Breaking speciation barriers through random and CRISPR/Cas9-directed mutagenesis in plants

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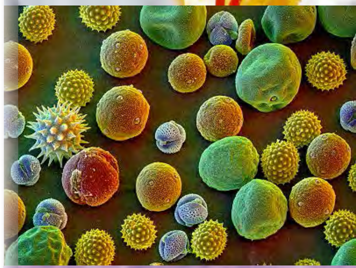
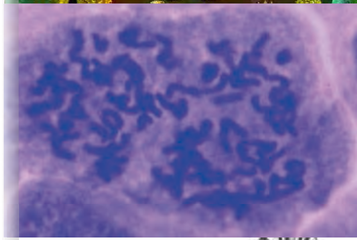
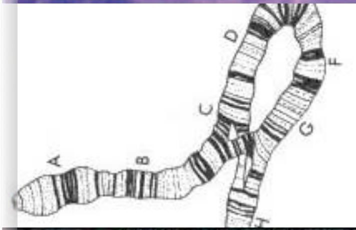
www.ub.edu/portal/web/dp-bsma/stress

Reproductive isolation (RI) barriers in plants

Pre-zygotic: - pre-pollination barriers
- pollen recognition

Post-zygotic: - polyploidy
- large chromosome rearrangements
- epistatic interactions between parental alleles (Dobzhansky-Muller genetic incompatibilities)


The Dobzhansky-Muller (D-M) model of genetic incompatibilities

STUDIES ON HYBRID STERILITY. II. LOCALIZATION OF STERILITY FACTORS IN DROSOPHILA PSEUDOOBSCURA HYBRIDS

TH. DOBZHANSKY

California Institute of Technology, Pasadena, California

Received October 19, 1935

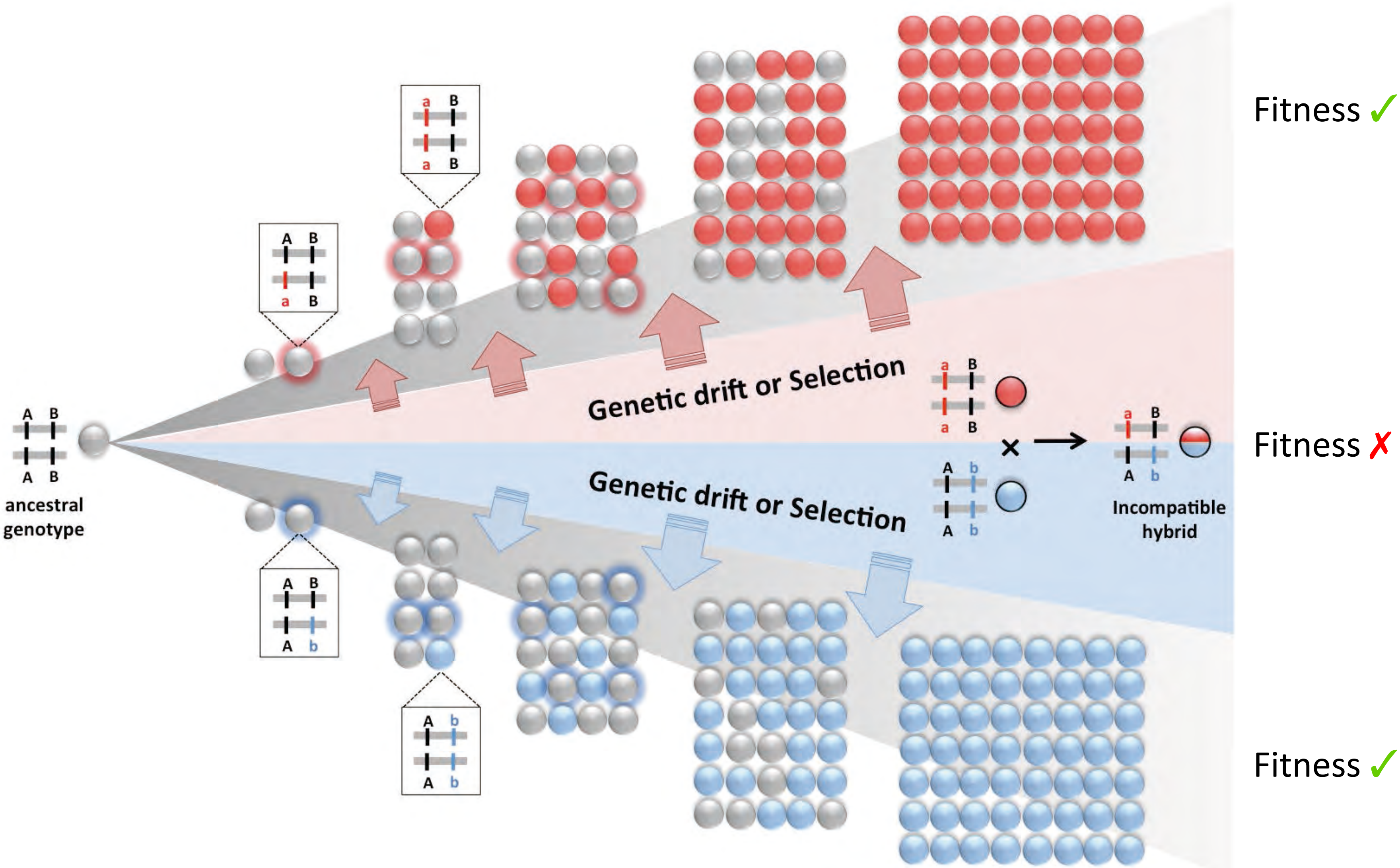
THE PROBLEM

THE causation of hybrid sterility has long been one of the unsolved problems of biology. To date, probably the greatest advance in this field has been made by FEDERLEY who discovered a failure of the meiotic chromosome pairing in the sterile hybrids between moths of the genus Pygaera. This finding has since been amply corroborated by other investigators in sterile hybrids both in animals and in plants. Naturally enough it became tempting to suppose that the failure of the meiotic pairing is the cause of hybrid sterility. The restitution of the normal meiotic pairing as well as of fertility following the reduplication of the chromosome complement in allotetraploid hybrids seems to be further evidence in favor of this view. And yet, this view proves to be inadequate as a general explanation of hybrid sterility. Two difficulties deserve particular attention. First, some hybrids are sterile despite the fact that the meiotic pairing in their gametogenesis is apparently normal (MICHAELIS 1933 in Epilobium, DOBZHANSKY 1934 in some crosses in *Drosophila pseudoobscura*); while in other sterile hybrids the gametogenesis does not reach the meiotic stages (KERKIS 1933, in *Drosophila melanogaster* × *D. simulans*). Second, the failure of the meiotic pairing in sterile hybrids is usually attributed to an "incompatibility" of the chromosomes of species or races entering the cross. This, clearly, is a restatement of facts and not a causal explanation. It remains possible that suppression of meiotic pairing may be caused by different mechanisms in different cases, and that sometimes there is no cause and effect relation between the failure of pairing and the sterility.

In my previous publications (DOBZHANSKY 1933, 1934) a hypothesis was suggested according to which there exist at least two different types of hybrid sterility—the chromosomal type and the genic type. The chro-

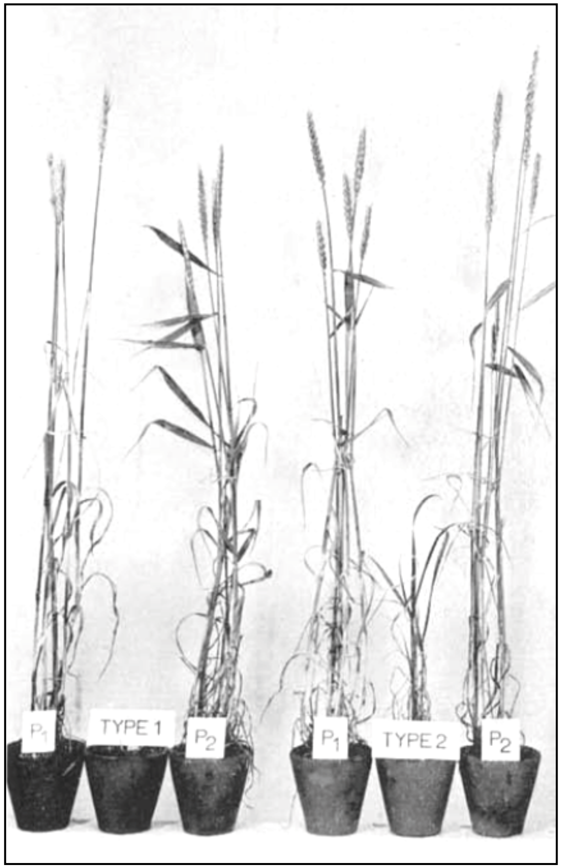
Theodosius Dobzhansky
1900 - 1975

The Dobzhansky-Muller (D-M) model of genetic incompatibilities



The study of RI in plants: from crops to model species

Blat, Trigo, Wheat



Euphytica 16 (1967) : 134-162

Euphytica 16 (1967): 134–162

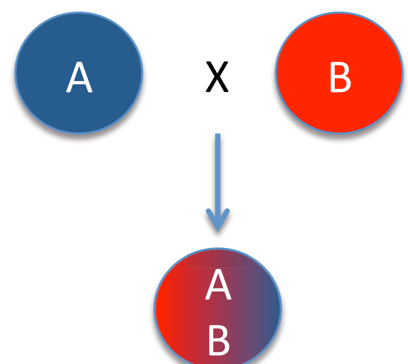
HYBRID DWARFNESS IN WHEAT

J. G. TH. HERMSEN
Institute of Plant Breeding, Wageningen, The Netherlands

With 5 figures

Received 3 Jan. 1967

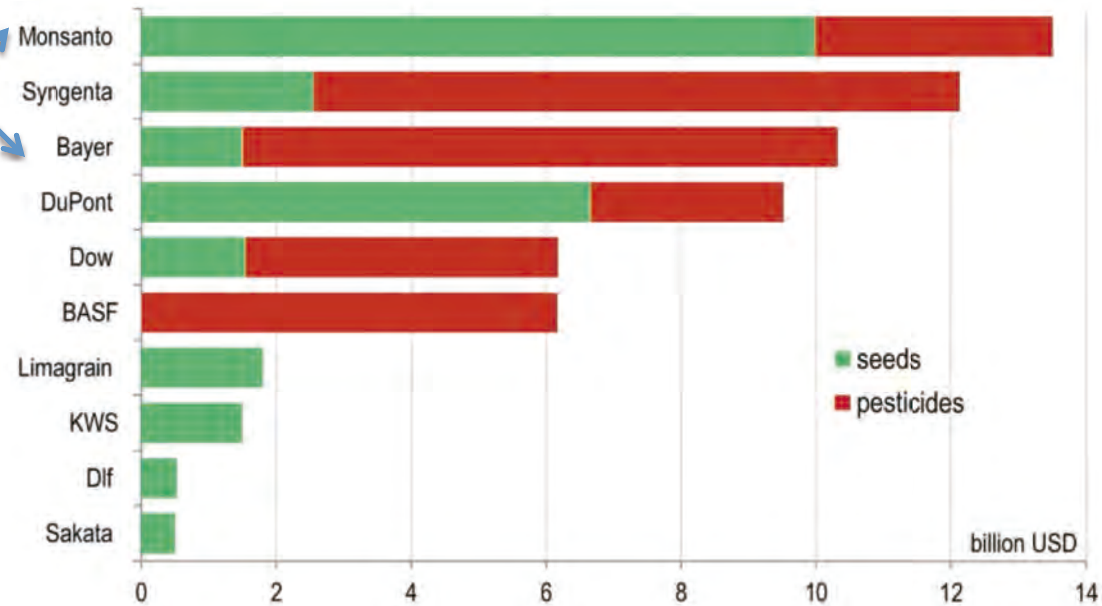
Problem for the development of new varieties (by crossing).



Seed and Agrochemical Industry Market distribution

La major part de la indústria de les llavors (biotecnologia vegetal) és <u>farmacèutica</u>.

Crop protection.



sustainability MDPI

Review

Corporate Concentration and Technological Change in the Global Seed Industry

Sylvie Bonny

Economie Publique, AgroParisTech, INRA, Université Paris-Saclay, 78850 Thiverval-Grignon, France; sylvie.bonny@inra.fr

2016

Monsanto acepta la oferta de compra de Bayer y crearán un gigante mundial

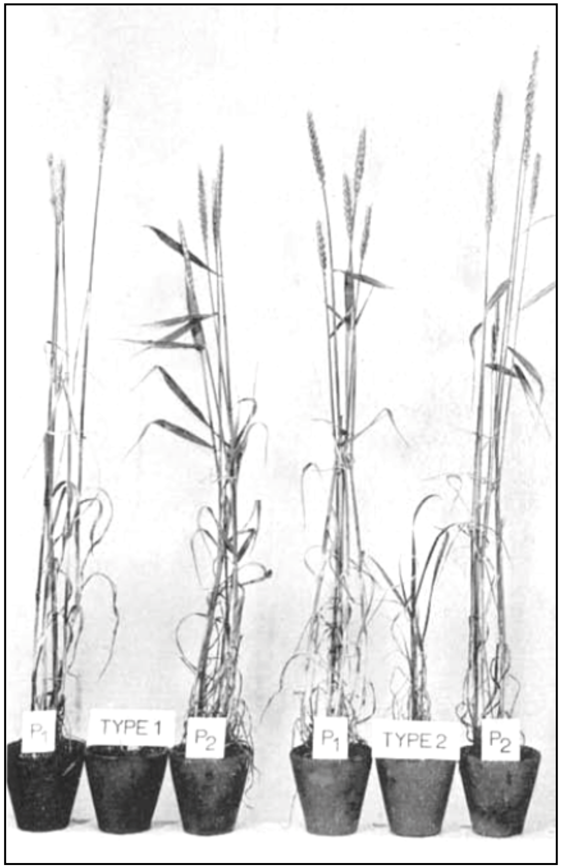
La compañía que emerja de la operación, valorada en 58.500 millones de euros, dominará el negocio de semillas y de pesticidas a escala global

SANDRO POZZI

Nueva York - 16 SEP 2016 - 08:53 CEST

The study of RI in plants: from crops to model species

Blat



Euphytica 16 (1967) : 134-162

Arabidopsis thaliana

Uk-1/Uk-3 F_1 hybrid

Bomblies et al., 2007 PLoS Biology

L*er*/Kas-2 F_2 hybrid

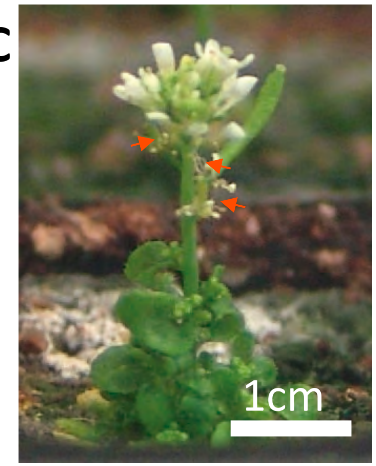
Alcázar *et al.*, 2009 PNAS 106:334-339
Alcázar *et al.*, 2010 Nature Genetics 42:1135-1139

Reproductive isolation between Landsberg erecta (L*er*) and Kashmir-2 (Kas-2)

L*er* Landsberg *erecta* (Poland)
Kas-2 Kashmir-2 (India)

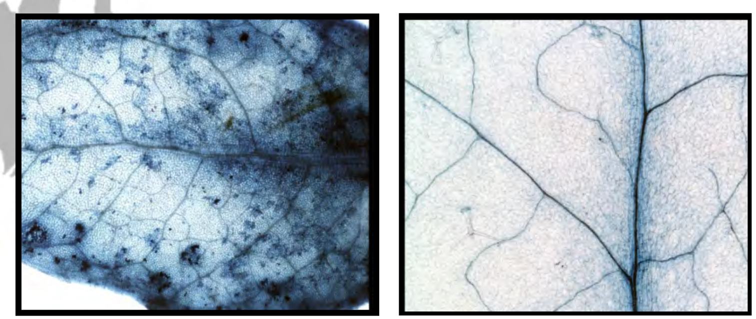
Sterility

14°C



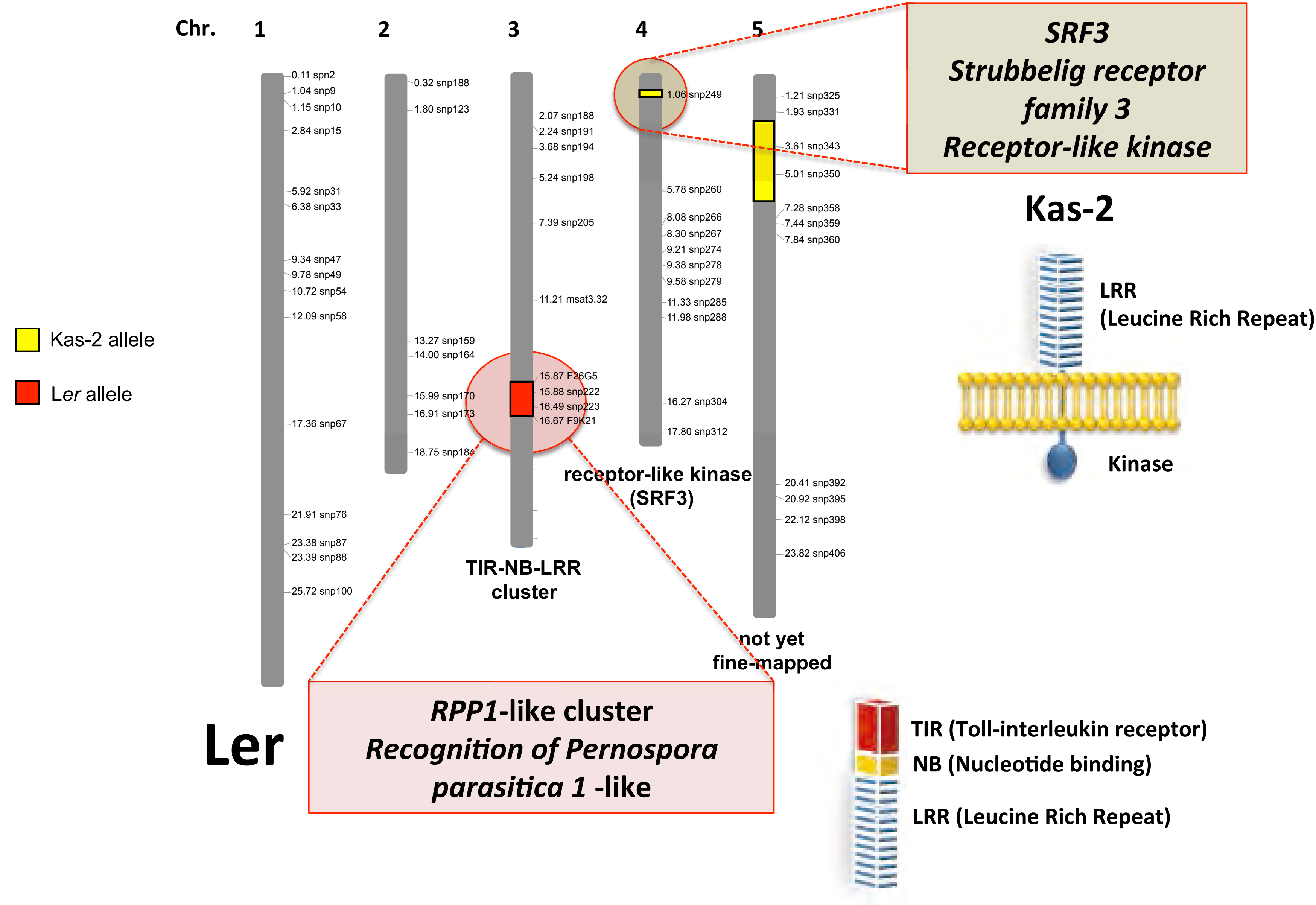
Cell death

14°C **20°C**



Síndrome autoimmune

Causal loci for the Ler/Kas-2 hybrid incompatibility

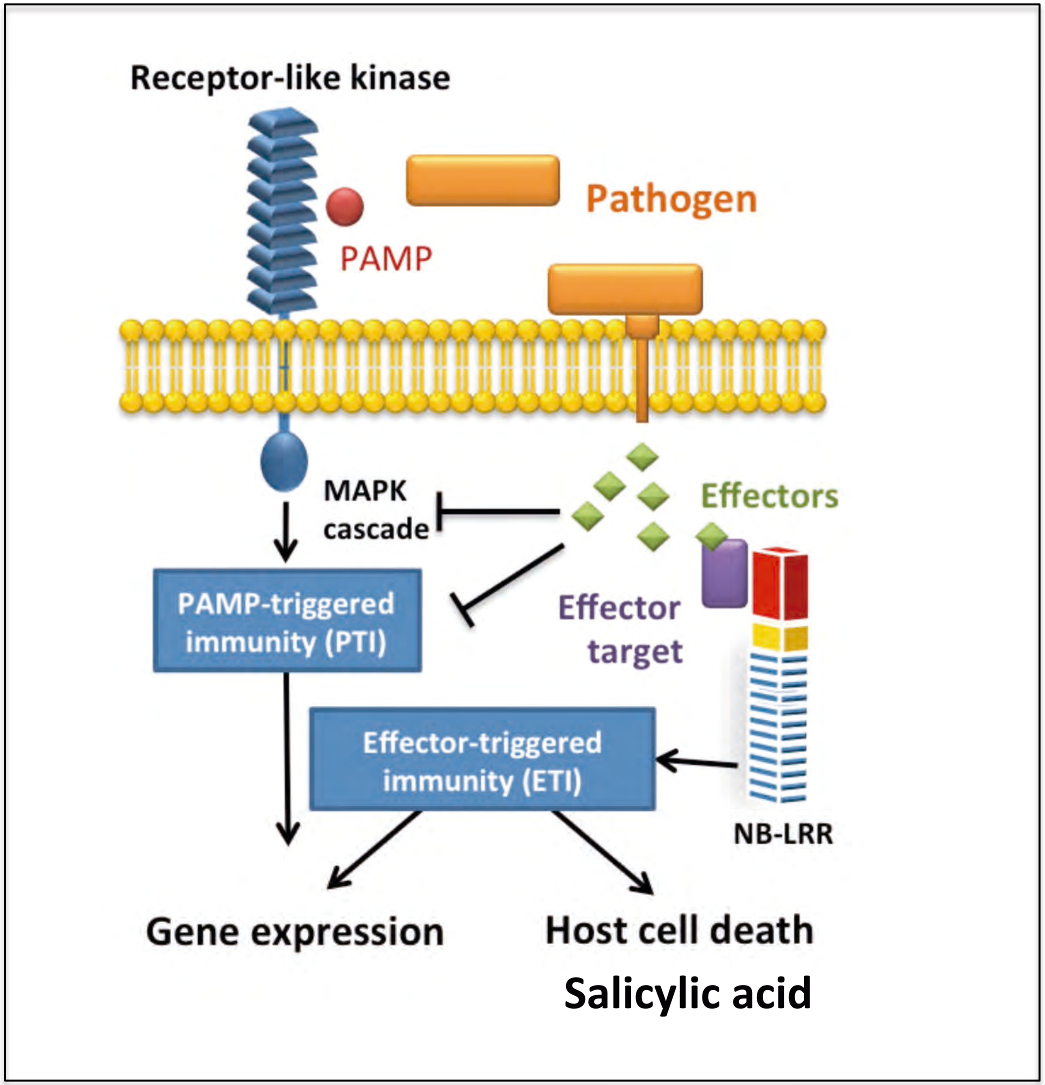


Molecular models of pathogen recognition

Plant immune system:
- ✓ Innate
- ✓ Non-adaptive

Animal immune system:
- ✓ Innate
- ✓ Adaptive

Plant immune system



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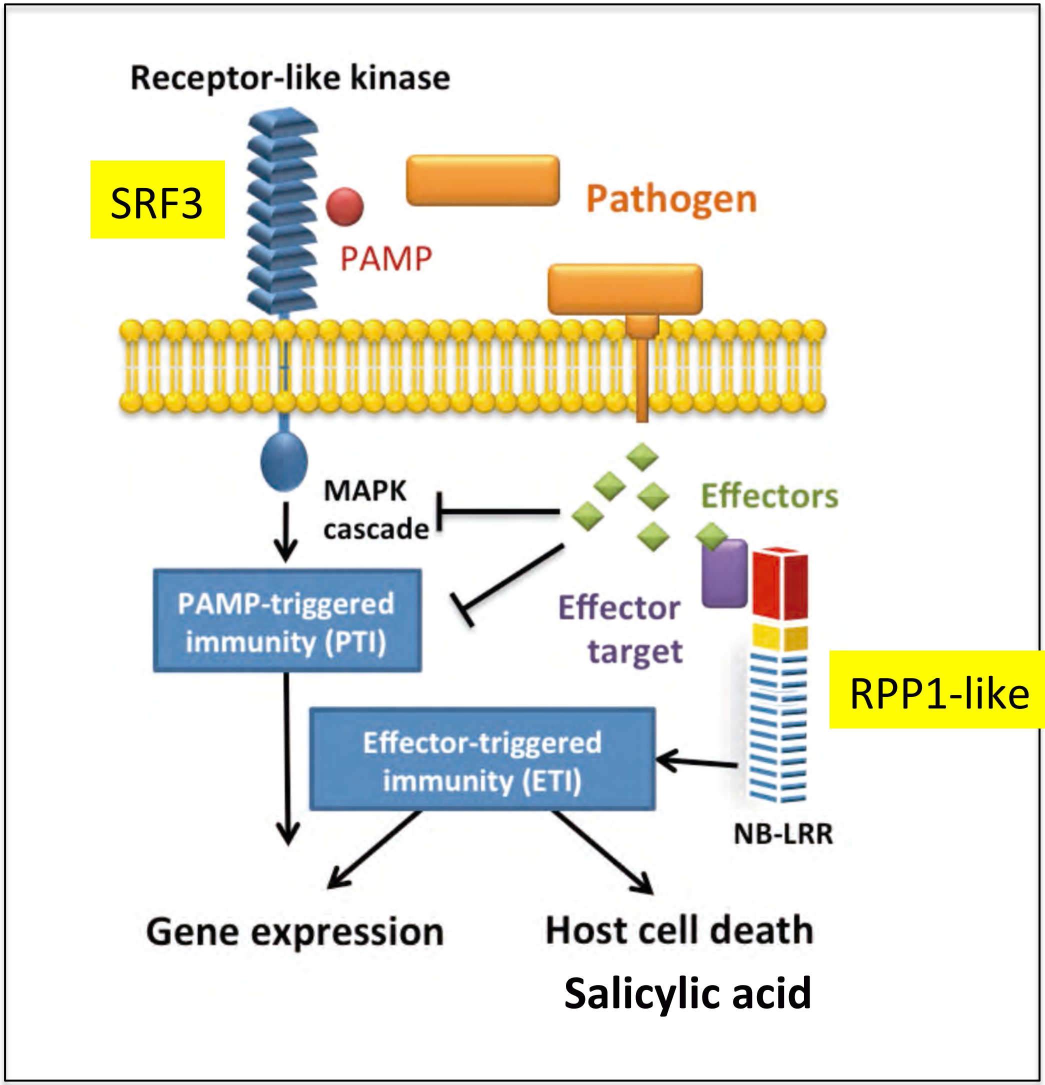


RPP1-like genes: what do they do?

The Plant Cell, Vol. 10, 1847–1860, November 1998, www.plantcell.org © 1998 American Society of Plant Physiologists

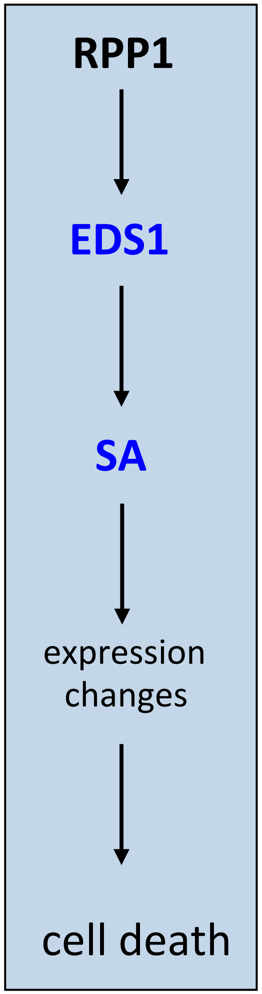
Three Genes of the Arabidopsis *RPP1* Complex Resistance Locus Recognize Distinct *Peronospora parasitica* Avirulence Determinants

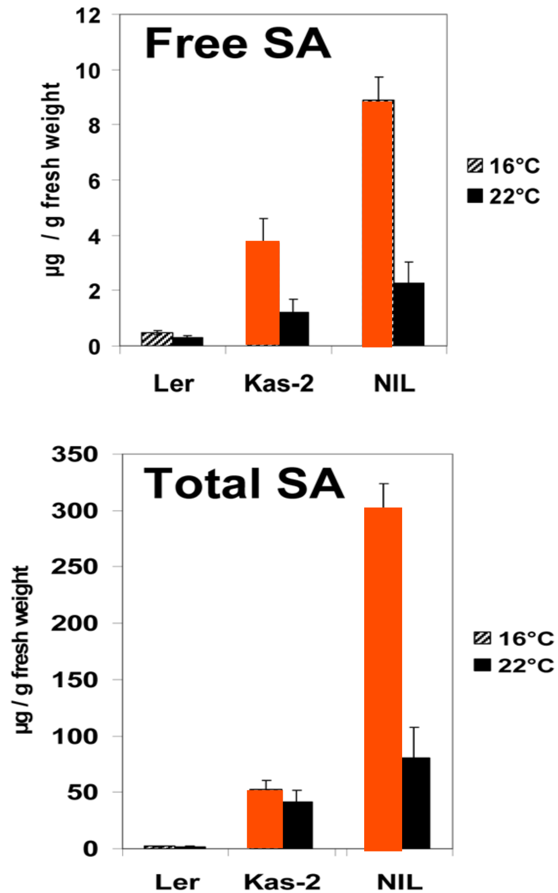
Miguel A. Botella,[a,1] Jane E. Parker,[a] Louise N. Frost,[a] Peter D. Bittner-Eddy,[b] Jim L. Beynon,[b] Michael J. Daniels,[a] Eric B. Holub,[b] and Jonathan D. G. Jones[a,2]

[a]Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich, NR4 7UH, United Kingdom
[b]Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF, United Kingdom

downy mildew
(mildiú)

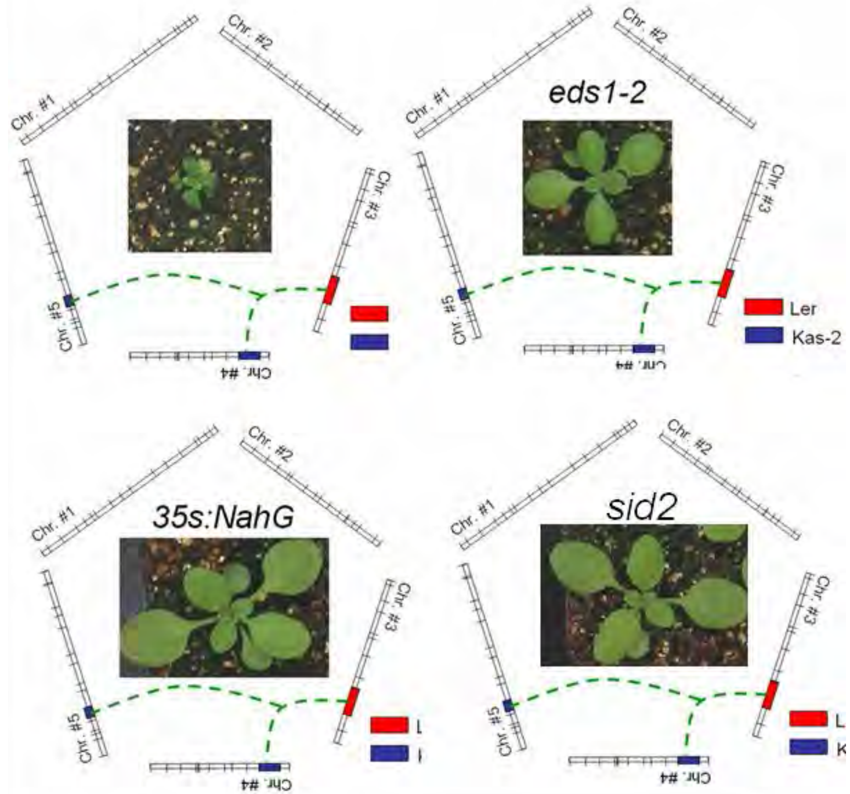
Ler/Kas-2 incompatibility requires *EDS1* and salicylic acid (SA) competence





incompatible line

incompatible line in *EDS1* loss-of-function mutant



incompatible line transformed with NahG

incompatible line in *SID2* loss-of-function mutant

Salicylic acid —NahG→ Cathecol

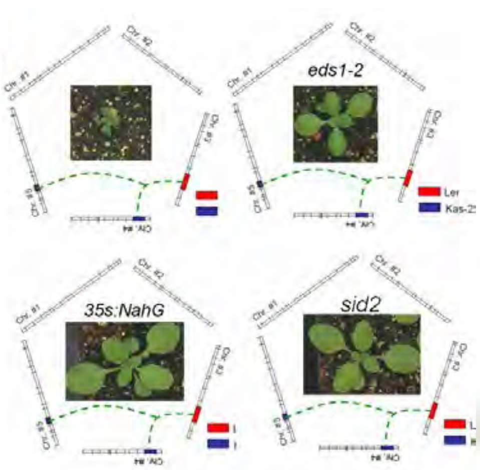
EDS1: enhanced disease susceptibility 1
SID2: isochorismate synthase 1

Yes! we can

restore growth

Ups! we can't.

keep resistance against pathogens.

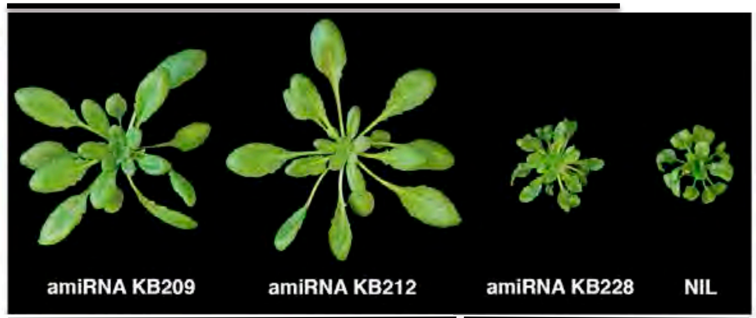


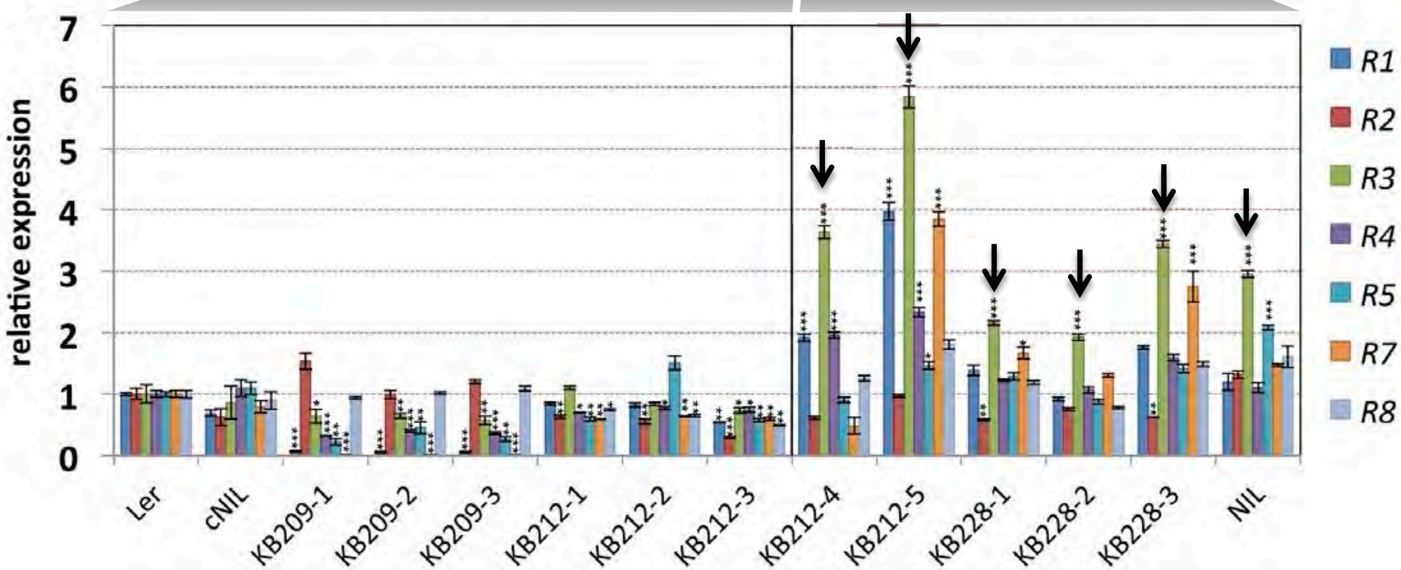
artificial microRNA (amiRNA) silencing of *RPP1*-like Ler genes

amiRNA lines suppress incompatibility

amiRNA lines

non-transformed Ler/Kas-2 hybrid (NIL)

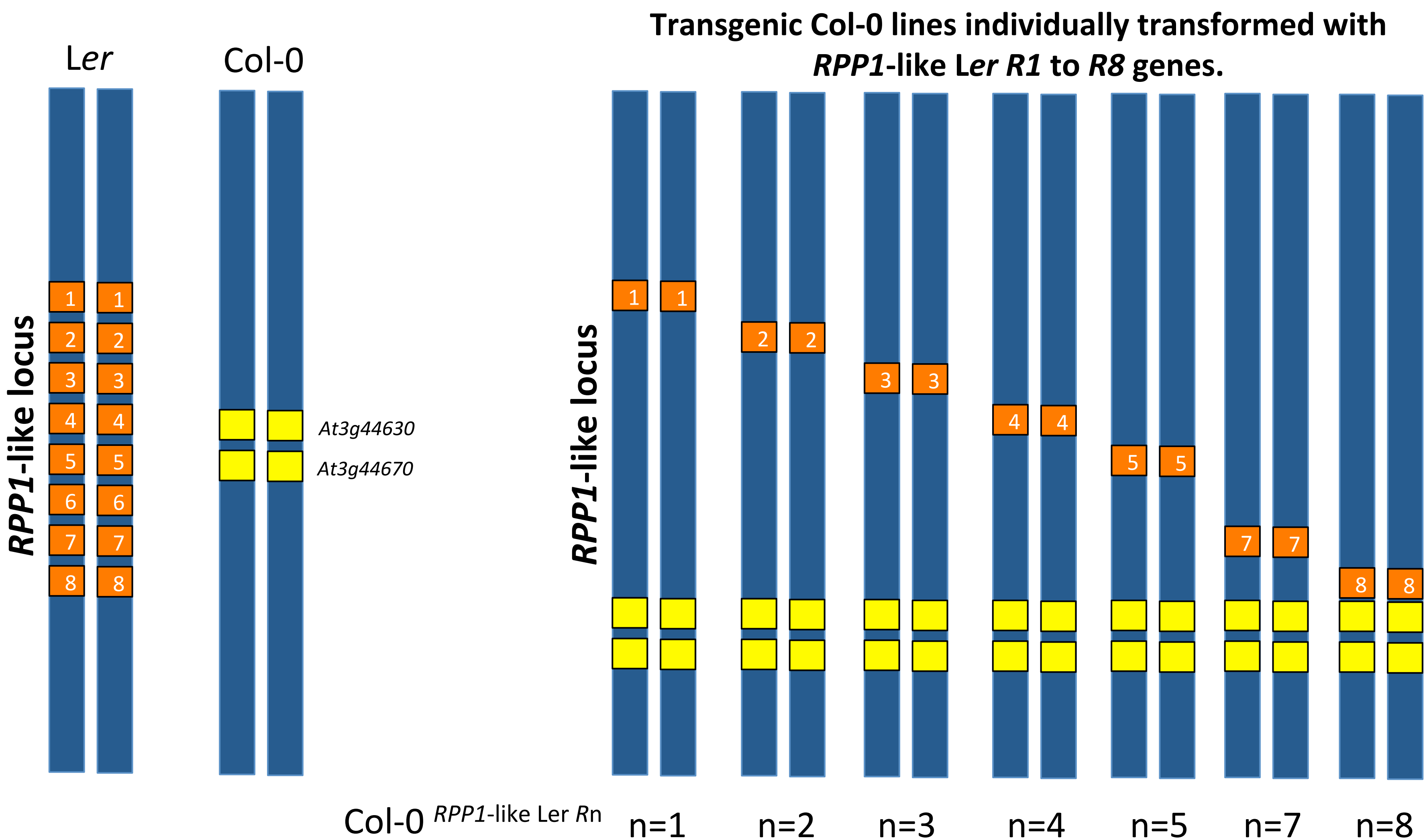




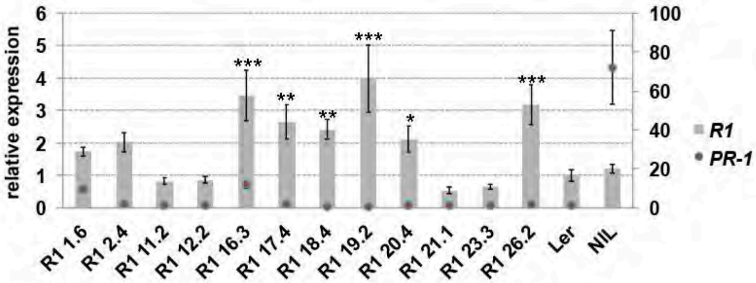
Many genes in the *RPP1*-like L*er* cluster are silenced in suppressed lines.

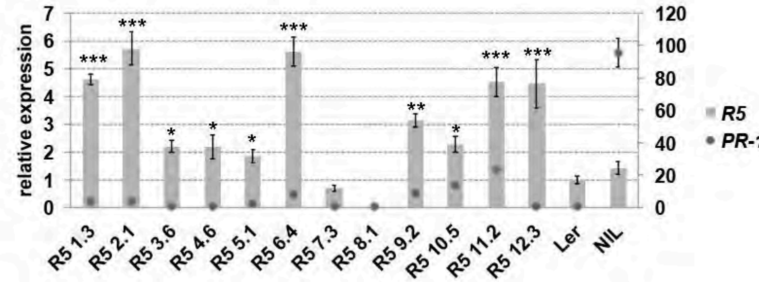
Higher *R3* expression associates with occurrence of incompatibility

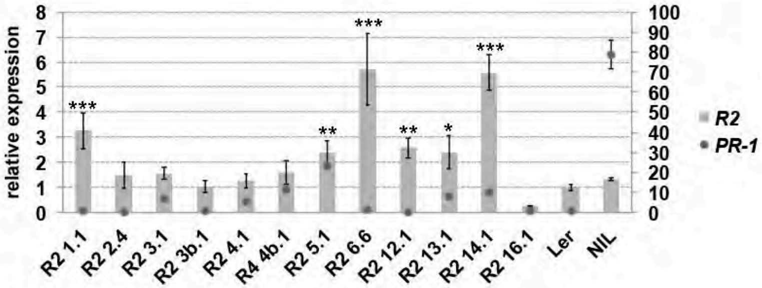
Reconstitution of incompatibility. Using a neutral background.

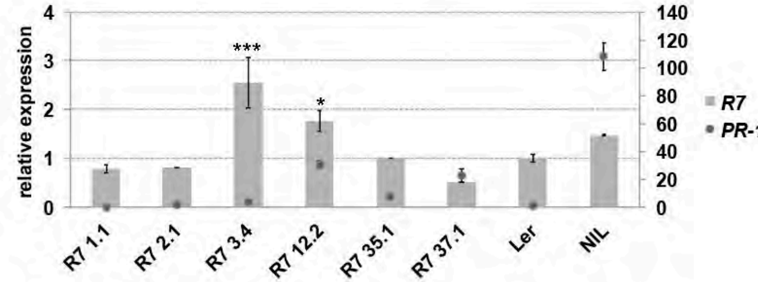


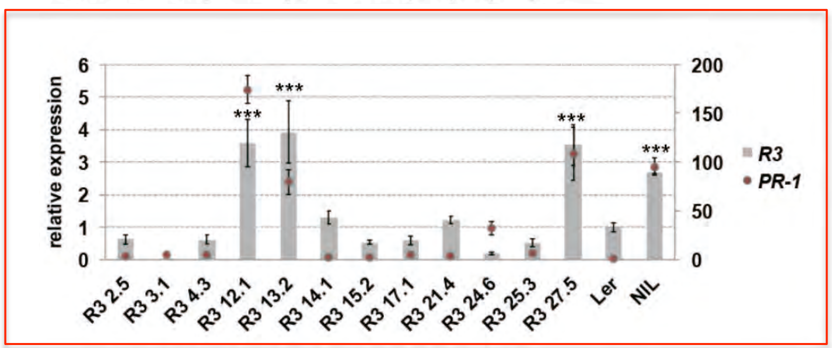
Variable transgene expression in different Col *RPP1*-like Ler lines

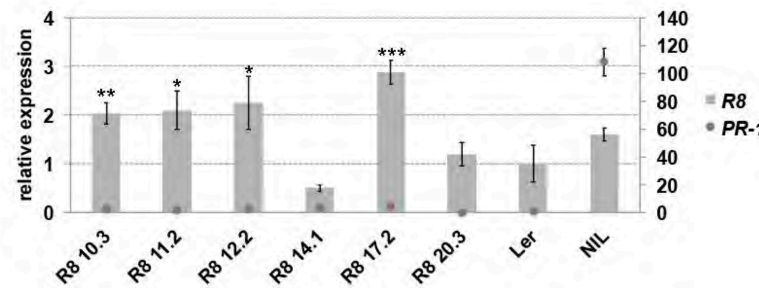


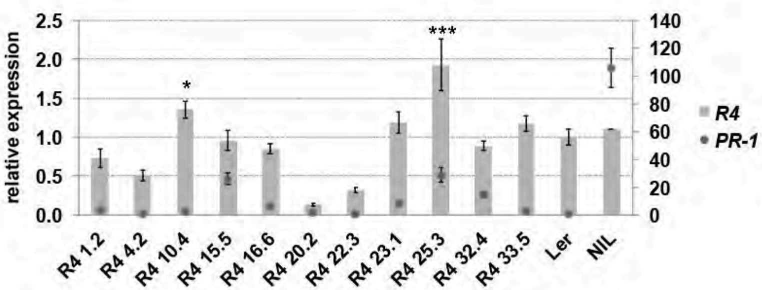






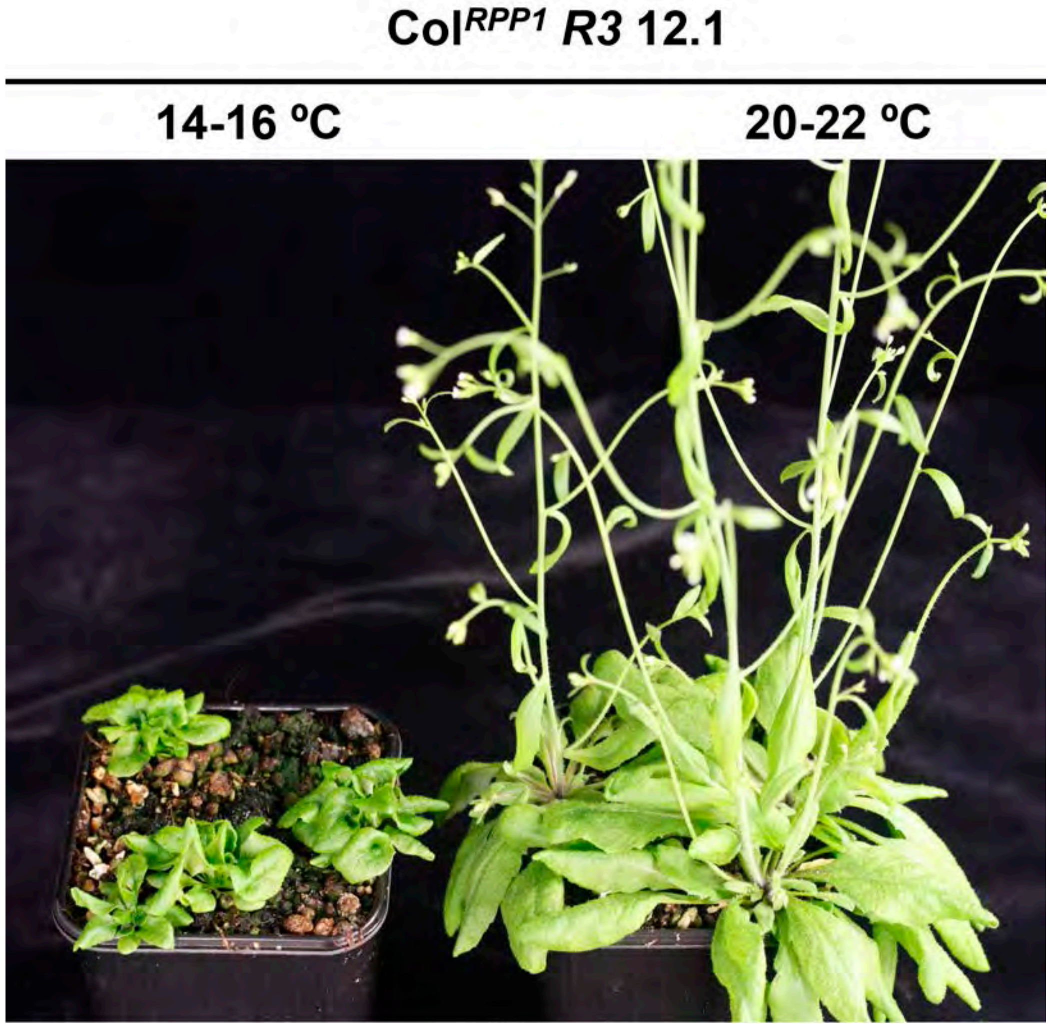




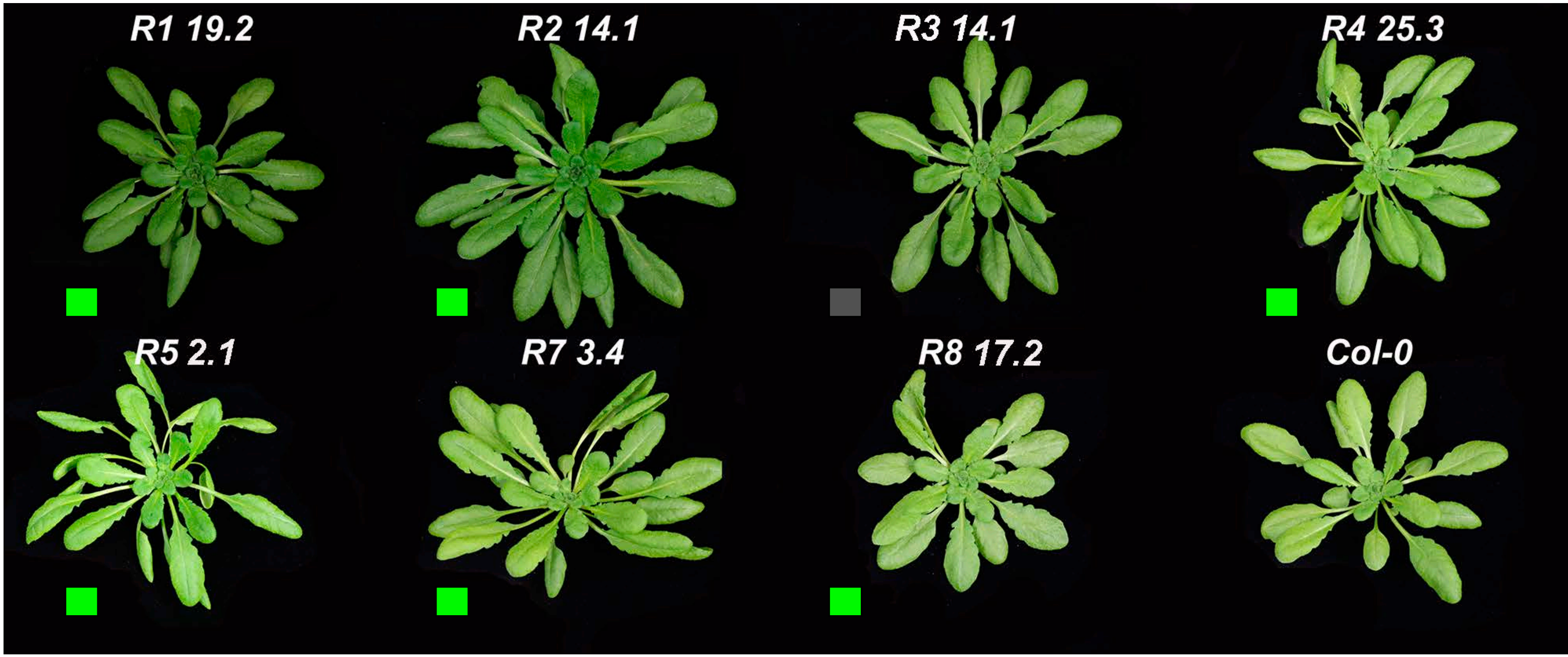


***PR1* is a marker for SA-pathway activation**

R3 overexpression (> 3-fold) induces incompatible phenotypes

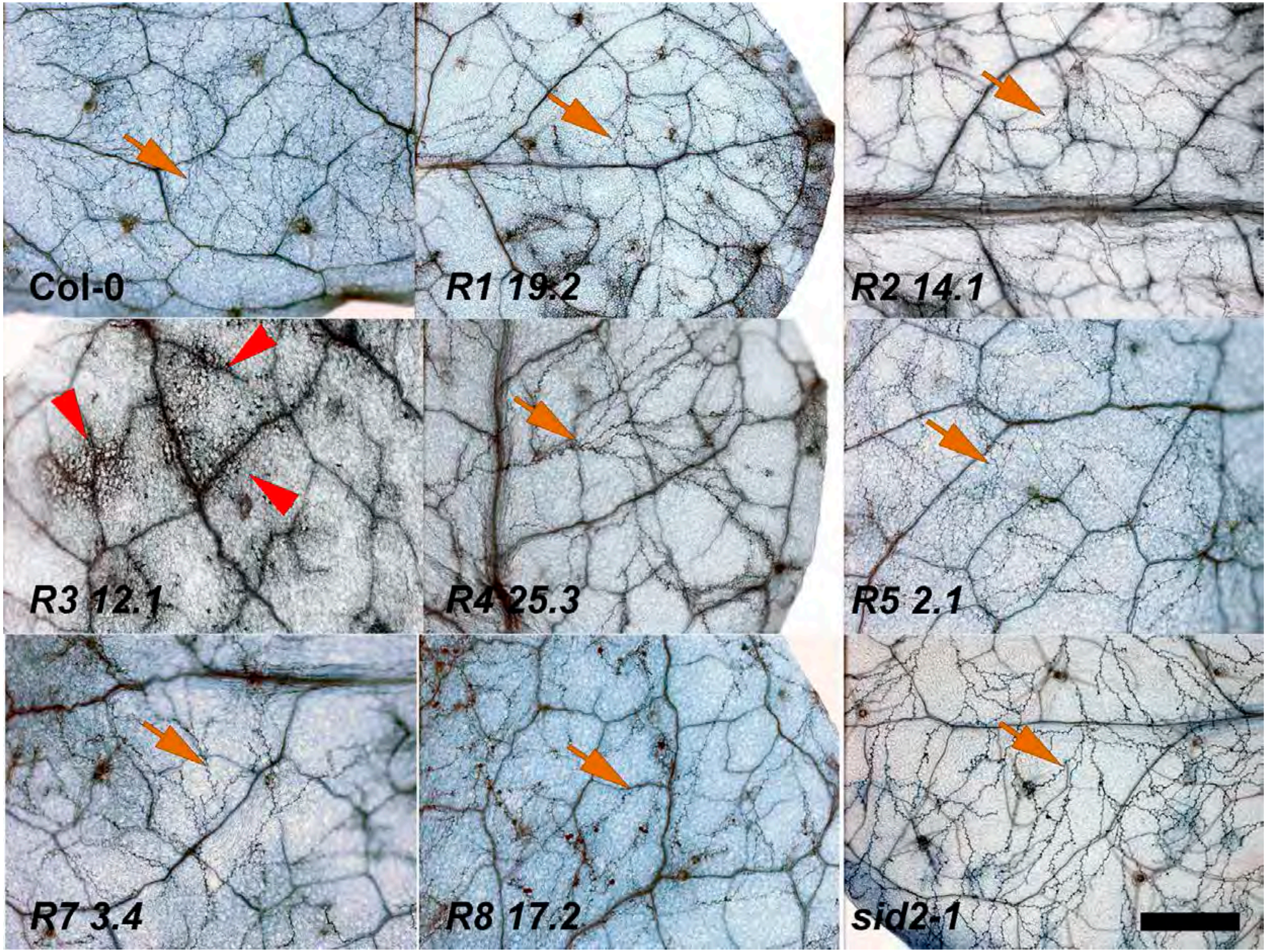


Overexpression of other *RPP1*-like genes in Col-0 does not induce incompatible phenotypes.

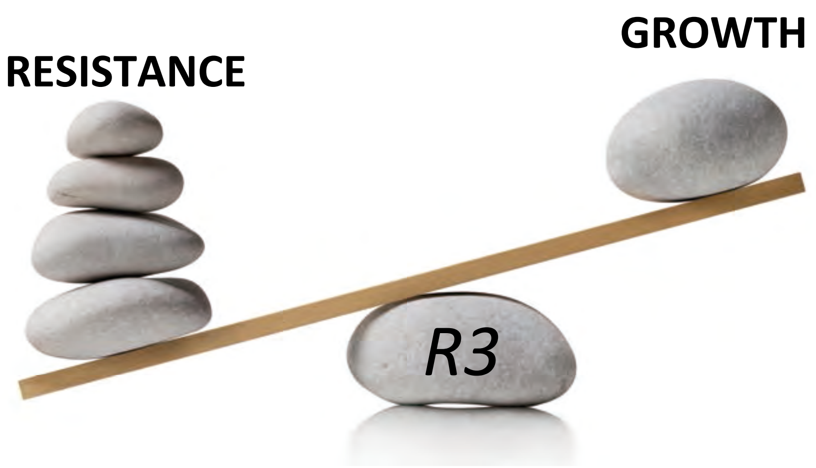


0 4.0 - fold

R3 overexpressor lines are resistant to *Hyaloperonospora arabidopsidis* (Noco)



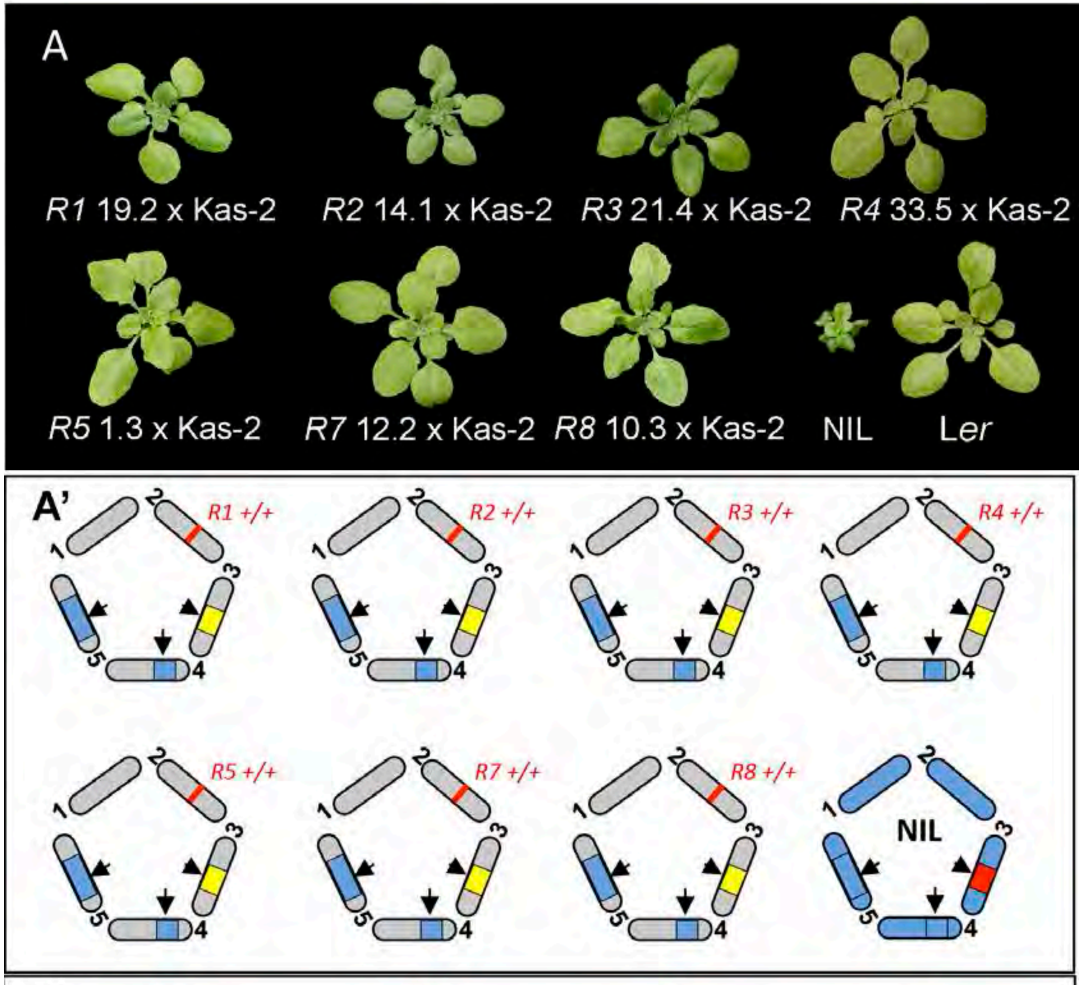
R3 expression conditions the balance between growth and disease resistance



TO GROW OR TO RESIST

but we want to GROW and TO RESIST

... but R3 is not sufficient to trigger incompatibility with Kas-2 when expression is at wild-type levels



Co-action of two or more RPP1-like Ler genes is required for incompatibility.

Screen for suppressors of Ler/Kas-2 incompatibility (*sulki*) mutants

25.000 seeds Ler/Kas-2 NIL

↓ EMS (mutagenesis)

M_1 Population

↓

M_2 Population

20 *sulki* mutants identified

↓ x 5 backcrosses to NIL

BC_5F_1

Whole genome sequencing

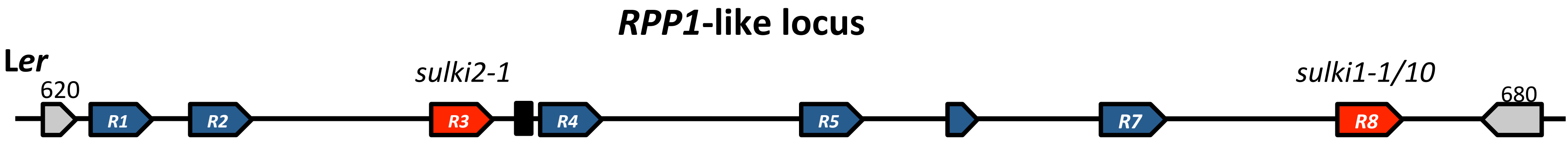
Illumina

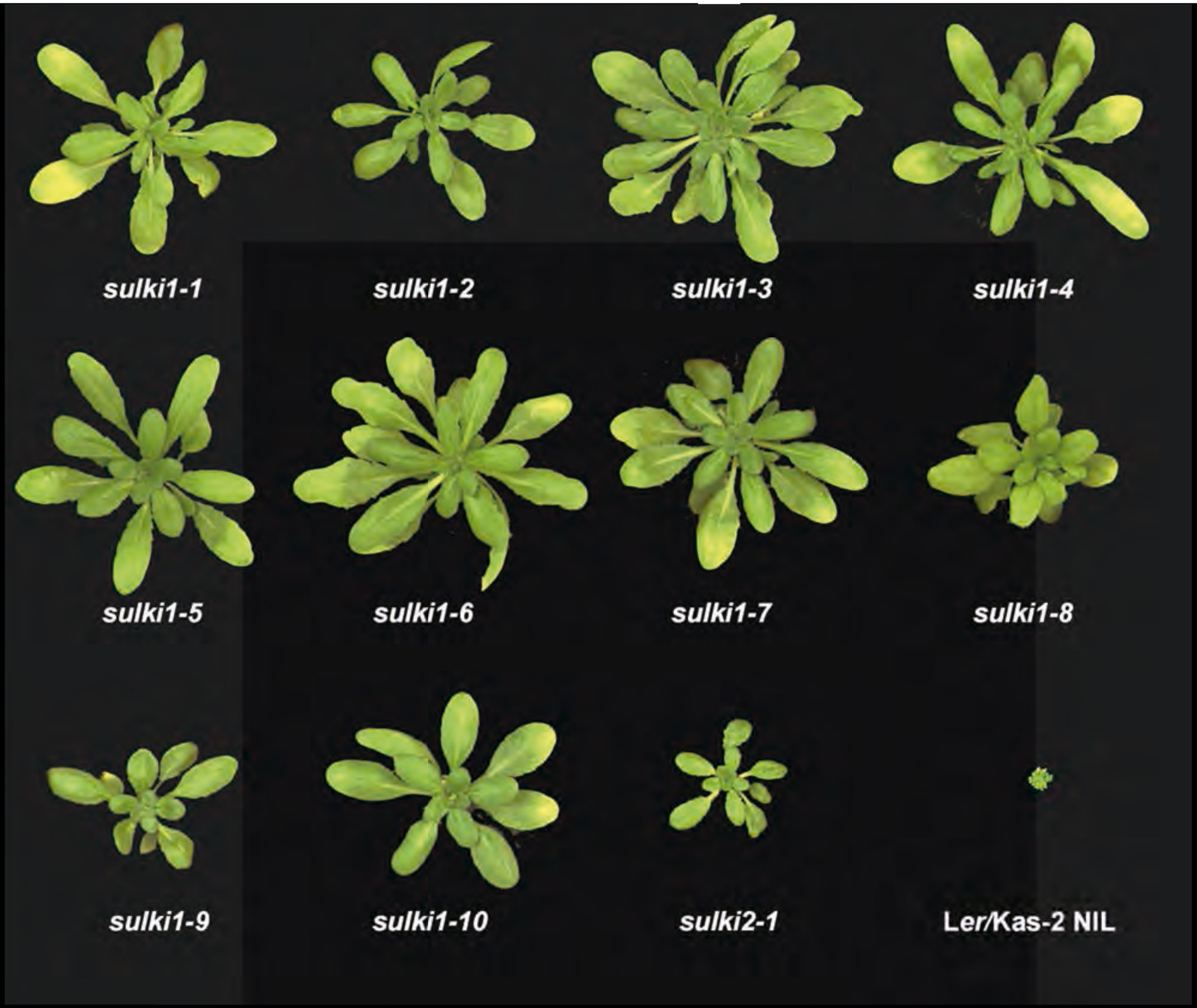
Next generation sequencing

→ 11 intragenic mutations, 10 allelic

→ 9 extragenic mutations

Screen for suppressors of Ler/Kas-2 incompatibility (*sulki*) mutants

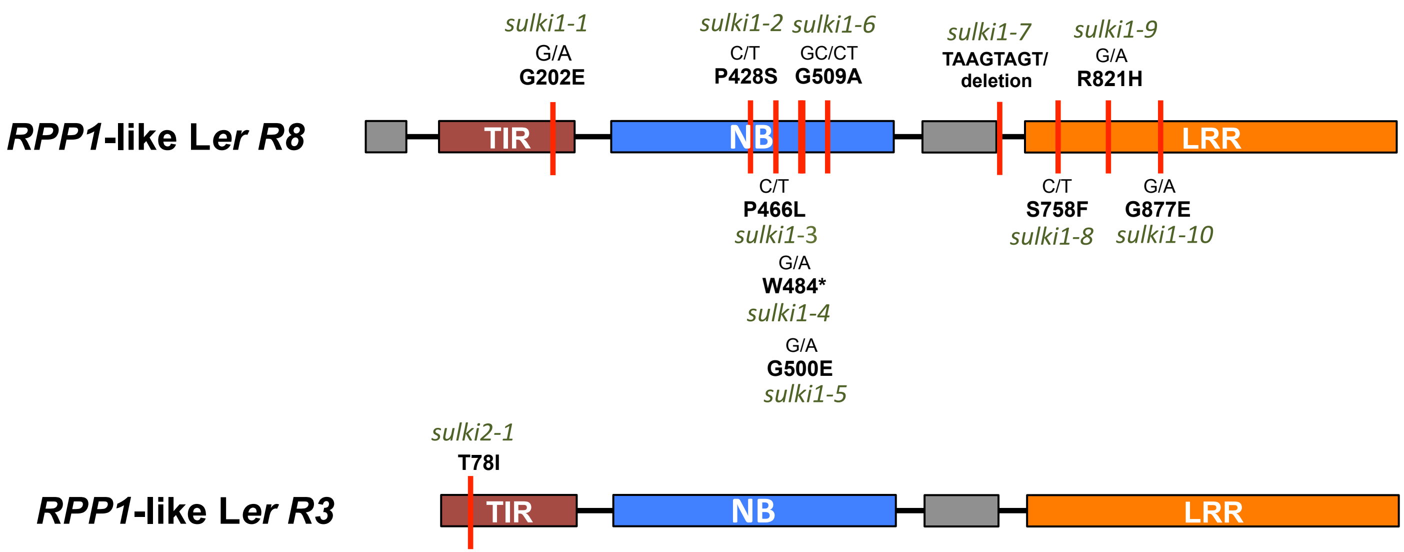




Screen for suppressors of Ler/Kas-2 incompatibility (*sulki*) mutants

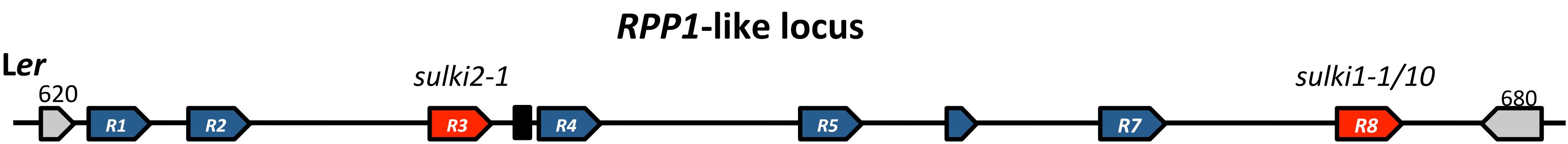
RPP1-like locus

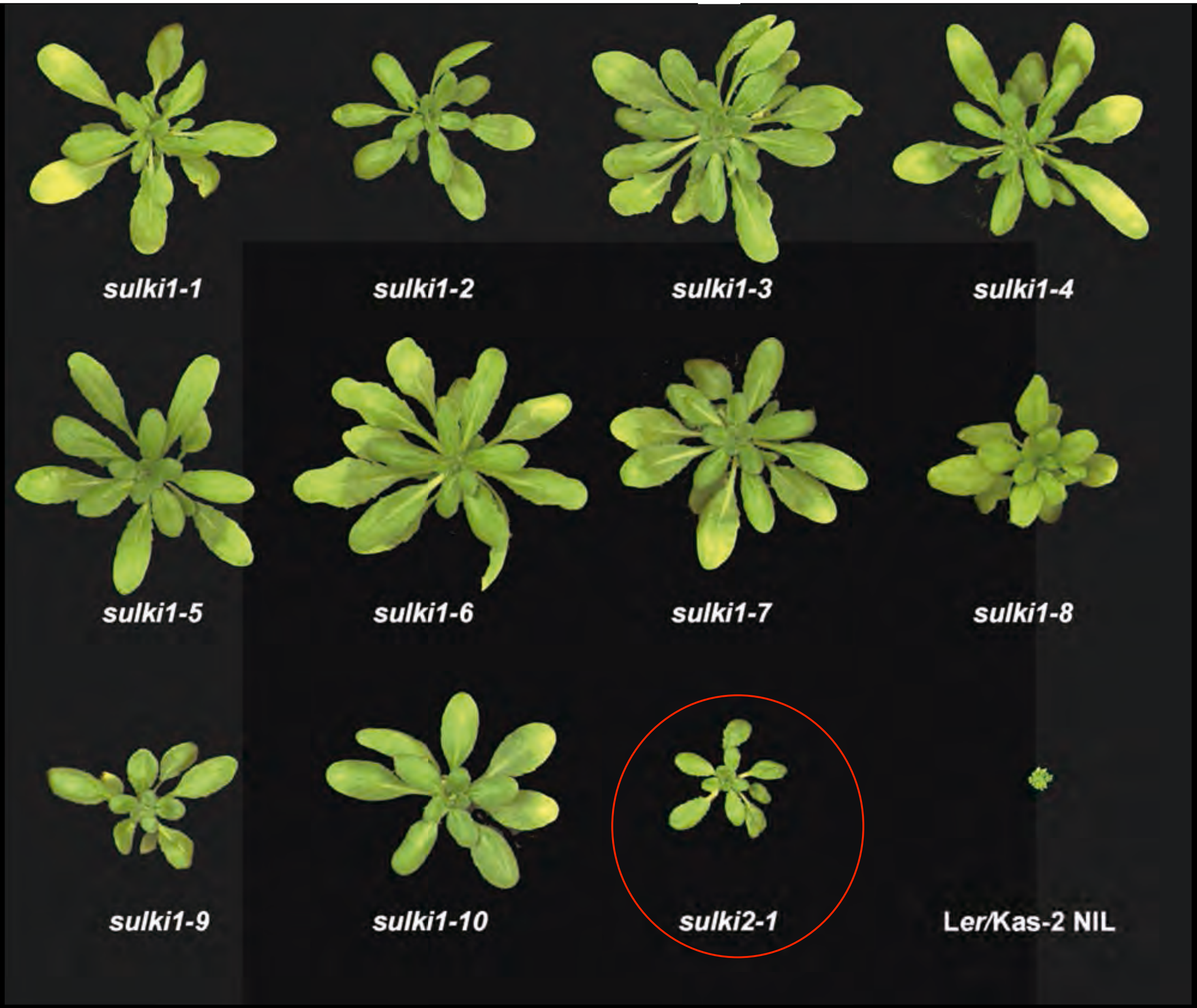
Mutations in invariable or highly conserved residues



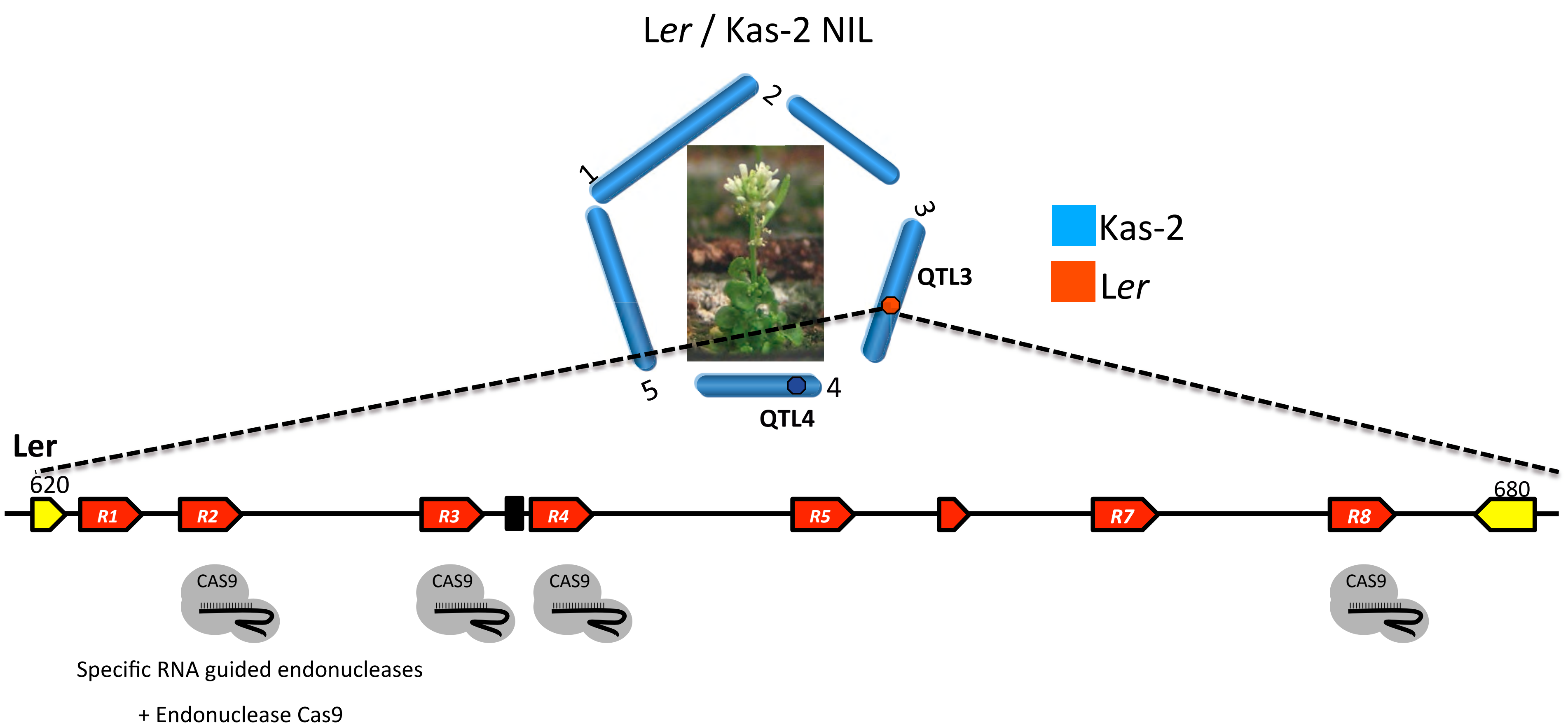
Not only the phenotype is restored, but also the **transcriptional (RNA-seq)** and **primary metabolism (GC/MS)** changes induced by the hybrid incompatibility.

Screen for suppressors of Ler/Kas-2 incompatibility (*sulki*) mutants

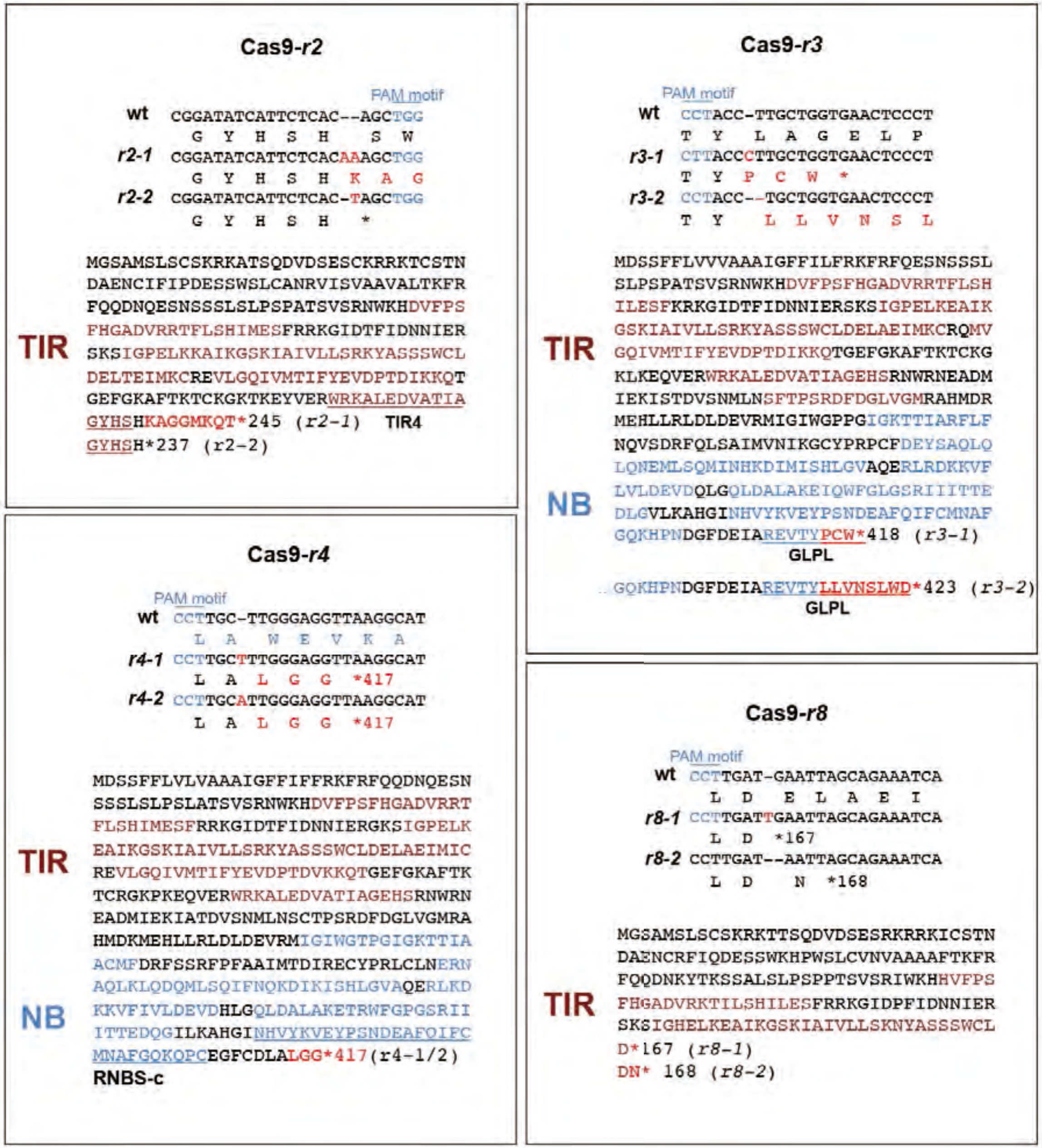




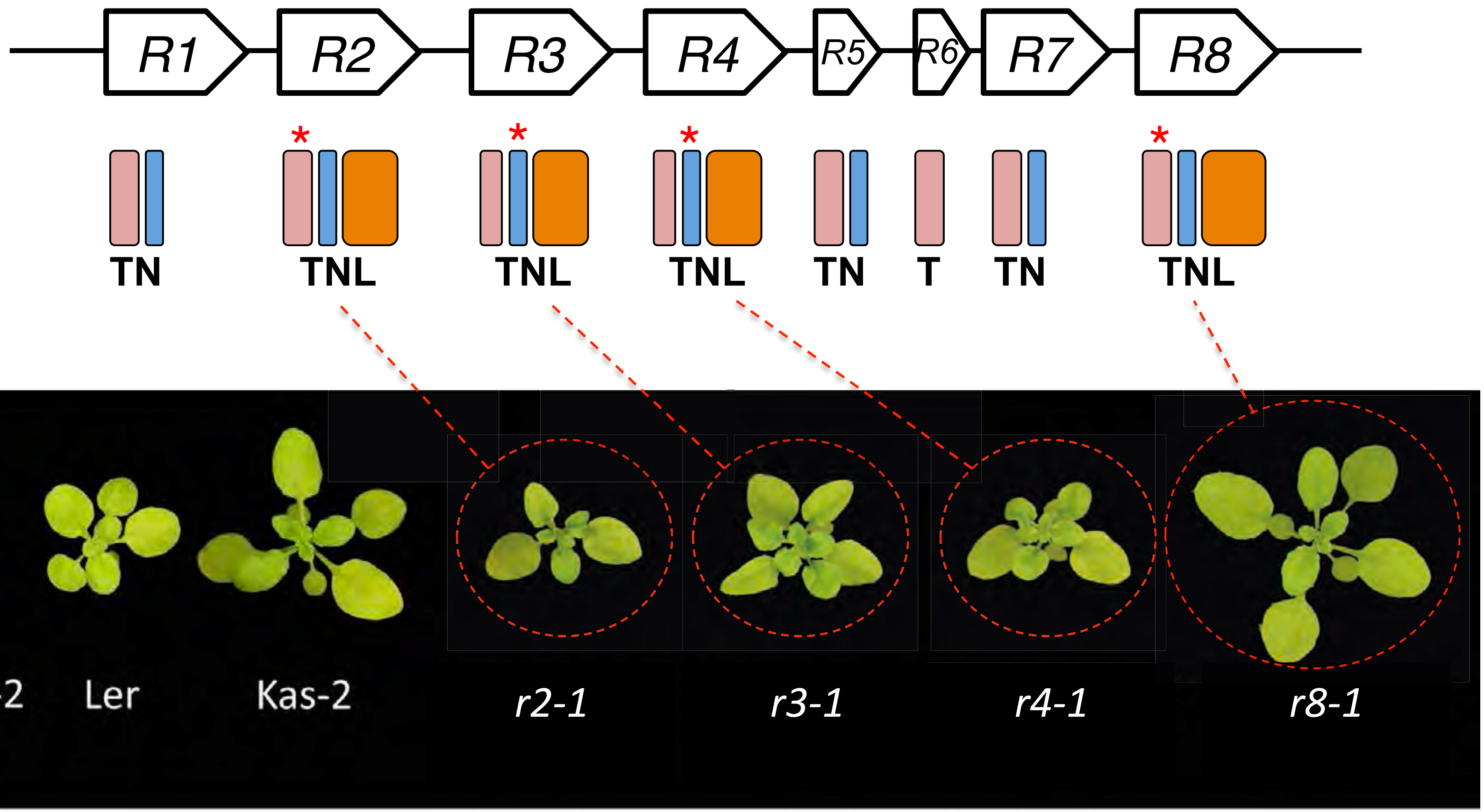
Directed mutagenesis by CRISPR/Cas9



Precision breeding

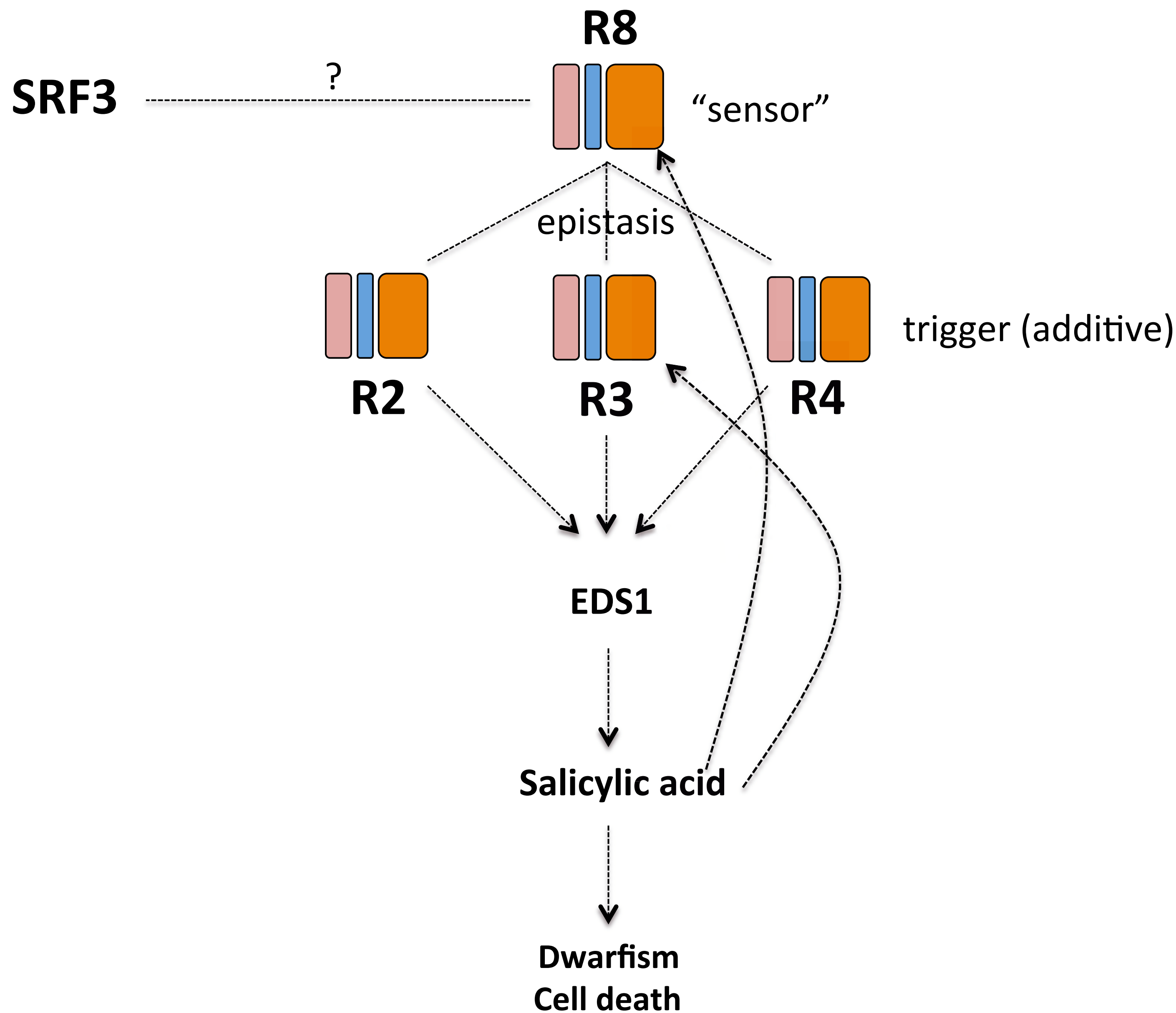

Cas9-r2
PAM motif
wt CGGATATCATTCTCAC--AGCTGG
G Y H S H S W
r2-1 CGGATATCATTCTCACAAAGCTGG
G Y H S H K A G
r2-2 CGGATATCATTCTCAC-TAGCTGG
G Y H S H *
TIR
MGSAMSLSCSKRKATSQDVDSESCKRRKTCSTN
DAENCIFIPDESSWSLCANRVISVAAVALTKFR
FQQDNQESNSSSLSLPSPATSVSRNWKHDVFPS
FHGADVRRTFLSHIMESFRRKGIDTFIDNNIER
SKSIGPELKKAIKGSKIAIVLLSRKYASSSWCL
DELTEIMKCREVLGQIVMTIFYEVDPTDIKKQT
GEFGKAFTKTCKGKTKEYVERWRKALEDVATIA
GYHSHKAGGMKQT*245 (r2-1) TIR4
GYHSH*237 (r2-2)
Cas9-r3
PAM motif
wt CCTACC-TTGCTGGTGAACTCCCT
T Y L A G E L P
r3-1 CTTACCCTTGCTGGTGAACTCCCT
T Y P C W *
r3-2 CCTACC--TGCTGGTGAACTCCCT
T Y L L V N S L
TIR
NB
MDSSFFLVVVAAAIGFFILFRKFRFQESNSSSL
SLPSPATSVSRNWKHDVFPSFHGADVRRTFLSH
ILESFKRKGIDTFIDNNIERSKSIGPELKEAIK
GSKIAIVLLSRKYASSSWCLDELAEIMKCRQMV
GQIVMTIFYEVDPTDIKKQTGEFGKAFTKTCKG
KLKEQVERWRKALEDVATIAGEHSRNWRNEADM
IEKISTDVSNMLNSFTPSRDFDGLVGMRAHMDR
MEHLLRLDLDEVRMIGIWGPPGIGKTTIARFLF
NQVSDRFQLSAIMVNIKGCYPRPCFDEYSAQLQ
LQNEMLSQMINHKDIMISHLGVAQERLRDKKVF
LVLDEVDQLGQLDALAKEIQWFGLGSRIIITTE
DLGVLKAHGINHVYKVEYPSNDEAFQIFCMNAF
GQKHPNDGFDEIAREVTYPCW*418 (r3-1)
GLPL
...GQKHPNDGFDEIAREVTYLLVNSLWD*423 (r3-2)
GLPL
Cas9-r4
PAM motif
wt CCTTGC-TTGGGAGGTTAAGGCAT
L A W E V K A
r4-1 CCTTGCTTTGGGAGGTTAAGGCAT
L A L G G *417
r4-2 CCTTGCATTGGGAGGTTAAGGCAT
L A L G G *417
TIR
NB
MDSSFFLVLVAAAIGFFIFFRKFRFQQDNQESN
SSSLSLPSLATSVSRNWKHDVFPSFHGADVRRT
FLSHIMESFRRKGIDTFIDNNIERGKSIGPELK
EAIKGSKIAIVLLSRKYASSSWCLDELAEIMIC
REVLGQIVMTIFYEVDPTDVKKQTGEFGKAFTK
TCRGKPKEQVERWRKALEDVATIAGEHSRNWRN
EADMIEKIATDVSNMLNSCTPSRDFDGLVGMRA
HMDKMEHLLRLDLDEVRMIGIWGTPGIGKTTIA
ACMFDRFSSRFPFAAIMTDIRECYPRLCLNERN
AQLKLQDQMLSQIFNQKDIKISHLGVAQERLKD
KKVFIVLDEVDHLGQLDALAKETRWFGPGSRII
ITTEDQGILKAHGINHVYKVEYPSNDEAFQIFC
MNAFGQKQPCEGFCDLALGG*417(r4-1/2)
RNBS-c
Cas9-r8
PAM motif
wt CCTTGAT-GAATTAGCAGAAATCA
L D E L A E I
r8-1 CCTTGATTGAATTAGCAGAAATCA
L D *167
r8-2 CCTTGAT--AATTAGCAGAAATCA
L D N *168
TIR
MGSAMSLSCSKRKTTSQDVDSESRKRRKICSTN
DAENCRFIQDESSWKHPWSLCVNVAAAAFTKFR
FQQDNKYTKSSALSLPSPPTSVSRIWKHHVFPS
FHGADVRKTILSHILESFRRKGIDPFIDNNIER
SKSIGHELKEAIKGSKIAIVLLSKNYASSSWCL
D*167 (r8-1)
DN* 168 (r8-2)

CRISPR/CAS9 – based mutagenesis of RPP1-like Ler genes in Ler/Kas-2 NIL

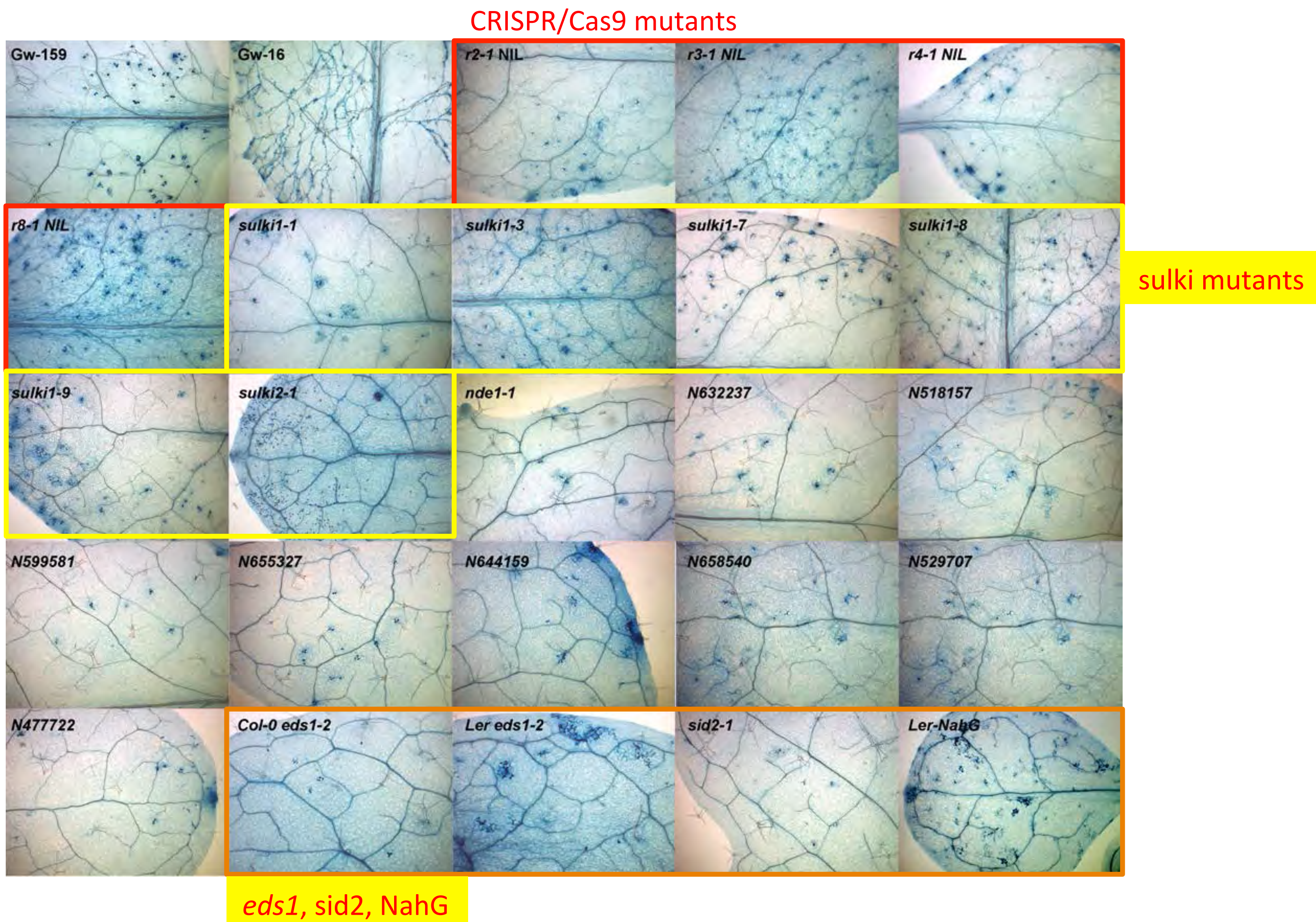


DOMAINS
T: TIR (Toll Interleukin Receptor), N: NB (Nucleotide binding), L: LRR (Leucine Rich Repeat).

Current genetic model



CRISPR/Cas9 or sulki mutations suppressing incompatibility do not compromise *Hpa* disease resistance



Yes! we can

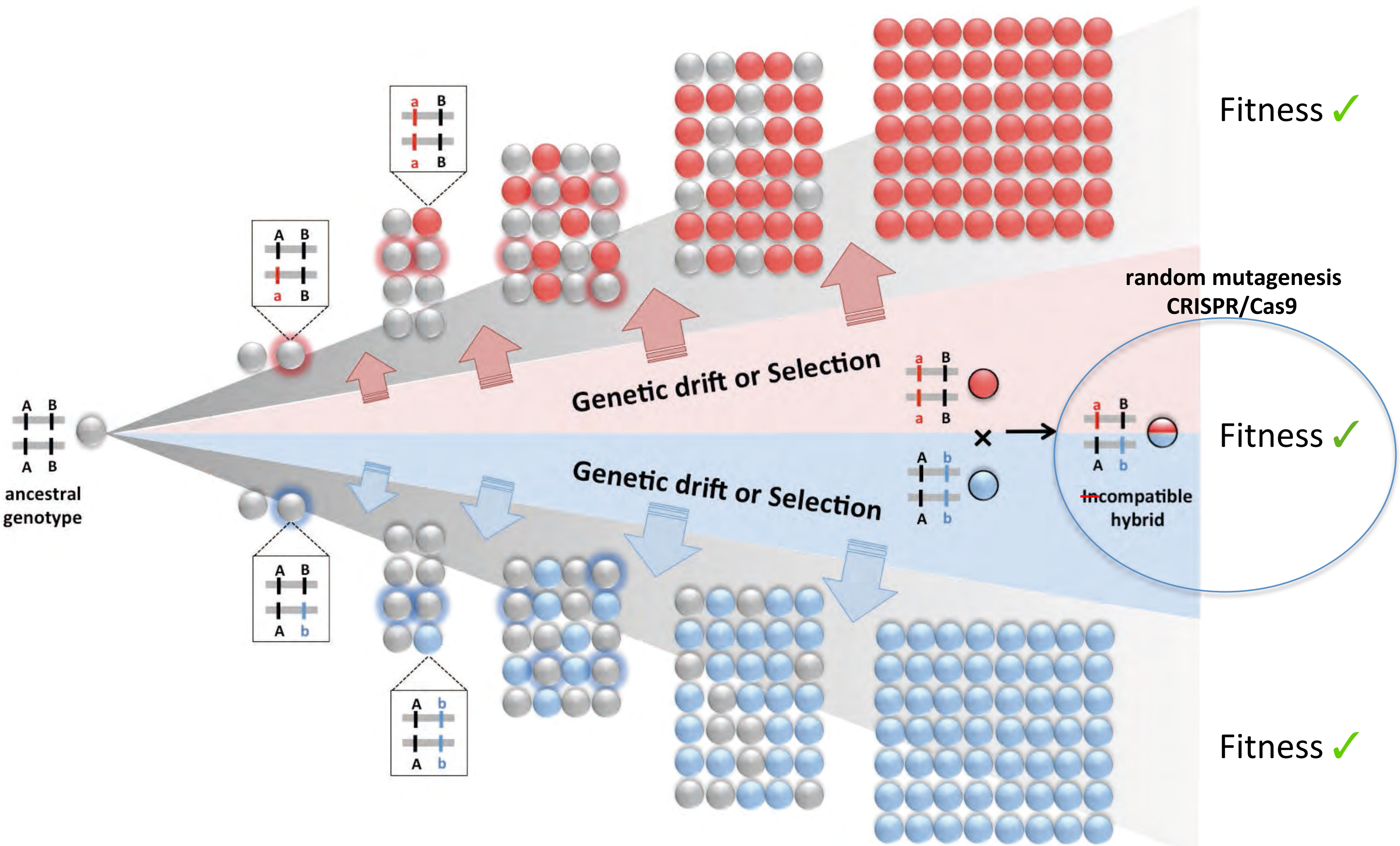
restore compatibility

Yes! we can

maintain resistance against pathogens.

Random and CRISPR/Cas9 directed mutagenesis enables the suppression of Dobzhansky-Muller genetic incompatibilities

The Dobzhansky-Muller (D-M) model of genetic incompatibilities



Main conclusions

Speciation events can be triggered by **single nucleotide mutations.**

The hybrids exhibit **fitness loss** associated with **constitutive activation of defense**.

Suppression of defense rescues compatibility, but **induces susceptibility**.

Through **random or directed** (when genes are known) mutagenesis, artificially-induced mutations can be selected that **restore compatibility at no cost on defense**.

This should enable the development of **new varieties** for which speciation (triggered by D-M incompatibilities) is a barrier.