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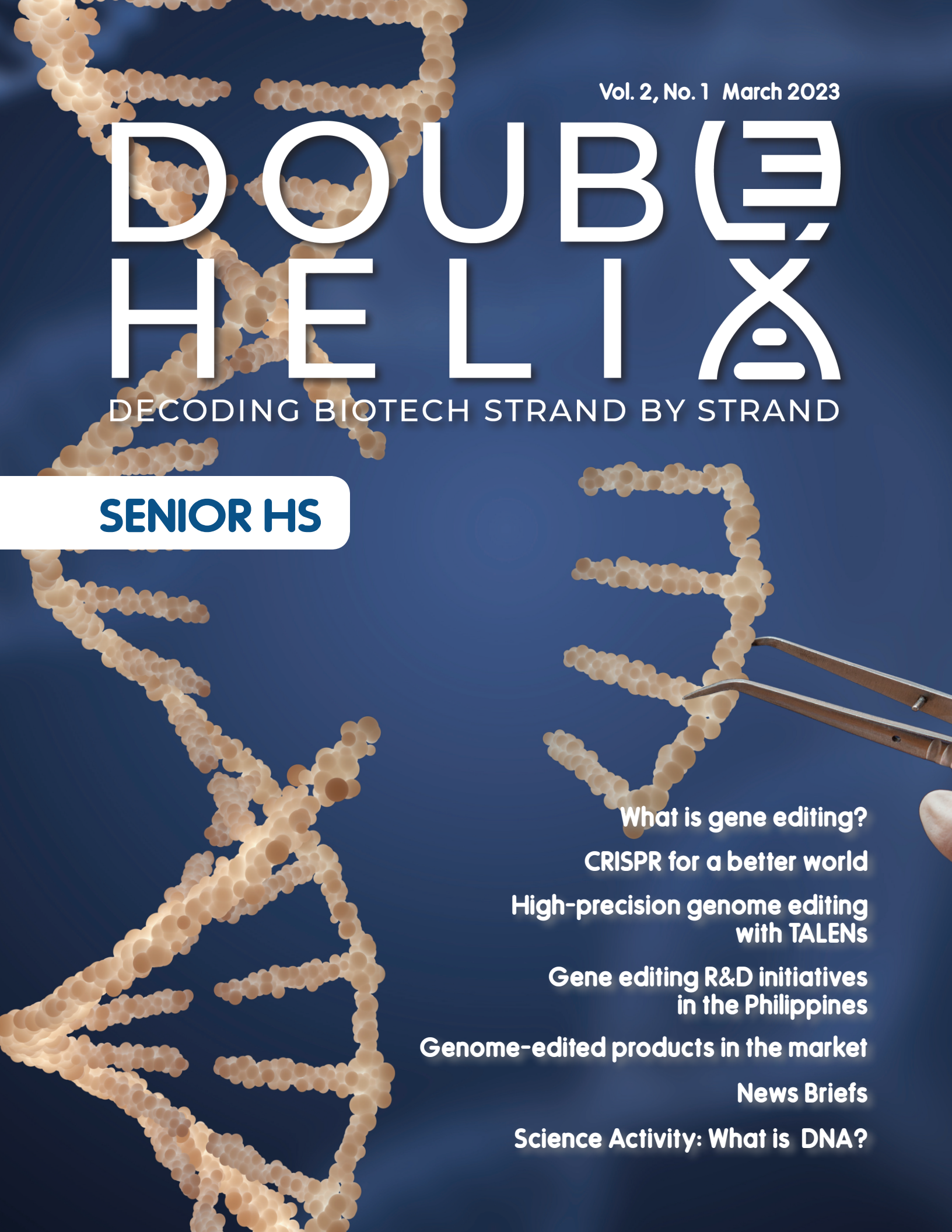
DOUBLE HELIX

DECODING BIOTECH STRAND BY STRAND

SENIOR HS



DOUBLE HELIX
DECODING BIOTECH STRAND BY STRAND

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Welcome!

Dear Teachers, Students, and Readers,

Welcome to the second issue of the *Double Helix*, the only magazine supplement on agricultural biotechnology for senior high school students in the Philippines!

The world is facing major challenges to food production as the global population increases and climate change affects agriculture. Innovative tools such as gene editing allows scientists to develop crops and animals with more precision and speed.

This issue of the *Double Helix* presents gene/genome editing, two editing tools (CRISPR and TALENs), R&D initiatives in the Philippines, and some of the genome-edited products available in the market today. We also included a fun science activity for all of you to enjoy in school or at home.

Through the *Double Helix*, we hope to share with you how innovative technologies such as gene/genome editing help in enhancing crops and animals for food production, health, and the environment.

If there are topics that you want to be covered in the *Double Helix*, send us a message at double.helix@isaaa.org. Please share the *Double Helix* with your family and friends!

— *Double Helix* Editorial Team

NEWS BRIEFS

TOMATOES IN CUSTOMIZED COLORS THANKS TO CRISPR

Using CRISPR-Cas9, scientists from China developed tomatoes that come in yellow, light yellow, yellow-green, light green, brown, and pink. They applied rapid breeding strategy and CRISPR-Cas9 mediated multiplex gene editing on a conventional red tomato cultivar to come up with colorful tomato lines. The tomatoes were developed in less than a year and have the same qualities and agronomic traits of the original cultivar. The same approach can possibly be used on other horticultural crops.

GENE-EDITED LEAFY GREENS TO HIT U.S. MARKETS SOON

American fruit company Pairwise aims to build a healthier world by developing better fruits and vegetables using gene editing. In March 2022, they launched nutritious and flavorful leafy green varieties called Conscious Greens. These are nutrient-dense, leafy greens in rich green and deep purple colors that go well with different kinds of salads. Conscious Greens is expected to hit US grocery stores as packaged salads in 2023.

AGRI STARTUP TO GROW RICE IN THE OCEAN USING CRISPR

Alora, a Canadian start-up company, is aiming to grow rice on land in salty waters by activating dormant genes from seagrasses using CRISPR tools. They found a particular pattern of eight genes in seagrasses and mangroves that work together to allow rice to defend itself against the saline environment and use it to power growth. These genes could be activated in terrestrial crops such as rice, wheat, and corn in the same pattern as these marine plants without introducing foreign DNAs from any other organism. Alora plans to move growing rice on floating platforms off the coasts of African and Asian countries using this technology.

BRAZIL AND COLOMBIA APPROVE FIRST DROUGHT TOLERANT GENE-EDITED SOYBEANS

In December 2022, Brazil approved the first gene-edited soybean developed by the Argentine company GDM. The soybean plant is able to thrive in temperate and tropical climates after researchers isolated one of its genes responsible for sensing water scarcity and influencing growth response in dry environments. After a month, the same company announced the approval of another type of gene-edited soybeans in Colombia that is easier to digest. These soybeans are low in raffinose and stachyose sugars which are indigestible by humans and animals like poultry and pigs.

SPINELESS FISH DEVELOPED VIA GENOME EDITING IN CHINA

After identifying the gene that regulates bone growth in the bream fish, a team of scientists in China produced the world's first patented comprehensive fishbone gene expression. They used gene editing technology to knock out 50 candidate genes one by one to determine the one that affects bone growth. Once the gene was identified, they were able to develop spineless zebrafish with spineless offspring. They tested these genome-edited fish and found that the meat quality, amino acid content, and fatty acid content were more or less the same with that of an ordinary fish.

TALENs USED TO EDIT POTATO

Researchers from Japan were able to conduct genome editing in potato by infecting it with *Agrobacterium tumefaciens* harboring TALEN-expression vector targeting *sterol side chain reductase 2 (SSR2)* gene and regenerated shoots without selection. This resulted in regenerated lines with disrupted-*SSR2* gene and without transgene of the TALEN gene, indicating transient gene expression. They found that Agrobacterial mutagenesis has the potential to accelerate the use of genome-editing technology to modify heterozygous plant genomes.

What is gene editing?

By Kristine Grace N. Tome

As the world population grows, we continue to search for ways to improve crops, animals, and other living organisms to provide food and improve life. From the time when people started domesticating crops, wild plants have changed significantly. Fast forward to the present, scientists can now modify the characteristics of crops with more precision and come up with better varieties in less time. One of the new breeding techniques for crop improvement is referred to as gene editing.

Gene editing is the process of making a targeted change in the DNA of a living organism. It's like editing a Word document on your computer. You can change a portion of that document by finding a certain word or phrase. You can either delete that word, replace it with a better word, or add another word to improve your document. This find-and-replace function is similar to how experts edit genomes.

Gene editing can be done using different tools and processes. The most famous tool is CRISPR-Cas9, which was adapted by scientists from some bacteria's natural gene editing system that they use in response to invading pathogens. Other tools such as TALENs, zinc-finger nucleases, and meganucleases are also available.

Because of its ease of use and versatility, gene editing has numerous applications. One of the first products of gene editing is the heart-healthy soybean oil called Calyno. Using TALENs, the researchers increased the oleic acid content of soybean oil by 80% and reduced saturated fat by 20%. It has been sold in the U.S. market since 2019 and has been well-received by the food industry. More and more experts are using gene editing in their research, especially in improving plant quality traits.

The future of food looks promising. Thanks to gene editing.

ABC KNOW YOUR ACRONYMS

DNA: deoxyribonucleic acid

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

Cas9: CRISPR-associated (Cas) endonuclease

TALENs: Transcription Activator-like Effector Nucleases

LEARN MORE! SCAN THIS!

CRISPR for a better world

By Kristine Grace N. Tome

The most popular tool used by researchers to edit genes is called CRISPR (pronounced as crisper), which stands for Clustered Regularly Interspaced Short Palindromic Repeats. This tool was discovered by Dr. Jennifer Doudna from the University of California Berkeley and Dr. Emmanuel Charpentier from Max Planck Unit for the Science of Pathogens. Their paper published in *Science* journal in 2012 reported that CRISPR-Cas9, a bacterial immune system, could be repurposed as a tool for editing genes.

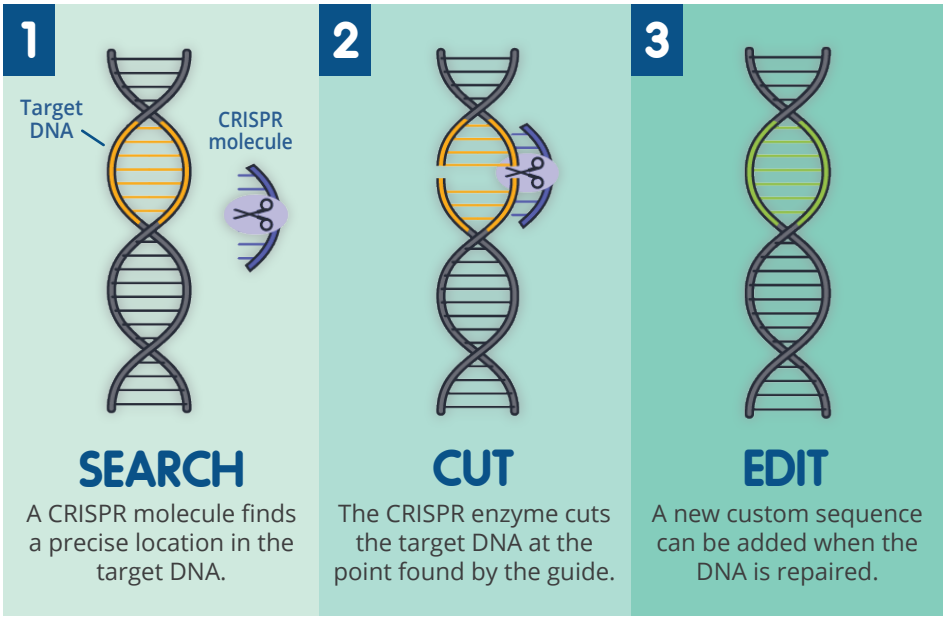


Photo Credit: https://innovativegenomics.org/

Since its discovery, CRISPR has paved the way for a multitude of breakthroughs in healthcare, food, agriculture, and bioenergy. In 2022, a CRISPR-based treatment successfully wiped out a 13-year-old's stubborn leukemia in the UK. Several crops have been developed to withstand pest attacks and diseases. It has also been used to make plants and animals adapt to climate change.

If you are wondering how CRISPR works, here's a quick explanation. It all starts with a desired change in mind of the researcher. Then he identifies the exact sequence of the DNA that needs to be changed. A protein combo containing a guide molecule and a molecular cutter is inserted into a cell. The guide molecule finds the target DNA strand where the change is needed to happen. The molecular cutter chops off the target DNA strand. The damaged DNA strand can either self-repair or be replaced with a short or long healthy DNA copy that can be attached in the protein combo before it is inserted into the cell.

In the Philippines, the government has formulated a specific set of rules to follow for gene editing and its products. Most of these are not considered genetically modified organisms (GMOs), especially if there is no foreign DNA inserted to the genome. The Department of Agriculture oversees the evaluation and monitoring of gene-edited products.

If you want to learn more about CRISPR and other gene editing tools, subscribe to **BIOTECH UPDATES:**

isaaa.org/subscribe

Read more about CRISPR

Plant Breeding Innovation: CRISPR-Cas9
https://bit.ly/CRISPRPK

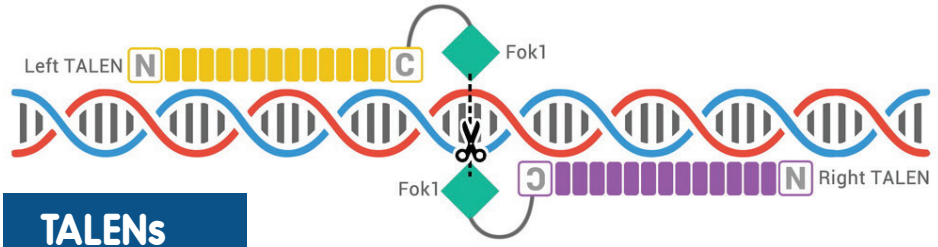
NCBP Policy on Plant Breeding Innovations or New Breeding Techniques
https://bit.ly/PBIPolicyBrief

Another CRISPR First: New Treatment Wipes Out Teen's Cancer
https://bloom.bg/42MwbYh

What is CRISPR and Why is it Important?
https://bit.ly/CRISPRWhatIs

High-precision genome editing with TALENs

By Zabrina J. Bugnosen



TALENs

As the world's population increases every year, scientists are continuously trying to find ways to combat challenges such as climate change and make sure that there is enough food to feed everyone. Those who study biology and agriculture are working with new tools and techniques to do just that. Some of them use precision breeding that involves sensors, detectors, and robotics to achieve accurate and more efficient production system control to develop better crops, and one of these precision breeding tools is called TALENs.

TALENs is short for transcription activator-like effector nucleases. It is a gene editing tool that is extremely precise in targeting any DNA sequence. TALENs are protein combinations made up of two parts. The first part is called TALE and it targets the protein to a specific DNA sequence. The second part is a nuclease (N) that cuts DNA. The most used nuclease for TALENs is called Fok1. What sets it apart from other gene editing tools like CRISPR and zinc finger nucleases is that it can target any DNA sequence with fewer errors. It can tell the difference between DNA modifications that affect a gene's expression. TALENs can also be used to modify DNA found in the mitochondria and chloroplasts.

How do TALENs work? TALE-nuclease fusions cut the DNA resulting in a specific double-strand break, like scissors cutting a string. The break then activates the plant cell's natural DNA repair mechanisms. The repair either removes DNA bases around the cut or puts in new DNA at the position of the break. This can result in either small insertions or deletions that create sequence diversity leading to new functions or inactivating existing ones. The resulting sequence diversity is not unconventional as the same effect is often found in nature. TALENs only helps it to happen with fine-tune traits in a targeted, predictable fashion rather than having it occur randomly in the genome.

CROPS DEVELOPED USING TALENs

POTATOES
Less browning, reduced acrylamide

SOYBEANS
High oleic oil, low polyunsaturated fats

WHEAT
Powdery mildew-resisant

RICE
Bacterial blight-resistant

TALENs have been incredibly useful in developing crops with favored traits that makes their yields more appealing to eat or make the plant itself resistant to certain diseases. An example is rice which is resistant to bacterial blight disease. Using TALENs, researchers edited a region in the rice genome that is essential for the *Xanthomonas* bacteria to infect the plant. This prevents the rice plant from being infected with bacterial blight while maintaining the heritability of the resistance. Scientists also used TALENs to knock out the potato's vacuolar invertase gene to come up with potato varieties with a reduced level of cancer-causing acrylamide and an appealing light color. And more recently, TALENs have been used to precisely edit the mitochondrial DNA of living cells. This opens opportunities for developing treatments for mitochondrial-associated diseases like muscular dystrophy or Alzheimer's disease.

TALENs is a useful gene editing tool to improve plants and other organisms. It will be exciting to see its progress to increase food security and improve human welfare in the coming years.

LEARN MORE! SCAN THIS!

Gene editing R&D initiatives in the Philippines

By Dr. Reynante Ordonio

The Philippines had its first policy on products derived from New Plant Breeding Techniques (NBT) or Plant Breeding Innovations (including gene editing) in 2020-2022 and this is expected to stimulate local modern biotech research and facilitate international trade involving gene-edited plants. The release of the policy is timely as several gene editing initiatives in the country are expected to give rise to non-GMO plants which could be overregulated if subjected to the same protocol used for Bt Talong and Golden Rice. Currently, gene editing initiatives in the Philippines are only on plants and research is mostly for crop improvement as shown in the following traits.

Improved photosynthesis

In 2017, researchers from the International Rice Research Institute (IRRI) used CRISPR-Cas9 and Cas12a to decrease the stomatal number of rice by knocking out the *EPFL9* gene. This is a milestone in the C4 Rice Project, which aims to develop rice with better photosynthetic capacity that can result in up to 50 percent higher grain production while using less water and nutrients.

Resistance to diseases

In 2018, IRRI researchers reported the development of rice tungro spherical virus (RTSV)-resistant rice using CRISPR-Cas9 that targeted the *eIF4g* gene. This was followed by the development of rice with bacterial leaf blight (BLB) resistance using CRISPR-Cas9 and TALEN to make the rice sugar transporter genes (*SWEET11*, *13* and *14*) insensitive to the pathogen *Xanthomonas oryzae*. Since 2021, through the support of the Department of Agriculture (DA), a similar gene editing research on RTSV and BLB but using other varieties is being done at the Philippine Rice Research Institute (PhilRice) to capacitate the newly established DA Crop Biotechnology Center (CBC).

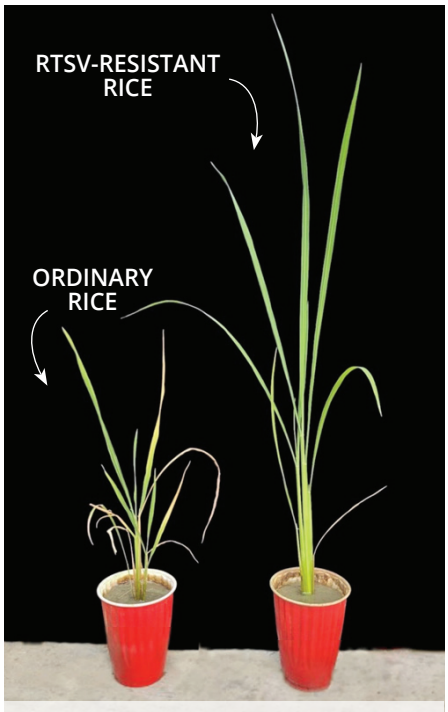


Photo Source: PhilRice

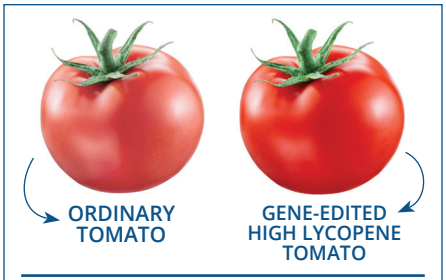
Improved quality

To reduce the amylose content and the associated hardness of stored cooked rice, DA CBC is targeting the *OsWaxy* gene of some varieties. Another is the knockout of *BadH2* gene to induce aroma in some rice varieties. On the other hand, the University of the Philippines Los Baños (UPLB) is knocking out eggplant's *polyphenol oxidase* (*Ppo*) gene to reduce browning in cut fruits.

Improved grain yield

An unpublished gene editing research conducted at IRRI successfully showed that a loss-of-function mutation in *Thousand grain weight 6* (TGW6) and *grain number 1A* (GN1A) genes using CRISPR-Cas12a resulted in increased grain weight and grain number using IR64 rice variety.

Improved drought resistance Current research at DA CBC strives to improve root branching and elongation to better adapt to drought conditions by knocking out the *Weg1* gene of rice.



The accumulation of lycopene results in redder tomatoes.

Illustration of ordinary vs. gene-edited tomato.

Improved nutrient value

The UPLB Institute of Plant Breeding has been working on reducing the phytic acid content of corn to improve its nutritional value by targeting the *ZmIPK1* gene. They are also developing tomato rich in lycopene by knocking out lycopene cyclase genes that convert lycopene to carotenes. The accumulation of lycopene results in redder tomatoes.

These researches are still in the development stage and further breeding may be needed to produce uniform and transgene-free candidate lines. Also, in order for the candidate lines to be exempted from the existing GMO regulation, the developer must first request for a technical consultation for evaluation and determination (TCED) through the DA Bureau of Plant Industry. It is important to note that while local gene editing researches are increasing, other gene-edited crops developed overseas may come in and could possibly be commercialized earlier. This is the case with reduced-browning banana developed by Tropic Biosciences UK, Ltd., which has undergone TCED in January 2023 and approved in March 2023, so is expected to be released soon.

Dr. Reynante Ordonio is a Senior Science Research Specialist and Scientist I at the Philippine Rice Research Institute.

Genome-edited products in the market

By Clement Dionglay

A report published by Rabobank in September 2022 revealed that the first batch of gene-edited crops that will hit the market will include fruits and vegetables with output traits, such as flavor, color, nutrients, and shelf life. Before the report was published, four genome-edited products have been approved for commercial sale in the United States and Japan.

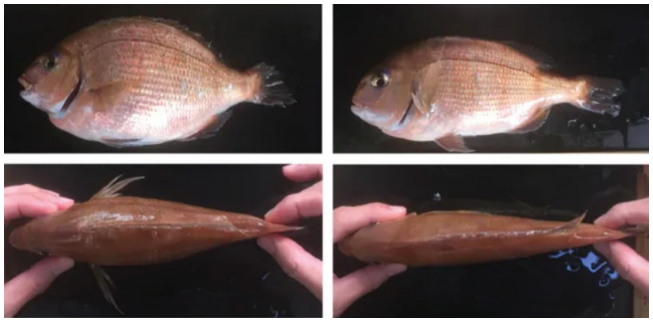
In 2019, Calyno™ High Oleic Soybean Oil produced by Calyxt, Inc. became the first genome-edited product to be sold in the U.S.. Calyno Oil was developed using TALEN® technology to produce healthier and more sustainable ingredients. The scientists turned off two genes involved in soybean's fatty acid synthesis to produce oil with 80% higher oleic acid, 20% less saturated fats, 0 trans fats, is less greasy, has three times fry-life, and a longer shelf-life compared to other soybean oils in the market.

In Japan, Sanatech Seed Co., Ltd. and partners launched the world's first genome-edited tomato in May 2021 with commercial sales following in September. Developed using CRISPR-Cas9, the Sicilian Rouge High GABA tomato contains high levels of gamma-aminobutyric acid (GABA), an amino acid that could help relaxation and lower blood pressure. The genome-edited tomato contains four to five times more GABA than a regular tomato.

In October and November 2021, two genome-edited fishes were approved for commercial sale in Japan. Kyoto-based start-up Regional Fish Co., Ltd. started selling genome-edited "Madai" red sea bream and "22-seiki fugu" tiger puffer. Both fishes were edited to grow bigger than their conventional counterparts.

Red sea bream is the 'king of fish' in Japan because of its elegant appearance, color, and superior taste. Regional Fish Co. and its partners developed the genome-edited "Madai" red sea bream using CRISPR to knock out myostatin, a protein that suppresses muscle growth. The fish lacking the myostatin gene has 1.2-1.6 times more edible parts and Japanese experts confirmed the safety of the fish.

Genome-edited high GABA tomatoes. Photo Source: Sanatech Seed



The gene-edited red sea bream (left) compared to unedited versions (right).

Photo Source:
Dr. Masato Kinoshita, Kyoto University and Dr. Keitaro Kato, Kindai University

Puffers such as globefish or blowfish are a luxury food in Japan despite some species being extremely toxic. Using CRISPR, Regional Fish Co. edited the popular pufferfish "torafugu" to increase the speed of growth. Out of the torafugu's 400 million genes, the scientists removed four leptin receptor genes that control appetite to boost their appetite and weight gain. The genome-edited pufferfish grows faster and are 1.9 times heavier than the conventional puffers in the same growing period. This will allow production and faster shipping than conventional tiger puffers which require more than two years to grow.

While gene editing is a fully-debated issue in some countries, these products in the market will pave the way for the acceptance of future products that scientists and researchers are currently working on, including genome-edited maize, wheat, potatoes, rice, cattle, and chicken.

For further reading

Specialty Crops to Hit the Market as First Gene-edited Entrants
https://bit.ly/RabobankGEd

Gene-edited High Oleic Soybean Oil Now Available in the US
https://bit.ly/CalynoOil

Japan Starts Sale of Genome-Edited High-GABA Tomato
https://bit.ly/HighGABATomato

Japan's Three Genome-Edited Food Products Reach Consumers
https://bit.ly/GEdFishes

DOUBLE HELIX
DECODING BIOTECH STRAND BY STRAND

SCIENCE ACTIVITY

What is DNA?

Plants and animals have a chemical recipe in their cells that dictates the appearance and the role of each cell and thus, the organism as a whole. This recipe is called DNA, short for deoxyribonucleic acid. Biotechnology is changing one of their ingredients in this recipe to make the dish better.

DNA contains two strands wrapped around each other in a helix, and these strands are connected by molecules called bases.

Do you want to see how DNA looks like?

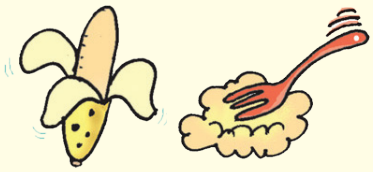
You can do this simple DNA extraction exercise in your class or at home! This activity is similar to what scientists do in the laboratory.

Prepare the following materials:

- ❒ 1 pc banana (cold)
- ❒ ½ cup cold water
- ❒ pinch of salt
- ❒ 1 tbsp liquid detergent
- ❒ ½ tsp pineapple juice
- ❒ cold ethyl alcohol (70-95%)
- ❒ 3 pcs clear plastic cup
- ❒ toothpick
- ❒ blender
- ❒ coffee filter
- ❒ rubber band
- ❒ fork
- ❒ saucer

What does DNA look like?

1 Peel a piece of banana and then mash.



2 Mix mashed banana, water, and salt in a blender for 15 seconds at high speed.

3 Cover one plastic cup (A) with a coffee filter. Secure with a rubber band. Pour the banana mixture on the filter.

4 Remove the coffee filter and add the liquid detergent. Mix gently to avoid forming bubbles. Leave it for 10 minutes.

5 Slowly pour 1/3 of the mixture on another cup (B). Add the pineapple juice. Slightly tilt the cup then pour 1/8 cup of ethyl alcohol through the side of the cup.

6 Wait for 5 minutes or until a cloudy substance appears. That substance is the DNA of a banana! Scoop it using a toothpick.

FOR MORE COOL SCIENCE ACTIVITIES, SCAN THIS!