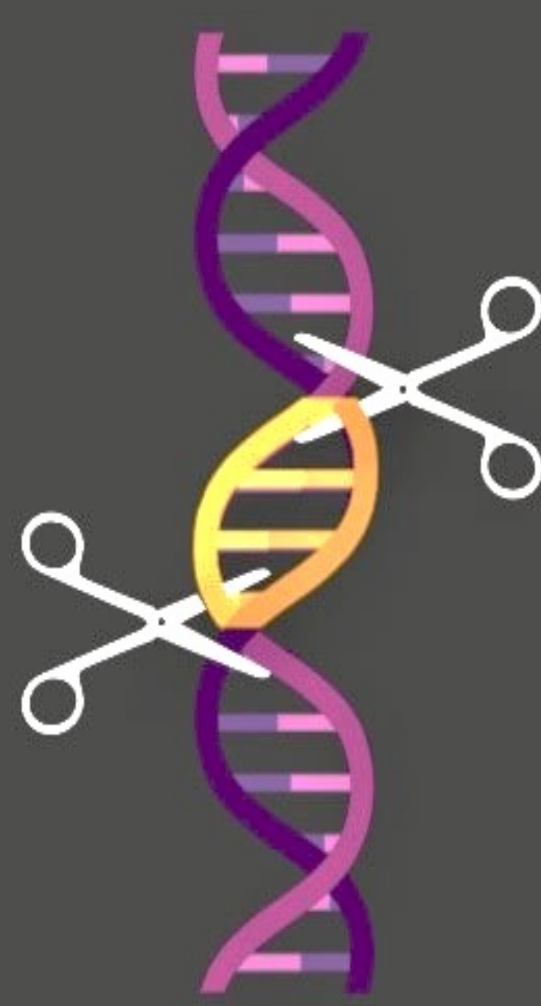


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## Abstract

Agriculture has supported human life through civilizations despite several biotic (pests, pathogens) and abiotic (drought, cold) stresses posing a challenge to meet the ever-increasing global food demand. Understanding the laws of genetics led to conventional plant breeding in the early 20<sup>th</sup> century giving rise to improved plant varieties. In the past 50 years, the understanding of cellular and molecular mechanisms in plants led to novel innovations in biotechnology for introducing desired genes/traits through plant genetic engineering. Commercial development of genetically modified crops such as tomatoes, corn and soybeans led to 185.1 million hectares of global land use in 2016 thereby, accelerating the process of conventional breeding to sustain the nutritional need of humans. Most recently, targeted genome editing technologies such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems have emerged as powerful tools for crop improvement. For example, genome editing with CRISPR is proving to be an efficient, simple process that involves low cost, can target multiple genes and that could be applicable to most plants. CRISPR is currently being used to engineer plant metabolic pathways to create resistance to viral, bacterial, or fungal pathogens, or abiotic stresses such as drought and cold in preparation for the global nutritional and food security. These novel genome editing technologies are poised to meet the UN sustainable development goals such as zero world hunger and good human health and well-being. In addition, these technologies could be more efficient in developing transgenic crops and speed up the mandatory regulatory approvals and risk assessments conducted by the US Departments of Agriculture (USDA), Food and Drug Administration (FDA) and Environmental Protection Agency (EPA).



## Transgenic Crops

Transgenic crops, economically important plants that have their genomes edited often with foreign DNA, have been developed for the betterment of agriculture, increased crop yield, and crop improvement in general. Over the years, 5 distinct categories of transgenic crops that have been commercialized are Herbicide-Tolerance, Insect-Resistance, Abiotic-Stress-Tolerance, Disease-Resistance, and Nutritional Enhancement in Crops (Figure 1).

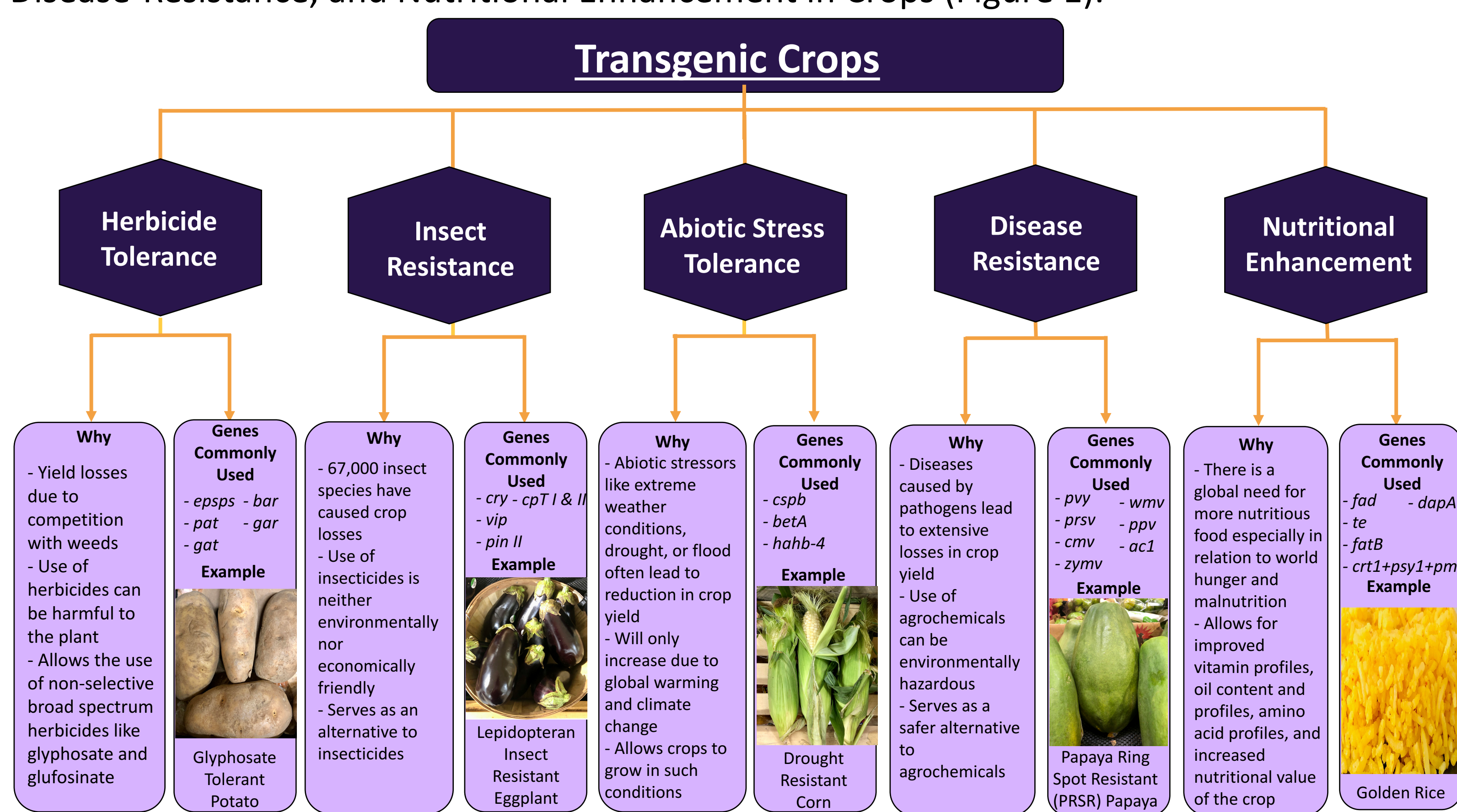


Figure 1. Different Types of Transgenic Crops: Why and How They Were Developed. Figure 1 provides a comprehensive summary of the different groups of transgenic crops.

## Genome Editing Technologies

Currently, there are many genome editing technologies that are being used in the field of agriculture. These include meganucleases, pentatricopeptide repeat protein (PPR), RNA Interference (RNAi), oligonucleotide-directed mutagenesis (ODM), and cisgenesis and intragenesis. However, the most commonly used technologies are ZFN, TALEN, and CRISPR/Cas9 (Figure 2):

### Zinc-Finger Nucleases (ZFN)

ZFNs were developed in 1985. ZFNs are composed of a zinc-finger based DNA recognition domain and the DNA-cleavage domain of the FokI restriction enzyme. Each zinc-finger domain binds to a nucleotide triplet allowing target sequences to range from 6 to 18 bp.

### Transcription Activator-Like Effector Nucleases (TALEN)

TALENs were developed in 2011. TALENs are composed of transcriptional activator-like effector (TALE) repeats and the FokI restriction enzyme. Each TALE repeat targets a single nucleotide which allows for a more flexible target design and increases the number of target sites.

### Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / Cas9 System

CRISPR was developed in 2012 after TALENs. They are composed of CRISPR in association with a single-guide RNA and a DNA endonuclease called Cas9 forming the CRISPR/Cas9 system. This system is simple, efficient, low cost, and can target multiple genes.

Like all fields, even the field of genome editing technologies is evolving rapidly especially in relation to agriculture. Recent literature has shown novel breakthroughs in the field of biotechnology such as base editing and CRISPR/Cpf1 system that can be more efficient than current technologies.

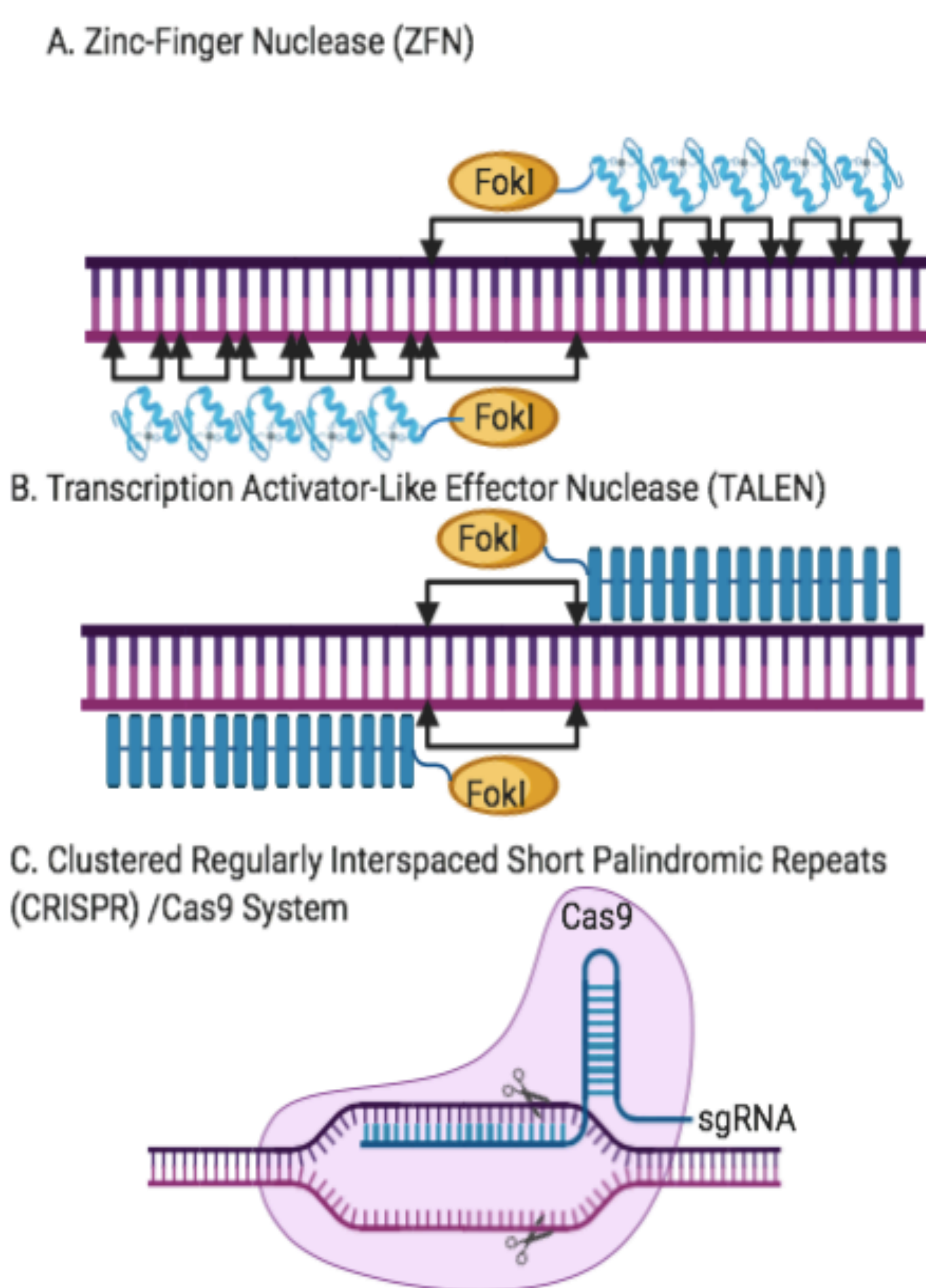


Figure 2. Structure and Functionality of the Three Main Genome Editing Technologies in Agriculture. A.) Zinc Finger Nucleases (ZFN) B.) Transcription Activator-Like Effector Nucleases (TALEN) C.) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) / Cas 9 System

## Advancement and Commercialization of Transgenic Crops

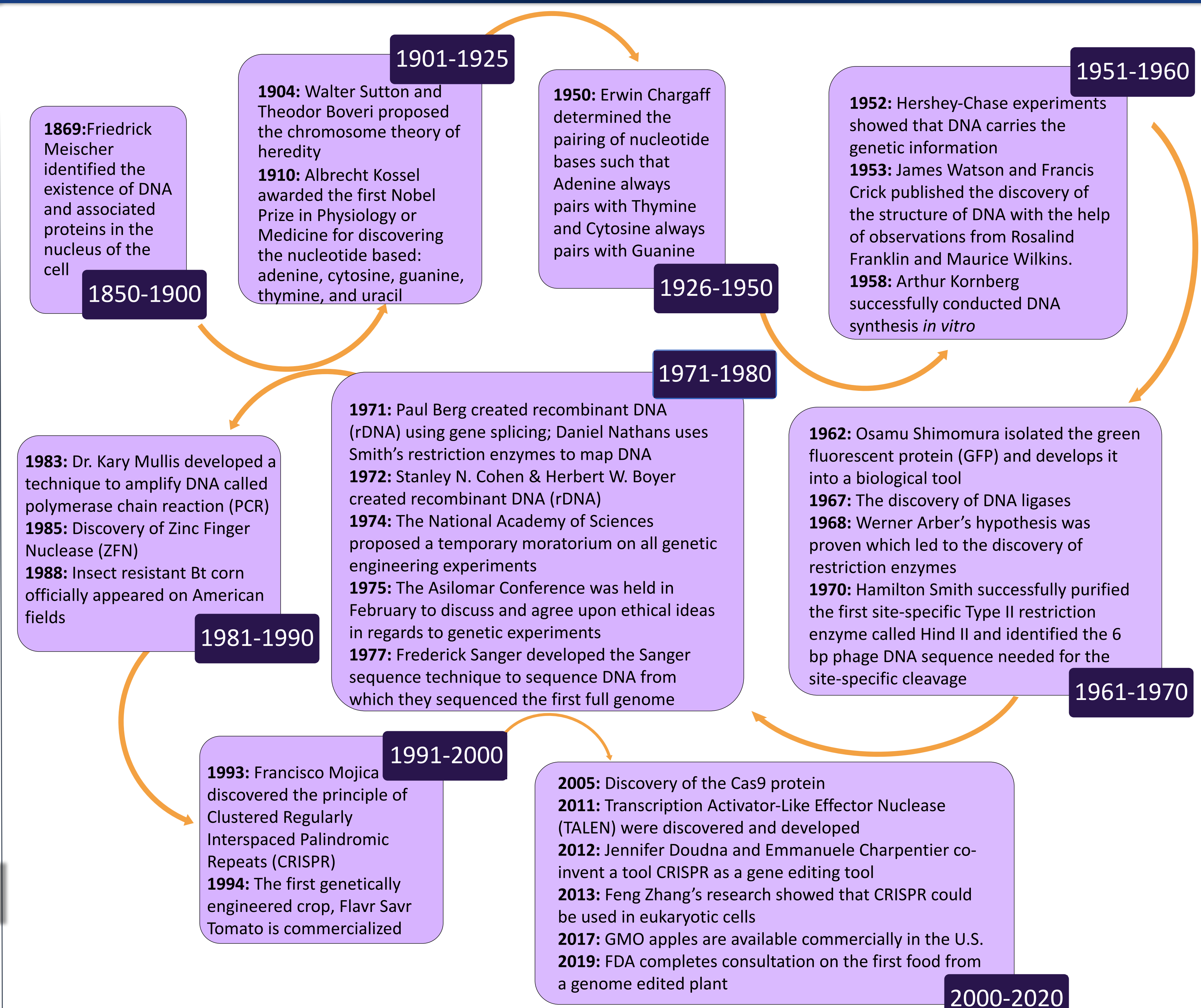


Figure 3. Events Leading Up to Genome Editing Technologies and Advancement & Commercialization of Transgenic Crops. Figure 3 provides a complete timeline of essential and major events that occurred leading to genome editing technologies and transgenic crops.

There were many important discoveries in science that led to the development of genome editing technologies which eventually led to the advancement and commercialization of transgenic crops (Figure 3).

### Advancement of Transgenic Crops:

- Allowed for efficient manipulation of crop genome leading to the advancement of transgenic crops by having the ability to introduce gene modifications, such as insertions, deletions, substitutions, in specific locations of the chromosomes
- Led to crop improvement by introducing agronomic traits or improving the functionality of already existing ones including fungal blast resistant rice and development of aromatic rice
- Lower cost for farmers while increasing crop yield
- Reduced negative environmental impacts like greenhouse gas emissions

### Commercialization of Transgenic Crops:

- Has allowed for the regulatory process to speed up making it easier for transgenic crops to be commercialized
- USDA has judged that crops produced by technologies such as CRISPR are not considered GMOs
- Often treated in the same manner as those developed through conventional breeding methods which lowers development and approval costs
- Positive impacts, like higher yield in less time and inputs, have motivated farmers to cultivate such crops for commercialization
- Boosts individual income and the agricultural sector for better economy

## Conclusions

Transgenic crops have existed long before genome editing technologies, but genome editing technologies have allowed transgenic crops to flourish and commercialize in ways that it could not in the past. Currently, one can see that genome editing technologies have changed the agricultural landscape and led to the rise of transgenic crops both of which would aid in meeting global needs like creating food and nutritional security.

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## References

Brookes, G. & Barfoot, P. (2018). Farm income and production impacts of using GM crop technology 1996-2016. *GM Crops & Food*, 9(2), 59-89. doi: 10.1080/21645698.2018.1464866  
Kniss, A.R. (2018). Genetically engineered herbicide-resistant crops and herbicide weed evolution in the United States. *Weed Science*, 66, 260-273. doi: 0.1017/wsc.2017.70