Can gene editing provide solutions to plant pathogen sensitivity ?

Erik Andreasson

SLU Alnarp

Resistance biology

Acknowledgements

Awais Zahid

Nam Kieu Phuong

Marit Lenman

Svante Resjö

Naga Charan

Milla Karlsson

Frida Meijer Carlsen (KU)

Bent L Petersen (KU)

All others at the resistance biology unit

MISTRA Biotech

novo nordisk fonden

FORMAS

KUNGL. FYSIOGRAFISKA SÄLLSKAPET I LUND

SCIENCE AND EDUCATION FOR SUSTAINABLE LIFE

Resistance genes (R-genes) – make the plants more resistent

- Reception of the pathogen
- Co-evolvement with pathogens can give resistance loss
- For many pathogens no correponding R genes are known

Suseptiblity genes (S-genes) -make the plant more sensitive to infection

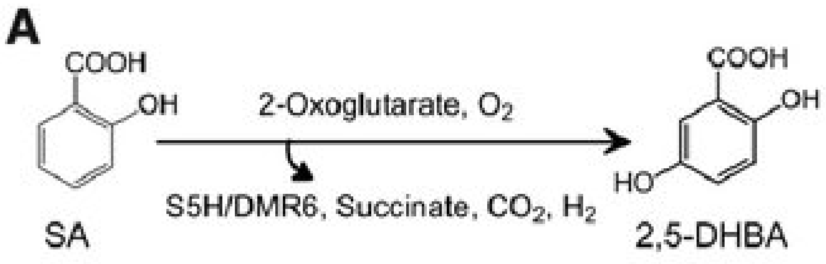
- Negative regulation of defence (often salicylic acid (SA) related)
- Transport of nutrients to pathogens
- Inactivation gives recessive resistance
  - Genomeditering (CRISPR/Cas9) or looking for mutants

  Negative effects by mutations?

Function of DMR6 –A known sensitivity gene

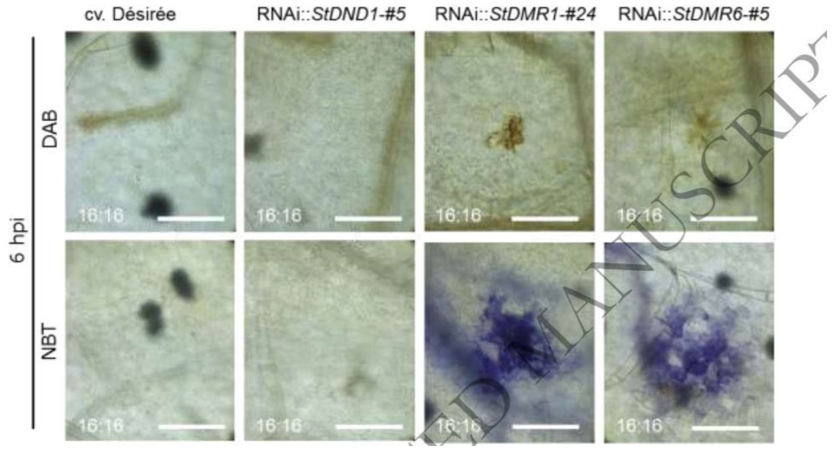
- DMR6 catalyze the conversion of SA to 2,5-DHBA on Arabidopsis and Tomato

(Zang el al. 2017; Thomazella DPT et. al. 2021)

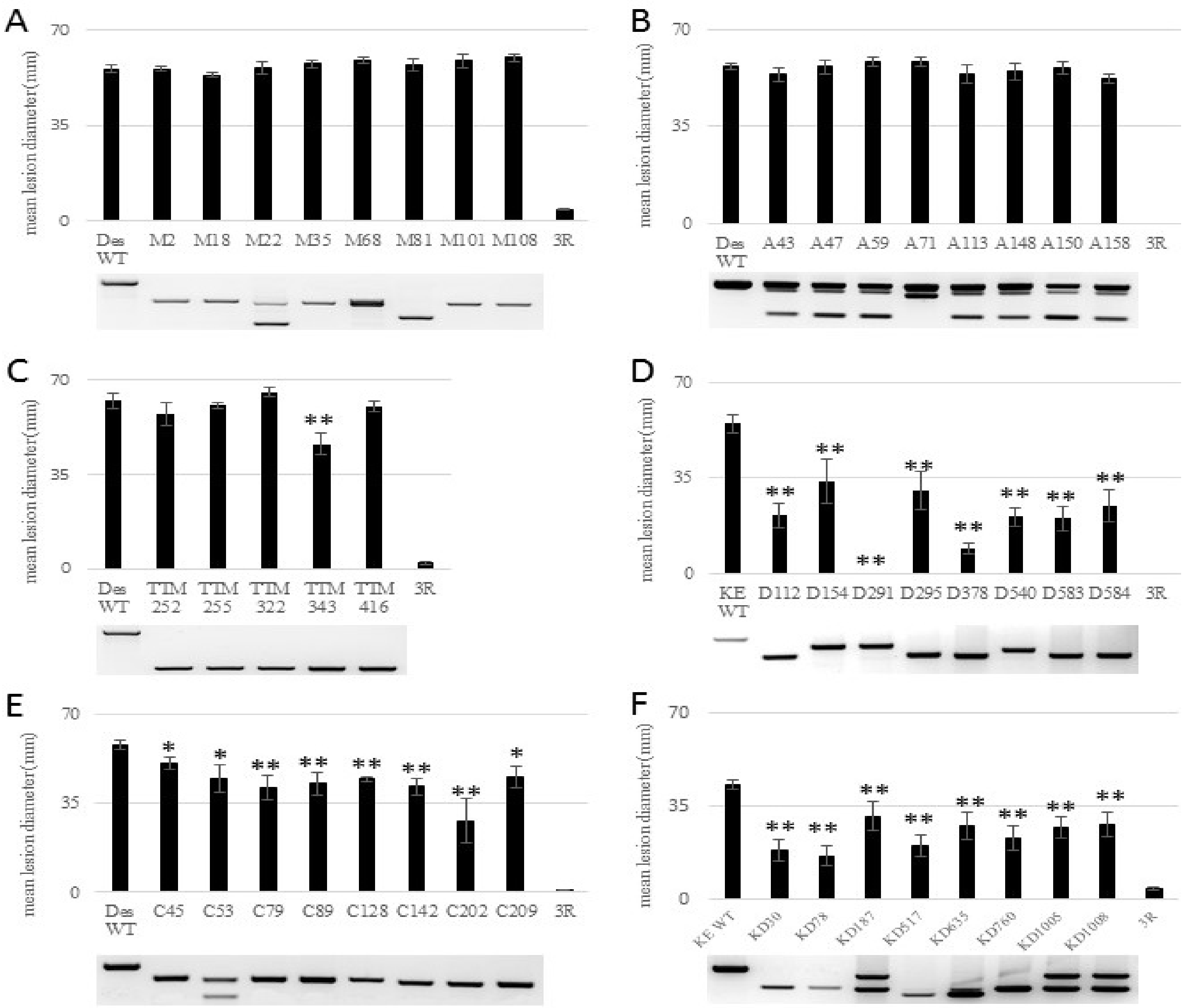


- StDMR6-silenced plants display early ROS accumulation after late blight attack

(Sun K et. al. Manuscript 2022)

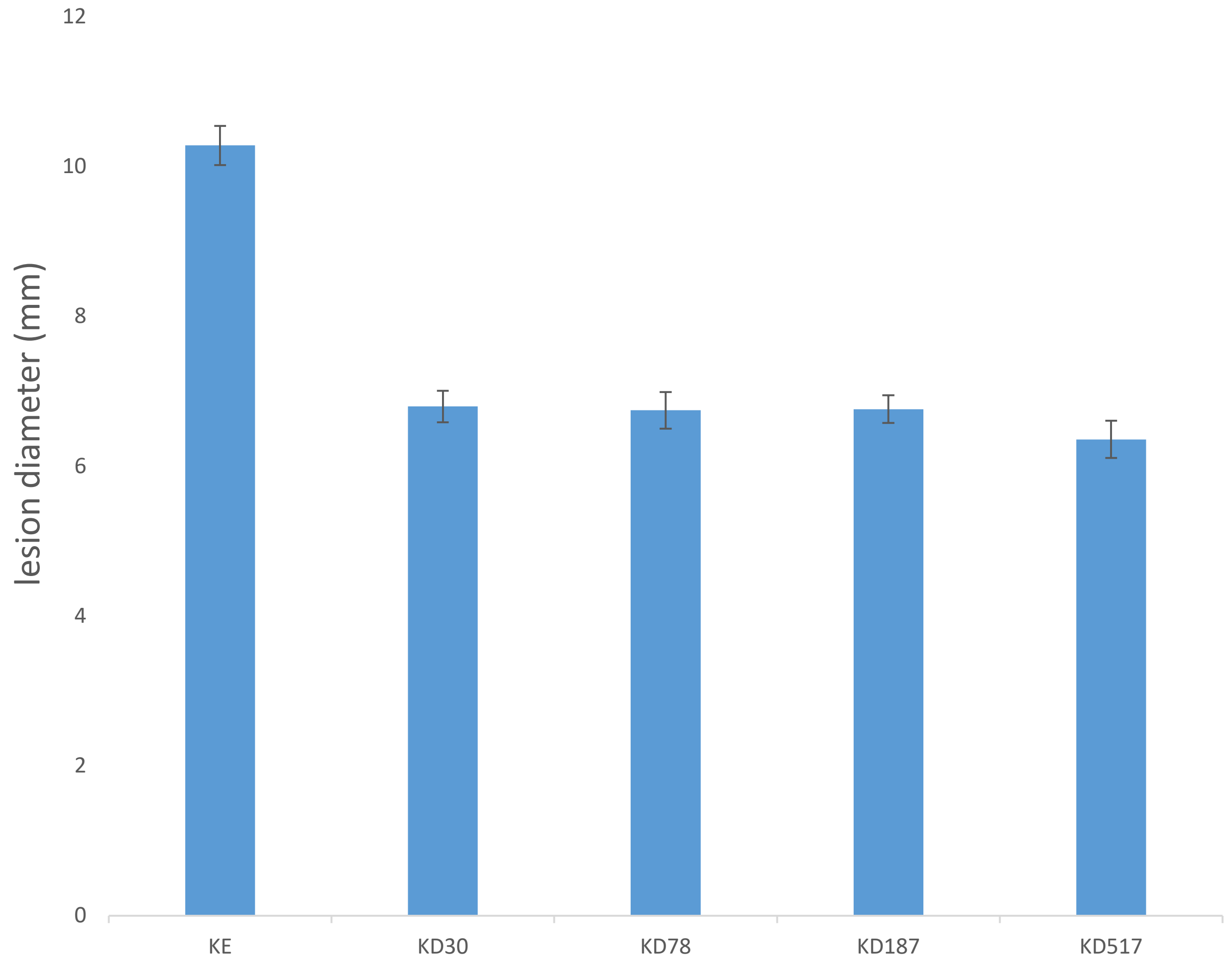


Screening of CRISPR/Cas9 generated S gene deletions in King Edward potato by lesion diameter of late blight



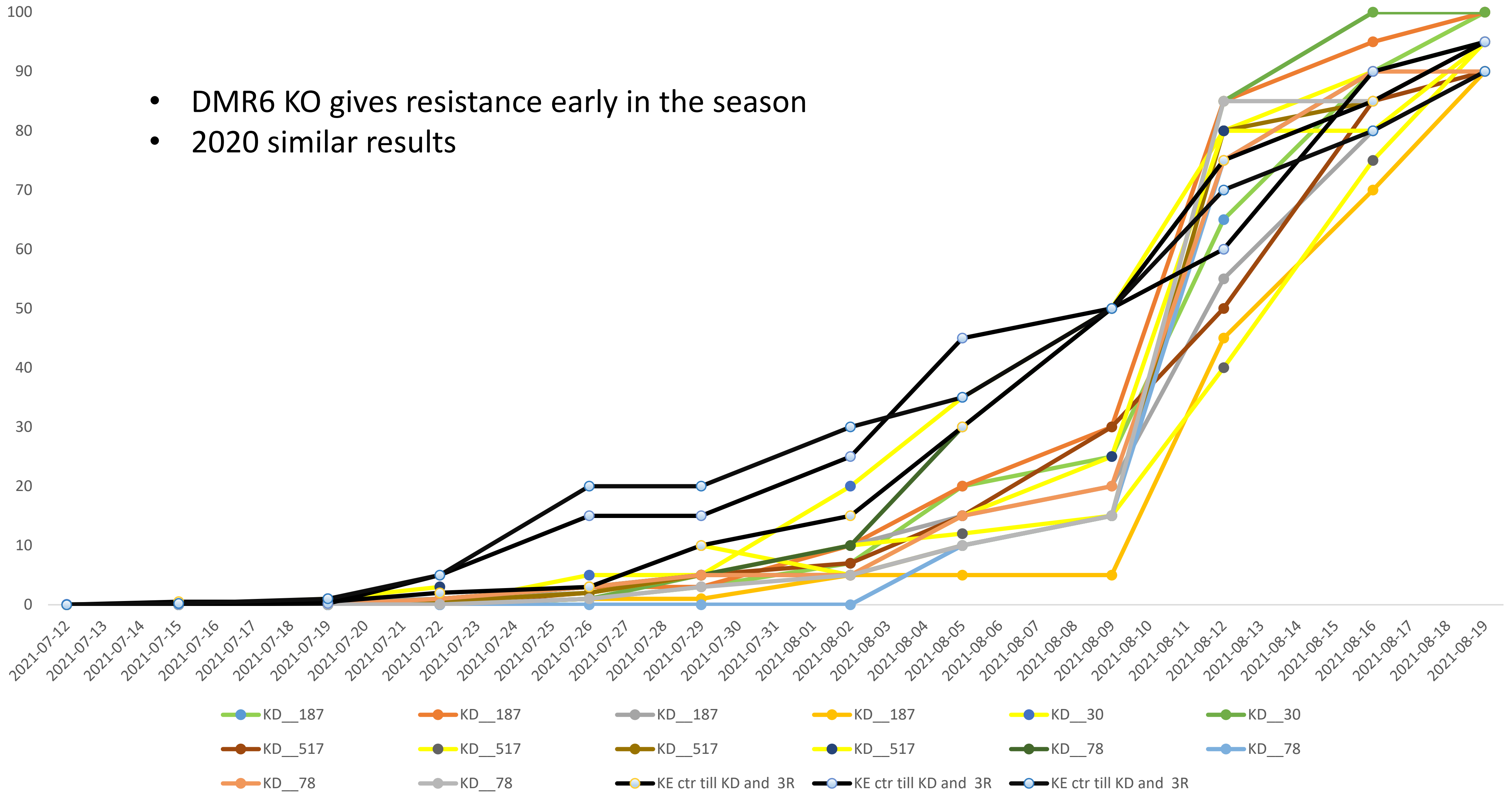
Scientific Reports Kieu et al 2021

DMR6 KO (KD) result in less Early blight (Alternaria solani) in controlled conditions



Late blight scoring of field trials in DMR6 CRISPR KO King Edward potato 2021 (black are controls)

- DMR6 KO gives resistance early in the season
- 2020 similar results

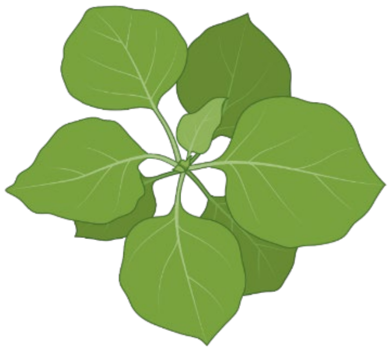


New susceptibility gene -From laboratory to field

Awais Zahid

Proteomics experiment

in potato leaves



Functional study of

proteomics candidate in a

model plant *Nicotiana*

benthamiana

Deletion of a new susceptibility

Gene via CRISPR-cas9 in

Potato

2020

Potato field trial

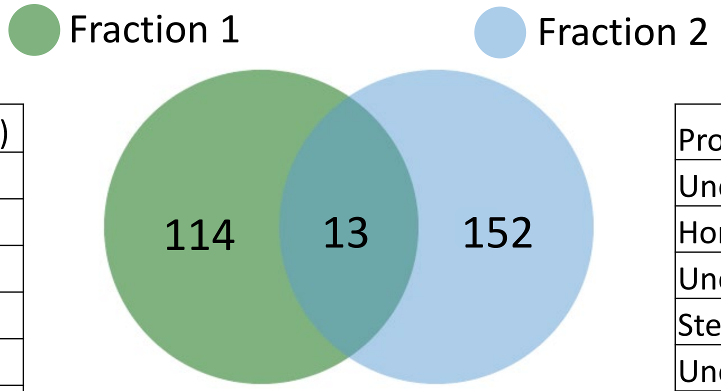
Borgeby, Sweden

2021

Potato proteomics after activation of immunity reactions

Fraction 1

Protein Names	Fold change (log$_2$)
Mitochondrial SBP40	4.0
SRC2-like protein	3.6
Rop guanine nucleotide exchange factor	3.4
Sucrose synthase	3.2
Alliin lyase	3.2
CC-NBS-LRR protein	3.2
Uncharacterized protein	3.1
30S ribosomal protein S8	2.5
Integrase core domain	2.3
PSI-H	2.2
Chlorophyll a-b binding protein (LHCB3)	-5.6
ATP synthase subunit c	-2.8
Hydrolase	-2.2
Photosystem II reaction (PSII-L)	-2.0
GDP-mannose 4,6-dehydratase	-1.9
32 kDa thylakoid membrane protein	-1.8
Thioredoxin-dependent peroxiredoxin	-1.7
Chlorophyll a-b binding protein	-1.7
NAD(P)H-quinone oxidoreductase	-1.6
Uncharacterized protein	-1.6



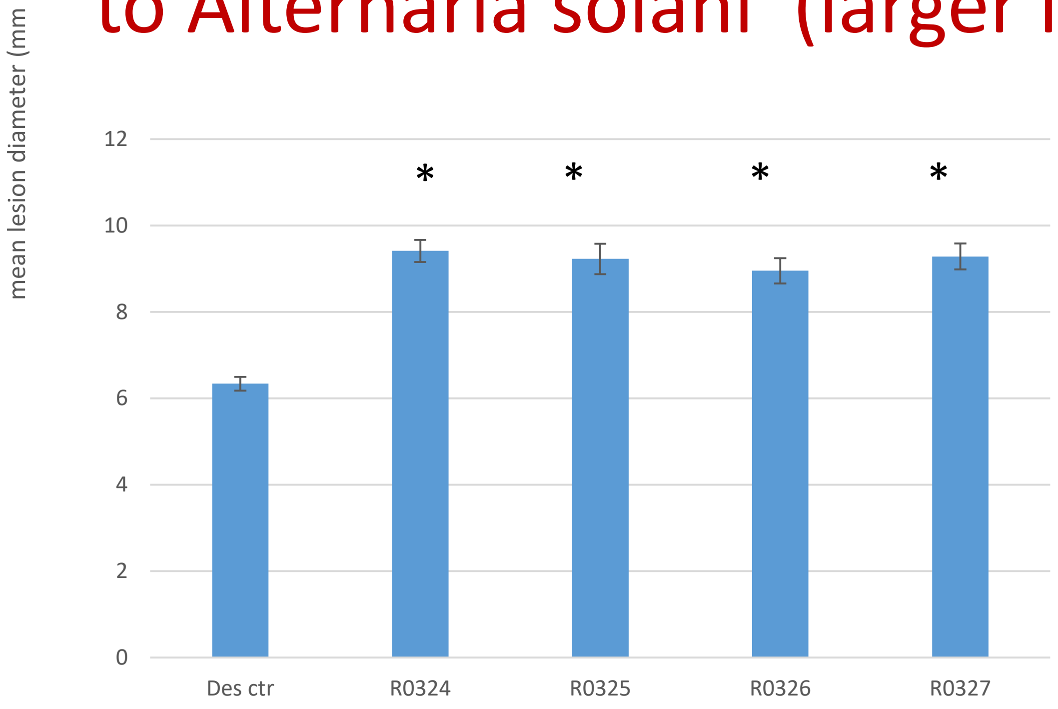
Fraction 2

Protein names	Fold change (log$_2$)
Uncharacterized protein	5.6
Homeobox-leucine zipper protein	4.7
Uncharacterized protein	4.1
Stem 28 kDa glycoprotein	3.3
Uncharacterized protein	3.2
PS II reaction center protein L	3.0
3-isopropylmalate dehydratase	2.9
4F5 protein family protein	2.8
4F5 domain-containing protein	2.8
Plastocyanin	2.8
5'-3' exoribonuclease	-6.2
NAD_Gly3P_dh_N	-4.6
Mitogen-activated protein kinase	-2.5
Chlorophyll a-b binding protein	-2.4
Histone H2B	-2.3
BZIP domain-containing protein	-2.2
Hexose transporter	-2.1
3-oxoacyl-CoA synthase	-2.0
Poly(RC)-binding protein	-2.0
Pre-mRNA-splicing factor cwc23	-1.9

New S genes and functions

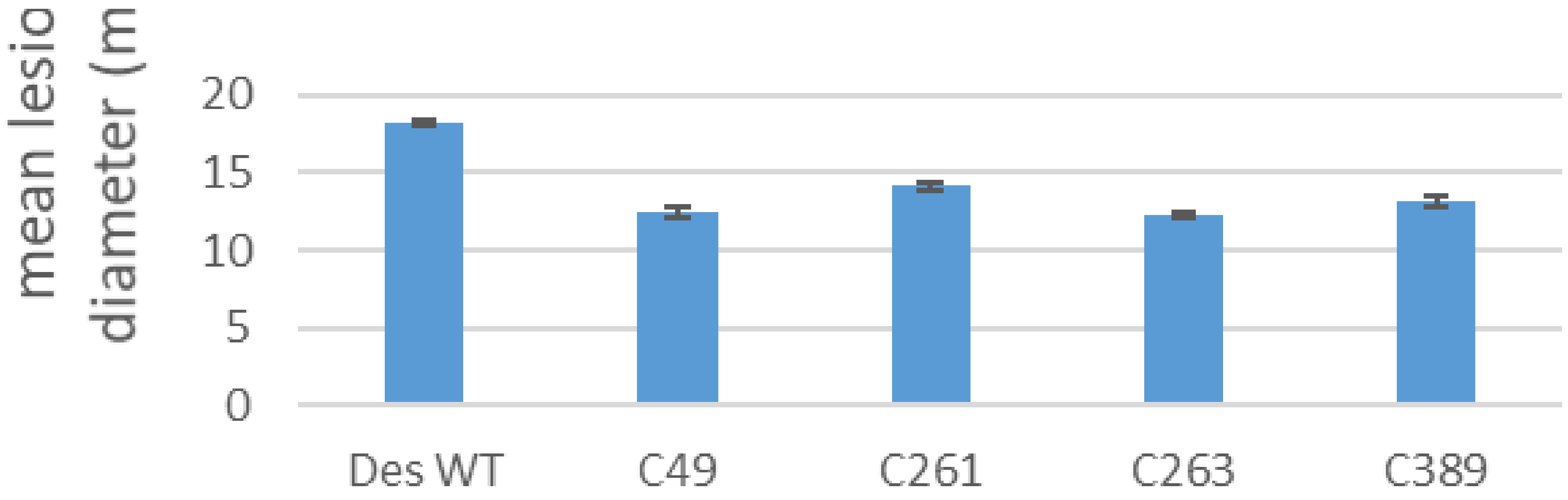
- DMR6 affects salicylic acid degradation, and have stress markers activated without stress
- One (SAs1) in the apoplast (unpublished, only Alternaria?)
- One (SPi1) in the thylakoid lumen (patent application) Broad spectrum resistance and in crops and also salt stress

Potato overexpressing SAs1 have a suseptible phenotype to Alternaria solani (larger lesions)

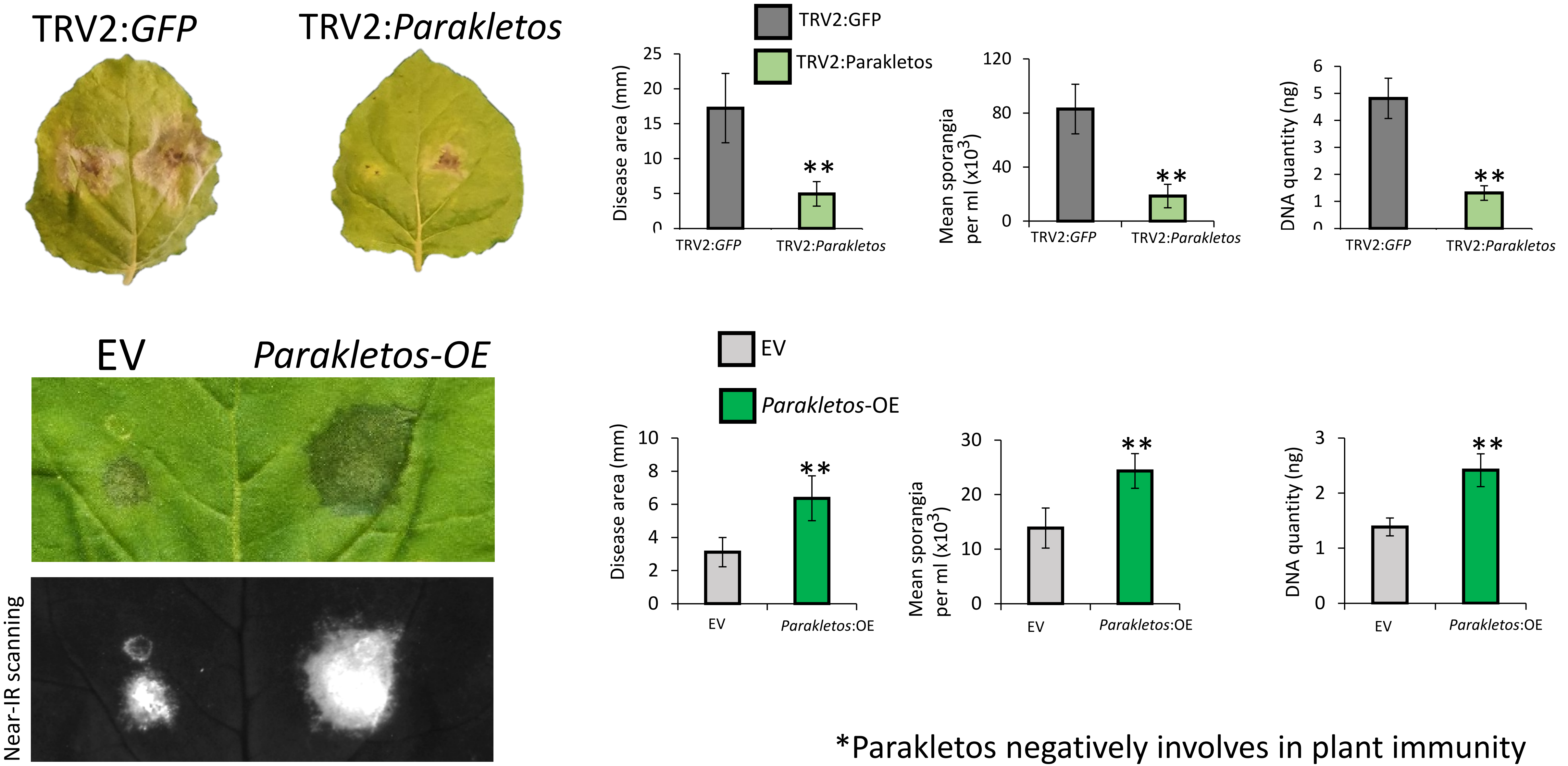


Des WT R0324
Leaf no.3 (bottom to top)

CRISPR-SAs1 Knock out Potato plants inoculated with Alternaria solani are more resistant to Early blight (torrfläckssjuka)

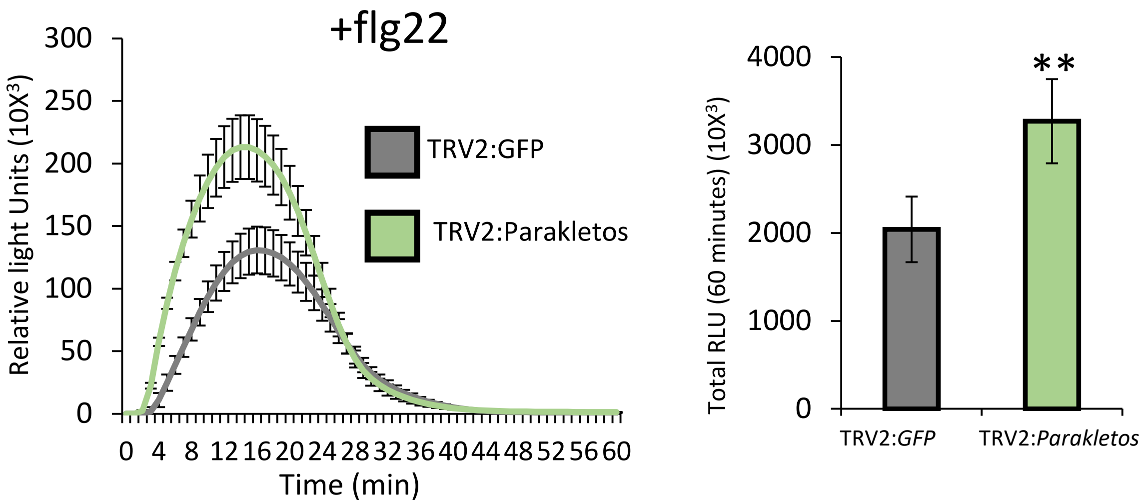


Functional test of candidate proteins reveals Spi1

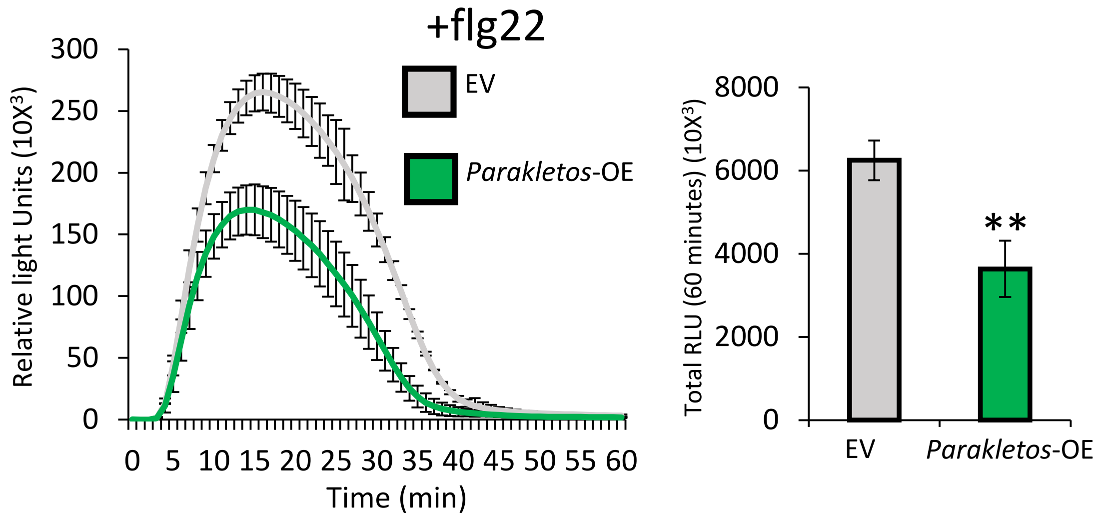


*Parakletos negatively involves in plant immunity

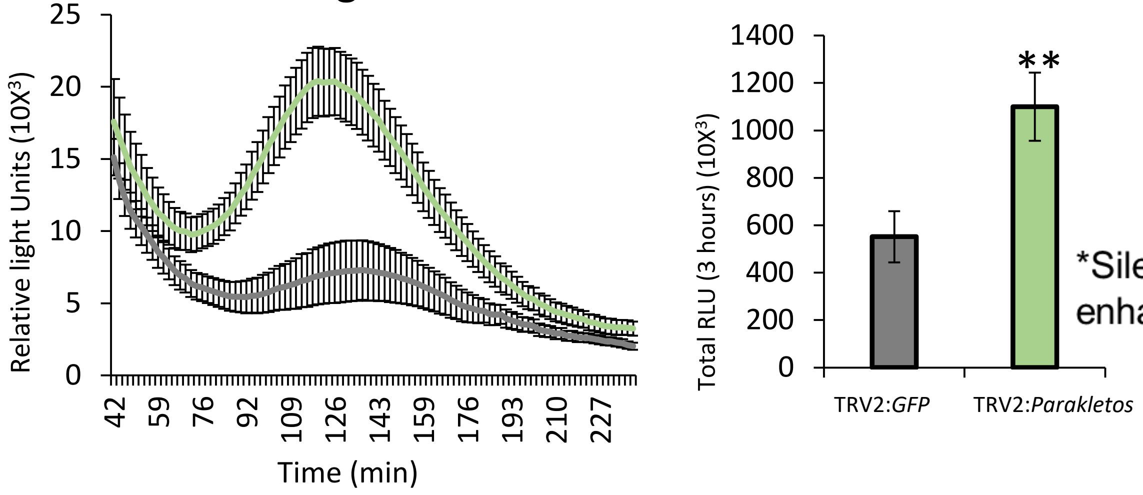
Spi1 suppresses ROS burst



*Silencing of Parakletos enhances 1st ROS burst

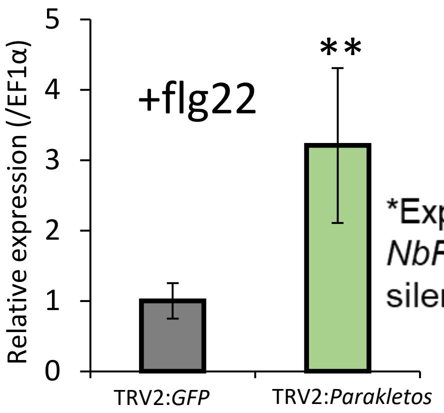


**Parakletos*-OE suppresses 1st ROS burst



*Silencing of Parakletos enhances 2nd ROS burst

NbRBOHB



*Expression of ROS generating *NbRBOHB* increases in Parakletos silenced plants

Summary

- Gene editing by CRISPR/Cas9 of potato is efficient

- Increased resistance to pathogens and salt have been achieved by inactivation of S genes (susceptibility genes)

- So far no negative agricultural effects of mutation of these S genes has been detected in potato