Genome editing:
Promises and limitations for plant breeding

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Plant Reproduction and Development, ENS de Lyon

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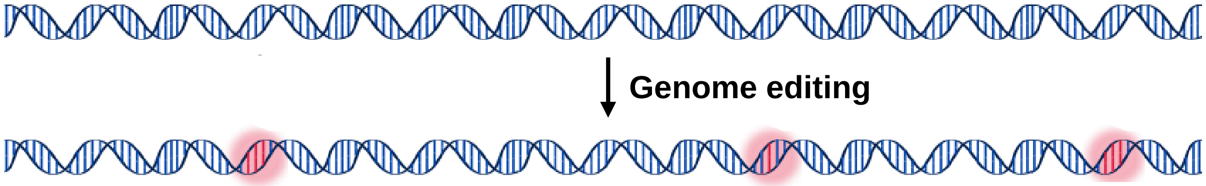
What is genome editing?

- Definition
  - Modification of the genome sequence (replacement, insertion, deletion) at one or several predetermined positions



☞ Genome editing generates mutants

Steps of genome editing

- Design and production of the editing tool (CRISPR-Cas9)
  - Knowledge on gene function or favorable SNP
    - Which gene(s) to modify to obtain a trait of interest?
    - Which modification(s)?
  - Knowledge of the genome sequence to avoid off target effects
  - State of the art design tools

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-22417-4 OPEN

CRISPECTOR provides accurate estimation of genome editing translocation and off-target activity from comparative NGS data

Ido Amit[1,5], Ortal Iancu[2,5], Alona Levy-Jurgenson[3], Gavin Kurgan[4], Matthew S. McNeill[4], Garrett R. Rettig[4], Daniel Allen[2], Dor Breier[2], Nimrod Ben Haim[2], Yu Wang[4], Leon Anavy[1], Ayal Hendel[2✉] & Zohar Yakhini[1,3✉]

nature biotechnology — REVIEW ARTICLE

https://doi.org/10.1038/s41587-020-0490-7

Design and analysis of CRISPR–Cas experiments

Ruth E. Hanna and John G. Doench✉

W242–W245 *Nucleic Acids Research, 2018, Vol. 46, Web Server issue* *Published online 14 May 2018*

doi: 10.1093/nar/gky354

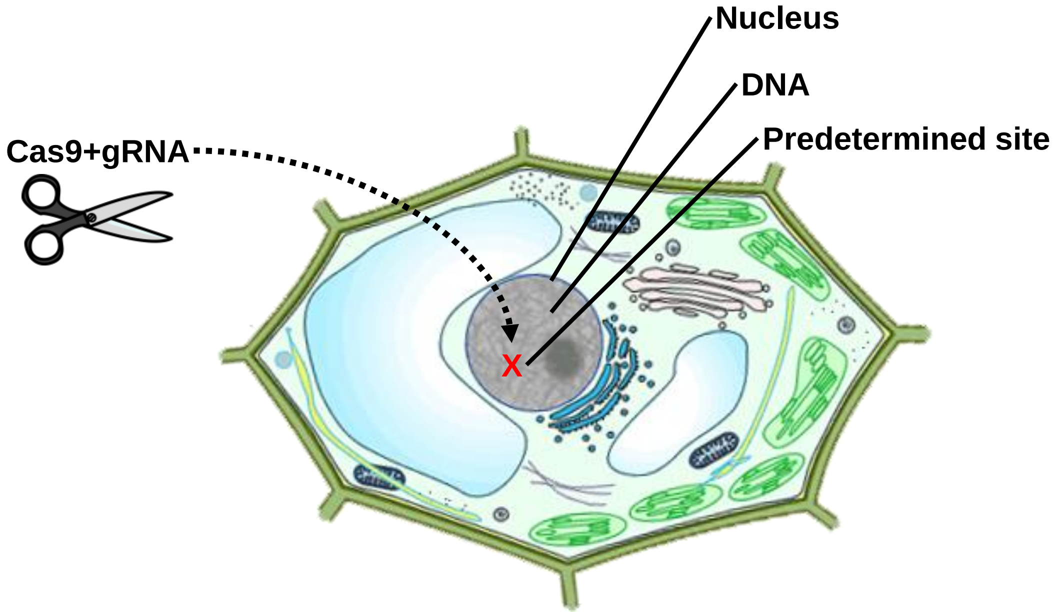
CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens

Jean-Paul Concordet[1] and Maximilian Haeussler[2,*]

☞ Genome editing requires upfront knowledge

Steps of genome editing

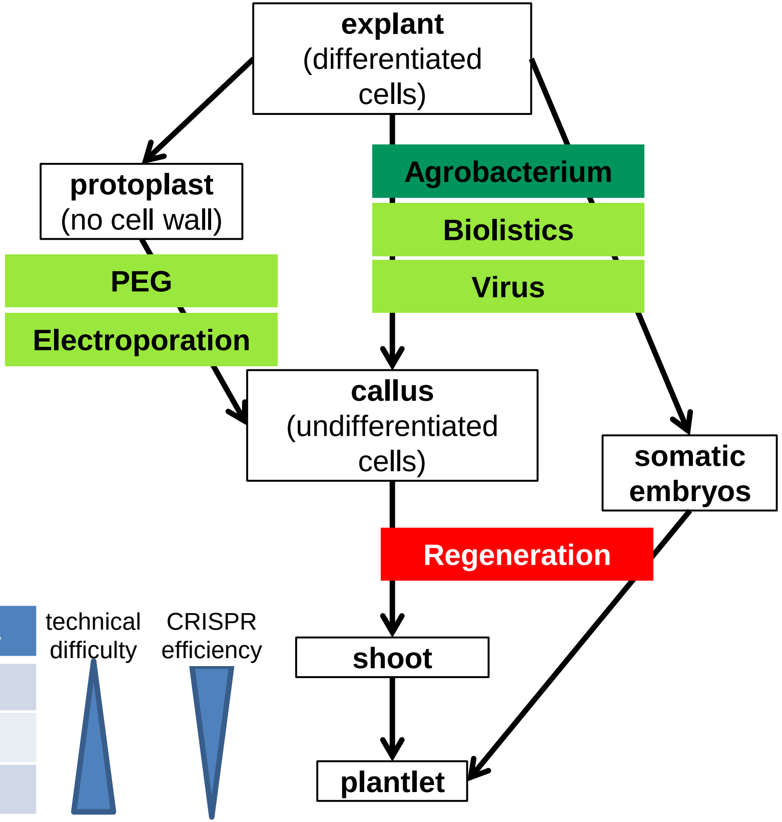
- Design and production of editing tool (CRISPR-Cas9)
- Introduction of the tool into the plant cell and the nucleus



☞ Genome editing requires mastery of plant transformation

Steps of genome editing

- Design and production of editing tool
- Introduction into the plant cell and nucleus
  - Transient transformation
    - DNA
    - Ribonucleoprotein (RNP) complex (Protein/RNA)
  - Stable transformation
    - Genetic segregation of the transgene
    - Excision of the transgene
  - Plant regeneration
    - Explant dependent
    - Genotype dependent
    - Species dependent
      - 35 plant genera edited
      - Problems with legumes

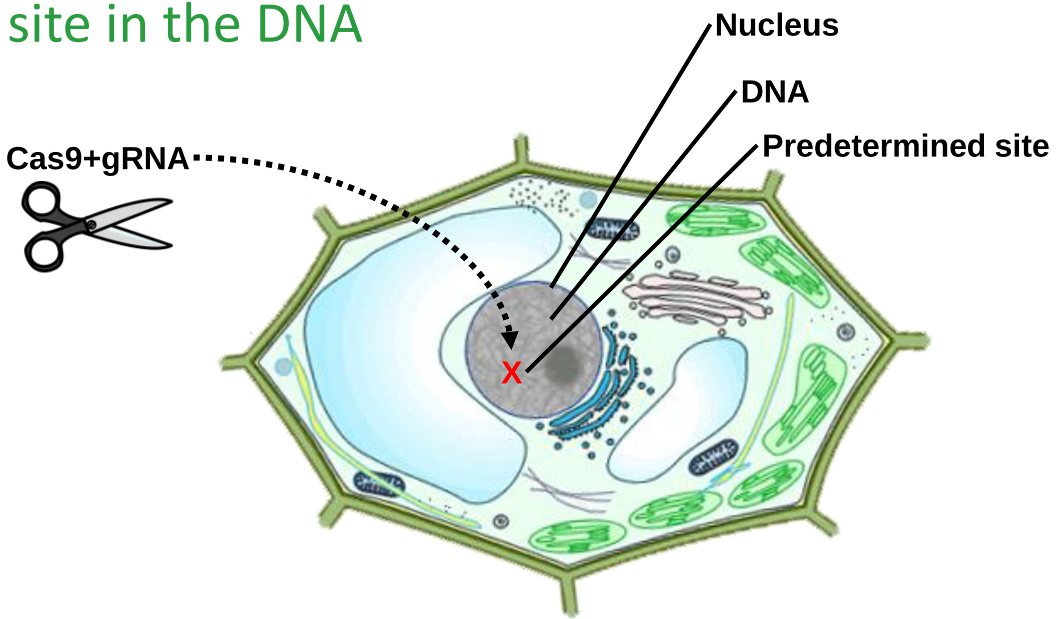


Cas9	sgRNA
DNA	DNA
DNA or RNA	RNA
Protein	RNA

☞ The bottle neck is generally not DNA or RNP transfer but regeneration

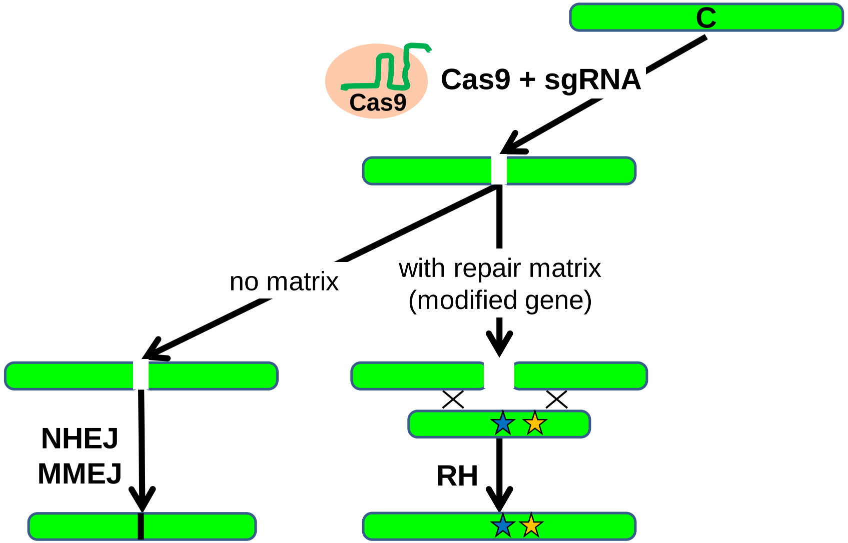
Steps of genome editing

- Design and production of editing tool (CRISPR-Cas9)
- Introduction of tool into the plant cell and the nucleus
- Recognition of a predetermined site in the DNA
- (DNA cleavage)
- DNA repair by the cell



☞ Genome editing allows to modify DNA at a unique, predetermined site

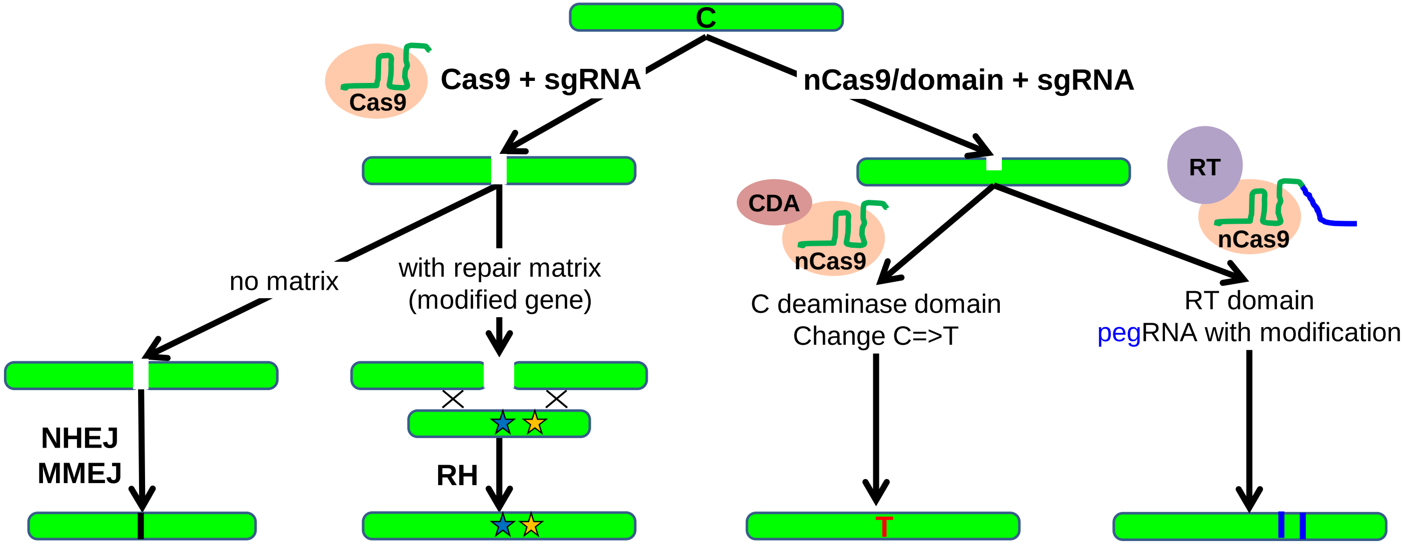
Targeted mutagenesis, gene editing, base editing, prime editing



	Targeted mutagenesis	True gene editing
actual use	97%	3%
Site in the genome	predetermined	predetermined
Nature of the modification	random	determined by repair matrix
Efficiency	excellent	low

☞ The vast majority of present publications concern targeted mutagenesis and not true gene editing

Targeted mutagenesis, gene editing, base editing, prime editing

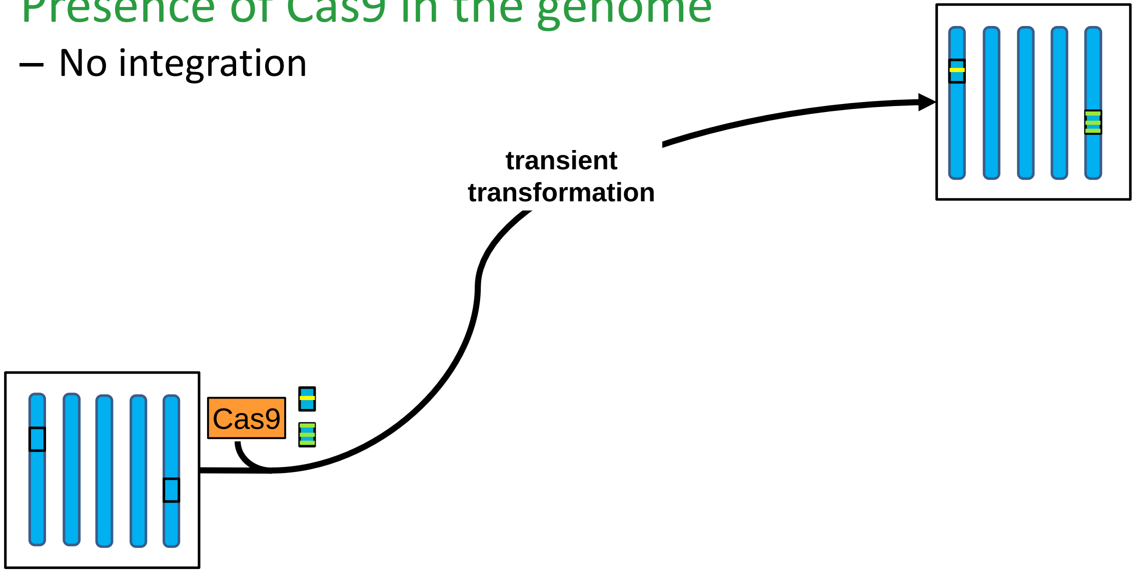


	Targeted mutagenesis	True gene editing	Base editing	Prime editing
actual use	97%	3%	growing	new
Site in the genome	predetermined	predetermined	predetermined	predetermined
Nature of the modification	random	determined by repair matrix	determined by enzyme	determined by pegRNA
Efficiency	excellent	low	very good	okay

☞ Targeted mutagenesis and base editing, but not true gene editing, are mastered in plants

Genome editing strategies

- Presence of Cas9 in the genome
  - No integration



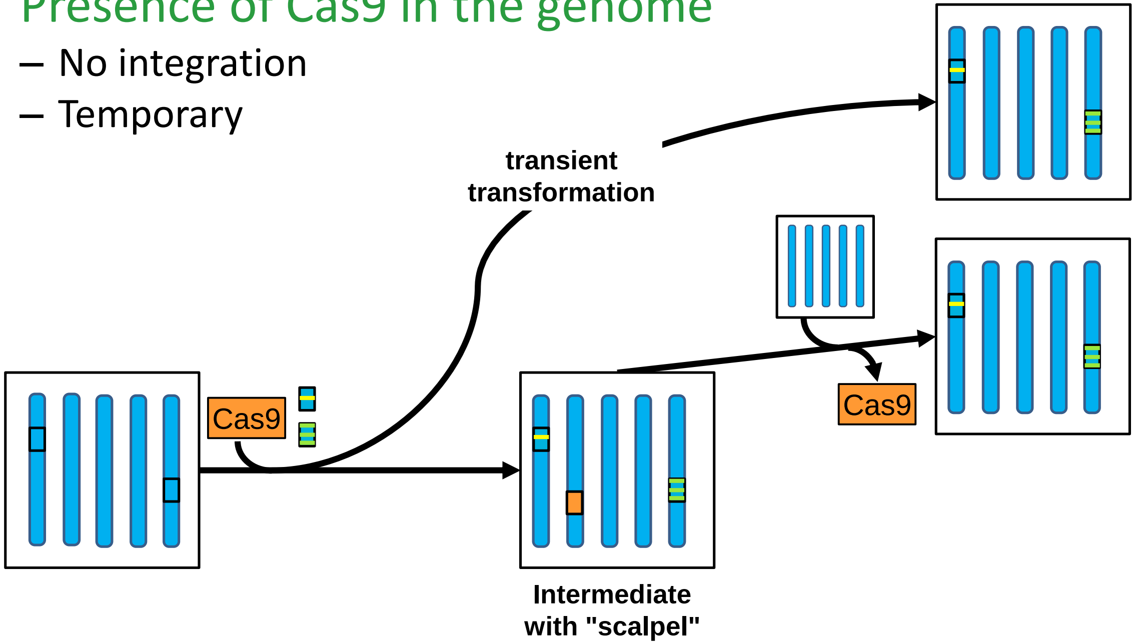
Appellation	Process	Product
non-transformed	no introduction of foreign DNA in the genome	mutant

☞ Transient transformation important for perennials and crops with vegetative propagation

☞ Non-integration of CRISPR-Cas9 into the plant genome may have consequences on regulation

Genome editing strategies

- Presence of Cas9 in the genome
  - No integration
  - Temporary

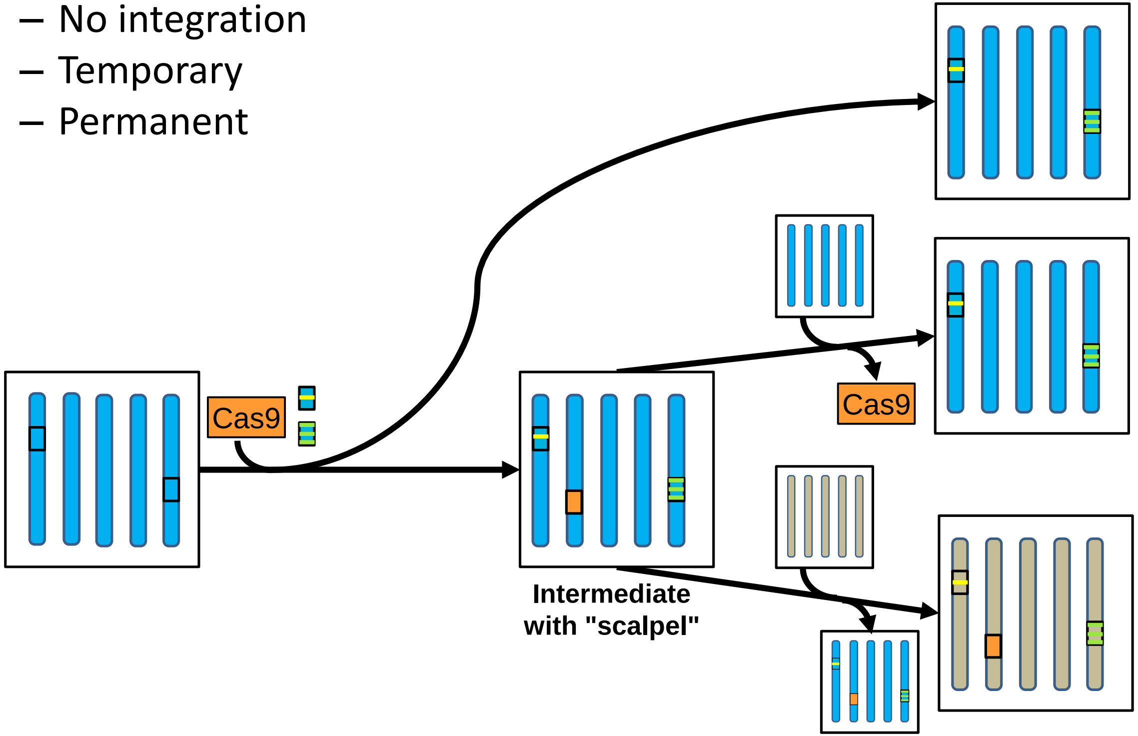


Appellation	Process	Product
non-transformed	no introduction of foreign DNA in the genome	mutant
nul segregant	intermediate with Cas9 in the genome	mutant

☞ Stable transformation and segregation of Cas9 is presently the most widely used strategy

Genome editing strategies

- Presence of Cas9 in the genome
  - No integration
  - Temporary
  - Permanent



Appellation	Process	Product
non-transformed	no introduction of foreign DNA in the genome	mutant
nul segregant	intermediate with Cas9 in the genome	mutant
gene drive	intermediate with Cas9 in the genome	Cas9 in the genome and mutant

☞ Gene drive is to be considered as a classical GMO

Genome editing and other breeding techniques

- Conventional breeding (phenotypic, marker assisted, genomic selection)
  - Average 1.6% annual yield increase in major crops over the last 20 years
  - Based on germplasm collections representing natural variation (1 mutation per 100 Mbp per generation)
- Mutation breeding (EMS, azide, irradiation)
  - Over 3500 varieties in the catalogs since 1930
  - Based on random enlargement of the gene pool
- Transgenesis (GMO)
  - Success for two traits (herbicide tolerance, insect resistance) in 4 species (maize, soybean, cotton, oilseed rape)
  - Based on targeted enlargement of the gene pool

What can be done with conventional breeding and does not need genome editing?

What can only be done with genome editing?

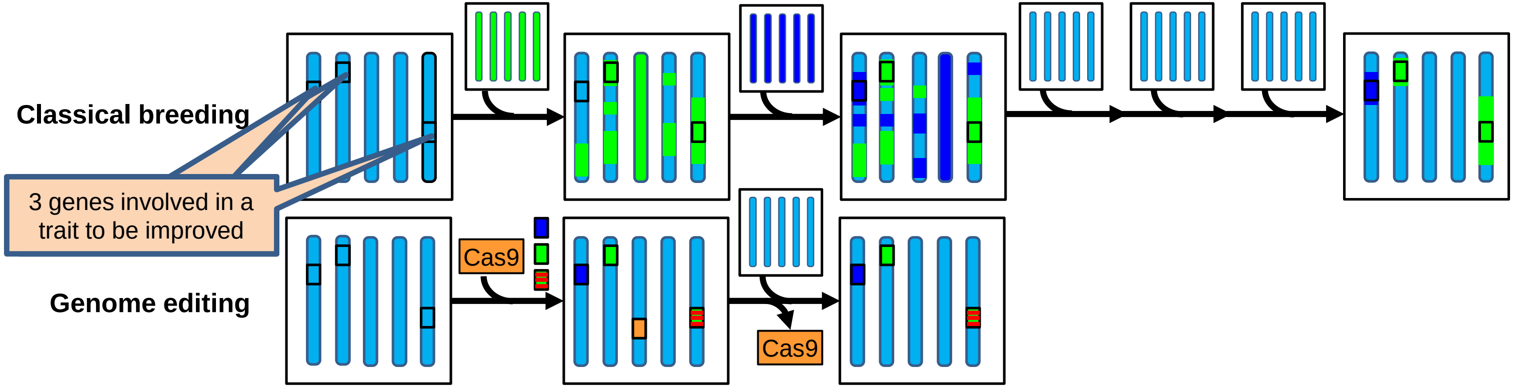
What cannot be done with genome editing

- Drought tolerance in maize
  - Aquamax (conventional)
  - DroughtGuard (GMO)
  - ARGOS8 (Genome editing)

☞ There is no strict link between trait and technique

Genome editing and classical breeding

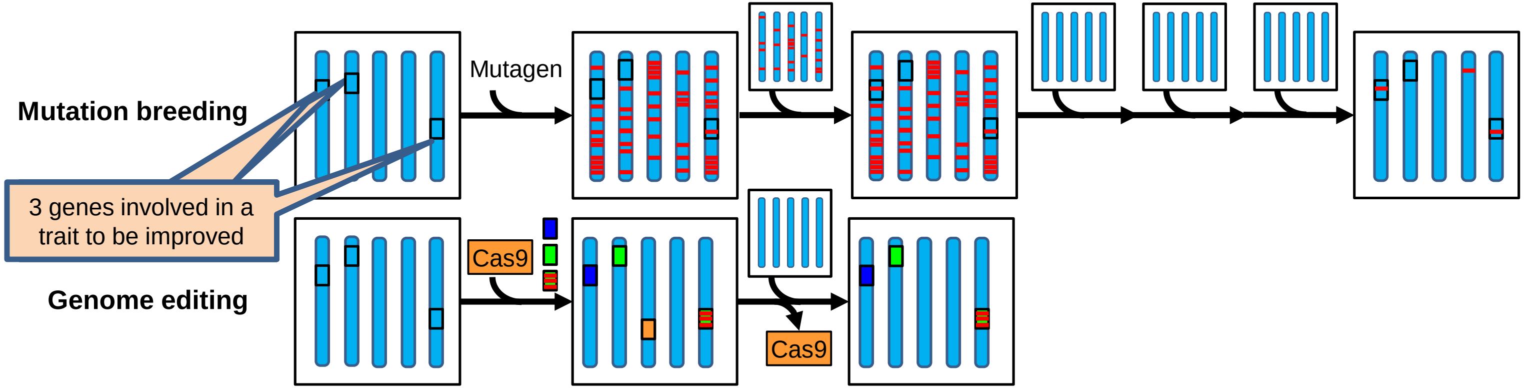
	Genome editing	Classical breeding
Interest	Trait improvement	Trait improvement
Precision	Precise modification of genes	Genetic drag of introgressed regions
Speed	1 to 2 generations	5 to 6 generations
Advantages	Enlargement of natural variation	Applicable to any species or variety
Limitations	Need for upfront knowledge Need for transformation system	Limited to natural variation of the species
Status in Europe	GMO	not GMO



☞ Genome editing enlarges the gene pool but requires knowledge

Genome editing and mutation breeding

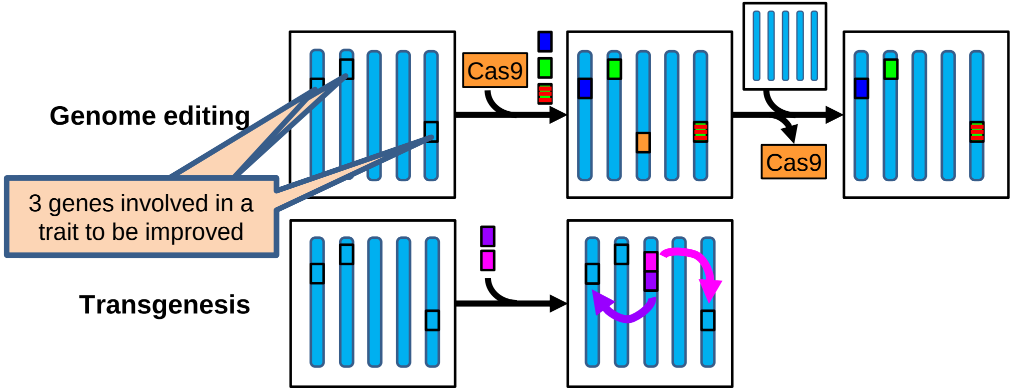
	Genome editing	Mutation breeding
Interest	Enlarged gene pool	Enlarged gene pool
Precision	Predetermined sites No unwanted sites (off target)	Random sites Approximately 300 sites per genome
Speed	1 to 2 generations	5 to 6 generations
Advantages	Multiple mutations in a gene	Applicable to any species or variety
Limitations	Need for transformation system	Only transitions and deletions, field space
Status in Europe	GMO	not GMO



☞ Genome editing is more rapid, precise and versatile than random mutagenesis

Genome editing and transgenesis

	Genome editing	Transgenesis
Interest	Enlarged gene pool	Enlarged gene pool
Precision	Predetermined sites	Random site
Speed	1 to 2 generations	1 generation
Advantages	No additional DNA in the genome	Introduction of novel genes Introduction of entire pathways
Limitations	Modification of existing genes	Co-existence with endogenous genes
Status in Europe	GMO	GMO



Limited modification of an existing DNA at a precise site of the genome

Insertion of an additional DNA at a random site of the genome

☞ Genome editing is more precise but also more limited than transgenesis

Genome editing: proof of concept

- Agronomic value
  - Yield (seed number, seed weight, inflorescence size, seed shattering,)
  - Growth characteristics (plant height, tillering, branching, flowering time, erect panicle, seedling size/vigour/speed, male sterility, NUE, fruit colour)
  - Storage characteristics (cold storage, improved shelf life)
- Food and feed quality
  - Altered fatty acid content, no amylose, high starch, less phytate, less black spots, less bitter taste, reduced heavy metal content, reduced gluten
- Biotic stress tolerance
  - Resistance to fungi, bacteria, viruses
- Herbicide tolerance
  - Basic research (selection for knockin events), applied research
- Industrial
  - reduced lignin content, oil composition
- Abiotic stress tolerance
  - Drought tolerance, salt tolerance, arsenic/cadmium/caesium tolerance

Review Article

Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture

Agnès Ricroch[1,2], Pauline Clairand[1] and Wendy Harwood[3]

SYSTEMATIC MAP — Open Access

What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map

Dominik Modrzejewski*, Frank Hartung, Thorben Sprink, Dörthe Krause, Christian Kohl and Ralf Wilhelm

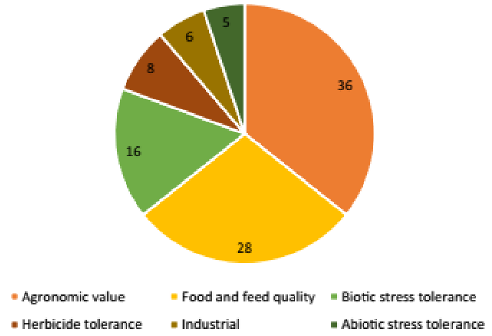


Fig. 6 Distribution of market-oriented applications of crops with nutritionally, agriculturally or industrially relevant traits (January 1996–May 2018)

☞ A large panel of traits can be improved by genome editing

Nutrition: proof of concept

- High oleic acid soybean
  - Commercialised by Calyxt
  - Loss-of-function of fatty acid desaturase2 (FAD2) catalysing oleic acid => linoleic acid
  - oleic acid (non essential omega 9) => beneficial effect on cancer, autoimmune and inflammatory diseases
  - Also in camelina, pennycress and peanut
- High GABA tomato
  - Commercialised by Sanatec
  - Loss of C-terminal autoinhibitory domain of glutamate decarboxylase (GAD), a key enzyme in GABA biosynthesis
  - γ-aminobutyric acid (GABA) => lower blood pressure

February 26, 2019

First Commercial Sale of Calyxt High Oleic Soybean Oil on the U.S. Market

Calyxt successfully markets Calyno™ High Oleic Soybean Oil as a premium, high-quality food ingredient

First commercial sale of High Oleic Soybean Meal as a premium non-GMO feed ingredient for livestock

Minneapolis-St. Paul, Minn. – February 26, 2019 – Calyxt, Inc. (NASDAQ: CLXT) a

Japan Launches World's First Genome-Edited Tomato

March 24, 2021

☞ The first two commercialised genome editing products concern nutrition

Yield: proof of concept

- Higher grain yield (rice)
  - Loss-of-function of amino acid transporter OsAAP3
  - More bud outgrowth, increased tillering,
  - Higher grain yield confirmed in field trials
- High grain yield (rice)
  - Gn1a (Os01g0197700) => increased grain number
  - DEP1 (Os09g0441900) => dense erect panicles
  - GS3 (Os03g0407400) => increased grain size
  - IPA1 (Os08g0509600) => increased tillering
  - All four effects on yield confirmed in field trials
- Larger ear diameter (maize)
  - Promoter editing of ZmCLE7 (signal peptide acting on meristem size)

Plant Biotechnology Journal
Plant Biotechnology Journal (2018) 16, pp. 1710–1722 doi: 10.1111/pbi.12907

Blocking amino acid transporter *OsAAP3* improves grain yield by promoting outgrowth buds and increasing tiller number in rice

Kai Lu[1,†], Bowen Wu[1,†], Jie Wang[1], Wei Zhu[1], Haipeng Nie[1,2], Junjie Qian[1,2], Weiting Huang[1] and Zhongming Fang[1,2,*]

frontiers in Plant Science
ORIGINAL RESEARCH
published: 30 March 2016
doi: 10.3389/fpls.2016.00377

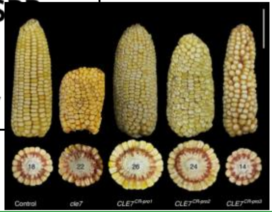
Reassessment of the Four Yield-related Genes *Gn1a*, *DEP1*, *GS3*, and *IPA1* in Rice Using a CRISPR/Cas9 System

Meiru Li[1,2†], Xiaoxia Li[3†], Zejiao Zhou[3], Pingzhi Wu[1,2], Maichun Fang[1,2], Xiaoping Pan[1,2], Qiupeng Lin[3], Wanbin Luo[3], Guojiang Wu[1,2*] and Hongqing Li[3*]

nature plants
LETTERS
https://doi.org/10.1038/s41477-021-00858-5

Enhancing grain-yield-related traits by CRISPR-Cas9 promoter editing of maize *CLE* genes

Lei Liu[1], Joseph Gallagher[2], Edgar Demesa Arevalo[1], Richelle Chen[1], Tara Skopelitis[1], Qingyu Wu[1,3], Madelaine Bartlett[2] and David Jackson[1]



☞ Several yield effects confirmed by field trials

Sustainability: proof of concept

- Yield stability under drought stress (maize)
  - Promoter editing (replacement) of ZmARGOS8 => reduced ethylene sensitivity => stress tolerance

Plant Biotechnology Journal
Plant Biotechnology Journal (2016), pp. 1–10 doi: 10.1111/pbi.12603

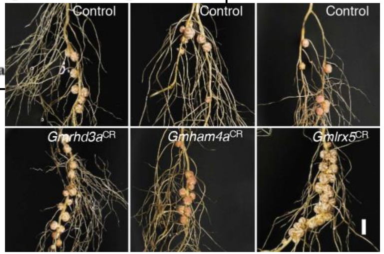
ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions

Jinrui Shi*, Huirong Gao, Hongyu Wang, H. Renee Lafitte, Rayeann L. Archibald, Meizhu Yang, Salim M. Hakimi, Hua Mo and Jeffrey E. Habben

- Higher root nodule number (soybean)
  - Loss-of-function of GmRHD3a/b or GmHAM4a or GmLRX5 (targets of rhizobial effectors) => increased nodule number

Rhizobial tRNA-derived small RNAs are signal molecules regulating plant nodulation

Bo Ren[1*], Xutong Wang[1*], Jingbo Duan[1], Jianxin Ma



- Tolerance to salinity (rice)
  - Loss-of-function of OsDRL1 or DRL2 (auxin responsive genes) => shallow root growth angle => enhanced yield in saline paddies

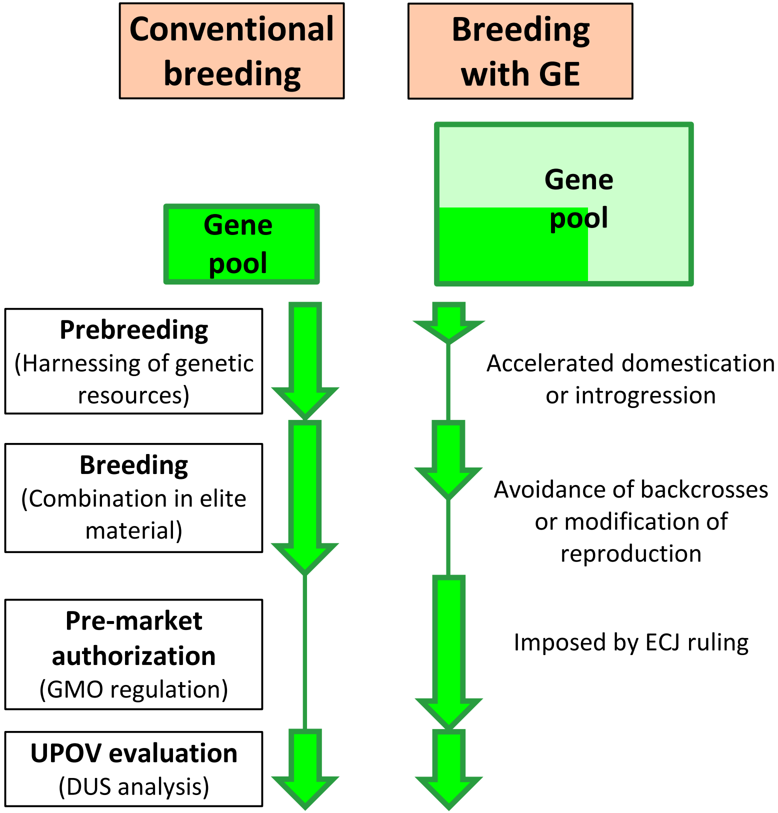
Root angle modifications by the *DRO1* homolog improve rice yields in saline paddy fields

Yuka Kitomi[a], Eiko Hanzawa[b], Noriyuki Kuya[a], Haruhiko Inoue[c,d], Naho Hara[c], Sawako Kawai[a,1], Noriko Kanno[a], Masaki Endo[c], Kazuhiko Sugimoto[a], Toshimasa Yamazaki[e], Shingo Sakamoto[f], Naoki Sentoku[c], Jianzhong Wu[a], Hitoshi Kanno[g], Nobutaka Mitsuda[f], Kinya Toriyama[g], Tadashi Sato[g,2,3], and Yusaku Uga[a,2,3]

☞ Complex sustainability traits can be addressed by genome editing

Genome editing: Limitations and promises

- Prerequisites
  - Mastery of cellular engineering
  - Upfront knowledge (gene function, favorable SNP . . .)
    - Which gene(s) to modify to obtain a trait of interest?
    - Which modification(s)?
- Promises
  - Powerful tool for basic research
  - Production of plants with new characteristics
    - Novel alleles by knowledge based enlargement of the gene pool
    - Accelerated domestication
    - Shorter breeding times



☞ Genome editing requires knowledge and knowhow, enlarges the gene pool

Thank you for your attention