Inside the Biotech Lab:

How to Genetically Engineer a Plant?

ISAAA
INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS

Biotechnology is one of the most controversial fields of science and technology. Some people think that it is a highly complicated field of science, with questions raised against how things are done by scientists in the lab and what their real intentions are in developing genetically modified organisms (GMOs). The fear of the unknown sometimes becomes an excuse to exercise the precautionary principle, which could block the progress of biotech innovations.

This booklet seeks to dispel fears about biotechnology by showing images of what actually happens inside the biotech laboratory. The process of developing a genetically engineered (GE) plant is explained using simple terms and images.

The images in this booklet with ISAAA icon are available in a photo database for public use. Journalists and other media practitioners are encouraged to use the photos to better represent biotechnology in their articles and replace fear-mongering images that are widespread in the media, especially online.

How to genetically engineer a plant?

Genetic engineering enables direct transfer of gene/s between closely or distantly related organisms. Though modifications may also be done by removing DNA sequences to switch on/off of genes, this booklet focuses on gene insertion. The two most common processes used in plant genetic transformation are particle bombardment and *Agrobacterium tumefaciens*-mediated transformation. These methods require a gene of interest that codes for a specific favorable trait (such as insect resistance, herbicide tolerance, and drought tolerance, to name a few) and a target plant that needs the trait.

Gene cloning

Cutting out the gene of interest from the DNA would have been easy if we're dealing with something made of paper, but we're not. In the lab, scientists use molecular scissors called restriction enzymes that have the ability to recognize a specific DNA sequence containing the gene of interest and then precisely cut it from the whole DNA. The isolated portion of the DNA would then have an unpaired nucleotide base, which have sticky ends.

The gene of interest is then placed into a circular piece of DNA called plasmid. This is possible because the cut genes have sticky ends that can be matched to the sticky ends of the plasmid. With advances in molecular biology, scientists can already engineer specific DNA sequences, synthesize them, and insert them in the plasmid.

The plasmid can be introduced into a bacterium such as *Escherichia coli* to make multiple copies of the DNA using the bacterium's natural amplification system. The multiplied DNA is isolated from the bacterium and used for particle bombardment.

Another kind of plasmid (binary vector plasmid) introduced into *Agrobacterium tumefaciens* can be used to transfer the gene of interest to the target organism.

Particle bombardment

The biotech maize MON810 was modified for resistance to certain insect pests by inserting genes through particle bombardment. This mechanical technique uses a gene gun to propel the gene of interest into the plant.

Take a look inside the genetic engineering laboratory at the Institute of Plant Breeding, University of the Philippines Los Baños, where a fabricated gene gun is used for plant transformation.

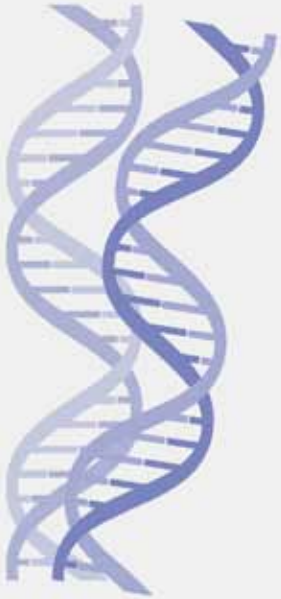
In most countries, genetic engineering is governed by biosafety regulations that require certain protocols to ensure the safety of the workers and prevent contamination of materials and equipment used in the process.

According to the Philippine Biosafety Guidelines, a biohazard signage must be placed on the access door of the laboratory work area to indicate that organisms containing recombinant DNA molecules are in use in the laboratory.

Similar to other laboratories, personnel are required to wear lab gowns, closed-toe shoes made of synthetic leather or any material that resists penetrations of spilled liquids or sharp objects, rubber gloves, goggles, surgical mask, and other protective clothing. This is to prevent any contamination that may affect the genetic engineering process.

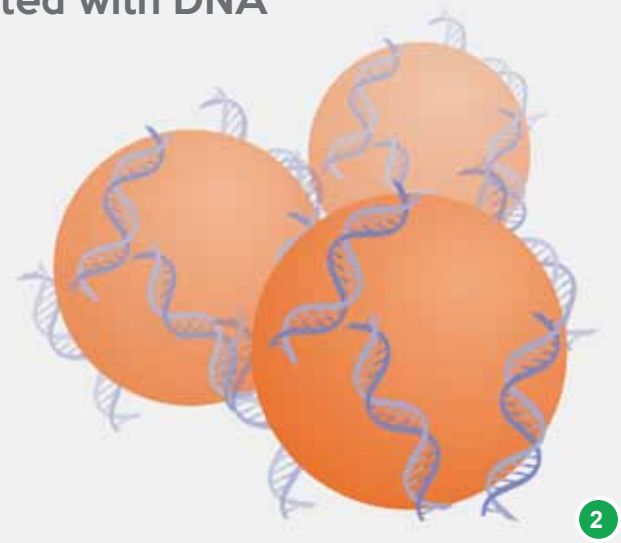
The development of genetically engineered crops involves several steps and complicated techniques, which can be summarized in a few basic steps:

Gene cloning



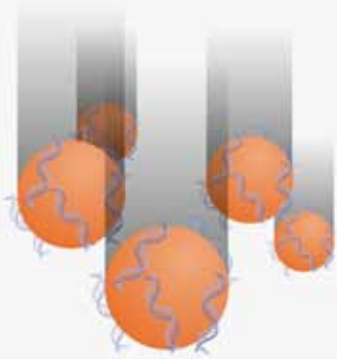
1

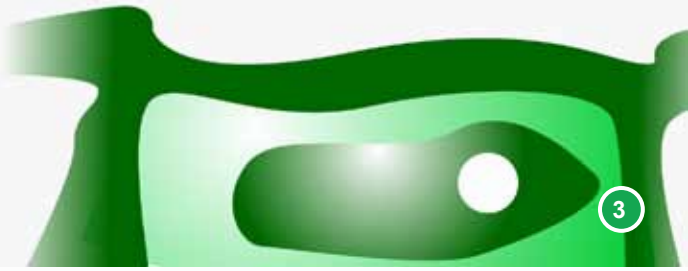
Gold/tungsten microparticles coated with DNA



2

Cells fired with gene gun and DNA incorporated into the target plant cells



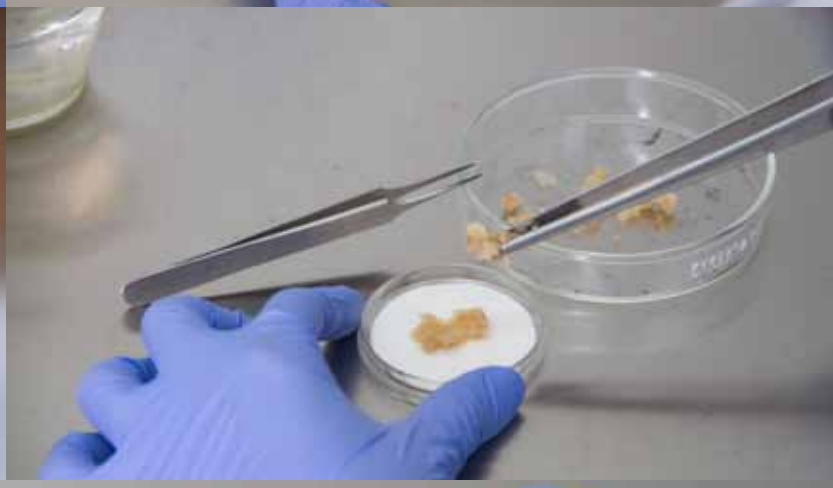


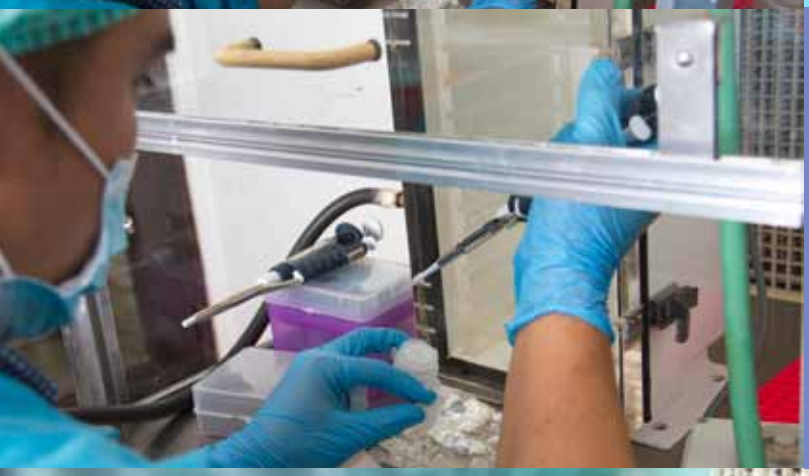
3

Plants regenerated and screened for transgene

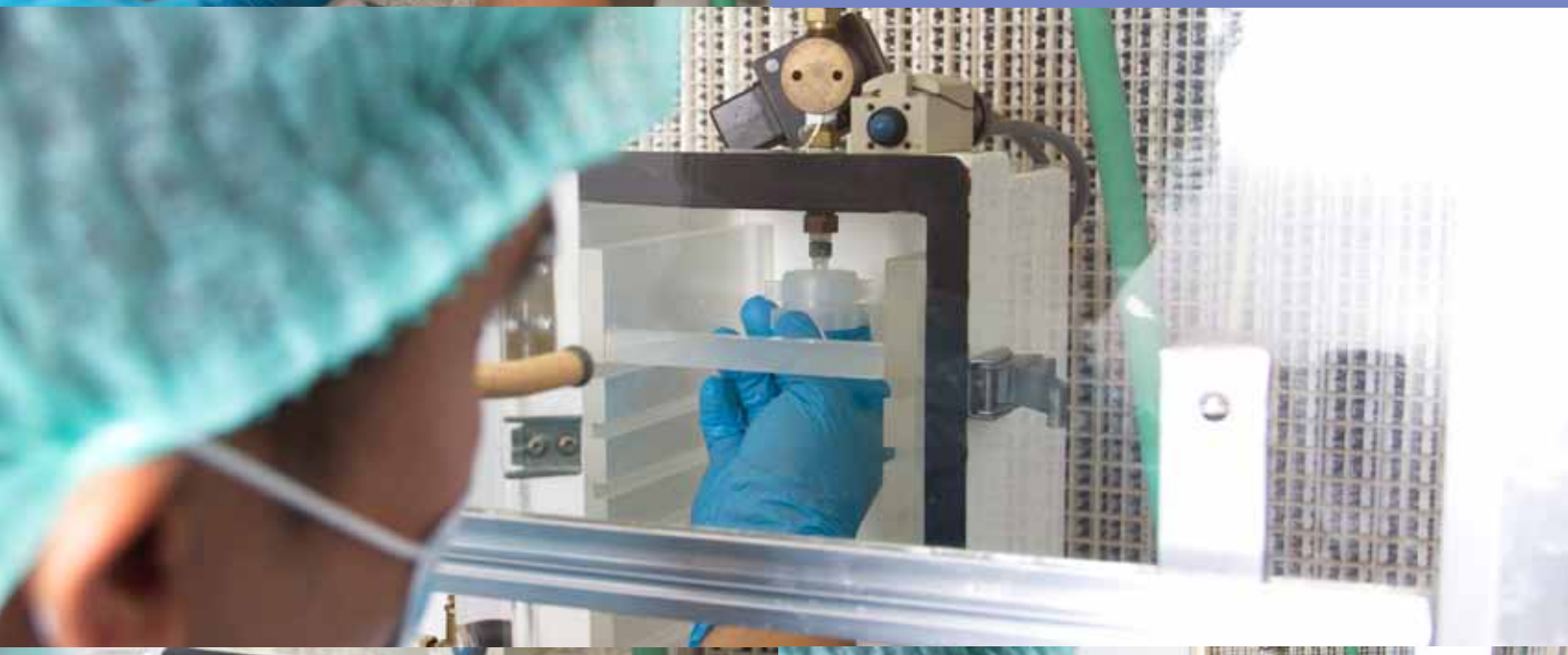
4

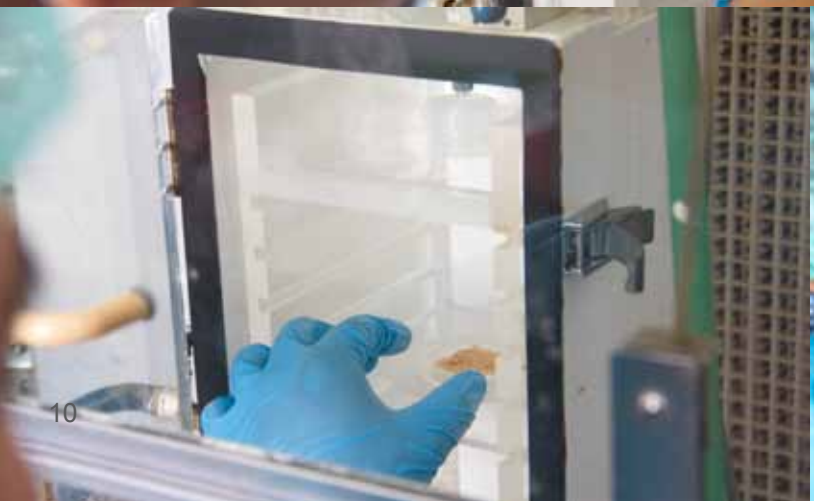
The initial step involves preparation of the target plant cells. This is done by generating plant calli or clumps of undifferentiated cells which are derived from highly-dividing plant cells (found in the roots, shoot buds, and floral plant parts) grown on a special medium.

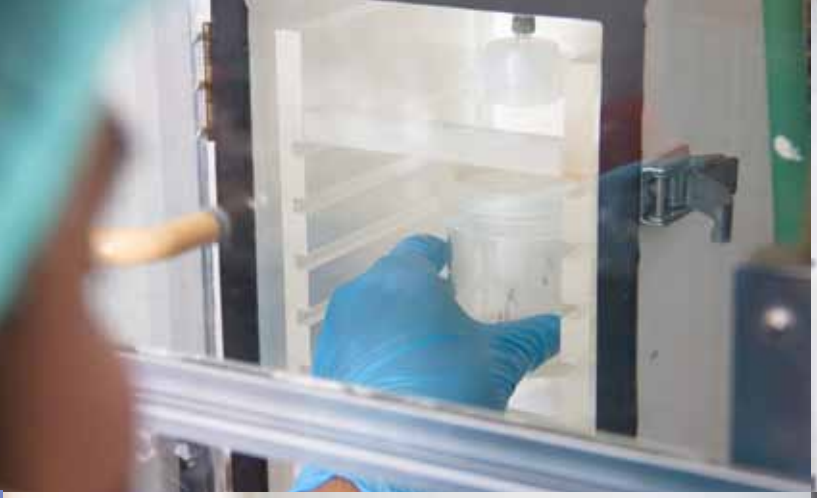


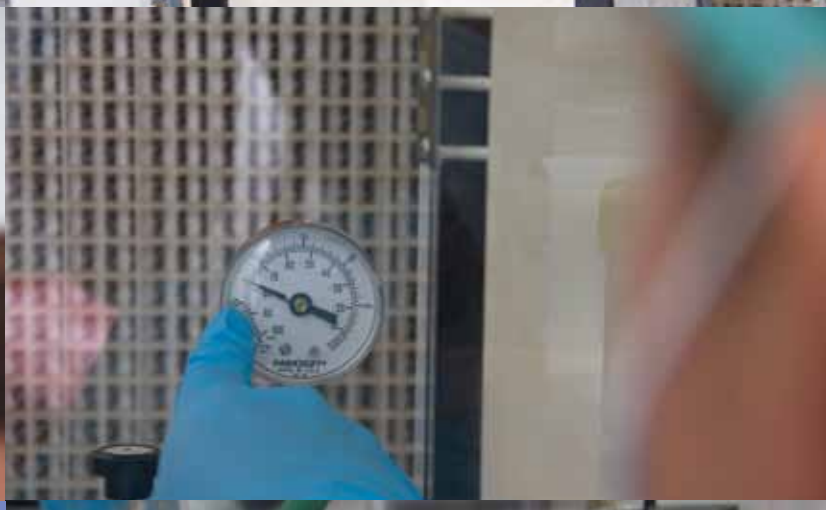


The gold or tungsten microparticles are coated with DNA and placed inside the gene gun chamber. The chamber must be in vacuum. Then pressurized helium gas is used to fire the microparticles into the plant cells.









The cells are set aside for hours to let the DNA fragment get inside the nucleus and integrated into the plant genomic DNA. The tungsten/gold particles are eventually eliminated from the cells which are then selected for the presence of the transgene. Selected calli are regenerated into plants and tested again for the presence of transgene.

Agrobacterium tumefaciens-mediated transformation

The biotech soybean MON-877Ø8-9 was developed to have dicamba and glyphosate herbicide tolerance through *Agrobacterium tumefaciens*-mediated transformation. This method of transformation uses the power of a bacterium to infect cells for transformation.

Agrobacterium tumefaciens is a bacterium from the soil that causes crown gall disease. The bacterium's successful infection is indicated by the formation of "tumors" usually on the roots and lower branches of the plants.

Aside from infecting plants, *Agrobacterium* has an amazing ability of forming a bridge to the plant cell and transfer some of its own genes across the plant cell wall, then across the membrane, and into the nucleus. Thus, it is known as nature's genetic engineer. Molecular biologists use this special ability of the bacterium to improve crops. They removed the gene that cause the formation of tumors and then replaced them with genes coding for favorable characteristics. This process is summarized in the following steps:

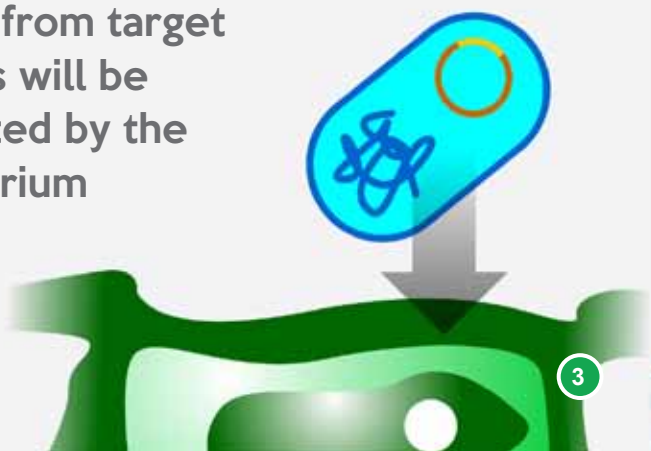
Design the plasmid vector with the gene of interest

1

The plasmid is transformed into the bacterium

2

Cells from target plants will be infected by the bacterium



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Regeneration of transformed plants

4

Development of GM crops

Gene discovery

1

GM crop development

2

Trait test in greenhouse

3

Confined field trial

4

Multi-location trial

5

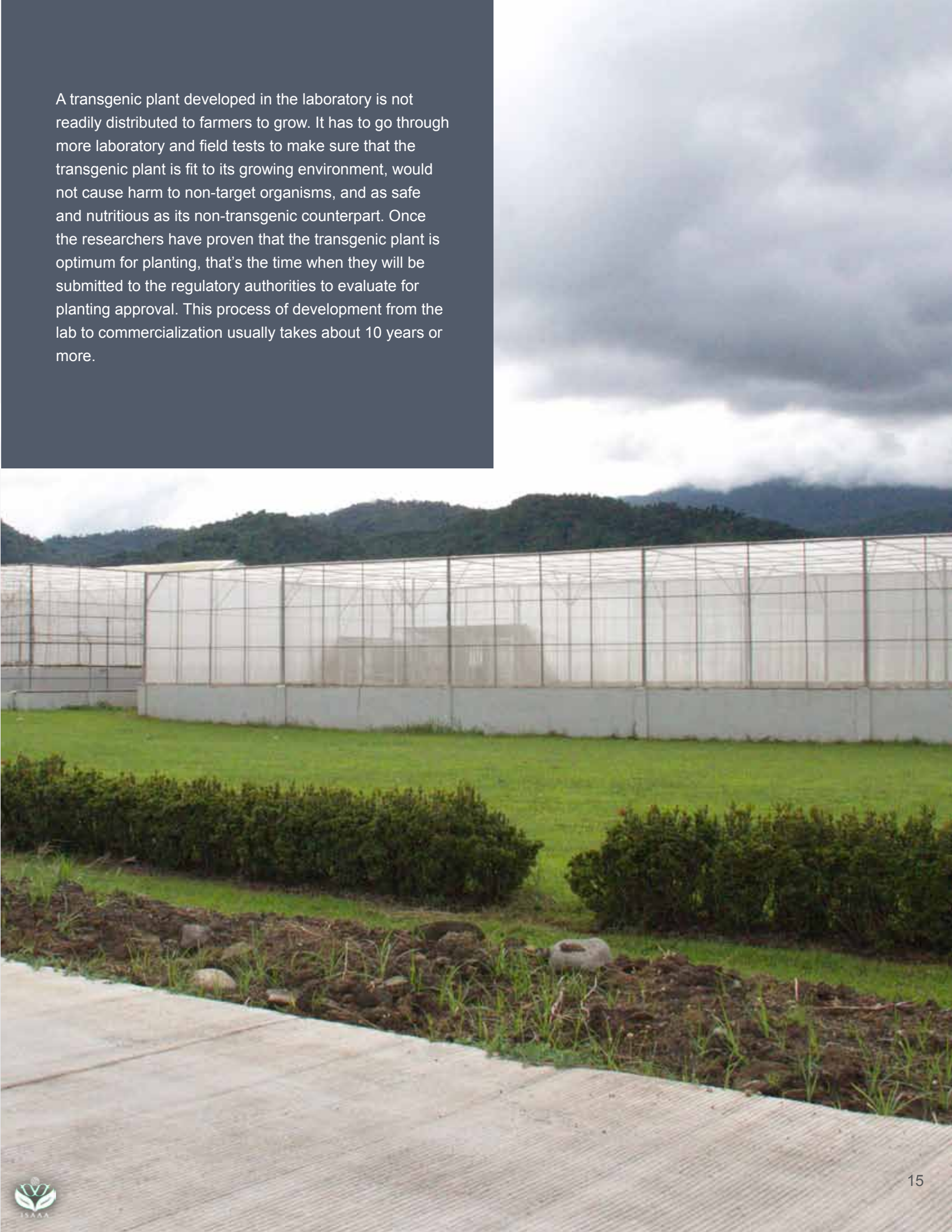
Safety assessments

6

Available in the market

7

A transgenic plant developed in the laboratory is not readily distributed to farmers to grow. It has to go through more laboratory and field tests to make sure that the transgenic plant is fit to its growing environment, would not cause harm to non-target organisms, and as safe and nutritious as its non-transgenic counterpart. Once the researchers have proven that the transgenic plant is optimum for planting, that's the time when they will be submitted to the regulatory authorities to evaluate for planting approval. This process of development from the lab to commercialization usually takes about 10 years or more.



Once the farmer takes hold of the biotech crops, they would have more options for farming. The traits incorporated into transgenic crops are usually determined based on the common problems and needs of the farmers. Among their concerns are insect pests, diseases, weeds, drought, and other uncontrollable factors. Scientists strive to find a way to help the farmers make the best out of their fields. In the end, the scientists fulfill their mission, and the farmers find peace of mind.

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Text: Kristine Grace Natividad-Tome
Illustrations & layout: Eric John Azucena

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ISAAA *SEAsia*Center
c/o IRRI, Los Baños, Laguna
DAPO Box 7777
Metro Manila, Philippines
isaaa-seasia@isaaa.org

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