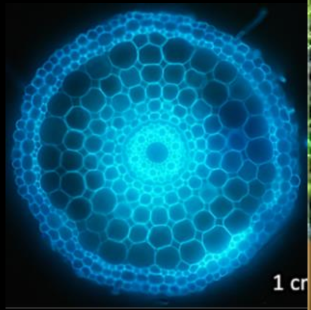
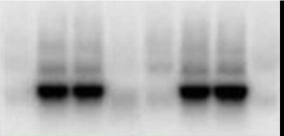
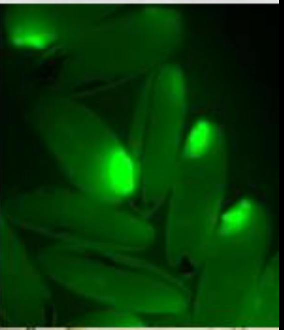
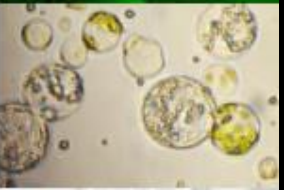
cirad
AGRICULTURAL RESEARCH FOR DEVELOPMENT

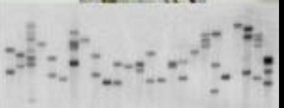


agAP
INSTITUTE
Genetic Improvement and Adaptation of Mediterranean and Tropical Plants

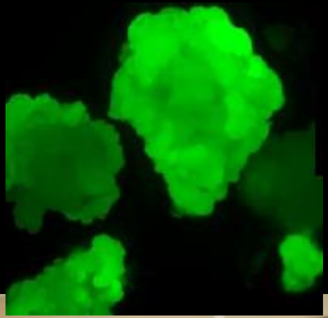


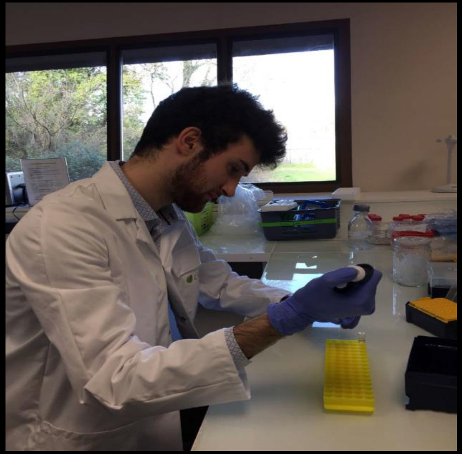






CIRAD
Genome Editing platform
Overview





Anne-Cécile Meunier. AFEG Platform. CIRAD Montpellier

27/09/2023 at University of the Philippines LOS BAÑOS

CIRAD Genome Editing platform overview

- AGAP - CIRAD Unit in few words
- AFEG and InCell platforms expertise and links
- Actual projects involving AFEG/InCell staff
- AGAP plant species / teams interested in genome editing / for future collaboration?

- Genome Editing Tools available on AFEG platform
- GE related technologies / know-how

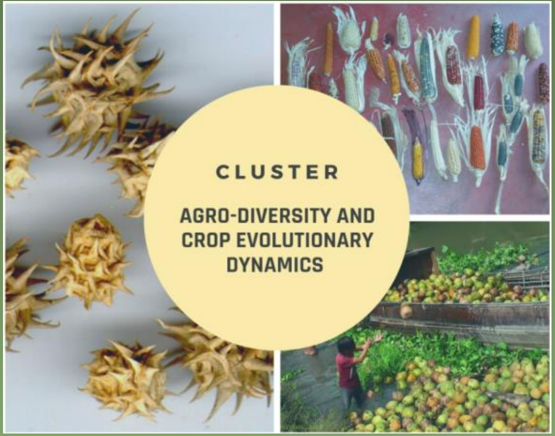
AGAP Research Unit : Genetic Improvement and Adaptation of Mediterranean and tropical plants

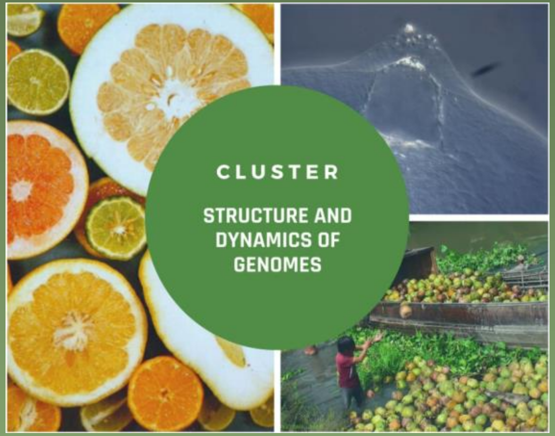
- More than 300 employees grouped in 13 research teams

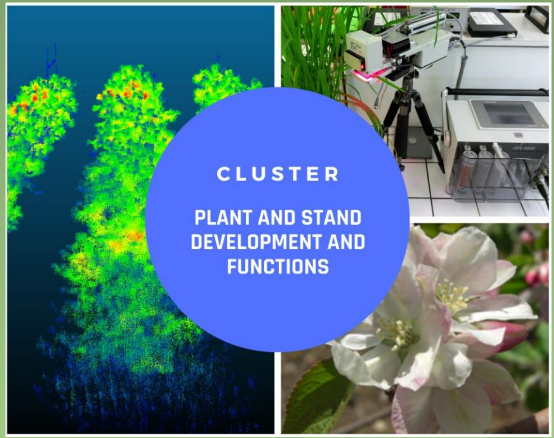
- 11 technical platforms and 3 Biological Ressources Centres

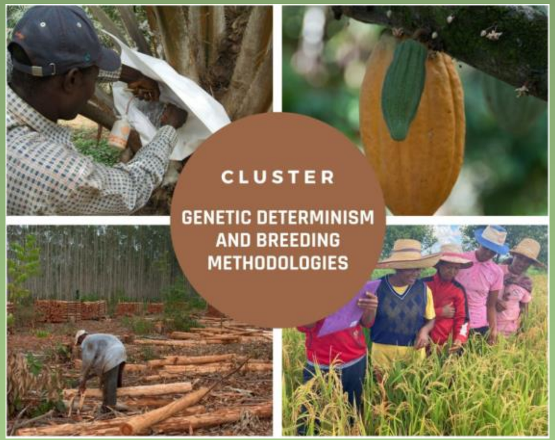
- Functional Analysis and Genome Editing platform
- Plant cultivation platform
- Molecular cytogenetic platform
- Bioinformatics platform
- Molecular biology platform (Guadeloupe)
- Seed characterization platform
- Technical platform of Roujol (Guadeloupe)
- Cryopreservation platform
- Ecophysiology platform
- Robotic sequencing genotyping platform
- Cell engineering platform
- Mycology platform
- Biochemical phenotyping platform

- 4 thematic clusters









AGAP Research Unit : Genetic Improvement and Adaptation of Mediterranean and tropical plants

agAP INSTITUTE
Genetic Improvement and Adaptation of Mediterranean and Tropical Plants

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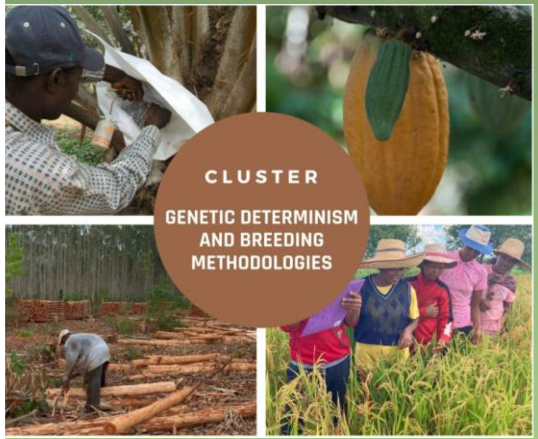
- **Mycology platform**
- **Biochemical phenotyping platform**

*Identify key traits and processes involved in
> **development and in adaptation** to the environment

*Elucidate the key mechanisms involved in the
> elaboration of **traits of interest** and
> their Genetic and Environmental variation

***Compare** mechanisms between species



AFEG and InCell platform expertise and links

AFEG
ANALYSE FONCTIONNELLE
& ÉDITION DES GÉNOMES

AFEG Platform

Classical molecular biology platform / Genome Editing Expertise

Provides plant scientists with the environment and tools to conduct studies to elucidate gene function.

Expertise fields : Tropical and Mediterranean species , Genome Editing tools

Resp. Anne-Cécile Meunier

- Vector cloning
- Bacteriology

- Molecular characterization of produced plants

- DNA /RNA /Protein working tools and methods

Close functional links AFEG and InCell platforms

InCell

InCell Platform

In Vitro Culture Platform

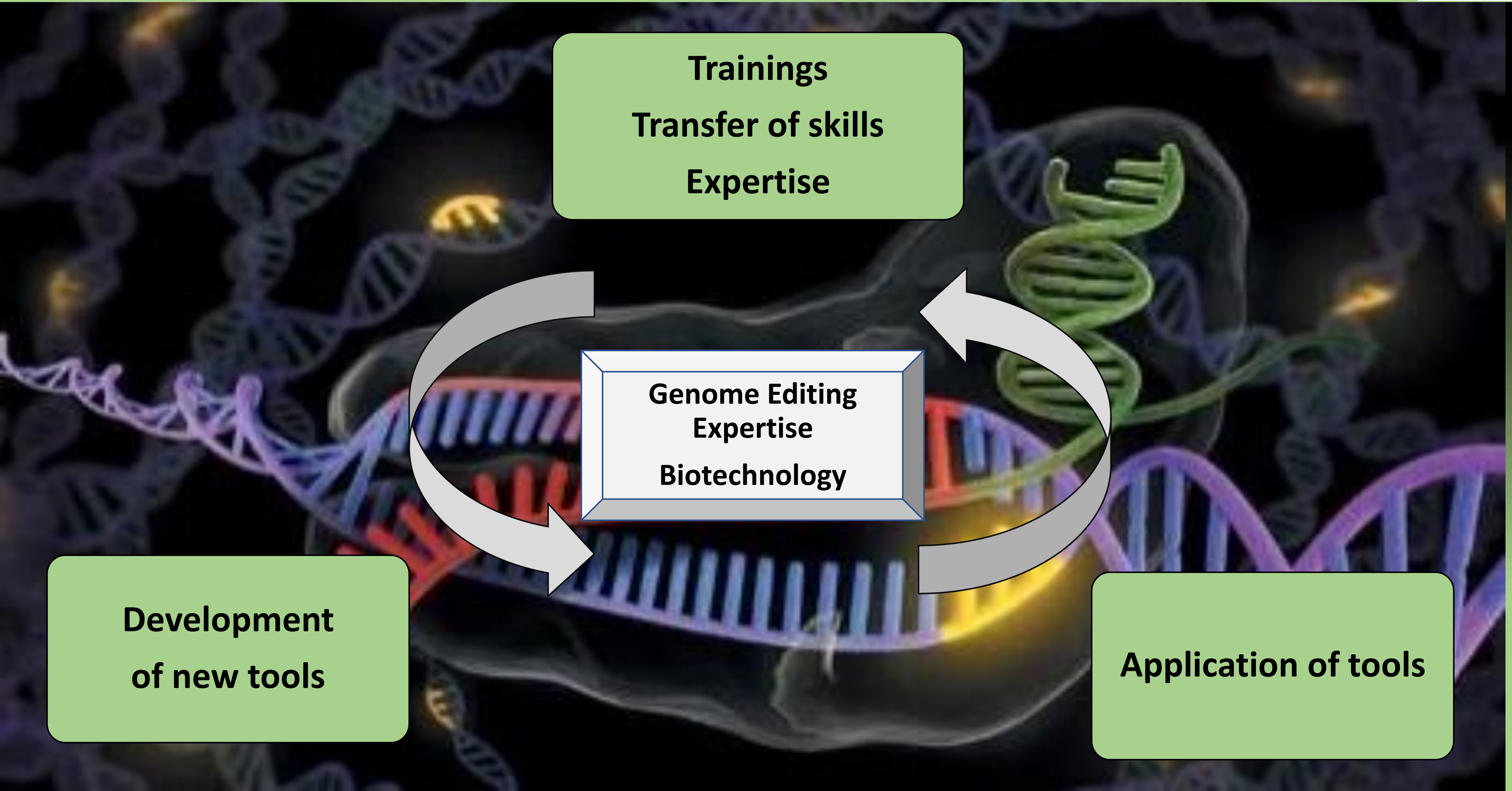
Masters of different cell engineering approaches applied to the improvement of tropical and Mediterranean species.

- Plant genetic transformation

- Culture In Vitro (Multiplication, cryoconservation, somatic hybridization)

Resp. Aurore Vernet

Scientific missions of the AFEG staff:



Trainings

Transfer of skills

Expertise

Genome Editing Expertise

Biotechnology

Development of new tools

Application of tools

ACTUAL PROJECTS INVOLVING AFEG/INCELL STAFF:

Sometimes only an expertise or training participation

<u>> External sollicitation</u>

Trainings

Transfer of skills

Expertise

UPLB Philipinnes

AFEG platform

<u>Training and expertise</u>

FEXTE project : Technical assistance to strengthen R&D for more climate-resilient agriculture in the Philippines

AFEG platform

<u>expertise</u>

NORCE Consortium (Norvegian Research center)

DARWIN : Development of new generation of innovative and reliable DNA-based analytical detection <u>methods for NGT detection in food</u>

Development of new tools

Application of tools

ACTUAL PROJECTS INVOLVING AFEG/INCELL STAFF:

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Trainings

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Most of the time, the 3 aspects are requested from AFEG/InCell Staff for Gene Functional Analysis projects:

- Study of genes involved in development and adaptation/response to abiotic stresses
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> Working with AGAP Research teams

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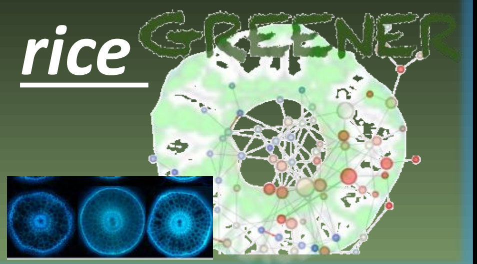
<u>> Working with AGAP Research teams</u>

French National project (ANR)

AFEG collab with DARS research team (Development and Adaptation of Rice and Sorghum)

Identifying major genes and networks involved in root tissue development and drought tolerance.

<u>rice</u>



UESC, Embrapa, UFV Brazil

AFEG collab with SEAPAG research team (Citrus evolution, polyploidie and breeding)

Genome Editing Optimisation for <u>citrus</u>

CAPES-COFECUB

French National project (PLANT ALLIANCE)

EDENNES Project

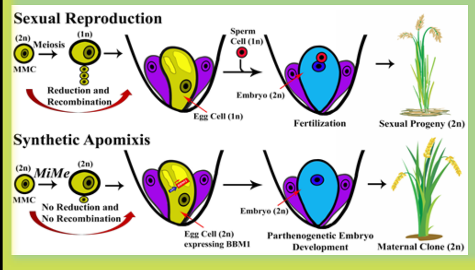
AFEG collab with DAAV research team (Diversity, Adaptation and Breeding of Grapevine)

Precise edition of the <u>grapevine</u>

Development of innovative processing methods for transformation, regeneration

French National project (PEPR)

DIVEDIT Project



Promoting AgrobioDIVersity through genome EDITing

<u>rice</u>

AFEG collab with GIV research team (Genetic and Variety Innovation)

ACTUAL PROJECTS INVOLVING AFEG/INCELL STAFF:

Sometimes only an expertise or training participation

> External sollicitation

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> Working with AGAP Research teams

Development of new tools

AFEG platform is also interested in developping innovative genome editing tools, to answer internal or partners needs

> Methodological projects

Application of tools

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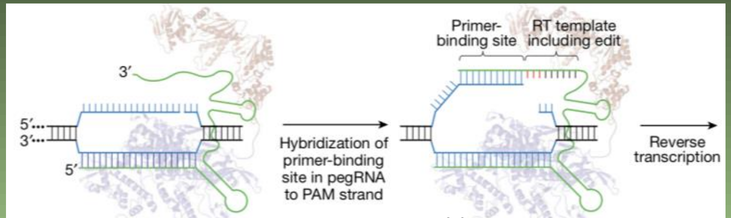
French National project (PEPR)

TYPEX
TOOLS FOR INNOVATION



*** Anzalone et al. 2019*

Toward highlY Predictable Editing of the plant genome leXicon.

Prime Editing optimisation

Rice *(as a model)*

AFEG collab with DARS research team (Development and Adaptation of Rice and Sorghum)

Development of new tools

AFEG platform is also interested in developping innovative genome editing tools, to answer internal or partners needs

> Methodological projects

Application of tools

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AGAP PLANT SPECIES / TEAMS INTERESTED IN GENOME EDITING:

Sometimes only an expertise or training participation

> External sollicitation

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> Working with AGAP Research teams

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> Methodological projects

Summary of PLANTS that AFEG/InCell platform are working with in Genome Editing Project :

RICE (DARS team, GIV collab)
GRAPEVINE (DAAV collab)
CITRUS (SEAPAG collab)

Other teams are interested in Genome Editing project in AGAP unit but have no project or work without AFEG/InCell Staff:

BANANA (GABA research team. Banana Genetics and Breeding)
RUBBER TREE (GSP research team. Genome and Selection of Perennials)
CACAO (GSP research team. Genome and Selection of Perennials)
SORGHUM (DARS research team. Development and Adaptation of Rice and Sorghum)
COFFEE (Coffee Adapt (DIADE CIRAD Unit))
PALM OIL (F2F (DIADE CIRAD Unit))

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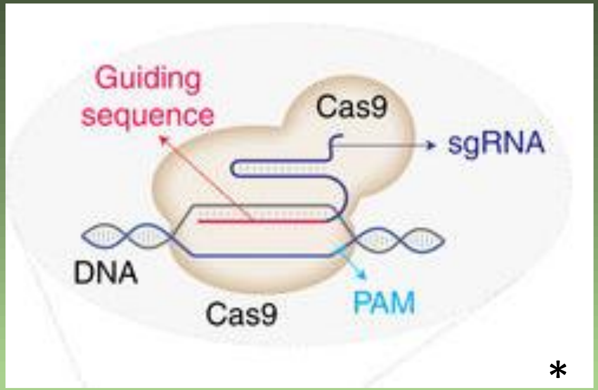
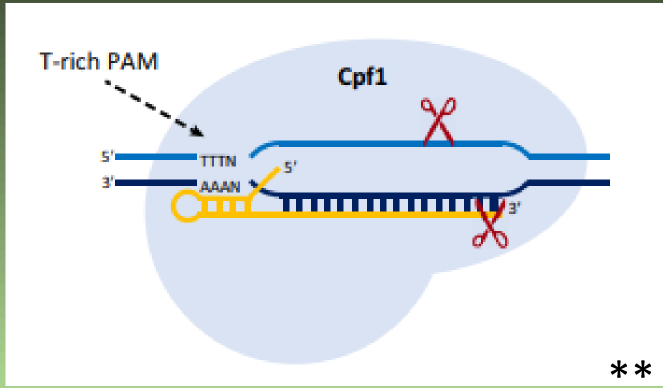
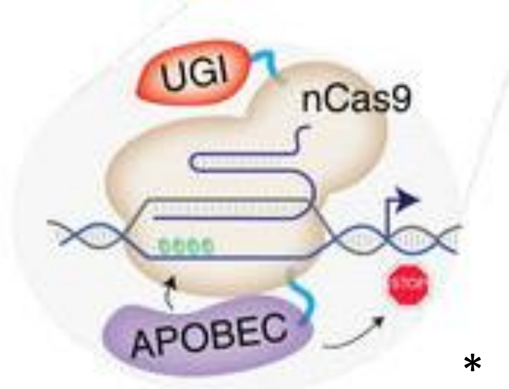
TOOLS AVAILABLE ON AFEG PLATFORM



CRISPR/Cas9



CRISPR/Cpf1



Base Editing

** Modified from Mazhar Adli,* 2018

*** Modified from Zaidi et al,* 2017

Examples of application:

- **Study of genes involved in development and adaptation/response to abiotic stresses**
- **Study of genes involved in the response to biotic stresses (pathogenic)**
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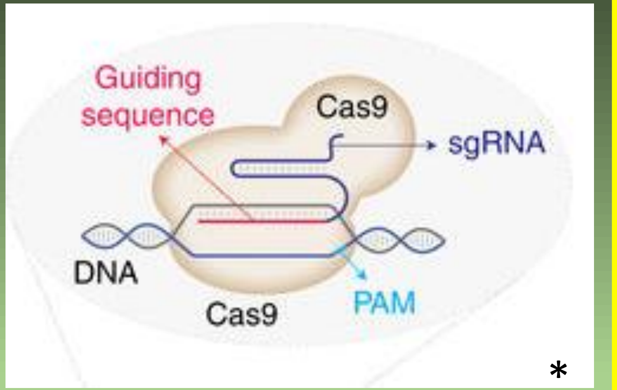
AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

TOOLS AVAILABLE ON AFEG PLATFORM



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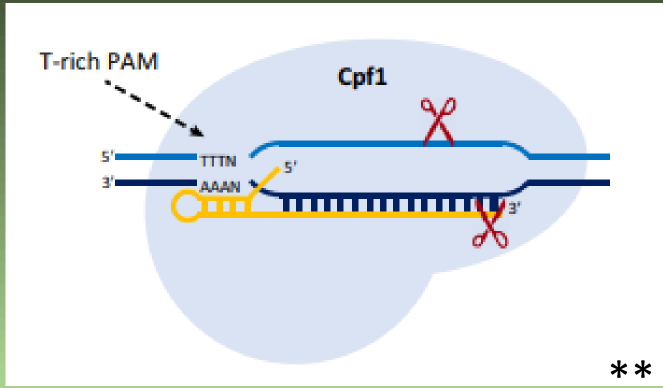

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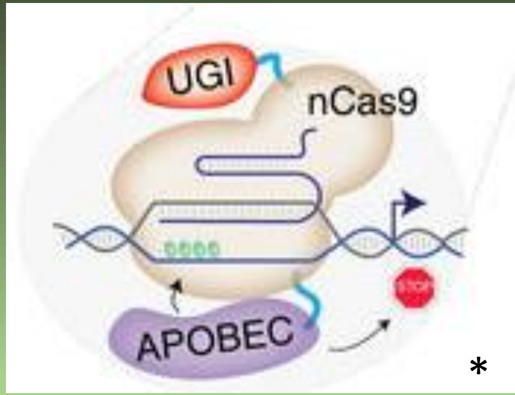

Base Editing

Gene KnockOut

** Modified from Mazhar Adli*, 2018

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Examples of application:

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AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9

CRISPR/CAS9 advantages:

- **Very Effective**

Assessment of the roles of SPO11-2 and SPO11-4 in meiosis in rice using CRISPR/Cas9 mutagenesis

Fayos et al, 2020

Table 1. Summary of sequence analysis at sites targeted by CRISPR/Cas9 mutagenesis in primary transformants

sgRNA	Number of edited plants	Number of unedited plants	Mutation frequency	Edited plants with a single nucleotide change	Single nucleotide change frequency
SPO11-1_ATG	9	3	75%	7	78%
SPO11-1_CDS	3	0	100%	0	0%
SPO11-2_ATG	15	4	79%	7	47%
SPO11-2_CDS	18	2	90%	10	56%
SPO11-4_ATG	28	5	85%	12	43%
SPO11-4_CDS	7	6	54%	2	29%
Total	80	20	80%	38	48%

TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9

CRISPR/CAS9 advantages:

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- **Precise**

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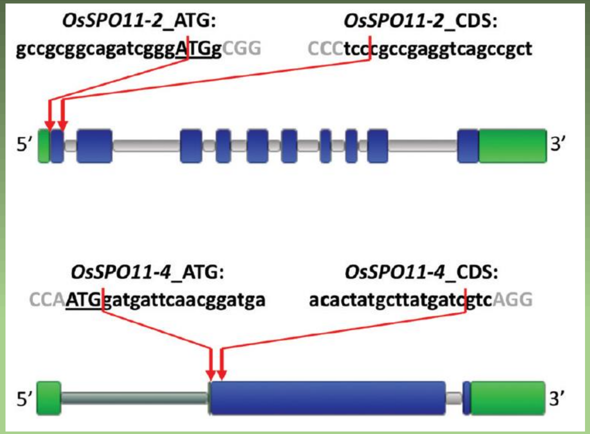

Meiogenix tuning genomes

AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9

CRISPR/CAS9 advantages:

- **Very Effective**
- **Precise**
- **Allelic series:**

(confirmation of phenotypes)

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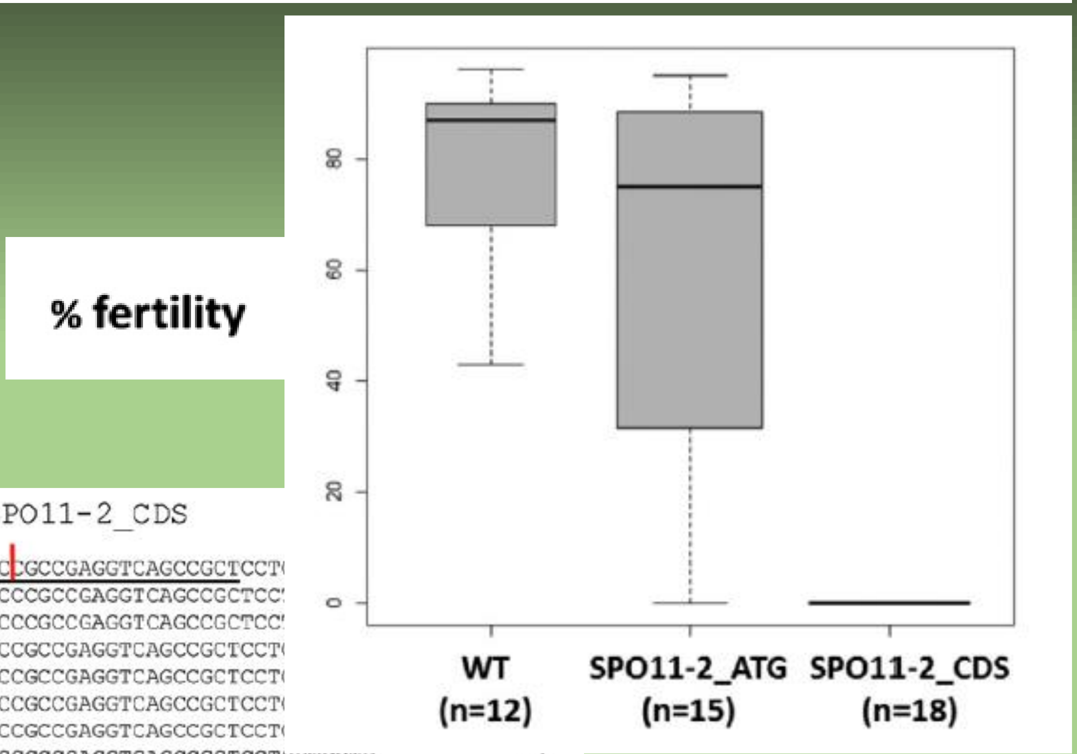
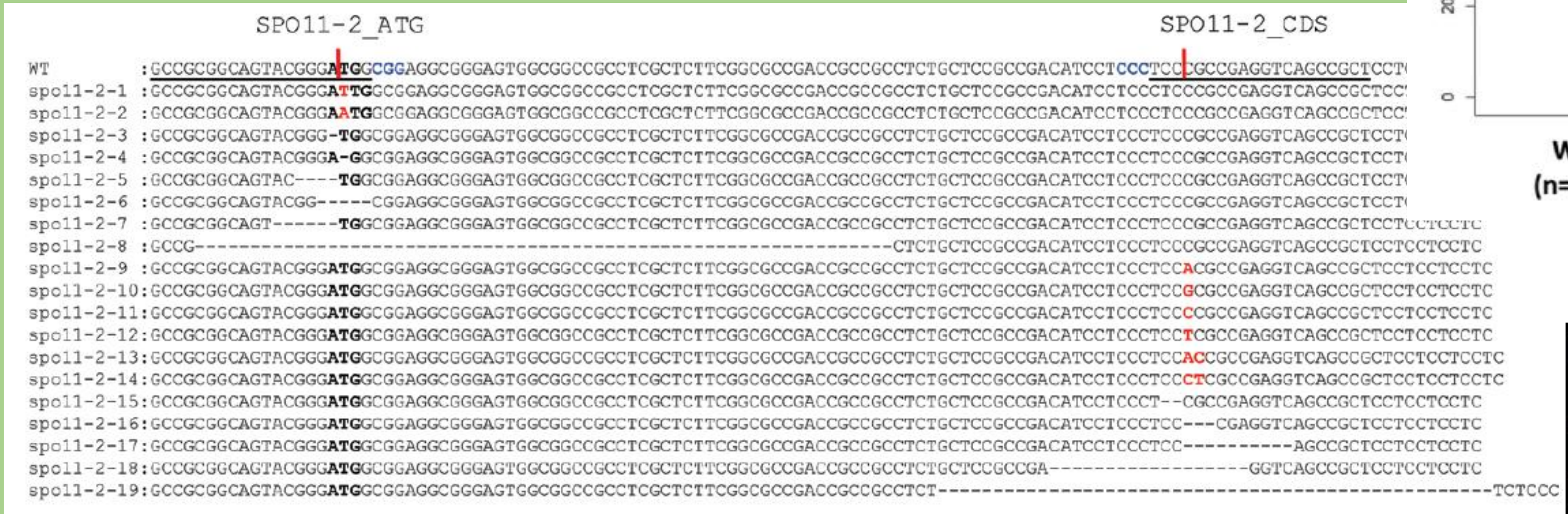
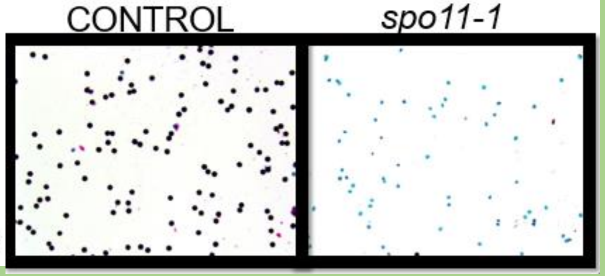




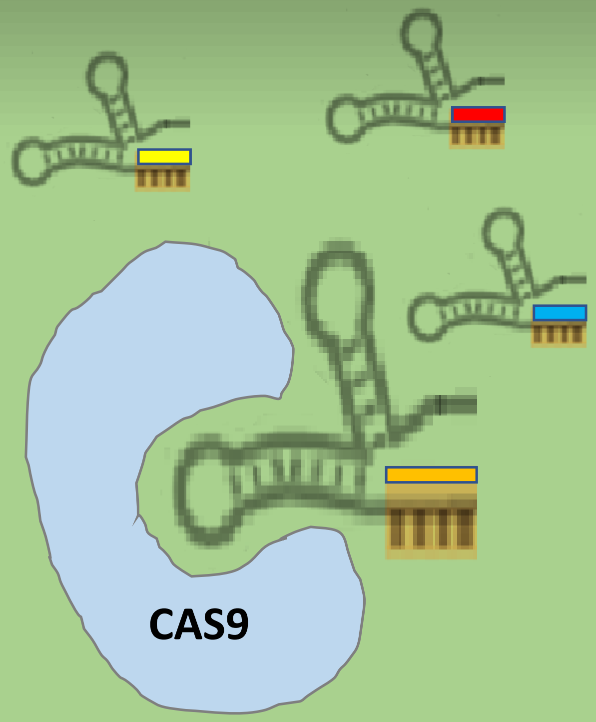
Pollen viability



TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9 - multiplexing

CRISPR/CAS9 advantages:

➢ **<u>Multiplexing possibilities (sequence deletion, multi-locus targetting)</u>**



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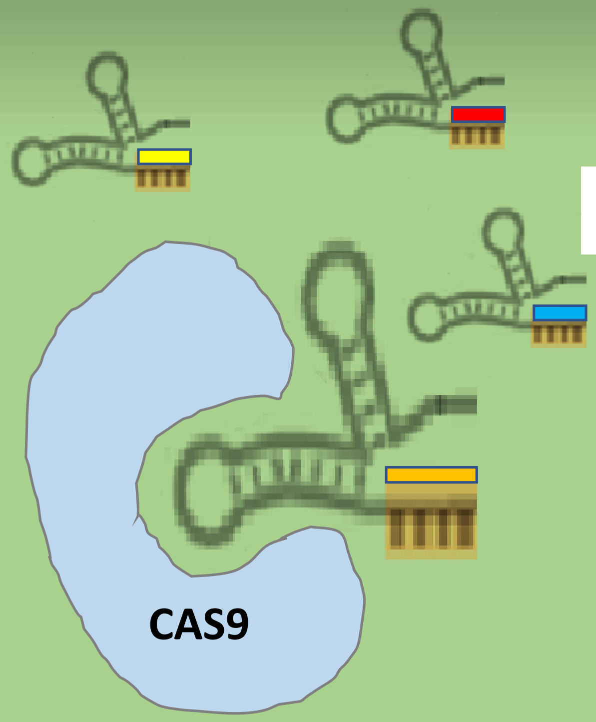
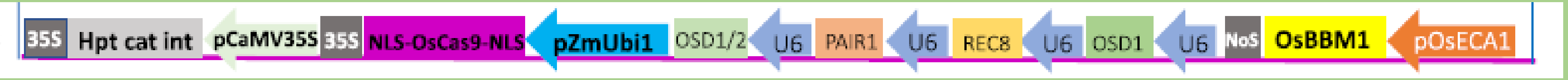


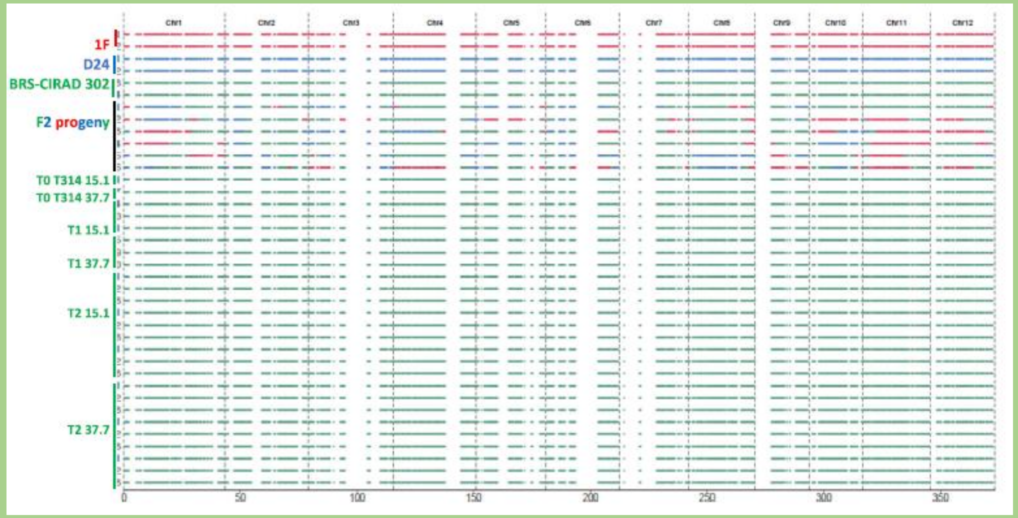
High-frequency synthetic apomixis in hybrid rice

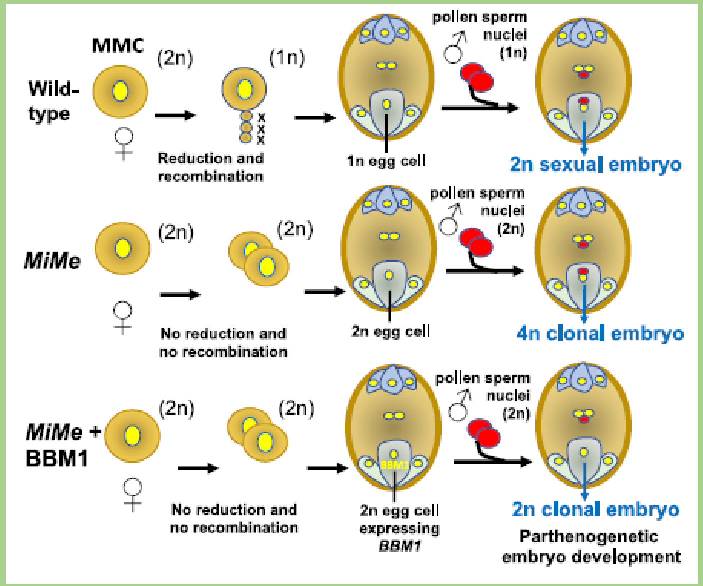
Vernet et al, 2022



In this study : Multiplexing of 4 guides + surexpression of a gene :

Apomictic rice in a Elite line (= no meiotic recombination)





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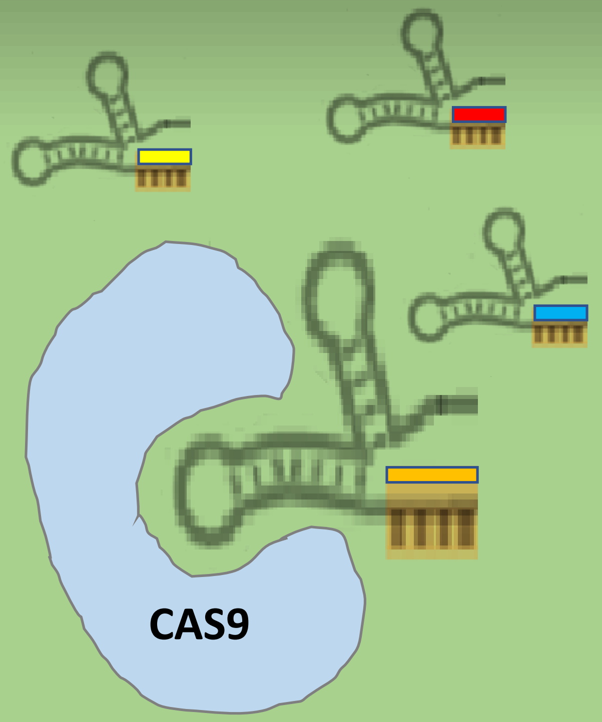

Genius

2012-2017

- **Study of genes involved in development and adaptation/response to abiotic stresses**
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TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9 - multiplexing

CRISPR/CAS9
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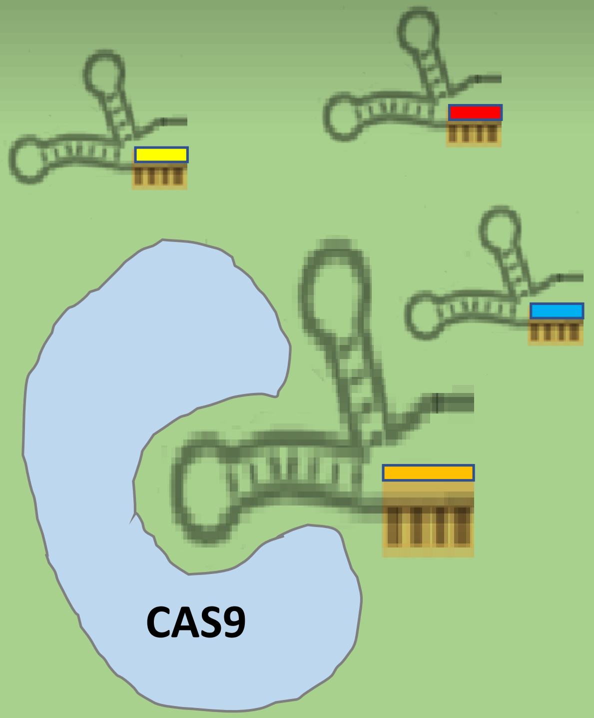
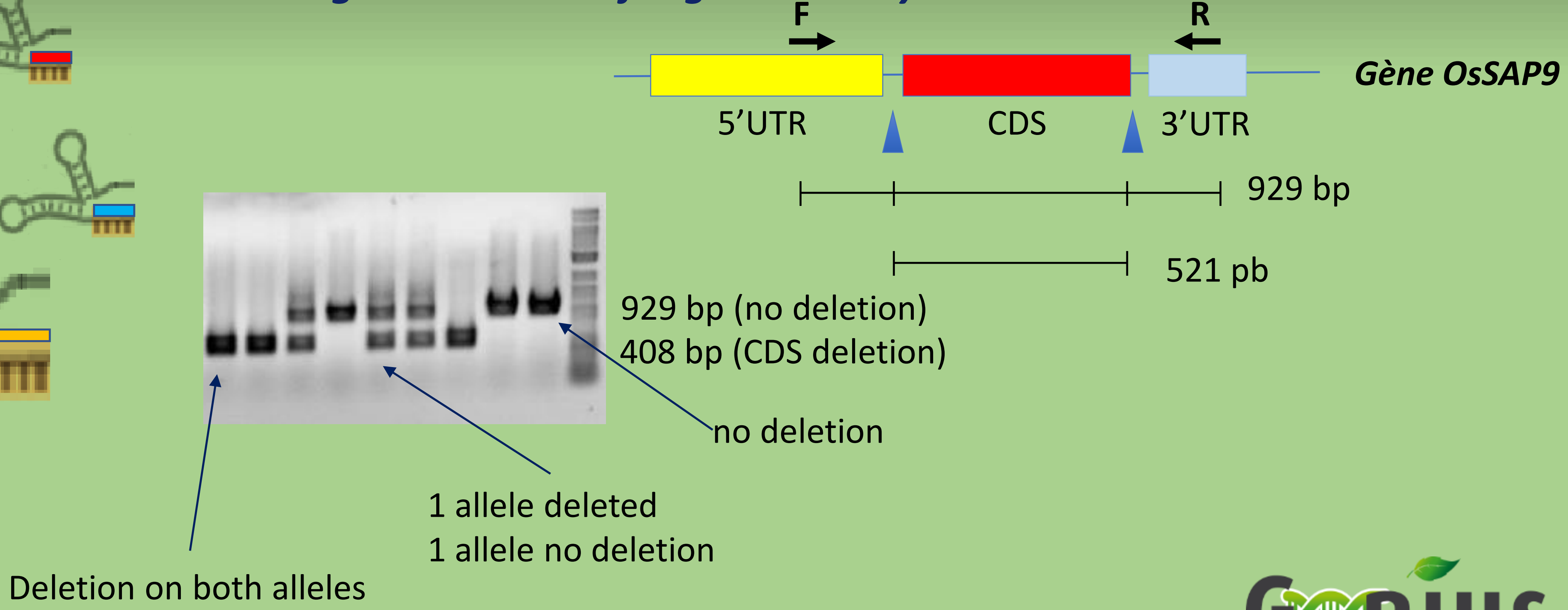


OsSAP9 gene deletion of regions one by one



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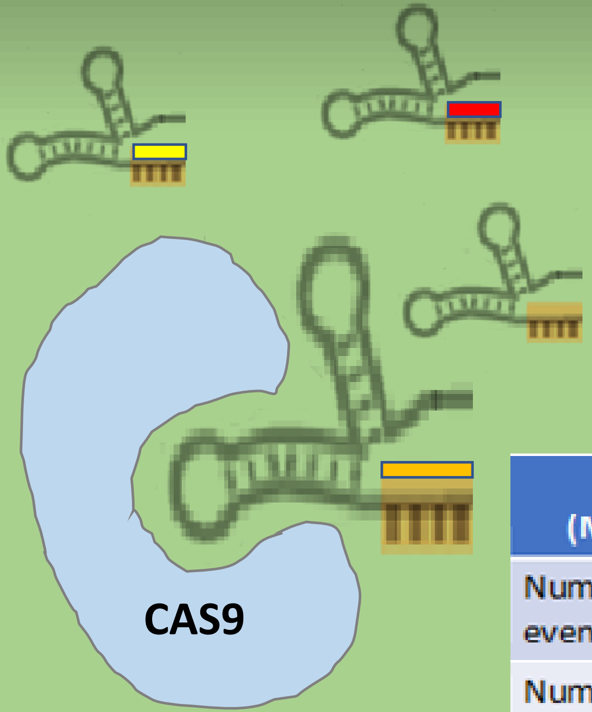
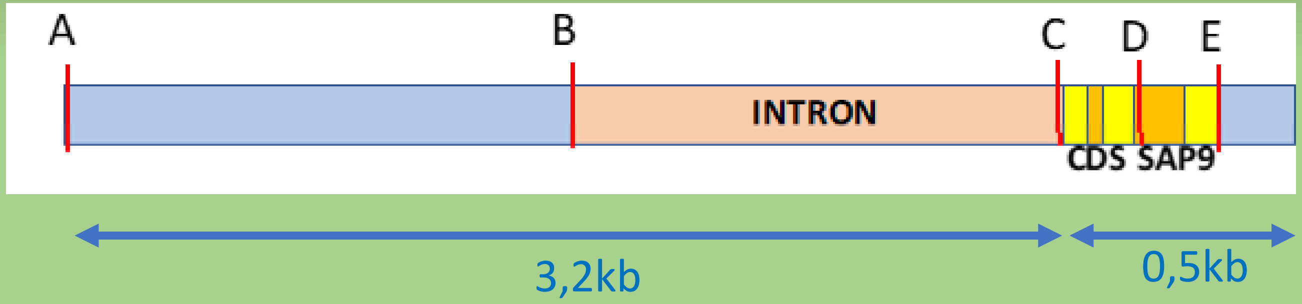


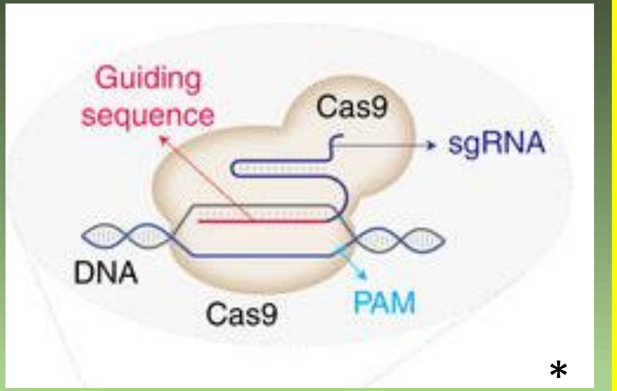


Target region (Multiplex Construct)	DE (T117)	CE (T135)	AB (T186)	BE (T125)	AC (T116)	AE (T175)
Number of independent events with successful PCR	41	24	69	96	39	45
Number of events with at least one deleted allele (sequence ascertained)	16	5	3	17	3	1
% deletion	39%	21%	4%	18%	8%	2%

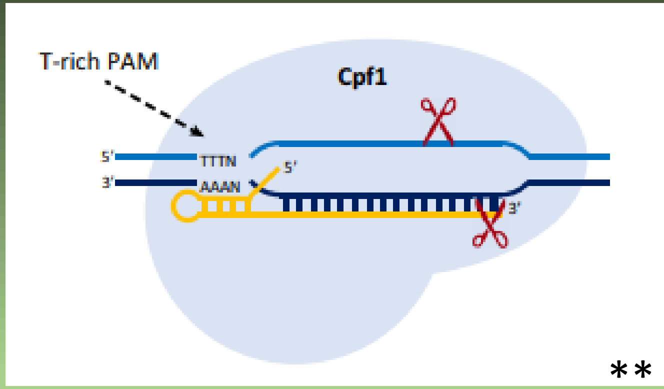
- Gets harder when region size increase

Genius

TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9



CRISPR/Cas9



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** Modified from Mazhar Adli, 2018*

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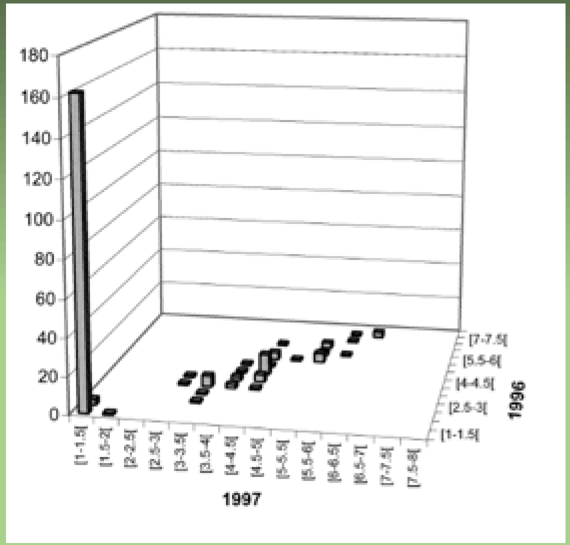
AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

TOOLS AVAILABLE ON AFEG PLATFORM : CRISPR/Cas9

Brown rust
Puccinia melanocephala



700 progeny of R570 (selfed) evaluated:

a major resistance gene

Daugrois et al, 1996, TAG;
Grivet et al, 1996, Genetic.
Asnaghi et al 2004, TAG

- ***Cultivar R570 bears a major gene (Bru1) that provides resistance to brown rust (1996)***
- ***Bru1 confers durable resistance to brown rust and explains resistance in many cultivars worldwide***

Research of rust resistance genes by CRISPR/Cas mutagenesis in a resistance QTL

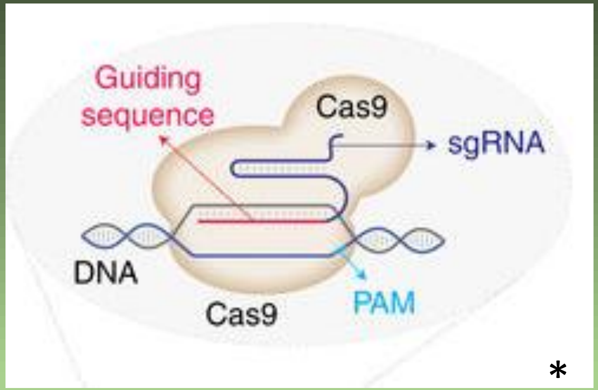
<u>sugar cane</u>

Collab :
Structure and Evolution of Genomes team (AGAP)

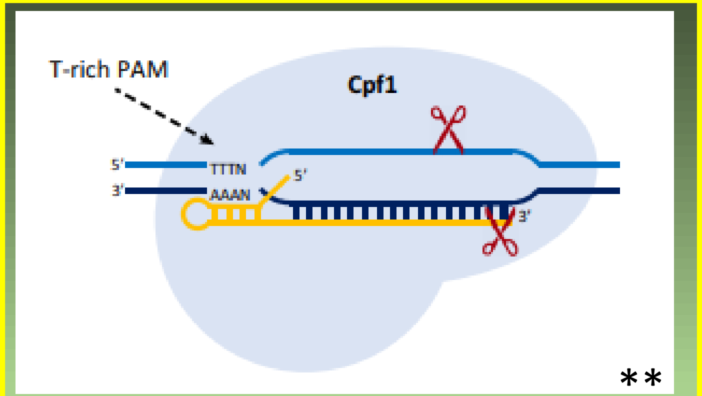
CHACRA EXPERIMENTAL AGRÍCOLA SANTA ROSA **(Argentina)**

AFEG platform
(CRISPR design and construct only)

TOOLS AVAILABLE ON AFEG PLATFORM

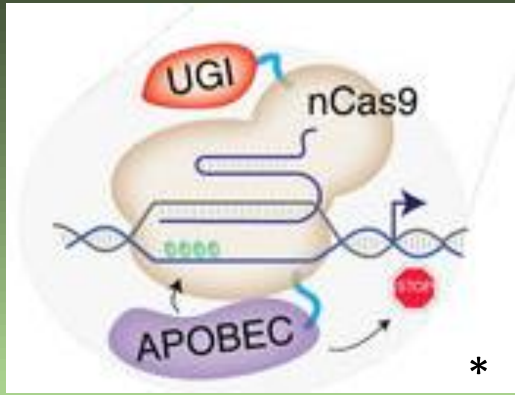


CRISPR/Cas9



CRISPR/Cpf1

Cas12a



Base Editing

** Modified from Mazhar Adli, 2018*

*** Modified from Zaidi et al, 2017*

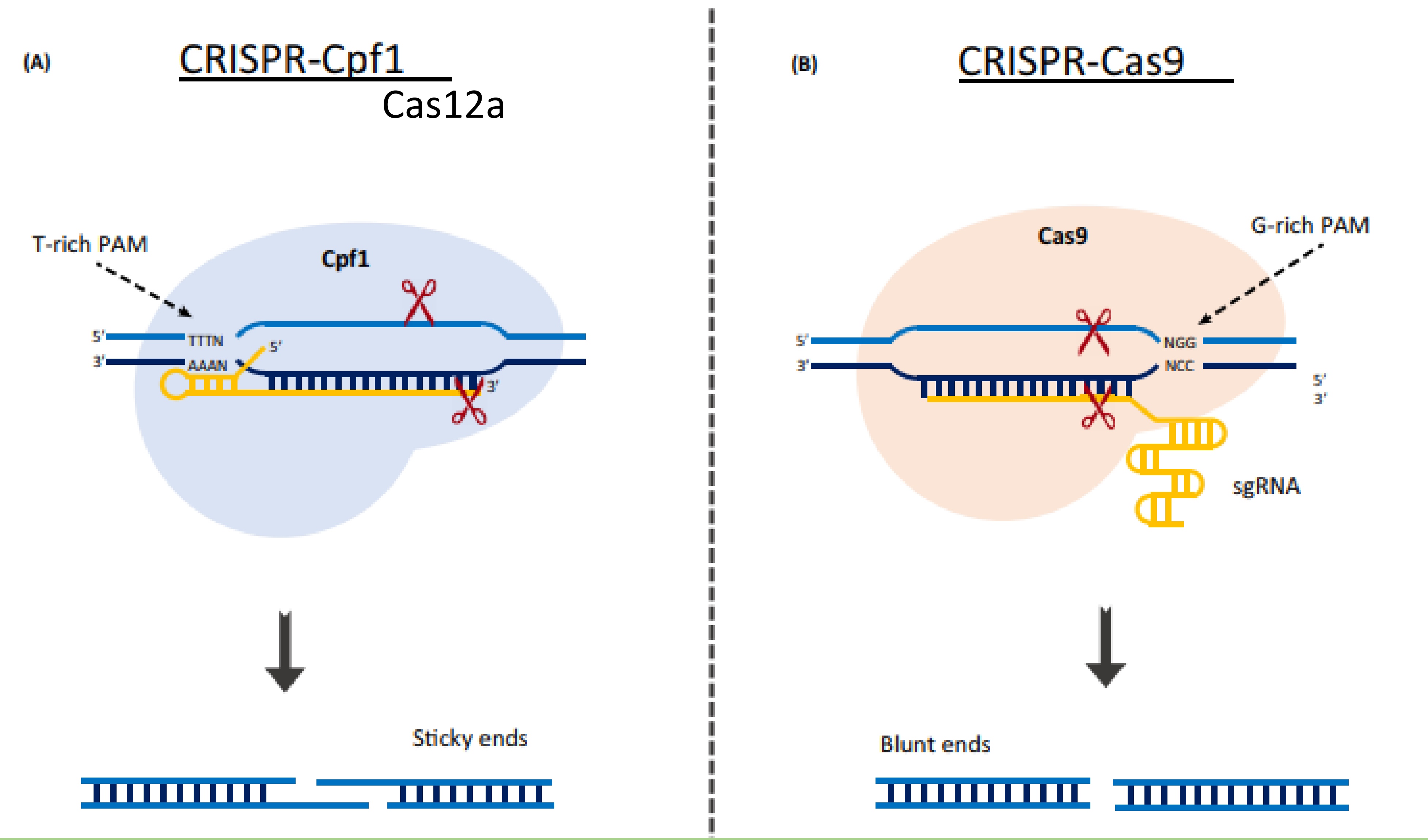
Examples of application:

- **Study of genes involved in development and adaptation/response to abiotic stresses**
- **Study of genes involved in the response to biotic stresses (pathogenic)**
- **Study of genes involved in meiotic recombination (genetic diversity)**
- **Methodological projects**

Genius

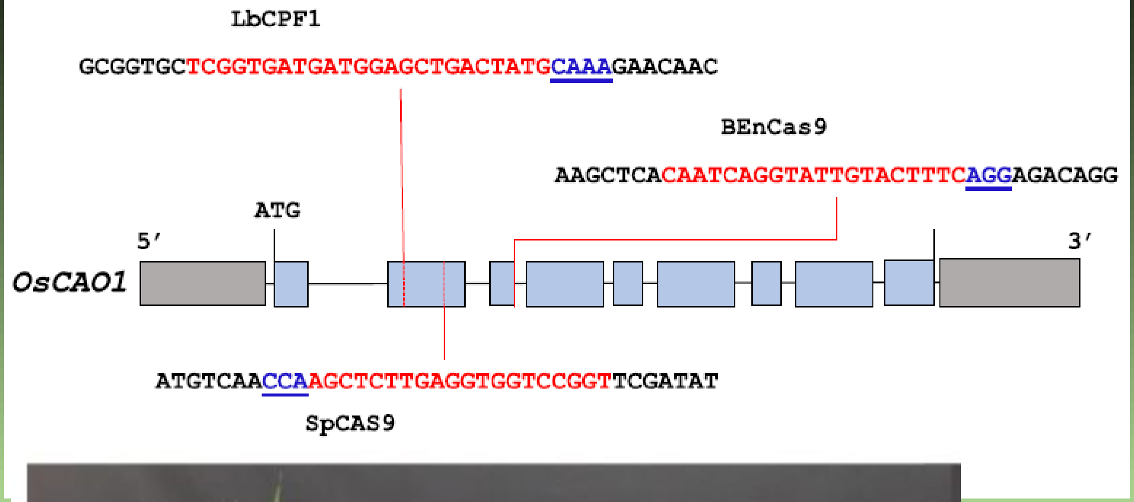
AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

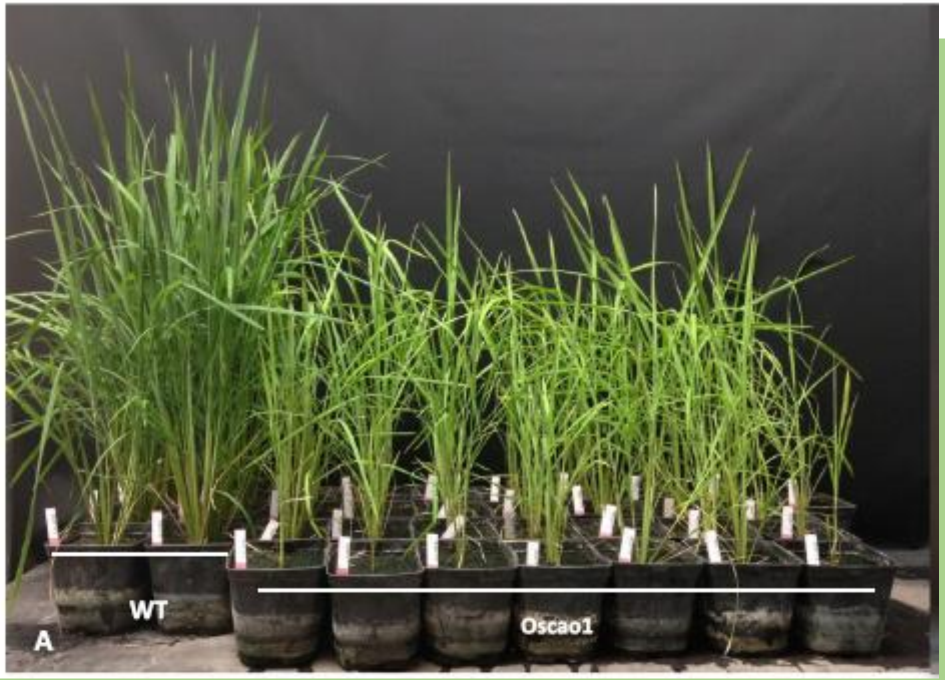
TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1

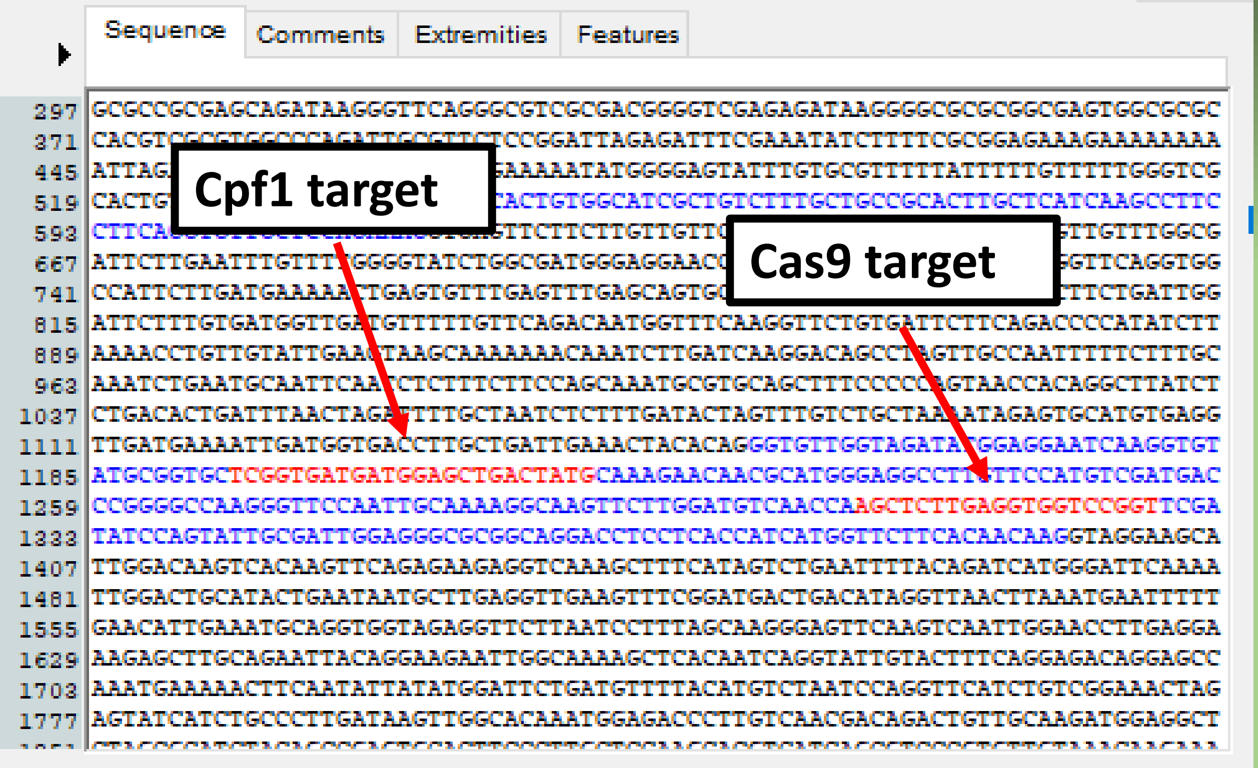


Zaidi et al, 2017

TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1







Herbert et al, 2020

TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1

Table 1 Homozygous, heterozygous, biallelic and wild-type plants produced using SpCAS9, LbCPF1 and BE_nCAS9D10A expressed as % of analyzed regenerated plants

	Homozygous	Biallelic	Heterozygous	Wild type
SpCAS9 (*n* = 35)	37.1% (13)	45.7% (16)	11.4% (4)	5.7% (2)
LbCPF1 (*n* = 25)	12% (3)	40% (10)	16% (4)	28% (7)

Herbert et al, 2020

Cas9: Better cut-off efficiency than Cpf1

- More mutants
- More often on 2 alleles

TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1

Obtained with CAS9

```
ATATCGAACCGGACCACCTCAAGAGCTTGGTTGACATCCAAGAA WT     [x4]
ATATCGAACCGGACCACCTCAAGnAGCTTGGTTGACATCCAAGAA  +1   [x28] (16A,5G,4T,3C)
ATATCGAACCGGACCACCTCAAG--CTTGGTTGACATCCAAGAA   -2   [x4]
ATATCGAACCGGACCACCTCA-GAGCTTGGTTGACATCCAAGAA   -1   [x3]
ATATCGAACCGGACCACCTCAA---CTTGGTTGACATCCAAGAA   -3   [x2] (homo)
ATATCGAACCGGACCACCTCAAGA-------------------A   -19  [x2] (homo)
ATATCGAACCGGACCACCTCAAGAcgaac//ttatcGCTTGGTT   +415 [x2] (homo)
ATATCGAACCGGACCACCTCAAGAcgcttggtgtcACATCCAAGAA +2 (-9,+11)
ATATCGAACCGGACCACCTCAgcgagGCTTGGTTGACATCCAAGAA +2 (-3,+5)
ATATCGAACCGGACCACCTCAAagaAGCTTGGTTGACATCCAAGAA +2 (-1,+3)
ATATCGAACCGGACCACCTCAAGAGCTTGGcTTGACATCCAAGAA  +1
ATATCGAACCGGACCACCTCAgagcGCTTGGTTGACATCCAAGAA  +1 (-3,+4)
ATATCGAACCGGACCctccCAAGAGCTTGGTTGACATCCAAGAA   +0 (-4+4)
ATATCGAACCGGACCACCTCAAGA-CTTGGTTGACATCCAAGAA   -1
ATATCGAACCGGACCACCTCAAGAGCTTGGTT-ACATCCAAGAA   -1
ATATCGAACCGGACCACCTCAAGAGa--GGTTGACATCCAAGAA   -2 (-3+1)
ATATCGAACCGGACCACCTCAAGAG---GGTTGACATCCAAGAA   -3
ATATCGAACCGGACCACCTCAgcga---GGTTGACATCCAAGAA   -3  (-7,+4)
ATATCGAACCGGACCACCTCAAGAttggttg---CATCCAAGAA   -3 (-10,+7)
ATATCGAACCGGACCACCTCgcttc----GTTGACATCCAAGAA   -4  (-9,+5)
ATATCGAACCGGACCACCTC----GCTTGGTTGACATCCAAGAA   -4
ATATCGAACCGGACCACCTCgcttcgcttag----ATCCAAGAA   -4 (-15,+11)
ATATCGAACCGGACCACCTgggtgagc---------TCCAAGAA   -9 (-17,+8)
GGATATCcaa----------------//-------GAACTTGCCT  -32 (-35+3)
ATATCGAACCGGACCACCTCAt---//--- CTCCCATGCGTT   -76 (-77+1)
ATATCCAATTTTG----//--------------TTCTTTGCATA   -251
```

A

Obtained with CPF1 (Cas12a)

```
GTTGTTCTTTGCATAGTCAGCTCCATCATCACCGAGCACCGCATACACCTTGA WT  [x4]
GTTGTTCTTTGCATAGTCAGCTCCATCA---CCGAGCACCGCATACACCTTGA -3  [x10] 2 homo
GTTGTTCTTTGCATAGTCAGCTC----------GAGCACCGCATACACCTTGA -10 [x2]    homo
GTTGTTCTTTGCATAGTCAGCTCCAT-------GAGCACCGCATACACCTTGA -7  [x2]
GTTGTTCTTTGCATAGTCAGCTCCATCA-------GCACCGCATACACCTTGA -7  [x2]
GTTGTTCTTTGCATAGTCAGCTCCAT--------AGCACCGCATACACCTTGA -8  [x2]
GTTGTTCTTTGCATAGTCAGCTCCAT----ACCGAGCACCGCATACACCTTGA -4
GTTGTTCTTTGCATAGTCAGCTCCATCA-----GAGCACCGCATACACCTTGA -5
GTTGTTCTTTGCATAGTCAGCTCCATC-----CGAGCACCGCATACACCTTGA -5
GTTGTTCTTTGCATAGTCAGCTCCA--------GAGCACCGCATACACCTTGA -8
GTTGTTCTTTGCATAGTCAGCTCCAT---------GCACCGCATACACCTTGA -9
GTTGTTCTTTGCATAGTCAGCTCCA----------GCACCGCATACACCTTGA -10
GTTGTTCTTTGCATAGTCAGCTC------------GCACCGCATACACCTTGA -12
GTTGTTCTTTGCATAGTCAGCTCC-------------ACCGCATACACCTTGA -13
GTTGTTCTTTGCATAGTCAGCTCC--------------CCGCATACACCTTGA -14
GTTGTTCTTTGCA----------------CACCGAGCACCGCATACACCTTGA -16
GTTGTTCTTTGCATAGTCAGCTCCA------------------TACACCTTGA -18
GTTGTTCTTTGCA-----------------------------ATACACCTTGA -26
```

B

Herbert et al, 2020

Different kind of mutations

TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1

	InFrame	OutFrame
SpCAS9	10% (6)	90% (56)
LbCPF1	43% (13)	57% (17)

Herbert et al, 2020

Allelic frequency for « +1nt »

Cas9: 42,4% (28 alleles over 66)
Cpf1: 0%

“simple” CAS9:
Knock out genes (high frequency frame shift)

VS

“simple” CPF1 (Cas12a):
Deletion of a protein domain/amino acid group without frame shift
Targeting regulatory/promoter regions... etc

TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1

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Herbert et al, 2020

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VS

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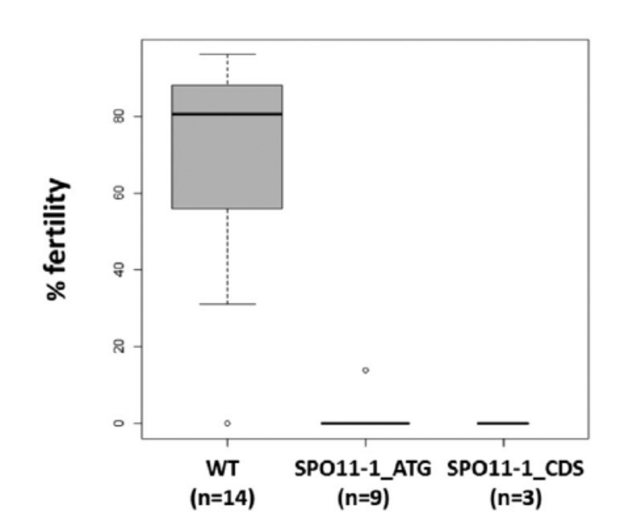
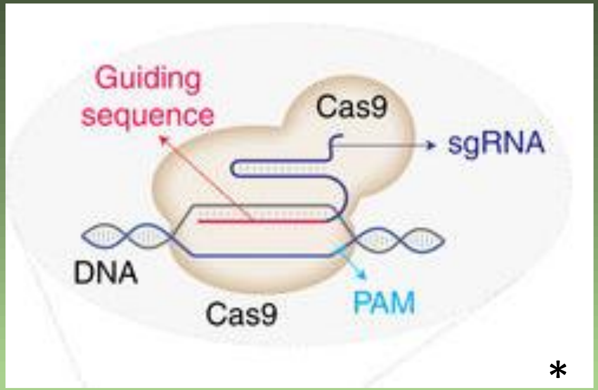
Targeting regulatory/promoter regions... Etc

Gene Knock out for allele engendering sterility (to obtain progeny / nuls segregants…)

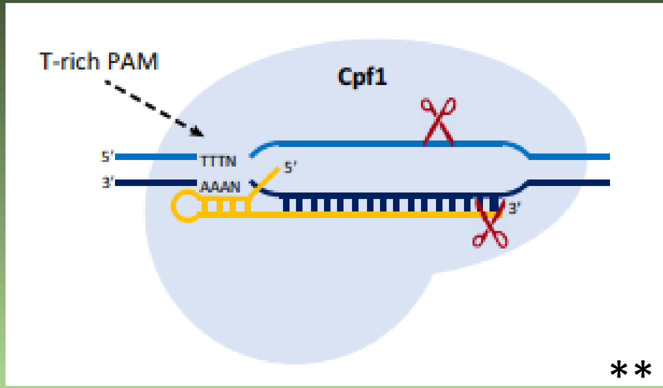


Fayos et al, 2020

TOOLS AVAILABLE ON AFEG PLATFORM – CRISPR/Cpf1



CRISPR/Cas9



CRISPR/Cpf1

Base Editing

** Modified from Mazhar Adli,* 2018

*** Modified from Zaidi et al,* 2017

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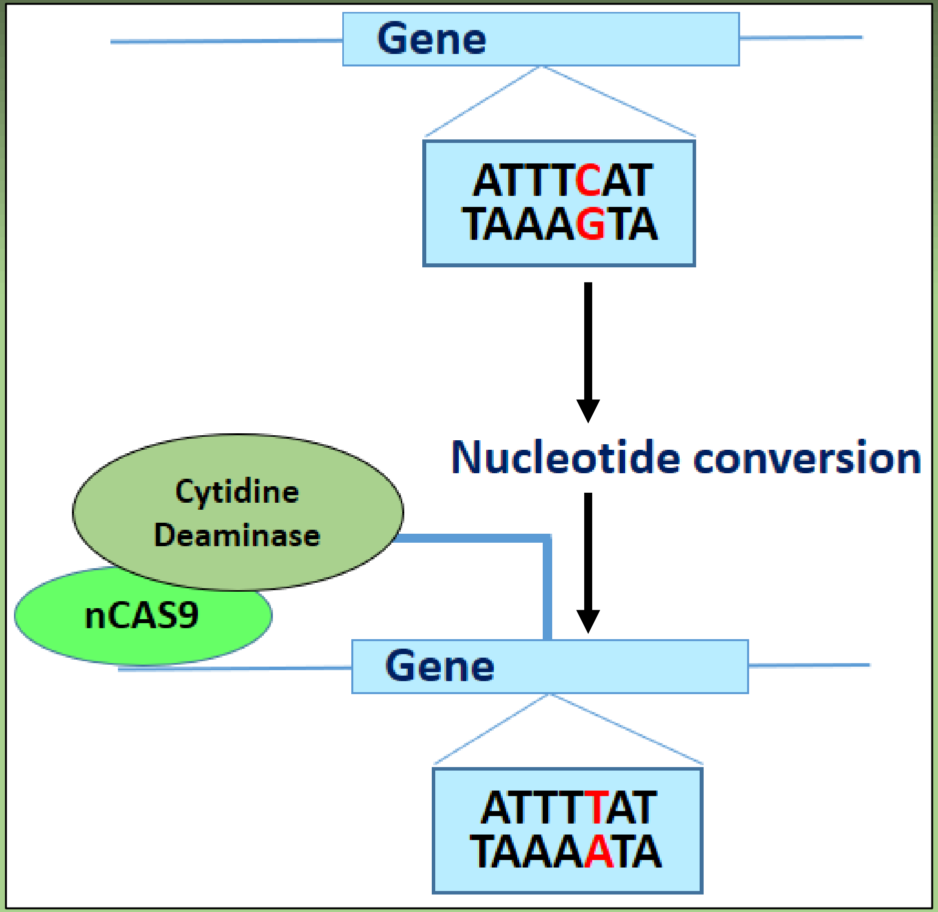
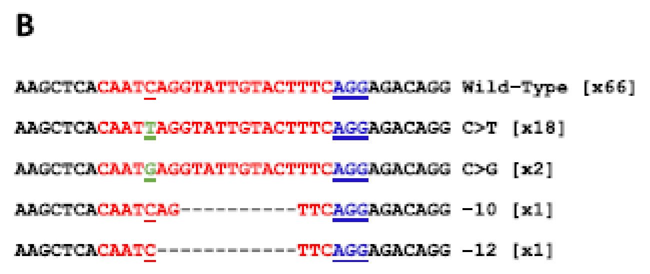
TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing

Nucleotide substitution
(no frame shift)



TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing



The crRNA for BECAS9 was designed to introduce a stop in a CAO *(Chlorophyll A Oxygenase)* reporter gene

36,4% of correct edited plant (6Ho + 10He over 44)

CAO1 mutant plant

Table 1 Homozygous, heterozygous, biallelic and wild-type plants produced using SpCAS9, LbCPF1 and BE_nCAS9D10A expressed as % of analyzed regenerated plants

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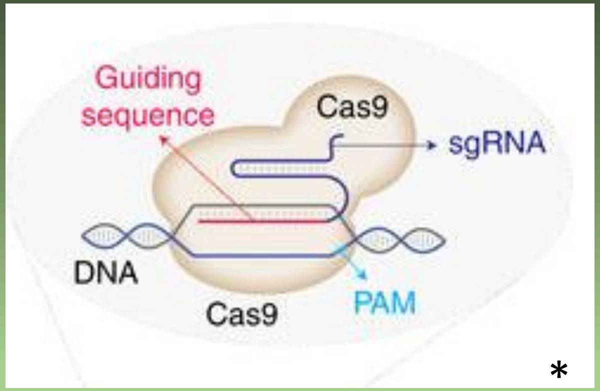
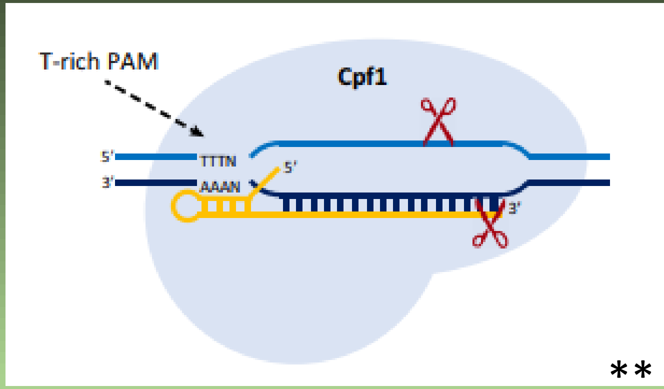
LbCPF1 (*n* = 25)	12% (3)	40% (10)	16% (4)	28% (7)
BE_nCAS9D10A (*n* = 44)	13.6% (6)	0% (0)	22.7% (10)	63.6% (28)

Herbert et al, 2020

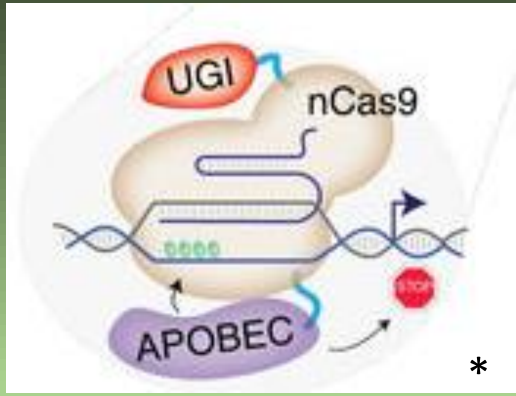
TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing



CRISPR/Cas9



CRISPR/Cpf1



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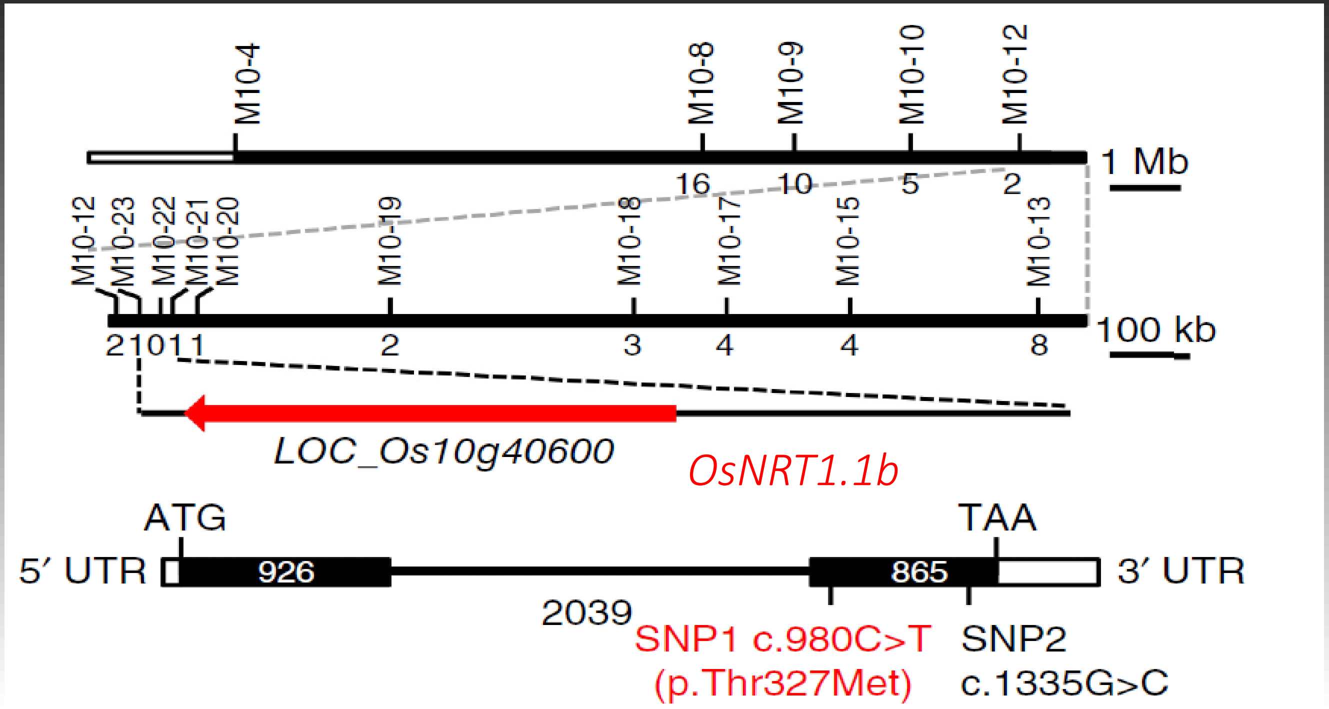
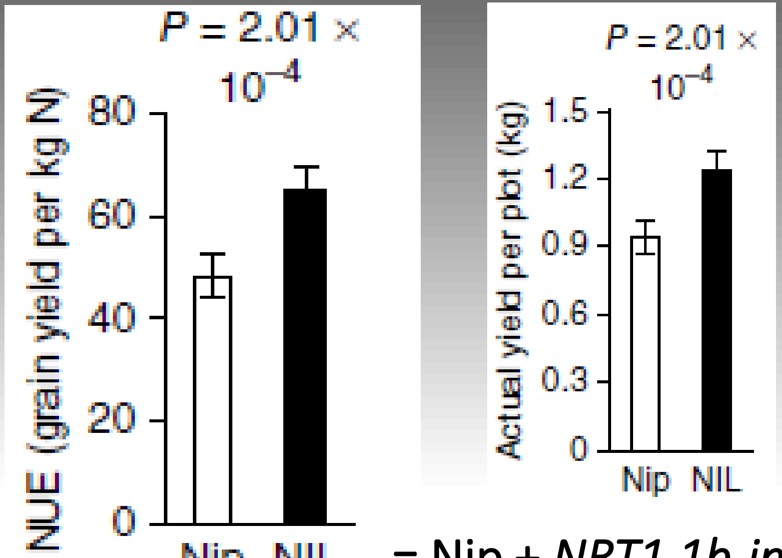
AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing



Hu et al., 2015

Oryza sativa (rice)
2 subspecies: indica and japonica
Indica better NUE than japonica



= Nip + *NRT1.1b-indica*

japonica

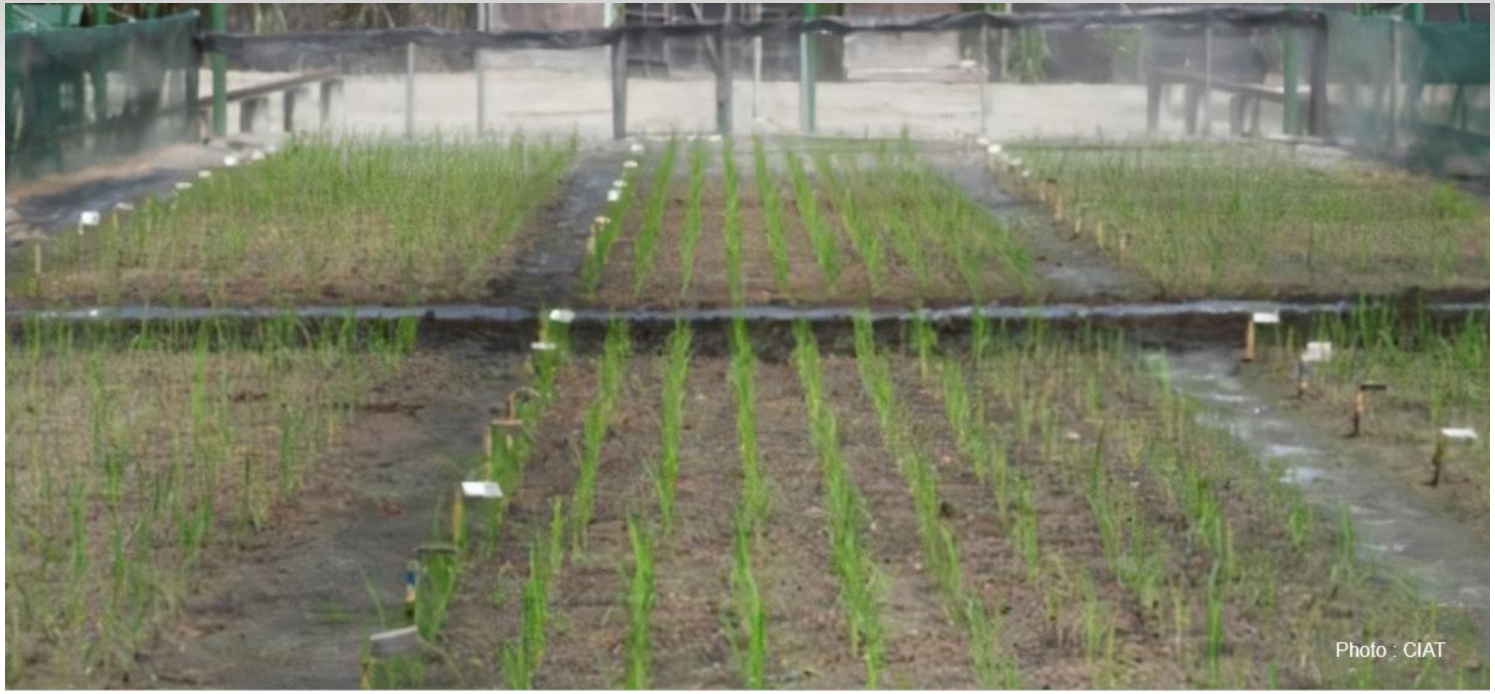
➢ ***Nipponbare cultivar***

TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing

Thesis Léo Herbert / Dr Christophe Périn
(Agropolis Foundation Project "GeneRice" 2017-2019):

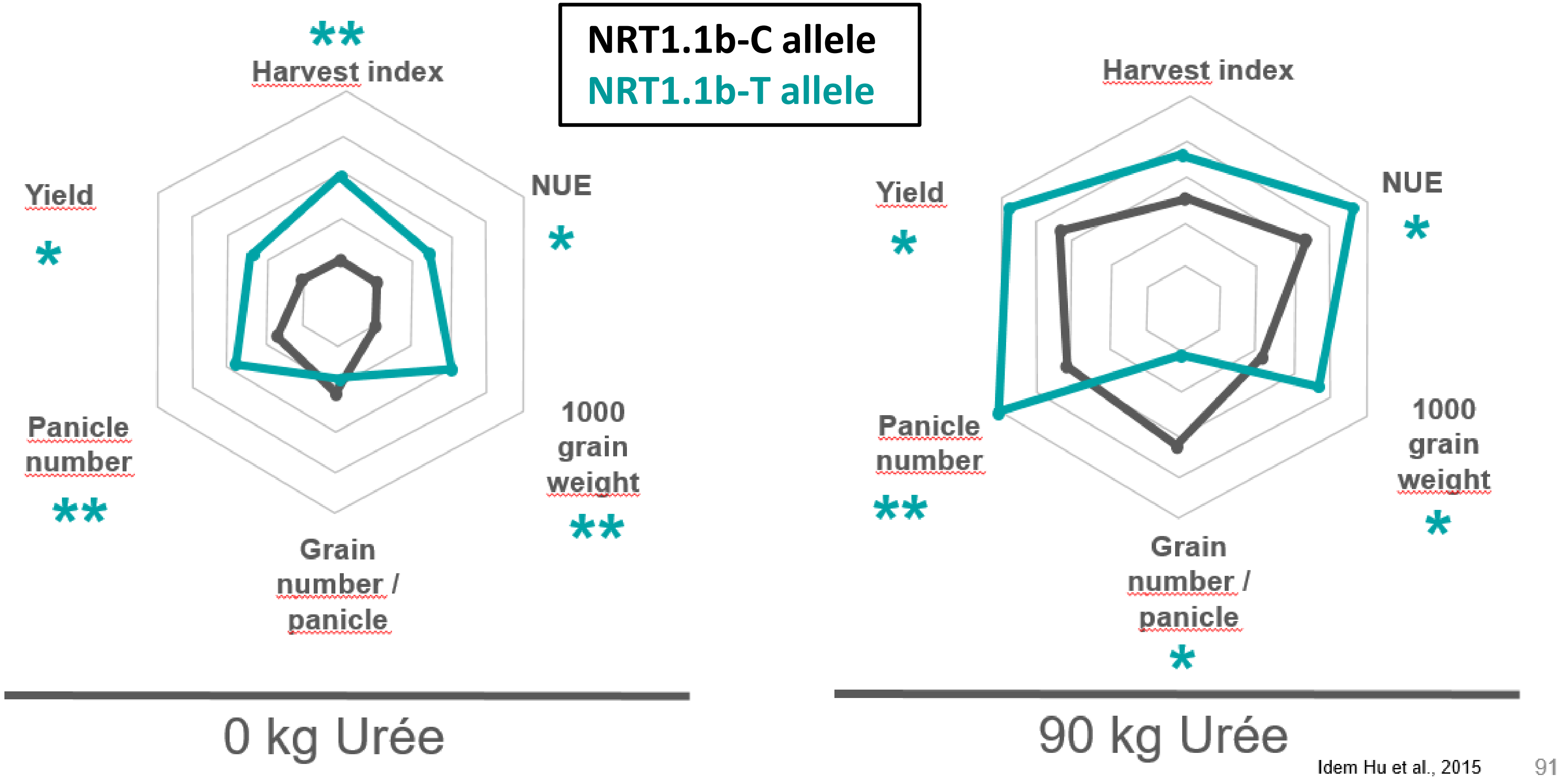
- Precise targeting with Base Editor of the cytosine responsible for the worst NUE in a japonica fund GROWN in Madagascar: Chhomrong DamThen
- Phenotyping in the field (Colombia CIAT) under conditions of high and low nitrogen concentration

>Measurement of various agronomic parameters



TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing

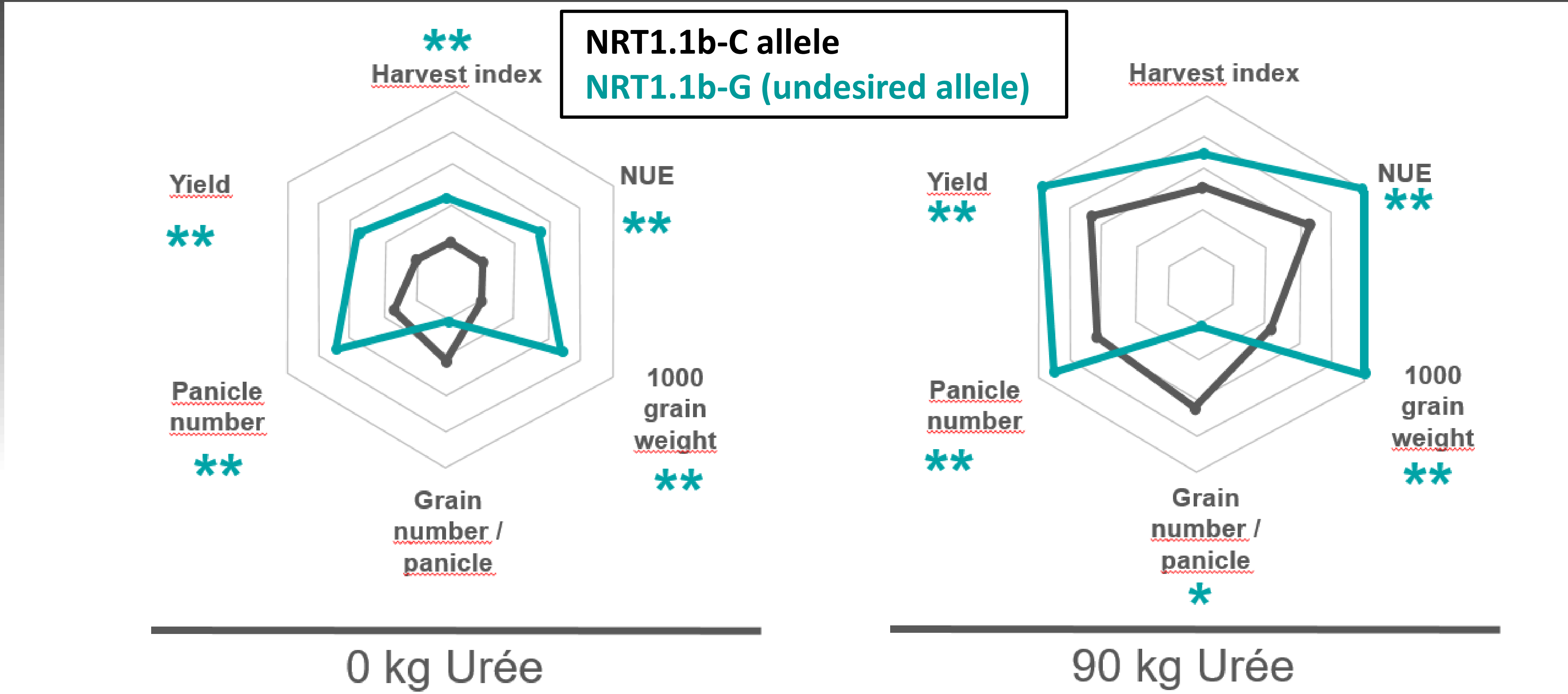
RADAR Agronomic parameters



Idem Hu et al., 2015

TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing

RADAR Agronomic parameters

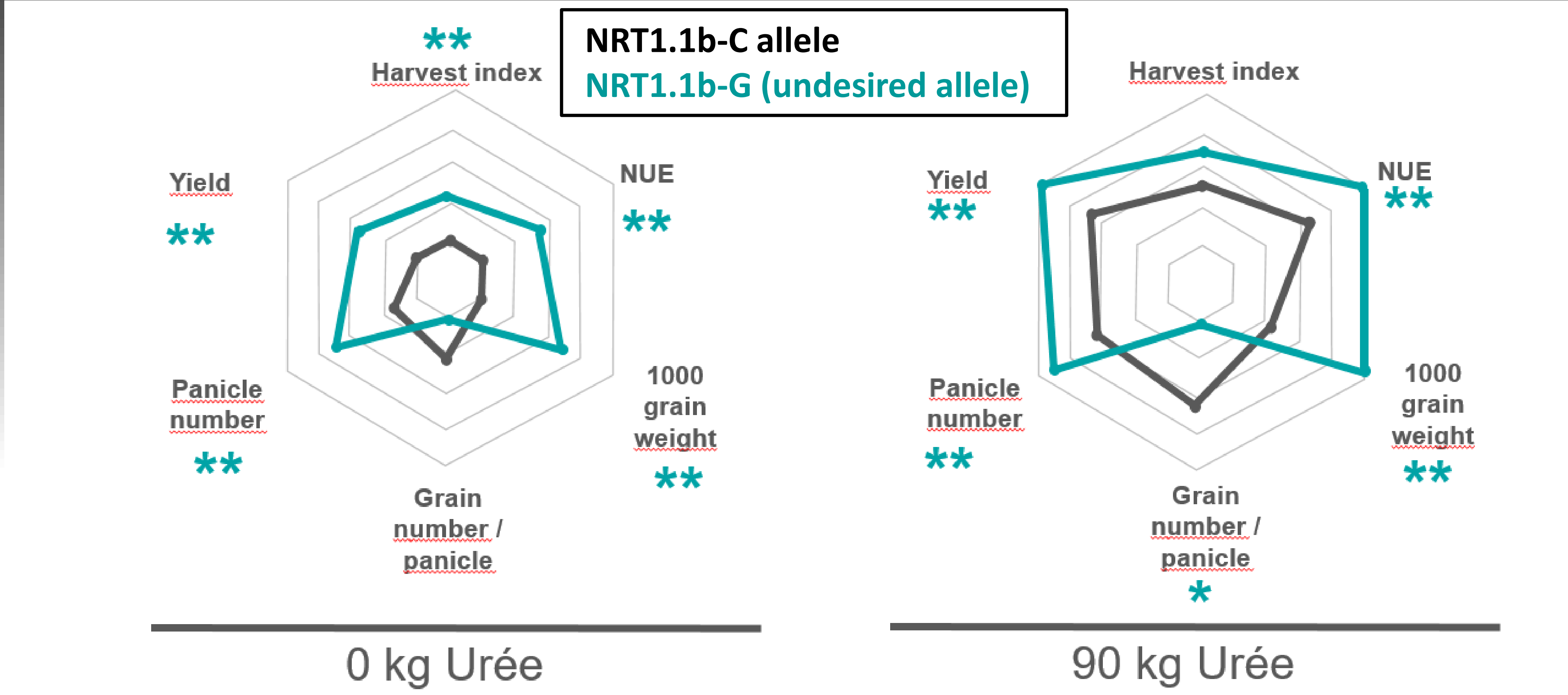


Agropolis Fundation project « GeneRice » 2017-2019

Léo Herbert Thesis

TOOLS AVAILABLE ON AFEG PLATFORM – Base Editing

RADAR Agronomic parameters

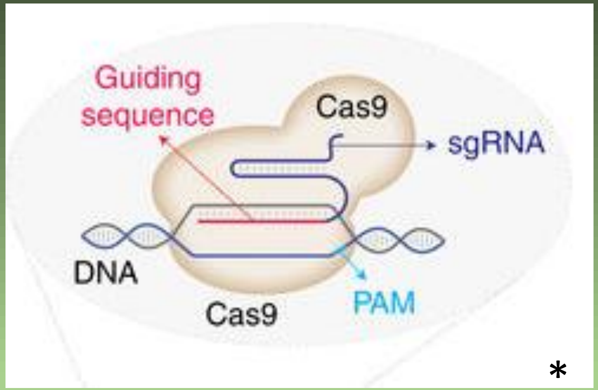


Unfortunately Phenotype not confirmed…

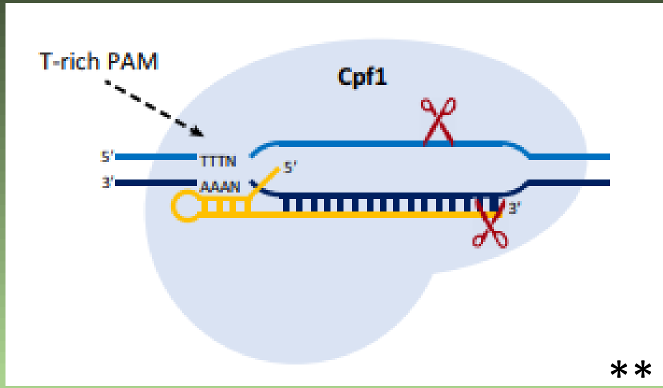
Agropolis Fundation project « GeneRice » 2017-2019

Léo Herbert Thesis

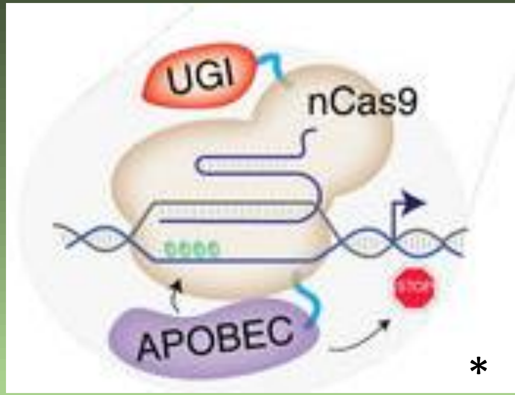
TOOLS AVAILABLE ON AFEG PLATFORM



CRISPR/Cas9



CRISPR/Cpf1



Base Editing

** Modified from Mazhar Adli,* 2018

*** Modified from Zaidi et al,* 2017

Some tools are available for Monocotyledone (Model species *Oryza sativa* (Rice)) or Dicotyledone (model species *Arabidopsis thaliana* / under implementation – 2024-2025)
Multiplexing

Under MTA signature for research use only

AFEG
ANALYSE FONCTIONNELLE
& ÉDITION DES GÉNOMES

TOOLS AVAILABLE ON AFEG PLATFORM

Annex 4: Terms and conditions of use

Terms and Conditions of use

1. The **RECIPIENT** and the **RECIPIENT SCIENTIST** agree that the **MATERIAL:**

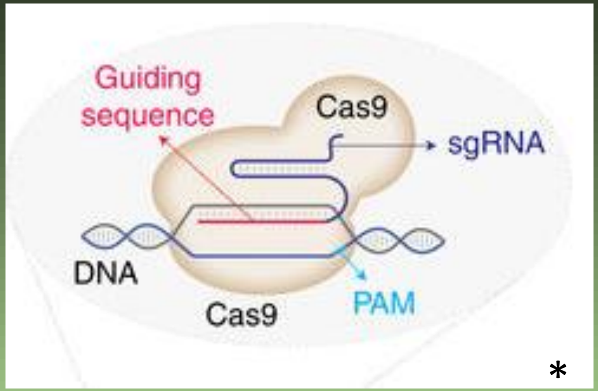
(a) is to be used solely for teaching and academic research purposes;

(b) will not be used in human subjects, in clinical trials, or for diagnostic purposes involving human subjects without the written consent of the **PROVIDER;**

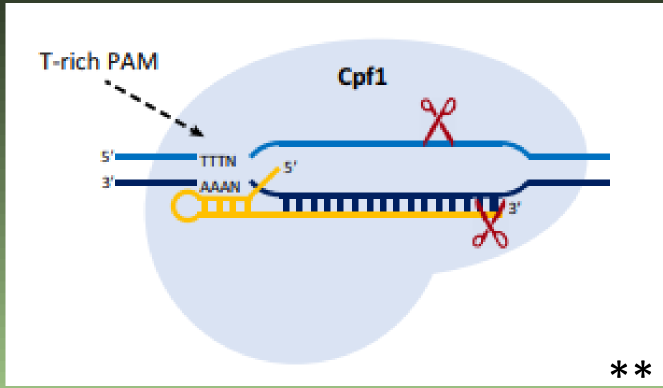
(c) is to be used only at the **RECIPIENT** organization under the direction of the **RECIPIENT SCIENTIST** or others working under his/her direct supervision;

AFEG
ANALYSE FONCTIONNELLE & ÉDITION DES GÉNOMES

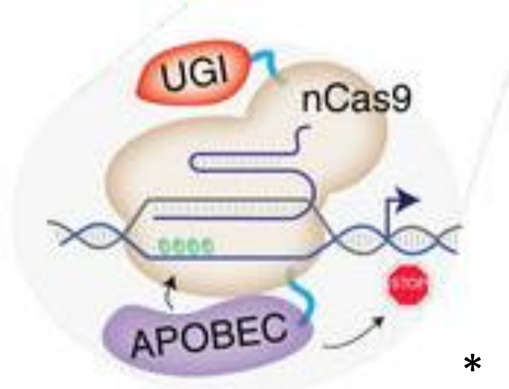
TOOLS AVAILABLE ON AFEG PLATFORM



CRISPR/Cas9



CRISPR/Cpf1



Base Editing

TOOLS UNDER DEVELOPMENT ON AFEG PLATFORM

TYPEX
TOOLS FOR INNOVATION

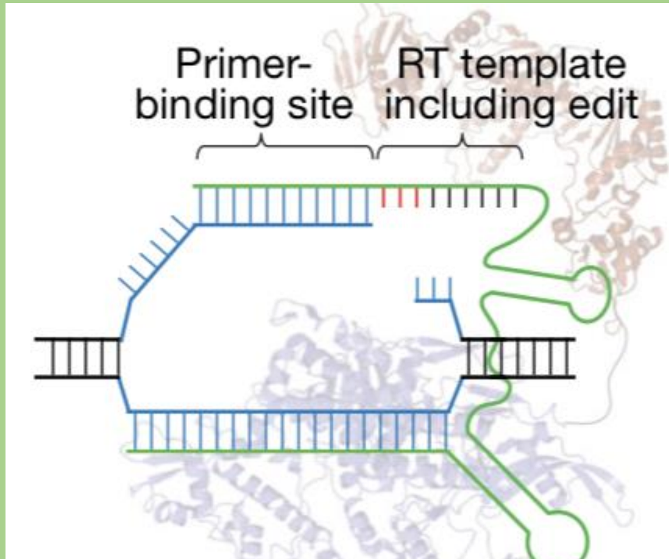
INVESTIR L'AVENIR

PEPR d'une Stratégie Nationale
Projet ciblé
2022

PEPR Sélection végétale avancée

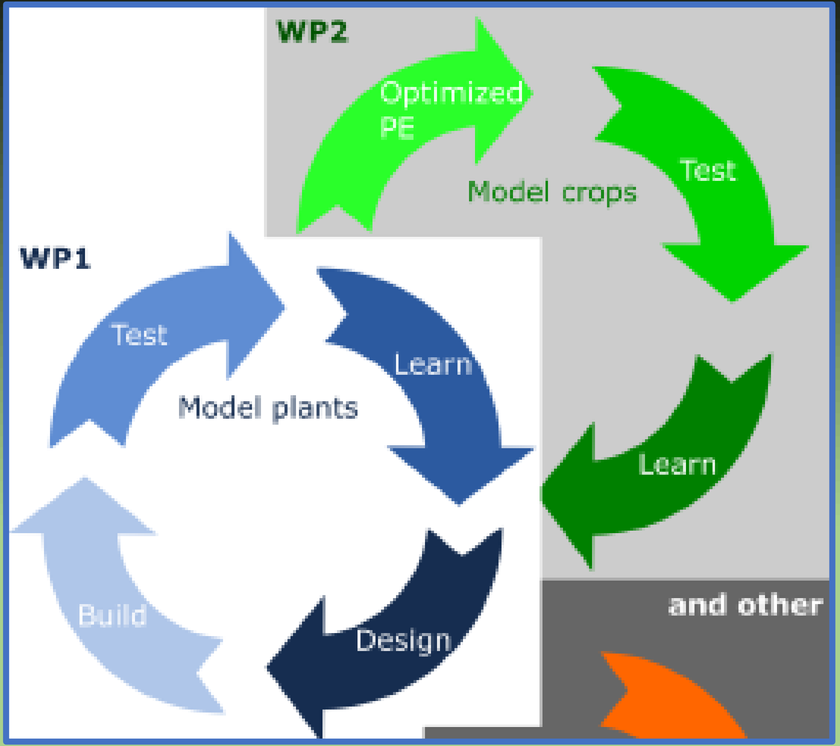
Toward highlY Predictable Editing
of the plant genome leXicon.

Prime Editing Optimisation



Anzalone et al, 2019

TYPEX PROJECT

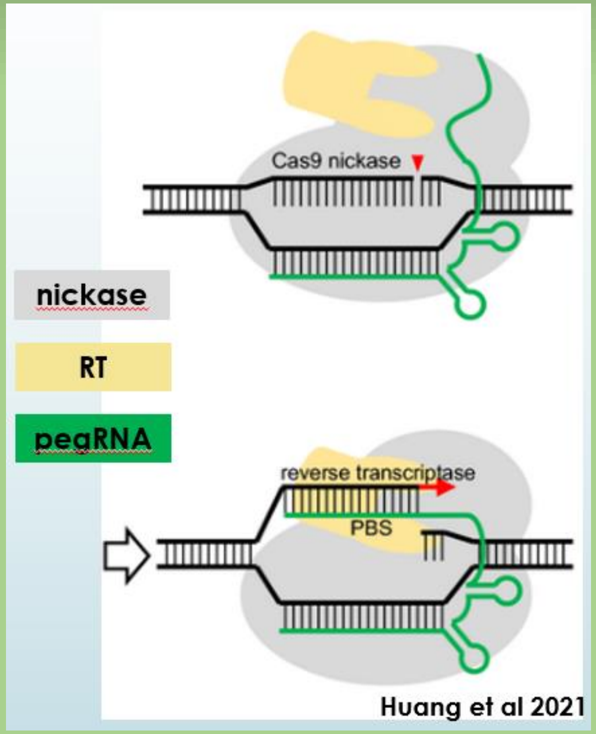


WP2 - Deploying prime-editing in crops

Rice Wheat Maize Tomato Poplar

R&D for prime editing in high-throughput model species

Physcomitrium Marchantia Arabidopsis



Prime Editing Optimisation

<u>TYPEX Project:</u>
Toward highlY Predictable Editing of the plant genome leXicon.

TYPEX
TOOLS FOR INNOVATION

CIRAD Genome Editing platform overview

cirad AGRICULTURAL RESEARCH FOR DEVELOPMENT

agAP INSTITUTE Genetic Improvement and Adaptation of Mediterranean and Tropical Plants

- AGAP - CIRAD Unit in few words
- AFEG and InCell platforms expertise and links
- Actual projects involving AFEG/InCell staff
- AGAP plant species / teams interested in genome editing / for future collaboration?

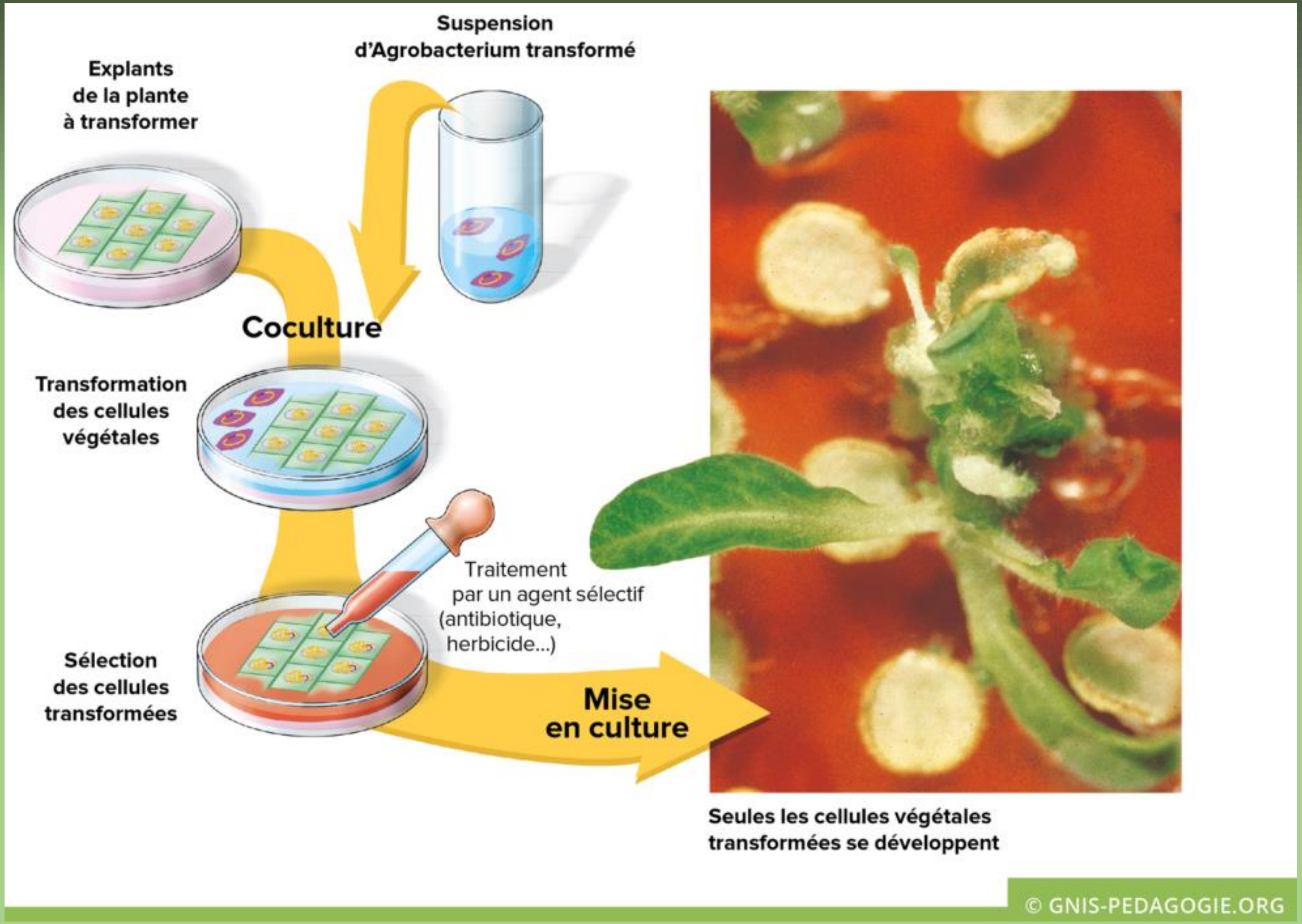
- Genome Editing Tools available on AFEG platform
- GE related technologies / know-how
    - Transformation methods / Transgenesis
    - Mutation detection and analysis

TECHNOLOGIES* AFEG/INCELL PLATFORMS

STABLE GENETIC TRANSFORMATION

*USEFULL FOR GENOME EDITING PROJECT

Biological or indirect transfer
Via *Agrobacterium tumefaciens*



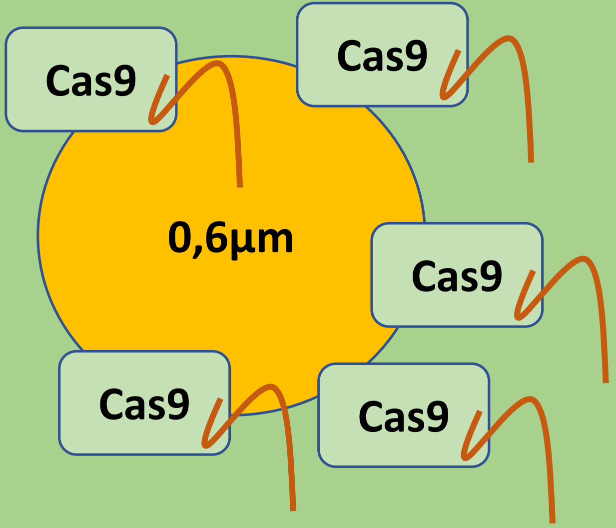
TECHNOLOGIES* AFEG/INCELL PLATFORMS

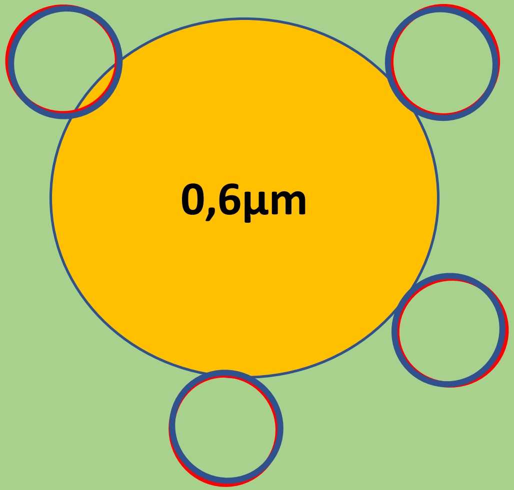
STABLE OR TRANSITORY GENETIC TRANSFORMATION

***USEFULL FOR GENOME EDITING PROJECT**

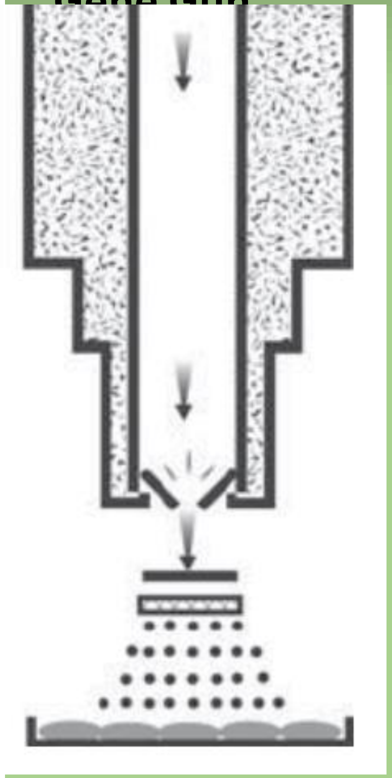
Direct transfer : Biolistic

microparticles (tungsten or gold)
coated with DNA or proteins to be projected into cells.





Gene Gun



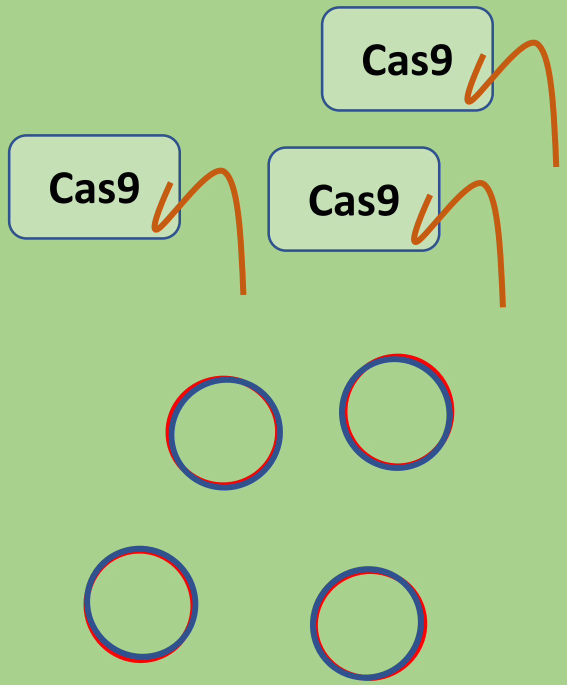
TECHNOLOGIES* AFEG/INCELL PLATFORMS

STABLE OR TRANSITORY GENETIC TRANSFORMATION

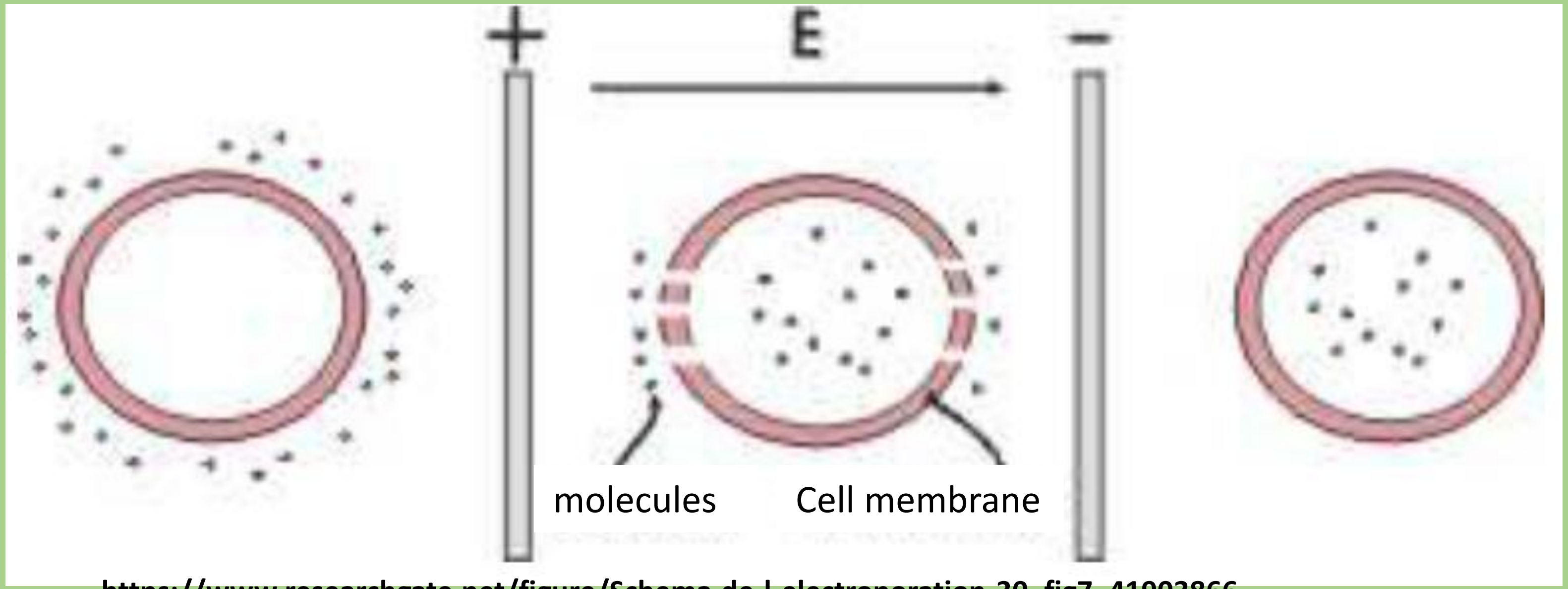
*USEFULL FOR GENOME EDITING PROJECT

Direct transfer : Electroporation or Chemical transfer (PEG)

DNA or proteins to be integrated into cells.



Électroporation



https://www.researchgate.net/figure/Schema-de-l-electroporation-30_fig7_41903866

TECHNOLOGIES* AFEG/INCELL PLATFORMS

TEAMS USING CIV PLATFORM	Agrobacterium	Biolistic	Protoplast regeneration	Protoplast for assays (no regeneration)
Rice. DARS team* / GIV team*	Routinely used	Routinely used		Routinely used
Sorghum. DARS team *	*In progress*			Routinely used
Rubber tree. GSP team*	Routinely used			
Grapevine. DAAV team*	*In progress ++*			Routinely used
Banana. GABA team*	*Interested*			
Cacao. GSP team*	*Interested*			
Citrus. SEAPAG team*	*In progress*		*In progress (No Tg)*	Routinely used
Apple tree. AFEF team*	*In progress*			?
Palm Oil. F2F team *			*In progress*	Routinely used
Coffee. COFFEEADAPT team *	Routinely used			*In progress ++*

*CIRAD AGAP Unit / *CIRAD DIADE Unit

CIRAD Genome Editing platform overview

cirad
AGRICULTURAL RESEARCH FOR DEVELOPMENT

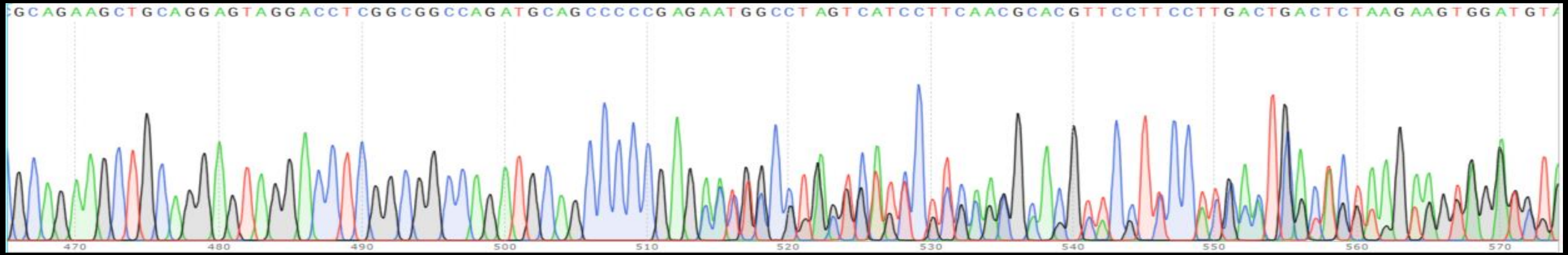
agAP INSTITUTE
Genetic Improvement and Adaptation of Mediterranean and Tropical Plants

- AGAP - CIRAD Unit in few words
- AFEG and InCell platforms expertise and links
- Actual projects involving AFEG/InCell staff
- AGAP plant species / teams interested in genome editing / for future collaboration?

- Genome Editing Tools available on AFEG platform
- GE related technologies / know-how
    - Transformation methods / Transgenesis
    - Mutation detection and analysis

TECHNOLOGIES* AFEG/INCELL PLATFORMS

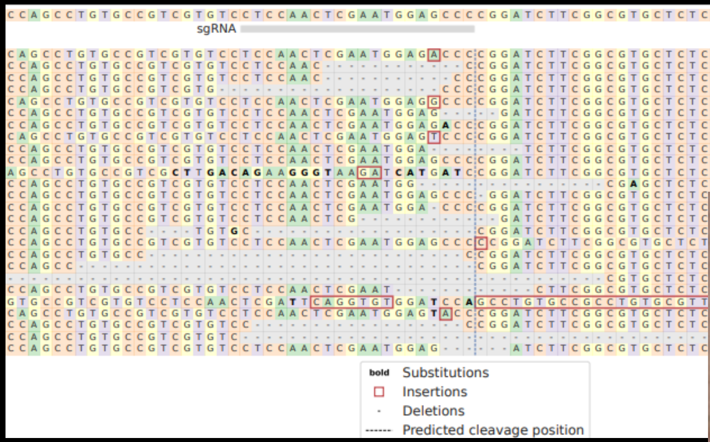
*USEFULL FOR GENOME EDITING PROJECT



SANGER sequencing and deconvolution analysis

AFEG platform (CIRAD - AGAP)

Genotyping platform (CIRAD- AGAP)



NGS amplicon deep sequencing and analysis

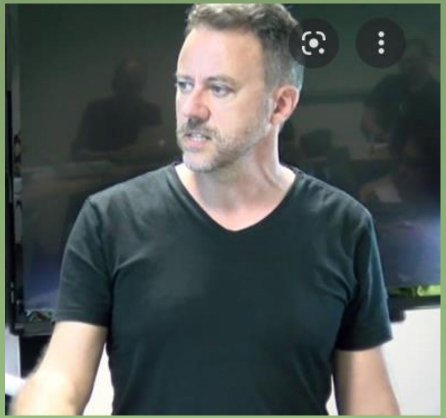
Bioinformatic platform (CIRAD- AGAP)

THANK YOU FOR YOUR ATTENTION

Léo Herbert (GIV -Meiogenix)

Ian Fayos (GIV -Meiogenix)

AFEG/InCell Staff



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