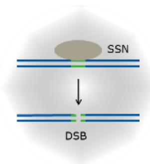
Opportunities of New Plant Breeding Techniques

Jan Schaart



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Plant Breeding

- Important for crop improvement
- Combining of genetic variation
- Based on the steps of crossing and selection
- Limitations
  - polyploidy,
  - heterozygosity
  - self-incompatibility
  - long generation time
  - …

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Breeding projects at WUR-Plant Breeding

- Food: potato, tomato, cabbage, lettuce, onion, apple, quinoa, mushroom,…
- Bio-based: crambe, camelina, hemp, miscanthus…
- Ornamental: lily, tulip, rose…

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Improved breeding using New Plant Breeding Techniques

http://edepot.wur.nl/357723

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New Plant Breeding Techniques (NPBT)

- New genetic techniques for crop improvement
- Similar breeding goals as conventional plant breeding but, faster, easier
- All NPBT have <u>process</u> that make use of a genetic modification (GM) step
- For most techniques: Ultimate <u>products</u> are free of genes that are not present in the species gene pool

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Process & products

Plant

↓

Genetic modification (application of recombinant DNA)

↓

Screening for desired genomic modifications

↓

Removal of recombinant DNA

↓

Improved plant

Products with three levels of genomic modifications

1. Addition of an extra copy of a existing gene to the plant genome
2. Modifications to an gene that is present in the plant genome
3. No direct modification to the genome (process used to facilitate breeding steps)

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Range of techniques and concepts

- Cisgenesis
- Sequence-specific nuclease technology (ZFN, TALENs, CRISPR-Cas)
- Oligo-directed mutagenesis
- RNA-dependent DNA-methylation
- Grafting on a GM-rootstock
- Reverse breeding
- Induced early flowering
- CENH3-mediated haploid induction

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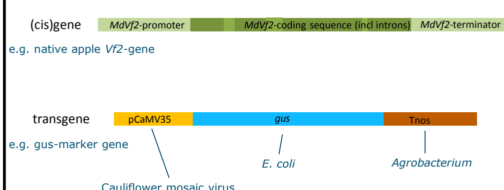
Cisgenesis vs transgenesis

- Transformation concept introducing gene sequences that originate from the species itself
  - Cisgenesis: addition of an extra copy (allele) of a native gene



Cisgenesis

- Extra copy of a gene: superior allele
- Agrobacterium-mediated or direct gene transfer
- Examples:
  - Apple: Scab resistance
    Red flesh
  - Potato: Phytophthora resistance

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Scab in apple

- Apple scab is major disease in apple
- Common culture: 20-30 sprays/season

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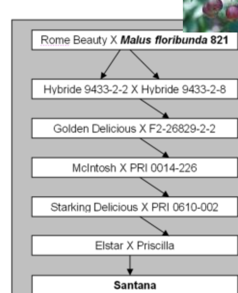
Classical introgression of *Vf*-gene in apple

- Different sources of scab-resistances available
  - *Malus floribunda Vf*-gene
- Classical breeding is extremely time-consuming
  - >50 years to breed *Vf*-gene in commercial cultivar



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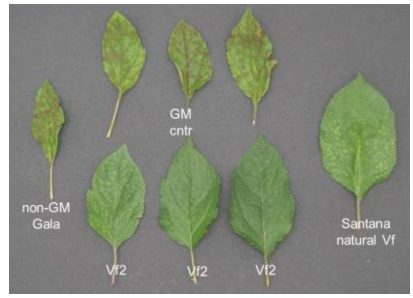
Introduction of *Vf*-gene in apple through cisgenesis

- At WUR-Plant breeding: validation of *Vf2* as suitable scab-resistance gene
- Introduction in Gala together following transgenic approach
- Greenhouse scab-resistance test


Scab-resistance test



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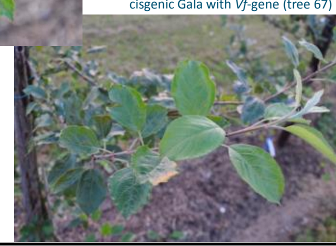
Cisgenic Gala with *Vf*

cisgenic Gala with *Vf*-gene (tree 67)

Gala (tree 21)



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Next step: Durable resistance by gene stacking *Vf2+V25+Vr2*

- Gene stacking: Combining *Vf2* with other scab resistance genes:
  - *Vr2,* cloned from *M. pumila*, tested in transgenic plants, ready for cisgenesis
  - *V25,* cloned from selection *1980-015-025* and currently being tested
  - *Vf, V25* and *Vr2* different type of genes, different mode of action

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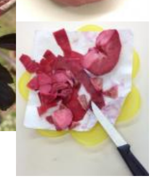
Cisgenic red-fleshed Gala apple

- Red flesh: *MdMyb10*-gene from apple cultivar '*Red Field*'







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Cisgenesis in potato for durable *Phytophthora* disease resistance

- Stacking of three broad-host resistance genes from reated wild species:
  - *Rpi-sto1* (from *S. stoloniferum*)
  - *Rpi-blb3* (from *S. bulbocastanum*)
  - *Rpi-vnt1.1* (from *S. venturii*)

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Triple R-genes against *Phytophthora infestans*

Field test

before after

Tuber test

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DuRPh

Field trial August 2015

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Durable potato cultivation

- Improved variety lines with various combinations of resistance genes
- Cultivation rotation scheme to support durable disease management

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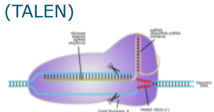
Sequence-Specific Nucleases (SSNs)

- Pre-designed restriction enzymes
- Cut at predefined DNA target sequence (so at your favourite sequence)
- Different types:
  - Meganucleases
  - Zinc Finger Nuclease (ZFN)
  - ~~TAL Effector Nuclease (TALEN)~~
  - CRISPR-Cas9



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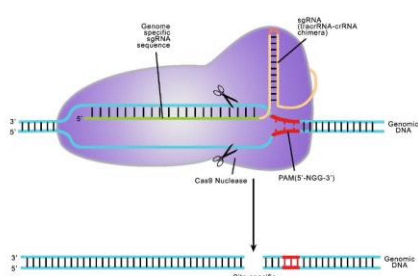
CRISPR-Cas9



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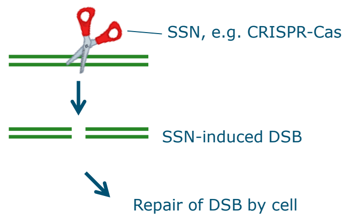
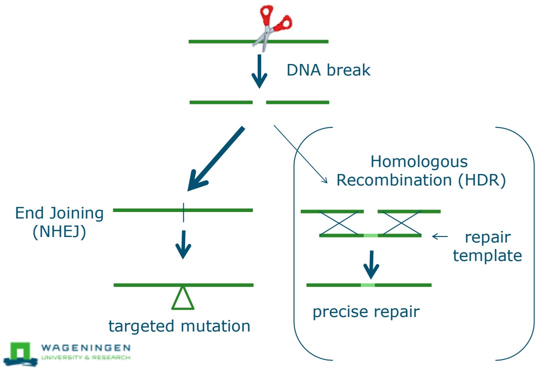
What are SSNs doing?

- SSNs induce double strand breaks (DSB) at a specific target site in genomic DNA



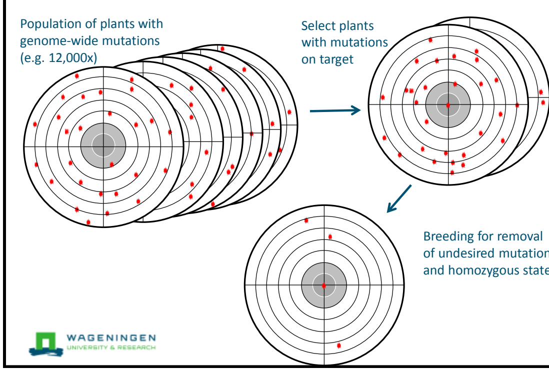
DNA repair: two outcomes



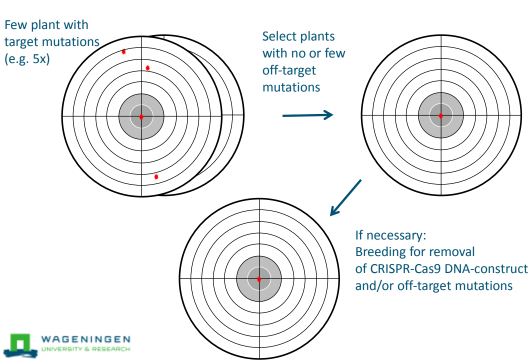
Applications: targeted mutagenesis

- Direct mutation of target genes
  - no mutagenized populations required; no laborious and expensive screening methods (Tilling)
- Powerful tool to make mutations in multiple alleles: polyploids
  - very difficult using traditional mutation methods

Traditional mutagenesis



Directed mutagenesis



Many crops have a polyploid genome

- Polyploids have multiple allelic versions of each gene eg:
  - Potato, tetraploid (4x)
  - Wheat, chrysanthemum, hexaploid (6x)
  - Strawberry, octoploid (8x)
- Knock-out mutation: all alleles have to be targeted

- Multi-allelic mutation with CRISPR-Cas!

Directed mutagenesis-projects at WUR-PBR

- CRISPR-Cas for mutagenesis in different crops:
  - -potato (4x): S-genes, starch, carotenoids
  - -tomato (2x): S-genes, taste attributes
  - -camelina, crambe (6x): oil composition, anti-nutritional factors
  - -bread wheat (6x): gluten
  - -chrysanthemum (6x): haploid induction
  - -nicotiana (4x): tests

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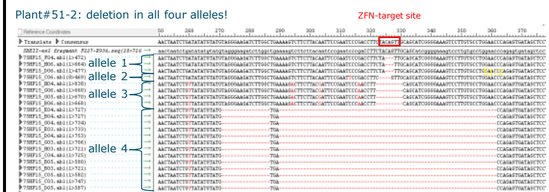
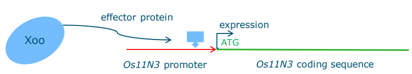
ZFN-induced deletion in tetraploid potato

- ZFNs targeted towards *sbe*II
- All four alleles targeted in a single experiment



Os11N3 susceptibility gene

- S-gene for rice bacterial blight (caused by *Xanthomonas oryzae* pathovar *oryzae* (Xoo)
- Xoo produces effector-proteins that activate the expression of *Os11N3*



- RNAi: Silencing of *Os11N3*-expression: Xoo resistance! ...but *Os11N3* is essential gene in rice. Silencing results in stunted plants.

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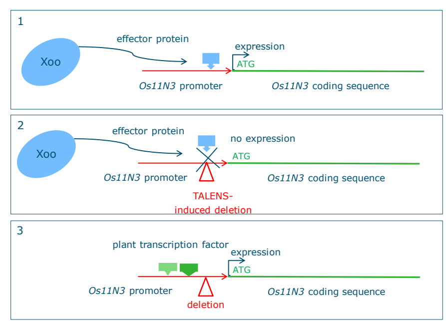
Deletion of *Os11N3* promoter



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TALENs-induced mutations in *Os11N3* promoter

```
                                                                              WT
CTTCCTTCCTAGCACTATATAAAcccccctccaaccaggtgcTAAGCTCATCAAGCCTTCAAGC              –55a
-----------------------------------------gtgcTAAGCTCATCAAGCCTTCAAGC           –7/+3
CTTCCTTCCTAGCACTATATAAAcccccctc--AAA-- gtgcTAAGCTCATCAAGCCTTCAAGC             –32a
CTTCCTTCCTA ---------------------------------- AGCTCATCAAGCCTTCAAGC           –18
CTTCCTTCCTAGCACTATATAAAcccccct -------------------CATCAAGCCTTCAAGC            –22/+5
CTTCCTTCCTAGCACTATATAAA-------GGATC------------ CTCATCAAGCCTTCAAGC            –9a
CTTCCTTCCTAGCACTATATAAAccc ---------- aggtgcTAAGCTCATCAAGCCTTCAAGC           –5b
CTTCCTTCCTAGCACTATATAAAccc ----- aaccaggtgcTAAGCTCATCAAGCCTTCAAGC           –4b
CTTCCTTCCTAGCACTATATAAAcccccctccaa ---- gtgcTAAGCTCATCAAGCCTTCAAGC           –3a
CTTCCTTCCTAGCACTATATAAAcccccctcc --- caggtgcTAAGCTCATCAAGCCTTCAAGC           +9
CTTCCTTCCTAGCACTATATAAAcccccctccaaccagGTTTATATAgtgcTAAGCTCATCAAGCCTTCAAGC
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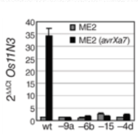

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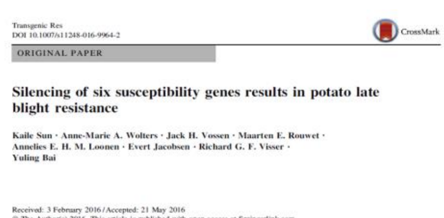
S-genes for *Phytophthora*


Transgenic Res
DOI 10.1007/s11248-016-9964-2

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ORIGINAL PAPER

Silencing of six susceptibility genes results in potato late blight resistance

Kaile Sun · Anne-Marie A. Wolters · Jack H. Vossen · Maarten E. Rouwet · Annelies E. H. M. Loonen · Evert Jacobsen · Richard G. F. Visser · Yuling Bai

Received: 3 February 2016 / Accepted: 21 May 2016
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- New source of resistance??

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Induced early flowering

- E.g. in fruit trees: generation time 5-10 years
- Shortening juvenile phase: accelerated breeding
- Induction of early flowering:
  - Over-expression of 'Flowering locus T' gene (*AtFT*)
  - Silencing of 'Terminal Flower 1' gene (*MdTFL1*)

Source: H. Flachowsky; JKI

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Application of early flowering

- Flachowski et al. (2011) in apple
  - Overexpression of BpMADS4 gene (silver birch): breaking of juvenility: early flowering
  - Speed breeding
- Scorza et al. (2011) in plump
  - Overexpression of FT (populus)
  - FasTrack breeding

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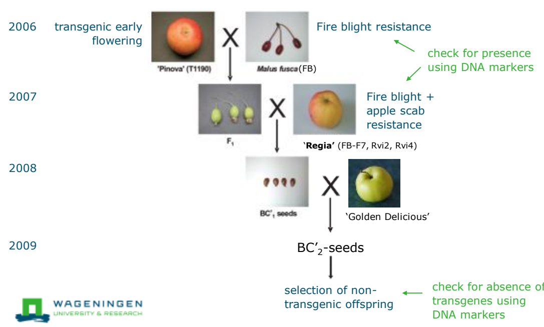
Stacking of resistance genes by speed breeding

Flachowsky et al. 2009 & 2011 (adapted)



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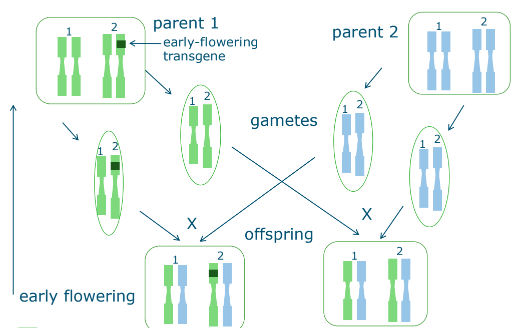
'Speed-breeding' scheme



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Viral delivery of early flowering genes

- Yamagishi et al (2013)
  - expression of early flowering genes using apple latent spherical virus (ALSV)
  - Infection of seedlings: 90% produced fertile flowers in 1.5-3 months
  - virus is not transmitted through seed

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Conclusion

- New genetic techniques for crop improvement
- Similar breeding goals as conventional plant breeding
  - faster
  - easier
  - new possibilities
- Products often indistinguishable from products of traditional breeding

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Related Issues

- All NPBT have <u>process</u> that make use of a genetic modification (GM) step
- For most techniques: Ultimate <u>products</u> are free of genes that are not present in the species gene pool
- Products often indistinguishable from products of traditional breeding

- Are products from these New Plant Breeding Techniques GMOs or not
- Consumer acceptance

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Thank you for your attention!

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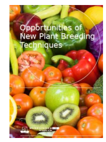

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