PlantEd

COST Action CA18111
Genome Editing in Plants

Novel disease resistance approaches – are they durable ?

Jeremy Sweet

JT Environmental Consultants

Cambridge

UK

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

Novy Sad 6 Nov 2019

Gene editing for disease resistance and management

* **GE techniques can be used to boost molecular immunity and resistance against different plant pathogens.**
* **Gene sequencing techniques advanced to stage where there is good info on both plant and pathogen genes and transcriptomes to allow targeting of SDNs.**
* **improved understanding of complex interactions between plants and pathogens and related Cross Talk at RNA level**

The Genome Editing (GE) techniques, include SDN techniques :

- Clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) system,
- Transcription activator-like effector nucleases (TALENs),
- Zinc-finger nucleases (ZFNs)
- Meganucleases (LAGLIDADG homing endonucleases), mostly found in the mitochondria and chloroplasts of eukaryotic unicellular organisms.

ZFNs :

ZFNs made little impact on editing host plant genes involved in disease development as they are complex to engineer and difficult to multiplex. (Khandagale and Nadaf, 2016;Ruiz de Galarreta; Lujambio, 2017; Jaganathan et al., 2018).

Artificial zinc finger proteins (AZPs) used for antiviral resistance in plants by targeting and blocking specific DNA binding sites of viral protein replication (Sera,2005; Takenaka et al., 2007).

Examples : Multiple resistance against begomoviruses:
Tomato yellow leaf curl virus (TYLCCNV)
Tobacco curly shoot virus (TbCSV) (Chen et al 2014)

TALENS

- **Transcription activator-like effector nucleases**
  transcription factors translocated by Xanthomonas bacteria
. through their type III secretion system into plant cells
  (*Boch and Bonas, 2010*).
- TALEs engineered to bind any desirable DNA sequence that when fused to a nuclease (TALEN) cause DNA breaks to induce mutations through NHEJ
  e.g. Rice blight : *Xantho oryziae*
  Begomoviruses TomYLCV and TobCSV (Cheng 2015)

- Effective against both RNA and DNA viruses.

- Can be imprecise and can create mutations at targeted sites and loss-of-function. (*Joung and Sander, 2013*).

CRISPR/Cas9 :

CRISPR/Cas9: robust and versatile toolkit
uses the simplified **single-guide RNA** (sgRNA) to direct the Cas9 protein to bind and cleave a particular DNA sequence for genome editing.

- sgRNA-engineered nucleases make precise modifications at specified locations in the genome.

Disease resistance in plants can be achieved by editing

- genome of pathogen or
- plant genes encoding susceptibility factors (S-genes)

CRISPR/Cas9

Shan et al.,(2013) compared the frequency of mutation induced by CRISPR/Cas9 and TALENs, and indicated that CRISPR/Cas9 is more efficient for inducing sequence-specific mutations in plants. **Prime editing** even more specific …….

Mushtaq et al (2019) : CRISPR/Cas9 leads other GE techniques (TALENs and ZFNs) due to its high efficiency, accurate targeting, relative simplicity and low risk of off-target effects.

However important to determine that inducing resistance to one pathogen e.g. Phytophthora does not increase susceptibility to others e.g. Botrytis

CRISPR/Cas9

Targeting Susceptibility Genes : S Genes in Plants

Bacterial S Genes : Xanthomonas : rice, Citrus,
Pseudomonas : Tomato

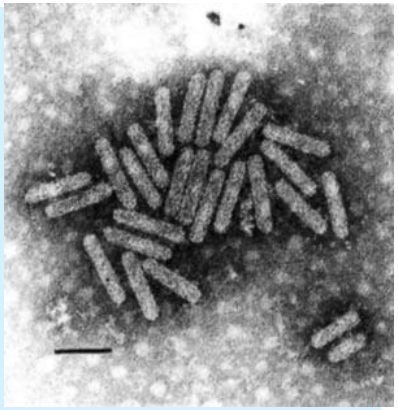
Fungal S Genes : Powdery mildew: Wheat and Grape
Downy mildew : Tomatoes
Phytophthera: Tomato, potato
Botrytis : tomato, fruits
Megaporthe : Rice Blast

- some activity against genes in guard cells to inhibit pathogen passage Tomato SlJA72 <> Pseudomonas

Useful for studying gene functions in host plants.

e.g. MLO R genes in wheat
Phytophthera pathogenicity in soy

CRISPR/Cas9



Targeting Pathogen genome:

- <u>Virus genome</u>: targeting genes associated with virus replication
- Large range of RNA and some DNA viruses
  e.g Gemini and Begemoviruses

Wide range of crops including Banana, Cotton, Cereals, Fruits, etc…

PlantEd

COST Action CA18111
Genome Editing in Plants

CRISPR Base editing (*Hess et al 2017*)

- Causes point mutations and used to target stop codons in genes for protein replication in viruses.
  (*Kuscu et al, Billon et al 2017)* .

- avoids DSBs and exploits mismatch repair pathways

- Caused targeted point mutations at multiple endogenous loci in rice and wheat (*Li et al., 2018*).

“ base editing can be employed to develop plants with immunity against different single and multiple pathogens by targeting and modifying the genome. Thus, base editing can open up new avenues for plant genome engineering. “
(Mushtaq et al 2019)

CRISPRi - CRISPR interference

Mutation of active sites of both nuclease domains, RuvC and HNH of Cas9, = dead endonuclease (dCas9)
retains the aptitude of DNA binding at sites defined by the guide RNA sequence and the PAM.
dCAS9 can be fused to regulatory elements such as proteins or RNA molecules for blocking transcription, binding of RNA polymerase, or transcription factor.

This technique is CRISPR interference (CRISPRi) : controls activation or down-regulation of transcription which depends on the specific site(s) recognized by the complex dCas9–guide RNA. (*Wally & Punja 2010*)

CRISPRi - CRISPR interference

- used to develop HIGS for targeting virulence genes and related systems in necrotrophic fungi such as production of oxalic acid in Sclerotinia and tricothecene mycotoxin in Fusarium
  (Wally and Punja 2010) .

- CRISPRi can target specific receptor and pathogenicity genes.

- Alternative to some GM RNAi (HIGS) systems exploiting cross talk between plant and pathogen RNA systems .
  (*Rodriguez-Marino et al. 2017*)

Activity and Efficacy

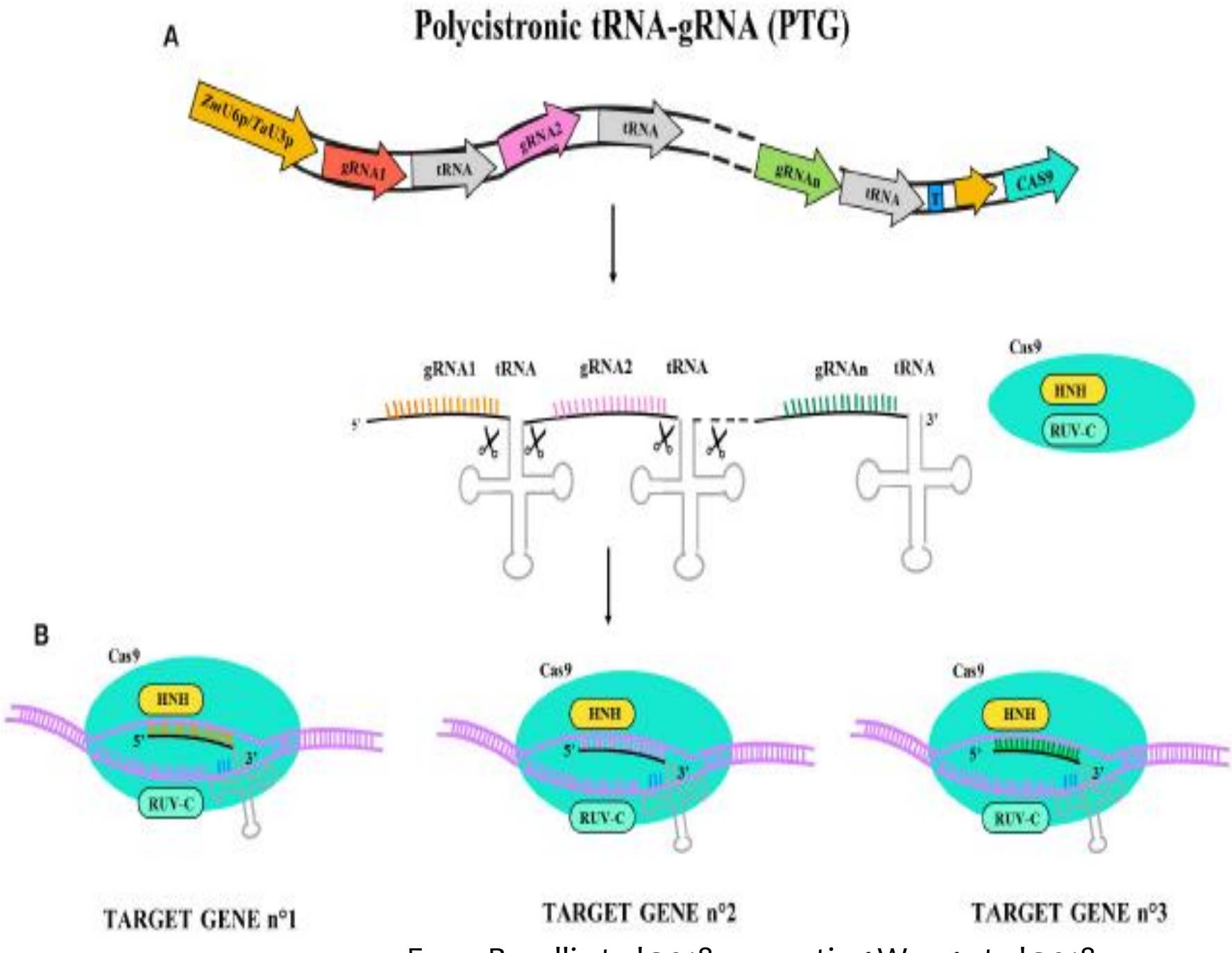
- These various CRISPR systems allow multiple approaches to pathogen control > multiple approaches for each pathogen .

  AND

- Combining approaches for a range of pathogens

- **Multiplexing** of systems using several guide RNAs and targeting of several pathogens.

- See fig


A
Polycistronic tRNA-gRNA (PTG)
ZmU6p/TaU3p
gRNA1
tRNA
gRNA2
tRNA
gRNAn
tRNA
T
CAS9
gRNA1 tRNA gRNA2 tRNA gRNAn tRNA
5'
3'
Cas9
HNH
RUV-C
B
Cas9
HNH
5'
3'
RUV-C
TARGET GENE n°1
TARGET GENE n°2
TARGET GENE n°3

Activity and Efficacy

Can also be combined with:

- **Traditional resistance breeding techniques**
- **GM pest and herbicide resistance**
- **GM RNAi systems targeting both plant genes and pathogen genes.**
- **These can be HIGS and SIGS : examined in iPlanta COST action CA15223**

- **SDNs : Limited range of target pathogens at present but increasing ……**

IMPACTS OF GE DISEASE RESISTANCE SYSTEMS

IMPACT DEPENDS ON EFFICACY, DURABILITY, COST, ENVIRONMENTAL IMPACT AND ACCEPTANCE

EFFICACY : to be determined case by case

DURABILIY: depends on MANAGEMENT :

- **Multiplexing systems so that they operate both through the plant and the pathogen and target different genes and loci in both .**
- **Need to vary systems in new crop variety successions and across landscapes.**

€/$: Research, Development and Commercialisation: case by case but is it likely to be higher ?

IMPACTS OF GE DISEASE RESISTANCE SYSTEMS

- **Environmental Impact : case by case but usually same as conventional breeding. Reduce use of pesticides and fungicides.**

- **Acceptance : GE resistance systems fit well with integrated Pest and Disease management systems (IPM) and into Sustainable agriculture .**

PlantEd

COST Action CA18111
Genome Editing in Plants

Thank you **Хвала**

jeremysweet303@aol.com

cost
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY