

Opportunities of New Plant Breeding Techniques

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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

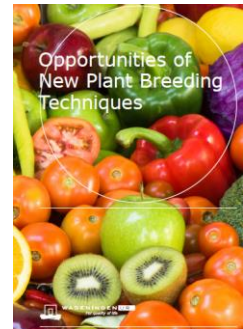
- Food: potato, tomato, cabbage, lettuce, onion, apple, quinoa, mushroom,...
- Bio-based: crambe, camelina, hemp, miscanthus...
- Ornamental: lily, tulip, rose...



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Improved breeding using New Plant Breeding Techniques



<http://edepot.wur.nl/357723>

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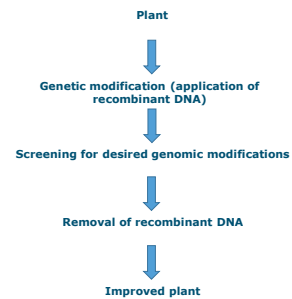
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New Plant Breeding Techniques (NPBT)

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


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Process & products



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

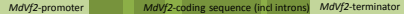


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Cisgenesis vs transgenesis


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

(cis)gene



e.g. native apple Vf2-gene

transgene




e.g. gus-marker gene

Cauliflower mosaic virus


E. coli

Agrobacterium



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







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

Rome Beauty X *Malus floribunda* 821

Hybride 9433-2-2 X Hybride 9433-2-8

Golden Delicious X F2-26829-2-2



McIntosh X PRI 0014-226

Starking Delicious X PRI 0610-002

Elistar X Priscilla

Santana

Het schurftresistente ras Santana

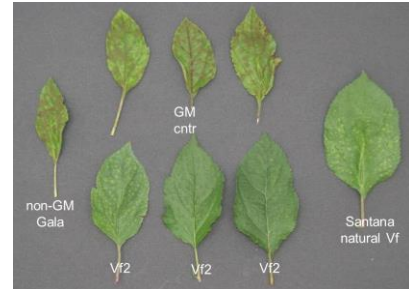



Introduction of *Vf*-gene in apple through cisgenesis

- 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*



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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 related 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 Tuber test

before after

DuRPh

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Field trial August 2015



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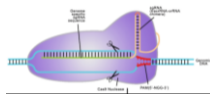
Durable potato cultivation

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

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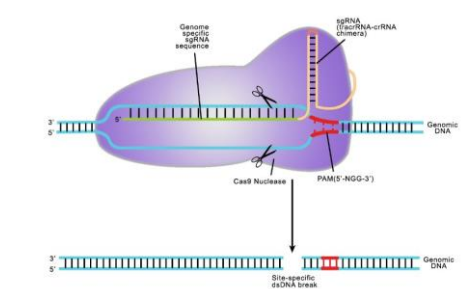
Sequence-Specific Nucleases (SSNs)

- 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



Genome specific sgRNA sequence

sgRNA (CRISPR/Cas9 chimera)

Genomic DNA

Cas9 Nuclease

PAMIS'-NGG-3'

Site-specific dsDNA break

Genomic DNA

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What are SSNs doing?

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

SSN, e.g. CRISPR-Cas

SSN-induced DSB

Repair of DSB by cell

DNA repair: two outcomes

DNA break

End Joining (NHEJ)

targeted mutation

Homologous Recombination (HDR)

repair template

precise repair

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

Population of plants with genome-wide mutations (e.g. 12,000x)

Select plants with mutations on target

Breeding for removal of undesired mutations and homozygous state

Directed mutagenesis

Few plant with target mutations (e.g. 5x)

Select plants with no or few off-target mutations

If necessary: Breeding for removal of CRISPR-Cas9 DNA-construct and/or off-target mutations

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

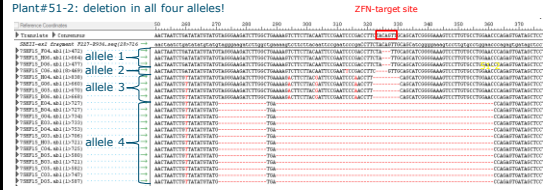
- CRISPR-Cas for mutagenesis in different crops:
 - potato** (4x): S-genes, starch, carotenoids
 - tomato** (2x): S-genes, taste attributes
 - camelina, crambe** (6x): oil composition, anti-nutritional factors
 - bread wheat** (6x): gluten
 - chrysanthemum** (6x): haploid induction
 - nicotiana** (4x):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

ZFN-induced deletion in tetraploid potato

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



Plant#51-2: deletion in all four alleles!



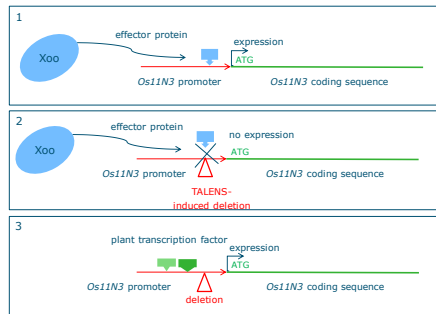
Os11N3 susceptibility gene

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

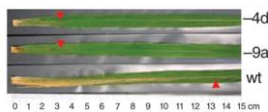
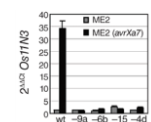


- RNAi: Silencing of *Os11N3*-expression: Xoo resistance! ...but *Os11N3* is essential gene in rice. Silencing results in stunted plants.

Deletion of *Os11N3* promoter



TALENs-induced mutations in *Os11N3* promoter



S-genes for *Phytophthora*

Transgenic Res
DOI 10.1007/s11228-016-9964-2



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. Loumen · Evert Jacobsen · Richard G. F. Visser · Yufeng Bai

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- New source of resistance??

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*)



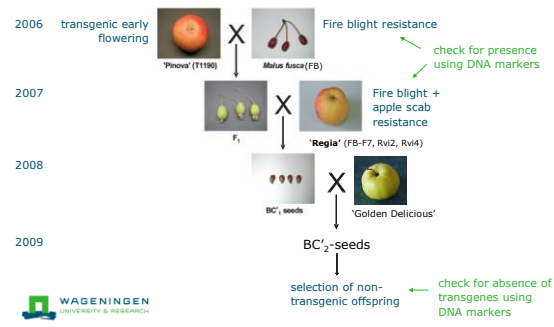
Source: H. Flachowsky, 2011

Application of early flowering

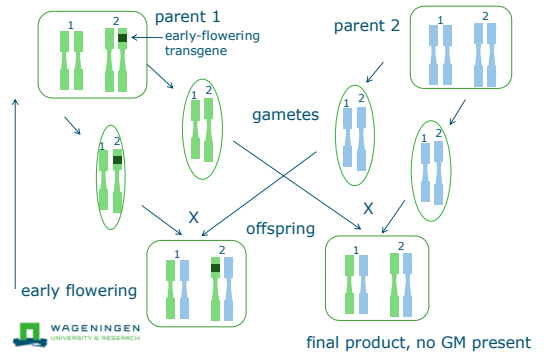
- Flachowsky et al. (2011) in apple
 - Overexpression of *BpMADS4* gene (silver birch): breaking of juvenility: early flowering
 - Speed breeding
- Scorza et al. (2011) in plump
 - Overexpression of *FT* (populus)
 - FasTrack breeding

Stacking of resistance genes by speed breeding

Flachowsky et al. 2009 & 2011 (adapted)



'Speed-breeding' scheme



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



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

Related Issues

- All NPBT have process that make use of a genetic modification (GM) step
- For most techniques: Ultimate products 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

Thank you for
your attention!

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