Food Security and Biotechnology in Africa

This project is financed by the European Union and implemented by the ACP Secretariat

MODULE 2
BIOTECHNOLOGY: HISTORY, STATE OF THE ART, FUTURE.

LECTURE NOTES: UNIT 4
FUTURE TRENDS AND PERSPECTIVES OF AGRICULTURAL BIOTECHNOLOGY

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Final version, February 2017

*This **Unit 4 of Module 2** is an integral part of the **six Master's level course modules** (each of 20 hrs) in the field of agricultural biotechnology as elaborated by the EDULINK-FSBA project (2013-2017) which are:*

- *Module 1: Food security, agricultural systems and biotechnology*
- ***Module 2: Biotechnology: history, state of the art, future***
- *Module 3: Public response to the rise of biotechnology*
- *Module 4: Regulation on and policy approaches to biotechnology*
- *Module 5: Ethics and world views in relation to biotechnology*
- *Module 6: Tailoring biotechnology: towards societal responsibility and country specific approaches*

PRESENTATION OF MODULE 2

INTRODUCTION

Achieving food security in its totality (food availability, economic and physical access to food, food utilization and stability over time) continues to be a challenge not only for the developing nations, but also for the developed world. The difference lies in the magnitude of the problem in terms of its severity and proportion of the population affected. According to FAO statistics, a total of 842 million people in 2011–13, or around one in eight people in the world, were estimated to be suffering from chronic hunger. Despite overall progress, marked differences across regions persist. Africa remains the region with the highest prevalence of undernourishment, with more than one in five people estimated to be undernourished. One of the underlying causes of food insecurity in African countries is the **rapid population growth (**Africa's population is expected to reach 2.4 billion in 2050) **that makes t**he food security outlook worrisome. According to some projections, Africa will produce enough food for only about a quarter of its population by 2025. How will Africa be able to cope with its food security challenge? Is biotechnology is key to food security in Africa?

Biotechnology's ability to eliminate malnutrition and hunger in developing countries through production of crops resistant to pests and diseases, having longer shelf-lives, refined textures and flavors, higher yields per units of land and time, tolerant to adverse weather and soil conditions, etc, has been reviewed by several authors. If biotechnology per se is not a panacea for the world's problems of hunger and poverty, it offers outstanding potentials to increase the efficiency of crop improvement, thus enhance global food production and availability in a sustainable way. A common misconception being the thought that biotechnology is relatively new and includes only DNA and genetic engineering. So, agricultural biotechnology is especially a topic of considerable controversy worldwide and in Africa, and public debate is

fraught with polarized views and opinions. Therefore, working at the sustainable introduction of biotechnology for food security in Africa requires a strong conceptual understanding by the learner (stakeholders and future stakeholders) of what is biotechnology.

GENERAL OBJECTIVE OF THE MODULE:

The main objective of this module is to offer a broad view of biotechnology, integrating historical, global current (classical and modern) and future applications in such a way that its applications in Africa and expected developments could be discussed based on sound knowledge of processes and methods used to manipulate living organisms or the substances and products from these organisms for medical, agricultural, and industrial purposes.

SPECIFIC OBJECTIVES:

On successful completion of this module, the learner should be able to:

- Demonstrate knowledge of essential facts of the history of biotechnology and description of key scientific events in the development of biotechnology
- Demonstrate knowledge of the definitions and principles of ancient, classical, and modern biotechnologies.
- Describe the theory, practice and potential of current and future biotechnology.
- Describe and begin to evaluate aspects of current and future research and applications in biotechnology.
- Select and properly manage information drawn from text books and article to communicate ideas effectively by written, oral and visual means on biotechnology issues.
- Demonstrate an appreciation of biotechnology in Africa especially in achieving food security.

COURSE STRUCTURE

The content of the course is organized in five units as followed:

- Unit 1: Introduction to biotechnology, history and concepts definition
- Unit 2: The Green Revolution: impacts, limits, and the path ahead
- Unit 3: Agricultural biotechnology: the state-of-the-art
- **Unit 4: Future trends and perspectives of agricultural biotechnology**
- Unit 5: Biotechnology in Africa: options and opportunities

UNIT 4:
FUTURE TRENDS AND PERSPECTIVES OF AGRICULTURAL BIOTECHNOLOGY
(04 HOURS)

PRESENTATION

Objective

The main objective of this Unit is to present the degree to which new plant breeding techniques are developed and adopted; and discussed future prospects. The drivers (technical potential and economic advantages) and the constraints (efficiency, availability, cost, safety and regulatory issues) are analyzed focusing on new plant breeding techniques.

Content

This unit is structured in 3 sections:

1. New plant breeding techniques (*approx. 02 hours*)
2. Examples of applications of new breeding techniques (*approx. 01 hour*)
3. Current challenges and future perspectives (*approx. 01 hour*)

Course Delivery

Lecture Slides

The slides used in lectures are summaries that have as main objective to guide the learner in his personal work (mainly reading the selected literature).

- ***Reading the slides is not an adequate substitute for attending lectures. The slides do not contain anything that the instructor says, writes on the board, or demonstrates during lectures.***

Lecture Notes

The Lecture notes offer an overview of a subject (you will need to fill in the detail) and detailed information on a subject (you will need to fill in the background). It encourages taking an active part in the lecture by doing reference reading. ***Links to useful didactic videos are given***.

To continue

The learner may be interested in:

- Module 4 of FSBA course on "Regulation on and policy approaches to biotechnology"
  This should allow the learner to better understand the regulatory processes of new techniques and to understand the difference between biotechnology products that should be called GM and non-GM

NEW PLANT BREEDING TECHNIQUES

Despite the wide range of biotechnology methods that are already in use, new developments are expected especially in the field of food and nutrition to meet the global challenges of population growth, climate change and the increase in awareness of the people about health and bio safety issue. Several public surveys have shown that one of the major concerns about GM food among the general public is the combination of genetic elements derived from different organisms. This unit examines new alternatives strategies and approaches to transgenesis; and should highlight what is GMO and what is not! It focuses on recent developments and future challenges of techniques applied in plant breeding. Promising techniques like Synthetic biology and Nanotechnology are presented. At completion, challenges for developing countries are discussed on the opportunities to direct the great potentials of biotechnology towards ensuring food security and economic development in the developing world.

New breeding techniques are emerging rapidly from advances in genomic research, for application in crop improvement. They enable precise, targeted, reliable changes in the genome (and, thus, are different from genetically modified organisms –GMOs-, produced previously) and have significant potential for the sustainable intensification of agriculture and food security (European Academies' Science Advisory Council, 2).

- ***For several of the techniques, the resultant plant product is free from genes foreign to the species and would not be distinguishable from the product generated by conventional breeding techniques. This calls into question what is meant by genetic modification and raises issues for the modernization of regulatory frameworks.***

The new breeding techniques include:

- Cisgenesis & Intragenesis
- Targeted mutagenesis
- Transient introduction of recombinant DNA
- RNA-induced DNA methylation gene silencing
- Reverse breeding
- Grafting non-GM scion onto GM rootstock
- Synthetic Genomics
- Genome editing techniques

Cisgenesis & Intragenesis

Genetically Modified Organisms (GMO) could be the answer for many relevant problems affecting crops. However, improving crops through GMO is also often associated with safety concerns, environmental risks and health issues due to the presence of foreign DNA. These limitations have prompted the development of alternative technologies including cisgenesis and intragenesis.

Cisgenesis and intragenesis are the restriction of transgenesis to DNA fragments from the species itself or from a cross-compatible species. In the case of cisgenesis, the inserted genes, associated introns and regulatory elements are contiguous and unchanged. In the case of intragenesis, the inserted DNA can be a new combination of DNA fragments from the species itself or from a cross-compatible species. Both approaches aim to confer a new property to the modified plant (see Fig. 1/4).

➲ ***So far, application of cisgenesis and intragenesis as alternatives to conventional transgenesis are limited to a few species, mainly due to the lack of knowledge of the regulatory sequences required.***

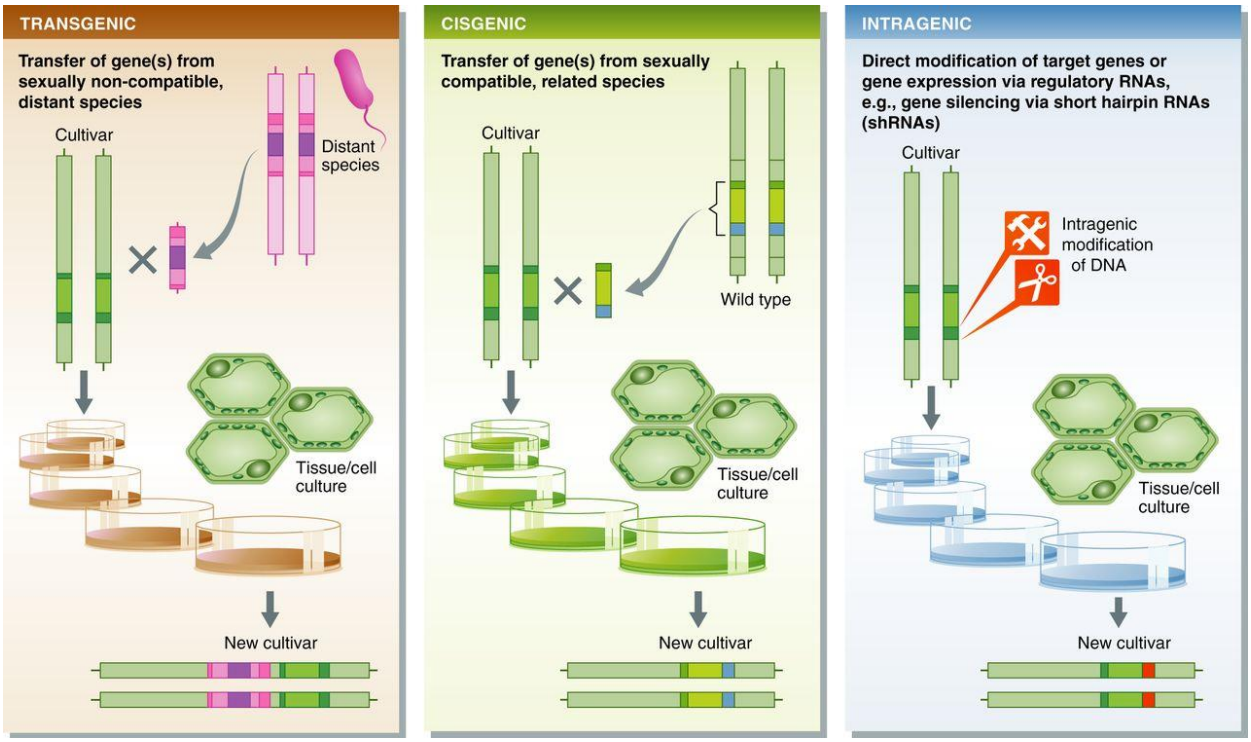


Fig. 1/4: Comparative scheme of transgenic, cisgenesis and intragenesis

See more on Cisgenesis & Intragenesis at:

a) http://www.isca.in/AGRI_FORESTRY/Archive/v1/i10/4.ISCA-RJAFS-2013-061.pdf
b) http://www.isb.vt.edu/news/2013/Jul/HolmeWendtHolm.pdf
c) https://www.omicsonline.org/open-access/transgenic-cisgenic-intragenic-and-subgenic-crops-2329-8863-1000e123.pdf

See a video at: https://hstalks.com/t/2853/transgenics-in-agriculture/

Targeted mutagenesis/Zinc finger nuclease (ZFN)

Zinc-finger nucleases (ZFNs) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. ZFNs are proteins which have been custom-designed to cut at specific deoxyribonucleic acid (DNA) sequences. They consist of a "zinc finger" domain (recognising specific DNA sequences in the genome of the plant) and a nuclease that cuts double stranded DNA. The rationale for the development of ZFN technology for plant breeding is the creation of a tool that allows the introduction of site-specific mutations in the plant genome or the site-specific integration of genes (see Fig. 2/4).

Zinc finger nucleases are useful to manipulate the genomes of many plants and animals including arabidopsis, tobacco, soybean, corn, *Drosophila melanogaster*, *C. elegans*, *Platynereis dumerilii*, sea urchin, silkworm, zebrafish, frogs, mice, rats, rabbits, pigs, cattle, and various types of mammalian cells. Zinc finger nucleases have also been used in a mouse model of haemophilia and a clinical trial found CD4+ human T-cells with the CCR5 gene disrupted by zinc finger nucleases to be save as a potential treatment for HIV/AIDS. ZFNs are also used to create a new generation of genetic disease models called isogenic human disease models.

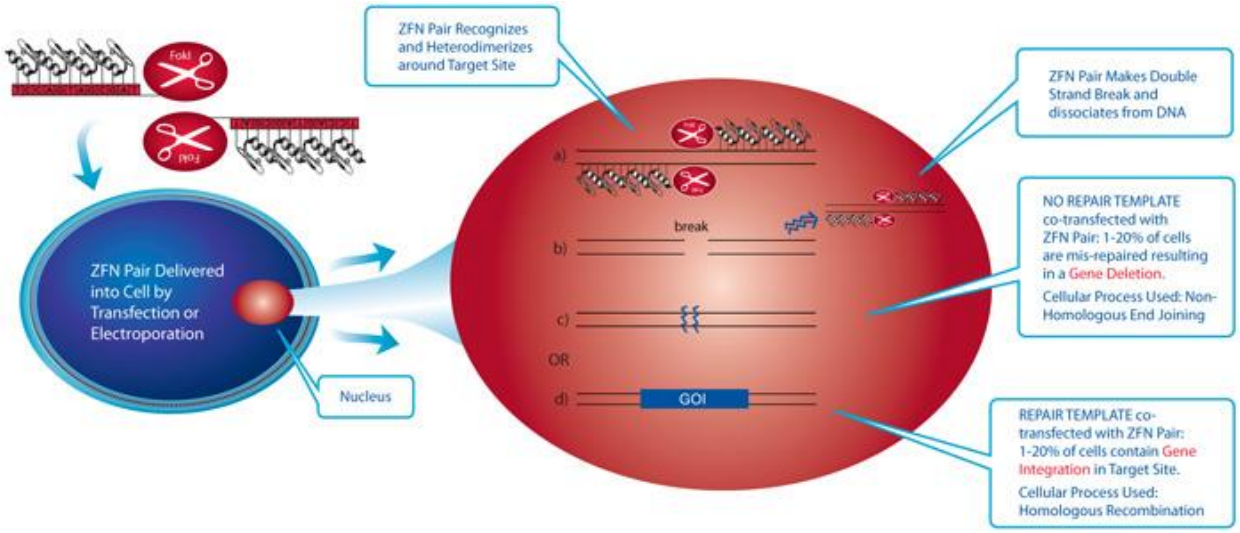


Fig. 2/4: Framework of Targeted mutagenesis/Zinc finger nuclease (ZFN)

See more on targeted mutagenesis/Zinc finger nuclease at:

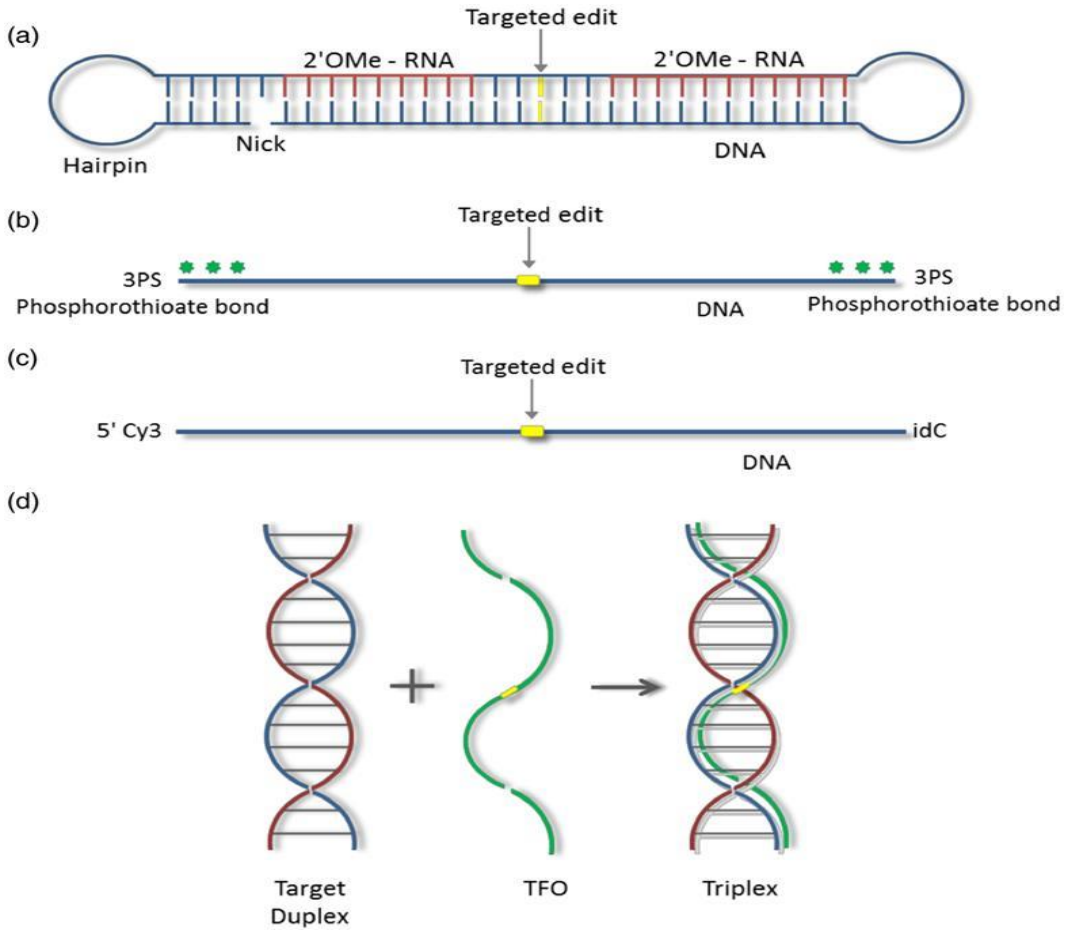
a) http://ww2.biol.sc.edu/~awaldman/Zincfingernucleases.pdf
b) http://ko.cwru.edu/publications/Miller.pdf
c) http://genetics.wustl.edu/bio5491/files/2013/03/Remy-et-al.-2009.pdf

See a video at: https://www.youtube.com/watch?v=aattpw-oVSI

Transient introduction of recombinant DNA/Oligonucleotide directed mutagenesis (ODM)

Differences in gene sequences, many of which are single nucleotide polymorphisms, underlie some of the most important traits in plants. With humanity facing significant challenges to increase global agricultural productivity, there is an urgent need to accelerate the development of these traits in plants. Oligonucleotide-directed mutagenesis (ODM), one of the many tools of Cibus' Rapid Trait Development System (RTDS™) technology, offers a rapid, precise and non-transgenic breeding alternative for trait improvement in agriculture to address this urgent need.

ODM is based on the use of oligonucleotides for the induction of targeted mutations in the plant genome, usually of one or a few adjacent nucleotides. The genetic changes that can be obtained using ODM include the introduction of a new mutation (replacement of one or a few base pairs), the reversal of an existing mutation or the induction of short deletions (see Fig. 3/4 on oligonucleotide designs).



Source: http://onlinelibrary.wiley.com/doi/10.1111/pbi.12496/full

Fig. 3/4: Framework of Oligonucleotide designs.

Note: (a) chimeraplast schematic showing regions of DNA (blue) and RNA (red; 2′-O-methyl modified), a nick and hairpin (total chimeraplast is ~68 nucleobases). (b) A single-stranded oligonucleotide modified with 3PS (3 phosphorothioate bonds) at both the 5′ and 3′ ends (total oligonucleotide length is 41, 101 or 201 nucleobases). (c) A single-stranded oligonucleotide modified with a Cy3 dye at the 5′ end and a reverse base (idC) at the 3′ end (total oligonucleotide is 41 nucleobases). (d) Triplex-forming oligonucleotide (TFO). The target duplex homopurine and homopyrimidine strands are shown in blue and red. The TFO, which binds the homopurine strand, is indicated in green. The location of the targeted nucleotide in all oligonucleotides is shown in yellow.

See more on targeted mutagenesis/Zinc finger nuclease at:

a) https://croplife.org/wp-content/uploads/pdf_files/ODM-position-paper.pdf
b) http://www.nbtplatform.org/background-documents/factsheets/factsheet-oligo-directed-nuclease.pdf
c) http://nabc.cals.cornell.edu/Publications/Reports/nabc_26/26_2_3_Gocal.pdf

See a video at: https://www.youtube.com/watch?v=WYAvlvx786w&t=44s

RNA-induced DNA methylation gene silencing

DNA cytosine methylationis an important epigenetic process that is correlated with transgene silencing, transposon suppression, and gene imprinting. In plants, small interfering RNAs (siRNAs) can trigger DNA methylation at loci containing their homolog sequences through a process called RNA-directed DNA methylation (RdDM). RNA-dependent DNA methylation (RdDM) allows breeders to produce plants that do not contain foreign DNA sequences and in which no changes or mutations are made in the nucleotide sequence but in which gene expression is modified due to epigenetics. RdDM induces the transcriptional gene silencing (TGS) of targeted genes via the methylation of promoter sequences.

In canonical RdDM, 24 nucleotide (nt) siRNAs (ra-siRNAs) will be loaded into their effector protein called ARGONAUTE 4 (AGO4) and subsequently targeted to RdDM loci through base-pairing with the non-coding transcripts produced by DNA-directed RNA Polymerase V. Then, the AGO4-ra-siRNA will recruit the DNA methyltransferase to catalyze de novo DNA methylation (see Fig. 4/4).

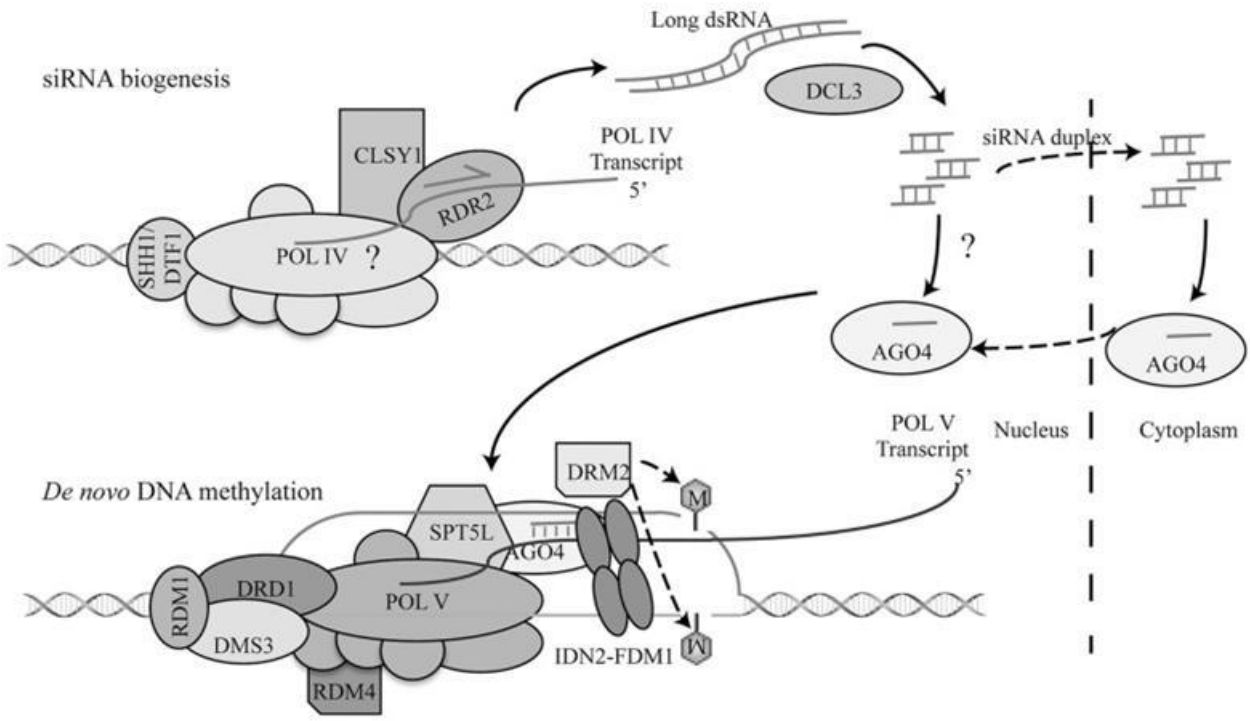


Fig. 4/4: Framework of canonical RNA-induced DNA methylation

See more on targeted mutagenesis/Zinc finger nuclease at:

- **a)** http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.334.5372&rep=rep1&type=pdf
- **b)** http://agents.cirad.fr/pjjimg/estelle.jaligot@cirad.fr/Meyer_book_chapter.pdf
- **c)** http://www.fundacion-barcelo.com.ar/oncologia-molecular/Bibliografia Complementaria sesion 1/Munoz - Proto oncogenes y oncogenes/baylin - Epigenetic gene silencing in cancer.pdf

See a video at: https://www.youtube.com/watch?v=J-OjEzCaacY

Reverse breeding

Reverse breeding (RB) is a novel plant breeding technique designed to directly produce parental lines for any heterozygous plant, one of the most sought after goals in plant breeding. RB generates perfectly complementing homozygous parental lines through engineered meiosis. The method is based on reducing genetic recombination in the selected heterozygote by eliminating meiotic crossing over. Male or female spores obtained from such plants contain combinations of non-recombinant parental chromosomes which can be cultured in vitro to generate homozygous doubled haploid plants (DHs). From these DHs, complementary parents can be selected and used to reconstitute the heterozygote in perpetuity. Since the fixation of unknown heterozygous genotypes is impossible in traditional plant breeding, RB could fundamentally change future plant breeding.

To sum up, reverse breeding is a method in which the order of events leading to the production of a hybrid plant variety is reversed. It facilitates the production of homozygous parental lines

that, once hybridised, reconstitute the genetic composition of an elite heterozygous plant, without the need for backcrossing and selection (see Fig. 5/4).

➲ ***The reverse breeding technique makes use of transgenesis to suppress meiotic recombination. In subsequent steps, only non-transgenic plants are selected.***

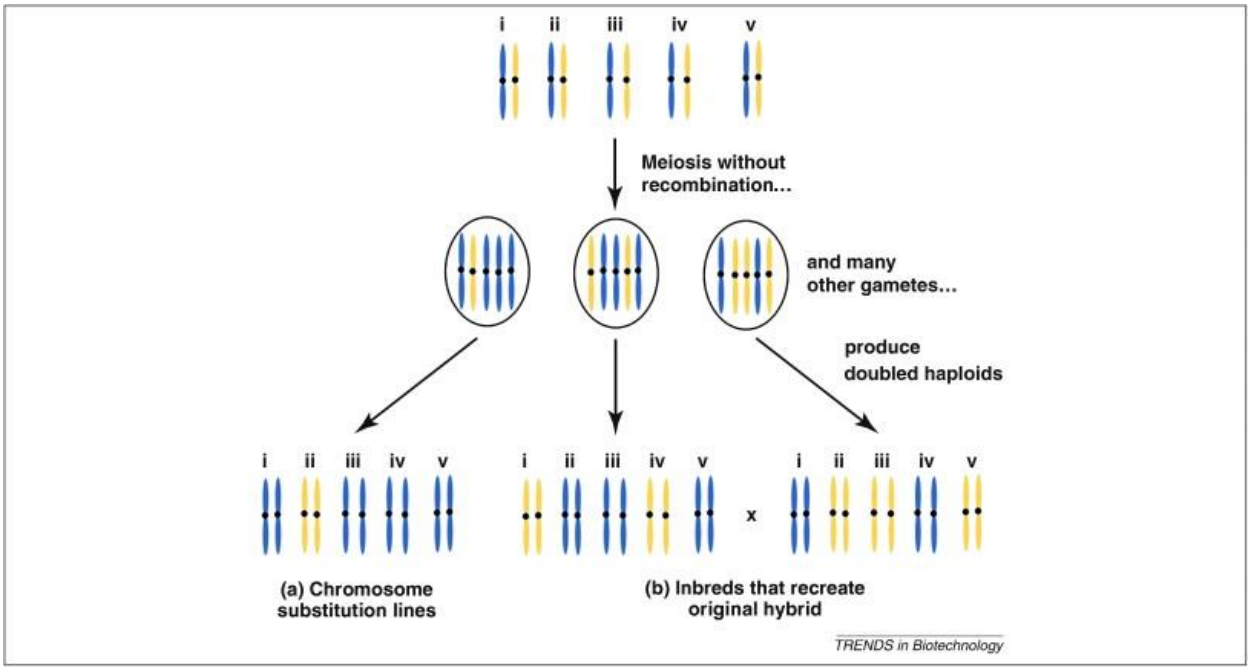


Fig. 5/4: Framework of Reverse breeding

See more on targeted mutagenesis/Zinc finger nuclease at:

- **a)** http://www.nbtplatform.org/background-documents/factsheets/factsheet-reverse-breeding.pdf
- **b)** https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2784905/
- **c)** http://www.vib.be/en/about-vib/plant-biotech-news/Documents/vib_facts_series_fromplanttocrop_ENG.pdf

See a video at: https://www.youtube.com/watch?v=-RwuNos_kZc

Grafting non-GM scion onto GM rootstock

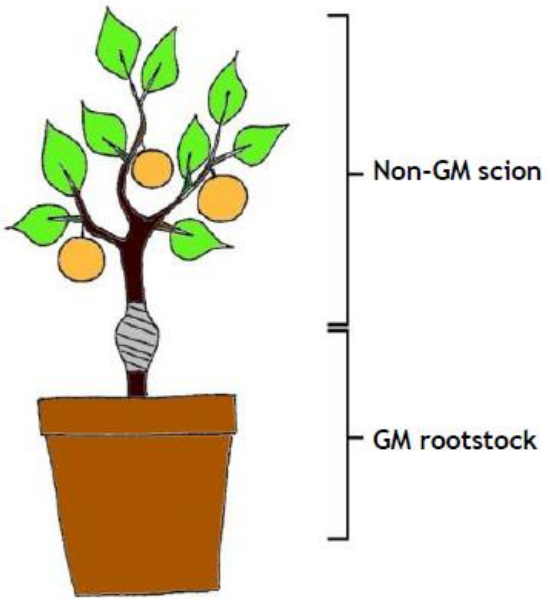
Grafting is a method whereby the above ground vegetative component of one plant (also known as the scion), is attached to a rooted lower component (also known as the rootstock) of another plant to produce a chimeric organism with improved cultivation characteristics. Normal vascular flow (e.g. nutrient flow) is established between scion and rootstock if grafting is successful, allowing for the growth and development of the scion.

The shoots of the rootstock are usually eliminated, so that all the aerial parts of the grafted plant bear the characteristics of the scion. Grafting is commonly used to combine the quality of the harvested products of the scion with beneficial characteristics of the rootstock, such as resistance to soil-borne disease or more efficient nutrient uptake resulting in a higher yield. Grafting of a non-GM scion onto a GM-rootstock works in the same way, utilising the required and/or

beneficial characteristics of a specifically selected GM rootstock (see Fig. 6/5). Transgenesis, cisgenesis and a range of other techniques can be used to transform the rootstock and/or scion.

Although the rootstock is regarded as GM, and will likely require cultivation approval, it has been established that heritable genetic material is not transmitted from the rootstock to the scion. Signalling molecules can be exchanged that affect the growth and development of the scion or rootstock, but these effects are transient in nature and not heritable. Therefore, any materials harvested from the non-GM scion are regarded as non-GM.

- ***If a GM scion is grafted onto a non-GM rootstock, then stems, leaves, flowers, seeds and fruits will be transgenic***



Source*: *NBT Factsheet Grafting on GM rootstock (drafted 2013)

Fig. 6/4: Simplified illustration of grafting non-GM scion onto GM rootstock.

See more on targeted mutagenesis/Zinc finger nuclease at:

a) http://www.nbtplatform.org/background-documents/factsheets/factsheet-grafting-on-gm-rootstock.pdf
b) http://www.epsoweb.org/file/2180
c) https://www.researchgate.net/publication/281735158_Grafting_of_Genetically_Engineered_Plants

See a video at: https://www.youtube.com/watch?v=S2FE3WJLLzM

Synthetic Genomics

New organisms and biological systems designed to satisfy human needs are among the aims of synthetic genomics and synthetic biology. Synthetic biology seeks to model and construct biological components, functions and organisms that do not exist in nature or to redesign existing biological systems to perform new functions. Synthetic genomics, on the other hand,

encompasses technologies for the generation of chemically-synthesized whole genomes or larger parts of genomes, allowing to simultaneously engineering a myriad of changes to the genetic material of organisms. Engineering complex functions or new organisms in synthetic biology are thus progressively becoming dependent on and converging with synthetic genomics. While applications from both areas have been predicted to offer great benefits by making possible new drugs, renewable chemicals or clean energy, they have also given rise to concerns about new safety, environmental and socio-economic risks – stirring an increasingly polarizing debate

Synthetic biology

Giving an unequivocal definition of synthetic biology is challenging, even to the various actors in the field (references). Rather than constituting a strictly defined field, synthetic biology may be best described as an engineering-related approach to rationally design and construct biological compounds, functions and organisms not found in nature, or to redesign existing biological parts and systems to carry out new functions. It integrates different scientific disciplines, including molecular and systems biology, chemistry, (bio-)physics, computer-aided modeling and design as well as an engineering-based notion of generating and using interchangeable "biological parts" (such as regulatory DNA and RNA elements, or coding sequences for proteins/protein domains). Compared to "traditional" genetic engineering, which mostly enhances existing biological functions or transfers them between organisms based on the modification or transfer of one or very few genes, synthetic biology work may be characterized as involving the combination of multiple genes, newly constructed "biological parts" or the use of non-natural molecules to enhance traits or to construct new biological pathways and functions – and (in the future) entire organisms.

Synthetic genomics

Synthetic genomics has been defined as "the engineering of biological components and systems that do not exist in nature and the re-engineering of existing biological elements; it is determined on the intentional design of artificial biological systems, rather than on the understanding of natural biology" (Synbiology, 2006).

Another definition of synthetic genomics is "the engineering and manipulation of an organism's genetic material on the scale of the whole genome, based on technologies to design and chemically synthesize pieces of DNA and to assemble them to long, chromosome-sized fragments". These can serve as entire genomes of viruses or bacteria. Compared with traditional genetic engineering, where typically only very few nucleotides or genes in an organism are altered (mostly based on recombinant DNA technology), synthetic genomics thus allows to

simultaneously change a large number of nucleotides or gene loci all over the genome by gene synthesis.

Thanks to the technological level reached by genetic engineering and the current knowledge regarding complete genomes sequences, large functional DNA molecules can now be synthesised efficiently and quickly without using any natural template. The production of biofuels, pharmaceuticals and the bioremediation of environmental pollution are expected to constitute the first commercial applications of this new technique.

➲ ***No research relevant to the use of synthetic genomics in plant breeding is under way***

See more on targeted mutagenesis/Zinc finger nuclease at:

a) https://science.energy.gov/~/media/ber/berac/pdf/Syn_bio.pdf
b) http://www.currentscience.ac.in/Volumes/107/12/1975.pdf
c) http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-genomics-report/Commissioned-Papers-Synthetic-Genomics-Governance.pdf
d) https://www.neb.com/~/media/NebUs/Files/News Items/Synthetic_Genomics.pdf

See a video at: https://www.youtube.com/watch?v=5NtVOB9D8PY

Genome editing techniques/CRISPR/Cas

The discovery of a bacterially derived programmable nuclease termed clustered regularly interspaced palindromic repeats (CRISPR)-associated protein 9 (Cas9) has revolutionized the field genome-editing because of its versatility and wide applicability. CRISPR genome editing relies on Cas9 and a single guide RNA (sgRNA). sgRNA is a custom, synthetic, single-stranded RNA that contains an 18–25-nucleotide sequence specific to the target DNA, followed by a scaffold sequence that complexes with Cas9. Hybridization of sgRNA-Cas9 complex to the targeted locus creates a conformational change that activates Cas9 nuclease activity, resulting in a DNA double-strand breaks (DSB).

To sum up, the CRISPR-Cas9 system consists of two key molecules that introduce a change (mutation) into the DNA:

1. an enzyme called Cas9. This acts as a pair of ‘molecular scissors’ that can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed.
2. a piece of RNA called guide RNA (gRNA) located within a longer RNA scaffold. The scaffold part binds to DNA and the pre-designed sequence ‘guides’ Cas9 to the right part of the genome. This makes sure that the Cas9 enzyme cuts at the right point in the genome (see Fig. 7/4).

CRISPR-Cas9 is a powerful tool for genome editing because the sgRNA can be quickly designed and synthesized to target specific genomic sites. Another advantage of the sgRNA targeting mechanism is that multiple genes can be targeted simultaneously. This strategy has been used to perform genome-wide knockout screens and identify mutations involved in complex biological processes.

- ***CRISPR-Cas9 (is a unique technology that enables geneticists and medical researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence***

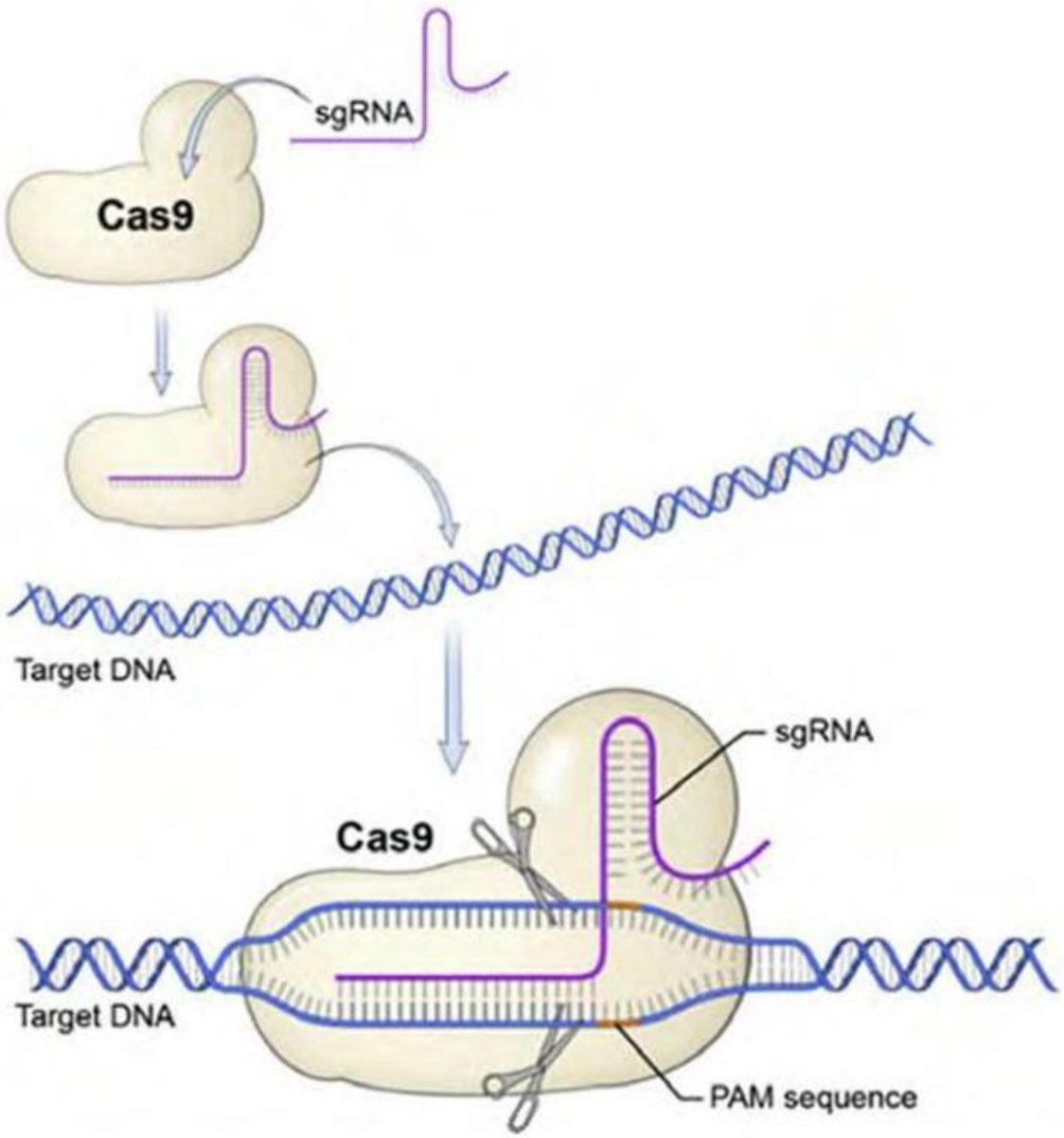


Fig. 6/4: Framework of CRISPR-Cas9–sgRNA genome targeting.

Note: sgRNA complexes with Cas9 nuclease to hone in on the targeted genomic site containing an adjacent PAM sequence. Nucleotide hybridization of sgRNA-Cas9 complex to targeted loci creates a conformational change that activates Cas9 nuclease activity, resulting in DNA double-strand breaks.

Cas9=CRISPR-associated protein 9; CRISPR=clustered regularly interspaced palindromic repeats; PAM= protospacer adjacent motif; sgRNA, single guide RNA.

See more on Genome editing techniques/CRISPR/Cas at:

- **a)** http://www.hos.ufl.edu/sites/default/files/faculty/gamoore/crispr1.pdf
- **b)** http://arep.med.harvard.edu/pdf/Yang_CPMB_2014.pdf
- **c)** http://www.origene.com/assets/documents/CRISPR-CAS9/CRISPR_manual.pdf

See a video at: https://www.youtube.com/watch?v=2pp17E4E-O8

EXAMPLES OF APPLICATIONS OF NEW BREEDING TECHNIQUES

Since the turn of the century several new tools and techniques have been invented or conceived and are being implemented to facilitate breeding of new improved crop varieties. Compared to traditional breeding these techniques reduce the time and effort needed to produce new crop varieties. These techniques are referred to as 'new plant breeding techniques'. Several of these new plant breeding techniques result in improved plants that can be obtained with traditional breeding as well (although through a very time consuming process). Table 1/4 summarizes the classification of the improved plants obtained with the different new plant breeding techniques.

Table 1/4: Classification of final products from new plant breeding techniques.

	What is the final product after breeding?		
Technique	Improved plant 1: Plant with new genes at new chromosomal locus	Improved plant 2: Plant without new genes but with a mutation	Improved plant 3: Plant without new genes or modificati-ons
Cisgenesis	X[1]		
Intragenesis	X[1]		
Sequence-specific nuclease technology (SSN-1, SSN-2)		X	
Sequence-specific nuclease technology (SSN-3)	X[2]	X[3]	X[4]
Oligo-directed mutagenesis (ODM)		X	
RNA-dependent DNA methylation			X
Reverse breeding			X
Induced early flowering			X
Grafting on GM rootstock			X

***Source*: http://edepot.wur.nl/357723**

1) new DNA is originating from the same or closely related species, 2) for targeted integration of cisgenes or intragenes at a specified location, 3) for gene replacement with a modified (artificially changed) allele (modified cisgene), 4) for gene replacement with a natural allele (cisgene)

Herbicide-tolerant oilseed rape

The company Cibus has used gene editing technology for a product that does not integrate foreign genetic material (Anon., 2015). This commercial crop, the variant has been planted in the USA in spring 2015 and has authorization to be cultivated in Canada.

➲ ***German authorities have said that they would not consider products created by gene editing as GM but rather as products of conventional breeding, but that this judgement would change if the European Commission decides otherwise.***

Fig. 7/4: Pictures of Herbicide-tolerant oilseed rape vs. control.

Potato with reduced bruising, browning and reduced propensity to generate acrylamide

The USDA and FDA have approved a potato variant developed by the company Simplot that contains no foreign DNA…Elements were transferred from sexually compatible wild potato and uses RNA interference to reduce the level of several enzymes including polyphenol oxidase responsible for bruising and browning. This variant, by lowering the level of the amino acid asparagine and of reducing sugars, also has reduced ability to generate the potentially carcinogenic metabolite acrylamide at high temperatures.

Fig. 8/4: Pictures of modified vs. conventional potatoes.

Other examples of potential applications of New Plant Breeding Techniques

1. Late blight (Phytophthora)-resistant potato using cisgenesis
2. Bacterial leaf blight resistance in rice using genome editing 20
3. Powdery mildew resistance in wheat by genome editing 21
4. Improving oil quality by genome editing (by TALENs) 21
5. Resistance to AHAS (ALS)-targeting herbicides 22
6. Induction of early flowering in trees

Read the document at: http://edepot.wur.nl/357723

Read also the "Swiss Federal Office for the Environment" Baseline report (2012) on New Plant Breeding Techniques at:
http://www.awel.zh.ch/internet/baudirektion/awel/de/biosicherheit_neobiota/gvo/Neue_Pflanzenzuchtverfahren/_jcr_content/contentPar/downloadlist/downloaditems/735_1479897633551.spooler.download.1479897341538.pdf/NPBT_translation_updated+report2016_final+version.pdf

CURRENT CHALLENGES AND FUTURE PERSPECTIVES

Advances in understanding plant biology, novel genetic resources, genome modification, and omics technologies generate new solutions for food security and novel biomaterials production under changing environmental conditions. The combination of novel molecular tools, screening technologies, and economic evaluation should become the main goal of the plant biotechnological revolution in agriculture…

Current challenges

While agricultural production advanced impressively during past decades due, among other factors, to the implementation of biotechnological tools, several remaining important issues must be addressed. The major current challenges of plant and agricultural biotechnology are:

- Contribution of new plant biotechnological tools to advanced crop breeding ;
- bottlenecks holding back the translation of genomic data to crop plant traits (genotype–phenotype gap);
- Crucial importance of plant adaptation and tolerance to abiotic and biotic stress;
- Role and significance of epigenetics for plant development under changing environmental conditions;
- plant biomaterials and biofuels as a novel scope of agricultural biotechnology

Read the document on the current challenges at:
https://www.researchgate.net/publication/274407041_Current_challenges_and_future_perspectives_of_plant_and_agricultural_biotechnology

Perspectives

Future directions, the prospects for which seem promising, should be aimed at solving the current major hurdles to agricultural biotechnology. (i) Bridging the genotype–phenotype gap by improving quantitative and automated selection and screening methods that focus on whole-plant physiology (e.g., transpiration, photosynthesis) and quality traits. These traits, combined with decision-making algorithms, will enhance the release of newly bred varieties to farmers and avoid long development phases and large-scale field studies. (ii) Bridging the genome–environment gap: since many desired plant traits depend on the interaction of many genes and metabolic pathways with the environment, enhanced adoption of translational and interactome research at all R&D stages (viz., continuously relating molecular data and breeding parameters to field performance) should preferably use more model crop plants. (iii) More attention should be given to epigenetic molecular events that are evolutionarily most relevant to plant adaptation to changing environments. (iv) Improving the biotechnological procedures of novel biomaterial production. (v) Promoting transparent dialog between molecular biologists and plant physiologists on the one hand and farmers, breeding companies, and the public on the other hand to solve jointly the economic, sociological, legal, and ethical hurdles. We thus urge the adoption of a systems bioagriculture integrated approach (as in systems biology), also considering the plant microbiome, to achieve substantial progress in plant biotechnology and agriculture in the 21st century.

CONCLUSION

Further advances in plant biotechnology and agriculture depend on the efficient combination and application of diverse scientific inputs (Figure 9/4) as the ingredients going into the biotechnology processing funnel: cell biology, biochemistry, and metabolism, the various omics, systems and synthetic biology approaches, and other, enabling techniques (e.g., tissue culture, transformation, informatics). Additional major achievements in plant biology are the new methods of plant genome engineering. For example, the bacterial RNA-directed CRISPR–Cas9 endonuclease is a versatile tool for site-specific genome modification in eukaryotes.

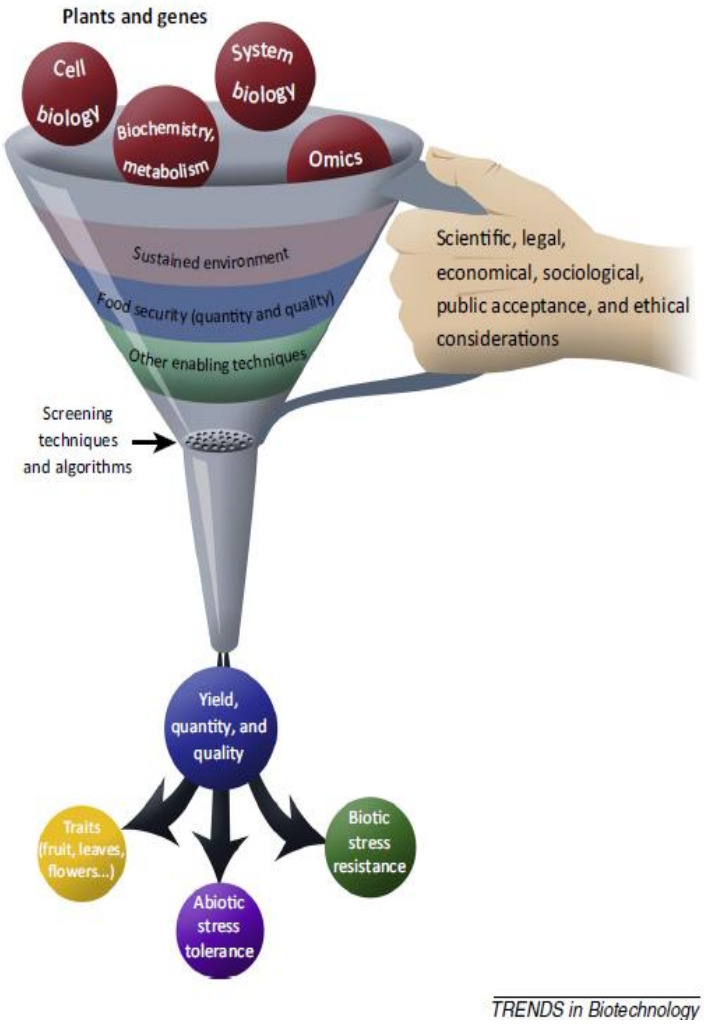


Fig. 9/4: The agricultural biotechnology processing and screening funnel.

Agricultural biotechnology processing and screening funnel

The agricultural biotechnology landscape is presented here as a processing and screening funnel comprising the major targets of plant and agricultural biotechnologies. The funnel is nourished by the various 'ingredients'; that is, diverse scientific inputs in addition to plants and their genomes. Following appropriate screening techniques, the various agricultural products and traits are expressed and released for consumers. The hand holding the funnel emphasizes that all biotechnological applications should be evaluated with respect to their contribution to global food security and judged by economic, sociological, legal, and ethical criteria.

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