiplication. Using CRISPR-Cpf1, they disrupted gene Gn1a to increase grain weight in rice. With the same editing tool, a rice gene was knocked out to reduce stomata number, and the edited lines are now undergoing bioassay for water use efficiency. IPB on the other hand is conducting genome editing to improve corn and tomato quality. The team of Dr. Antonio Laurena aims to reduce phytic acid in corn to increase its nutritional value by deactivating a phytate synthesis gene using CRISPR-Cas9. To intensify red color in the ripe tomato fruit, the IPB team is editing lycopene genes for higher lycopene accumulation. Meanwhile, PhilRice is gearing to develop rice lines with tungro resistance, BLB resistance, and optimized grain amylose content through genome editing.

Vietnam

Vietnam is a relatively newcomer in GM crop cultivation, but it can be considered an early bird in genome editing. GM corn cultivation in Vietnam started in 2015. Since then, 225,000 hectares have been planted to maize containing GM traits in Vietnam and in 2019, it accounted for 10.2% of the total maize crop. The technology gave Vietnamese farmers higher yields from better pest and weed control than conventional varieties (Brookes and Dinh, 2021).

The government and private sector have collaborated in finding a suitable regulatory treatment for genome editing. In 2020, the Vietnam Academy of Agricultural Sciences (VAAS) and the Vietnam Seed Trade Association (VSTA) in cooperation with the US Grains Council held a workshop on Genome Editing: Global Perspectives and Potential for Vietnam. The workshop brought to the fore the distinction between gene editing and genetically modified organisms (GMOs) — no genes from other organisms remain in the final edited products. The VSTA strongly recommends the application of genome editing, which they regard as similar to yet faster than traditional breeding. The VAAS contends that editing plant characteristics is much safer than gene modification, and new regulations for gene editing technology and products is unnecessary because the new plant varieties are not genetically different from those created by traditional techniques (Oahn, 2020). This qualification specifically applies to SDN-1 and SDN-2 type products.

The Agriculture Genetics Institute (AGI) argues that gene editing has the potential to significantly improve the efficiency and timelines of breeding programs. However, the present uncertain regulatory status of gene editing products is a barrier to investments and applications of editing techniques in crop breeding. The AGI therefore recommends to revise and/or update the current GMO regulatory system, especially clarifying the scope of the definition of GMO, in order to facilitate genome editing R&D and product development in Vietnam (Phuong et al., 2020).

While the regulatory policy is still under development, a local gene editing project has been accomplished. Through the genome editing system CRISPR-Cas9, Vietnamese scientists from the Institute of Biotechnology created soybean types with low indigestible sugar content in seeds through directed mutations, a first study in the world (Le et al., 2020).

Indonesia

Indonesia lies at the doorstep for GM crop cultivation and is inclined to follow the global trend in genome editing. The national biosafety regulatory framework of Indonesia was established in 2005. However, GM crop varieties have not been commercialized due to the lack of post-monitoring guidelines. This gap was recently filled with the issuance of Regulation 50/2020 establishing a post-monitoring scheme for genetically engineered (GE) crops. The monitoring scheme, to be conducted by an independent survey agency or university, is required in the first three years of cultivation with focus on the impact of the GM crop on the health of livestock or the environment (ISAAA Crop Biotech Update, February 10, 2021).

Following the lifting of the last hurdle to GM crop cultivation, regulators also lend an optimistic view on the potential of genome editing technology in Indonesia. The technology is regarded as an efficient tool to bolster food security, and will be a boost for the utilization and value-adding of the country's rich biodiversity (Prasetya webinar, June 2021).

The Indonesian Biosafety Committee of Genetically Engineered Products sets the parameters for policy formulation on genome editing as follows: 1) presence of foreign gene in the end product, 2) assessment of molecular characterization and phenotype. The Technical Committee of Biosafety makes the determination whether the final product contains the transgene. If not, the product is categorized as non-GMO and is then endorsed to non-GM or common scheme of registration. This automatically applies to SDN-1 as the edits only involve a few base deletions; but not to SDN-2 that uses a repair template to introduce new sequences, which may contain foreign gene sequences.

Several research centers in Indonesia have undertaken genome editing research, namely: 1) Biotechnology and Genetic Resources, Ministry of Agriculture; 2) Institut Pertanian Bogor University; 3) State University Jember; and 4) Gajahmada University. The focus crops are rice, orange, chili, sugarcane, oil palm, Artemisia species, and Amorphophallus species (Prasetya webinar, June 2021).

Malaysia

Malaysia has been open to crop biotechnology for more than a decade and is likely to be receptive to genome editing products. The principal policy for GMOs in Malaysia is the Biosafety Act 678 issued in 2007. Technical risk assessment of applications is conducted by the Genetic Modification Advisory Committee and the final approval is granted by the Ministry of Environment and Water. Since 2007, 50 biotech events in five crops have been approved for direct use as food, feed, and for processing. No applications for commercial planting of GM crops have been submitted.

In the meantime, contained use and open release activities for genome editing are regulated by the existing regulatory framework. Gene edited crops will be regulated as LMOs/GMOs if they fall under the definitions of the current policy. The policy is process-based with the use of modern biotechnology as the trigger, such as in vitro techniques which involve the manipulation of genes, use of exogenous DNA, and characterized by a novel genetic combination that results in LMO. It is possible that simplified procedures may be developed in the near future for NBT and applied on a case-to-case basis (Abdullah webinar, June 2021).

Thailand

The scientific community in Thailand has long supported agricultural biotechnology. As early as 1983, the country's National Center for Genetic Engineering and Biotechnology was founded, and the first field trial approval for GM crop was granted to Flavr Savr tomato in 2004. However, resistance from civil society and trading pressures from the large European market have effectively restrained the biotechnology policy. Thus, GM cultivation in farmers' fields has not

been realized and only a handful of GM crops have been allowed for direct use.

While Thailand remains cognizant of the potential of biotechnology, naming it a vital cog in achieving the fourth industrial revolution or the so-called Thailand 4.0 policy, there are no signs that the guidelines will loosen and pave the way for greater GM use and cultivation. It also appears to be a passive observer of genome editing. The country needs to be more proactive and assess its competitiveness amid the technological innovations in the region and global arena. The rapid developments in GM cultivation and great interest in genome editing in its neighbors Vietnam and Myanmar, and the intense genome editing research sweeping across the northern neighbor China, can pose a threat in global trade of common commodities, and present regulatory problems on the biotech products that inevitably cross the border. Furthermore, the global market may accommodate more genome-edited products. A review and appropriate adjustments in their biotechnology guidelines is necessary for the country to keep pace with its neighbors and for its farmers and consumers to enjoy the benefits of plant breeding innovations (Napasintuwong, 2019).

Summary

Previous familiarity and experience with the use and cultivation of GM crops provides a positive vantage point in the acceptance and regulation of genome-edited products. GM user/grower countries that immediately started or laid down the groundwork for genome editing R&D, such as India, the Philippines, Vietnam, and Indonesia, may be the most receptive to genome-edited products and are likely to be the first ones to establish an enabling genome editing policy environment. GM user countries with reservations on GM cultivation, such as Pakistan, Thailand, and Malaysia, may find genome editing less threatening to the environment and thus be more amenable to genome editing cultivation.

The emerging trend points to a consensus that SDN-1 type is clearly out of the main GMO regulation in the countries, and so is most if not all of the SDN-2 type. Most of the SDN-3 type tend to be considered GMOs by most countries, except the Philippines where it is proposed that products from SDN-3 with inserts from the same species be considered conventional.

The process of developing new guidelines, however, faces hurdles from legal, political, and to a certain extent socio-economic considerations.

Legal: the current GM policy in the country may be broad and encompassing that can be interpreted as covering genome-editing. To make it suitable to the less intrusive genome editing, amendments through legislative or executive actions may be required. This is especially true for process-based policies as genome editing uses recombinant DNA.

Political: the current government and heads of agriculture and environment agencies may be passive, neutral, precautionary, or even sympathetic to anti-biotech activists, and thus are not prioritizing the formulation of new guidelines.

Socio-economic: biotech products are not considered by influential social scientists and economic managers as being more advantageous in sustaining the export market, and in raising incomes and general well-being of farmers compared to conventional varieties. Thus, only clear, significant socio-economic advantages of biotech products can encourage adoption.

Finally, countries like most of the rest of the South and Southeast Asian region that never had experience with GMOs may find genome editing a direct extension of the current conventional breeding methods. Provided a modest seed industry, and basic molecular and tissue culture expertise and laboratories are present, they may directly engage in genome editing research and trade. If necessary, minimal adjustments can be made to their regulatory framework for conventional crops to accommodate genome editing.

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New breeding innovations are among key technologies that Africa will rely on to boost agricultural production and achieve food security and nutrition to match the rising demand for food and feed.

Prospects of New Breeding Innovations in Africa

By MARGARET KAREMBU, PhD and GODFREY NGURE

Introduction

Africa is projected to be among the main beneficiaries of new breeding innovations. As the continent approaches the close of the first quarter of the 21st century, it has seen a gradual rise in both challenges and opportunities in building sustainable communities and a thriving society. On one hand, the human population in sub-Saharan Africa has been growing steadily, with a growth rate of 2.6 percent and 1.136 billion inhabitants by the year 2020 (World Bank Data, 2021). This is coupled with rising demand for food, undernutrition, diminishing natural resources, and worsening effects of climate change on the environment and agricultural production (Coulibaly et al., 2020).

On the other hand, the continent has recorded a positive trend in the adoption of science, technology, and innovations (ST&I) in addressing intractable societal challenges. Primarily, manufacturing and service economic sectors are now benefiting more than ever from innovative solutions of biological, chemical, physical, engineering, computer and information sciences. This has in part been inspired by formulation and implementation by governments, of policies that

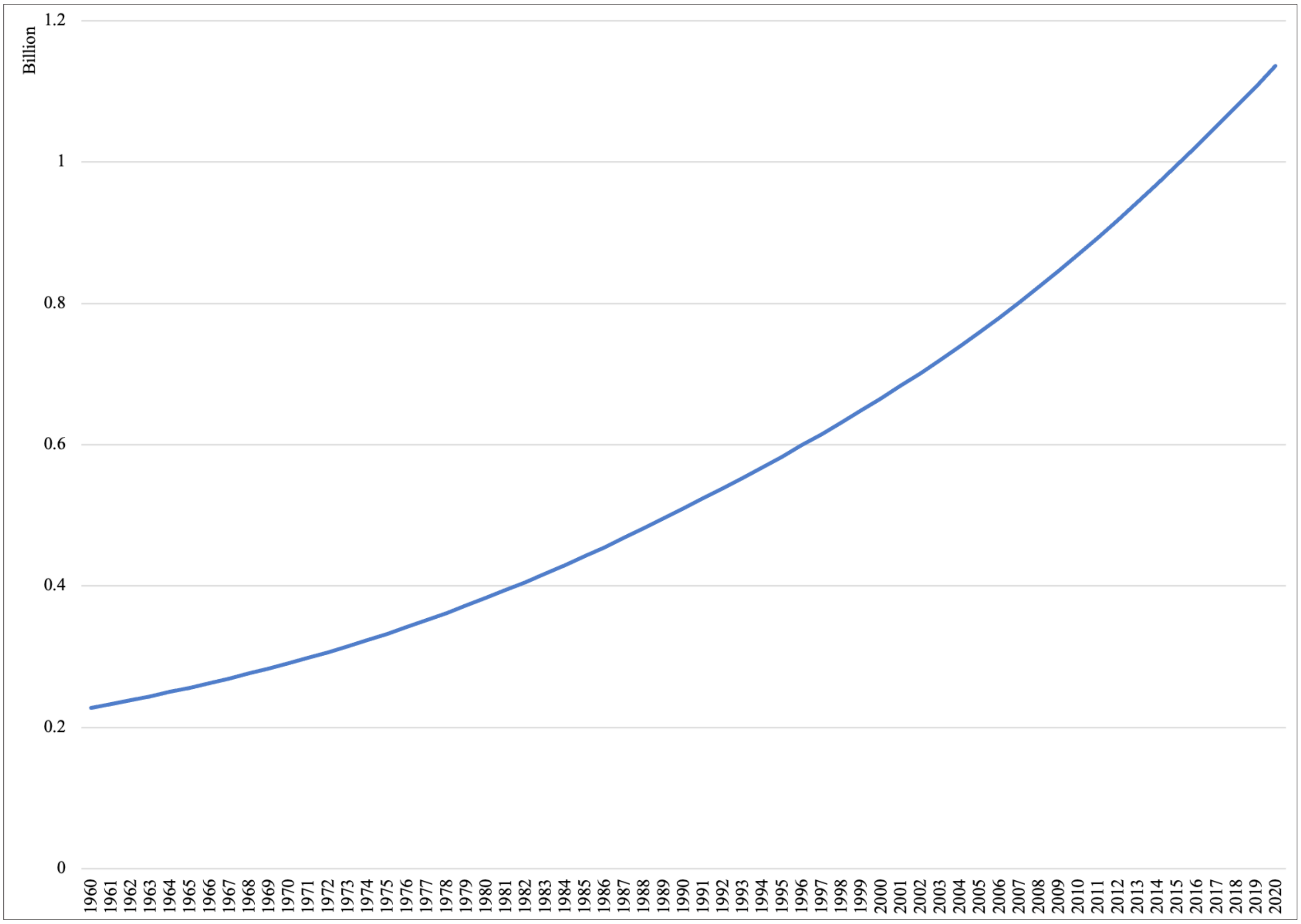

Figure 1. Human Population in sub-Saharan Africa by 2020 (World Bank Data, 2021)

promote harnessing the power of ST&I in not only solving problems but also spurring socio-economic growth and development.

New breeding innovations are among the key technologies that Africa is eyeing to boost agricultural production and achieve food security and nutrition to match the rising demand for food. In the past decades, Africa has been slow in adopting modern food production technologies. The onset of the Agricultural Transformation Agenda I in Africa in the continent was based on selecting plant and animal varieties/breeds with higher yields, resistant to biotic and abiotic stresses and adaptable to changing climatic conditions. In crops, African breeders have been selecting for better varieties using conventional breeding techniques of introgressing agronomic traits between sexually compatible species. The selection process of crossing and backcrossing varieties to generate better hybrids have produced most of the crop varieties being cultivated by African farmers today.

Conventional breeding is however cumbersome, expensive in time and labor, and is limited by linkage drag – where the resultant cultivars bear deleterious genes inherited alongside the targeted beneficial genes (Peleman and Van der Voort, 2003). In addition, conventional breeding is heavily reliant and therefore limited by genetic variability and sexual compatibility within a select gene pool. Moreover, the long generation time characteristic of conventional breeding is out of touch with the current urgency to develop better crop varieties to match the growing demand for food and feed in a challenging, rapidly changing agro-ecological environment in sub-Saharan Africa.

Therefore, the advent of new breeding innovations has presented Africa with an additional, more efficient tool for improving agricultural productivity. In crop and animal breeding, these innovations will improve the ease, speed, precision, cost and generation time of higher yielding, superior varieties and breeds with durable resistance to pests, diseases, efficient use of water and nutrients, and adaptable to climate change. However, deploying new breeding innovations in the continent will rely heavily on development and implementation of policies that foster an enabling environment for research, development, and adoption.

Primary Focus of Research, Development, and Adoption

Primary focus of new breeding innovations in sub-Saharan Africa will respond to the prevailing specific agricultural production challenges, as well as improvements in crops and animals that will enhance food and feed security. This requires extensive consultation between researchers, farmers, government ministries, and private sector players in the agri-food sector. Research must be preceded by a thorough needs assessment to identify gaps and Africa context-specific challenges. Preliminary consultations have identified the following research focus areas for new breeding innovations in sub-Saharan Africa (Table 1).

Trailblazer Projects: Genome Editing in Africa's Agriculture

African researchers are on the frontline of employing new breeding innovations to provide solutions to challenges in agricultural production. Particularly, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 genome editing technology is being used by research teams to improve crops and livestock. Research is primarily being focused on developing crops and animals resistant to economically important diseases and pests endemic in the continent. In this paper, we highlight some of the trailblazer genome editing projects in Africa.

1. *Evaluation of Striga resistance in Low Germination Stimulant 1 (LGS1) mutant sorghum*

Parasitic weed Striga is a huge constraint to the production of sorghum and other cereal crops. Most cultivated cereals, including maize, millet, sorghum, and rice, are parasitized by at least one Striga species, leading to enormous economic losses. The Striga genus has over thirty species

Prof. Steven Runo of Kenyatta University in a Striga-infested crop field in Kenya (Photo by Joel Masanga, KU)

Table 1. Preliminary research focus for new breeding innovations in sub-Saharan Africa.

Focus	*Challenge	Intervention
Crop Breeding	Crop diseases (viral, bacterial, fungal)	Breeding disease-resistant, farmer-preferred varieties
	Crop pests (insect pests and parasitic weeds)	Breeding insect-resistant, farmer-preferred varieties
	Abiotic stress – heat, drought, salinity	Breeding stress-tolerant, climate-resilient varieties
Animal Breeding	Animal diseases (ASF, AAT, ECF, CBPP, CCPP, Brucellosis)	Breeding disease-resistant breeds
	Low productivity (meat, milk, eggs)	Breeding high-producing, fast-growing breeds
	Heat stress	Building heat-stress tolerance in livestock

MLN screening facility in Naivasha, Kenya (Photo by L.M. Suresh, CIMMYT–Kenya)

distributed over 50 countries in sub-Saharan Africa (SSA), causing an estimated 7 billion dollars worth of crop losses every year. This project is evaluating LGS1 gene knock-out in conferring Striga resistance in sorghum. Preliminary results show that mutant alleles at the LGS1 locus drastically reduce Striga germination stimulant activity. The project is being led by Prof. Steven Runo, Professor of Molecular Biology at Kenyatta University (KU), Kenya.

2. *Genome editing to control maize lethal necrosis in Africa for improved maize productivity and grain harvests*

Maize lethal necrosis (MLN) disease causes severe losses to maize in Kenya and neighboring countries. Traditional breeding approaches are time-consuming and disrupt the favorable characteristics of elite varieties, whereas genome editing can achieve MLN resistance without altering desirable traits and performance of the target susceptible elite lines and varieties.

This project is working to introduce resistance against MLN disease directly into parent inbred lines of popular commercial maize varieties, which are currently susceptible to the disease, and reintroduce them into the farmers' fields in Kenya with possible scaling out to other countries in East Africa. The collaborative project is being led by Dr. James Karanja, a senior research scientist and Head of MLN section at Kenya Agricultural and Livestock Research Organization (KALRO). Project partners include Corteva Agriscience, International Maize and Wheat Improvement Center (CIMMYT), and the United States Department of Agriculture – Agricultural Research Service (USDA-ARS).

3. *CGIAR research program on roots, tubers and banana (CRP-RTB)*

The Consortium of International Agricultural Research Centers (CGIAR) research program on roots, tubers, and bananas (CRP-RTB) is working to harness the untapped potential of crops in order to improve food security, nutrition, income, climate change resilience, and gender equity of

Dr. Leena Tripathi at IITA Research Facility in Nairobi, Kenya (Photo by Jaindra Tripathi)

smallholders. One aim of the project is to use genome editing to target disease susceptibility loci of popular roots, tubers, banana varieties, and promising breeding stocks. In Africa, the project being implemented in Ethiopia, Nigeria, Tanzania, Cameroon, DR Congo, Ghana, Kenya, Malawi, Mozambique, Rwanda, Uganda, Zambia, Burundi, Congo, and Ivory Coast. In Kenya, the program is led by Dr. Leena Tripathi, Principal Scientist at the International Institute of Tropical Agriculture (IITA).

4. *Application of reproductive biotechnologies to develop a transgenic goat as a model for genetic control of animal diseases*

Animal African Trypanosomiasis is one of the diseases that cause huge losses to livestock-dependent communities in sub-Saharan Africa. Efforts for its control and eradication have not been successful. Scientists have discovered a gene (Apolipoprotein L1 or APOL1) in primates that encodes proteins that cause lysis of trypanosomes in the body, hence making the primates resistant to trypanosomiasis. A group of scientists from New York State University (Jayne Raper and team) have developed a synthetic version of the ApoL 1 gene that is compatible with the caprine genome. This gene could be transferred to livestock to develop genetically resistant animals through transgenesis. This project is investigating the feasibility of introducing a synthetic *APOL1* gene into the genome of a group of goats and evaluating resistance to trypanosomiasis. The project is being conducted by Wilkister Nakami, a Ph.D. Graduate Fellow at the International Livestock Research Institute (ILRI).

5. *The Mzima cow project*

In partnership with the Centre for Tropical Livestock Genetics and Health, ILRI is applying genome editing in a research project - The Mzima cow, aimed at improving cattle production in Africa that are resistant to trypanosomes, the parasite responsible for African sleeping sickness in humans. The disease, prevalent in 36 countries of sub-Sahara Africa is caused by extracellular protozoan parasites – Trypanosoma that are

Dr. Nakami Wilkister a PhD fellow working on genome edited goat embryos in ILRI labs, Nairobi, Kenya.

transmitted between mammals by Tsetse flies (*Glossina* sp.). In cattle, trypanosome is a major constraint on livestock and agricultural production in Africa that costs US$ 1 billion loss annually.

6. ***Modulation of energy homeostasis in maize to develop lines tolerant to drought, genotoxic, and oxidative stresses***

Maize is the most important staple food crop in sub-Saharan Africa, consumed by over 300 million Africans (Badu-Apraku and Fakorede, 2017). Over 40% of Africa's maize-growing area faces occasional drought, resulting in yield losses of 10–25% (Fisher et al., 2015). This project focused on metabolic engineering of Poly-ADP-ribosylation pathway (a stress response pathway) to broaden stress tolerance in plants by maintaining energy homeostasis during stress conditions. Knock-down of the maize PARP gene expression using CRISPR-Cas9 genome editing was employed as a strategy for abiotic and genotoxic stress tolerance. The work was conducted by Dr. Elizabeth Njuguna, former Doctoral Fellow at VIB-UGENT Center for Plant Systems Biology, Ghent University, Belgium.

7. *Improving oil qualities of Ethiopian mustard (Brassica carinata) through the application of CRISPR-CAS 9-based genome editing*

The level of erucic acid in Ethiopian germplasm materials, as well as in *Brassica carinata* varieties released earlier, is in the range of 31-51% of total fatty acid, much beyond the nutritionally acceptable level (<5%). The emergence of novel genome editing tools such as CRISPR-Cas9 has opened a good opportunity for improving the quality of *B. carinata* through editing targeted genes so that the crop can be applicable for both food/feed and oleochemical industries. This project is developing *B. carinata*

In 2018, ILRI's Mzima livestock project was expanded to include goats. Goats are significant contributors to the livelihoods and nutritional outcomes in smallholder households. (Photo by ILRI)

genotypes with low erucic and glucosinolate for food and feed application. The project is being led by Prof. Teklehaimanot Haileselassie Teklu, Associate Professor at the Institute of Biotechnology, Addis Ababa University, Ethiopia.

8. ***Developing sal1-mutant drought-tolerant wheat using CRISPR-Cas genome editing***

Drought is one of the primary stresses that limit crop productivity and cause huge economic losses. The development of abiotic stress-tolerant crops like wheat is an important avenue to mitigate these problems and enable good agricultural yields, despite environmental challenges. This project is employing CRISPR-Cas9 genome editing techniques to generate drought stress tolerant wheat by inactivating *sal*1 gene, a negative regulator of drought tolerance. The project is led by Prof. Naglaa Abdallah, Professor of Genetics at the Department of Genetics, Cairo University, Egypt.

Regulatory Oversight for New Breeding Innovations in Africa

Different countries are responding in different ways as to whether products of genome editing should be regulated as GMOs or not. So far, biosafety regulatory authorities in various countries are considering the premise that genome editing, in cases where no novel combinations of genetic material have been created, should be no more regulated than a product of conventional mutagenesis (Komen et al., 2020).

In Kenya, for instance, the National Biosafety Authority has developed guidelines that will distinguish regulation of genome editing from that of GMOs. By June 2021, Kenya's Biosafety Authority had granted approval to seven research projects applying genome editing. They include:

- Banana resistant to Banana Streak Virus (BSV);
- Nutritionally enhanced and diseases resistant yam;

Tumaini, ILRI's cloned Born bull, and his offspring. Mzima cow project seeks to introduce resistance to trypanosomes in cattle via genome editing. (Photo by ILRI)

- Development of vaccines for the control of African Swine Fever in pigs;
- Trypanosome resistant goats;
- Nutritionally enhanced grass peas;
- Sorghum resistant to Striga weeds; and
- Cassava with induced early flowering trait.

Preliminary stakeholder consultations affirm that products of genome editing that do not contain a novel combination of genetic material will not be regulated under the Biosafety Act. Evaluation will be done on a case-by-case basis. In Nigeria, discussions are ongoing on establishing similar guidelines after the country's Biosafety Agency through a parliamentary amendment of the Biosafety Act incorporated genome editing for regulatory oversight. Undoubtedly, the two countries will set the stage for Africa's regulatory approach for new breeding innovations. The African Union Development Agency-New Partnership for Africa's Development (AUDA-NEPAD) and biotechnology stakeholders are also working closely with state agencies to establish regulatory guidelines for genome editing that align with best international practices and the Africa Union's ST&I Agenda.

Communicating about New Breeding Innovations in Africa

Although new breeding innovations present great opportunities in improving agricultural production, communication approaches will either hamper or facilitate their uptake. Dialogue on how products of genome editing should be regulated has already sparked in several sub-Saharan African states. In this light, ISAAA *Afri*Center dedicated the 3rd Africa Biennial Biosciences Communication (ABBC2019) symposium held in Pretoria, South Africa, to conversations on genome editing in the region. Running under the theme *"Getting it Right: Communicating about Genome Editing"*, the symposium provided an opportunity to address key components that will lay the foundation for uptake of genome editing in Africa.

The Symposium's overall objective was to interrogate best communication practices

Dr. Elizabeth Njuguna at VIB-UGENT Center for Plant Systems Biology, Ghent University, Belgium (Photo by Dr. Elizabeth Njuguna)

that will facilitate informed decision-making on genome editing. Stakeholder engagement needs to keep pace with rapid advancements in research, to avoid the adoption of restrictive regulatory frameworks. Key players in genome editing research, development, policies, and regulations must embrace constructive dialogue about the technology early. The following are the recommendations from ABBC 2019:

i. To work together in improving bioscience communication, including the use of new and emerging strategies to ensure effectiveness.
ii. To foster open and transparent dialogue with all stakeholders, including those with divergent views on genome editing, in an effort to build consensus and common understanding.
iii. To encourage public participation in research direction and policy formulations on genome editing.
iv. To create awareness among the policy and decision-makers on genome editing.
v. To establish an African Coalition for Communicating about Genome Editing.

The African Coalition for Communicating about Genome Editing was officially launched in September 2021 during ABBC2021, which ran as a hybrid, in-person in six African countries – Ethiopia, Ghana, Kenya, Malawi, Nigeria, and Uganda while the rest of the international community joined virtually. The leadership of six African universities and the African Union Development Agency (AUDA-NEPAD), among other actors pledged their support for the Coalition, describing it as necessary in shaping the narrative and public perceptions on emerging gene technologies in Africa. To initiate the Coalition, country chapters will customize their communication plans modeled on a blueprint communication strategy agreed upon by member states. Other organizations have added to the concerted efforts of communicating about new breeding innovations in Africa by engaging with key stakeholders. These include the Network of African Science Academies, Cornell Alliance for Science, African Seed Trade Association, the Consortium of International Agricultural Research Centres, among others. These efforts

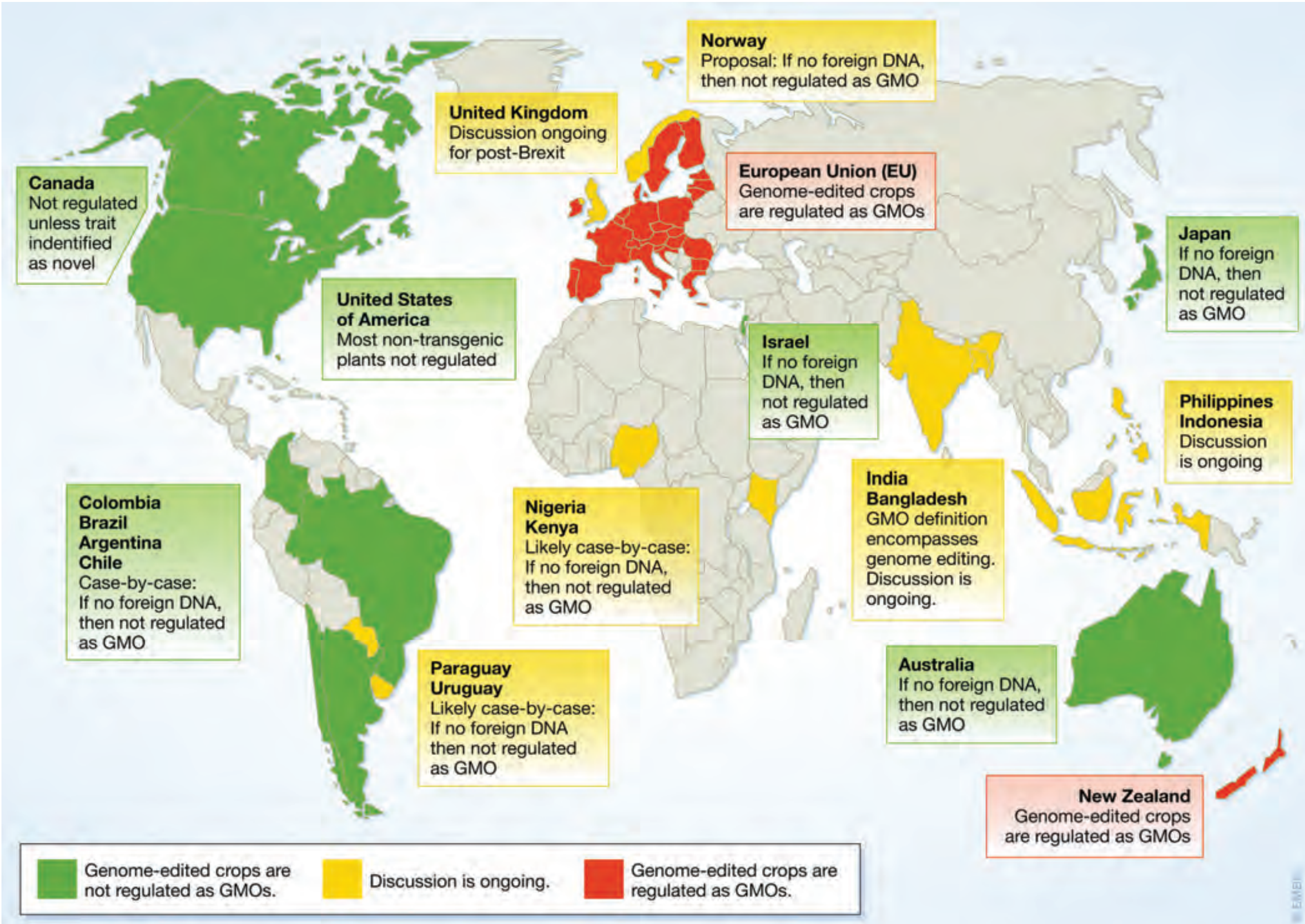

Figure 2. Regulatory approaches for genome editing in various countries (Schmidt et al., 2020)

will build a common public understanding of new breeding innovations and their application essential in shaping the dialogue for cultivating political goodwill.

Conclusion

The worsening impacts of climate change on food production, coupled with the increasing demand for food and feed due to the burgeoning population has seen an increased prevalence of undernutrition. In 2019 alone, prior to the COVID-19 pandemic, almost 690 million people (8.9% of the global population) were undernourished (WFP Hunger Map, 2020). Globally, the first quarter of the 21st century has seen a major increase in undernourishment (World Food Programme, 2020). Without fast and efficient interventions, the number of hungry people will reach 840 million by 2030. In Africa, over 250 million people (20% of the population) are undernourished. This situation has necessitated for rapid adoption of science, technology, and innovations that improve the way food is produced.

New breeding innovations have greatly increased the efficiency of improving crops with disease and pest resistance, abiotic stress tolerance, and improved nutritional content. In Africa, CRISPR-Cas9 genome editing is already being used by researchers to address various crop production challenges. Due to its ability to generate genome-edited crops similar to those developed via conventional breeding, the technology is now regarded as one of the versatile tools for improving agricultural productivity to feed the rapidly growing population amidst climate change and dwindling arable land.

Modern biotechnologies are projected to play a critical role in building sustainable agricultural systems able to accommodate the rapidly growing demand for food. Breeding of 'climate-change ready' and adaptable crop varieties and animal breeds is now more than ever critical in transforming agricultural productivity and ensuring global food security and nutrition. African scientists are moving fast to harness the potential of new breeding innovations in developing crop varieties suited for the continent's modern agriculture. This spells a promising future where the inevitable impacts of climate change and the growing population are well mitigated through technology-supported, sustainable agricultural systems.

Abbreviations

ASF	African Swine Fever
AAT	Animal African Trypanosomiasis
ECF	East Coast Fever
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
WFP	World Food Programme

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Do we need different communication strategies for genome editing technology, drawing lessos from our past experience in GM technology?

Communicating Genome Editing: Editing the Bloopers from the Past Science Communication Strategies

By MAHALETCHUMY ARUJANAN, PhD

The excitement of scientists over emerging technologies is often reciprocated by the public with concerns and fear. The mismatch does not end here. Scientists, on the other hand, reciprocate public concerns on technologies with loads of unintelligible data.

The year 2021 marks the silver jubilee for the adoption of genetically modified (GM) crops, yet the concerns, fear, pseudoscience, and conspiracy theories surrounding these crops have not subsided. In fact, critics of the technology keep raising the bar and demanding “nice-to-know” information that has no bearing on GM food safety.

While GM technology is still making its way into many countries with 29 cultivating countries so far and 72 have issued 4,485 regulatory approvals (ISAAA, 2019), we have another new tool in the toolkit – genome editing. Scientists have found an almost perfect set of tools to repair or delete faulty DNA; or make existing genetic makeup confer beneficial traits, hence, it is called “genome editing”. In many instances, genome editing may not involve insertion of foreign genes at all, which is the cause of excitement among scientists.

The golden questions – will genome edited crops and foods face the same fate in the eyes of regulators, politicians, and consumers? Is scientists' hope that this technology will bypass regulations and public opposition far from reality? Do we need different communication strategies for genome editing technology, drawing lessons from our past experience in GM technology?

The need to unlearn and relearn to communicate genome editing

There is a need to revisit the current communication models and adapt them to suit recent public sentiments, ideologies, information sources, science knowledge, and social behaviors.

A quick look on science communication models and their impact is presented in Table 1.

From the dawn of the genetic modification era, scientists have been very accustomed to the deficit model when engaging with the public. A "top-down" linear transmission of knowledge from experts to lay public is the preferred model for scientists, though it is deemed to be ineffective and creates a barrier between the experts and the public. Here is how Craig Cormick (2019), a well-known science communicator describes deficit model:

> *Put simply, the model implies that people make 'wrong' decisions or have 'wrong' attitudes to science simply because they don't have the right information. And if only the right information was given to them, they would think more positively about the science under consideration. They have a deficit of correct information.*

Cormick further says people have their own ideas and beliefs and knowledge and are not 'empty vessels' waiting to be filled with science information. This is probably the most important aspect scientists who communicate emerging technologies like gene editing have to unlearn.

Table 1. The Different Communication Models

Communication Model	*Challenge	Intervention
Deficit	• The public is perceived to have gaps in their knowledge of science • It is the duty of experts to teach the public and fill in these gaps through outreach programmes • Information flow is one-way	• Creates distrust among the public due to lack of transparency and empathy • The knowledge and concerns of public are ignored • Widens the barrier between experts and the public
Dialogue	• Two-way communication • Experts are still viewed as the authority for science but the public has the opportunities to ask questions, respond and play a more active role in shaping the political and social repercussions of science	• Builds trust and connections • Concerns from the public are addressed
Participative	• Creation of shared identity and sense of equality among participants • Seeks to embed science in society	• Equal participation of experts and the public • Democratising science • May create opposition to science
Lay expertise	• Gives importance to local knowledge, sometimes known as "lay knowledge" • Seeks to empower	• May lead to pseudoscience • May create opposition to science

They need to relearn that while science may still be new knowledge to many sectors of the public, society is becoming more vocal, opinionated, and has access to diverse sources of information that shapes their opinions.

The problem does not seem to end there. Anti-science activists, on the other hand, prefer a more extreme model (lay expertise) that empowers lay public to be the experts that leads to pseudoscience and opposition to science. This has given rise to the post-truth, post-trust, and post-expert era where many public opinions on science stand at odds with scientific evidence.

There is a clear trend among scientists and anti-biotechnology activists in their choice of communication models and this has contributed to polarized discussions on genetic modification. This has to be fixed in the gene-editing era. It is time experts move towards the dialogue model where the strategies could be described as:

- Experts see the public's diverse needs;
- Public view is sought;
- The public talks back; and
- Experts address the issues.

The ultimate goal of the dialogue model is to build public trust through transparency. Previous experience in communicating GM technology has proven that no amount of science can effectively change the public's ideology, value and behaviour on science-related matters.

Editing the Bloopers from the Era of Genetic Modification

Many grave and costly mistakes were made in communicating GM crops. These mistakes will serve as great lessons in developing effective communication strategies for gene-edited crops.

Blooper 1: Good science needs good communication and not propaganda rhetoric
The propaganda rhetoric of advocates of GM technology might be alienating the sectors of the public who are risk averse. Public engagement on gene technologies often starts with the conversation of opposing views between experts and the public, which creates a lot of mental noise, distrust, and cognitive dissonance. Cognitive dissonance is described as mental discomfort

when a person has two or more opposing beliefs or is presented with values that do not align with his/hers. Individuals face strong psychological pressure to conform their risk perceptions to values that align to theirs and reject competing information (Kahan, 2012).

Imagine the pressure to reject strong propaganda such as "GM crops alleviate poverty" and "GM crops are needed to feed the global population" among the naysayers who oppose GM as a result of cognitive dissonance. The easiest way to resolve the conflicting views is by rejecting views that are against ours. This is exactly what our critics do when we present them with the benefits of GM technology.

The biotechnology critics' mental noise and dissonance have always been ignored when experts engage them, creating a greater barrier. Experts keep drumming their views to the opponents who are then pushed back to their own values and ideology and in fact, come back stronger in opposing the technology.

This has to be rectified in the era of gene editing. It is time to move away from extreme and overpromising propaganda. Benefits of gene editing should be packaged in a way that does not oversell the technology, and risks are openly discussed to build trust.

Blooper 2: Lack of shared values

Shared values are the basis of trust. In the past, conversation between experts and the public did not start with shared values. The public was preoccupied with their concerns on GM technology and the experts with the benefits of it. Both were typically speaking different languages.

Start the conversation with shared values and not the benefits of the technology which is the point of contention. Sharing examples that the public could immediately relate and see the impact of GM technology might open room for deeper conversation. Insulin is a good example. Cheese is a GM product that is unknown to many. Rennet used in cheese making is found in the lining of the calf stomach. Why kill calves for the enzymes if these enzymes can be produced through genetic modification by bacteria and yeast? How GM technology supports animal welfare and provides an option to vegetarians are values to be shared. Can we find similar values for gene editing?

Blooper 3: Communication is not just about giving information

Experts are used to giving information instead of asking questions. Asking questions makes the audience evaluate their claims and accusations against GM technology. A question I often pose to my audience is if they have seen tomatoes, chilies, wheat or any crops that we cultivate growing wildly in the forest when they go hiking. Not coming across these crops simply means all the crops that we cultivate are genetically modified in one way or the other. Claims do not have to be rebutted by the experts. Allow the audience to justify their claims.

Blooper 4: Data that lacks soul

Experts are used to crunching and sharing data. Science lacks storytelling. Scientists tend to be too cerebral, too literal-minded, and too unlikeable. To top it off, they are poor storytellers and poor listeners (Olson, 2009).

Data reaches the heads whereas emotion reaches the hearts. We tend to think with our hearts when presented with conflicting views and emotive arguments cannot be fought with scientific reasoning. What is needed are anecdotes and real-life stories relating the benefits of the technology and not just numbers.

Blooper 5: Too much farmer-centric messaging

The first generation GM crops brought more benefits to farmers and indirectly to consumers. As much as the public is concerned about farming, food security, and sustainable development, no one wakes up in the morning worrying about these issues. Knowledge shared must be relevant to the audience. Reduced pesticides would mean less pesticide residues in our food and water. Reframing the messages and making it relevant to the general public would make gene-editing part of everyone's life.

Elements for communicating gene-editing

Where the gap between the expert and the public is big and shrouded with distrust and ambiguity, the first step in communication is to bridge the gap by building trust and showing genuine intention to alleviate public concerns. Most technologies like aviation, automotive and pharmaceutical drugs are not fully understood by the public, yet they are used without grueling questions. The reason is because of the trust the public has on its regulations, authorities and experts. The same level of trust and transparency has to be developed for gene editing. Once this is done, messages have to be developed for individual sectors of the public and delivered in channels that they trust and are available to them in a story telling manner using real anecdote. This approach will bridge the current gap between experts and the public.

1. ***Trust:*** Speak as a community member and not just as an expert. Adding human factor like sharing personal anecdotes creates the connection with the audience.
2. ***Transparency:*** Be open about risks and shortcomings of the technology.
3. ***Storytelling:*** Storytellers rule the world and critics of the technology are doing a great job on this compared to experts.
4. ***Customization:*** There is no cookie-cutter approach in communication. Each target audience needs customized messages.
5. ***Social media:*** One of the best information source for the public yet underutilized by scientists.

Gene editing is an exciting technology that offers many great messages that will resonate better with the public and if these are packaged well, the opposition from the public could be minimized. Some examples are:

1. The technology is more accessible to public sector institutes and smaller companies, crushing the earlier accusation that gene technologies are monopolized by multinational companies.
2. New traits are more consumer-centric.
3. A number of gene editing technologies might not have any foreign gene inserted, making them non-GMO.

The Way Forward

Gene editing is an extension of a natural process and has huge application in agriculture, medicine and environment that could lead us to sustainable development. It is based on a natural DNA repair mechanism that could improve traits in crops and animals to provide higher yield; reduce waste; support animal welfare; reduced usage of agricultural outputs like fertilizers, water and pesticides; and reduce the burden on the environment to produce more for the growing population. In medicine, it is possible to repair genetic disorders in children. It is painful to see the planet ailing and congenital defects among children. This technology has the answer provided the public allow its potential to be explored and adopted.

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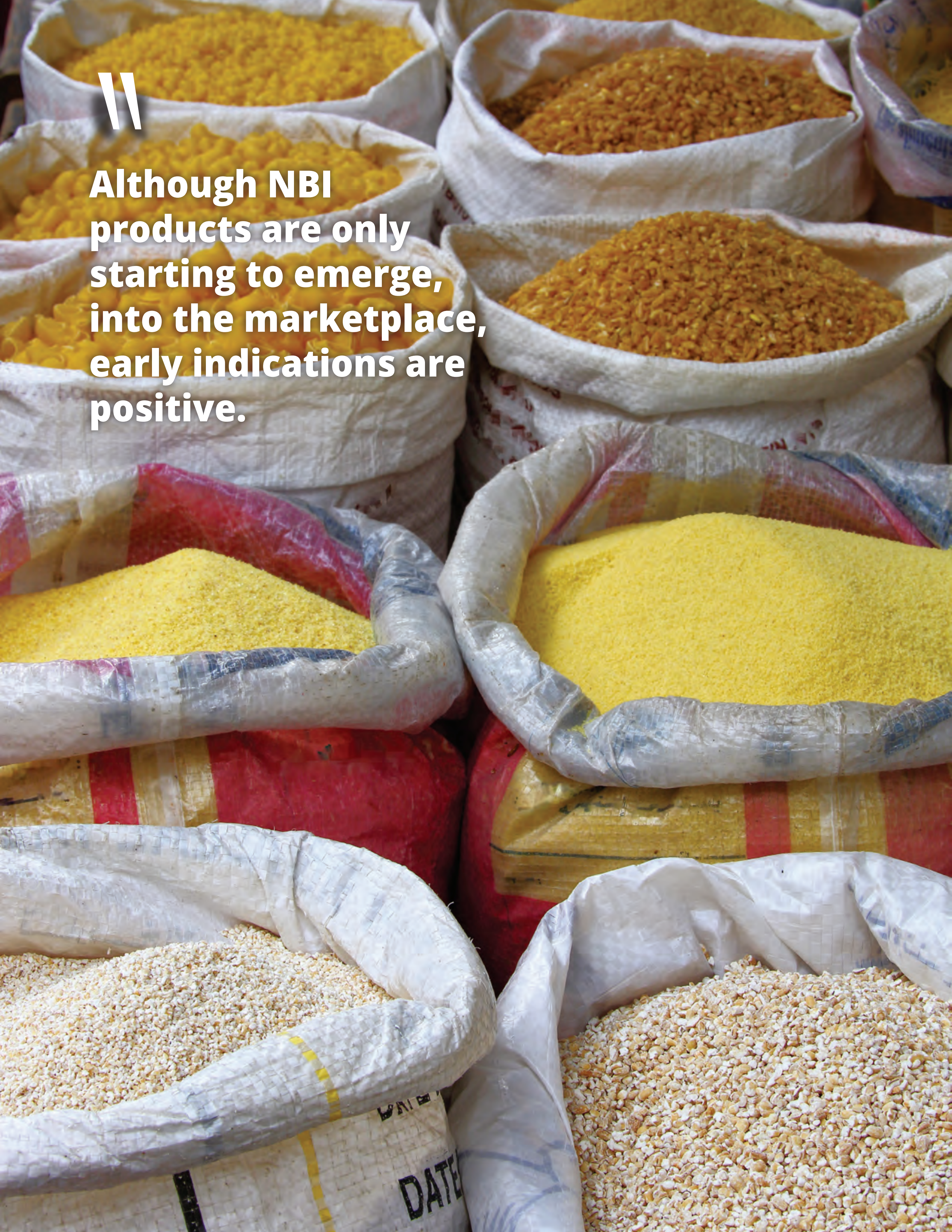

Although NBI products are only starting to emerge, into the marketplace, early indications are positive.

Potential Contributions of New Breeding Innovations in Food Security

By PAUL S. TENG, PhD

Introduction

The world is facing major challenges to feed itself! According to the Food and Agriculture Organization of the United Nations (FAO), there is a need to produce at least 50% more food to meet the demands of the human population by 2050 (FAO, 2017). This comes at a time when the challenges to food production have increased in intensity and prevalence, such as climate-change related severe weather events, changes in the seasonal behavior of rainfall, loss of arable land and freshwater resources, and a declining workforce in agriculture driven by the economic structural transformation process in many developing countries. Additionally, rapid urbanization and changing demographics means that consumers are increasing their demand for sustainably-produced food with minimal pesticide use and higher nutrition. Indeed the world is forced into producing more and higher quality food with less chemicals, less land, less water, and less labor, and under increased stress from variable weather patterns (Montesclaros & Teng, 2021).

In this chapter, the role of the New Breeding Innovations (NBI) described elsewhere in this Primer is discussed with respect to food security. Since food security is a multidimensional phenomenon today, it is necessary to first explain what these dimensions are before describing how NBIs can result in crop traits to assure each of the dimensions.

Food security dimensions with potential genetic solutions

To understand the complexities of food security in a modern world, the scope of food security is captured by the definition proposed by the FAO in 1996, that it is a condition "when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (FAO, 1996). The FAO definition may be interpreted to suggest that food security can only be achieved if the dimensions of availability, physical and economic access, and utilization are simultaneously met. The classification of food security into four dimensions also enables governments to tackle the issue using an approach where each dimension is necessary for overall food security, but may weigh in differently in different settings, such as rural versus urban (Teng and Lassa, 2016). What this also means is to improve the food availability dimension, for example, the smallholder farmer will be the most important in Asia as this group of producers are responsible for the bulk of food produced in Asia (Montesclaros and Teng, 2021). The four dimensions of food security are schematically shown in Figure 1.

Food Availability: Food is commonly made available at a country level through production by farmers, imports through trade or releases from stockpiles (Figure 1). An imperative of this dimension is raising agricultural productivity, particularly for countries with a large agricultural sector. A number of factors impact food production. Changes in supply and price of material inputs such as fertilizers and seeds affect production

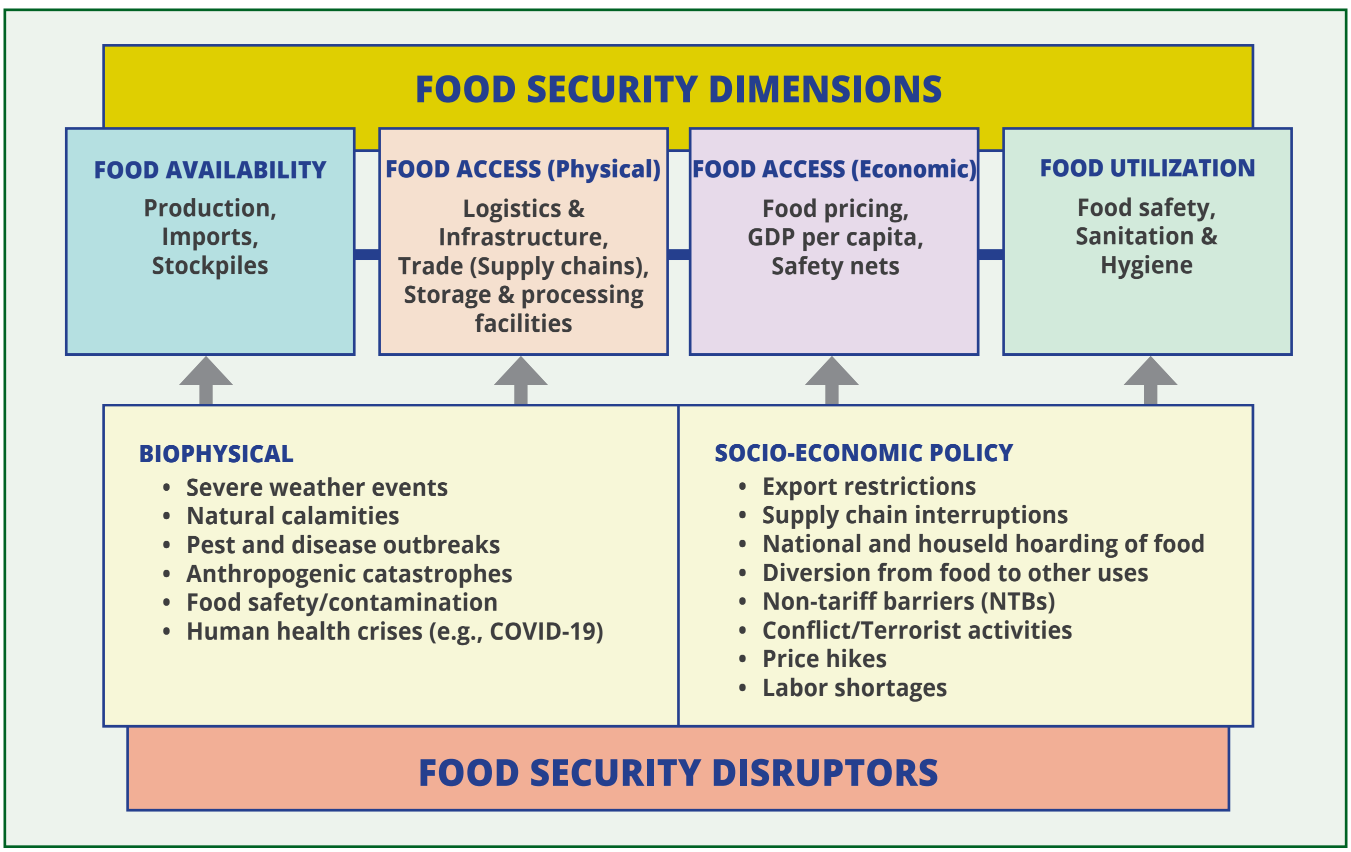

Figure 1. Schematic showing the four food security dimensions and their disruptors.

levels. External factors such as the state of agro-ecosystems, climate change and competition for land will change crop yields, fish catch, and livestock production.

For net food-importing countries that are predominantly urban such as Singapore and Hong Kong, imports and reserves play a larger role. Commodity transactions with supply chain participants can also alter the level of food availability. Unexpected occurrences of natural calamities such as typhoons also affect availability by destroying food crops and livestock (Teng et. al., 2015).

The food availability dimension of food security is the one most affected by farming and farmer expertise, through crop productivity. Seeds with high yielding potential and resistance to stresses, developed using modern breeding techniques will give varieties to farmers which allow them to achieve higher yields (Fischer et al., 2014).

Food losses and wastage, which occur across the food supply chain, further add pressure on food availability. It has been estimated that about one-third of food produced for human consumption, or 1.3 billion tons annually, is unused or discarded globally (FAO, 2011). Hence, reducing food losses and wastage is crucial in improving the efficiency of the food supply chain and increasing food availability. As will be discussed in a later section, NBIs have potential to result in food products that have delayed decay, and therefore reduce the amount of fresh produce that is thrown away.

Physical and Economic Access: The second and third dimensions of food security are access to food, both physical and economic (Figure 1). Countries which import food require reliable supply chains between food exporting and importing countries. Within countries, consumers, and in particular, vulnerable households, must be able to physically reach food supplies, mainly through the marketplace, and the food must be affordable. Non-biological factors that affect access include poor infrastructure, inadequate logistics for food distribution, market imperfections, and war and conflict (Teng and Escaler, 2014).

The importance of infrastructure and technology is seen at various points along the supply chain,

including post-harvest, storage, processing, marketing and distribution. Policies or frameworks that drive up transportation and marketing costs are another concern. For example, regulations affecting inter-island shipping between islands in Indonesia keep transportation prices high. For urban populations, market supply chains are the main distribution channels for food. Thus, in cities, raising the efficiency of market supply chains to deliver food to consumers is a primary concern.

It should also be noted that economic access weighs more heavily in an urban setting where poorer consumers spend a significant proportion of their household budget on food. Factors that influence economic access include employment and income security, macroeconomic policies such as tariffs on food commodities, and market prices. Managing economic access is key since any small increase in price can result in fewer meals a day for the more vulnerable sectors of society, and become a catalyst for civil disobedience. One way to improve economic access is to ensure a plentiful, stable supply of food produced efficiently using modern crop varieties such as those from new breeding technologies.

Food Utilization: The fourth dimension of food security is utilization which is typically reflected in the nutritional status of an individual (Figure 1). Utilization refers to the general diversity and nutritional value of food as well as food safety and proper sanitation. A household may have the capacity to purchase all the food it needs but it may not always have the ability to utilize that capacity to the fullest. There is now greater emphasis on the unique nutritional or health attributes of foods based on the genetic characteristics of the food ingredients. Rapid urbanization and rising incomes have also led to an increase in demand for high-value and nutritious food.

Factors disrupting food security dimensions:
As shown in Figure 1, factors disrupting food security may be divided into two major groups, biophysical, and socio-economic. Each dimension may potentially be affected by a set of factors, for example, food production in food availability (Figure 1) is potentially affected by severe weather events, pests and diseases, natural calamities, and human-induced catastrophes, as well as supply chain interruptions which prevent

agricultural inputs (seed, fertilizer, etc.) getting to farmers to grow their crops.

Some factors may disrupt more than one dimension, for example, severe weather events may directly disrupt availability and physical access, and consequently also affect economic access (by increasing food prices) and nutrition (delays in transporting food from farm to retail shop may reduce the nutritional value of food). The above discussion has pointed out which of the food security disruptors lend themselves to genetic improvement using NBIs and these are the disruptors which directly affect the seed or its subsequent development into crops and eventually harvestable products. The main ones are severe weather events, pest and disease outbreaks, natural calamities and anthropogenic catastrophes (Figure 1).

To help farmers deal with these main disruptors, scientists have used plant breeding techniques to produce crop varieties with specific traits that can allow crops to resist or tolerate the disruption.

Traits from NBIs

A central question that still exists is – How can the NBIs help farmers, especially smallholder farmers, contribute to improving food security by producing more food under all the situations described in the Introduction? One answer lies in giving the farmer the best seeds derived from the latest science and technology because seeds are the foundation on which high crop yields can be expected (Fischer et al., 2014); and these seeds should preferably possess one or more traits.

Higher potential yield: All seed genotypes have a potential yield which is embedded in the seed's DNA and represents the highest possible yield if there were no constraints during the crop growth period (Figure 2, Nutter et al., 1993). However, farmers don't usually get the potential yield in their fields because of the many stresses in the environment. Instead actual yields in farmers' fields may be as low as half of the potential yield and even at best reach about 80% of the potential yield (Fischer et al., 2014). Potential yield may sometimes be achieved in small plots under experimental conditions. However, in the context

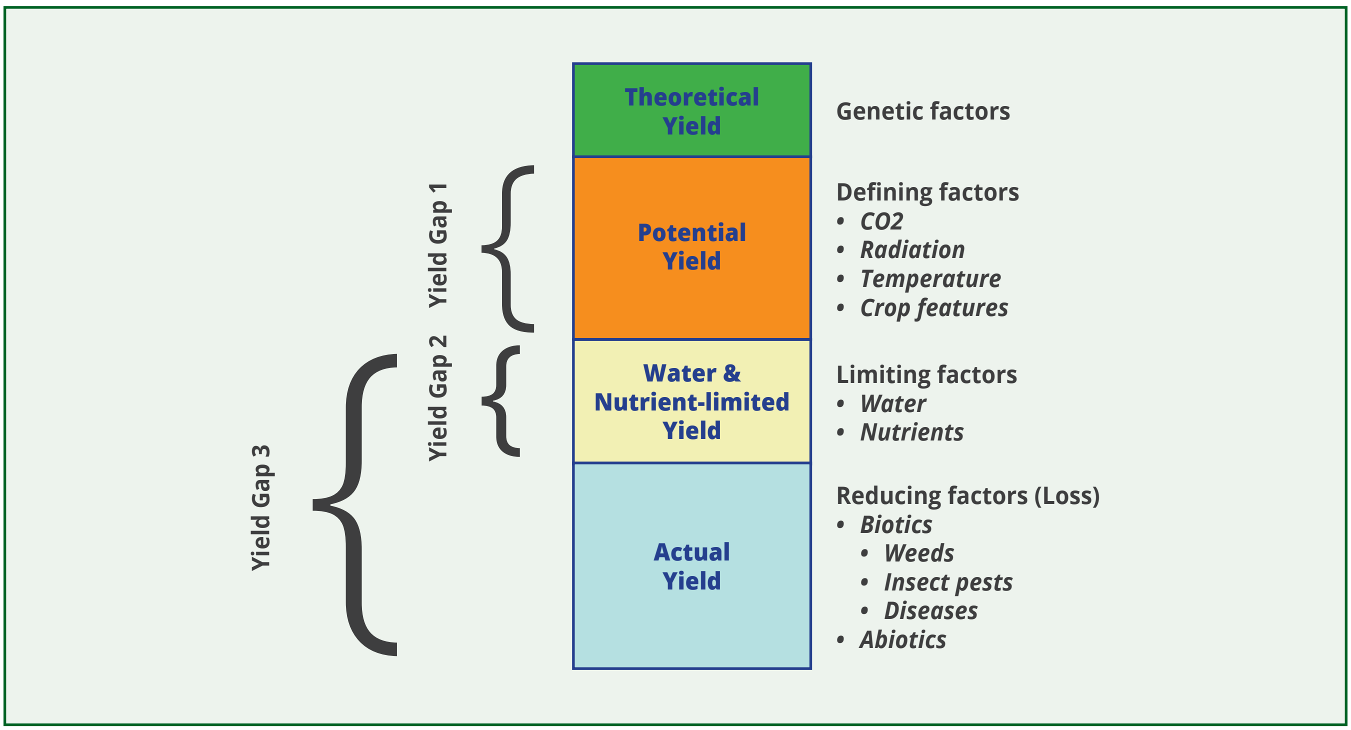

Figure 2. Conceptualization of crop yield levels and yield gaps.

Golden Rice grain with beta carotene-rich foods. Part of the image collection of the International Rice Research Institute (IRRI).

of food security, what is of more interest is the theoretical yield, as the higher this is, the higher would be the corresponding potential yield. Biotechnological approaches such as those represented by NBIs offer opportunities to use existing crop genomes to change the potential yield to approximate theoretical yields. Higher farmer's yields would have a profound effect on raising crop production overall and contribute to food security.

Tolerance to environmental (abiotic) stresses: Environmental stresses and limitations of water and nutrients in the environment are responsible for causing yield gaps between potential and actual yields (Figure 2, Yield Gap 2, 3). The environmental stresses are often described as abiotic, and represent traits to tolerate flooding (submergence) and drought, both of which are known to be multigenically-determined and have been difficult to breed using traditional phenotypic breeding and screening. There is much hope that the use of NBIs such as gene editing alone or in combination with other techniques may quicken development of genotypes with strong tolerance to both. The other aspect of environment tolerant traits is associated with breeding genotypes (or varieties) which use less water and nutrients to achieve the same or faster level of growth and development (Yield Gap 2). This has become even more important as freshwater resources decline due to pollution or overuse, and excessive nutrients delivered through fertilizers contaminate groundwater or make soil unsuitable for cropping (Teng and Oliveros, 2015).

Resistance to insect pests and diseases: Annually, insect pests and diseases are estimated to cause between 30-50% crop losses in many crops, contributing to yield gaps in farmers' fields (Savary et al., 2015). Traditional breeding techniques using Mendelian genetics have been successful with the insect pests and pathogens which have relatively simple modes of infestation/ infection on crops, but many pests and diseases continue to plague crops all around the world. Older transgenic techniques have shown that it is possible to produce crop varieties with sufficient resistance to insect pests and confer value to smallholder farmers, and these have been adopted in millions of hectares worldwide (International Service for the Acquisition of Agri-biotechnology Applications, 2019) NBIs such as genome editing with induced gene silencing (GeiGSTM), a RNAi technique, offers opportunities to build on earlier successes at pest management with biotechnology plants, and enable scientists to tackle some of the most serious diseases and confer resistance to severe diseases such as Panama Wilt on bananas or Blast on rice (https://www.tropicbioscience.com/).

Modified nutritive value or flavor: One of the goals of plant breeding, apart from high yields, is to modify the nutritive value of crops. The highly visible effort to produce the "Golden Rice" is an example of modifying rice to address the Vitamin A deficiency problem in many developing countries (https://www.irri.org/golden-rice). While this uses a different kind of biotechnology, there are now efforts to use the NBI gene editing to change the nutritive value and flavor of food and beverages, for example, the nature of coffee beans to reduce caffeine content (https://www.tropicbioscience.com/).

Delayed decay or senescence: Food loss and waste due to decay is responsible for as much as 30% of food not being consumed (FAO, 2011). This problem has become even more serious as temperatures rise and food supply chains get longer. Although it is genetically possible to delay ripening of fruits and senescence of vegetables, past efforts have used genetic engineering techniques which face heavy regulatory control. There is anticipation that gene editing will enable the development of horticultural crops with fruits which have delayed ripening (Martin-Pizarro and Pose, 2018). Experimental protocols have been developed and in the near future the anticipation is that delayed ripening will become a common feature in fruits, together with delayed senescence in leafy vegetables. Both will help greatly reduce food waste.

Conclusion: Benefits to food security from NBIs

To be better than current or older methods of plant breeding, NBIs need to demonstrate clearly that they can confer benefits to farmers and consumers by addressing the traits previously discussed. Although NBI products are only starting to emerge into the marketplace, early indications are positive.

Early indications from several countries (U.S.A., Australia, Japan, and others) are further, that the crop varieties and seeds produced using NBIs do not need to undergo the complicated regulatory approval processes such as with the older biotechnology crops, as long as no transgenes are incorporated. This would mean that yield gaps faced in many crops and losses caused by abiotic and biotic factors could be drastically reduced by the new NBI varieties, and all in a shorter time frame. This last aspect is critical to global efforts to ensure that food security is still possible by 2050 (FAO, 2017). Ultimately, NBIs have the potential to positively affect all four dimensions of food security.

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