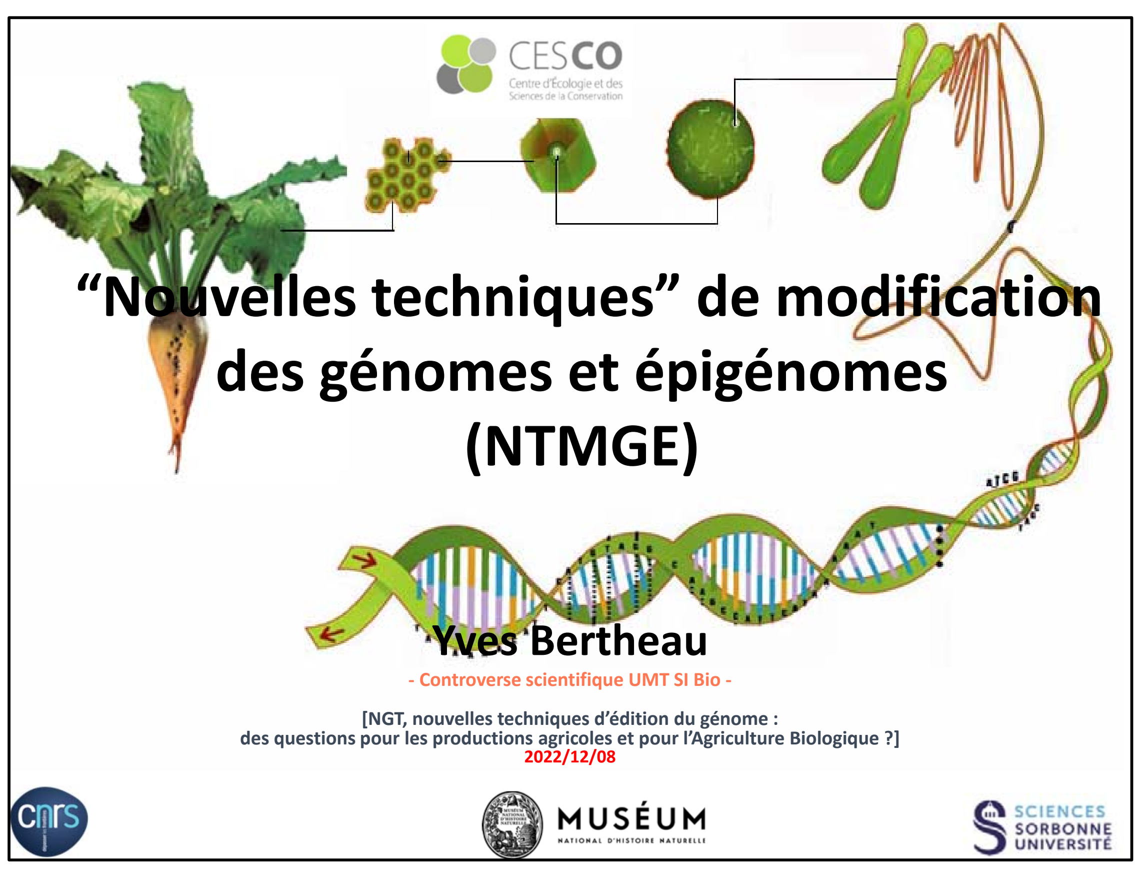

CESCO
Centre d'Écologie et des Sciences de la Conservation
“Nouvelles techniques” de modification des génomes et épigénomes (NTMGE)
Yves Bertheau
- Controverse scientifique UMT SI Bio -
[NGT, nouvelles techniques d’édition du génome :
des questions pour les productions agricoles et pour l’Agriculture Biologique ?]
2022/12/08
cnrs
MUSÉUM
NATIONAL D'HISTOIRE NATURELLE
SCIENCES
SORBONNE
UNIVERSITÉ

Albert Camus,
Prix Nobel de littérature,
écrivait en 1944:

« MAL NOMMER UN OBJET, C'EST AJOUTER AU MALHEUR DE CE MONDE"

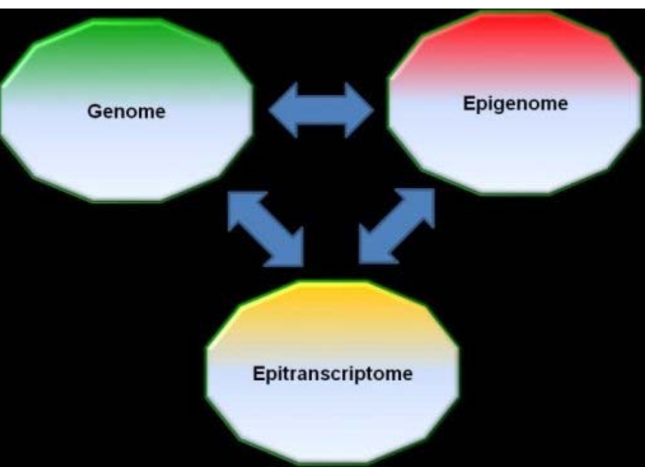
paru dans *Poésie 44*, (*Sur une philosophie de l'expression*).

Sélection variétale et mutagénèses modifient les génomes, épigénomes et épitranscriptomes nucléaires et des organites

- Génomes nucléaire et d'organites
- Épigénome : méthylation de l'ADN et modifications d'histones (protéines): un génome peut avoir plusieurs épigénomes.
- Épitranscriptome : modifications chimiques des ARN (codant et non-codant)

Epitranscriptome : https://www.nature.com/collections/nrdmjjhwky

Des interactions complexes entre domaines encore très mal connus



La modification de l'un d'eux peut entraîner des effets sur les autres difficilement discernables.

D'où l'intérêt essentiel du principe de Précaution

En raison des incohérences persistantes de corrélation génotype-phénotype

Associations pangénomiques (GWAS) encore peu fiables

NB : prépondérance du fonds génétique

4

Breeding-assisted genomics. Jesse Poland. Current Opinion in Plant Biology 2015, 24:119–124

LA SÉLECTION VÉGÉTALE

Sélection variétale

- Effet initial des herbivores autour des points d'eau sur la sélection des espèces,
- Domestication: origine des plantes cultivées, **syndrome**,
- Sélection par les agriculteurs: variétés traditionnelles,
- Lignées pures et sélection massale : cultivars (traits qualitatifs: 1 ou peu de « gènes »)
- Fécondation contrôlée (pédigrés, idéotype, hétérosis),
- Mutagénèse induite au hasard, *in vivo* (physique)
- Transgénèse,
- Mutagénèse induite au hasard *in vitro* (variation somaclonale, chimique, biologique), culture d'embryons, doublement haploïde, fusion de protoplastes,
- Sélection assistée par marqueurs (cartographie, Tilling pour génomique fonctionnelle)
- Sélection génomique (recherche de stabilité du génome et de plasticité épigénétique)
- Génétique réverse,
- Mutagénèse dirigée (OdM, RNAi, meganucleases, ZFN, TALEN, Crispr-Cas et très nombreuses variantes) et NBT en général : greffe, ségrégants négatifs,
- Speed breeding,
- Rewilding, redomestication,

Cultivars: lignées pures, variétés populations, hybrides, clonales, apomictiques et multilignées

Tilling : McCallum, C. M., L. Comai, et al. (2000). "Targeting induced local lesions IN genomes (TILLING) for plant functional genomics." Plant Physiol **123**(2): 439-442.
Signatures d'agents mutagènes :

- Bertheau, Y. (2021). Advances in identifying GM plants. Toward the routine detection of "hidden" and "new" GMOs. Developing smart-agrifood supply chains: using technology to improve safety and quality. L. Manning, Burleigh Dodds Science Publishing**:** In Press.
- Gulfishan, M., T. A. Bhat, et al. (2015). Mutants as a Genetic Resource for Future Crop Improvement. Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. J. M. Al-Khayri, S. M. Jain and D. V. Johnson. Cham, Springer International Publishing**:** 95-112.
- Enhanced crop production is seriously needed to meet the challenges of food security imposed by the rapidly increasing human population around the globe. Unpredictable climatic conditions, depleting water resources and finite arable land limit crop production. Thus the development of new crop varieties with improved yield and resistant to biotic and abiotic stresses will make a vital contribution to food security. Induced mutagenesis has played a pivotal role in ensuring food security by creating 3218 mutant varieties around the world. Mutagenesis combined with advanced molecular biology techniques and in vitro culture methods have resulted in enhanced food production. Mutant germplasm resources have been developed for different crop plants and are freely available to speed up crop improvement programs. These mutant resources are also being used for functional genomics studies, molecular

breeding and a greater understanding of the molecular basis of other biological process.

- Acquaah, G. (2015). Conventional Plant Breeding Principles and Techniques. Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. J. M. Al-Khayri, S. M. Jain and D. V. Johnson. Cham, Springer International Publishing: 115-158.

Conventional plant breeding is the development or improvement of cultivars using conservative tools for manipulating plant genome within the natural genetic boundaries of the species. Mendel's work in genetics ushered in the scientific age of plant breeding. The number of genes that control the trait of interest is important to breeders. Qualitative traits (controlled by one or a few genes) are easier to breed than quantitative traits (controlled by numerous genes). General steps in breeding are: objectives, creation/assembly of variability, selection, evaluation and cultivar release. Breeders use methods and techniques that are based on the mode of reproduction of the species self-pollinating, cross-pollinating, or clonally propagated. The general strategy is to breed a cultivar whose genetic purity and productivity can be sustained by its natural mating system. There are six basic types of cultivars: pure line, open-pollinated, hybrid, clonal, apomictic and multilines. The common methods for breeding self-pollinated species include mass selection, pure line selection, pedigree, bulk population, single seed descent, backcrossing, multiline and composite. Methods for breeding cross-pollinated species include mass selection, recurrent selection, family selection and synthetics. Hybrid cultivar breeding exploits the phenomenon of heterosis, and is applicable to both self- and cross-pollinated species. Polyploids have complex genetics. Hybridization of parents is often accompanied by infertility of the hybrid. Mutation breeding may be resorted to when the gene of interest is non-existent in nature and may be induced. Also, sometimes, the desired trait is found in wild relatives of the species and may be introgressed into cultivated species through pre-breeding.

synopsis en ligne 2021/11/08

This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording “hidden” GMOs and “new” GMOs as defined by the French NGOs and farmers’ union at the origin of the 2018 European Court of Justice ruling.

résumé accepté

In 2018 the Court of Justice of the European Union recalled that organisms with genomes modified by artifactual techniques should be considered GMOs under European regulations. GMOs derived from cultures of cells isolated in vitro or from

new genomic techniques must therefore be traceable. This chapter reviews the various technical steps and characteristics of those techniques causing genomic and epigenomic scars and signatures. These intentional and unintentional traces, some of which are already used for varietal identification, and are being standardized, can be used to identify these GMOs and differentiate them from natural mutants. The chapter suggests a routine procedure for operators and control laboratories to achieve this without additional costs.

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The growing knowledge in genetics, epigenomics, epitranscriptomics, and the 3D - or even 4D - genome structure provides an increasing number of detection targets that can be used to identify species or genetic lines, whether modified or not. Biotic and abiotic stresses also induce numerous unintentional genetic, epigenetic, and epitranscriptomic modifications. Those changes are transmissible and can be ordered in regions and classified. The detection target is characterised by the mutagenesis technique used. For instance, the detection of transgenic GMO or SDN3 modification of New Breeding Techniques (NBTs) will target their insertion's junction fragments into the genome. Each insertion induces epigenetic, and probably epitranscriptomic, changes which can also be targeted. In addition, one group of markers is linked to the trait(s) introduced or modified by the breeder whose sequence could be used in quantification and "screening". The other target will be a subset of the elements of a matrix approach (as described in the previous chapter). General selection markers, such as those used for plant breeding, together with mutagenesis techniques specific markers, could differentiate genetically modified organisms (GMOs) of any origin. They can be used to quantify and certify, through a global approach to the organism, that the trait modification is artefactual and not "natural." The growing mastery of single-cell sequencing techniques should soon make it possible to differentiate the modifications due, for example, to each step of a Crispr-Cas transformation of cells in culture. This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording “hidden” GMOs and “new” GMOs as defined by the French NGOs and farmers’ union at the origin of the 2018 European Court of Justice ruling.

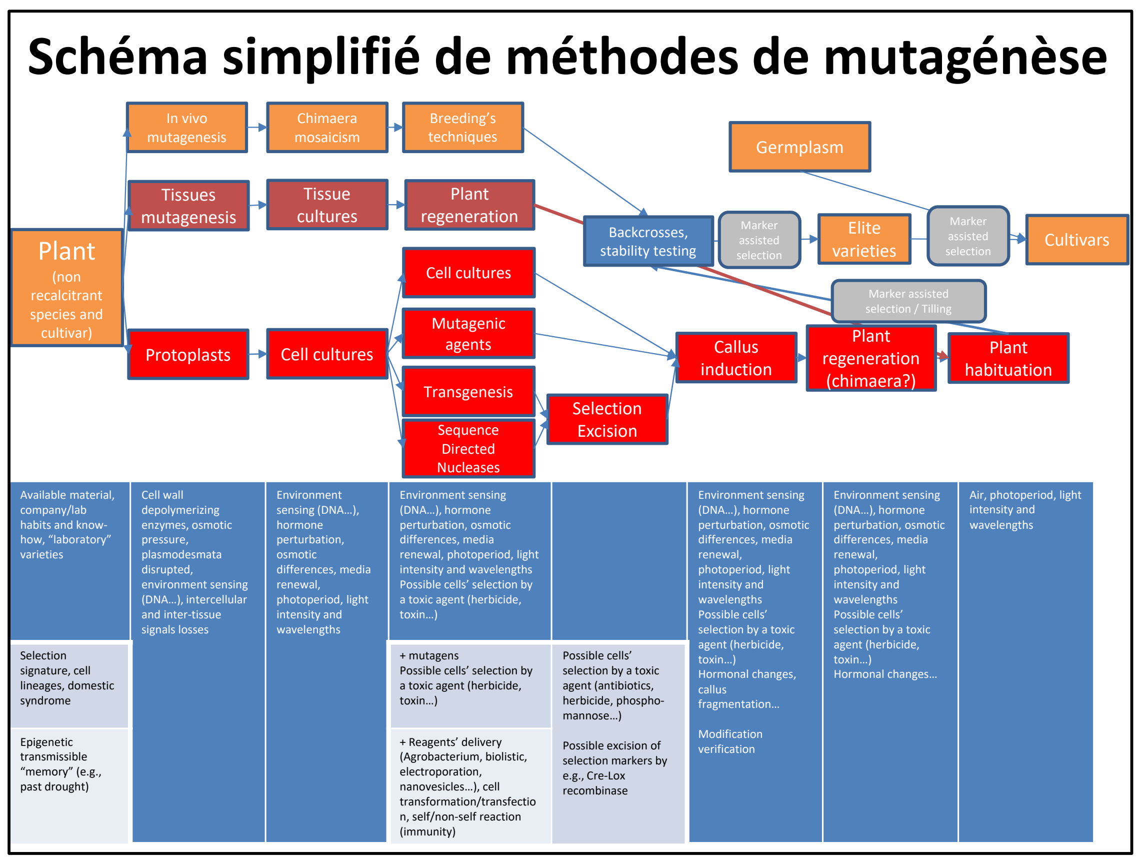

Schéma simplifié de méthodes de mutagénèse
In vivo mutagenesis
Chimaera mosaicism
Breeding's techniques
Germplasm
Tissues mutagenesis
Tissue cultures
Plant regeneration
Backcrosses, stability testing
Marker assisted selection
Elite varieties
Marker assisted selection
Cultivars
Plant (non recalcitrant species and cultivar)
Cell cultures
Marker assisted selection / Tilling
Protoplasts
Cell cultures
Mutagenic agents
Callus induction
Plant regeneration (chimaera?)
Plant habituation
Transgenesis
Selection Excision
Sequence Directed Nucleases
Available material, company/lab habits and know-how, "laboratory" varieties
Cell wall depolymerizing enzymes, osmotic pressure, plasmodesmata disrupted, environment sensing (DNA…), intercellular and inter-tissue signals losses
Environment sensing (DNA…), hormone perturbation, osmotic differences, media renewal, photoperiod, light intensity and wavelengths
Environment sensing (DNA…), hormone perturbation, osmotic differences, media renewal, photoperiod, light intensity and wavelengths Possible cells' selection by a toxic agent (herbicide, toxin…)
Environment sensing (DNA…), hormone perturbation, osmotic differences, media renewal, photoperiod, light intensity and wavelengths Possible cells' selection by a toxic agent (herbicide, toxin…) Hormonal changes, callus fragmentation…
Modification verification
Environment sensing (DNA…), hormone perturbation, osmotic differences, media renewal, photoperiod, light intensity and wavelengths Possible cells' selection by a toxic agent (herbicide, toxin…) Hormonal changes…
Air, photoperiod, light intensity and wavelengths
Selection signature, cell lineages, domestic syndrome
+ mutagens Possible cells' selection by a toxic agent (herbicide, toxin…)
Possible cells' selection by a toxic agent (antibiotics, herbicide, phospho-mannose…)
Epigenetic transmissible "memory" (e.g., past drought)
+ Reagents' delivery (Agrobacterium, biolistic, electroporation, nanovesicles…), cell transformation/transfection, self/non-self reaction (immunity)
Possible excision of selection markers by e.g., Cre-Lox recombinase

Facteurs à prendre en compte

- Fonds génétique dominant, d'où « variétés de laboratoire » pour les biotechs, croisements complexes à partir des « Elite » et introduction de nouveaux caractères, effet ancestral (cf. thérapies géniques),
- Traits qualitatifs mais majoritairement quantitatifs (gènes candidats…), multifactoriels (génétiques et épigénétiques, épistasie, pleïotropie),
- Rétrocroisements n'éliminent qu'une partie des séquences indésirables,

Microbiotes oubliés dans sélection variétale,

- Distance génétique entre 2 variétés Elite de maïs équivaut à celle entre chimpanzé et homme
- Backcross de blé : à 98% de pureté théorique, encore des millions de base non « nettoyées », likage drag, haplotypes…

Bertheau, Y. (2019). New breeding techniques: detection and identification of the techniques and derived products. Encyclopedia of Food Chemistry, Reference Module in Food Science. R. H. Stadler, Elsevier**:** 320-336.

Since the commercial releases of GMOs in the 90s, new genetic modification tools known as New breeding techniques have been developed for e.g. gene silencing or more precise genomic modifications such as Crispr-endonuclease based systems. As for GMOs several consumers view may prevail about the societal interest in agricultural production and food of such genetic modification. Ensuring the freedom of choice to consumers needs to develop detection tools which could infer the NBT nature of the modification technique used. This article reviews all the elements which could allow the identification and detection of such techniques and products.

Bertheau, Y. (2021). Advances in identifying GM plants. Toward the routine detection of "hidden" and "new" GMOs. Developing smart-agrifood supply chains: using technology to improve safety and quality. L. Manning, Burleigh Dodds Science Publishing**:** In Press.

This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability

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In 2018 the Court of Justice of the European Union recalled that organisms with genomes modified by artifactual techniques should be considered GMOs under European regulations. GMOs derived from cultures of cells isolated in vitro or from new genomic techniques must therefore be traceable. This chapter reviews the various technical steps and characteristics of those techniques causing genomic and epigenomic scars and signatures. These intentional and unintentional traces, some of which are already used for varietal identification, and are being standardized, can be used to identify these GMOs and differentiate them from natural mutants. The chapter suggests a routine procedure for operators and control laboratories to achieve this without additional costs.
The growing knowledge in genetics, epigenomics, epitranscriptomics, and the 3D - or even 4D - genome structure provides an increasing number of detection targets that can be used to identify species or genetic lines, whether modified or not. Biotic and abiotic stresses also induce numerous unintentional genetic, epigenetic, and epitranscriptomic modifications. Those changes are transmissible and can be ordered in regions and classified. The detection target is characterised by the mutagenesis technique used. For instance, the detection of transgenic GMO or SDN3 modification of New Breeding Techniques (NBTs) will target their insertion's junction fragments into the genome. Each insertion induces epigenetic, and probably epitranscriptomic, changes which can also be targeted. In addition, one group of markers is linked to the trait(s) introduced or modified by the breeder whose sequence could be used in quantification and "screening". The other target will be a subset of the elements of a matrix approach (as described in the previous chapter). General selection markers, such as those used for plant breeding, together with mutagenesis techniques specific markers, could differentiate genetically modified organisms (GMOs) of any origin. They can be used to quantify and certify, through a global approach to the organism, that the trait modification is artefactual and not "natural." The growing mastery of single-cell sequencing techniques should soon make it possible to differentiate the modifications due, for example, to each step of a Crispr-Cas transformation of cells in culture. This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording “hidden” GMOs and “new” GMOs as defined by the French NGOs and farmers’ union at the origin of the 2018 European Court of Justice ruling.

- Complexité caractères et erreurs de sélection : exemple de la tolérance à la sécheresse du maïs aux USA ou du blé en Argentine (évitement de la sécheresse versus tolérance)

Li, P., B. Ma, et al. (2021). "Wheat breeding highlights drought tolerance while ignores the advantages of drought avoidance: A meta-analysis." European Journal of Agronomy **122**: 126196.

Crop tolerance and avoidance are critical adaptation mechanisms to cope with drought stress but they contribute differently to grain yield formation. Little is known about the different roles of these two mechanisms in long-term crop breeding. A meta-analysis was conducted to determine the different effects of drought tolerance and avoidance mechanisms on the drought adaptation of wheat crops. The meta-analysis summarized the results of 283 published papers in international journals, and illustrated that primitive wheat genotypes, including wild, cultivated diploids, tetraploids and old hexaploids, preferentially showed a drought avoidance strategy, as evidenced by large root biomass, small leaf area, and reduced stomatal conductance under water deficits. Modern hexaploid genotypes showed stronger drought tolerance advantages, such as high leaf water potential and osmotic adjustment, with a small root system. The meta-analysis indicated that the breeding process of dryland wheat has been continuously enhancing drought tolerance while weakening drought avoidance. Under severe water deficits, old hexaploid wheat genotypes with drought avoidance characteristics showed lower reduction of aboveground biomass and yield than modern genotypes with stronger drought tolerance features, while under mild and moderate water deficits genotypes with stronger drought tolerance features had higher yields and aboveground biomass. The meta-analysis provide information for making decisions on the direction of modern crop breeding and the implementing of managing practices to cope with drought stress, which frequency and severity is increasing with the advent of climate change.

Gillam, C. (2012). Science group finds drought-tolerant GMO corn lacking. Reuters.

Report challenges effectiveness of Monsanto corn

Says no improved water efficiency

Monsanto says new corn mitigates risk of yield loss in drought

Field trials ongoing this year

New genetically altered corn aimed at helping farmers deal with drought offers more hype than help over the long term.

This is according to a report issued by a science and environmental advocacy group. The Union for Concerned Scientists (UCS) said the only genetically altered corn approved by regulators and undergoing field trials in the United States has no improved water efficiency, and provides only modest results in only moderate drought conditions.

"Farmers are always looking to reduce losses from drought, but the biotechnology industry has made little real-world progress on this problem," said Doug Gurian-Sherman, a plant pathologist and senior scientist for UCS. "Despite many years of research and millions of dollars in development costs, DroughtGard doesn't outperform the non-engineered alternatives."

UCS used data generated by Monsanto, the developer of biotech "DroughtGard" corn approved by regulators in December and an analysis by the US Department of Agriculture (USDA). It said Monsanto's corn "does not appear to be superior to several recent classically bred varieties of drought-tolerant corn."

Conventional breeding techniques and improved farming practices have helped boost drought tolerance of corn planted in the United States by about 1% per year

over the past several decades. The group calculated this was roughly equal to or better than what the new GMO corn has demonstrated.
Monsanto said its new drought-tolerant corn “can help farmers mitigate the risk of yield loss when experiencing drought stress, primarily in areas of annual drought stress, which in the US historically has been the Western Great Plains region.”
Monsanto spokesman Thomas Helscher said, “Specifically, these hybrids with the drought trait can use less water during severe drought stress and have more kernels per ear.”
Monsanto’s Drought Gard corn hybrids are in the final phase before commercialization in on-farm field trials. The company hopes to roll the product out commercially next year.
Drought is a significant problem for agriculture in the United States and globally. Last year, extreme drought in Texas and throughout the US South wiped out crops and left livestock without pasture or hay, with damages to the agriculture industry calculated at more than $5 billion.
Monsanto, DuPont, and other biotech companies have touted crops that perform better in drought as a means to help farmers combat water shortages. The UCS report said that classical and other forms of breeding are more cost efficient and effective than genetic engineering.
“An exaggerated expectation about the capacity of genetic engineering at the expense of other approaches risks leaving farmers and the public high and dry when it comes to ensuring that the United States and other nations can produce enough food, and have enough clean freshwater, to meet everyone’s needs,” the report said.
UCS said that rather than relying on private industry research, Congress and the USDA should substantially increase support for public crop-breeding programs to improve drought tolerance, and should use conservation programs funded under the federal Farm Bill to expand the use of available methods for improving drought tolerance and water use efficiency.
A spokeswoman for the biotechnology industry said genetically modified drought-tolerant crops could still prove valuable.
“It’s too early to make assumptions about drought tolerant technology, while it is still being tested,” said Karen Batra, spokeswoman for the Biotechnology Industry Organization (BIO).
“It’s absurd to assume it’s an either/or debate. With growers all over the world dealing with climate change and increased demand due to overpopulation, we need to turn to all the means available — including improved seeds and biotechnology to address these challenges.”

Ashraf, M. (2010). "Inducing drought tolerance in plants: recent advances." Biotechnology Advances **28**(1): 169-183.
Undoubtedly, drought is one of the prime abiotic stresses in the world. Crop yield losses due to drought stress are considerable. Although a variety of approaches have been used to alleviate the problem of drought, plant breeding, either conventional breeding or genetic engineering, seems to be an efficient and

economic means of tailoring crops to enable them to grow successfully in drought-prone environments. During the last century, although plant breeders have made ample progress through conventional breeding in developing drought tolerant lines/cultivars of some selected crops, the approach is. in fact, highly time-consuming and labor- and cost-intensive. Alternatively, marker-assisted breeding (MAB) is a more efficient approach, which identifies the usefulness of thousands of genomic regions of a crop under stress conditions, which was, in reality, previously not possible. Quantitative trait loci (QTL) for drought tolerance have been identified for a variety of traits in different crops. With the development of comprehensive molecular linkage maps, marker-assisted selection procedures have led to pyramiding desirable traits to achieve improvements in crop drought tolerance. However. the accuracy and preciseness in QTL identification are problematic. Furthermore, significant genetic x environment interaction, large number of genes encoding yield, and use of wrong mapping populations, have all harmed programs involved in mapping of QTL for high growth and yield under water limited conditions. Under such circumstances, a transgenic approach to the problem seems more convincing and practicable, and it is being pursued vigorously to improve qualitative and quantitative traits including tolerance to biotic and abiotic stresses in different crops. Rapid advance in knowledge on genomics and proteomics will certainly be beneficial to fine-tune the molecular breeding and transformation approaches so as to achieve a significant progress in crop improvement in future. Knowledge of gene regulation and signal transduction to generate drought tolerant crop cultivars/lines has been discussed in the present review. In addition, the advantages and disadvantages as well as future prospects of each breeding approach have also been discussed. (C) 2009 Elsevier Inc. All rights reserved.

Lobell, D. B., M. J. Roberts, et al. (2014). "Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest." Science **344**(6183): 516-519.
A key question for climate change adaptation is whether existing cropping systems can become less sensitive to climate variations. We use a field-level data set on maize and soybean yields in the central United States for 1995 through 2012 to examine changes in drought sensitivity. Although yields have increased in absolute value under all levels of stress for both crops, the sensitivity of maize yields to drought stress associated with high vapor pressure deficits has increased. The greater sensitivity has occurred despite cultivar improvements and increased carbon dioxide and reflects the agronomic trend toward higher sowing densities. The results suggest that agronomic changes tend to translate improved drought tolerance of plants to higher average yields but not to decreasing drought sensitivity of yields at the field scale.

- microbiotes
Favela, A., M. O. Bohn, et al. (2021). "Maize germplasm chronosequence shows crop breeding history impacts recruitment of the rhizosphere microbiome." The

ISME Journal **15**(8): 2454-2464.
Recruitment of microorganisms to the rhizosphere varies among plant genotypes, yet an understanding of whether the microbiome can be altered by selection on the host is relatively unknown. Here, we performed a common garden study to characterize recruitment of rhizosphere microbiome, functional groups, for 20 expired Plant Variety Protection Act maize lines spanning a chronosequence of development from 1949 to 1986. This time frame brackets a series of agronomic innovations, namely improvements in breeding and the application of synthetic nitrogenous fertilizers, technologies that define modern industrial agriculture. We assessed the impact of chronological agronomic improvements on recruitment of the rhizosphere microbiome in maize, with emphasis on nitrogen cycling functional groups. In addition, we quantified the microbial genes involved in nitrogen cycling and predicted functional pathways present in the microbiome of each genotype. Both genetic relatednesses of host plant and decade of germplasm development were significant factors in the recruitment of the rhizosphere microbiome. More recently developed germplasm recruited fewer microbial taxa with the genetic capability for sustainable nitrogen provisioning and larger populations of microorganisms that contribute to N losses. This study indicates that the development of high-yielding varieties and agronomic management approaches of industrial agriculture inadvertently modified interactions between maize and its microbiome.
Melotto, M., M. T. Brandl, et al. (2020). "Breeding Crops for Enhanced Food Safety." Frontiers in Plant Science **11**(428).
An increasing global population demands a continuous supply of nutritious and safe food. Edible products can be contaminated with biological (e.g., bacteria, virus, protozoa), chemical (e.g., heavy metals, mycotoxins), and physical hazards during production, storage, transport, processing, and/or meal preparation. The substantial impact of foodborne disease outbreaks on public health and the economy has led to multidisciplinary research aimed to understand the biology underlying the different contamination processes and how to mitigate food hazards. Here we review the knowledge, opportunities, and challenges of plant breeding as a tool to enhance the food safety of plant-based food products. First, we discuss the significant effect of plant genotypic and phenotypic variation in the contamination of plants by heavy metals, mycotoxin-producing fungi, and human pathogenic bacteria. In addition, we discuss the various factors (i.e., temperature, relative humidity, soil, microbiota, cultural practices, and plant developmental stage) that can influence the interaction between plant genetic diversity and contaminant. This exposes the necessity of a multidisciplinary approach to understand plant genotype × environment × microbe × management interactions. Moreover, we show that the numerous possibilities of crop/hazard combinations make the definition and identification of high-risk pairs, such as Salmonella-tomato and Escherichia coli-lettuce, imperative for breeding programs geared toward improving microbial safety of produce. Finally, we discuss research on developing effective assays and approaches for selecting desirable breeding germplasm. Overall, it is recognized that although breeding programs for some human

pathogen/toxin systems are ongoing (e.g., Fusarium in wheat), it would be premature to start breeding when targets and testing systems are not well defined. Nevertheless, current research is paving the way toward this goal and this review highlights advances in the field and critical points for the success of this initiative that were discussed during the Breeding Crops for Enhanced Food Safety workshop held 5–6 June 2019 at University of California, Davis.

LES LIMITES DES SÉQUENÇAGES,
ASSEMBLAGES ET ANNOTATIONS

La progression des stratégies

- Organismes modèles initialement proposés : trop d'inconnues,
- Second ensemble de propositions : 1 001 génomes par organisme pour définir les pangénomes,
- Propositions récentes : 10 000 génomes et épigénomes
- Recul des espoirs sur les synténies et micro-synténies inter-espèces, voire entre variétés (importance du fond génétique),
- Reconnaissance des pangénomes et des structures 3D/ 4D des génomes

(Koch, 2016)

Synténie (Wikipedia) :
La **synténie** est la présence simultanée sur le même chromosome de deux ou plusieurs loci, indépendamment de leur liaison génétique. La notion de synténie est de plus en plus utilisée pour décrire la conservation de l'ordre des gènes entre deux espèces apparentées.
Dans ce cas, la localisation de plusieurs gènes peut être prédite grâce à un modèle de données. Les comparaisons entre espèces phylogénétiquement éloignées révèlent une augmentation de la perte de synténie.

Conclusion :
à la découverte de nouvelles
Terra incognita…
Mais avons-nous les boussole, astrolabe et portulan pour ces aventures ?

Les NTMGE...
Mais comment parvient-on aux fabuleux changements annoncés?

Majorité de brevets portant sur des plantes tolérantes aux herbicides et quelques insectes (continuité des OGM de transgénèse car retours sur investissements rapides)

Greffes de 40 fruits sur un arbre (université de Syracuse)
https://www.youtube.com/watch?v=5kO6-PpgZ1M
Sam Van Aken, an art professor at Syracuse University, grafted the tree over nine years into something of biblical proportions. The "Tree of 40 Fruits" contains peaches, plums, nectarines and apricots, all of which are readily edible. Jeff Glor reports.

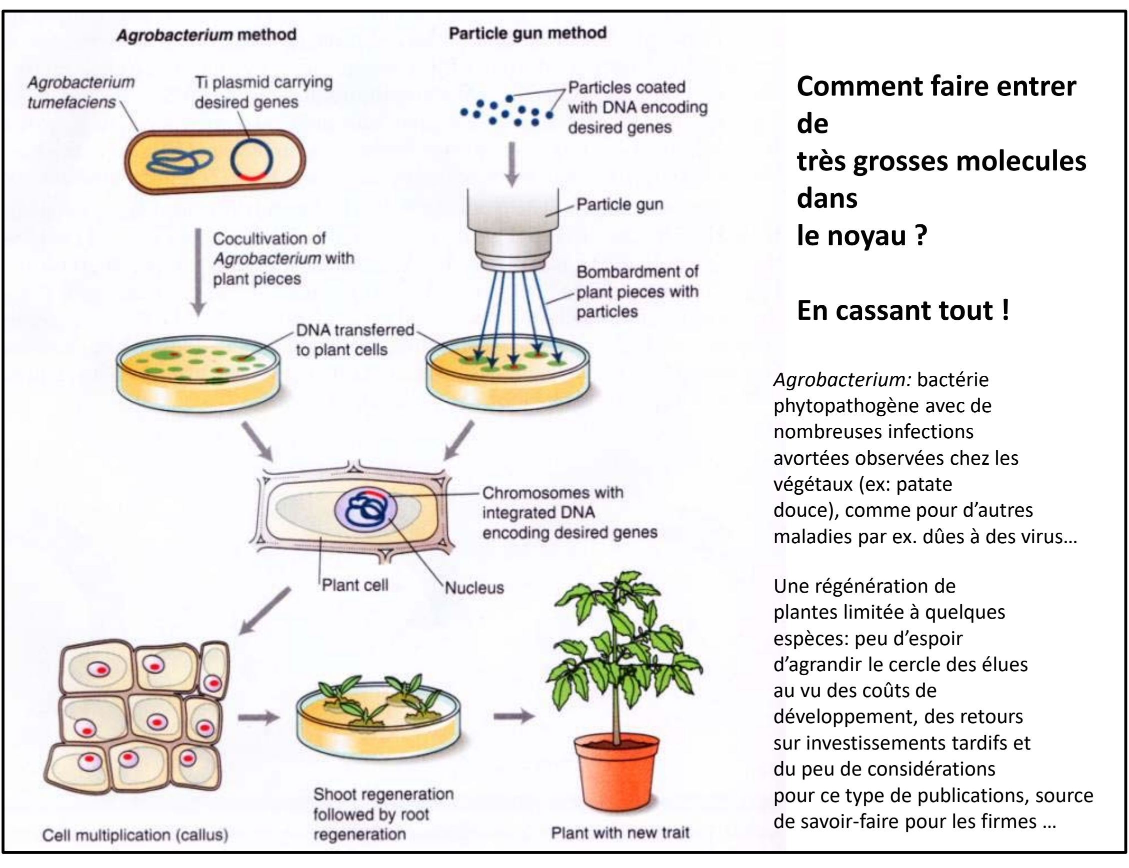
Céréales avec plus de fibres pour les américains alors qu'il suffirait de consommer des farines complètes

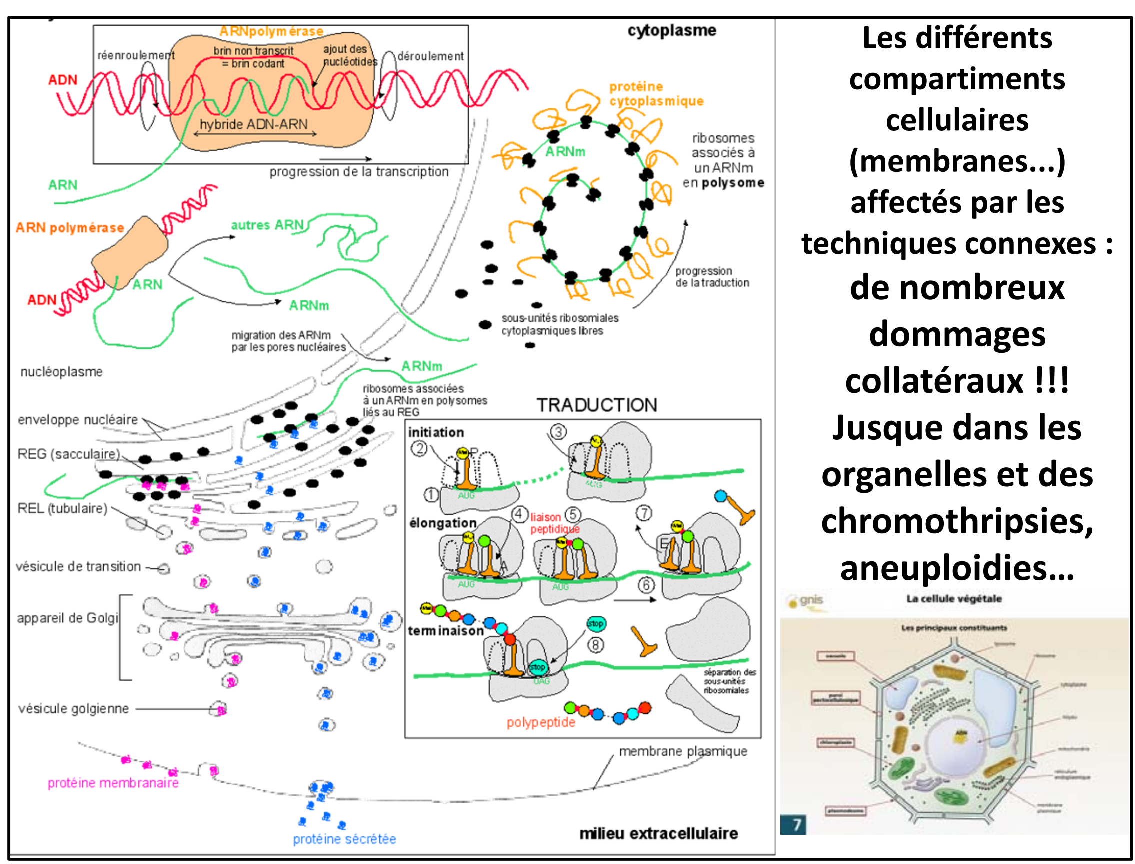
Pomme ou champignon brunissant plus lentement : comment choisir ses fruits et légumes sur un étal si le vieux paraît aussi frais que ceux récemment cueillis avec leurs vitamines encore présentes ? Pertes de repères des consommateurs après des millénaires d'apprentissage de recherche du frais avec vitamines et sels minéraux…

Pomme de terre avec moins d'asparagine (transformation en acrylamide à des températures élevées): il suffit de cuire moins fort, et surtout la consommation en très grosses quantités de frites concerne majoritairement les américains.

ÉTAPES INDISPENSABLES:
LES « TECHNIQUES CONNEXES »,
UN « DÉTAIL » SOUVENT OUBLIÉ
DANS LES PRÉSENTATIONS DES NTMGE

(OU COMMENT FAIRE PARVENIR LES RÉACTIFS NTMGE AUX SITES D'ACTION, LES FAIRE AGIR, SÉLECTIONNER LES QUELQUES CELLULES TRANSFORMÉES, ÉLIMINER LES SYSTÈMES DE SÉLECTION ET TENTER DE RÉGÉNÉRER DES PLANTES...)


Agrobacterium method
Particle gun method
Agrobacterium tumefaciens
Ti plasmid carrying desired genes
Particles coated with DNA encoding desired genes
Particle gun
Cocultivation of Agrobacterium with plant pieces
Bombardment of plant pieces with particles
DNA transferred to plant cells
Chromosomes with integrated DNA encoding desired genes
Plant cell
Nucleus
Cell multiplication (callus)
Shoot regeneration followed by root regeneration
Plant with new trait
Comment faire entrer de très grosses molecules dans le noyau ?
En cassant tout !
Agrobacterium: bactérie phytopathogène avec de nombreuses infections avortées observées chez les végétaux (ex: patate douce), comme pour d'autres maladies par ex. dûes à des virus…
Une régénération de plantes limitée à quelques espèces: peu d'espoir d'agrandir le cercle des élues au vu des coûts de développement, des retours sur investissements tardifs et du peu de considérations pour ce type de publications, source de savoir-faire pour les firmes …



Chromothripsie : https://theconversation.com/la-chromothripsie-quand-nos-genes-subissent-un-cataclysme-a-lorigine-de-cancers-tres-agressifs-157169

Les techniques connexes: sources de faux positifs et négatifs et de contaminations en ADN

Les NTMGE nécessitent le recours aux « vieilles techniques » utilisées pour la transgenèse des OGM déjà commercialisés:

- protoplastisation, vectorisation (virus, particules de biolistique, bactéries comme *Agrobacterium*…), cultures cellulaires, systèmes de sélection des cellules modifiées et leur élimination, régénération des plantes non récalcitrantes (d'où un spectre toujours limité d'espèces)…
- Toutes techniques stressantes inductrices de mutations et épimutations (jusqu'à 35% pour les cultures cellulaires)
  - mal repérables (logiciels et génomes de référence fiables manquant) car souvent mutations ponctuelles ou indels, surtout dans des régions répétées ou non codantes, problèmes des translocations et inversions…
  - Difficilement éliminables (rétrocroisements par les firmes en nombre insuffisant, co-ségrégations selon les caractères, régions à hérédité non mendélienne) laissant des millions de pb non « apurés » et peu / mal contrôlables (cf. point logiciels et génomes de référence),
  - Chromothripsie ex. cultures cellulaires de pomme de terre, aneuploidie
- Ex. : colza Cibus, faillite société Genta après 1 milliards US$ essais cliniques

Colloque à Londres en Octobre 2016 : laboratoires recherchent désespérément bons chefs cuisiniers bien entraînés et regrettent le manque d'écoles pour former les futurs « chefs »… de ces techniques connexes.

17

Voir nouveaux à propos du colza Cibus, brevets sur technique RTDS (système OdM : Oligonucleotide Directed Mutagenesis) finalement devenue une variation smalconale après un intense lobbying auprès de la Commission européenne et nombreux brevets
Voir les articles de Inf'OGM:

https://www.infogm.org/5975-ogm-modifier-plante-pas-anodin
https://www.infogm.org/5982-ogm-modifier-plante-pas-anodin-suite

On old cooking recipes (past decades) see for instance Ledford 2016 Nature 539 (7627): 16-17 about the Conference held in October 2016 o those 'related technics'.

NBT labs are looking for trained chiefs but training are missing and thus experienced chiefs also…
In most – if not all - of the description of NBT, the related technics such as protoplast preparation or cell culture are omitted while their use in the genome editing technics, rdDM, OdM are all causes of unexpected effects.
We have to recall that most of theses related technics are also used for transgenesis and are the sources of some of the unintended genome changes also observed for the GMO obtained by transgenesis.

Protoplastes' preparation, cell culture and plant regeneration, somaclonal variation

are all inducing by stresses mutations and epimutations, chromosomes' rearrangements…

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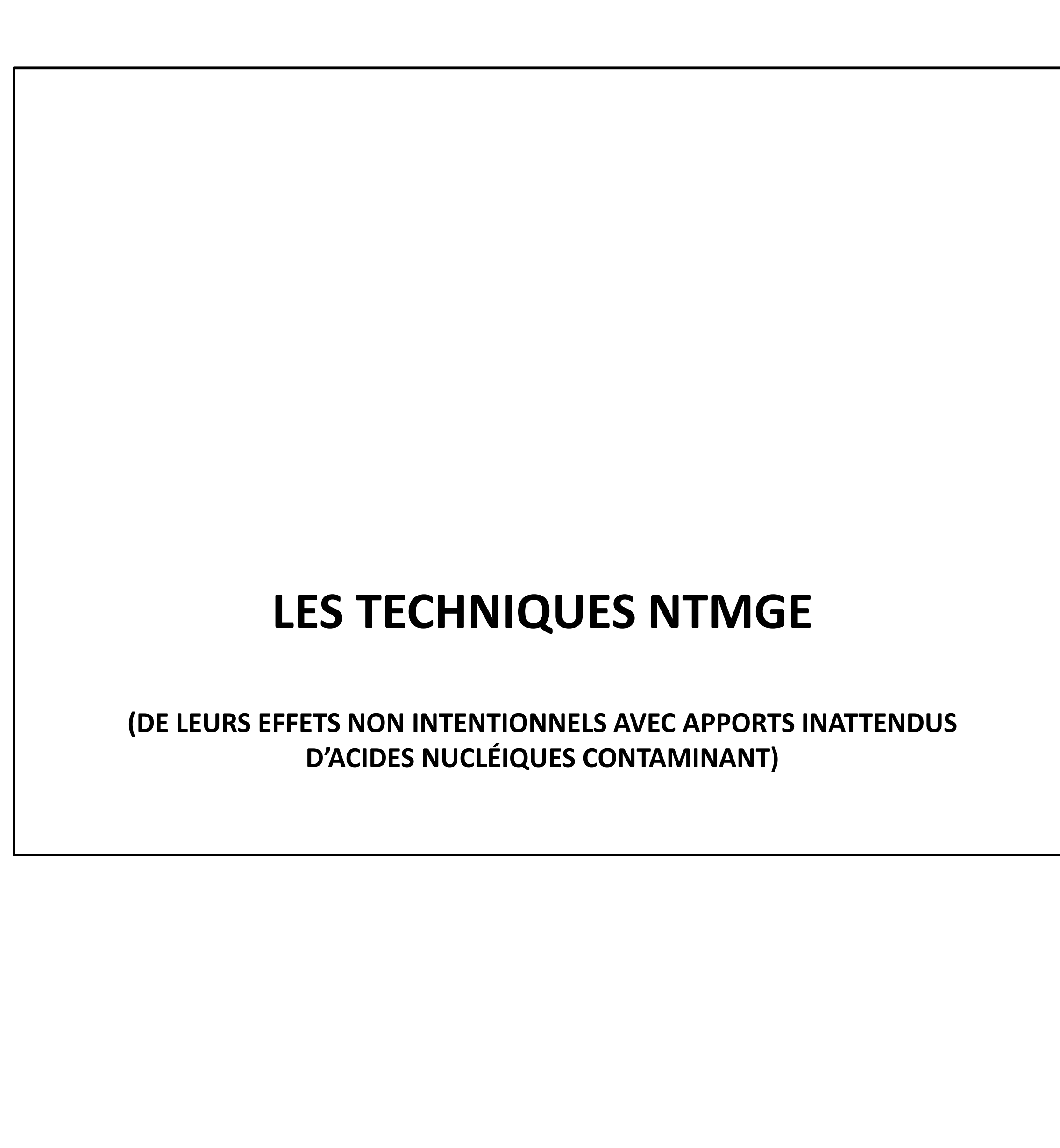

LES TECHNIQUES NTMGE
(DE LEURS EFFETS NON INTENTIONNELS AVEC APPORTS INATTENDUS
D’ACIDES NUCLÉIQUES CONTAMINANT)

Le travail communautaire initial sur les NTMGE (COGEM 2007)

- Zinc finger nuclease technology (ZFN1-ZFN3) + TALEN+ meganucleases
- Oligonucleotide Directed Mutagenesis (ODM)
- Cisgenesis/ Intragenesis vs. Transgenesis
- RNA-dependent DNA Methylation (RdDM)
- Grafting (GM rootstock / scion)
- Agro-infiltration (Agro-infiltration "*sensu stricto*", Agro-infection, Floral-dip *i.e.* plant transformation)
- Reverse breeding, negative segregants
- Synthetic biology

Un groupe de travail financé par la Commission (2007-2012)
Rapport confidentiel
mais commenté pour ses aspects « positifs » (ex: JKI) 19

A very large set of diverse unrelated different techniques used or not in combinations with several mechanisms but possible final characteristics.

The first version of the NBT set did not include some technics, such as CRISPR-Cas9, published in 2012,
A contrario agro-infiltration is thought by some Countries only as sensu-stricto and in confined areas, while some other ones are also considering agroinoculation and floral dip, and as disseminated organisms

Meganucleases: 1990s
Oligonucleotide-directed mutagenesis: late 1990s
Zinc finger nucleases (ZFNs): mid 2000s
Transcription activator like effector nucleases (TALENs): early 2010s
Clustered regularly spaced short palindromic repeats (CRISPR): 2012 but several previous papers of Bayer

A discourse targeting only plants as the technics do not appear to be ready for animals, to avoid fears about Human transformation, while plants will be used for "proof of concept".

A 2007 first memo from COGEM (Dutch advisory board on GMOs)

Lusser et al. 2011 New plant breeding techniques State-of-the-art and prospects for commercial development EUR 24760 EN

The importance of semantics and semiology…
Have a look to the wording “breeding technique” and the “New” adjective… more or restriction to plants, idea of improvement (techniques presented as the offspring of old and secure techniques)
The faith in backcrosses for “rapidly” coming back from GM organisms to non-GM plants (but 4 to 14 generations needed according to the location of and distance between off-target / unintended mutation and the targeted trait, not segregation
To decrease the duration of successive backcrosses: special greenhouses (see for instance Florimond Desprez investments: 5 M€ for reducing the 3 first generations to 14 months) with further impact on seeds’ prices…

 Zinc finger nucleases are chimeric proteins “Zinc finger” domain (recognising specific DNA sequence) & nuclease cutting double-stranded DNA

 ZFN-1 technology:
- delivery of genes encoding ZFNs (without repair template)
- site-specific double-strand break (DSB)
- natural repair process (through non-homologous end-joining NHEJ)
- mutation in one or a few bp, short deletions or insertions (site-specific but random change)

 ZFN-2 technology:
- delivery of genes encoding ZFNs & repair template (DNA stretch of a few kbp)
- site-specific double-strand break (DSB)
- natural repair process (through homomologous recombination HR)
- mutation in one or a few bp, short deletions or insertions (site-specific and specific change)

 ZFN-1 and -2
- ZFN gene expression transient
- or in the case of gene insertion, progeny carrying the transgene is segregated out

 ZFN-1
- mutation in one or a few bp, short deletions or insertions (site-specific but random change)
- mutation frequency varies, but usually rather low

ZFN-3 technology Leads to site-specific insertion of the target gene

Oligonucleotide directed mutagenesis (ODM)

� Oligonucleotides
- share homology with target sequence with the exception of the nucleotides to be modified
- chimeric oligonucleotides (mixed DNA and RNA bases)
- or single-stranded DNA oigonucleotides
- 20 to 100 pb long
� Oligonucleotides “target” the homologous sequence in the genome
� Create one or more mismatch base pairs corresponding to the non-complementary nucleotides

� Meganucleases
- Natural proteins acting as “DNA-scissors”
- Recognise specific DNA sequence (12-30 bp)
- Cause site-specific DNA break
� Possible applications as for ZFN technology

� Transcription activator–like effector (TALE) proteins
- Proteins that bind to DNA in a sequence-specific way
- Converted into “DNA-scissors” by binding to a nuclease (catalytic domain of FokI)
- Can be modified for targeting a given sequence
� Possible applications as for ZFN technology

Cisgenesis/Intragenesis
� Stable integration of cisgene
� In the case of *Agrobacterium*-mediated transformation, presence of T-DNA border sequences
� Possible unintended effects:
- Interruption of open reading frames (ORFs)
- Creation of new ORFs
- Deletion of host DNA
- Gene transfer can lead to modified levels of gene expression
- In the case of gene stacking, multiple recombination sites may cause chromosomal rearrangements
- In the case of use of selectable markers, they have to be recombined out and leave behind a recombination site

Intragenesis (additional effects)
- Possible unintended effects:
- New combinations of genes and regulatory sequences – more extensive modification of gene expression to be expected (compared to cisgenesis)
- In the case RNAi is used for gene silencing, effects on other genes and metabolic pathways possible

AGROINFILTRATION "SENSU STRICTO”

• Infiltration with suspension of Agrobacterium sp. containing the gene of interest
• Local, transient gene expression at high levels
Infiltration with construct containing the foreign gene in a full-length virus or Agrobacterium vector
Question: what is the status / classification of the progeny?

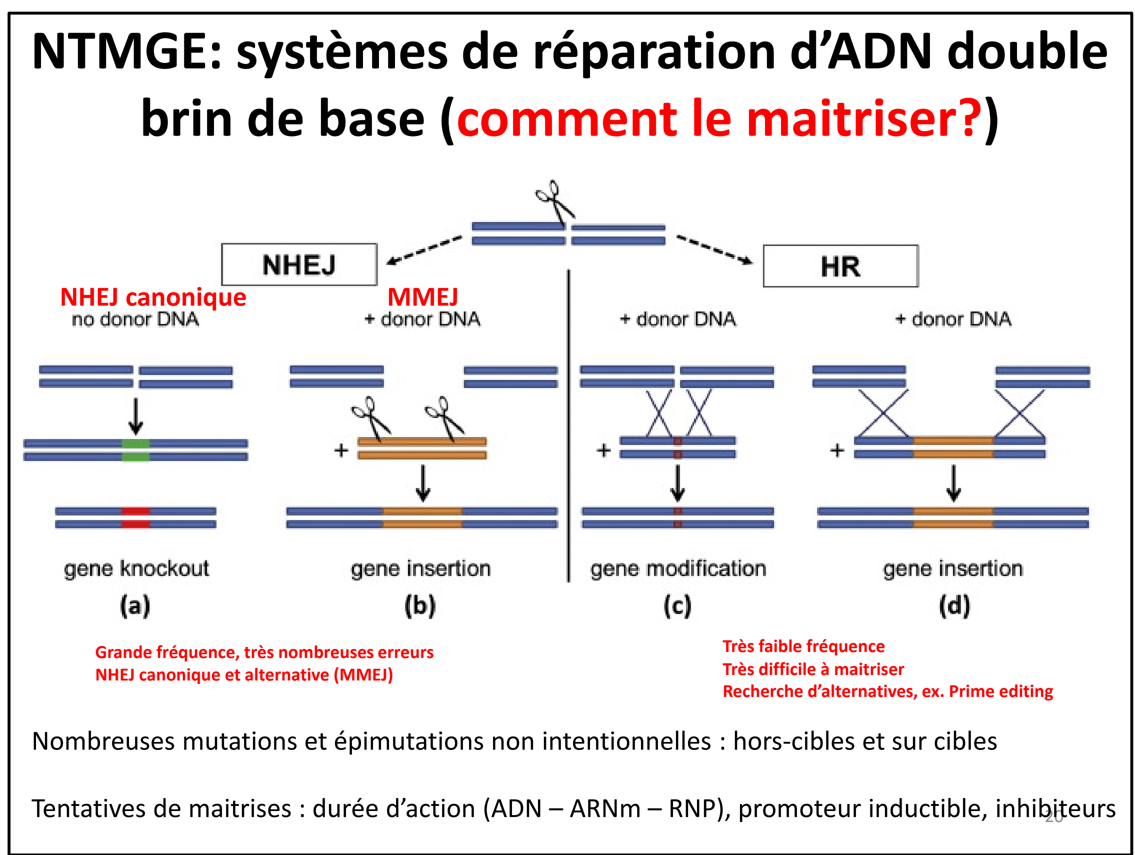
AGRO-INFECTION
• Infiltration with suspension of Agrobacterium sp. containing the gene of interest inserted into a full-length virus vector
• Facilitates the spreading and expression of the target gene in the plant

FLORAL DIP
• Germline tissue (typically flowers) is immersed into a suspension of Agrobacterium sp. containing a T-DNA construct to transform female gametocyte
• GM seeds are obtained

Agro-infiltration and Agro-infection
 Temporary expression of specific coding sequences
 No DNA integration in the plant genome
 Possible unintended changes:
Agro-infiltration:
- Integration of T-DNA cannot be excluded
Agro-infection:
- RNA viruses cause spreading of the gene construct
- Since spread via RNA molecules, no integration in plant genome

Grafting on GM rootstock
 Changes in the genome are targeted to root tissue
 Intended manipulation of gene expression (new trait) in the scion can be achieved through movement of specific proteins and/or RNA from roots to scion, e.g. RdDM
 Possible unintended changes:
- genetic exchange restricted to graft site
- unintended manipulation of gene expression through movement of specific proteins and/or RNA from roots to scion

NPBT are mainly applied for pest resistance and herbicide tolerance.
Some techniques (like cisgenesis) have been already tested in many crop plants, while others (like ZFN) have been tested mainly in model plants, commercial plants are available



Genome editing with site-specific nucleases. Double-strand breaks induced by a nuclease at a specific site can be repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR). (a) Repair by NHEJ usually results in the insertion...
Luisa Bortesi, Rainer Fischer Biotechnology Advances, Volume 33, Issue 1, 2015, 41–52

NBT as for "spontaneous" mutations & mutagenesis through ionizing radiation or chemical mutagens
Consequences can be
- chromosome breakage, translocation and elimination
- chromosome (segment) doubling

prevalent with irradiation

In plants, DSBs are mostly and typically repaired by the imprecise non-homologous end joining (NHEJ) DNA repair pathway, resulting in random deletions and insertions (indels) at the site of repair (Schröpfer et al., 2014). By leveraging the error-prone nature of NHEJ, many groups have reported using engineered nucleases to generate loss of function alleles in a target-specific manner (Carlson et al., 2012; Lor et al., 2014; Shan et al., 2015; Zhang et al., 2015).

NHEJ random DNA repair, the most frequent mechanism used by cells, means you

cannot predict what would happen. You thus have to transform numerous cell before having a chance to get the expected result on the target. It is thus still a “trial and error” system which explains whay Addgene is so important for supporting scientits as providing proofs it works in some instances.

“Precision genome editing”, on the other hand, relies on template-directed repair of DSBs using exogenously supplied double stranded DNA or ssODN and as such is more precise than NHEJ (Voytas, 2013). While groups have reported using oligonucleotides in combination with engineered nucleases to increase gene editing frequencies in fish, mammals and flies, in plants the challenge of making and tracking targeted genome edits from individual cells through to whole plants in a non-selectable manner remains a barrier.

- chromosome - single base-pair changes (substitution, insertion and deletion)

prevalent with chemical mutagens

 Further propagation or backcrossing and selection necessary

Cf. V*an de Wiel, C. et al., “Traditional plant breeding techniques”, Wageningen UR Plant Breeding, Report 338 (2010)*

Susan W.P. Wijnhoven and Harry van Steeg 2003 Toxicology 193 171–187
At least five main, partly overlapping damage DNA repair pathways operate in mammals: nucleotide excision repair (NER), base excision repair (BER), homologous recombination (HR), end joining (EJ) and mismatch repair (MMR) (reviewed in Hoeijmakers 2001)

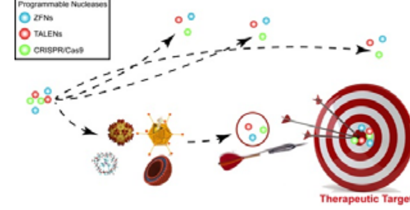
NHEJ (Non Homologous End Joining) DNA repair system of DSB (double stranded DNA) is also involved in transgenesis. See e.g. Jun Dai et al. 2010 Int J Biol Sci 6(7):756-768.

La **captation d'attention**: « hors-cibles » ou pas…

D'abord s'entraîner…

Ensuite se lancer… pour en rater pas mal…

- De nombreuses homologies totales ou partielles des ARN guide dans le reste du génome,
- Des considérations thermodynamiques,
- De nombreuses recettes de cuisine pour tenter de réduire le nombre de off-targets (facteur 1 500...)



En conséquence : des insertions / délétions ponctuelles ou non,
On targets: des réarrangements chromosomiques (inversions, translocations…) difficiles à prédire et détecter...

Au vu du coût des séquençage et assemblage : divers logiciels tentant de prédire où chercher les off-targets par PCR,

De nombreuses variantes aux effets encore inconnus

- Dead-Cas9: nickase
- Orthologues,
- Base editor, prime editor
- Cas-integrase

- De très nombreuses recettes de cuisine pour modifier les nucléases aux effets inconnus.

Bo Huang, biophysicien à l'université de Californie à San Francisco :

"Les gens n'ont tout simplement pas le temps de caractériser certains des paramètres très fondamentaux du système"

"Il y a une mentalité selon laquelle tant que cela fonctionne, nous n'avons pas besoin de comprendre comment ou pourquoi cela fonctionne".

NTMGE sans ADN étranger?

- Utilisation de plasmides ou virus vecteurs,
- Les réactifs NTMGE (ADN, ARNm et RNP) sont contaminés par les acides nucléiques des organismes hôtes initiaux,
- Les réactifs commerciaux ou internes sont contaminés par de l'ADN étranger

Il faut systématiquement séquencer en profondeur les organismes issus de NTMGE…

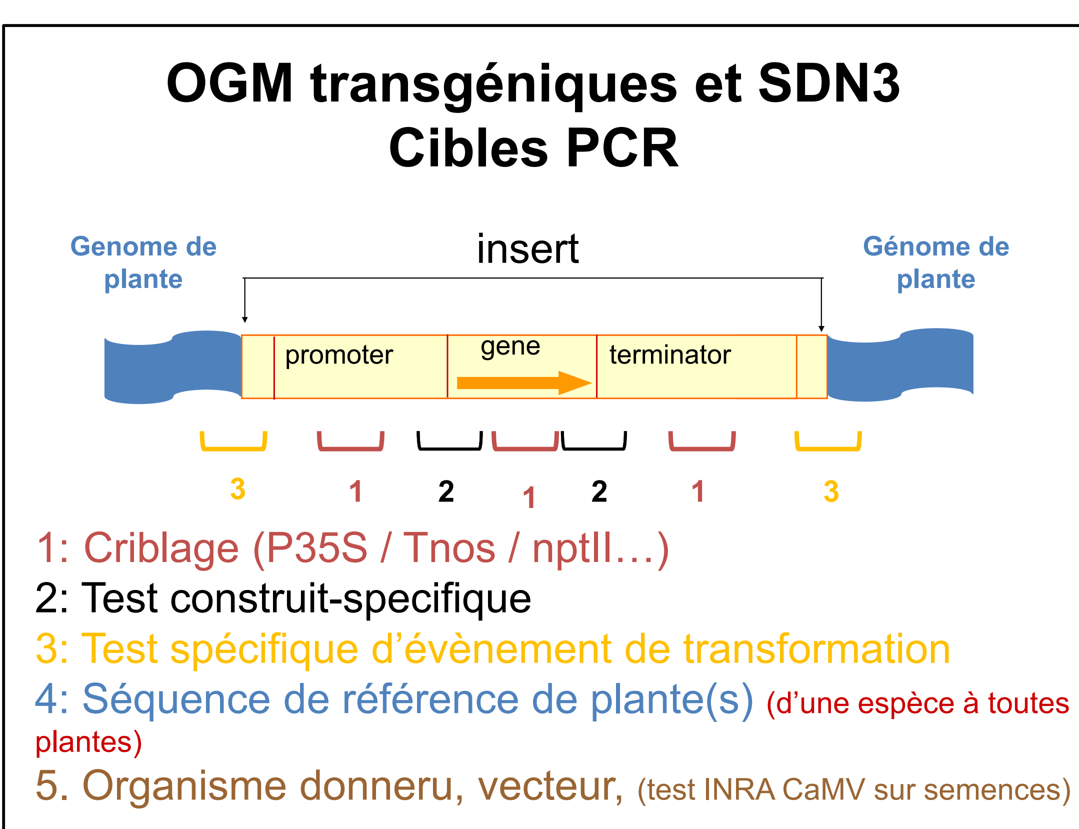
Et ne pas se contenter de PCR biaisées !

Cis / intragénèse

- Problèmes des micro-synténies
- Distance génétique entre variétés (Elites: chimpanzé-homme)
- Effets épistatiques, fonds génétique…
- Ex. effet ancestral en thérapie génique…

- **Les génomes et épigénomes ne sont pas constitués de briques interchangeables comme dans un mécano**

DÉTECTION ET IDENTIFICATION DES
PRODUITS NTMGE ET DES TECHNIQUES
À LEUR ORIGINE


OGM transgéniques et SDN3
Cibles PCR
Genome de plante
insert
Génome de plante
promoter
gene
terminator
3
1
2
1
2
1
3
1: Criblage (P35S / Tnos / nptII…)
2: Test construit-specifique
3: Test spécifique d'évènement de transformation
4: Séquence de référence de plante(s) (d'une espèce à toutes plantes)
5. Organisme donneru, vecteur, (test INRA CaMV sur semences)

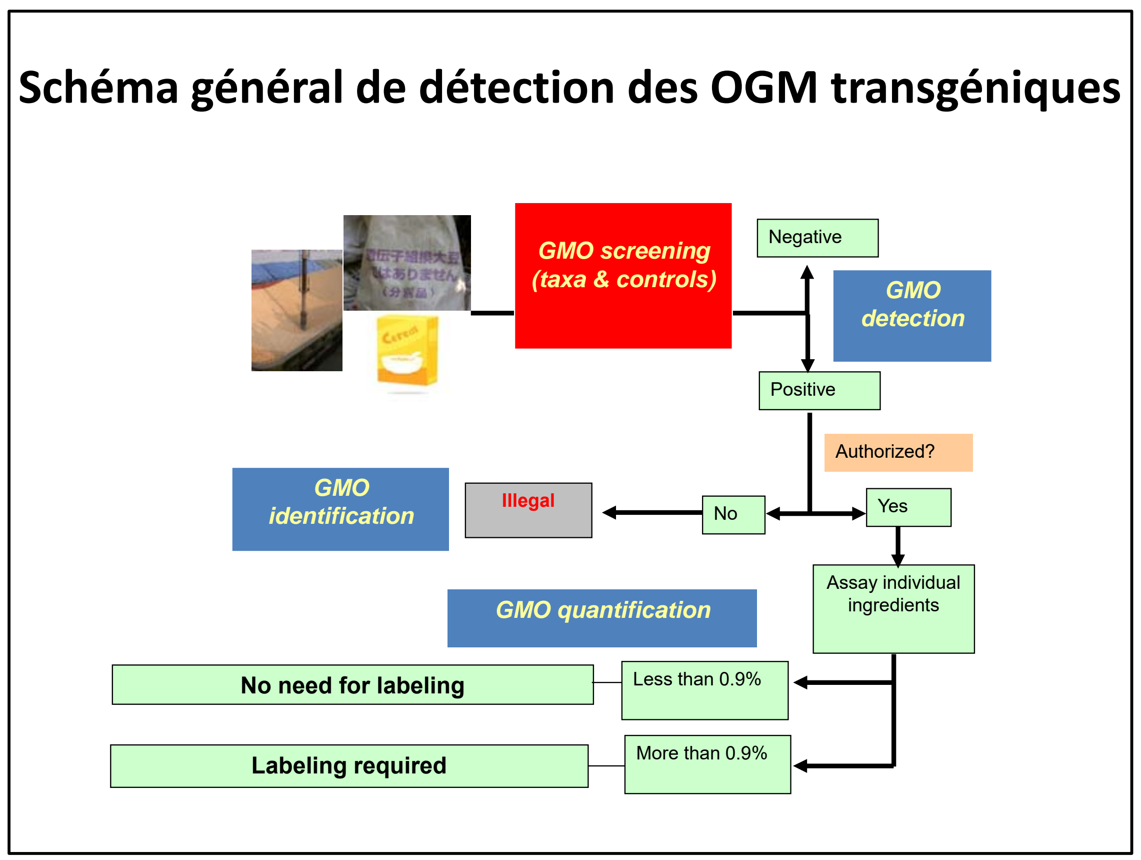
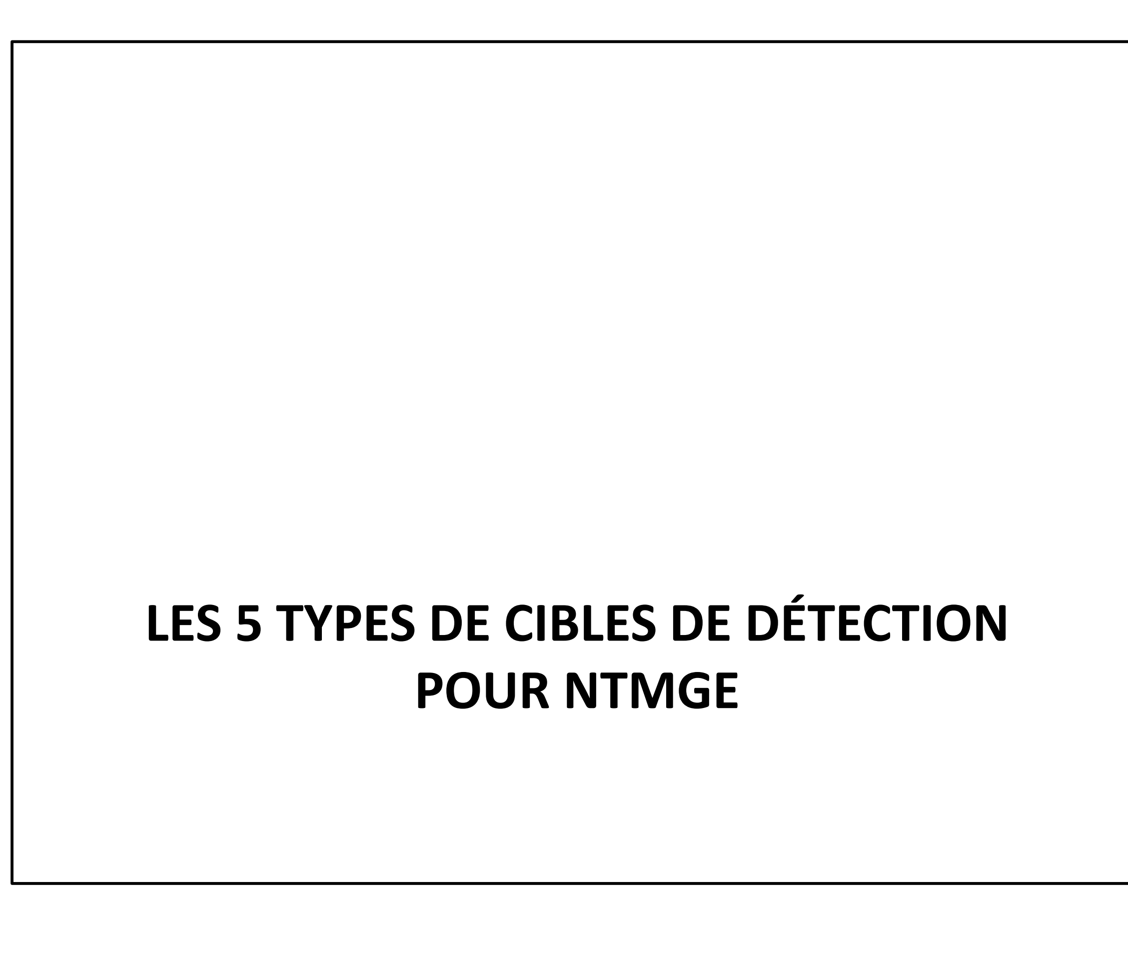


Schéma général de détection des OGM transgéniques
GMO screening
(taxa & controls)
Negative
GMO
detection
Positive
Authorized?
GMO
identification
Illegal
No
Yes
Assay individual
ingredients
GMO quantification
No need for labeling
Less than 0.9%
Labeling required
More than 0.9%


LES 5 TYPES DE CIBLES DE DÉTECTION
POUR NTMGE

Syndrome de domestication

(réduction de la diversité génétique)

- Aspects moléculaires (génétique et épigénétique) sur :
  - perte de dormance des graines
  - suppression des mécanismes de dispersion (indéhiscence)
  - maturité groupée
  - changement du système de reproduction
  - changement du niveau de ploïdie
  - gigantisme
  - changement du port de la plante
  - changement de forme de vie (annualisation des vivaces ou bisannuelles)
  - changements biochimiques
  - changements physiologiques (p ex modification de la photopériode)

(Chauvet, 2008)

Signatures des germoplasmes / variétés Elite

- Les sociétés utilisent majoritairement les mêmes fonds génétiques pour essayer de prédire l'effet de l'introduction d'un trait, d'où l'identification.
- Signatures de sélection (effet fondateur) : SNP, SMP utilisés à l'ISO et l'UPOV pour l'identification des cultivars (puces à ADN, logiciels…),

Ces signatures sont les premiers signes d'identification d'une variété modifiée ou non, même pour des OGM inconnus.

(Bertheau, 2021) (Saxena et al., 2018)
Cultivar : variété cultivée
Le fonds génétique est fondamental dans l'expression d'un trait introduit. D'où la nécessité de posséder de nombreuses variétés Elite.
Les schémas de croisement entre variétés Elite sont complexes et confèrent un type de signatures.

Signatures des laboratoires

- Mêmes types d'outils moléculaires utilisés dans un laboratoire (ex. souche d'*Agrobacterium*),
- Mêmes variétés de laboratoire,
- Signatures de constructions, de mutations apparues et propagées,
- Identification du laboratoire, voire de personnes par IA (intelligence artificielle)

(Alley et al., 2020; Kim et al., 2020; Nielsen and Voigt, 2018; Tong and Nikoloski, 2021)

Cicatrices des techniques connexes

- Mutagénèse induite au hasard : types et fréquences de mutations, localisations…,
- Transgénèse et mutagénèse dirigée :
  - Mutations et épimutations (variation somaclonale)
  - Réarrangements chromosomiques, chromothripsie, mobilisation des éléments transposables,
  - Morceaux de vecteurs (chromosome et plasmides d'*Agrobacterium*) ou petites tailles de plasmides (biolistique),
  - Méthode à base d'ADN la plus performante, avec nécessité d'inhibiteurs ou d'activateurs,
  - Séquences de l'organisme de préparation des plasmides et protéines,
  - Cicatrices d'excision de séquences de sélection (Cre-Lox/ antibiotique…) ou de séquences de sélection non excisées (mannose…)
- Contamination des réactifs (ADN, ARNm et RNP) par de l'ADN étranger (même les enzymes commerciales les plus purifiées sont contaminées).

Bertheau, Y. (2019). New breeding techniques: detection and identification of the techniques and derived products. Encyclopedia of Food Chemistry, Reference Module in Food Science. R. H. Stadler, Elsevier: 320-336.

Since the commercial releases of GMOs in the 90s, new genetic modification tools known as New breeding techniques have been developed for e.g. gene silencing or more precise genomic modifications such as Crispr-endonuclease based systems. As for GMOs several consumers view may prevail about the societal interest in agricultural production and food of such genetic modification. Ensuring the freedom of choice to consumers needs to develop detection tools which could infer the NBT nature of the modification

technique used. This article reviews all the elements which could allow the identification and detection of such techniques and products.

Bertheau, Y. (2022). Advances in identifying GM plants. Toward the routine detection of "hidden" and "new" GMOs. Developing smart-agrifood supply chains: using technology to improve safety and quality. L. Manning, Burleigh Dodds Science Publishing**:** 87-150.

synopsis en ligne 2021/11/08

This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording “hidden” GMOs and “new” GMOs as defined by the French NGOs and farmers’ union at the origin of the 2018 European Court of Justice ruling.

résumé accepté

In 2018 the Court of Justice of the European Union recalled that organisms with genomes modified by artifactual techniques should be considered GMOs under European regulations. GMOs derived from cultures of cells isolated in vitro or from new genomic techniques must therefore be traceable. This chapter reviews the various technical steps and characteristics of those techniques causing genomic and epigenomic scars and signatures. These intentional and

unintentional traces, some of which are already used for varietal identification, and are being standardized, can be used to identify these GMOs and differentiate them from natural mutants. The chapter suggests a routine procedure for operators and control laboratories to achieve this without additional costs.
=====

The growing knowledge in genetics, epigenomics, epitranscriptomics, and the 3D - or even 4D - genome structure provides an increasing number of detection targets that can be used to identify species or genetic lines, whether modified or not. Biotic and abiotic stresses also induce numerous unintentional genetic, epigenetic, and epitranscriptomic modifications. Those changes are transmissible and can be ordered in regions and classified. The detection target is characterised by the mutagenesis technique used. For instance, the detection of transgenic GMO or SDN3 modification of New Breeding Techniques (NBTs) will target their insertion's junction fragments into the genome. Each insertion induces epigenetic, and probably epitranscriptomic, changes which can also be targeted. In addition, one group of markers is linked to the trait(s) introduced or modified by the breeder whose sequence could be used in quantification and "screening". The other target will be a subset of the elements of a matrix approach (as described in the previous chapter). General selection markers, such as those used for plant breeding, together with

mutagenesis techniques specific markers, could differentiate genetically modified organisms (GMOs) of any origin. They can be used to quantify and certify, through a global approach to the organism, that the trait modification is artefactual and not "natural." The growing mastery of single-cell sequencing techniques should soon make it possible to differentiate the modifications due, for example, to each step of a Crispr-Cas transformation of cells in culture. This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording “hidden” GMOs and “new” GMOs as defined by the French NGOs and farmers’ union at the origin of the 2018 European Court of Justice ruling.

Broeders, S., et al. (2014). "Guidelines for validation of qualitative real-time PCR methods." Trends in Food Science & Technology **37**(2): 115-126.

• Guidelines for in-house and inter-laboratory validation of qualitative qPCR methods.
• Method acceptance and performance parameters to be evaluated.
• Acceptance criteria to be fulfilled for the above mentioned parameters.
• Detailed experimental setup for in-house and inter-

laboratory validation

As for many areas of molecular testing, detection of Genetically Modified Organisms (GMO) relies on the real-time Polymerase Chain Reaction (qPCR) technology. Due to the increasing number of GMO, a screening approach using qualitative screening methods has become an integrated part of GMO detection. However, specific guidelines for the validation of these methods are lacking. Here, a pragmatic approach to conduct in-house and inter-laboratory validation studies for GMO screening methods, is proposed. Such guidelines could be adapted to other areas where qualitative qPCR methods are used for molecular testing allowing to implement easily a more reliable screening phase where necessary.

Mühl, H., et al. (2010). "Activity and DNA contamination of commercial polymerase chain reaction reagents for the universal 16S rDNA real-time polymerase chain reaction detection of bacterial pathogens in blood." Diagnostic Microbiology and Infectious Disease **66**(1): 41-49.

Universal 16S rRNA gene polymerase chain reaction (PCR) is a promising means of detecting bacteremia. Among other factors, the PCR reagents play a prominent role for obtaining a high sensitivity of detection. The reagents are ideally optimized with respect to the amplifying activity and absence of contaminating DNA. In this study, it was shown in a universal 16S rDNA real-time PCR assay that commercial PCR reagents can vary greatly among each other in these characters. Only 1 of the 5 reagents tested met the criteria of sensitive detection of pathogen DNA with a minimum of false-positive results. The reagent was validated by the detection of pathogens at low titers using bacterial DNA extracted from blood that was spiked with various Gram-positive and Gram-negative bacteria.

Wu, Y., et al. (2018). "DNA decontamination methods for internal quality management in clinical PCR laboratories." Journal of Clinical Laboratory Analysis **32**(3): e22290.
Background The polymerase chain reaction (PCR) technique, one of the most commonly applied methods in diagnostic and molecular biology, has a frustrating downside: the occurrence of false-positive signals due to contamination. In previous research, various DNA decontamination methods have been developed to overcome this limitation. Unfortunately, the use of random or poorly focused sampling methods for monitoring air and/or object surfaces leads to the incomplete elimination during decontamination procedures. We herein attempted to develop a novel DNA decontamination method (environmental surveillance, including surface and air sampling) and quality management program for clinical molecular diagnostic laboratories (or clinical PCR laboratories). Methods Here, we performed a step-by-step evaluation of current DNA decontamination methods and developed an effective procedure for assessing the presence of decontaminating DNA via PCR analysis. Performing targeted environmental surveillance by sampling, which reached optimal performance over 2 weeks, and the decontamination process had been verified as reliable. Additionally, the process was validated to not affect PCR amplification efficiency based on a comparative study. Results In this study, effective guidelines for DNA decontamination were developed. The method employed ensured that surface DNA contamination could be effectively identified and eliminated. Furthermore, our study highlighted the importance of overall quality assurance and good clinical laboratory practices for preventing contamination, which are key factors for compliance with regulatory or accreditation requirements. Conclusions Taken together, we provided the evidence that the presented scheme ranged from troubleshooting to the elimination of surface contamination, could serve as critical foundation for developing regular environmental surveillance guidelines for PCR laboratories.

Mennerat, A. and B. C. Sheldon (2014). "How to deal with PCR contamination in molecular microbial ecology." Microbial Ecology **68**(4): 834-841.
Microbial ecology studies often use broad-range PCR primers to obtain community profiles. Contaminant microbial DNA present in PCR reagents may therefore be amplified together with template DNA, resulting in unrepeatable data which may be difficult to interpret, especially when template DNA is present at low levels. One possible decontamination method consists in pre-treating PCR mixes with restriction enzymes before heat-inactivating those enzymes prior to the start of the PCR. However, this method has given contrasting results, including a reduction in PCR sensitivity. In this study, we tested the efficiency of two different enzymes (DNase 1 and Sau3AI) as well as the effect of dithiothreitol (DTT), a strong reducing agent, in the decontamination procedure. Our results indicate that enzymatic treatment does reduce contamination levels. However, DNase 1 caused substantial reductions in the bacterial richness found in communities, which we

interpret as a result of its incomplete inactivation by heat treatment. DTT did help maintain bacterial richness in mixes treated with DNase 1. No such issues arose when using Sau3AI, which therefore seems a more appropriate enzyme. In our study, four operational taxonomic units (OTU) decreased in frequency and relative abundance after treatment with Sau3AI and hence are likely to represent contaminant bacterial DNA. We found higher within-sample similarity in community structure after treatment with Sau3AI, probably better reflecting the initial bacterial communities. We argue that the presence of contaminant bacterial DNA may have consequences in the interpretation of ecological data, especially when using low levels of template DNA from highly diverse communities. We advocate the use of such decontaminating approaches as a standard procedure in microbial ecology.

Champlot, S., et al. (2010). "An Efficient Multistrategy DNA Decontamination Procedure of PCR Reagents for Hypersensitive PCR Applications." PLoS ONE **5**(9): e13042.

Background PCR amplification of minute quantities of degraded DNA for ancient DNA research, forensic analyses, wildlife studies and ultrasensitive diagnostics is often hampered by contamination problems. The extent of these problems is inversely related to DNA concentration and target fragment size and concern (i) sample contamination, (ii) laboratory surface contamination, (iii) carry-over contamination, and (iv) contamination of reagents. Methodology/Principal Findings Here we performed a quantitative evaluation of current decontamination methods for these last three sources of contamination, and developed a new procedure to eliminate contaminating DNA contained in PCR reagents. We observed that most current decontamination methods are either not efficient enough to degrade short contaminating DNA molecules, rendered inefficient by the reagents themselves, or interfere with the PCR when used at doses high enough to eliminate these molecules. We also show that efficient reagent decontamination can be achieved by using a combination of treatments adapted to different reagent categories. Our procedure involves γ- and UV-irradiation and treatment with a mutant recombinant heat-labile double-strand specific DNase from the Antarctic shrimp Pandalus borealis. Optimal performance of these treatments is achieved in narrow experimental conditions that have been precisely analyzed and defined herein. Conclusions/Significance There is not a single decontamination method valid for all possible contamination sources occurring in PCR reagents and in the molecular biology laboratory and most common decontamination methods are not efficient enough to decontaminate short DNA fragments of low concentration. We developed a versatile multistrategy decontamination procedure for PCR reagents. We demonstrate that this procedure allows efficient reagent decontamination while preserving the efficiency of PCR amplification of minute quantities of DNA.

Ghogare, R., et al. (2021). "Genome editing reagent delivery in plants." Transgenic

Research **30**(4): 321-335.

Genome editing holds the potential for rapid crop improvement to meet the challenge of feeding the planet in a changing climate. The delivery of gene editing reagents into the plant cells has been dominated by plasmid vectors delivered using agrobacterium or particle bombardment. This approach involves the production of genetically engineered plants, which need to undergo regulatory approvals. There are various reagent delivery approaches available that have enabled the delivery of DNA-free editing reagents. They invariably involve the use of ribonucleoproteins (RNPs), especially in the case of CRISPR/Cas9-mediated gene editing. The explant of choice for most of the non-DNA approaches utilizes protoplasts as the recipient explant. While the editing efficiency is high in protoplasts, the ability to regenerate individual plants from edited protoplasts remains a challenge. There are various innovative delivery approaches being utilized to perform in planta edits that can be incorporated in the germline cells or inherited via seed. With the modification and adoption of various novel approaches currently being used in animal systems, it seems likely that non-transgenic genome editing will become routine in higher plants.

Chu, P. and S. Z. Agapito-Tenfen (2022). "Unintended Genomic Outcomes in Current and Next Generation GM Techniques: A Systematic Review." Plants **11**(21): 2997.

Le génie génétique classique et les nouvelles techniques d'édition du génome, notamment la technologie CRISPR/Cas, augmentent les possibilités de modification du matériel génétique des organismes. Ces technologies ont le potentiel de fournir de nouvelles caractéristiques agricoles, y compris des micro-organismes modifiés et des applications environnementales. Cependant, des préoccupations légitimes en matière de sécurité découlent des modifications génétiques involontaires (MG) qui ont été signalées comme des effets secondaires de ces techniques. Dans cet article, nous passons systématiquement en revue la littérature scientifique pour les études qui ont examiné les altérations génomiques involontaires dans les plantes modifiées par les techniques GM suivantes : Transfert de gènes médié par Agrobacterium tumefaciens, bombardement biolistique, et CRISPR-Cas9 délivré par transfert de gènes médié par Agrobacterium (basé sur l'ADN), bombardement biolistique (basé sur l'ADN) et sous forme de complexes ribonucléoprotéiques (RNP). Les résultats de notre revue de la littérature montrent que l'impact de ces techniques dans les génomes des hôtes varie de petits polymorphismes nucléotidiques à de grandes variations génomiques, telles que la duplication segmentaire, la troncature chromosomique, la trisomie, la chromothripsie, la rupture de pont de fusion, y compris de grands réarrangements des séquences de l'épine dorsale du vecteur ADN. Nous avons également examiné le type de méthode d'analyse appliquée pour étudier les altérations génomiques et avons constaté que seuls cinq articles utilisaient le séquençage du génome entier dans leurs méthodes d'analyse. En outre, les variations structurelles plus importantes détectées dans certaines études ne seraient pas possibles sans les stratégies de séquençage à longue lecture, ce qui montre une sous-estimation potentielle de ces effets dans la littérature. Les nouvelles technologies étant en

constante évolution, un examen plus approfondi des méthodes d'analyse prospectives devrait être mené à l'avenir. Cela permettra aux régulateurs travaillant dans le domaine des organismes génétiquement modifiés et édités de disposer d'informations précieuses sur la capacité à détecter et à identifier les interventions génomiques.

Classical genetic engineering and new genome editing techniques, especially the CRISPR/Cas technology, increase the possibilities for modifying the genetic material in organisms. These technologies have the potential to provide novel agricultural traits, including modified microorganisms and environmental applications. However, legitimate safety concerns arise from the unintended genetic modifications (GM) that have been reported as side-effects of such techniques. Here, we systematically review the scientific literature for studies that have investigated unintended genomic alterations in plants modified by the following GM techniques: Agrobacterium tumefaciens-mediated gene transfer, biolistic bombardment, and CRISPR-Cas9 delivered via Agrobacterium-mediated gene transfer (DNA-based), biolistic bombardment (DNA-based) and as ribonucleoprotein complexes (RNPs). The results of our literature review show that the impact of such techniques in host genomes varies from small nucleotide polymorphisms to large genomic variation, such as segmental duplication, chromosome truncation, trisomy, chromothripsis, breakage fusion bridge, including large rearrangements of DNA vector-backbone sequences. We have also reviewed the type of analytical method applied to investigate the genomic alterations and found that only five articles used whole genome sequencing in their analysis methods. In addition, larger structural variations detected in some studies would not be possible without long-read sequencing strategies, which shows a potential underestimation of such effects in the literature. As new technologies are constantly evolving, a more thorough examination of prospective analytical methods should be conducted in the future. This will provide regulators working in the field of genetically modified and gene-edited organisms with valuable information on the ability to detect and identify genomic interventions.

Wally, N., et al. (2019). "Plasmid DNA contaminant in molecular reagents." Scientific reports **9**(1): 1652.

Background noise in metagenomic studies is often of high importance and its removal requires extensive post-analytic, bioinformatics filtering. This is relevant as significant signals may be lost due to a low signal-to-noise ratio. The presence of plasmid residues, that are frequently present in reagents as contaminants, has not been investigated so far, but may pose a substantial bias. Here we show that plasmid sequences from different sources are omnipresent in molecular biology reagents. Using a metagenomic approach, we identified the presence of the (pol) of equine infectious anemia virus in human

samples and traced it back to the expression plasmid used for generation of a commercial reverse transcriptase. We found fragments of multiple other expression plasmids in human samples as well as commercial polymerase preparations. Plasmid contamination sources included production chain of molecular biology reagents as well as contamination of reagents from environment or human handling of samples and reagents. Retrospective analyses of published metagenomic studies revealed an inaccurate signal-to-noise differentiation. Hence, the plasmid sequences that seem to be omnipresent in molecular biology reagents may misguide conclusions derived from genomic/metagenomics datasets and thus also clinical interpretations. Critical appraisal of metagenomic data sets for the possibility of plasmid background noise is required to identify reliable and significant signals.

Signatures des techniques NTMGE

- Ex. Modifications par ZFN, TALEN et les Crispr-Cas et enzymes modifiées
- Rappel: phénomène aléatoire de réparations (NHEJ, MMEJ et HDR) avec séquences homologues aux cibles dans les génomes
- Hors-cibles repérables par:
  - homologies de séquences avec la séquence ciblée,
  - taille : fonction du type de système (ZFN vs. TALEN vs. Cas),
  - environnement en nucléotides,
- Sur cible: réarrangements chromosomiques
- Plus généralement : chromothripsie possible, aneuploidie
- PAM : proximité avec les zones modifiées par Cas, distance PAM-DSB

Modifications des nucléases, exemple: nucléase à coupure double brin (ex. Cas9, ou FokI de TALEN) transformées en nickase (coupure simple brin), ajout de séquences de désaminase pour en faire des « base éditeur » (transformant une base A ou C),, ou (prime editor) ajout de transcriptase inverse pour rétrotranscription inverse d'ARN (ajouté au sgRNA) en ADN.
dCAs (deadCas) : activité double brin des Cas inactivée
Enfin Cas insérée entre 2 bras de ZFN ou TALEN pour délimiter zones d'intervention
Nombreux effets hors cibles et sur-cibles, certains résultats montrant même un accroissement des modifications non intentionnelles.
Effet des constructions sur les modifications non intentionnelles ex. (Lei et al., 2021)

PAM- Protospacer Adjacent Motif (zone d'accrochage de la CasXX) de séquences différentes selon les Cas, et

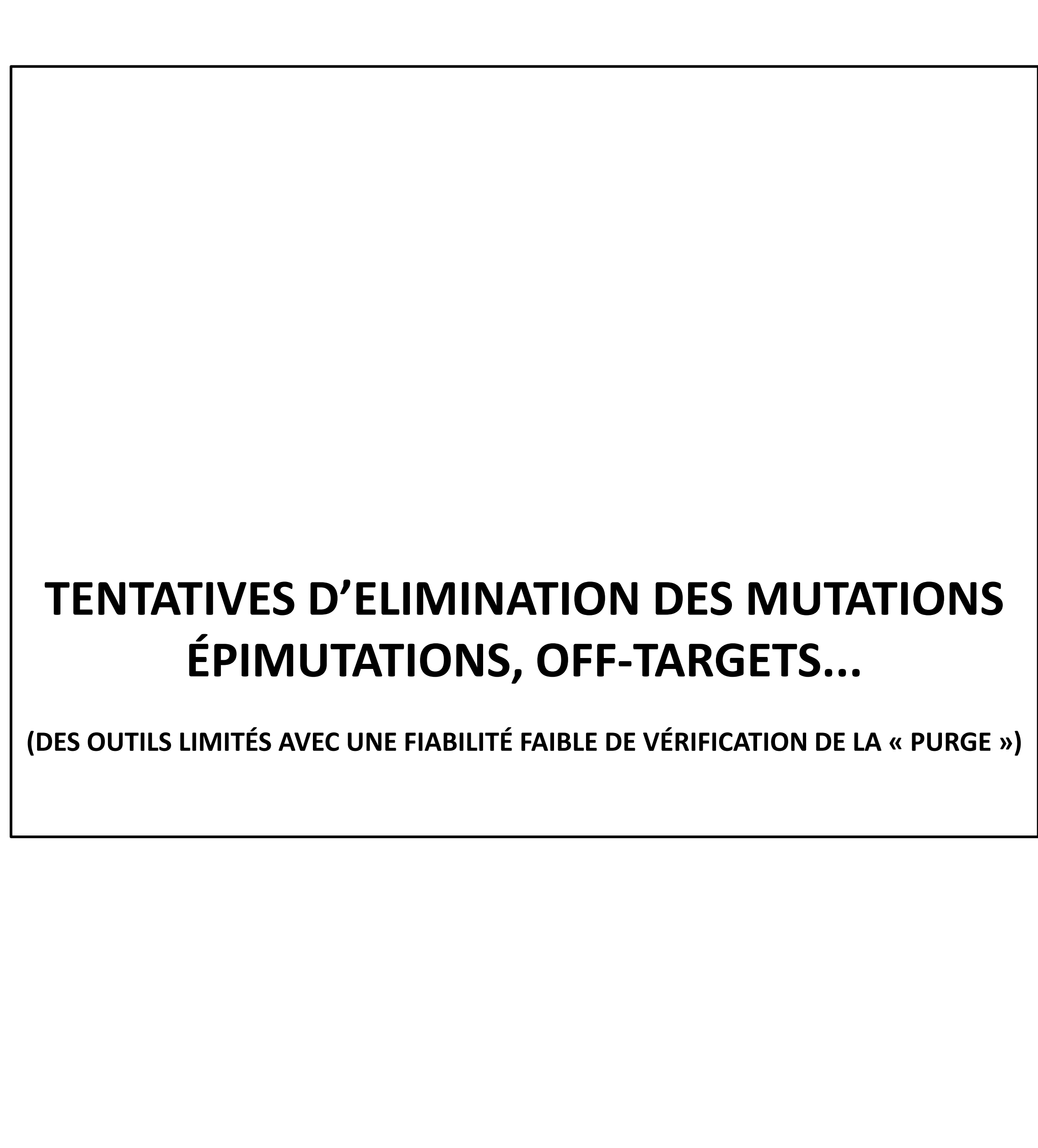
Apports récents du programme FELIX et du GEA

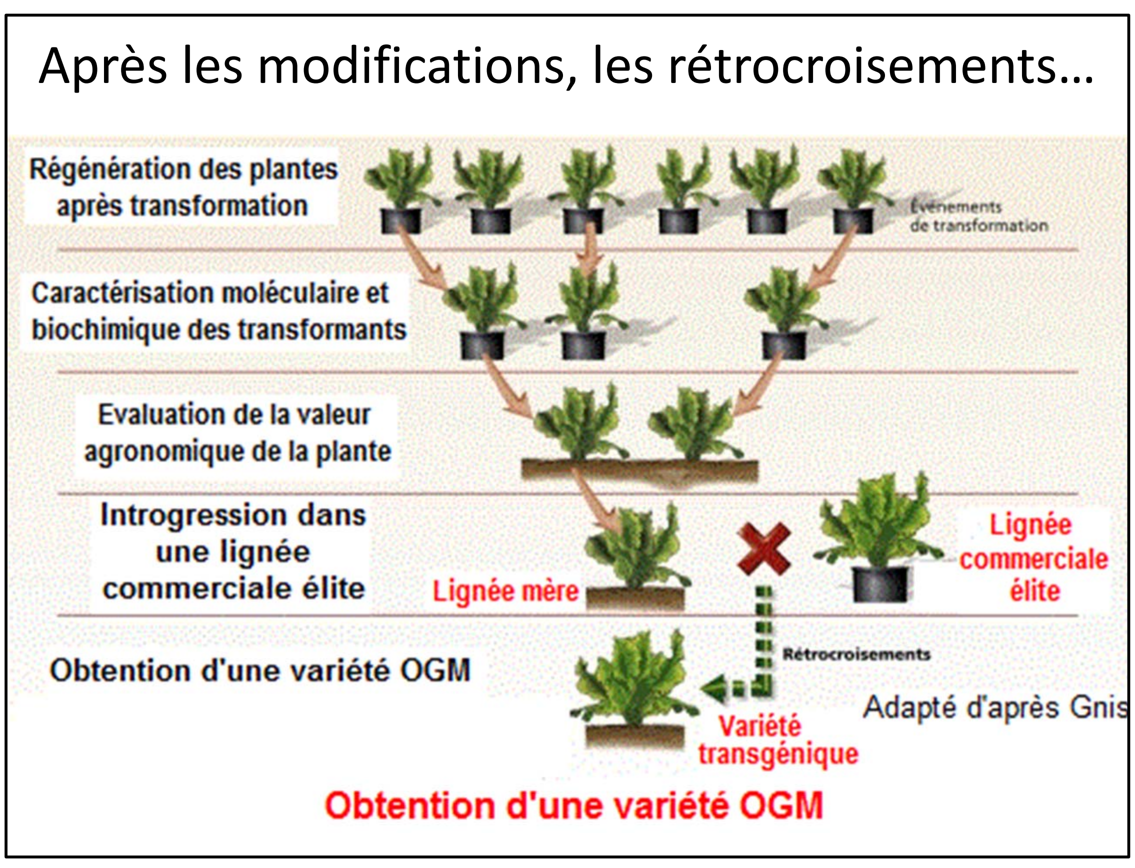
- FELIX: programme de recherche pour l'iARPA US ayant abouti en octobre 2022 à des outils de détection d'agents biologiques génétiquement modifiés inconnus.
- GEA challenge (*Genetic Engineering Attribution challenge*), un concours de science des données visant à identifier à l'aide de l'intelligence artificielle les séquences modifiées des génomes et leur source originale.

Questionnement sur l'accès aux outils développés par FELIX…

FELIX :
https://www.youtube.com/watch?v=XQjR3mmFLhk
2022/10/17 and other links.

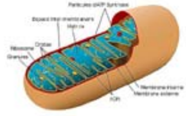
GEA challenge (Genetic Engineering Attribution challenge)
https://www.drivendata.org/competitions/63/genetic-engineering-attribution/ and
https://drivendata.co/blog/genetic-engineering-attribution-winners

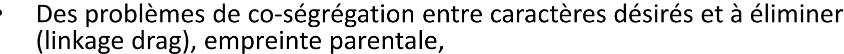

TENTATIVES D'ELIMINATION DES MUTATIONS
ÉPIMUTATIONS, OFF-TARGETS...
(DES OUTILS LIMITÉS AVEC UNE FIABILITÉ FAIBLE DE VÉRIFICATION DE LA « PURGE »)


Après les modifications, les rétrocroisements…
Régénération des plantes après transformation
Événements de transformation
Caractérisation moléculaire et biochimique des transformants
Evaluation de la valeur agronomique de la plante
Introgression dans une lignée commerciale élite
Lignée mère
Lignée commerciale élite
Obtention d'une variété OGM
Rétrocroisements
Adapté d'après Gnis
Variété transgénique
Obtention d'une variété OGM

Les rétrocroisements éliminent-ils les modifications non-intentionnelles ?

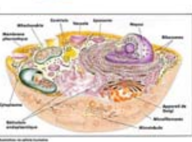
- Approche théorique phénotypique, jamais validée par séquençages comparés,
- Un nombre de rétrocroisements
  - très dépendant des tailles, complexité des génomes (polyploïdie, cf blé des dizaines de millions de nucléotides non « purgés »…) et nombre de loci différant entre plantes et inbred,
  - trop faible en pratique (généralement <6 au lieu de 8, stabilité génomique à étudier sur 5 générations [EFSA]),
  - organelles non considérés,
- Même avec le nombre requis de rétrocroisements, statistiquement (~95%) des quantités importantes d'ADN non « purgé » (ex. blé : 500 Mbp sur 17Bbp)





- Des problèmes de co-ségrégation entre caractères désirés et à éliminer (linkage drag), empreinte parentale,

- Des régions à comportement non mendélien (haplotypes),



- Des outils de séquençage insuffisants pour vérifier la qualité de la purge du génome et encore plus des épigénomes (ADN, ARN et protéines), sans compter les effets des sauts d'exon,

S'ils sont nécessaires (dixit les firmes) pourquoi vouloir modifier directement les variétés Elite comme préconisé par les firmes pour réduire le temps de développement des variétés commerciales ?

Le nombre de rétrocroisements vers les variétés Elite est généralement de l'ordre de 6 backcross (Ferreira et al., 2016). Le nombre de rétrocroisements nécessaires pour se rapprocher d'une variété Elite augmente avec le nombre de loci différant entre plantes et des groupes d'inbred dont sont issus Elite et variété transformée (Holland, 2007).

Transmission non mendélienne, mammifères ex: expansion de triplets, mitochondriale, empreinte parentale, digénisme.
Imprinting (Waters et al., 2013) (Rodrigues and Zilberman, 2015)
Organelles : (C. William Birky, 2001)

https://en.wikipedia.org/wiki/Backcrossing
Disadvantages
•Works poorly for quantitative traits
•Is more restricted for recessive traits
•In practice, sections of genome from the non-recurrent parents are often still present and can have unwanted traits associated with them
•For very wide crosses, limited recombination may maintain thousands of 'alien' genes within the elite cultivar
•Many backcrosses are required to produce a new cultivar which can take many years

Traçabilité des produits NTMGE: une confusion savamment entretenue

- Traçabilité (norme ISO) : facile et bon marché, ne dépend que du bon vouloir des traders et distributeurs : *Traceability* is the ability to identify and trace the history, distribution, location, and application of products, parts, materials, and services. A traceability system records and follows the trail as products, parts, materials, and services come from suppliers and are processed and ultimately distributed as final products and services.
- Détection : facile et relativement bon marché selon les techniques utilisées
  - Action ou procédé de découverte, mise en évidence ou de noter quelque chose.
  - Très facile à réaliser quand la cible est connue (veille, brevet, bases de données…), OGM inconnus : différentes méthodes aux résultats quelquefois de faible poids convergents et à l'utilisation facilitée par des DSS
  - Méthodes : phénotype (ex: plantule tolérante à un herbicides, méthodes immunologiques, PCR, LCR, Qβ replicase, SNPLex, LAMP, spectroscopie (méthodes pour omics)… au champ et au laboratoires selon ese méthodes (ex: PCR et LAMP au champ)
- Quantification:
  - Méthodes quantitative s
  - Pour les méthodes qualitatives : position envers un seuil avec des méthodes de sous-échantillonnage comme par exemple celles utilisées en certification de semences.
- Identification de la NTMGE utilisée / du propriétaire du produit : recherche de signatures utilisables en approche matricielle avec ou sans DSS et NGS
  - Approche matricielle initiale: profiles de mutations et épimutations (toutes techniques)
  - Signatures épigénétiques Crispr
  - Systèmes de structuration moléculaire…
  - Probables signatures univoques…
- Capacité à différencier produits issus de mutagénèse *in vitro* et *in vivo*

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Beyond the impossible obligation to GMO developers to mandatorily introduce signature into their GMOs.

Do not be confused by the wording, numerous parts of methods to know what you have in a sample are relatively easy to use or develop.

Data have to accumulated in e.g. Euginius database http://www.euginius.eu/euginius/pages/home.jsf and GMDD http://gmdd.shgmo.org/ and/or Genbank… depending on the data.

As for any complex detection, several strategies have be combined through e.g. the "matrix approach" with or without the help of DSS (Decision Support System).
Holst-Jensen et al. 2012 Biotech advances. 30(6):1318–35.
Holst-Jensen et al. 2016 Anal. Bioanal. Chem. 408(17): 4595–4614

Signature to be determined as for cancer, where several patterns could be distinguished (see for instance
Ehrich et al., 2005
Gravina et al., 2015
Laird, 2003
Shen and Waterland, 2007

Suzuki and Bird, 2008)

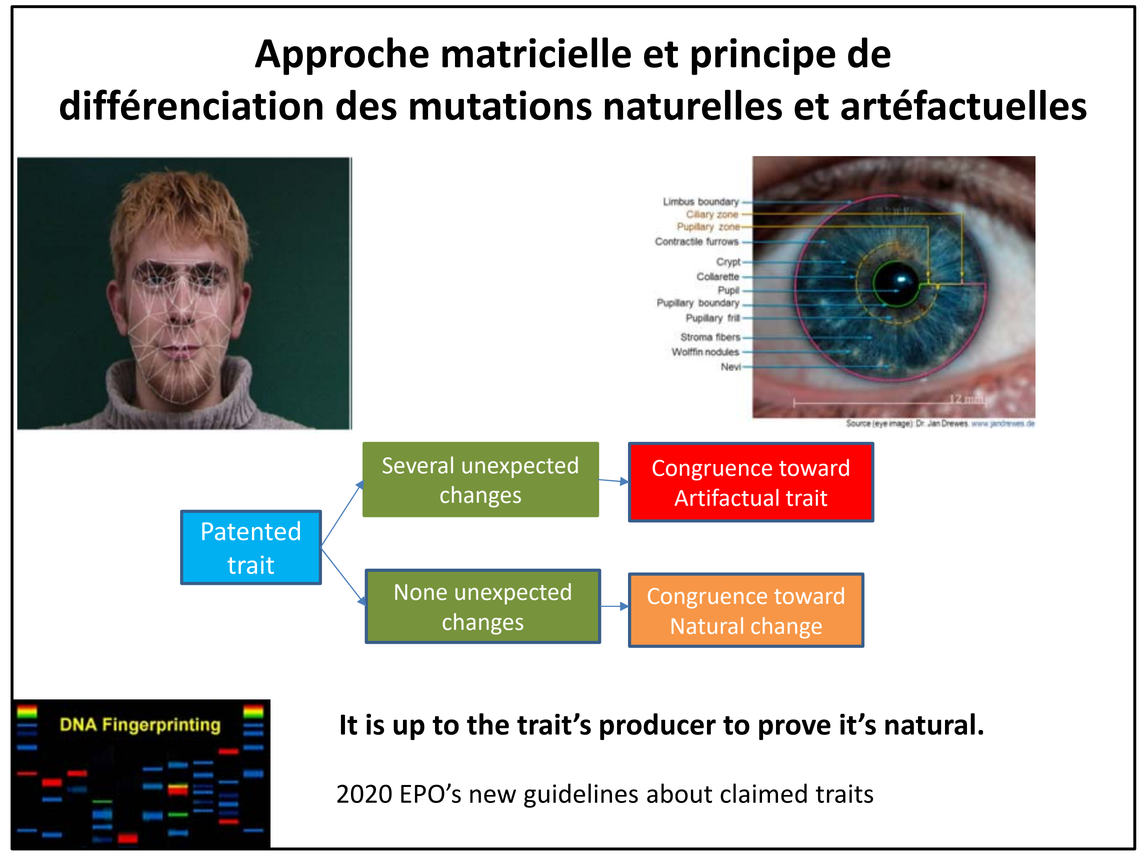
Polymorphism and epigenetics patterns
Mendizabal et al. 2014 Integrative and Comparative Biology 54(1): 31-42

All additional use of Crispr (and more generally speaking all DNA changes such as DNA insertion) could induce epigentics changes able to provide part of a signature at least for mammals (the best studies organisms) Sullivan et al. 2015 Biochemistry and Biophysics Reports 2: 143-152

Huge work to be performed before a safe use of Crispr. Hille and Charpentier 2016 Philosophical Transactions of the Royal Society B-Biological Sciences. 371: 1707

If we remind the FP5, FP6 and FP7 EU research funding for GMOs, clearly a political decision has been taken to avoid to provide detection / identification / quantification tools.

Taking the former old GMOs ways to cover this issue of ‘traceability’ would have provide solutions…



EPO: European Patent Office

Identification des produits et techniques NTMGE par les biomarqueurs

- En 2013 et à plusieurs autres reprises : proposition du réseau européen ENGL de travailler sur la détection des produits NBT, refus de la CE...
- Fondamentalement:
  - il s'agit du même type de détection / identification que celui des variétés : polymorphisme de divers marqueurs moléculaires, logiciels et tests statistiques...
  - En cours de normalisation à l'ISO, l'UPOV et testés par les laboratoires de l'ISTA,
  - Mais avec des marqueurs nettement plus faciles à détecter et combiner…

L'identification de la technique initiale et la traçabilité des produits NTGME sont possibles...
Mais la preuve de concept se heurte à des considérations politiques !

Détection et identification des produits NTGME et des techniques à leur origine : (Bertheau, 2019) (Bertheau, 2021a, b) (European Network of GMO Laboratories (ENGL), 2019)(Chhalliyil et al., 2020)

Bertheau, Y. (2019). New breeding techniques: detection and identification of the techniques and derived products. Encyclopedia of Food Chemistry, Reference Module in Food Science. R. H. Stadler, Elsevier**:** 320-336.

Since the commercial releases of GMOs in the 90s, new genetic modification tools known as New breeding techniques have been developed for e.g. gene silencing or more precise genomic modifications such as Crispr-endonuclease based systems. As for GMOs several consumers view may prevail about the societal interest in agricultural production and food of such genetic modification. Ensuring the freedom of choice to consumers needs to develop detection tools which could infer the NBT nature of the modification technique used. This article reviews all the elements which could allow the identification and detection of such techniques and products.

Bertheau, Y. (2021). Advances in identifying GM plants. Current frame of the detection of transgenic GMOs. Developing smart-agrifood supply chains: using technology to improve safety and quality. L. Manning, Burleigh Dodds Science Publishing**:** In Press.

synopsis 2021/11/08

Transgenic GMOs have been widely rejected in the European Union since their

commercialization in the late 1990s. A tolerance threshold for adventitious or technically unavoidable presence has been established with a standardized traceability system. The status of new techniques for modifying genomes and epigenomes (NBTs) and that of products with uncertain status has recently been the subject of decisions by the European Court of Justice and the French Conseil d'Etat. These products modified by techniques that could not justify in 2001 numerous applications nor show a long history of proven safety are subject to GMO regulations. Are GMO regulations applicable to products that some consider indistinguishable from those resulting from traditional breeding? This chapter presents the various tools, targets, and strategies that make it possible to unequivocally identify all GMOs. Finally, it proposes procedures for routine and control laboratories.

Résumé accepté
Transgenic GMOs were welcomed in the 1990s due to the difficulties distinguishing genetic and epigenetic modifications from random mutagenesis and their ability to insert new nucleic sequences more rapidly but still randomly. Their marketing in Europe has been accompanied by health and environmental risk assessments, specific monitoring and traceability procedures to preserve the free choice of consumers and allow the coexistence of different supply chains. This chapter reviews the regulations, detection techniques, strategies and standards that have been put in place in the European Union since 1996 to ensure the analytical traceability of these GMOs. The capacity of the matrix approach, initially targeted at transgenic GMOs, to trace other types of GMOs is discussed in an accompanying chapter.
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The discovery in the 1980s of the pathogenesis' mechanisms of *Agrobacterium tumefaciens* led to transgenesis, a technique for increasing the diversity of traits that could be used in plant breeding. Various other means of plant transformation were then implemented. This new technique came when in vitro mutagenesis was stalled due to the lack of mutations' screening systems until the description in 2000 of the Tilling technique which was then followed by various developments. Consumers received the genetically modified organisms (GMO) products resulting from these artefactual transformations in different ways in different countries. In European countries with a long culinary tradition and numerous products under official quality labels, the precautionary principle, which had previously prevailed in third countries, was introduced in the face of these new techniques which at the time had lacked any history of safe use. From then on, these GMOs were only produced and marketed after a risk assessment. In addition, labelling and traceability, according to the farm-to-fork approach, are required with specific and general post-market environmental monitoring. This chapter describes the scientific, technical and regulatory framework of this European traceability system,

which allows all European consumers to make informed choices about their food. Moreover, this traceability approach enables the coexistence of GM and non-GM supply chains and should thus make it possible to avoid mixing food products with those for pharmaceutical, functional food or industrial use.
The framework we describe in this chapter must be used to deal with the traceability of "new" GMOs and "hidden" GMOs. GMOs resulting from in vitro mutagenesis of isolated cells and NBT techniques, so named by the non-governmental organisations (NGOs) and farmers' union that brought the dispute before the French Conseil d'Etat in 2015 engendered a conflict that led to the European Court of Justice recalling the 2001/18 directive's definition of GMOs in 2018. The feasibility of this traceability of these "hidden" and "new" GMOs is discussed in the next chapter.

Bertheau, Y. (2021). Advances in identifying GM plants. Toward the routine detection of "hidden" and "new" GMOs. Developing smart-agrifood supply chains: using technology to improve safety and quality. L. Manning, Burleigh Dodds Science Publishing**:** In Press.

synopsis en ligne 2021/11/08

This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording "hidden" GMOs and "new" GMOs as defined by the French NGOs and farmers' union at the origin of the 2018 European Court of Justice ruling.

résumé accepté

In 2018 the Court of Justice of the European Union recalled that organisms with genomes modified by artifactual techniques should be considered GMOs under European regulations. GMOs derived from cultures of cells isolated in vitro or from new genomic techniques must therefore be traceable. This chapter reviews the various technical steps and characteristics of those techniques causing genomic and epigenomic scars and signatures. These intentional and unintentional traces, some of which are already used for varietal identification, and are being standardized, can be used to identify these GMOs and differentiate them from natural mutants. The chapter suggests a routine procedure for operators and control laboratories to achieve this without additional costs.

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The growing knowledge in genetics, epigenomics, epitranscriptomics, and the 3D - or even 4D - genome structure provides an increasing number of detection targets that can be used to identify species or genetic lines, whether modified or not. Biotic and abiotic stresses also induce numerous unintentional genetic, epigenetic, and epitranscriptomic modifications. Those changes are transmissible and can be ordered in regions and classified. The detection target is characterised by the mutagenesis technique used. For instance, the detection of transgenic GMO or

SDN3 modification of New Breeding Techniques (NBTs) will target their insertion's junction fragments into the genome. Each insertion induces epigenetic, and probably epitranscriptomic, changes which can also be targeted. In addition, one group of markers is linked to the trait(s) introduced or modified by the breeder whose sequence could be used in quantification and "screening". The other target will be a subset of the elements of a matrix approach (as described in the previous chapter). General selection markers, such as those used for plant breeding, together with mutagenesis techniques specific markers, could differentiate genetically modified organisms (GMOs) of any origin. They can be used to quantify and certify, through a global approach to the organism, that the trait modification is artefactual and not "natural." The growing mastery of single-cell sequencing techniques should soon make it possible to differentiate the modifications due, for example, to each step of a Crispr-Cas transformation of cells in culture. This chapter will focus mainly on detection targets based on nucleic acids, DNA, RNA, modified or unmodified, for their routine use in private and enforcement detection laboratories to comply with food labelling and European traceability rules. The chapter use the wording “hidden” GMOs and “new” GMOs as defined by the French NGOs and farmers’ union at the origin of the 2018 European Court of Justice ruling.

European Network of GMO Laboratories (ENGL) (2019). Detection of food and feed plant products obtained by new mutagenesis techniques (JRC116289). Luxembourg, European Commission, Joint Research Centre. **JRC116289:** 21.

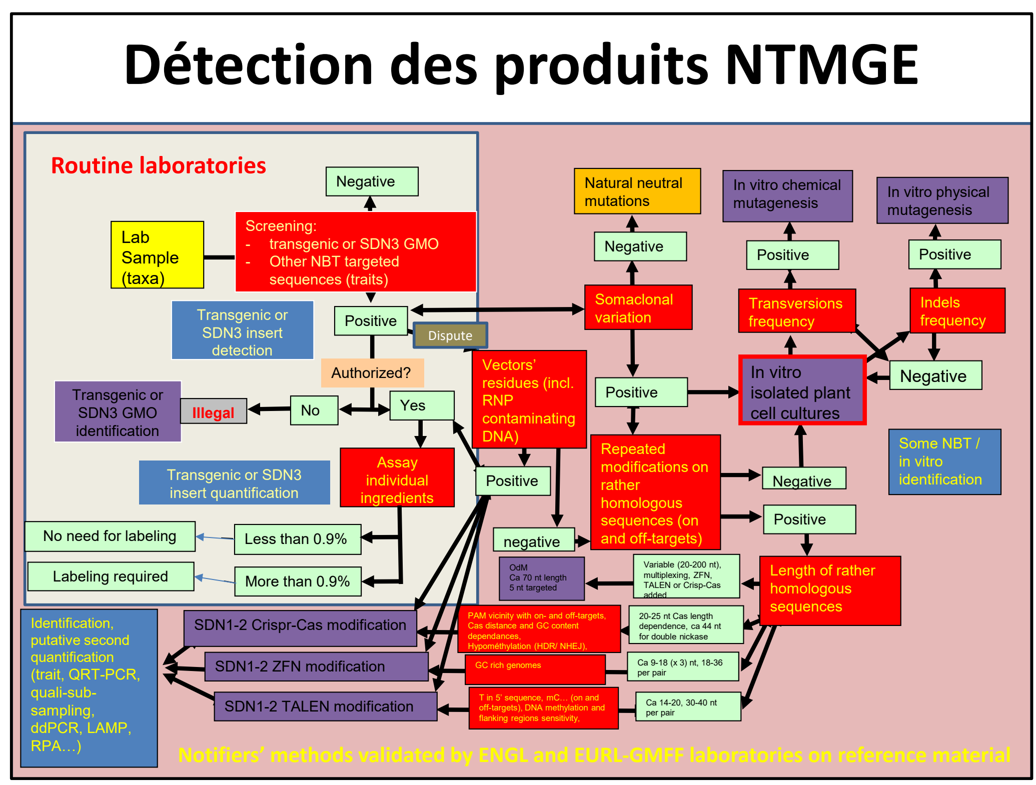
Executive Summary

The European Network of GMO Laboratories (ENGL) has reviewed the possibilities and challenges for the detection of food and feed plant products obtained by new directed mutagenesis techniques leading to genome editing. The focus of this report is on products of genome editing that do not contain any inserted recombinant DNA in the final plant.
The procedures for the validation of detection methods as part of the market authorisation application process for genome-edited plant products will in principle be the same as for the current conventional GMOs. It is, however, questionable if event-specific identification and quantitative detection methods can be developed readily for all genome-edited plants. For instance, detection methods for those plant products that are characterised by a non-unique DNA alteration will probably lack the specificity required to identify the genome-edited plant. Moreover, accurate quantification may be challenging if only changes of just one or a few basepairs are introduced.
The EU Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) assisted by the ENGL will need to review the minimum performance requirements that are applied for GMO method validations in view of the specific characteristics of genome-edited plants. This should provide further guidance to applicants for market authorisation and to the EURL GMFF for validation of the event-specific methods. For example, it is currently unclear how to demonstrate or assess the

specificity of the method if the mutation could also occur spontaneously or could be introduced by random mutagenesis techniques. Furthermore, it needs to be emphasised that specific detection methods would be required to cover all DNA alterations in a multi-edited plant.
For market control, considering the current knowledge and state of the art of GMO testing, it is highly improbable for enforcement laboratories to be able to detect the presence of unauthorised genome-edited plant products in food or feed entering the EU market without prior information on the altered DNA sequences. The PCR (polymerase chain reaction)-based screening methods that are commonly used to detect conventional GMOs cannot be applied nor could be developed for genome-edited plant products. The reason is that the currently used screening methods are targeting common sequences which are not occurring in genome-edited plants.
DNA sequencing may be able to detect specific DNA alterations in a product. However, this does not necessarily confirm the presence of a genome-edited plant product. The same DNA alteration could have been obtained by conventional breeding or random mutagenesis techniques, which are exempted from the GMO regulations.
In conclusion, validation of an event-specific detection method and its implementation for market control will only be feasible for genome-edited plant products carrying a known DNA alteration that has been shown to be unique. Under the current circumstances, market control will fail to detect unknown genome-edited plant products.
Several issues with regard to the detection, identification and quantification of genome-edited products are currently based on theoretical considerations only and lack any experimental evidence. Therefore, they will require further consideration.

Chhalliyil, P., H. Ilves, et al. (2020). "A Real-Time Quantitative PCR method specific for detection and quantification of the first commercialized genome-edited plant." Foods **9**(9): 1245.
Discussion regarding the regulatory status of genome-edited crops has focused on precision of editing and on doubts regarding the feasibility of analytical monitoring compliant with existing GMO regulations. Effective detection methods are important, both for regulatory enforcement and traceability in case of biosafety, environmental or socio-economic impacts. Here, we approach the analysis question for the first time in the laboratory and report the successful development of a quantitative PCR detection method for the first commercialized genome-edited crop, a canola with a single base pair edit conferring herbicide tolerance. The method is highly sensitive and specific (quantification limit, 0.05%), compatible with the standards of practice, equipment and expertise typical in GMO laboratories, and readily integrable into their analytical workflows, including use of the matrix approach. The method, validated by an independent laboratory, meets all legal requirements for GMO analytical methods in jurisdictions such as the EU, is consistent with ISO17025 accreditation standards and has been placed in the public domain. Having developed a qPCR method for the most challenging class of

genome edits, single-nucleotide variants, this research suggests that qPCR-based method development may be applicable to virtually any genome-edited organism. This advance resolves doubts regarding the feasibility of extending the regulatory approach currently employed for recombinant DNA-based GMOs to genome-edited organisms.



Janik, E., Niemcewicz, M., Ceremuga, M., Krzowski, L., Saluk-Bijak, J., and Bijak, M. (2020). Various Aspects of a Gene Editing System—CRISPR–Cas9. International Journal of Molecular Sciences *21*, 9604.
ZFN 9-12 bp
TALEN 8-31 bp
Crispr-Cas 17-20 bp

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5901495/
TALEN liaison cytosine méthylée Valton 2012
Pas Crispr-Cas Vojta 2016
TALEN reconnait une T en n terminal donc en 5'

TALEN: longueur et succession / proximité de nucleotides pour la modification du génome de mitochondries d'Arabidopsis transformées (C en T) par floral dip et Agrobacterium (Nakazato et al., 2021) https://www.nature.com/articles/s41477-021-00954-6

Cf.
https://www.medecinesciences.org/en/articles/medsci/full_html/2014/02/medsci20143002p186/medsci20143002p186.html
Demi-site 14-20nt – FokI 14-24 nt – demi site 14-20 nt
Donc 2 x 14-20 pour la detection des on- et off-targets

Gupta Musunuru 2014 Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9

ZFN recognition sites : x 3 nt multiples
Usually 9-18 nt
TALEN 10-30

ZFN

Farris et al. 2020 Detection of CRISPR-mediated genome modifications through altered methylation patterns of CpG islands

https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-020-07233-2#ref-CR30
Epigenetic changes inheritable
DNA methylation changes according to HDR vs NHEJ DNA damage repair mechanism used

Lee et al 2020 Epigenetic Footprints of CRISPR/Cas9-Mediated Genome Editing in Plants
https://www.frontiersin.org/articles/10.3389/fpls.2019.01720/full
Epigenetic changes due to Crisp-Cas9 induced modifications I Arabidopsis

Khalil 2020 The genome editing revolution: review

ZFN GC rich preferential
9-18 nt per ZFN monomer
18-36 per ZFN pair
TALEN : 14-20 per TALEN monomer
28-40 per TALEN pair
Cas 9 20 nt + 2 nt PAM
Up to 44 nt for double nicking

Ates 2020 Delivery Approaches for Therapeutic Genome Editing and Challenges

ZFN 18-36 nt

https://www.ptglab.com/news/blog/crispr-cas9-talens-and-zfns-the-battle-in-gene-editing/
ZFN 9-18

TALEN 30-40
CRISPR-Cas 22 + PAM

Jansing 2019 Genome editing in agriculture: technical and practical considerations
OdM 20-200 nt

Zhang 2019 Genome Editing with mRNA Encoding ZFN, TALEN, and Cas9
https://www-sciencedirect-com.insb.bib.cnrs.fr/science/article/pii/S1525001619300176

Okuzaki 2004
Chimeraplast (RNA/DNA hybrid) length of ca 68 nt, for 5 nt targeted

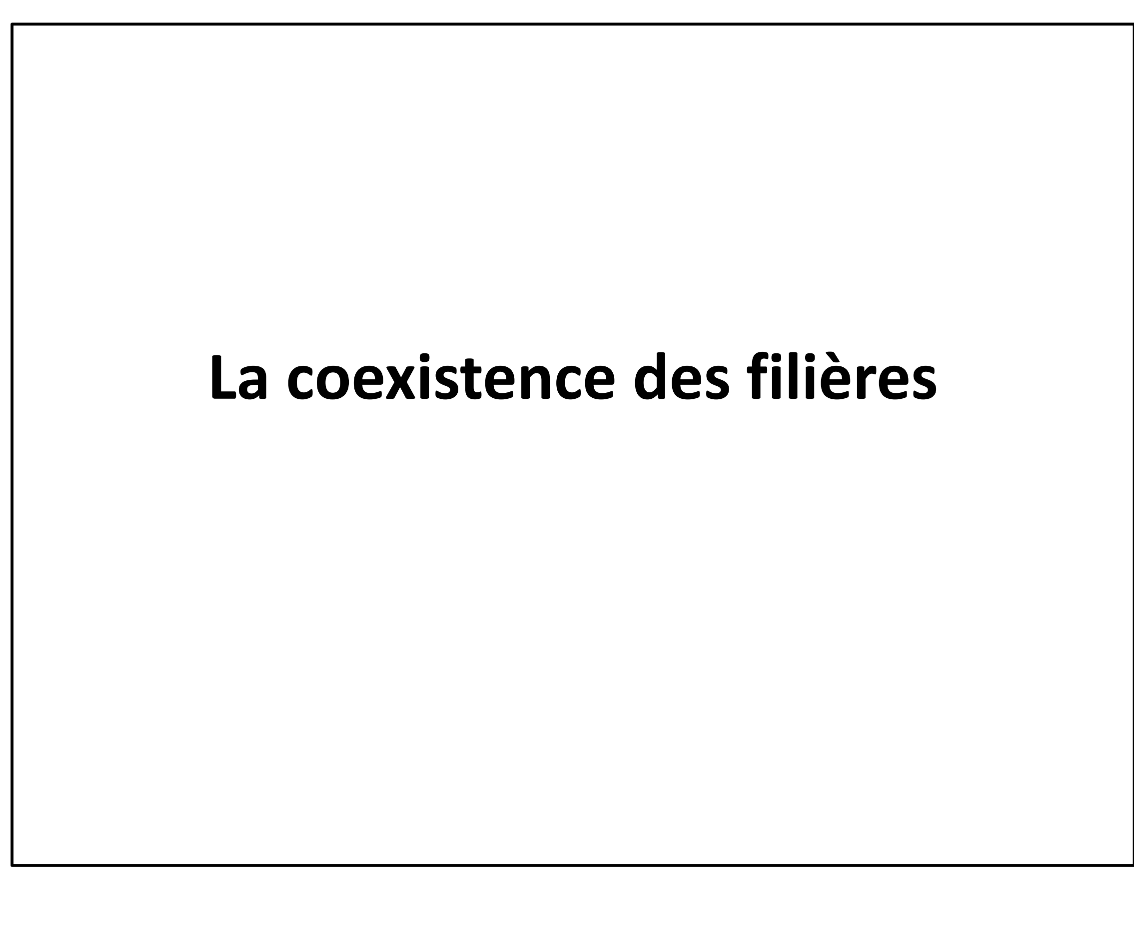
Differentiation of Cas: PAM vicinity distance; Abdullah et al. 2020 CRISPR base editing and prime editing: DSB and template-free editing systems for bacteria and plants
https://www.sciencedirect.com/science/article/pii/S2405805X20300624

L’absence de méthodes de détection des produits NTMGE ? Une volonté politique…

De nombreux biomarqueurs et techniques sont disponibles pour détecter et identifier les produits NBT, quelles que soient les techniques NBT utilisées. Les stratégies sont similaires à celles utilisées actuellement à l’ISO et à l’UPOV pour identifier les variétés végétales…

- Depuis 2007, date d’interrogation par le COGEM hollandais de la Commission européenne à propos des NBT, et au contraire de ce qui avait été initié au début du XXIème siècle pour les OGM issus de transgénèse, aucun programme de recherche n’a été initié visant à assurer aux producteurs, citoyens et entreprises la capacité d’appliquer la réglementation européenne en matière d’OGM et donc l’étiquetage nécessaire aux libres choix de chacun.
- La demande, il y a plusieurs années, du réseau ENGL de commencer à développer des outils de détection des produits NBT a été rejetée par la Commission européenne.
- Il est pour le moins étonnant qu’en 2018 un haut fonctionnaire européen ait trouvé le temps de participer à la rédaction d’un pré-rapport sur la détection des produis NTMGE, pré-rapport ensuite disparu des sites de la Commission…
- La lecture du rapport du JRC de 2019 révèle que les laboratoires de contrôles d’État ont les moyens pour identifier les produits NBT pourvu que les règlements 1829/03 et 1830/03 soit appliqués. Les firmes peuvent fournir les informations nécessaires aux identifications et les matériaux de référence.
- Un groupe de travail ENGL a été mis en place l’été 2022: un « trop grand nombre de volontaires » ne permettait pas au JRC-EURL GMFF de m’accepter comme membre de ce GT.

C’est donc bien une volonté politique qui a présidé et préside à l’assertion, fausse, que les produits NBT ne peuvent pas être tracés.


La coexistence des filières

La coexistence, stratégies

- La nécessité des coexistences: produits différenciés selon les choix des consommateurs , nécessités sanitaires (sans gluten…), besoin industriels, productions agricoles alimentaires (semences, commodities) enrichis ou non en un élément (cf. riz doré), tomate GABA) vs. produits à destination industrielle…
- Commence dès la production, puis méthode HACCP et quelques contrôles,
- Cas des OGM comme d'autres cultures :
  - Flexible
  - Zones de production dédiées
- De nombreuses contaminations de semences

Contaminations de semences inattendues, ex. Le Monde 2006/10/14

Aspects sociétaux et économiques

- Coexistence flexible : les voisins se coordonnent (décalage des semis, rotation de cultures, bordures de protection…)
- Coexistence par zones dédiées:
  - des régions entières choisissent de cultver GM ou non. Ex Coopétition en Alsace pour le maïs,
  - Impact sur les résidents non exploitants

Coopétition

Choix politiques

- Empilages de gènes: unité de mesure modifiée (ADN vs. graine ou poids) pour faciliter la coexistence (en diminuant les quantités) et éviter l'étiquetage
- EcoBureau européen
- Plus généralement désengagement de l'Etat (cf. USA : dicamba)
- USA : plus de travaux de l'AC21

"unité" ADN recommandée par ENGL Commission européenne choix après résultats Co-Extra d'une nouvelle unité: la graine qui aura l'avantage de diminuer el steneurs en OG et donc faciliter la coexistence et éviter étiquetage de produits
Particulièrement important avec les empilages de gènes.
USA: https://www.usda.gov/topics/farming/coexistence/coexistence-resources-and-statistical-data

Surveillance spécifique

- Surveillance uniquement dans la parcelle de phénomènes prévisibles : résistance d’adventices, d’insectes…
- Quelques lignes directrices,
- Quelques programmes publics d’études, majoritairement des questionnaires avec les nombreux biais inhérents,
- Aucun retour d’expérience en plus de 20 ans,
- Cas du maïs Mon810…

Surveillance générale

- Surveillance au champs et à l'extérieur (agroécosystèmes) de phénomènes inattendus
- Pas de lignes directrices, pas de réseau constitué de surveillance en 30 ans pour des raisons de coûts, surfaces concernées, types d'observation, rassemblement des informations (cartes et bases de données) et longue durée,
- Cas du coton « Bt » en Chine…

Nombreux lots de semences contaminés par des OGM
Ventes illégales de riz OGM (issues d'un laboratoire et d'essais au champs) en Chine : régions entières repiquées en OGM non autorisés, alertes européennes depuis plus de 15 ans, énormes difficultés à se procurer la méthode de détection et le matériel de référence,

(Bergé and Ricroch, 2010; Cloutier et al., 2008; Lang, 2006; Lu et al., 2010; Lu et al., 2008; Pearson, 2006; Wang et al., 2008; Wang et al., 2009; Whitehouse et al., 2007; Wu, 2007; Wu et al., 2003)
Coton OGM résistant à certains insectes, relative efficacité entrainant une diminution des quantités d'insecticides dans les champs de coton, bon pour la santé des cotoniculteurs, mais fort développement de miridés (sortes de punaises) qui se répandent sur les autres cultures. Forte augmentation des épandages d'insecticides sur ces autres cultures.
Quel avantage écologique des coton Bt ?
Lu, Y., K. Wu, et al. (2010). "Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China." Science **328**(28): 1151-1154.
Long-term ecological effects of transgenic Bacillus thuringiensis (Bt) crops on nontarget pests have received limited attention, more so in diverse small holder–based cropping systems of the developing world. Field trials conducted over 10 years in northern China show that mirid bugs (Heteroptera: Miridae) have progressively increased population sizes and acquired pest status in cotton and multiple other crops, in association with a regional increase in Bt cotton adoption.

More specifically, our analyses show that Bt cotton has become a source of mirid bugs and that their population increases are related to drops in insecticide use in this crop. Hence, alterations of pest management regimes in Bt cotton could be responsible for the appearance and subsequent spread of nontarget pests at an agro-landscape level.
Wang, Z.-J., H. Lin, et al. (2009). "Bt cotton in China: are secondary insect infestations offsetting the benefits in farmer fields?" Agricultural Sciences in China **8**(1): 83-90.
1997. It has effectively controlled the bollworm. However, in recent years, concern has surfaced about the emergence of secondary insect pests, particular mirids, in Bt cotton fields. This study measures the patterns of insecticide use based on farm-level from 1999 to 2006, the analysis demonstrates a rise in insecticide use to control mirids between 2001 and 2004, secondary insect infestations is largely related to the rise of mirids, but this rising did not continue in more than half of sample villages studied in 2004-2006. Moreover, the increase in insecticide use for the control of secondary insects is far smaller than the reduction in total insecticide use due to Bt cotton adoption. Further econometric analyses show that rise and fall of mirids is largely related to local temperature and rainfall.

Autres exemples :
Fok, M. (2010). "Autant en emporte la culture du coton transgénique aux Etats-Unis…." Cahiers Agriculture **19**(4): 292-298.
Les conférences 2010 du Beltwide cotton ont apporté une vision nouvelle sur les conséquences de 15 années d'utilisation massive des variétés de coton transgénique aux Etats-Unis. L'observation des changements dans les complexes d'ennemis des cultures de cotonnier et les solutions recherchées pour y faire face montrent que les effets
positifs proclamés de l'utilisation de ces variétés se sont évanouies en termes d'efficacité du contrôle des ennemis des cultures, de réduction du coût et d'antagonisme entre voie chimique et voie biotechnologique de ce contrôle. La durabilité technique et économique des variétés transgéniques dépend de l'approche systémique et coordonnée
de leur utilisation.
Gone with the wind of transgenic cotton use in the United States
A vision of the discussions and presentations at the New Orleans
(Louisiana, USA) "Beltwide Cotton Conferences" in January 2010
The Cotton Beltwide Conferences 2010 have provided a new vision of the consequences
of about 15 years of widespread and non-coordinated use of transgenic cotton in the United States. The observation of the shift in pest complexes on one hand, and, on
the other, the adaptation solutions being explored today show that the positive effects
initially claimed look like vanished illusions in the areas of pest control

effectiveness,
cost reduction, and antagonism between chemical and biotech means. The technical
and economic sustainability of transgenic varieties lies in a systemic and coordinated
approach of their use.

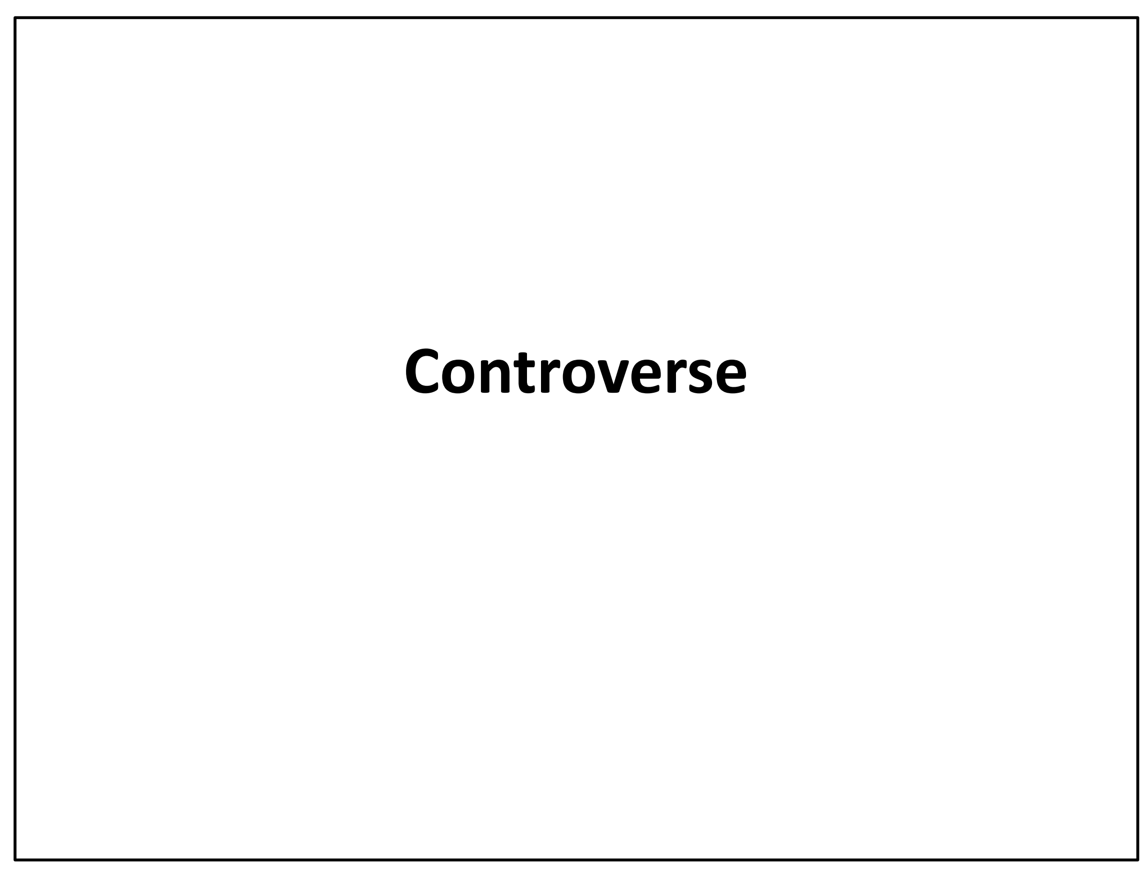
Fok, M. (2011). "Gone with transgenic cotton in the USA. A perception of the presentations and interactions at the Beltwide Cotton Conferences, New Orleans (Lousiana, USA), 4-7/01/2010." Biotechnology, Agronomy, Society and Environment **15**(4): 545-552.
The 2010 Beltwide Cotton Conferences provided a new vision of the consequences of about 15 years of widespread and uncoordinated cropping of transgenic cotton in the United States. Insect resistant and/or herbicide-tolerant cotton varieties modified parasite complexes, namely those of insects and weeds damaging cotton crops. The Conferences have revealed that the adaptation solutions so far proposed make illusory the expectations at the launch of transgenic cotton, in terms of effective pest control, cost reduction, and antagonism between chemical and biotech methods.
The USA case points out that the technical and economic sustainability of transgenic varieties must lie in a systemic and coordinated approach.

Cloutier, C., S. Boudreault, et al. (2008). "Impact of Colorado potato beetle-resistant potatoes on non-target arthropods: A meta-analysis of factors potentially involved in the failure of a Bt transgenic plant." Cahiers Agricultures **17**(4): 388-394.
The relatively high specificity of transgenic plants based on Cry toxins of Bacillus thuringiensis (Bt) implies the possibility of upward agroecosystemic cascades toward new equilibria among arthropods associating with cultivated plants. We examine the hypothesis that exclusion of the Colorado potato beetle from potato expressing the Cry3a toxin increases the abundance of non-target herbivores, which indirectly favours the abundance of herbivore-dependent predators and omnivores foraging on agricultural plants. We examined the impact of Bt potato on non-target arthropod taxa, based on impact studies conducted during development of the Newleaf(R) Bt potato in North America. Of 32 field tests comparing Bt potato to non-transgenic controls, 14 (42%) revealed a significant, positive effect on the abundance of sucking insects (aphids, leathoppers, mirids, thrips). Among 72 tests on generalist predators that were simultaneously monitored, 14 (similar to 20%) also revealed significant positive effects. Such positive effects on predators can best be explained by their abundance being increased as a result of greater productivity due to overabundance of sucking insect prey, which are selectively favoured by the high specificity of the Cry3a Bt toxin. Our results support the idea that development of the Bt potato may have been hampered in part by its positive effects on sucking insect pests, and underline the importance of conserving the natural enemies of secondary pests that are indirectly favoured.

Brevets et aspects juridiques

- Effet des brevets princeps et d’applications sur les agriculteurs et entreprises,
- Financiarisation du système de brevets et « trolls »,
- Effacement progressif du droit devant la morale scientiste,

Comme le remarquait en 1990 le juriste Oppetit « *à la source de la modernité se trouve l’ambition prométhéenne, pour reprendre la formule cartésienne, de devenir maître et possesseur de la nature grâce à la science et à la technique : or l’occident, en devenant moderne, a déchaîné de tels appétits de domination et de découverte qu’il ne cesse de se détruire, à mesure qu’il avance, et donc il recule* ».



(Chateauraynaud et al., 2013)
(Demortain, 2004, 2008, 2010, 2017)(Demortain and Borraz, 2015; Everson and Vos, 2009)

Chateauraynaud, 2018, rapport pour l'Anses : **Une nouvelle saison dans la série des controverses sur les biotechnologies en agriculture** Francis Chateauraynaud (GSPR- EHESS) Version du *26 février 2018*

Understanding causality and uncertainty in volcanic observations: An example of forecasting eruptive activity on Soufrière Hills Volcano, Montserrat
Invasive species
THE POWER OF BIAS IN ECONOMICS RESEARCH*
River management: dams erections and leveling
Urbanization d
flooding risks
Policy decisions making:
experts, lawyers, practitioners, stakeholders…
Italy earthquake experts charged with manslaughter
Smog / air pollution
Playing on an iceberg
Worst decision EVER
ALMOST
50%
OF EARTHS
ORIGINAL
FOREST COVER
IS GONE
WITHIN THE PAST
THREE DECADES
NBT status as an expertise crisis?

"Naturalité" des modifications par les SDN

- La notion de nature est extrêmement polysémique et le manque de définition de ce mot pour les techniques NBT est un classique de la rhétorique,
- Tout est possible et devient donc naturel sur des échelles de milliers ou millions d'années, pas en quelques semaines sauf évènement exceptionnel,
- Conserver une approche holistique: une mutation naturelle est soit neutre soit éliminée si elle n'apporte pas de meilleure adaptation, elle ne peut être naturelle si de nombreuses autres mutations concomitantes sont artéfactuelles,
- Qu'une modification biochimique finale ne nous fasse pas passer d'une chimie du carbone à une chimie de la silice ne prouve pas que la modification est naturelle,

Le raisonnement implicite sollicité tendrait à démontrer que la tour Montparnasse est naturelle car constituée de sable comme la grande dune du Pilat…

ECONOMIE DE PROMESSES

VERS UNE ACCÉLÉRATION DE LA SÉLECTION (ENTRE AUTRES) ?

Société Genta: 1 milliard US$ d'essais cliniques avant la faillite à cause du produit Genasense (mauvaise interprétation initiale des premiers résultats)
Therenos et diagnostic…

Intervention de scientifiques comme par exemple au SAM (Scientific Advice Mechanism) européen, un bon exemple d'« ultracrépidarianisme» : le fait, parce qu'on est spécialiste d'un domaine – ici par exemple les Mathématiques –, de penser qu'on est fondé à posséder une expertise sur d'autres sujets à propos desquels on n'a pas de compétence particulière – les NBT.

Nourrir le monde…

- Modélisations et observations actuelles de croissance de la population,
- Principales causes de famine et carences (ordre décroissant):
  - Guerres, conflits et déplacements de population,
  - Pertes et très importants gaspillages tout au long des filières, dont chez les consommateurs,
  - En raison des surproductions et exportations subventionnées, recherche de meilleurs revenus par les exploitants agricoles: biocarburants, cultures à quota subventionnées…
  - Pertes de surfaces agricoles (urbanisation, pollutions…) : 10^6 km^2 perdus…
  - Cultures de rentes dans des pays en développement,

(Bertheau, 2016)

Nourrir le monde…
Le gaspillage alimentaire en chiffres
En France, ce gâchis représente chaque année plusieurs milliards d'euros.
Il concerne tous les acteurs, du producteur au consommateur.
PRÈS DE 50 MILLIONS DE REPAS JETÉS À LA POUBELLE CHAQUE JOUR DANS NOTRE PAYS
10 MILLIONS de tonnes de nourriture consommable sont gaspillées chaque année.
Soit l'équivalent de 18 MILLIARDS de repas mis à la poubelle.
Dans le monde, 1/3 DES ALIMENTS destinés à la consommation humaine est gaspillé.
Chaque Français jette chez lui plus de 20 kg de nourriture par an, dont 7 kg d'aliments encore emballés.
UN GÂCHIS SUR L'ENSEMBLE DE LA CHAÎNE
32% lors de la PRODUCTION
Fruits et légumes abîmés, poissons trop petits rejetés morts en mer…
21% lors de la TRANSFORMATION
Processus ou conditionnement défectueux en usine…
14% lors de la DISTRIBUTION
Casse, rupture de la chaîne du froid, produits abîmés ou invendus…
33% lors de la CONSOMMATION
dont
14% pour la restauration collective et commerciale
Déchets de préparation, restes de repas, mauvaise gestion des stocks…
19% pour la consommation à domicile
Restes de repas jetés, produits périmés…
DES ENJEUX ÉCONOMIQUES ET ENVIRONNEMENTAUX
Le coût annuel du gaspillage alimentaire en France est estimé à 16 MILLIARDS D'EUROS.
L'impact carbone des pertes et gaspillages est évalué à 15,3 millions de tonnes équivalent CO2, soit 3% de l'ensemble des émissions de l'activité nationale.
A-t-on vraiment besoin de produire plus dans des espaces agricoles de plus en plus réduits par l'urbanisation galopante ?
Ou de produire mieux ?

NTMGE et sélection variétale: une dépendance au sentier

- Les NTMGE: une révolution vraiment ?
- Un plus en sélection variétale?
  - Des applications chez toutes les espèces ?
  - Réduction du temps de criblage, sélection et d'intégration de nouveaux traits ?
  - Nouveaux caractères ? Homologies et variations entre espèces et cultivars ? Traits mono / oligo / multigénique? QTL ? Une variation dans une variété ne s'exprime pas forcément de la même manière dans une autre (effet du fond génétique, de l'environnement…), l'épigénétique des conditions de culture des parents influence l'expression de caractères dans les descendances (cf. tolérance à la sécheresse…),
  - Une capacité à améliorer la résilience des cultures face au changement climatique? Aux maladies et parasites émergents ?
  - Des produits orientés consommateurs plutôt que producteurs ?

Les NTMGE: la meilleure façon d'épuiser rapidement la variabillité utile en agriculture?
Une biologie moléculaire mécaniste des années 1970
Une économie de promesses comme les OGM des années 90
et le clonage il y a 20 ans
Un verrou technologique supplémentaire
Une longue bataille de brevets avant les accords croisés dans des « clubs »
L'évolution de l'exemption de la recherche…

57

Par exemple on vient très récemment de découvrir que 1% du génome du maïs, résidant dans la chromatine ouverte, serait responsable d'au moins 40% des variations phénotypiques des traits agronomiques de cette espèce (Rodgers-Melnick et al., 2016). Parmi ces 98% de matière noire… Observations similaires de réseaux de régulation importants dans le génome humain (Chi, 2016; Fulco et al., 2016).

Jeffrey Sander (Pioneer, Johnston, Iowa) :

« *En plus, le génome des plantes présente une extrême diversité*, souligne Jeffrey Sander, chercheur au département d’ingénierie moléculaire. *Entre deux variétés de maïs, il y a la même distance génétique qu’entre un homme et un singe*. »

In « Dans la fabrique des plantes du futur », Nathaniel Herzberg, *Le Monde Sciences & Médecine 2017/06/07 page sch4*

Autres promesses

- Santé (gluten, acrylamide des pommes de terre…),
- Réduction des polluants, et intrants eutrophisant basés sur les énergies fossiles ou produits miniers en cours d'épuisement (phosphate avec du cadmium…),
- Répondre aux défis du changement climatique(semis plusieurs mois à l'avance, tolérance multifactorielle…),
- Innovation et économie…

Implication pour la médecine personnalisée...

« Compte tenu de la complexité des interactions entre mutations, Craig Venter estime qu'il faudrait séquencer 10 millions d'individus pour identifier la quasi-totalité de la composante génétique des maladies et de nos caractéristiques phénotypiques. Son programme de séquençage devrait dépasser 1 million d'individus par an, pour lesquels il dispose d'un dossier médical électronique de grande qualité, grâce à un accord avec des assureurs santé. Le but avoué est de créer un logiciel permettant d'optimiser la prise en charge des patients et d'augmenter leur espérance de vie. »

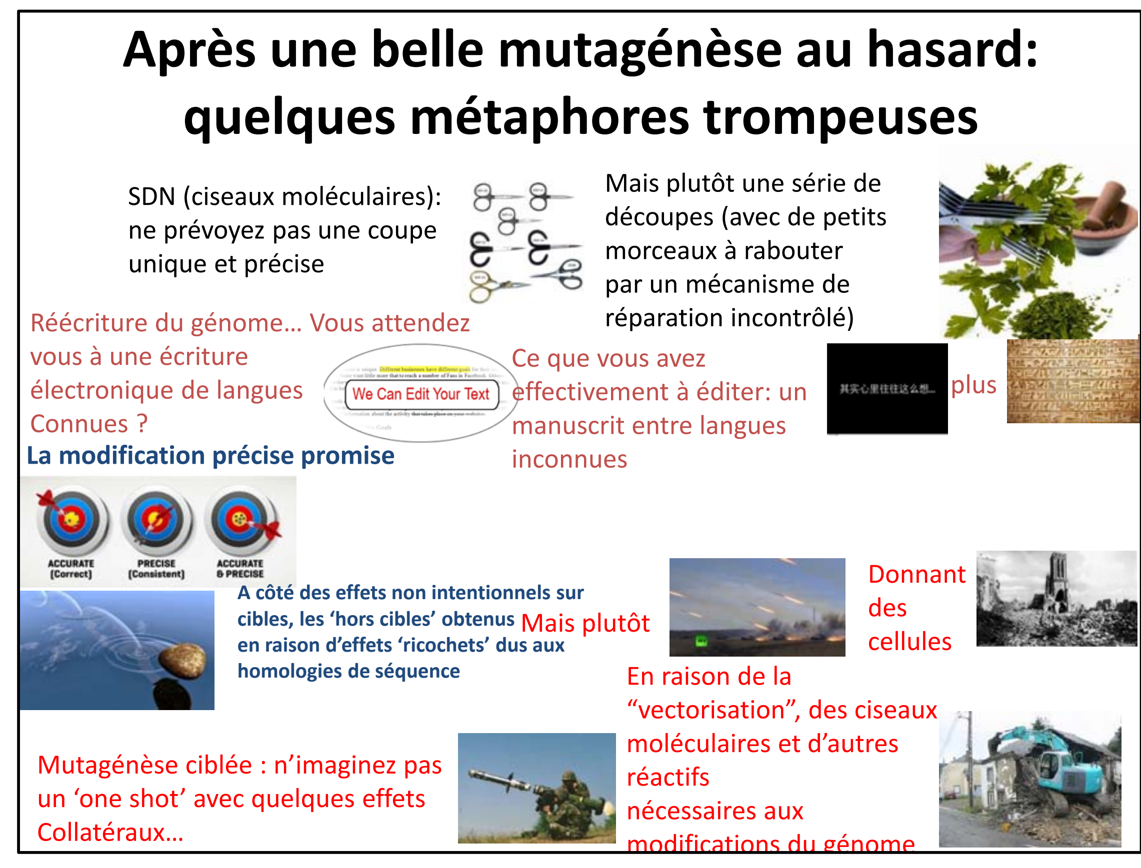
(Laurent Alexandre, 2016)

In « La bataille de l'ADN », Laurent Alexandre, *Le Monde, Sciences & Médecine,* 2016/06/08 page SCH7

MÉTAPHORES, COMMUNICATION
ET RHÉTORIQUE

Considérations diverses

- Mal nommage: nucléases dirigeables vers des séquences, il ne s’agit pas de site repéré par un GPS,
- Mots creux jamais définis mais répétés en boucle,
- Mensonge par omission (techniques connexes quasiment jamais mentionnes dans les articles de vulgarisation…),
- Course en avant avec un déni de réalité,
- Captation de l’attention,
- Changements incessants de terminologie pour faire oublier les OGM.



And not forget the border between our 2 jobs: scientist and expert. A lot of them forget it.

As (almost) said by Pierre Gilles de Gennes (Nobel Prize): experts are almost similar to militaries, they know very well the pas wars, but cannot anticipate the new ones.

Keep 'critical mind' (officially a part of the training of scientists) in front of any assertion of a lack of hazards. Beyond the usual financial conflicts of interest, there are numerous other links of interest: institutional, politics, economics…

Numerous scientists are unaware of professional ethics…

As already by sociologists: the scientist does not leave its opinions at the door of the lab or the expertise room.

Be careful with metaphors: they are often useful for some initial explanations but when repeatedly used it will drastically change your perception, your wayd of thinking and then of acting.

Look at the wording/ semantic used.

First example:
gene or genome editing.

You think rapidly about this image as with a “copy and paste” or “cut and paste” in your favorite computer located word processor?

But why did you imagine

1) That you are using a computer with word processing system?
1.1 In fact you may be using hand written text, which means you will cross-out text…
1.2. It might also bee a type written text. With such old type writer you can inly cut paper, around sentences, you will then use scotch tape and work on in order to hidden the changes at the photocopier. You can also have to completely reformat pages and thus make mistakes I the successive pages…

2. That you know well the language used in this text?
Suppose it is a foreign language you do not know. How accurate and understandable could be your cut and paste?
Suppose you are reading hieroglyphs before Champollion used the Rosetta stone. What would make your “cut and paste” between eyes or birds differently located? No changes in the text’s meaning? Or in the poesy? The subtilities?

Second example.
Nuclease as molecular scissors
You are often facing the metaphor of endonuclease as a molecular scissor?
You thus spontaneously think to the scissors you have in your sewing box.

Forget it, you are using a multi-blades scissor, as the one you may be using for cutting herbs for cooking.

As you can see, never accept directly the metaphors and images somebody else is providing you… Look at what can be beyond the simple image, easily coming to your mind

Conclusion

- Les produits NTGME et les OGM de mutagénèse au hasard *in vitro* sont des OGM non exemptés…
- Il est préférable de continuer avec le système réglemntaire existant par ailleurs inconsistant et incomplet (aucune évaluation des modifications épigénomiques et des organites, surveillance générale post-commercialisation, disponibilité d'experts, coûts…) que de n'avoir aucune évaluation ex-post (cf. nanomatériaux, règlement REACH),
- Les produits NTMGE sont détectables et la technique d'origine identifiable…
- Les problèmes de coexistence ne sont toujours pas réglés, comme ceux de lignes directrices strictes pour les VrTH,
- Choix raisonné des consommateurs entre modifications pour la santé et modifications pour l'alimentation.

Le principe de précaution reste toujours et encore d'actualité!

Après des années dans des instances d'expertise, en particulier au HCB, j'aime rappeler cette phrase de Pierre Gilles de Gennes (prix Nobel) :
« Vous savez, les experts sont souvent comme les militaires. Ils sont experts de la dernière guerre mais pas de la prochaine… »
« Ayez le culte de l'esprit critique. Réduit à lui seul il n'est ni éveilleur d'idée, ni un stimulant de grandes choses. Sans lui tout est caduc. »
Louis Pasteur
MERCI POUR VOTRE ATTENTION