Exploring and exploiting resistance to *Globodera pallida* in potato

Ulrike Gartner

University of Idaho, 08 March 2019

The James Hutton Institute

Background

- 5.2 million tonnes of tuber production in UK in 2016 (44 tonnes/ha)

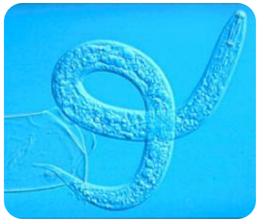
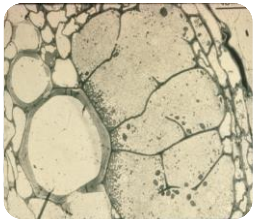
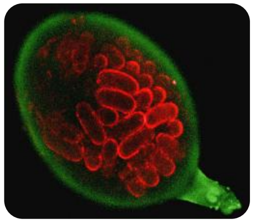
- 45% of land is used for the production of seed potatoes in Scotland

Healthy potato plants and plants affected with PCN

Tubers from various commercially available potato cultivars

Potato Cyst Nematodes

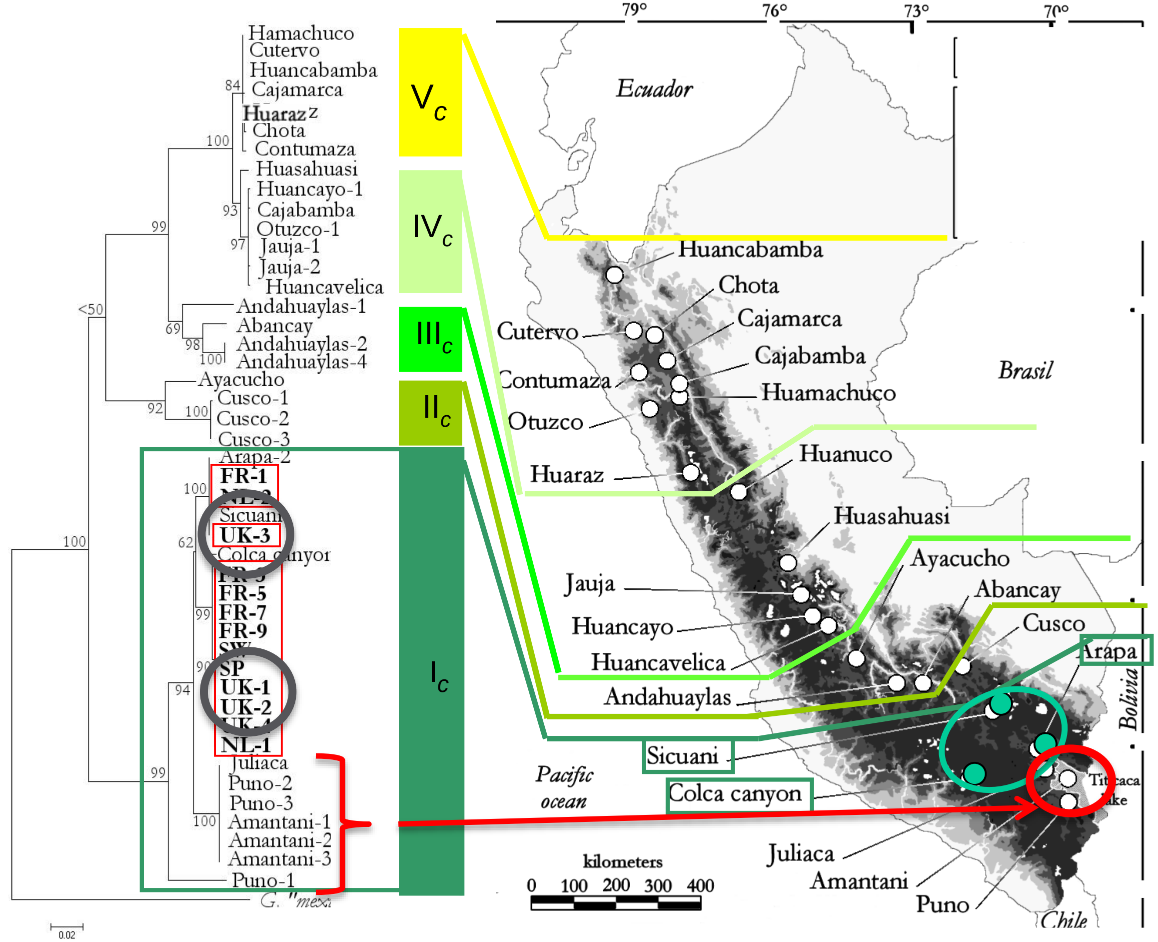
- Two species, *G. rostochiensis* and *G. pallida*, have been described in the UK
- Feed and develop in the root system
- Cysts fall into the soil and can survive there for many years
- Listed as quarantine organism and therefore subject to monitoring

1. **Characterisation of UK *G. pallida* populations and their distribution; finding markers to distinguish between pathotypes**

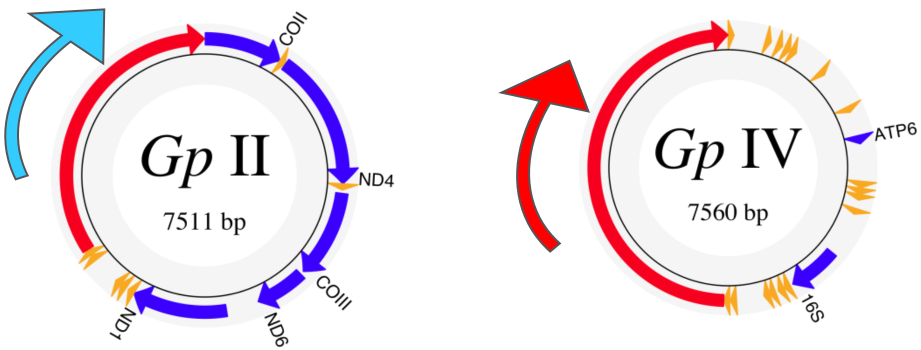
2. **Wild potatoes species as source of new resistance to *G. pallida***

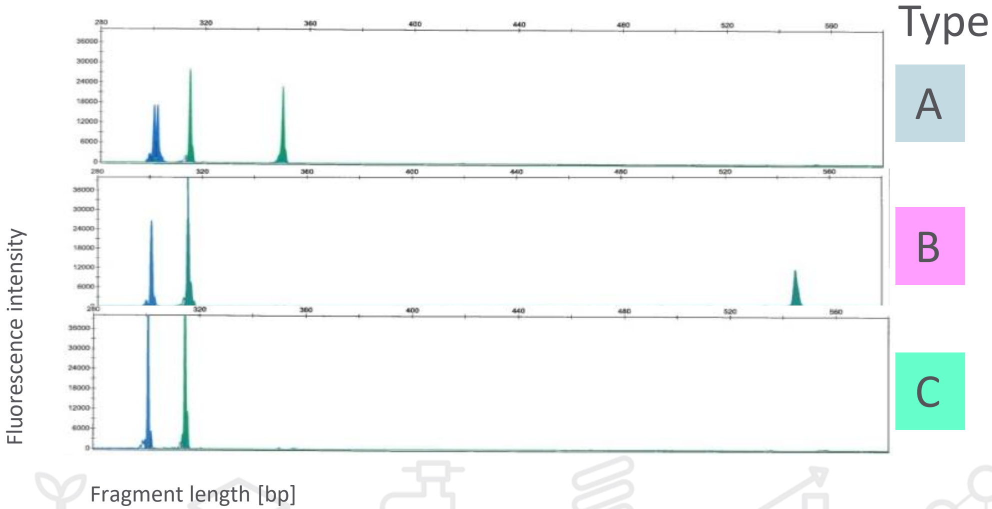
G. pallida populations in the UK originate from South America based on microsatellites and Cytb



Plantard *et al* (2008), *Molecular Ecology*

A mitochondrial marker can distinguish between the three introductions of *G. pallida*





PCR amplification of a non-coding region of mitochondrial circles II and IV with primers that are labelled with two different fluorophores, Taq1 digestion, electrophoresis and laser detection of fluorescent DNA fragments.

Composition of *G. pallida* from the historical JHI collection

Samples	Population	Type A	Type B	Type C	No. cysts analysed
JHI	Lindley	25 (96%)	1 (4%)		26
collection	Luffness field 1	2 (6%)	33 (94%)		35
	Pa1	4 (19%)	2 (10%)	15 (71%)	21

Composition of *G. pallida* from the historical JHI collection

Samples	Population	Type A	Type B	Type C	No. cysts analysed	
JHI	Lindley	25 (96%)	1 (4%)		26	"Pa2 end of Pa2/3"
collection	Luffness field 1	2 (6%)	33 (94%)		35	"Pa3 end of Pa2/3"
	Pa1	4 (19%)	2 (10%)	15 (71%)	21	"Pa1"

Composition of *G. pallida* from the historical JHI collection

Samples	Population	Type A	Type B	Type C	No. cysts analysed
JHI	Lindley	25 (96%)	1 (4%)		26
collection	Luffness field 1	2 (6%)	33 (94%)		35
	Pa1	4 (19%)	2 (10%)	15 (71%)	21

"Pa2 end of Pa2/3"
"Pa3 end of Pa2/3"
"Pa1"

Can these markers be used as pathotype markers?

Composition of *G. pallida* in historical and recent field samples

Samples	Population	Type A	Type B	Type C	No. cysts analysed
JHI	Lindley	25 (96%)	1 (4%)		26
collection	Luffness field 1	2 (6%)	33 (94%)		35
	Pa1	4 (19%)	2 (10%)	15 (71%)	21

“Pa2 end of Pa2/3”

“Pa3 end of Pa2/3”

“Pa1”

Composition of *G. pallida* in historical and recent field samples

The James Hutton Institute

Samples	Population	Type A	Type B	Type C	No. cysts analysed
JHI	Lindley	25 (96%)	1 (4%)		26
collection	Luffness field 1	2 (6%)	33 (94%)		35
	Pa1	4 (19%)	2 (10%)	15 (71%)	21
Field	Luffness field 1	7 (39%)	9 (50%)	2 (11%)	18
	Luffness field 2			2 (100%)	2
	Luffness field 3	4 (100%)			4
	Luffness field 5	1 (33%)		2 (67%)	3

"Pa2 end of Pa2/3"
"Pa3 end of Pa2/3"
"Pa1"

Composition of *G. pallida* in historical and recent field samples

Samples	Population	Type A	Type B	Type C	No. cysts analysed	
JHI	Lindley	25 (96%)	1 (4%)		26	“Pa2 end of Pa2/3”
collection	Luffness field 1	2 (6%)	33 (94%)		35	“Pa3 end of Pa2/3”
	Pa1	4 (19%)	2 (10%)	15 (71%)	21	“Pa1”
Field	Luffness field 1	7 (39%)	9 (50%)	2 (11%)	18	
	Luffness field 2			2 (100%)	2	
	Luffness field 3	4 (100%)			4	
	Luffness field 5	1 (33%)		2 (67%)	3	

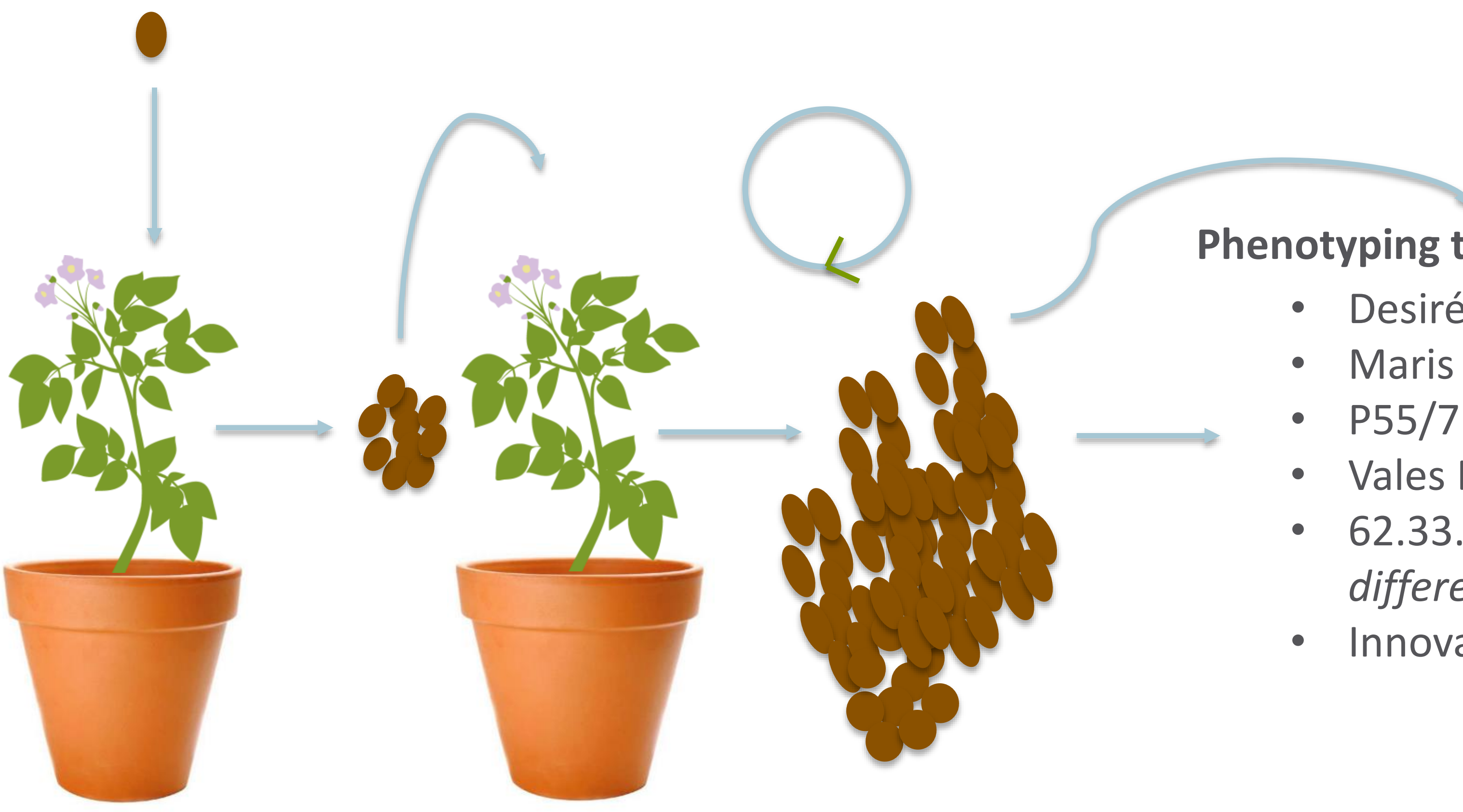
In Scotland the 3 *G. pallida* introductions are widespread

- All 3 types are widespread
- Most fields have 1 cytB type
- 1/5 fields contain mixtures
- <3% contain all three types
- 1 field has a 4^{th} type

Eves van den Akker *et al* 2015 *Molecular Ecology*

If UK fields contain multiple introductions of *G. pallida*, are they also mixtures of phenotypes?

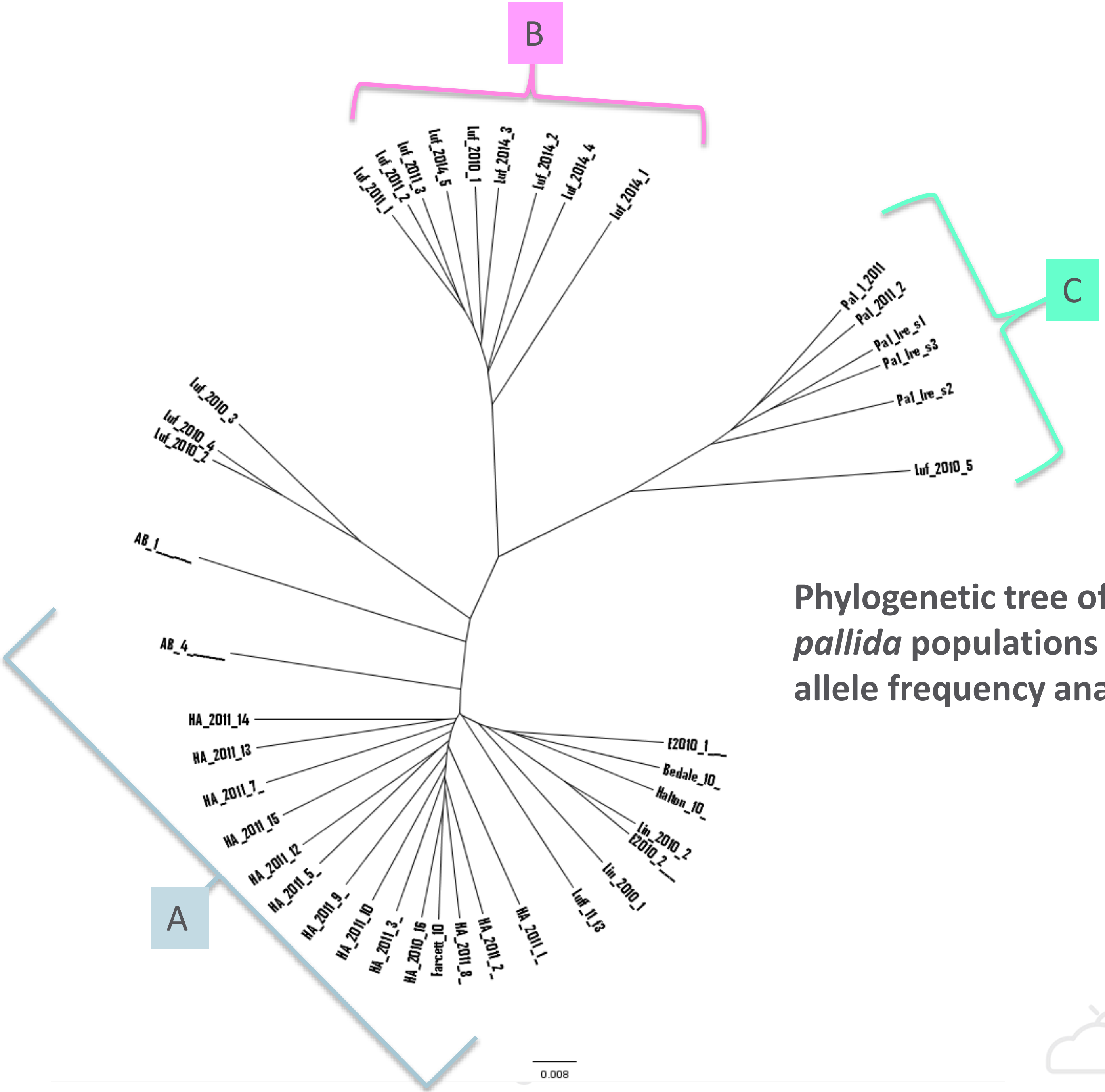
Generation of single cyst lines



Phenotyping test on

- Desirée
- Maris Piper (*H1*)
- P55/7 (*H2*)
- Vales Everest (*H3*)
- 62.33.3 (*Gpa5 different source*)
- Innovator (*Gpa5*)

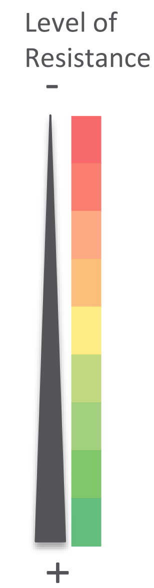
Year 1 | Year 2-4 | Year 3-5



Phylogenetic tree of different British *G. pallida* populations based on genome-wide allele frequency analysis of 1500 loci

Results

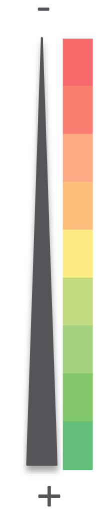
Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

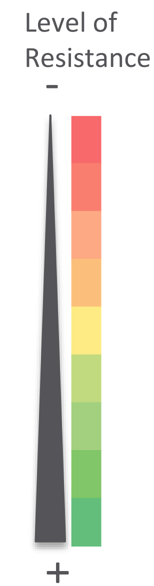
Level of Resistance



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

The James Hutton Institute



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

Level of Resistance

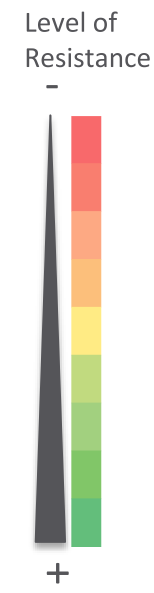
−

+

The James Hutton Institute

Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

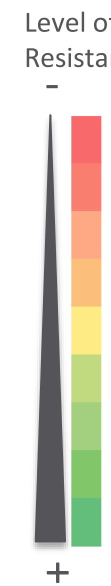


The James Hutton Institute

Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

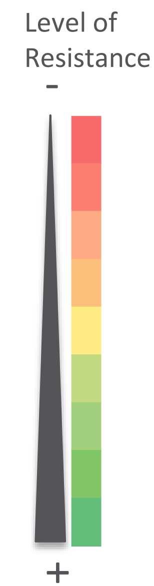
The James Hutton Institute



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

The James Hutton Institute



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
Lindley 2010 11	2016						
Lindley 2010 11	2017						
Lindley 2010 3	2017						
Lindley JHI pool	2016						
Lindley JHI pool	2017						
Luff 2011 3-8	2017						
Luff 2011 3-17(b)	2016						
Luff 2011 3-17(b)	2017						
Luff 2011 3-18(a)	2016						
Luff 2011 3-18(a)	2017						
Luff 2011 1-12	2016						
Luff 2011 1-12	2017						
Luff 2014 1-4	2017						
Luff 2014 1-19	2017						
Luff 2014 1-30	2017						
Lufness JHI pool	2016						
Lufness JHI pool	2017						
Pa 1 2011 3	2017						
Pa 1 2011 12	2017						
Pa1 2011 pool	2016						
Pa1 2011 pool	2017						

The James Hutton Institute

Level of Resistance

–

+

Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance						Mito-type	GBS Group
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator		
Lindley 2010 11	2016							A	A
Lindley 2010 11	2017							A	A
Lindley 2010 3	2017							A	A
Lindley JHI pool	2016							A	
Lindley JHI pool	2017							A	
Luff 2011 3-8	2017							A	A
Luff 2011 3-17(b)	2016							A	A
Luff 2011 3-17(b)	2017							A	A
Luff 2011 3-18(a)	2016							A	A
Luff 2011 3-18(a)	2017							A	A
Luff 2011 1-12	2016							B	B
Luff 2011 1-12	2017							B	B
Luff 2014 1-4	2017							B	B
Luff 2014 1-19	2017							B	B
Luff 2014 1-30	2017							B	B
Lufness JHI pool	2016							B	B
Lufness JHI pool	2017							B	B
Pa 1 2011 3	2017							A	C
Pa 1 2011 12	2017							A	C
Pa1 2011 pool	2016							C	C
Pa1 2011 pool	2017							C	C

Level of Resistance

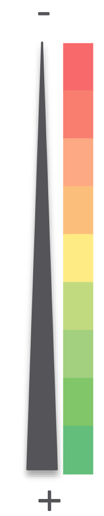
-

+

Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
AB A8-2	2016						
AB A8-2	2017						
AB Pb-3	2016						
AB Pb-3	2017						
AB Pb-12	2017						
HA 2011 9	2016						
HA 2011 9	2017						
HA 2011 32	2016						
HA 2011 32	2017						
HA 2011 12	2016						
HA 2011 12	2017						
HA 2011 27	2016						
HA 2011 27	2017						
HA 2011 34	2017						
HA 2011 49	2017						

Level of Resistance



The James Hutton Institute

Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
AB A8-2	2016						
AB A8-2	2017						
AB Pb-3	2016						
AB Pb-3	2017						
AB Pb-12	2017						
HA 2011 9	2016						
HA 2011 9	2017						
HA 2011 32	2016						
HA 2011 32	2017						
HA 2011 12	2016						
HA 2011 12	2017						
HA 2011 27	2016						
HA 2011 27	2017						
HA 2011 34	2017						
HA 2011 49	2017						

Level of Resistance
-
+

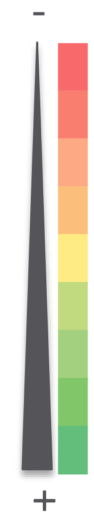
The James Hutton Institute

Results

The James Hutton Institute

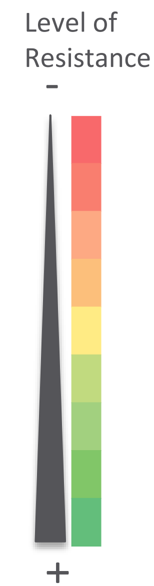
Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
AB A8-2	2016						
AB A8-2	2017						
AB Pb-3	2016						
AB Pb-3	2017						
AB Pb-12	2017						
HA 2011 9	2016						
HA 2011 9	2017						
HA 2011 32	2016						
HA 2011 32	2017						
HA 2011 12	2016						
HA 2011 12	2017						
HA 2011 27	2016						
HA 2011 27	2017						
HA 2011 34	2017						
HA 2011 49	2017						

Level of Resistance



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance					
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator
AB A8-2	2016						
AB A8-2	2017						
AB Pb-3	2016						
AB Pb-3	2017						
AB Pb-12	2017						
HA 2011 9	2016						
HA 2011 9	2017						
HA 2011 32	2016						
HA 2011 32	2017						
HA 2011 12	2016						
HA 2011 12	2017						
HA 2011 27	2016						
HA 2011 27	2017						
HA 2011 34	2017						
HA 2011 49	2017						



Results

Population ID	Year Tested	Potato Genotypes with Different Sources of Resistance						Mito-type	GBS Group
		Desirée	M. Piper	P55/7	VTN 62.33.3	V. Everest	Innovator		
AB A8-2	2016							A	
AB A8-2	2017							A	
AB Pb-3	2016							A	A
AB Pb-3	2017							A	A
AB Pb-12	2017							A	A
HA 2011 9	2016							A	A
HA 2011 9	2017							A	A
HA 2011 32	2016							A	A
HA 2011 32	2017							A	A
HA 2011 12	2016							B	A
HA 2011 12	2017							B	A
HA 2011 27	2016							B	A
HA 2011 27	2017							B	A
HA 2011 34	2017							B	A
HA 2011 49	2017							B	A

Level of Resistance

-

+

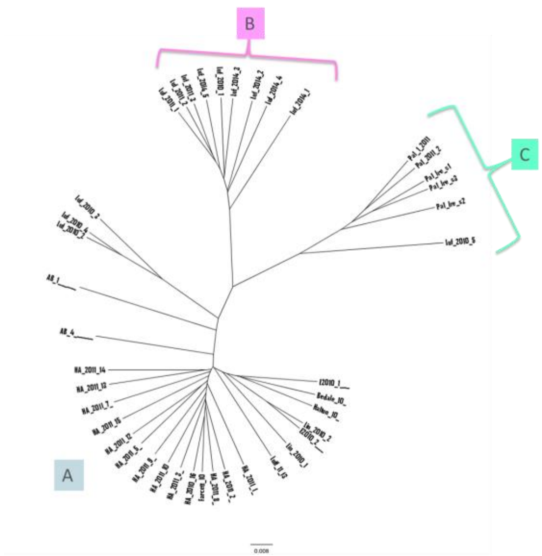
The James Hutton Institute

Characterisation of UK *G. pallida* populations and their distribution summary

- Our data suggest that PCN populations became more complex over time.
- Current *G. pallida* field populations are likely to comprise mixtures of up to the three different historical introductions from South America.
- VTN62.33.3, P55/7 and Vales Everest show the greatest variability in nematode multiplication.
- Innovator consistently provides a good level of resistance for all *G. pallida* populations. There is evidence that this resistance will be overcome. We need more resistant varieties, pyramiding of different resistance genes.
- The mitochondrial markers for the different introductions are not suitable as pathotype markers

Future Work: Development of molecular pathotype markers for *G. pallida*

Combining the SNP data obtained from the GBS analysis with the phenotypic data obtained for the single cyst lines and population pools provided SNP candidates in order to find robust molecular pathotype markers.



Single cyst line/population	Pheno type	Mito type	GBS group
HA 12	PA3	B	A
HA27	PA3	B	A
HA34	PA3	B	A
PB-12	PA2	A	A
Luff3-18	PA2	A	A
Luff 3-8	PA2	A	A
Luff1-12	PA3	B	B
Luff1-4	PA3	B	B
Luff1-30	PA3	B	B
Pa1-12	PA1	A	C
Pa1 pool	PA1	C	C
Pa1 ire 1	PA1	n/a	C

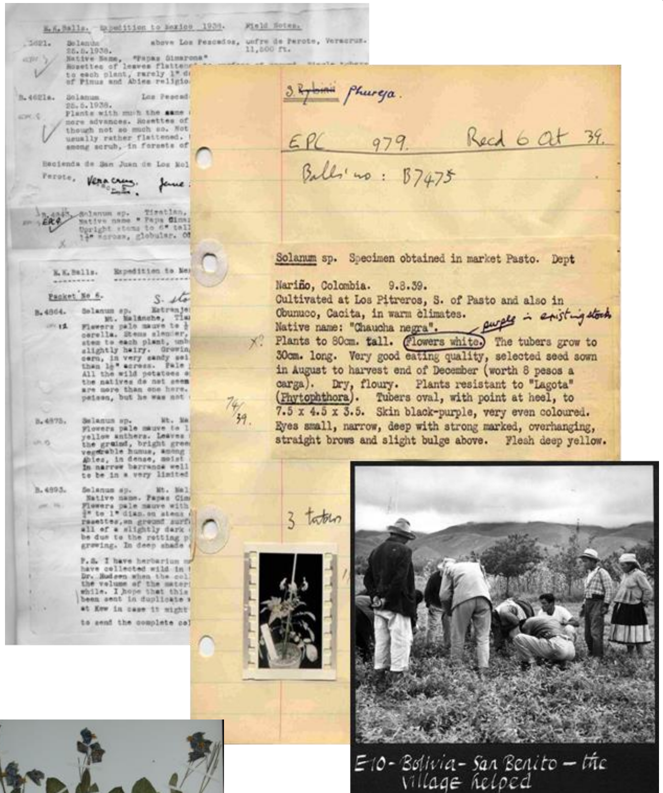
Future work: Candidate SNPs for molecular pathogen markers for *G. pallida*

	Allele1/2	% allele 1 in populations			
		PA2	PA3	PA1	
1	A/T	0.0	0.0	100.0	some populations sequenced, mixture of sequences.
2	T/C	0.0	9.1	100.0	some populations sequenced, SNPs found in Pa1 compared to Pa2/3
3	G/A	100.0	100.0	16.7	some populations sequenced, no pathotype specific SNPs found
4	G/A	100.0	100.0	16.7	primers designed, not sequenced yet
5	G/A	100.0	100.0	16.7	primers designed, not sequenced yet
6	T/C	0.0	9.1	100.0	
7	G/C	87.5	81.8	16.7	
8	T/C	100.0	9.1	100.0	
9	T/C	25.0	81.8	0.0	
10	T/A	37.5	90.9	0.0	
11	T/C	50.0	81.8	0.0	
12	G/A	75.0	90.9	16.7	

1. Characterisation of UK *G. pallida* populations and their distribution; finding markers to distinguish between pathotypes

2. **Wild potatoes species as source of new resistance to *G. pallida***

History of the Commonwealth Potato Collection (CPC)



- Discovery of blight resistant spontaneous hybrid between cultivated potato and the Mexican species *Solanum demissum*.
- Collecting expeditions to South America 1938-1939.
- Jack Hawkes.
- Empire Collection created, now CPC.

CPC today

- One of a network of international potato gene banks.
- Currently over 1500 accessions with over 80 different species.
- 2/3 wild tuber bearing species 1/3 primitive cultivated types.
- Collection held in true seed form.
- High health status.
- Working collection and base collection system.

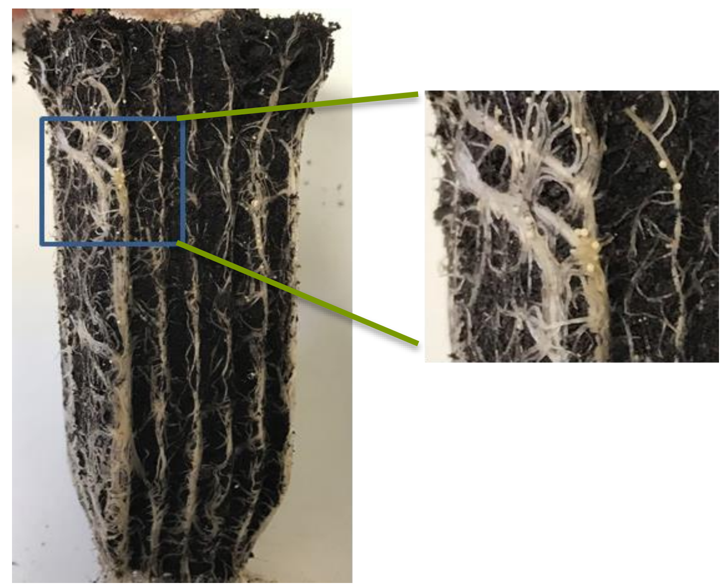
Identifying natural sources of resistance

- *S. spegazzinii*, acc. 7195, shows natural resistance to *G. pallida.*
- Diploid wild potato species.
- 1966 collected in Argentina, la Riocha, Fatima 1950 m altitude

- 2003: *S.spegazzinii* 7195(10) x *S. tuberosum* group phureja DB337(37)
- Scoring for resistance against PCN in progeny, selection of one resistant plant 02.F1.3a (35) as parent for my screen.
- *2013 backcross* 02.F1.3a (35) x *S. tuberosum* group phureja DB337(37) 13.A.02 ~1000 F1

Identification of resistant and susceptible progeny

200 F1 plants from 13.A.02 and their parents were scored for *G. pallida* population Lindley resistance/susceptibility in 4 replicate by counting infection the number of females on the surface 8 weeks post infection.



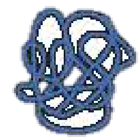
Mapping of genes conferring resistance to *G. pallida* in the wild potato species S. *spegazzinii*

The James Hutton Institute

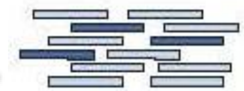
Crossing 13A02	*S. tuberosum* phureja DB327(37) susceptible		
Resistant clone 03.F1.3.35a from crossing *S. Spegazzinii CPC7195* x *S. tuberosum* phureja		r	r
	R	Rr	Rr
	r	rr	rr

Backcrossing resistant parent with susceptible parent screening of F1 and parents for resistance/susceptibility to PCN

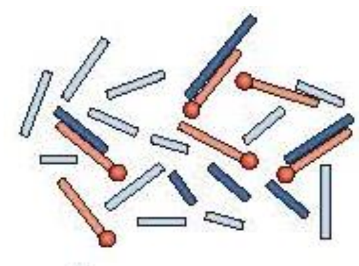
Bulking resistant and susceptible plants, preparation of genomic DNA



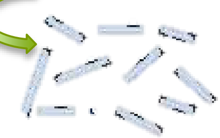
Construction of **indexed library**



Hybridisation with biotinylated RNA library baits,

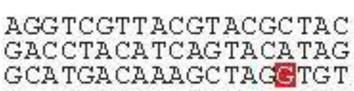


Pulldown, wash **Enrichment step**



Next Generation Sequencing

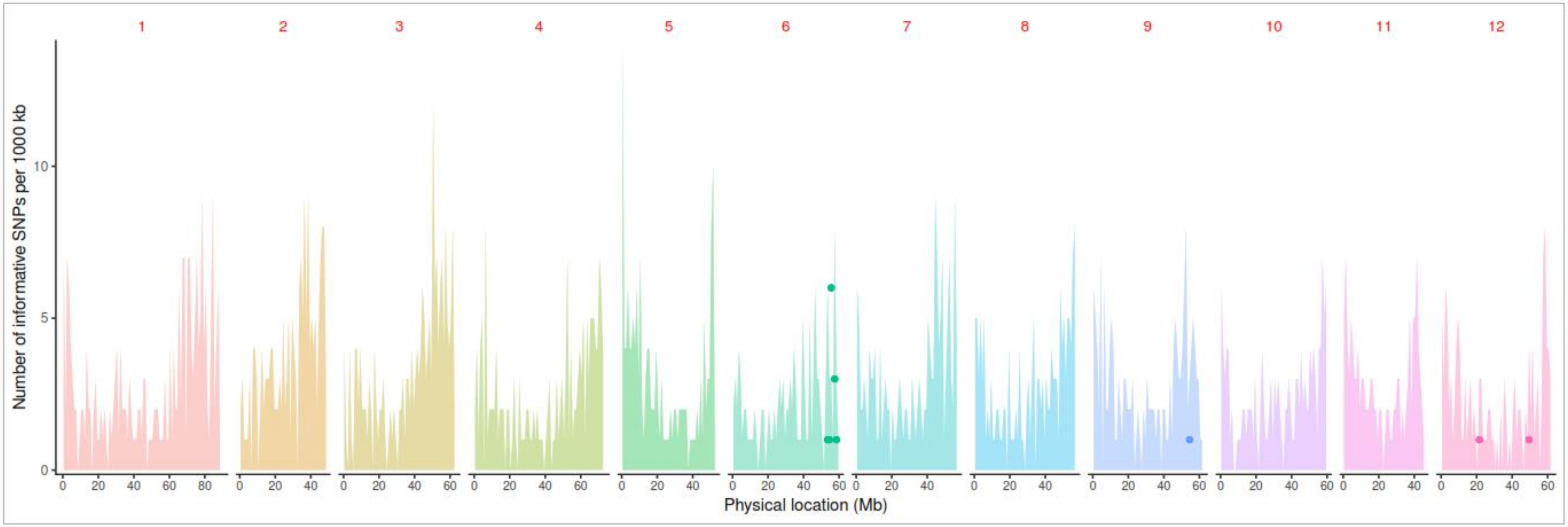
Analysing data



Mapping, alignment, **variant calling**

adapted from Michael J. Bamshad, Sarah B. Ng, Abigail W. Bigham, Holly K. Tabor, Mary J. Emond, Deborah A. Nickerson & Jay Shendure, Nature Reviews Genetics 12, 745-755 (November 2011)

Generic-mapping enrichment Sequencing (GenSeq)



Informative SNPs from GenSeq: enrichment of ~2000 single/low copy number genes: the background color on the y-axis show the areas covered by the enrichment, the dots indicate the number and location of informative SNPs, which are SNPs present in all resistant plants but not in any of the susceptible ones.

Development of KASP (Kompetitive Allele Specific PCR) markers

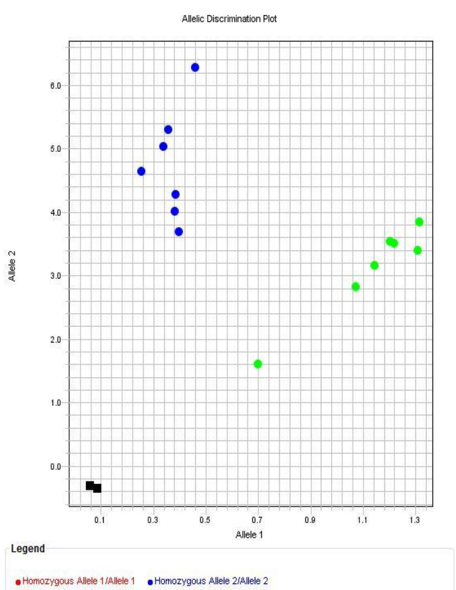
Flanking sequences (50 bp each upstream and downstream) around the SNP positions were used to design KASP primers (KASP by Design Oligos, LGC Genomics limited).

Markers were validated by testing them on the parents.

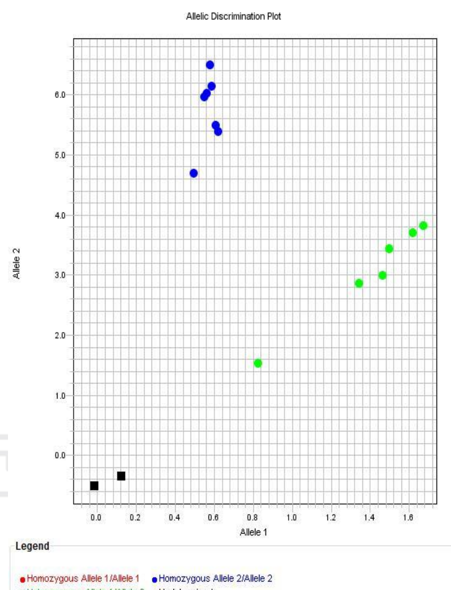
Resistant Parent green

Susceptible Parent blue

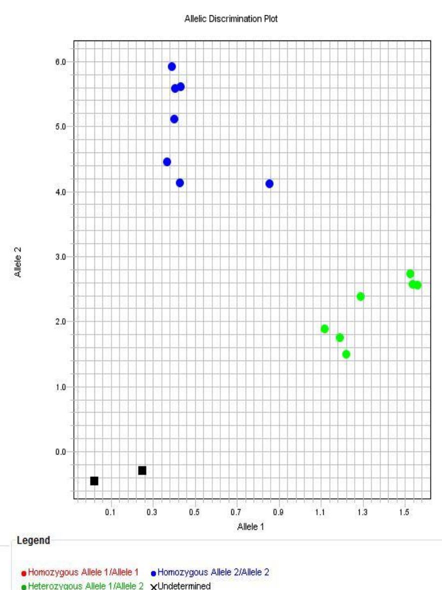
KASP 5



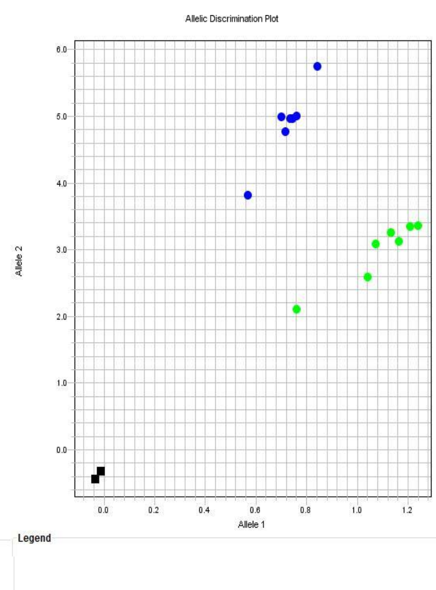
KASP 6



KASP 7



KASP 8



Graphical Genotyping

The James Hutton Institute

	SP	RP
K5	S	R
K6	S	R
K7	S	R
K8	S	R

K5 to K8 comprise 5.4 Mb

K5 to K7 comprise 3.4 Mb

	615	524	58	444	448	465	467	471	479	510	519	545	628	635	637	638	643	644	566
K5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
K6	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
K7	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
K8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

	A036	686	49	70	446	463	495	512	516	523	568	586	633	648	700	579	678	500
K5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

S	susceptible
R	resistant
	homozygot
	heterozygot

Work in progress

- Screening of all 1000 progenies of the crossing with marker K5 and K7, looking for recombinants
- Recombinants are also screened with K6 and new markers to narrow down position
- Recombinants will be screened of resistance/susceptibility

Introgressing the resistance to tetraploid cultivars and breeding clones.

The James Hutton Institute

- Diploid tetraploid crossing relies on unreduced gametes
- If successful, small berries with only a few seeds.
- Pyramiding of resistances
- Validating markers

Summary

- The wild potato species *S. spegazzinii*, acc. 7195, shows natural resistance to *G. pallida.*
- With enrichment sequencing we have identified a region on chromosome VI which contains SNPs linked to the resistance.
- Kasp markers were developed and the locus for this new resistance locus for *G. pallida* in *S. spegazzinii* could be mapped to a region of 3.4 Mb.
- More markers have been designed and should provide more information about the locus.
- Evaluation if markers can be used more universally
- Introgression into tetraploid breeding clones to pyramid resistances.

Acknowledgements

James Hutton Institute

Vivian Blok
Glenn Bryan
Ingo Hein
Miles Armstrong
Karen McLean
Brian Harrower
Katrin MacKenzie
Nematology lab
Potato-genetics lab
Glasshouse staff

Agriculture and Agri-Food Canada

Benjamin Mimee
Pierre-Yves Veronneau

SASA Edinburgh

Jon Pickup
Andrew Pitt
Nematology lab