Biotechnological Applications of Recombinant DNA

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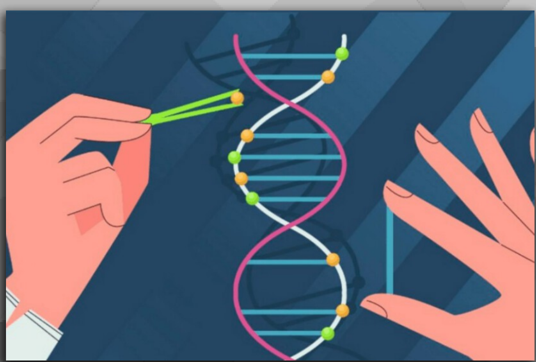

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I Plant improvement

1. Objectives

- Investigate genetic and biotechnological strategies to improve plant productivity and maximize crop yield.
- Explore biotechnology to create plant varieties with increased resistance to pests and diseases, reducing the reliance on chemical pesticides.

2. Introduction

Plants are a vital part of our ecosystem, providing us with oxygen, food, and resources. Over the years, scientists and farmers have been working on various methods to improve the growth and development of plants. Plant improvement involves a range of techniques aimed at enhancing desirable traits in plants, such as increased yield, disease resistance, and improved nutritional value. One common method of plant improvement is selective breeding. This process involves carefully selecting plants with desirable traits and cross-pollinating them to create offspring with a combination of these traits. Through selective breeding, farmers have been able to develop crop varieties that are more resistant to pests, drought, and other environmental stresses.

Another technique used in plant improvement is genetic engineering. This cutting-edge technology allows scientists to directly modify the DNA of plants, introducing specific genes to enhance desired characteristics. Genetic engineering has led to the development of genetically modified organisms (GMOs), which can have traits such as increased yield, pest resistance, and improved nutritional content.

In addition to selective breeding and genetic engineering, plant improvement also involves other methods such as tissue culture and marker-assisted selection. Tissue culture is a technique where small pieces of plant tissue, such as cells or embryos, are grown in a laboratory under controlled conditions. This method enables the mass production of plants with desirable traits, such as disease resistance. Marker-assisted selection, on the other hand, involves using molecular markers to identify plants with specific genes or traits of interest, allowing for more precise and efficient breeding. About 500 out of over one million plant species have been domesticated or brought under cultivation as crop plants through centuries of human selection*57*[57]*.

It turns out it is super common for plants to play around with polyploidy, the technical name for having more than two full sets of chromosomes. Even in the food we eat. Maybe especially in the food we eat.

- Potatoes can have four sets of chromosomes= 4n
- Kiwis can have six sets of chromosomes= 6n
- Strawberries can have eight sets of chromosomes= 8n

Extra copies of genes can be kind of a blank slate for evolution to play around with over time. With other copies of those genes still doing their thing, the extra copies of genes are then free to change things up and acquire new functions.

Future of Plant Breeding (cf. Handbook-of-Plant-Breeding-Case-Studies-from-Africa-2019.pdf)

3. Genetically modified organisms (GMOs)

Genetically modified organisms (GMOs) have become a significant topic of discussion in the field of plant improvement. Scientists have been using genetic engineering techniques to modify the DNA of plants to enhance their traits and improve their overall performance. This process involves introducing specific genes from other organisms into the plants, giving them new characteristics that they wouldn't naturally possess.

One of the main objectives of genetically modifying plants is to increase their resistance to pests and diseases. By inserting genes that produce toxins harmful to pests or make the plant less appealing to insects, scientists can reduce the need for chemical pesticides. This not only benefits the environment but also helps farmers protect their crops more effectively.

GMOs also offer the potential for increased crop yields. Through genetic modifications, scientists can enhance traits such as drought tolerance, nutrient uptake efficiency, and resistance to harsh environmental conditions. These modifications allow plants to grow better in challenging climates and produce higher yields, which can contribute to global food security58[58]*.

Another area where GMOs have shown promise is in improving the nutritional content of crops. Scientists can introduce genes that enhance the levels of essential nutrients in plants, such as vitamins and minerals. This has the potential to address nutritional deficiencies and improve the health outcomes of populations that rely on these crops as their primary food source. These advancements in plant improvement techniques are crucial for ensuring food security and addressing the needs of a growing global population.

Despite the potential benefits, GMOs also raise concerns. Some worry about the potential environmental impacts of genetically modified plants, such as the spread of modified genes to wild plant populations. Additionally, there are concerns about the long-term effects on human health, though extensive scientific research has shown no evidence of harm to date. To address these concerns, many countries have established regulations for the cultivation and labeling of GMOs. These regulations aim to ensure the safety of genetically modified crops and provide consumers with the information they need to make informed choices.

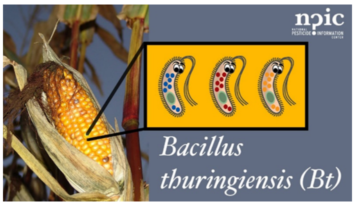
Exemple **: Golden Rice**

- Nutrient content of a crop plant can be improved by metabolic engineering (Rice, maize, wheat, tomatoes) in order to enhance the level of certain nutrients of beta carotene to have higher levels of Vitamin A.
- GM rice was accomplished by inserting phytoene synthase gene from *daffodils* and phytoene desaturase gene from *Erwinia* bacteria.

Golden Rice

Exemple **: BT crops**

Bt Crops are transgenic crops that produce the same toxin as the bacterium *Bacillus thuringiensis* in the plant cell, thereby, protecting the crops from pests. The bacterium secretes specific proteins known as “cry proteins” that are toxic to insects. A few of the Bt crops include cotton, brinjal, corn, etc.



BT crops

Exemple : Glucosinolate Broccoli

- Plant improvement led to some beneficial effects to the broccoli by exhibited higher levels of glucosinolate glucoraphanin resulting in improving the human metabolism and reducing the fatty acids associated with inflammation.

Exemple : Enhanced strawberries

- Strawberries have been modified to provide 3 times more of vitamin C.
- GalUR gene codes for an enzyme that converts a protein in the plant to vitamin C. Similarly, another gene was found in the Arabidopsis thaliana responsible for the same function.
- The incorporation A. thaliana gene into strawberry plant produces 3 times as much vitamin C.

Exemple : New compounds in plants

- Polyhydroxyalkanoates produced in plants, a way to synthesize a biodegradable polymer.
- Provitamin A carotenoids in food such as corn, rice, and wheat.
- Folate or vitamin B produced in tomato fruits.
- **Salt tolerant tomatoes.**
- **Enhanced carrots to produce a vaccine against hepatitis B.**

4. Application of Mutation Breeding

To understand the application of mutation breeding for improving plant products, it is essential to first grasp the concept of mutation. A mutation refers to a change in the genetic composition of an organism, which can occur naturally or be induced deliberately, as in plant breeding. When mutations are introduced, they can alter a plant's chromosomal structure, potentially leading to desirable traits. However, mutation breeding is a delicate process, as small errors in manipulation can result in crop failure or unintended characteristics that may compromise the plant's genetic integrity*59*[59]*.

Despite these challenges, mutation breeding has been successful, particularly in enhancing cereals and rice. For instance, dwarf varieties have been developed to shorten the growing period.

Biotechnology plays a crucial role in plant breeding by facilitating genetic transfers to improve plant species. Techniques like vectors and pathogens are now used to introduce genetic material into host cells. Common methods include*60*[60]*:

1. **Selection**: Genetically distinct progeny rows are evaluated, and their performance and yield are compared with commercial varieties.
2. **Hybridization**: Cross-pollination is employed to combine desirable traits from different plants.
3. **Polyploidy**: Chemicals are used to increase chromosome numbers in plants.

4. **Induced Mutation**: Radiation or chemicals are applied to induce mutations.

With advancements in biotechnology, essential minerals and components can be introduced into plants through biological methods. These techniques, when applied properly, can yield the desired plant traits while preserving the plant's natural characteristics.

(cf. Chemical mutagenesis in plants)

5. Application of genome editing technologies in the development of genetically modified crops

In the context of genetically modified crops, agriculture is a continuously evolving field that requires constant scientific advancements to meet societal needs. As part of this ongoing progress, genome editing (GE) technologies have emerged as crucial tools for crop improvement and are under continuous refinement.

5.1. Current genome editing technologies

Several GE technologies are currently applied in agriculture to enhance crop traits. Among the most prominent are Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system. Other notable techniques include meganucleases and Oligonucleotide-Directed Mutagenesis (ODM). In addition to these, RNA interference (RNAi) is a powerful method that, while not directly editing the genome, can regulate gene expression to improve crop characteristics.

a) Meganucleases

Meganucleases, also known as homing endonucleases, were the first class of site-specific enzymes applied in genome editing (GE). These enzymes have dual functional domains that enable them to bind to DNA and induce double-stranded breaks (DSBs) at specific target sequences. Meganucleases are typically restricted to sequences of 10–40 base pairs (bp), limiting their range of target sites *61*[61]*.

One of the earliest examples is the intron-encoded endonuclease I-SceI, isolated from *Saccharomyces cerevisiae*, which cleaves an 18 bp DNA sequence to induce DSBs (Stoddard, 2005; Takeuchi et al., 2011). However, most meganucleases are species-specific and do not function effectively across diverse biological kingdoms. Thus, in plant GE, meganucleases are often derived from plant species. For instance, the endonuclease I-CreI from *Chlamydomonas reinhardtii* has been utilized to introduce DSBs in various plant species*62*[62]*.

Despite their precision, meganucleases have a major limitation: they are restricted to a narrow range of target sequences. Protein engineering could potentially broaden their applicability, but this approach is labor-intensive and time-consuming. Therefore, more versatile GE technologies like Zinc-Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) are often preferred due to their flexibility and efficiency.

b) Zinc-Finger Nucleases (ZFNs)

Zinc-Finger Nucleases (ZFNs) were developed in the 1990s, marking an important advancement in gene editing by enabling targeted DNA cleavage. ZFNs consist of two key components: zinc-finger DNA-binding domains and the FokI nuclease, which cleaves the DNA. Each zinc-finger domain recognizes a specific DNA triplet, allowing ZFNs to bind sequences ranging from 6 to 18 base pairs *63*[63]*. However, ZFNs require a customized nuclease for each target, making the process expensive and time-intensive. This limitation restricts their use to single-gene modifications, which complicates efforts to edit multiple genes or complex traits.

Despite these challenges, ZFNs have been successfully employed to create transgenic crops like tobacco, maize, rice, and soybean. ZFNs have enabled precise gene insertions, facilitating the stacking of multiple desirable traits in crops, as demonstrated in maize. They have also been utilized to improve traits such as yield and stress resistance in crops like rice and maize, although more advanced GE tools have largely surpassed ZFNs in terms of efficiency and ease of use*64*[64]*.

c) Transcription Activator-Like Effector Nucleases (TALENs)

TALENs were developed about a decade after ZFNs, providing a more accessible tool for genome editing. TALENs consist of transcription activator-like effector (TALE) proteins, originally discovered in the bacterial plant pathogen *Xanthomonas*, and the FokI nuclease. Each TALE repeat binds to a specific nucleotide, offering more flexible target recognition than ZFNs. TALENs have broad applicability and have been used in various crops, including rice, maize, potato, and soybean *64*[64]*. For instance, TALENs were used to disrupt the *Ossweet14* gene in rice, creating bacterial blight-resistant plants. Additionally, TALENs have been employed to improve the nutritional profiles of crops, such as developing high-oleic acid soybeans by targeting fatty acid desaturase (fad) genes*65*[65]* . Despite their flexibility, TALENs are limited by their large size, which complicates their use in editing multiple genes simultaneously. Furthermore, the production of TALE repeats can be labor-intensive and has shown inconsistencies in targeting efficiency across species.

d) Oligonucleotide-Directed Mutagenesis (ODM)

Oligonucleotide-Directed Mutagenesis (ODM) is a non-transgenic genome-editing technique that introduces small changes to the DNA using synthetic oligonucleotides. ODM utilizes the cell's natural DNA repair mechanisms to correct mismatched base pairs after hybridization with a complementary single-stranded oligonucleotide . This method is particularly effective for introducing small insertions, deletions, or substitutions, but it is limited by low correction rates and the challenge of controlling the editing process. Despite these limitations, ODM has been successfully applied in crop biotechnology, as demonstrated by the discovery of the *aLs* gene in plants*70*[70]*.

e) CRISPR/Cas9 System

The development of the CRISPR/Cas9 system, introduced shortly after TALENs, revolutionized genome editing due to its simplicity and versatility . CRISPR/Cas9 uses a guide RNA (gRNA) to direct the Cas9 nuclease to a specific DNA sequence, where it induces DSBs. Compared to ZFNs and TALENs, CRISPR/Cas9 is more efficient, cost-effective, and capable of targeting multiple genes simultaneously*66*[66]*. This system has been widely applied in agriculture, generating transgenic crops such as rice, maize, wheat, and tomato. *67*[67]*CRISPR/Cas9 has been used to develop herbicide-resistant rice, drought-tolerant maize, and pathogen-resistant citrus*68*[68]*. Additionally, the technology has been employed to improve crop yield, nutritional quality, and stress resistance *69*[69]*.

5.2. Emerging Technological AdvancesAs with many areas in biotechnology, g

As with many areas in biotechnology, genome-editing (GE) techniques are constantly evolving, particularly in agriculture. Recent research highlights several groundbreaking advancements such as base editing, plastid genome modification, synthetic genomics, and DNA-free GE technologies.

a) DNA-Free Genome Editing Systems

DNA-free genome editing is a cutting-edge approach that minimizes the risk of unintended mutations, which can occur with traditional transgenic methods. This technique relies on methods such as protoplast-mediated transformation and particle bombardment, and has been successfully applied to various crops like tobacco, lettuce, rice, grapes, and apples . Additionally, particle bombardment-based DNA-free GE technology has been extensively used in wheat and maize *72*[72]*. In wheat, there has been progress combining both base editing and DNA-free genome editing *71*[71]* . If this combination becomes a single, commercialized technique, it could streamline base-editing applications in plant breeding and the development of transgenic crops.

6. Agronomic traits and transgenic crops

The adoption of transgenic crops, particularly those developed using genome-editing (GE) technologies, has brought both economic and environmental advantages. These benefits include higher crop yields, lower carbon dioxide emissions, increased income for farmers, and improved consumer health . Herbicide-tolerant crops have acquired additional agronomic traits beyond general pest resistance, such as the ability to boost net yield while maintaining pest resistance. The use of stacked traits, made possible through GE technologies, has significantly enhanced the agronomic qualities of herbicide-tolerant and other transgenic crops, transforming the market for both producers and consumers. Insect-resistant crops, in particular, have introduced valuable agronomic traits by improving yield potential and offering an alternative to insecticides, which can otherwise have harmful environmental impacts*73*[73]*.

7. Conclusion

One of the most transformative developments in plant biotechnology has been the ability to genetically modify plants through *Agrobacterium*-mediated transformation, a technique that has significantly impacted plant breeding and agricultural practices. Today, emerging gene-editing technologies are driving a new wave of innovation in crop improvement, at a time when humanity faces critical challenges such as food security and climate change. DNA-free genome editing, which utilizes pre-assembled ribonucleoprotein complexes and nanoparticles to achieve precise genetic modifications, shows great promise as an advanced method of genetic engineering. Additionally, the use of viral vectors presents a promising avenue for enhancing pathogen resistance and enabling genetic modifications. Traditionally, plant biotechnology has relied heavily on plant tissue culture, forming the foundational "triad" of plant biotechnology alongside transformation and molecular biology. However, the development of technologies that could bypass the need for tissue culture would be a significant breakthrough, simplifying and accelerating the genetic engineering process.

8. Exercice : Plant improvement

[solution n°1 p. 10]

Which of the following techniques revolutionized plant biotechnology by enabling the genetic transformation of plants?

Genetic transformation of plants

- CRISPR-Cas9
- Agrobacterium-mediated transformation
- Particle bombardment
- Tissue culture

DNA-free genome editing utilizes which of the following components for precise genetic modification?

DNA-free genome editing

- DNA plasmids and CRISPR-Cas9
- Pre-assembled ribonucleoprotein complexes and nanoparticles
- Viral vectors and bacterial plasmids
- RNA interference and Agrobacterium

Viral vectors are being explored in plant biotechnology for what potential application?

Viral vectors

- Targeted genome modification
- Facilitating protoplast-mediated transformation
- Enhancing pathogen resistance and genetic modification
- Improving yield and carbon sequestration

What percentage of global crop yield increase is attributed to transgenic crops, according to recent studies?

Percentage of global crop yield

What technique transformed plant biotechnology and agriculture?

Transformed plant biotechnology

What are the components used in DNA-free genome editing?

DNA-free genome editing

Solutions des exercices

Solution n°1

[exercice p. 9]

Which of the following techniques revolutionized plant biotechnology by enabling the genetic transformation of plants?

Genetic transformation of plants

- ○ CRISPR-Cas9
- ◉ Agrobacterium-mediated transformation
- ○ Particle bombardment
- ○ Tissue culture

DNA-free genome editing utilizes which of the following components for precise genetic modification?

DNA-free genome editing

- ○ DNA plasmids and CRISPR-Cas9
- ◉ Pre-assembled ribonucleoprotein complexes and nanoparticles
- ○ Viral vectors and bacterial plasmids
- ○ RNA interference and Agrobacterium

Viral vectors are being explored in plant biotechnology for what potential application?

Viral vectors

- ○ Targeted genome modification
- ○ Facilitating protoplast-mediated transformation
- ◉ Enhancing pathogen resistance and genetic modification
- ○ Improving yield and carbon sequestration

What percentage of global crop yield increase is attributed to transgenic crops, according to recent studies?

Percentage of global crop yield

22%

What technique transformed plant biotechnology and agriculture?

Transformed plant biotechnology

Agrobacterium-mediated transformation

What are the components used in DNA-free genome editing?

DNA-free genome editing

Ribonucleoprotein complexes, nanoparticles

Glossaire

Polymerase chain reaction (PCR)

A method widely used to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to amplify a very small sample of DNA (or a part of it) sufficiently to enable detailed study.

Abréviations

DNA : Deoxyribonucleic acid ; is the molecule that carries genetic information for the development and functioning of an organism.

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