

Evaluating the potential of *P. infestans* to genetically adapt to the Rpi-blb1 (RB) transgene

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Context?

- Complete impact assessment of GM crops relevant to Ireland
- Identify challenges
- Select crop traits of most relevance

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REVIEW ARTICLE

Relevance of genetically modified crops in light of future environmental and legislative challenges to the agri-environment

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10/07/09

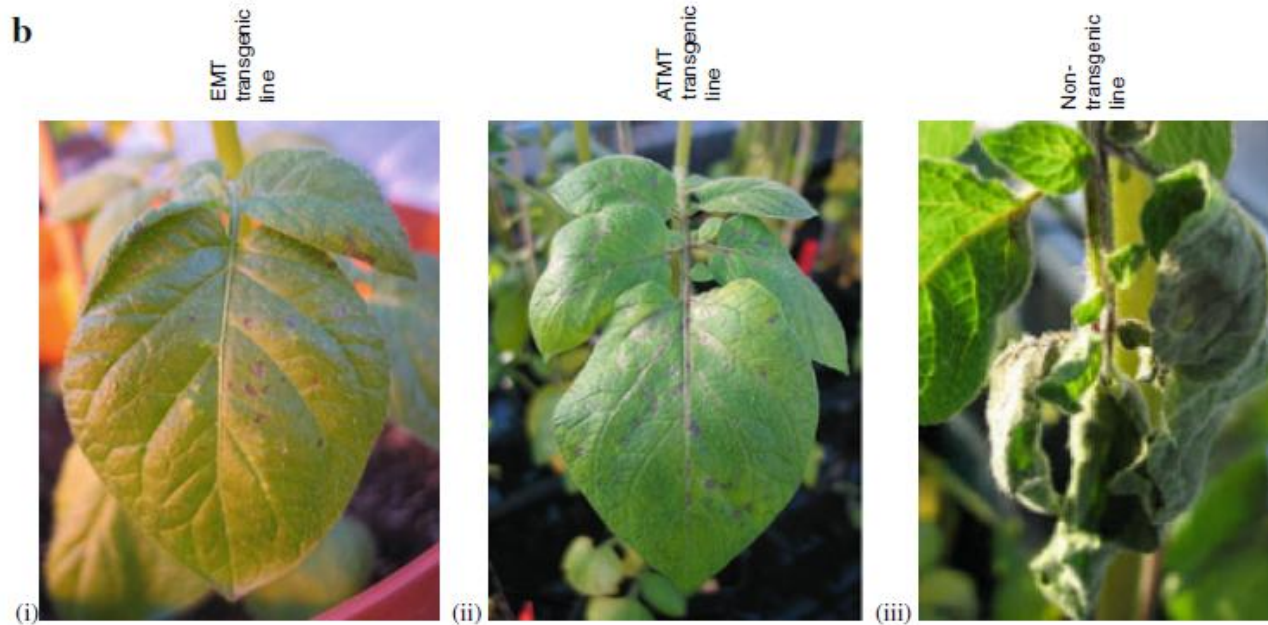


27/07/09



Context?

- How would Irish blight respond ?
- Sourced RB (Song et al, 2003) [*Rpi-blb1*]
- Generated transgenic cv. Desiree and Maris Peer



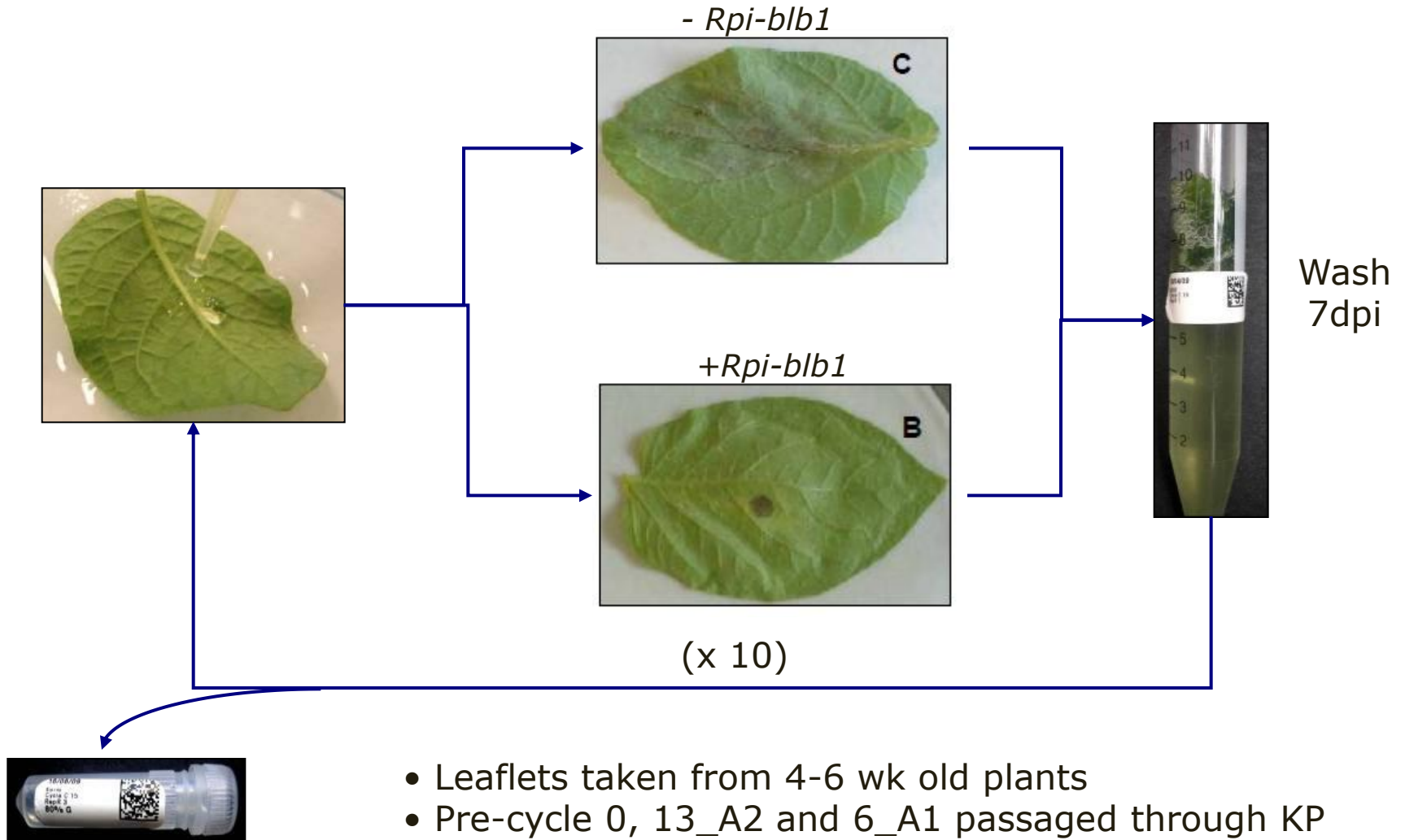
- Strong partial resistance (not immunity)
(as in Song et al. 2003, Van der Vossen et al. 2003, Chen and Halterman, 2010)

What do we know about the corresponding *blb1* effector?

- In 1998, *ipiO* (in planta induced) postulated as a pathogenicity factor (Van West et al. 1998)
- Identified as *avr-blb1* via effector profiling (Vleeshouwers et al. 2008)
- *ipiO* effector induces ETI in varieties equipped with *Rpi-blb1*
- Changes in *ipiO* can lead to loss of ETS = susceptibility
- *ipiO* gene family is highly diverse between strains, split into Class I, II and III (Champouret et al. 2009)
- All isolates with class I *ipiO* variants are avirulent on *Rpi-blb1*
- Aggressiveness correlated with increased *ipiO* diversity across *P. infestans* isolates (Halterman et al. 2010)

ipiO diversity within *P. infestans* isolates pre/post-infection?

- **13_A2** and **6_A1** isolates



- Leaflets taken from 4-6 wk old plants
- Pre-cycle 0, 13_A2 and 6_A1 passaged through KP
- 20uL of 2×10^4 sporangia/ml for disease initiation
- Replicated three times for each *P. infestans* genotype

Line	Rpi-blb1 copy number	Mean AULPC (\pm S.E)			
		6_A1		13_A2	
		cycle 1	cycle 10	cycle 1	cycle 10
MP100_4	5	85.95 (20.04)	0.00	515.63 (22.37)	344.51
MP100_5	3	201.36 (51.21)	1226.27	759.31 (41.56)	931.89 (51.14)
MP100_6	1	317.03 (45.83)	903.51	678.37 (31.14)	1055.85
MP100_9	1	54.27 (4.14)	1003.65 (44.82)	562.31 (31.58)	984.77
Maris Peer	0	647.19 (24.20)	1105.67 (27.52)	789.55 (42.47)	1050.96 (16.62)
DES RB4	1	787.90 (35.26)	1187.89 (9.53)	727.61 (42.22)	1158.23 (72.29)
Desiree	0	833.52 (18.49)	1082.26	772.43 (49.29)	1279.65

- DNA extracted from 6_A1 and 13_A2 isolates from C0 and C10 / interaction
- *ipiO* amplified via PCR
- PCR amplicons cloned in pGEM and sequenced (n=280)

Exciting result?

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Presence of the Potato Late Blight Resistance Gene *RB* Does Not Promote Adaptive Parasitism of *Phytophthora infestans*

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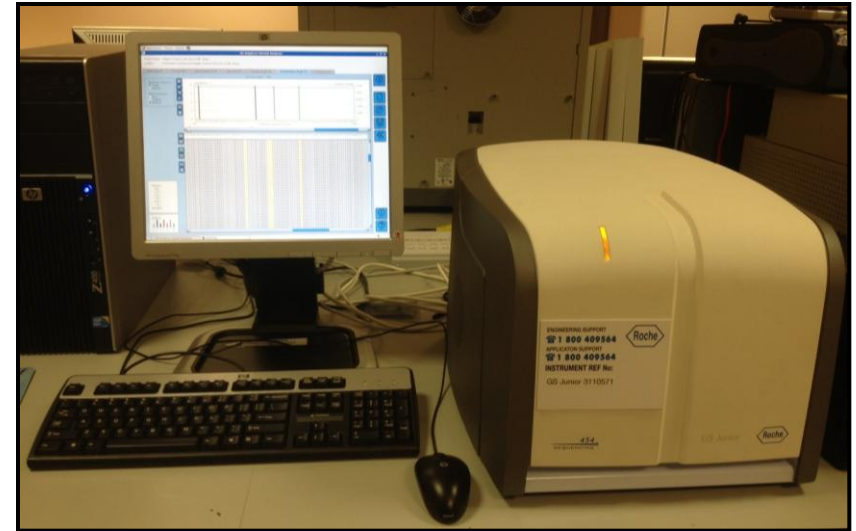
No aa mutations following
20 cycles of US-8 (A2) strain
through Kathadin +*Rpi-blb1*

PCR & clone sequencing limited by handling and cost

Up to 80,000 amplicons
read through next generation (454)
sequencing

Multiplexing possible
(e.g. 10 samples ~8,000 reads /
sample)

So, processed our passaged isolates on
GS Junior 454 platform

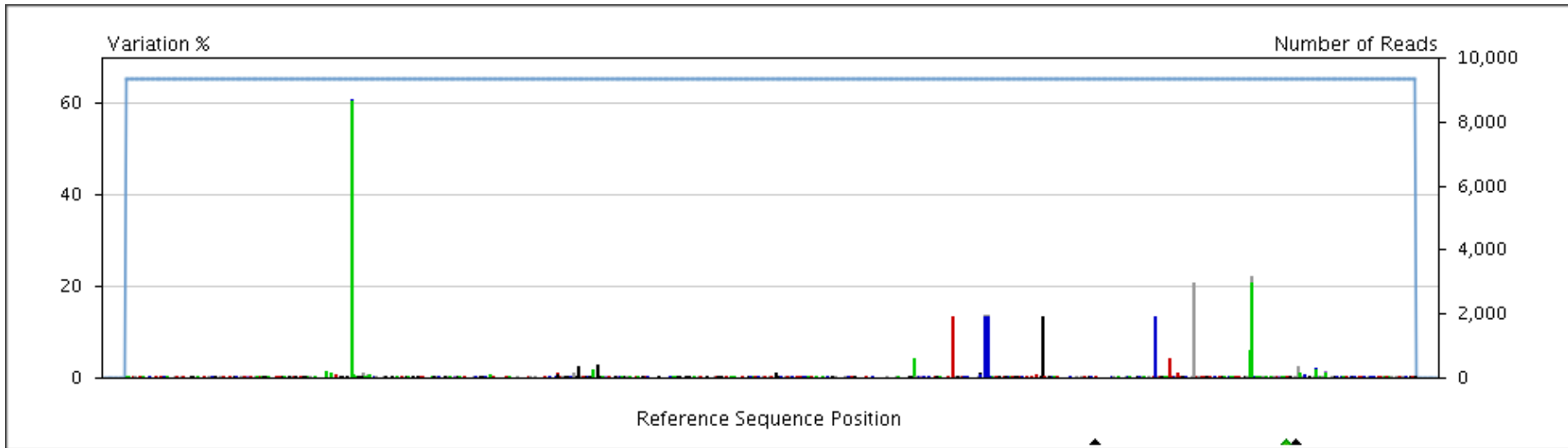


No. of *ipiO* reads for 6_A1?

P. inf isolate	Cycle	Host	Rep	#454 reads
6_A1	10	MP – Rpi blb1	R1	8,337
	10	MP – Rpi blb1	R2	8,662
	10	MP – Rpi blb1	R3	5,945
6_A1	10	MP + Rpi blb1	R1	7,711
	10	MP + Rpi blb1	R2	7,910
	10	MP + Rpi blb1	R3	5,895
6_A1	0	---	R1	7,789
	0	---	R2	5,356
	0	---	R3	6,027

Average ~7000 *ipiO* reads/sample

- Generated consensus of *ipiO* reads from 6_A1 cycle 0
- Reference sequence for 6_A1 cycle 10 (+/- *Rpi-blb1*)




- Variants only considered $>2\%$ frequency
- 12 'hotspots' present in 6_A1 isolates cycled through MP+ *Rpi-blb1*
- **BUT** also in 6_A1 isolates cycled through MP - *Rpi-blb1*
- Significance between isolates taken from MP +/- *Rpi-blb1* to be determined

Frequency of ipiO variation

Var #	Position	Variant	-blb1 R1	-blb1 R2	-blb1 R3	+blb1 R1	+blb1 R2	+blb1 R3
1	90	A inserted	59.91	57.01	57.19	53.09	56.8	57.03
2	163	G inserted	2.33	3.02	0.98	2.97	2.57	2.73
3	167	G inserted	2.83	3.47	1.8	3.67	3.2	3.2
4	278	T for C	13.45	12.73	13.49	14.62	31.61	13.87
5	289	C for T	13.46	12.78	13.49	14.61	31.68	13.87
6	290	C for A	13.47	12.77	13.49	14.61	31.68	13.87
7	304	G for T	13.34	12.66	13.41	14.54	31.54	13.81
8	340	C for G	13.46	12.72	13.47	14.55	31.62	13.73
9	343	T inserted	4.16	4.06	2.07	1.79	1.93	4.13
10	352	T deleted	20.56	18.71	17.8	15.81	21.86	18.79
11	367	A inserted	6	6.48	6.64	8.94	13.65	7.22
12	368	A for C	20.69	20.19	21.68	22.82	47.44	21.14

To conclude...

- Greater depth of sequencing = greater insight
- Much more going on than previously thought
- Data analysis currently tentative, further analyses required
- For example; how many variants <2% threshold (=100s)?
- Full impact on amino acid sequence
- Didn't expect equal no. variant hotspots in +/- *Rpi-blb1*
- What's the impact on other effectors?
- Something to investigate in  Assessing and Monitoring
the Impacts of Genetically modified plants on Agro-ecosystems
- MonPESC (2012 – 2017), all island survey of *P. infestans* for fungicide sensitivity, SSR, effector diversity, participate in Euroblight FTA

Thank you to...

Co-authors

and...



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An Roinn
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and...

You.