CRISPR/Cas9-mediated genome editing of *VvDMR6* confers dual stress tolerance in grapevine

Presented by Clara Holm

VITIS LABORATORY

Stellenbosch UNIVERSITY IYUNIVESITHI UNIVERSITEIT

Genetics Department 1925–2025

Background

South Africa **4th largest global exporter**

Challenges faced:

- Biotic (pathogens, diseases) and abiotic stress factors (drought, salinity, high temperatures)
- Sustainable production practices
- Consumer demands
- Highly competitive international market

Solution: Developing cultivars with combined resistance to biotic and abiotic stress



Image sources: SATI; WoSA; C Holm; WineBussinessMonthly; AWRI

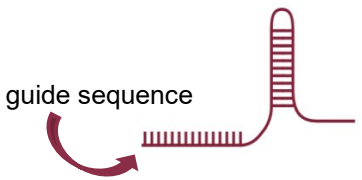
CRISPR/Cas9 genome editing technology

- Conventional breeding strategies introduce resistance traits from wild relatives
  Time consuming; up to 20-30 years

- New breeding technologies offers an efficient and more targeted approach to developing improved varieties

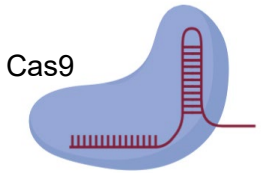
Clustered Regulatory Interspaced Short Palindromic Repeats/CRISPR-associated protein 9

1 Target design



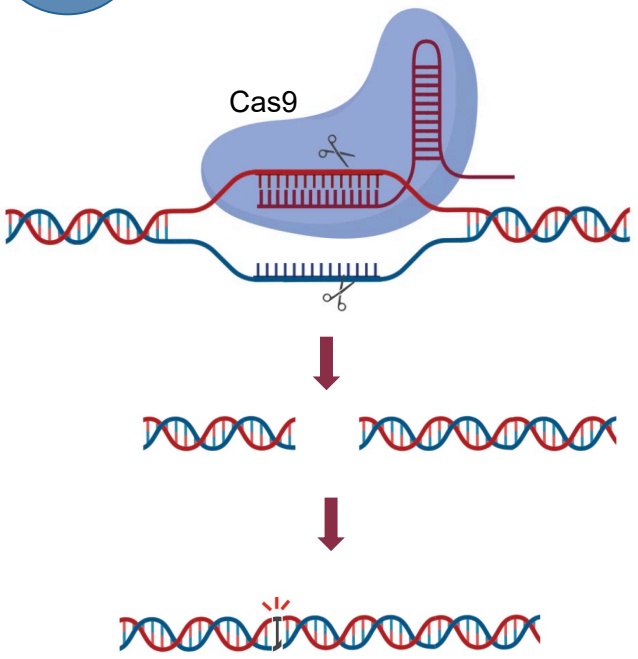
A "guide RNA" is designed in the lab that matches the grapevine DNA sequence that will be modified

2 Cas9 protein



The guide RNA is combined with a Cas9 protein, which acts as the molecular scissors to cut the DNA of interest, and is introduced into cell

3 CRISPR/Cas9 editing



The guide RNA leads the Cas9 protein to the grapevine target DNA sequence

Cas9 "cuts" the DNA

Small mutations are introduced naturally when the DNA repairs

Desired gene is "switched off"

The gene of interest

Downy Mildew Resistant 6 (*DMR6*) gene

Described as a **susceptibility factor**

Targeted inactivation leads to **broad-spectrum disease resistance** in tomato, potato, citrus, and grapevine

Evidence of its role during **abiotic stress**

DMR6-edited plants show improved tolerance to **drought** and **salinity stress** in tomato and potato

Promising target to achieve **dual-stress tolerance** in grapevine

Loss of function of a DMR6 ortholog in tomato confers broad-spectrum disease resistance

Daniela Paula de Toledo Thomazella[a,b], Kyungyong Seong[a], Rebecca Mackelprang[a], Douglas Dahlbeck[a,b], Yu Geng[a], Upinder S. Gill[c], Tiancong Qi[a,d], Julie Pham[b], Priscila Giuseppe[e], Clara Youngna Lee[a], Arturo Ortega[a,b], Myeong-Je Cho[b], Samuel F. Hutton[f], and Brian Staskawicz[a,b,1]

Mutations introduced in susceptibility genes through CRISPR/Cas9 genome editing confer increased late blight resistance in potatoes

Nam Phuong Kieu[1], Marit Lenman[1], Eu Sheng Wang[1], Bent Larsen Petersen[2] & Erik Andreasson[1]✉

Editing the *CsDMR6* gene in citrus results in resistance to the bacterial disease citrus canker

Saroj Parajuli[1], Heqiang Huo[2], Fred G. Gmitter Jr[3], Yongping Duan[4], Feng Luo[5] and Zhanao Deng[1,*]

Aim

Achieve CRISPR/Cas9 genome editing of *VvDMR6* in grapevine for enhanced downy mildew resistance, and investigate whether this extends to abiotic stress in grapevine

1 Objective

Generate *VvDMR6.1*-mutant lines through CRISPR/Cas9

2 Objective

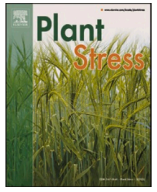
Assess resistance to downy mildew and physiological responses under water-limiting conditions

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Contents lists available at ScienceDirect

Plant Stress

journal homepage: www.sciencedirect.com/journal/plant-stress



CRISPR/Cas9-genome editing identifies the dual role of *VvDMR6.1* in downy mildew resistance and response to water limitation in grapevine

Clara Cornelia Holm [a], Minette Havenga [b], Justin Graham Lashbrooke [a], Johan Theodorus Burger [a], Manuela Campa [a,*]

[a] Department of Genetics, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa

CRISPR/Cas9 Genome Editing of *VvDMR6*

1 Target design and vector cloning

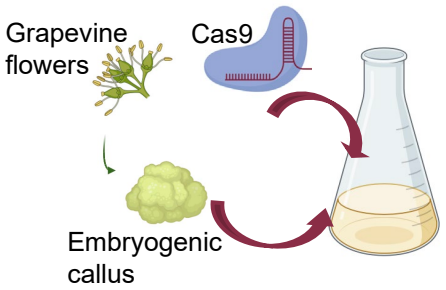


Guide sequence designed to target the *VvDMR6.1* gene in grapevine



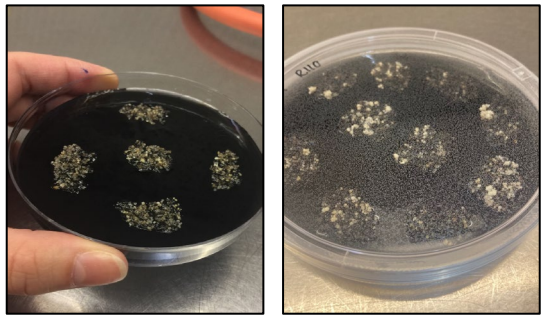
Cloning of *VvDMR6.1*-guide into a CRISPR/Cas9 vector

2 Delivery



The CRISPR/Cas9 system is delivered into undifferentiated grapevine cells (embryogenic callus) obtained from their flowers through *Agrobacterium*-mediated transformation

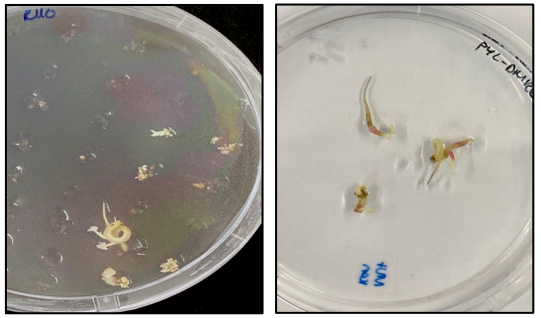
3 Selection of edited material



Transformed cells are monitored on selection media

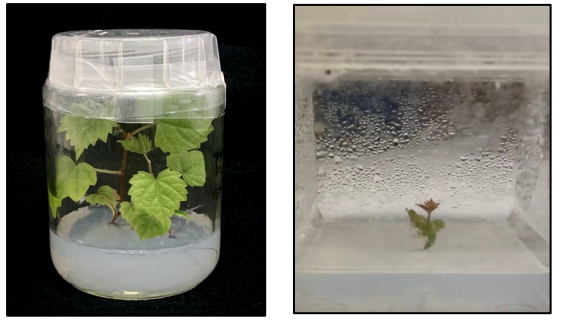
Edited cells will regenerate

4 Embryo formation



Embryos develop after 4-6 months and roots and shoots form

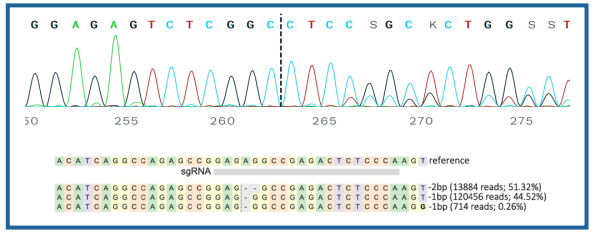
5 Plant regeneration



VvDMR6.1-edited lines regenerate

±12-14 months

6 Screening of edited lines



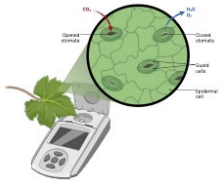
Lines are sequenced to determine the editing efficiency, and type of mutations induced

7 Assessments with edited lines



Downy mildew leaf disc assay

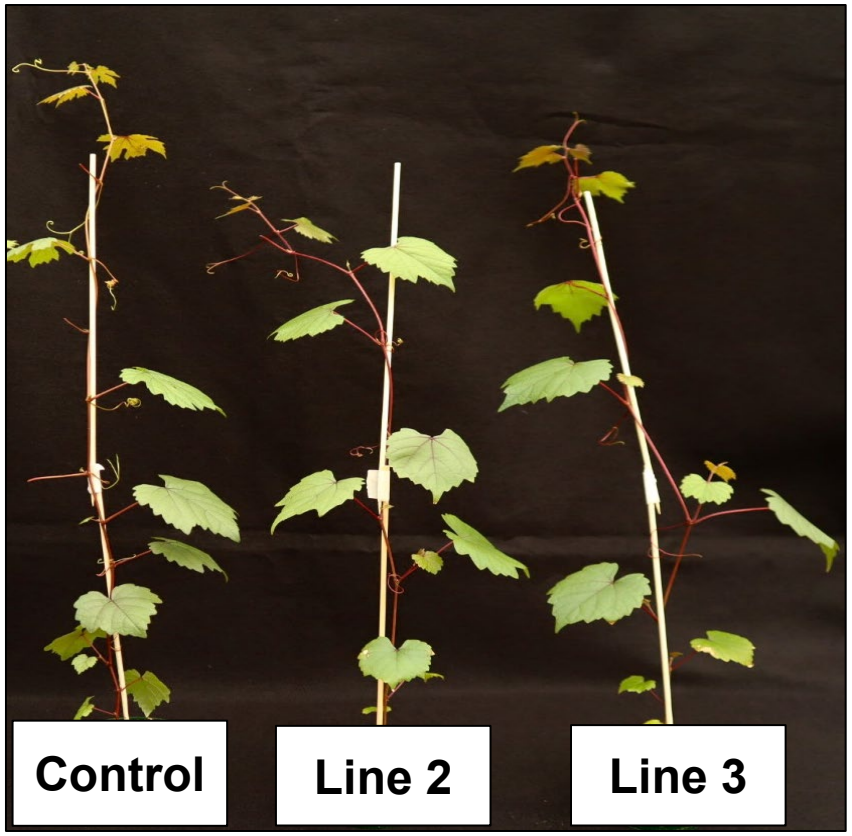
Disease
Drought
Resilience

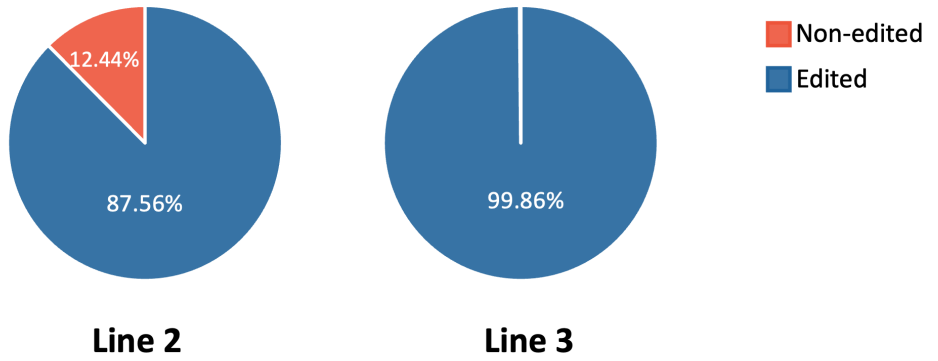


Water deficit analysis

Results

- Successful editing of *VvDMR6.1* in R110:





	Editing efficiency	Editing profile
Line 2	87.56%	-1bp;-2bp
Line 3	99.86%	-1bp; -2bp

Objective 1 Successfully generate *VvDMR6.1*-knockout lines through CRISPR/Cas9

Results

- *VvDMR6.1* knockout improves downy mildew resistance:

Control

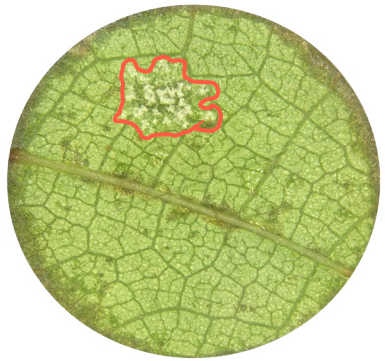


a

b

b

Line 2



a

Line 3



b

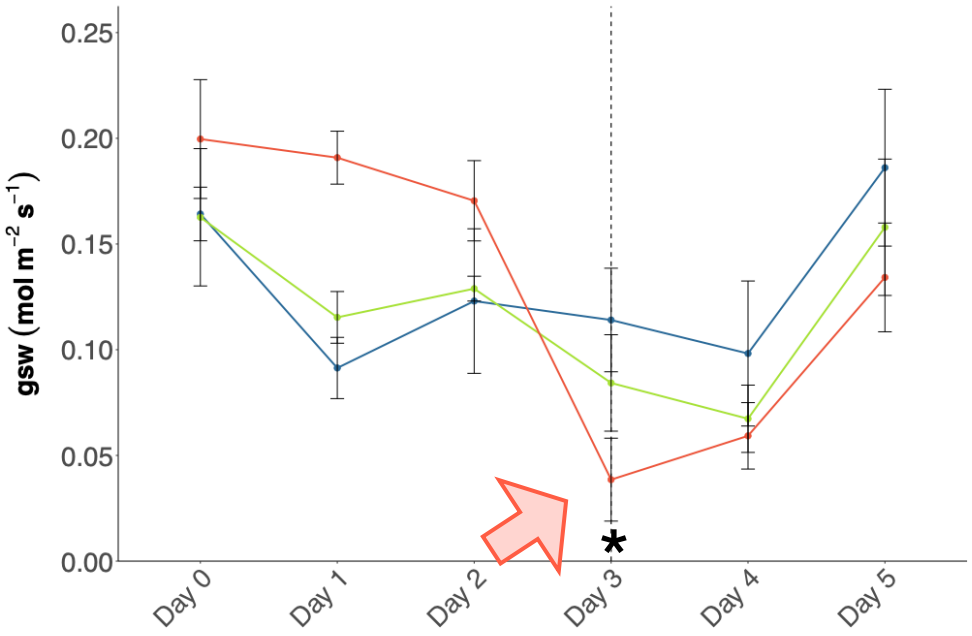
b

Objective 2

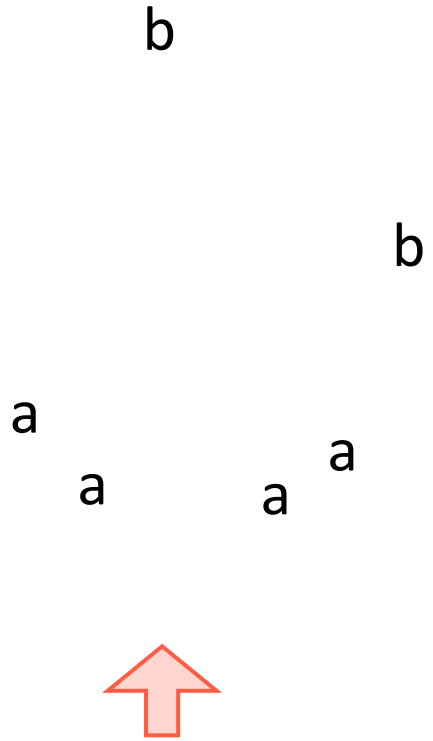
Knockout lines demonstrate improved resistance to downy mildew

Results

- Editing of *VvDMR6.1* alters stomatal conductance and ABA accumulation under water deficit conditions:



* control vs Line 3

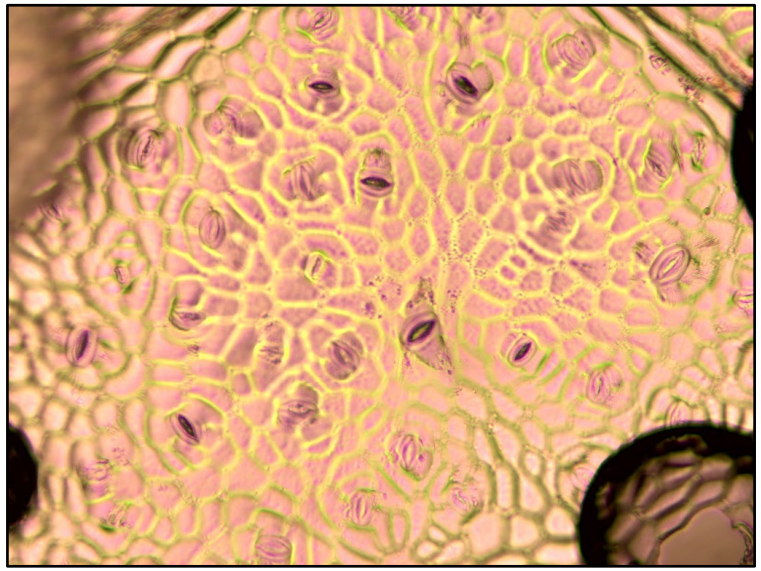


Objective 2 Knockout lines demonstrate preliminary dual-role in abiotic stress

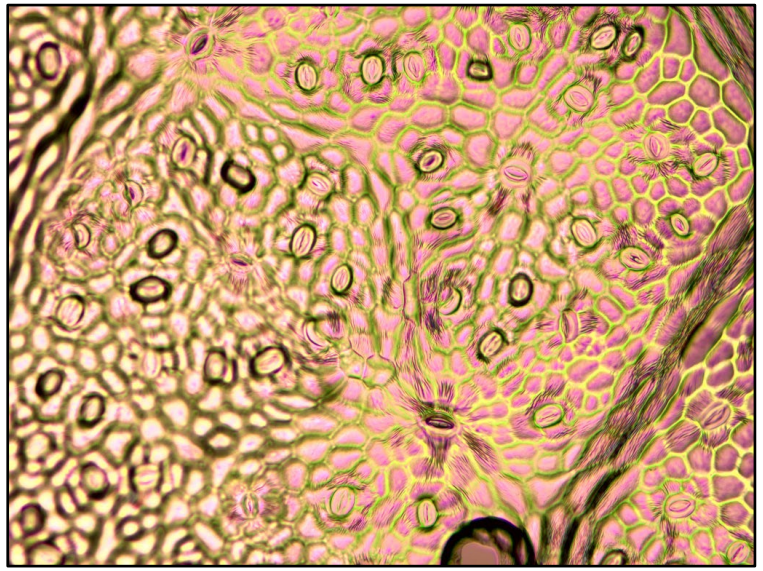
Results

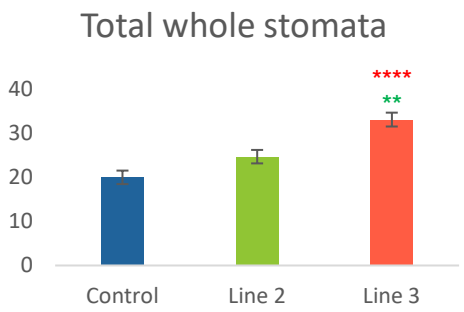
- Editing of *VvDMR6.1* alters stomatal morphology:

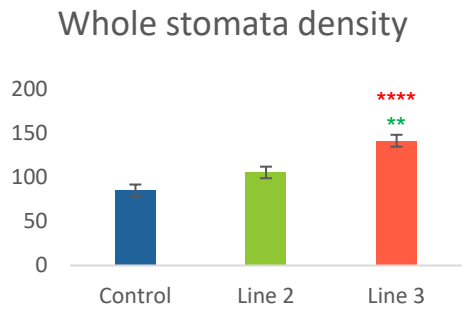
Control

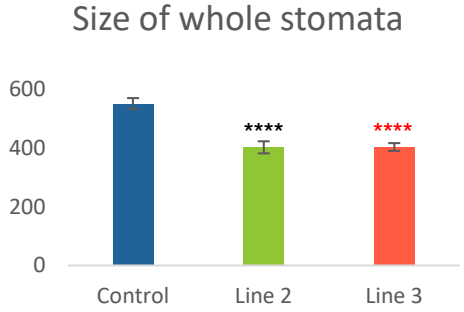


Line 3









* control vs Line 2

* control vs Line 3

* Line 2 vs Line 3

In Summary:

- *VvDMR6.1*-edited plants more sensitive to environmental stimuli
- First report identifying *VvDMR6.1* as promising target for developing dual-stress resilience in grapevine



Future research:

- Assess plants under field conditions
- Multi-stress resilience
- Downstream effects on growth and productivity
- Does the *VvDMR6.1*-edited rootstock confer benefits to the grafted scion?

Where do we go from here?

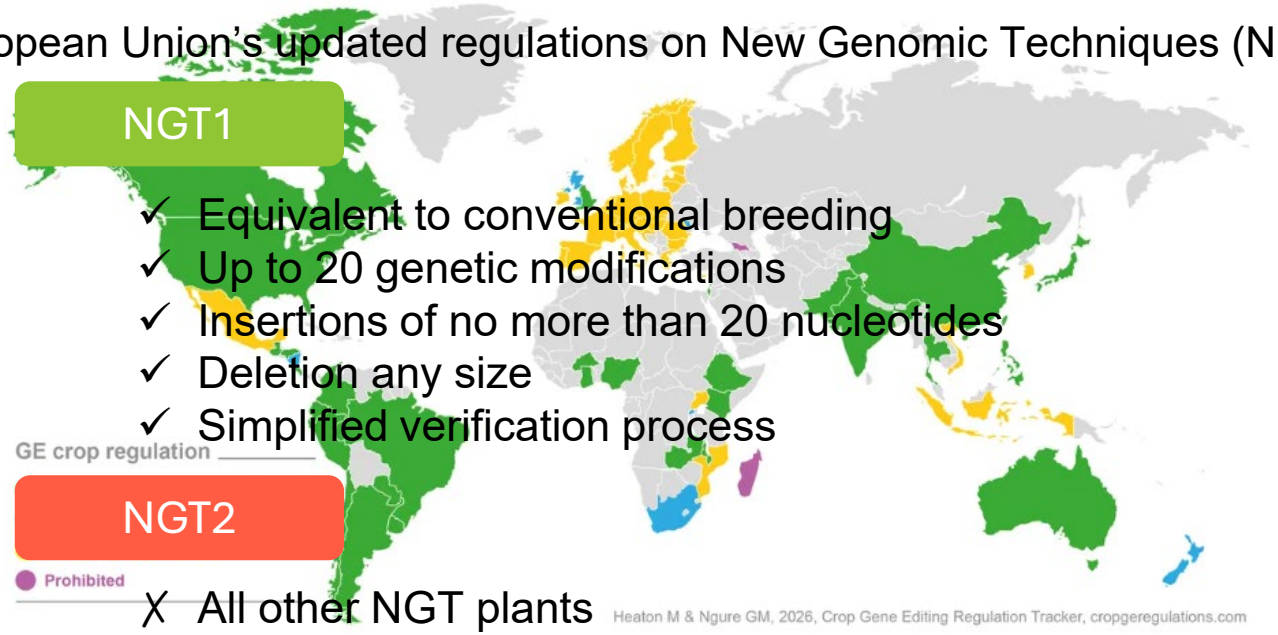
Current limitations:

- GM-status of edited plants

  Agrobacterium-mediated delivery

  Implementation of DNA-free methods

European Union's updated regulations on New Genomic Techniques (NGT)



- ✓ Equivalent to conventional breeding
- ✓ Up to 20 genetic modifications
- ✓ Insertions of no more than 20 nucleotides
- ✓ Deletion any size
- ✓ Simplified verification process
- ✗ All other NGT plants
- ✗ GMO-like authorization

Long-term implications

- Development of cultivars with greater resilience to combined stresses

Take home message

- First successful application of CRISPR/Cas9 in a woody crop in South Africa
- Major milestone for plant biotechnology in Africa
- Foundation for future research, expansion of genome editing
- New breeding technologies will become increasingly important - investment in biotech, innovation





