Base editing in potato: successes, limits and new tools
10 public units and 4 private companies

Objective: allow French researchers to acquire and maintain high level technical knowledge and expertise in the field of genome engineering in a variety of crop species, laying the basis for high throughput functional genomics and efficient plant breeding.

9 crops: wheat, maize, rice, oilseed rape, tomato, potato, poplar, apple, rose

2 model species: Brachypodium, Physcomitrella

Genome ENgineering Improvement for Useful plants of a Sustainable agriculture

Genius project

Leuven, 19/11/2019
Current breeding methods

Cross breeding (8–10 years):
- Elite variety (disease susceptible) × Donor variety (disease resistant)
- 5–7 cycles of backcross

Mutation breeding (8–10 years):
- Mutants
- Selection and backcross

Transgene breeding (8–12 years):
- Elite variety (disease susceptible)
- Foreign gene integration
- Genome
- Calli

Genome editing (4–6 years):
- Calli
- CRISPR
- Elite variety (disease susceptible)

Chen et al, 2019
Leuven, 19/11/2019
CRISPR-Cas9 barely increases the overall spontaneous mutation rate.

A given mutation can originate from different types of lesion and different repair pathways.

There is not a dedicated pathway to repair CRISPR-Cas9 mediated DNA alterations.
Random induced mutagenesis has been used in plant breeding for 70 years.

- More than 3200 varieties.
- Chemical or physical mutagens.

- Soybean, high protein content, 1984
- Wheat, good baking quality, 1969
- Tomato, high vitamin C content, 1977

What about CRISPR???

- Product based in USA, Argentina, Canada, Japan
- Process based in Europe...
CRISPR/Cas systems for genome editing

CRISPR/Cas9

CRISPR/Cpf1

CRISPR/CasX

DSB

Chen et al, 2019
CRISPR/Cas systems for genome editing

NHEJ repair pathway

1. Indel mutations
2. Gene deletion
3. Gene insertion/replacement

Cas9

Indels
Gene deletion
Multiplex gene knockout

Chen et al, 2019
CRISPR/Cas systems for genome editing

CRISPR/Cas9

CRISPR/Cpf1

CRISPR/CasX

DSB

NHEJ repair pathway

Indel mutations

Gene deletion

Gene insertion/replacement

HDR pathway

Gene correction

Gene insertion/replacement

Cas9

Indels

Gene deletion

Multiplex gene knockout

Chen et al, 2019
CRISPR/Cas base editing

CBE
Cytidine deaminases
rAPOBEC1/hAID
PmCDA1/hA3A

nCas9

ABE
Adenosine deaminases
ecTadA-ecTadA*

nCas9

Base editor

Amino acid change
Introducing stop codon
Modification of regulation site
Whole-gene screening

A A
T T

C-to-T substitution

C C
G G

A-to-G substitution

Chen et al, 2019

Leuven, 19/11/2019
CRISPR/Cas delivery methods

Chen et al, 2019
Genome editing in potato made in Ploudaniel

Leuven, 19/11/2019
Cultivated potato: theoretically not so easy to edit!

Potato belongs to the *Solanaceae* family, including some agriculturally important plant species like tomato, eggplant and pepper

World’s fourth most important food crop after rice, wheat and maize

Tetraploid genome (2n = 4x = 48), highly heterozygous, vegetatively propagated...

- 4 alleles for each gene
- Difficult guide design and genotyping
- No T-DNA segregation

**Potato genome**: 840 Mbp haploid size, 12 chromosomes, about 39000 protein-coding genes

- Reference sequence available from a double haploid
- Recent sequencing of cultivar Desiree by Nicolas Szydlowski (CNRS/University of Lille)
Agrobacterium-mediated transformation

4 week-old

Stem and shoot explants

Co-culture (48h)

Regeneration stage

Cetaxime, Timentin and Kana

Shoot isolation

Rooting on Kana

DNA extraction

Foreign DNA screening (triplex PCR)

HRM analysis

Sanger sequencing

Transgenic plants because the T-DNA cannot be segregated in potato

Leuven, 19/11/2019
ALS gene as an efficient lab tool

**ALS (Acetolactate synthase):** catalyzes the 1\textsuperscript{st} step in the synthesis of the branched-chain amino acids (valine, leucine and isoleucine)

Two homologs in potato with 96\% of similarity at protein level: *StALS1* and *StALS2*

**Dominant mutation:** 1 mutated allele (out of 8) is sufficient to confer chlorsulfuron resistance

**Amino acids substitutions in ** *StALS** can lead to chlorsulfuron resistance:** perfect tool to test base editors

- Pro 197
- Asp 376
- Asp 377 ?
- Trp 574

Leuven, 19/11/2019
**Agrobacterium-mediated base editing of StALS1**

Target: GGTCAAGTG
PAM: CCG

Wt: CCG : Pro 186

-20  -14 -13

Agrobacterium-mediated transformation using pDicAID (Shimatani et al, 2017)

Plant tissues are grown on chlorsulfuron so that only edited cells can survive

20 out of 20 regenerated plantlets are mutated in the target locus

Selection efficiency = 100%

Leuven, 19/11/2019
Agrobacterium-mediated base editing of StALS1

<table>
<thead>
<tr>
<th></th>
<th>Herbicide resistant</th>
<th>Mutated (HRM and Sanger)</th>
<th>Indels</th>
<th>Clean base edition</th>
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<tbody>
<tr>
<td>Nb of plants</td>
<td>20</td>
<td>20 (100%)</td>
<td>15 (75%)</td>
<td>5 (25%)</td>
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<td>4 (80%)</td>
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<td>5 (100%)</td>
<td>5 (100%)</td>
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<td>2 (40%)</td>
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<td>Plant #20</td>
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</table>

Leuven, 19/11/2019
**Agrobacterium-mediated base editing of StALS1**

Only transgenic plants (with or without mutation in the target)

Transgenic plants (with mutation in the target)

T-DNA free plants (with mutation in the target)

---

**Communication**

Transgene-Free Genome Editing in Tomato and Potato Plants Using *Agrobacterium*-Mediated Delivery of a CRISPR/Cas9 Cytidine Base Editor

Florian Veillet¹, Laura Perrot², Laura Chauvin¹, Marie-Paule Kermarrec¹, Anouchka Guyon-Debass³, Jean-Eric Chauvin¹, Fabien Nogué³ and Marianne Mazier², *ID

Leuven, 19/11/2019
Base editing of *StGBSSI*

Proof of concept of base editing using a single copy gene: *StGBSSI* (amylose synthesis)

Easy phenotyping

Starch

- 30% amylose
- 70% amylopectin

KTGGL: ADP-Glucose-binding site

Target

AAAACCTGTTGACTAGTTGATGTTCTTGGTTGGTGG

-17

Wt

CTA : L

TTA : L

ATA : I

GTA : V

Agrobacterium-mediated transformation

Shimatani et al, 2017
**Agrobacterium-mediated base editing of StGBSSI**

**HRM analysis**
**Editing efficiency = 90%**
(43 out of 48 plants)

**SANGER sequencing (StGBSSI, 4 alleles)**

18T.511.039
Desiree
18T.511.017

18T.511.073
18T.511.089
18T.511.061

**Sequencing reads**

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**PAM**

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**INRA**

**Leuven, 19/11/2019**
Agrobacterium-mediated base editing of StGBSSI

The *Solanum tuberosum* *GBSSI* gene: a target for assessing gene and base editing in tetraploid potato

Florian Veillet¹ · Laura Chauvin¹ · Marie-Paule Kermarrec¹ · François Sevestre²,³ · Mathilde Merrer¹ · Zoé Terret⁴,⁵ · Nicolas Szydlowski²,³ · Pierre Devaux⁶ · Jean-Luc Gallois⁴ · Jean-Eric Chauvin¹

Plant Cell Reports
https://doi.org/10.1007/s00299-019-02426-w

Leuven, 19/11/2019
New targets for pathogen resistance

SteIF4E : Eukaryotic translation Initiation Factor 4E : 1st step of eukaryotic mRNA translation, also required by many viruses to multiply by interaction with the VPg protein

Robaglia and Caranta, 2006

Jeang-Luc Gallois
New tools for genome editing
New CBE with less unwanted outcomes

Leuven, 19/11/2019

Low indels frequency...
But still C-to-G and C-to-A changes

Good compromise for diversifying edits
Development of new Cas9

Main limit of base editing:
PAM approximately 12-20 bp from the target base(s)

xCas9

Hu et al, 2018 (Nature)

Phage-assisted continuous evolution (PACE)
7 aa substitutions

Cas9-NG

Nishimasu et al, 2018 (Science)

Rationally engineered SpCas9
7 aa substitutions
Development of new Cas9 in plants

Cas9-NG Greatly Expands the Targeting Scope of the Genome-Editing Toolkit by Recognizing NG and Other Atypical PAMs in Rice
Bin Ren1,2,5, Lang Liu2,5, Shaofang Li1,3, Yongjie Kuang2, Jingwen Wang2, Dawei Zhang1, Xueping Zhou2,4, Honghui Lin1,4 and Huanbin Zhou2,4

Genome Engineering in Rice Using Cas9 Variants that Recognize NG PAM Sequences
Kai Hua1,3, Xiaoping Tao1, Peijin Han4, Rui Wang1,3 and Jian-Kang Zhu1,2,*

Improving Plant Genome Editing with High-Fidelity xCas9 and Non-canonical PAM-Targeting Cas9-NG
Zhaohui Zhong1,8, Simon Sretenovic2,8, Qirong Ren1,8, Lijia Yang1, Yu Bao3,4, Caiyan Qi1, Mingzhu Yuan1, Yao He1, Shishi Liu1, Xiaopei Liu1, Jiaheng Wang1, Lan Huang1, Yan Wang1, Dibin Baby2,5, David Wang2,6, Tao Zhang3,4, Yiping Qi2,7,* and Yong Zhang1,*

xCas9 and Cas9NG display less efficiency than Cas9 for canonical PAM NGG

Cas9NG is more efficient for non-canonical PAMs than xCas9

Leuven, 19/11/2019
SpCas9-NG expands the scope of gene editing in *Solanaceae*

<table>
<thead>
<tr>
<th>Target</th>
<th>PAM</th>
<th>SpCas9</th>
<th>xCas9 3.7</th>
<th>SpCas9-NG</th>
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<tbody>
<tr>
<td>5'-GGAGCGTTACCGGACCAGA-3'</td>
<td>AGG</td>
<td>2.6%</td>
<td>1.1%</td>
<td>0.4%</td>
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<tr>
<td>5'-GCCATCGAGAAGAAGCCAGGCG-3'</td>
<td>TGT</td>
<td>0%</td>
<td>0%</td>
<td>1.1%</td>
</tr>
<tr>
<td>5'-GACCAGAAGTGACCAGTCAT-3'</td>
<td>CGT</td>
<td>0%</td>
<td>0%</td>
<td>0.04%</td>
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<th>SpCas9</th>
<th>SpCas9-NG</th>
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<tbody>
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<td>5'-AGCAAAAACGGTGGACTAGGGTGA-3'</td>
<td>0% (n = 18)</td>
<td>0% (n = 21)</td>
<td>0% (n = 18)</td>
<td>10% (n = 21)</td>
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<th>SleIF4E2</th>
<th>Mutated</th>
<th>CGT PAM</th>
<th>GGA PAM</th>
<th>0-50% indels</th>
<th>51-100% indels</th>
</tr>
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<tbody>
<tr>
<td>5'-GGAATCGGATGATACCGCTTCTGGCTGGCTGGCATG-3'</td>
<td>56% (n = 268)</td>
<td>100% (n = 94)</td>
<td>49% (n = 94)</td>
<td>40% (n = 94)</td>
<td>60% (n = 94)</td>
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*Under review...*
SpCas9-NG expands the scope of base editing in Solanaceae

<table>
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<tr>
<th></th>
<th>StGBSSI 5′- AGCAAAACTGGTGGACTAGGTGA-3′</th>
<th>StDMR6-1 5′-GACCCGAATCCGATAGGCCACGT-3′</th>
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<tr>
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<td>SpCas9-NG</td>
<td>SpCas9-NG</td>
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<tr>
<td>Mutation efficiency</td>
<td>9% (n = 11)</td>
<td>64% (n = 11)</td>
</tr>
<tr>
<td>Clean base editing</td>
<td>100% (n = 1)</td>
<td>57% (n = 7)</td>
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<tr>
<td>Indels</td>
<td>0% (n = 1)</td>
<td>43% (n = 7)</td>
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<tr>
<td>Mutation efficiency</td>
<td>32% (n = 34)</td>
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<tr>
<td>Clean base editing</td>
<td>64% (n = 11)</td>
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<tr>
<td>Indels</td>
<td>36% (n = 4)</td>
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</table>

Under review...
Cas9 natural variant: ScCas9

**Microbiology**

Minimal PAM specificity of a highly similar SpCas9 ortholog

Pranam Chatterjee\(^1,2\)\(^{+}\), Noah Jakimo\(^1,2\)\(^{+}\), Joseph M. Jacobson\(^1,2\)

**New Results**

Robust Genome Editing of Single-Base PAM Targets with Engineered ScCas9 Variants

- **ScCas9**: PAM NNG
  - 1379 aa (4140 bp)

- **ScCas9 + (T1227K)**: PAM NNG
  - 1386 aa (4161 bp)
  - Higher efficiency

- **SpCas9**: PAM NGG
  - 1379 aa (4140 bp)

- **pUBI** → ScnCas9 dicot → PmCDA1-UGI

- **pUBI** → ScnCas9 dicot

- **pUBI** → ABE

Leuven, 19/11/2019
Cas9 natural variant: Nme2Cas9

A Compact, High-Accuracy Cas9 with a Dinucleotide PAM for In Vivo Genome Editing

Alireza Edraki,1 Aamir Mir,1,4 Raed Ibrahim,1 Ildar Gainetdinov,1 Yoonsoo Yoon,1 Chun-Qing Song,1 Yuaying Cao,1 Judith Gallant,1 Wen Xue,5,6 Jaime A. Rivera-Perez,7 and Erik J. Sontheimer,1,4,*

Discovery

Nme1Cas9
Nme2Cas9
Nme3Cas9
Other orthologs

>86% identity

Different PAMs:

N₄GATT
N₄CC
N₄CAAA

Features of Nme2Cas9

High accuracy
High target site density
All-in-one AAV

SpCas9
1379 aa (4140 bp)
PAM NGG

Nme2Cas9
1082 aa (3249 bp)
PAM NNNNCC
High specificity

pUBI → Nme2Cas9 dicot

Leuven, 19/11/2019
Search-and-replace genome editing without double-strand breaks or donor DNA

nCas9 H840A cuts the PAM-containing strand
Toward prime editing

Base editing
Transition substitutions (C→T, G→A, A→G and T→C)
Unwanted bystander mutations, PAM availability

Prime editing
Insertions, deletions and all types of base substitution...

Is this CRISPR tool efficient in plants???
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Leuven, 19/11/2019
“The power of Selection, whether exercised by man or brought into play under nature through the struggle for existence and the consequent survival of the fittest, **absolutely depends on the variability** of organic beings. *Without variability, nothing can be effected*.”

(Variation of Animals and Plants Under Domestication, 1868)