Renewal of the Mastenbroek differential set and creation of a new GM differential set for potato late blight

Suxian Zhu, Marjan Bergervoet, Maarten Nijenhuis, Jack H. Vossen,
Geert J.T. Kessel, Vivianne Vleeshouwers, Richard G. F. Visser, Evert Jacobsen

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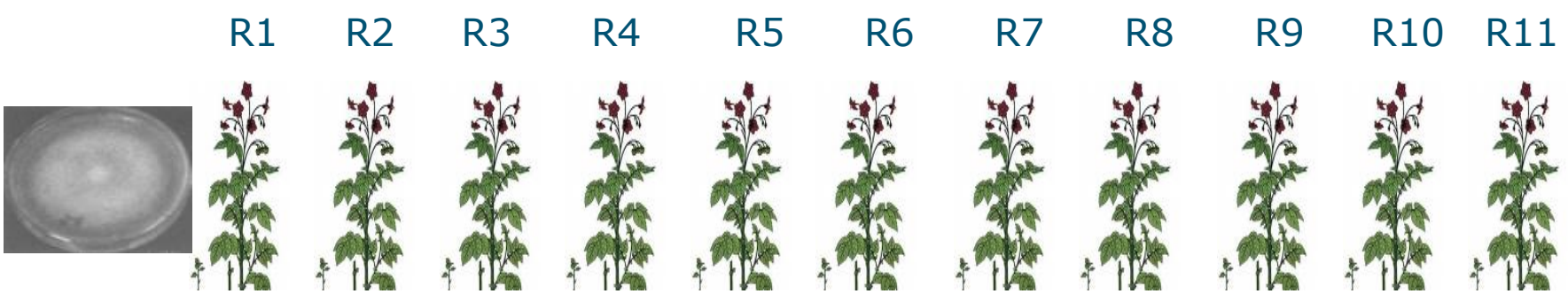
Potato Late blight

- Late blight pathogen
  - *Phytophthora infestans*
  - Oomycete
  - Hemi-biotrophic

Differential set

A set of genotypes, each containing a single *R* gene, used to define the pathogen virulence races based on the resulting susceptible and resistant reactions.



Race spectrum 2 4 5 7 10

Mastenbroek potato late blight differential set

11 genotypes, expected to contain 11 single *R* genes, from the hexaploid species *Solanum demissum.*

Ma*R1*
Ma*R2*
Ma*R3*
Ma*R4*
Ma*R5*
Ma*R6*
Ma*R7*
Ma*R8*
Ma*R9*
Ma*R10*
Ma*R11*

Disease test of the Dutch differential set against 16 in house isolates

Plant \ Isolate	IPO-0	PIC99177	PIC99189	PIC99183	EC1	88069	H30P04	Dinteloord	90128	VK98014	F95573	428-2	89148-09	K haar	IPO C	USA618
Desiree	S	S	S	S	S	S	S	S	S	S	SQ	S	S	S	S	S
Bintje	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ma*R1*	R	SQ	S	S	S	S	R	S	S	S	S	S	R	RQ	S	S
Ma*R2*	R	S	S	Q	R	R	R	Q	R	Q	R	Q	RQ	R	S	S
Ma*R3*	SQ	S	R	S	S	S	S	R	S	R	S	S	R	S	S	S
Ma*R4*	RQ	S	R	S	SQ	S	R	S	S	S	Q	S	R	S	S	R
Ma*R5*	R	R	RQ	R	R	R	R	R	R	R	R	R	R	R	SQ	R
Ma*R6*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S
Ma*R7*	Q	S	RQ	S	Q	S	S	Q	S	R	S	S	R	S	S	S
Ma*R8*	R	R	R	S	R	R	R	R	RQ	R	R	RQ	R	RQ	R	R
Ma*R9*	R	Q	R	Q	R	R	R	R	R	R	R	R	R	R	RQ	R
Ma*R10*	S	Q	S	S	S	SQ	SQ	R	S	R	S	Q	R	S	S	S
Ma*R11*	S	S	S	S	S	S	S	R	S	R	S	S	R	S	S	S

R, all inoculation spots showed resistance or hypersensitive response);
RQ, 6–7 out of eight spots on a leaf showed resistance;
Q, 3–5 out of eight spots on a leaf showed resistance;
SQ, 1–2 out of eight spots on a leaf showed resistance;
S, all spots on a leaf showed susceptibility.

Existence of additional *R* genes

Differential plant	Recently reported *R* gene content	Reference
Ma*R1*		
Ma*R2*		
Ma*R3*	*R3a*, *R3b*	Huang et al., 2005 Li et al., 2011
Ma*R4*		
Ma*R5*	*R1*	Trognitz and Trognitz, 2007
Ma*R6*	*R1*	Trognitz and Trognitz, 2007
Ma*R7*		
Ma*R8*	*R3a*, *R3b*, *R4*, *R8*	Kim et al., 2012
Ma*R9*	*R1*, *Rpi-abpt1*, *R3a*, *R3b*, *R4*, *R8*, *R9*	Kim et al., 2012 Trognitz and Trognitz, 2007
Ma*R10*		
Ma*R11*		

Additional *R* genes found via Agro-infiltration

Agro-infiltration with 7 *Avr* effectors on all members of the differential set

	Avr1	*Avr2*	*Avr3a*	*Avr3b*	*Avr4*	*Avr8*	*Avr10*	*R* gene content
Ma*R1*	+	-	-	-	-	-	-	*R1*
Ma*R2*	-	+	-	-	-	-	-	*R2*
Ma*R3*	-	-	+	+	-	-	-	*R3a, R3b*
Ma*R4*	-	-	-	-	+	-	-	*R4*
Ma*R5*	+	+	-	+	-	-	-	*R1, R2, R3b*
Ma*R6*	+	+	+	-	-	-	-	*R1, R2, R3a*
Ma*R7*	-	-	+	-	+	-	-	*R3a, R4*
Ma*R8*	-	-	+	-	+	+	-	*R3a, R4, R8*
Ma*R9*	+	+	+	+	+	+	-	*R1, R2, R3a, R3b, R4, R8*
Ma*R10*	-	-	-	+	-	-	+	*R3b, R10*
Ma*R11*	-	-	-	+	-	-	+	*R3b, R10*

'+' indicates recognition, '-' indicates absence of recognition.

Improved differential set

Name	Genotype	Plant species	*R*-gene content*
DS-R1	Ma*R1*	*S. demissum*	*R1*
DS-R2	Ma*R2*	*S. demissum*	*R2*
DS-R3a	SW8540-025	*S. demissum*	*R3a*
DS-R3b	SW8540-325	*S. demissum*	*R3b*
DS-R4	Ma*R4*	*S. demissum*	*R4*
DS-5	Ma*R5*	*S. demissum*	*R1*, *R2*, *R3b***
DS-6	Ma*R6*	*S. demissum*	*R1*, *R2*, *R3a*
DS-7	Ma*R7*	*S. demissum*	*R3a*, *R4*
DS-8	Ma*R8*	*S. demissum*	*R3a*, *R4*, *R8*
DS-R8	3020-330	*S. demissum*	*R8*
DS-9	Ma*R9*	*S. demissum*	*R1*, *Rpi-abpt1*, *R3a*, *R3b*, *R4*, *R8*, *R9***
DS-R9	3151-04	*S. demissum*	*R9***
DS-10	Ma*R10*	*S. demissum*	*R3b*, *R10*
DS-11	Ma*R11*	*S. demissum*	*R3b*, *R10*
DS-blb3	blb99-256-3	*S. bulbocastanum*	*Rpi-blb3*
DS-sto1	sto389-4	*S. stoloniferum*	*Rpi-sto1*
DS-blb1	blb8005	*S. bulbocastanum*	*Rpi-blb1***
DS-pta1	pta767-1	*S. papita*	*Rpi-pta1***
DS-blb2	blb2002	*S. bulbocastanum*	*Rpi-blb2***
DS-vnt1.1	vnt367-1	*S. venturii*	*Rpi-vnt1.1*
DS-chc1	chc543-5	*S. chacoense*	*Rpi-chc1***

- *R* gene content was determined using a combination of molecular marker analysis and *Avr* responsiveness.

** Indications are available for additional *R* genes in these plants, besides the *R* genes listed in this table.

Monogenic GM differential set of cv Desiree with 10 *R* genes

Name	Genotype	*R* gene	*R* gene donor	Donor species	Matching effector
DSD-R1	A(10-2-4)	*R1*	MaR1	*S. demissum*	*Avr1*
DSD-blb3	A03-142	*Rpi-blb3*	blb99-256-3	*S. bulbocastanum*	*Avr2*
DSD-R3a	A04-33	*R3a*	SW8540-025	*S. demissum*	*Avr3a*
DSD-R3b	A25-11	*R3b*	SW8540-325	*S. demissum*	*Avr3b*
DSD-sto1	A09-277	*Rpi-sto1*	sto389-4	*S. stoloniferum*	*Avr-blb1*
DSD-blb1	A01-20	*Rpi-blb1*	blb8005	*S. bulbocastanum*	*Avr-blb1*
DSD-pta1	A23-43	*Rpi-pta1*	Pta767-1	*S. papita*	*Avr-blb1*
DSD-blb2	A02-33	*Rpi-blb2*	blb2002	*S. bulbocastanum*	*Avr-blb2*
DSD-vnt1.1	A13-13	*Rpi-vnt1.1*	vnt367-1	*S. venturii*	*Avr-vnt1*
DSD-chc1	A17-27	*Rpi-chc1*	chc543-5	*S. chacoense*	*Avr-chc1*

DSD: differential set Desiree

All the transformants containing 1 copy of *R* gene, showed resistance against avirulent isolates in detached leaf assay.

Comparison of the GM and improved differential set

Genotype	*GM/Conventional*	*R* gene	*P. infestans* isolate							
			EC1	PIC99189	IPO-0	H3PO4	NL07434	NL08797	NL09129	NL11389
A01-20	*GM*	*blb1*	R	S	R	R	R	S	R	R
blb8005	*Conv.*	*blb1*	R	R	R	R	R	R	R	R
A09-268	*GM*	*sto1*	R	S	R	R	R	S	R	R
sto389-4	*Conv.*	*sto1*	R	SQ	R	R	R	RQ	R	R
A23-29	*GM*	*pta1*	R	S	RQ	R	R	S	R	R
pta767-1	*Conv.*	*pta1*	R	SQ	R	R	R	R	R	R
A02-33	*GM*	*blb2*	R	R	R	R	SQ	RQ	R	R
blb2002	*Conv.*	*blb2*	R	R	R	R	R	R	R	R
A03-142	*GM*	*blb3*	R	S	R	R	RQ	RQ	S	RQ
blb99-256-3	*Conv.*	*blb3*	R	SQ	R	R	R	R	S	R
A04-33	*GM*	*R3a*	S	R	R	S	S	S	S	S
SW8540-025	*Conv.*	*R3a*	S	R	R	S	S	S	S	S
A25-11	*GM*	*R3b*	S	S	S	S	S	S	S	S
SW8540-325	*Conv.*	*R3b*	S	S	S	S	S	S	S	S
A13-13	*GM*	*vnt1.1*	S	R	R	R	R	R	R	R
vnt367-1	*Conv.*	*vnt1.1*	Q	R	R	R	R	R	R	R
A17-27	*GM*	*chc1*	R	R	R	RQ	R	S	R	R
chc543-5	*Conv.*	*chc1*	R	R	R	R	R	SQ	R	R
MaR9	Conv.	*	R	R	R	R	R	R	RQ	R
Desiree	Conv.	-	S	S	S	S	S	S	S	S

R, all inoculation spots showed resistance or hypersensitive response);
RQ, 6–7 out of eight spots on a leaf showed resistance;
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Application of GM differential set in the field

P. infestans isolate	Trap plant of origin	*R* gene in trap plant	Detached Leaf Assay									
			A(10-23-2)	A01-20	A09-276$$$	A23-29	A02-33	A03-142	A04-33	A25-04	A13-13$$	A17-27$
			R1	*blb1*	*sto1*	*pta1*	*blb2*	*blb3*	*R3a*	*R3b*	*vnt1.1*	*chc1*
NL11452	A01-20	*blb1*	ND	S	S	S	R	R	ND	ND	R	R
NL09067	A01-84	*blb1*	S	S	S	ND	R	R	S	S	R	S**
NL11592	A09-277	*sto1*	ND	S	S	S	R	R	ND	ND	R	R
NL11593	A23-29	*pta1*	ND	S	S	S	R	R	ND	ND	R	R
NL09068	A02-33	*blb2*	SQ	R	R	ND	SQ	R	S	S	R	R**
NL09030	Bionica	*blb2*	S	R	S	ND	S	S	S	S	R	S**
NL10216	Toluca	*blb2*	ND	S	ND	S	ND	S	ND	ND	R	S
NL09300	Toluca	*blb2*	S	R	Q	ND	ND	R	S	S	R	R
NL11027	A03-142	*blb3*	ND	R	R	R	R	S	ND	ND	R	S
NL11052	A04-33	*R3a*	ND	S	S	S	R	R	ND	ND	R	S
NL11054	A25-11	*R3b*	ND	S	S	S	R	S	ND	ND	R	R
NL11479	A13-13	*vnt1.1*	ND	S	S	S	R	R	ND	ND	S	S
NL11480	A17-27	*chc1*	ND	S	S	S	R	R	ND	ND	R	S
NL08645	chc543-5	*chc1*	ND	S	S	R***	R	ND	ND	ND	R	S**

** *Rpi-chc1* containing plant chc543-5 was used instead of A17-27

*** Rpi-pta1 containing plant pta767-1 was used instead of A23-29

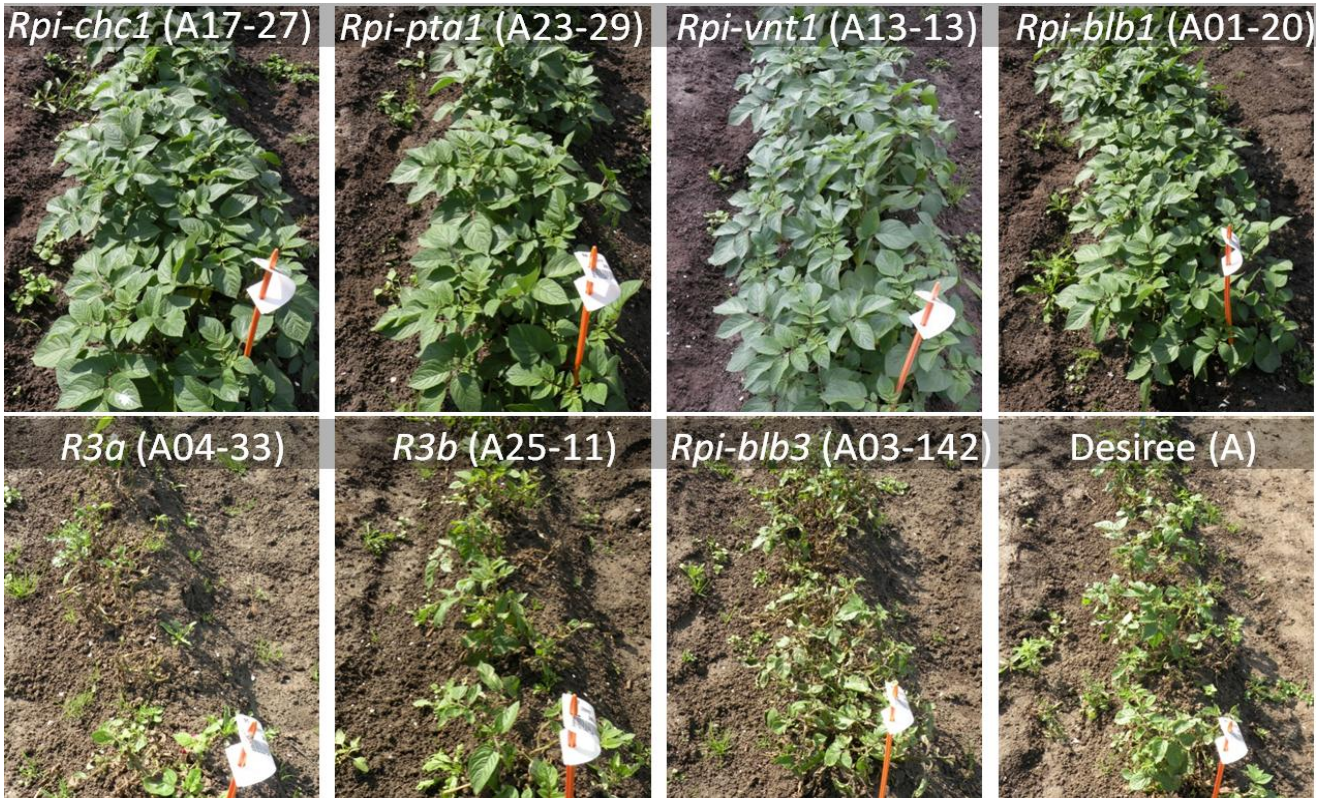
$ A17-28, an independent Desisree transgeneic event harbouring *Rpi-chc1*, instead of A17-27 was used for DLA of 2009 isolates.

$$ A13-1 instead of A13-13 was used for the characterization of the 2008 isolate.

$$$, plant A09-1 was used for 2008 isolates, and A09-7 was used for 2011 isolates.

R, no sporulation; RQ, sporulation occurred on around 25% of leaf surface; Q, around 50% of the leaf surface covered with sporulation; S, 100%;. ND: Not Done

Application of GM differential set in the field



GM differential set with natural infection in the Netherlands in 2011.
(pictures were taken 49 days after planting *in vitro* plants)

Take home message

1. Mastenbroek differential set was improved by extending with plants containing new *R* gene resources, and plants harboring less *R* genes compared to their original differential plants. However, some plants may still contain additional *R* genes.

2. GM differential set was set up with cv Desiree transformants harboring single *R* genes. This set is expected to bring more accurate characterization of *Pi* isolates. And these plants can be directly used in the field to investigate the functionality of inserted *R* genes in a specific region.

Thank you for your attention!