

Phytophthora infestans population in Peru

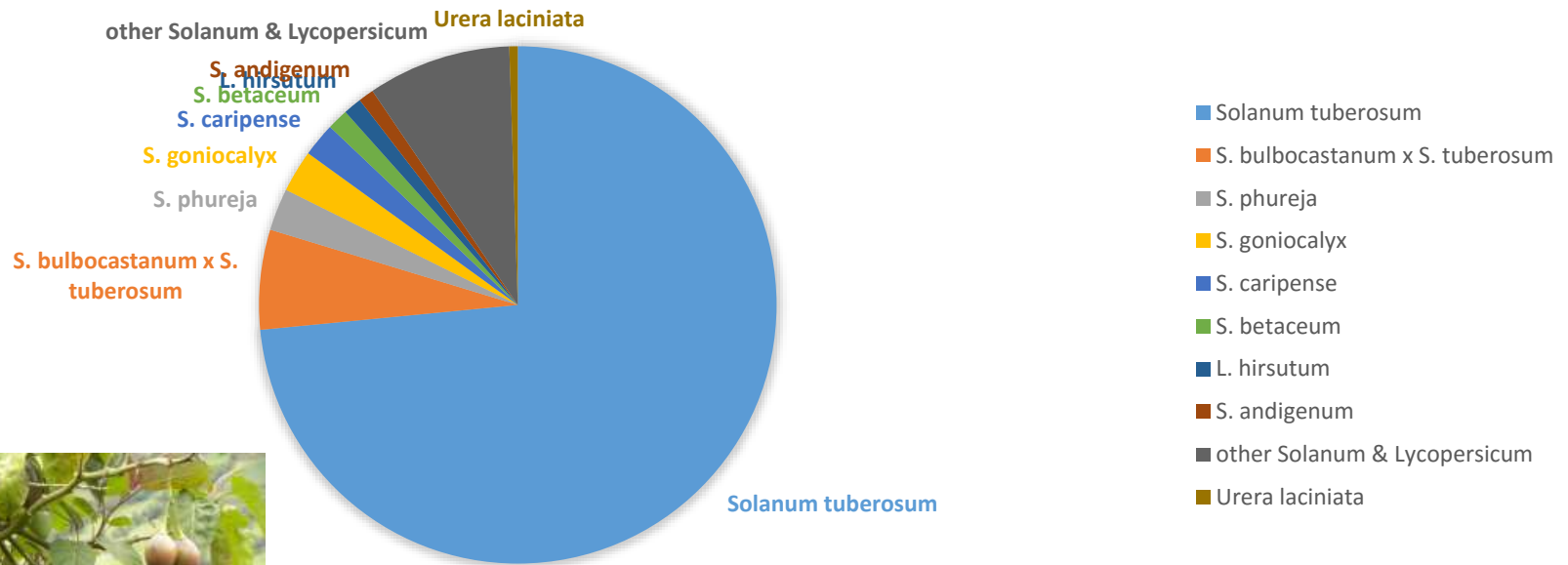
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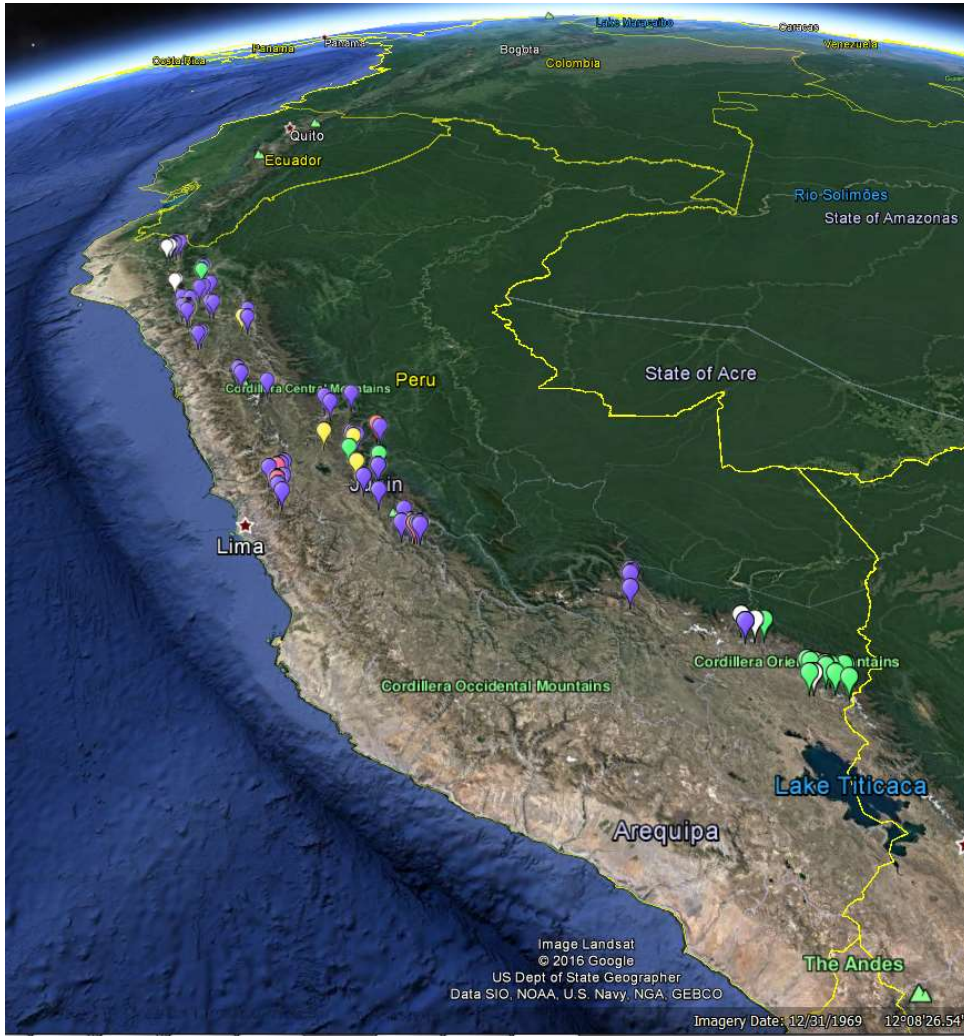
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HOST SPECIES OF PHYTOPHTHORA ISOLATES IN CIP COLLECTION





-  PE3
-  PE7
-  US1
-  EC1
-  new
-  PE8

P. infestans A1

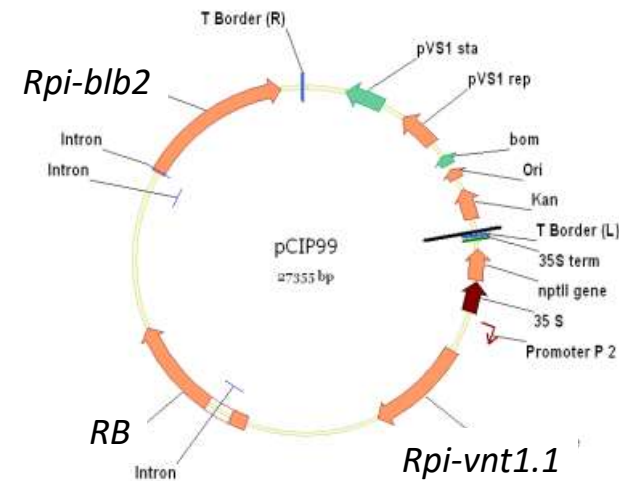
P. andina A2

Variability of effectors

Stacking *R* genes for extreme late blight resistance:

- Triple *R* gene construct:
 - *RB*
 - *Blb2*
 - *Vnt1*

- Recipient varieties:
 - Desiree (single gene and 3R gene)
 - Asante/Victoria (3R gene)

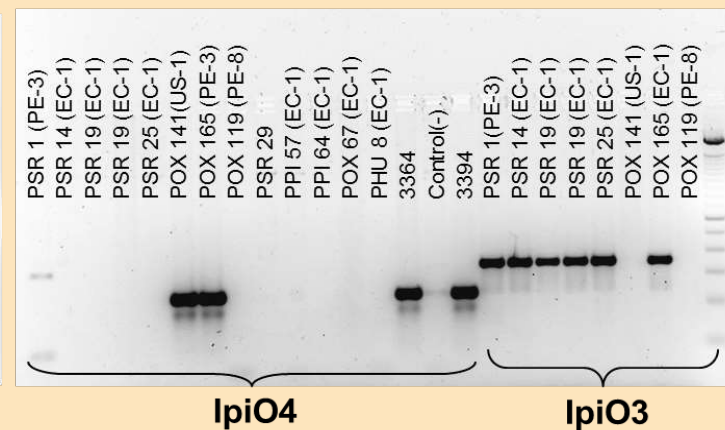
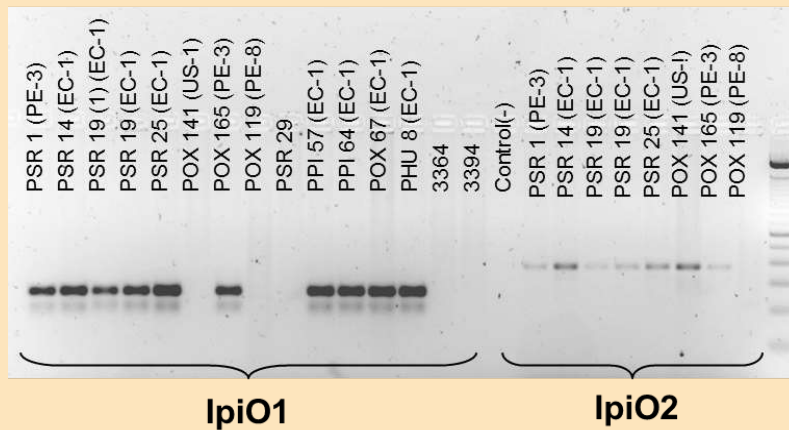


<i>R</i> gene	<i>Avr</i> gene
<i>RB</i>	<i>Avrblb1</i>
<i>Blb2</i>	<i>Avrblb2</i>
<i>Vnt1</i>	<i>Avrvnt1</i>

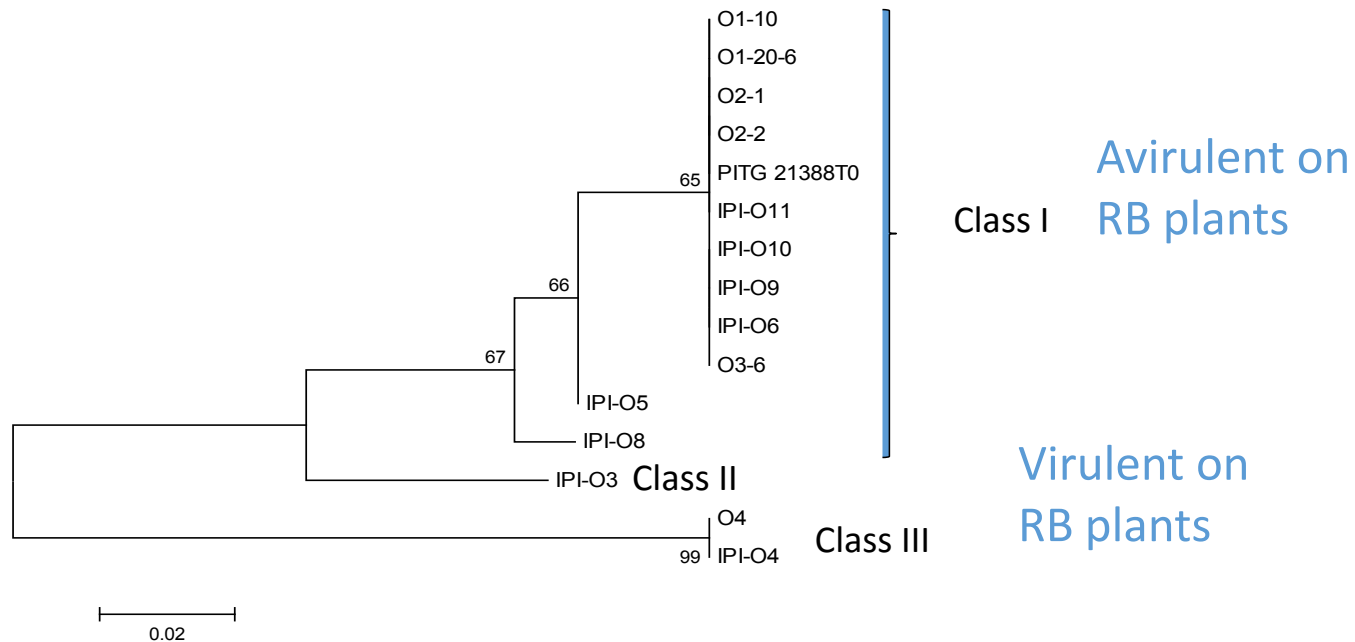
RB resistance: Test for Avrblb1 family effectors

<i>Avrblb1</i> class	Variant	Primers	Annealing temp. (°C)	Size band
Class I	IpiO1	ipio123F / ipio1R	61	~ 300 bp
Class I	IpiO2	RD6F / ASipio2	58	~ 390 bp
Class II	IpiO3	RD6F / ipio3R	62	~ 370 bp
Class III	IpiO4	ipio4F / ipio4R	62	~ 230 bp

95°C	2 min	} 32 Cycles
95°C	30s	
Annealing temp. (°C)	72°C	
72°C	7 min	



Avr-blb1 variation: 3 classes



Isolates that lack Class I variants and contain Class III variants can overcome resistance provided by RB

Avr-blb1 effector family variability in Peru

Lineage	Host	I	II	III
EC-1	<i>S. tuberosum</i> (10), <i>S. stenotonum</i> (2), <i>S. bulbocastanum</i> + <i>tuberosum</i>) (9), <i>S. grasilifrons</i> , <i>S. huancabambense</i> , <i>S.</i> <i>hypacrarthrum</i> , <i>S. phureja</i> , <i>S. hirsutum</i> , <i>S. peruvianum</i> (3)	*	*	
PE-3	<i>S. phureja</i> , <i>S. raquialatum</i> , <i>Solanaceae</i>	*	*	
PE-7	<i>S. hirsutum</i> , <i>S. peruvianum</i>	*		
	<i>S. peruvianum</i> , <i>S. medians</i>	*	*	
US-1	<i>S. tuberosum</i>	*	*	
	<i>S. cajamarquense</i> , <i>S. tuberosum</i> (2)		*	*

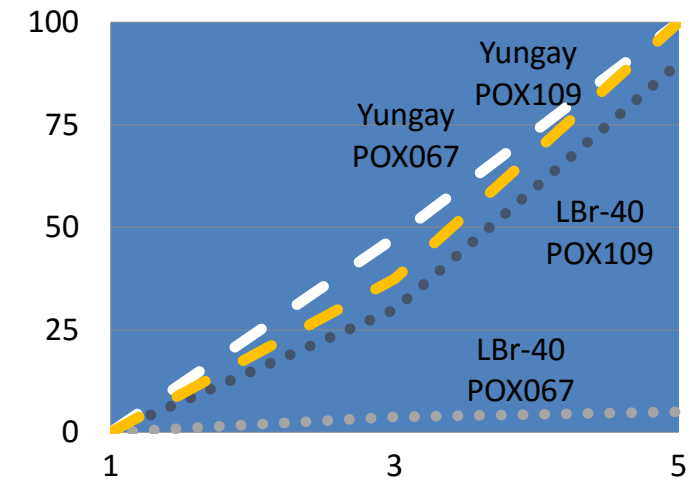
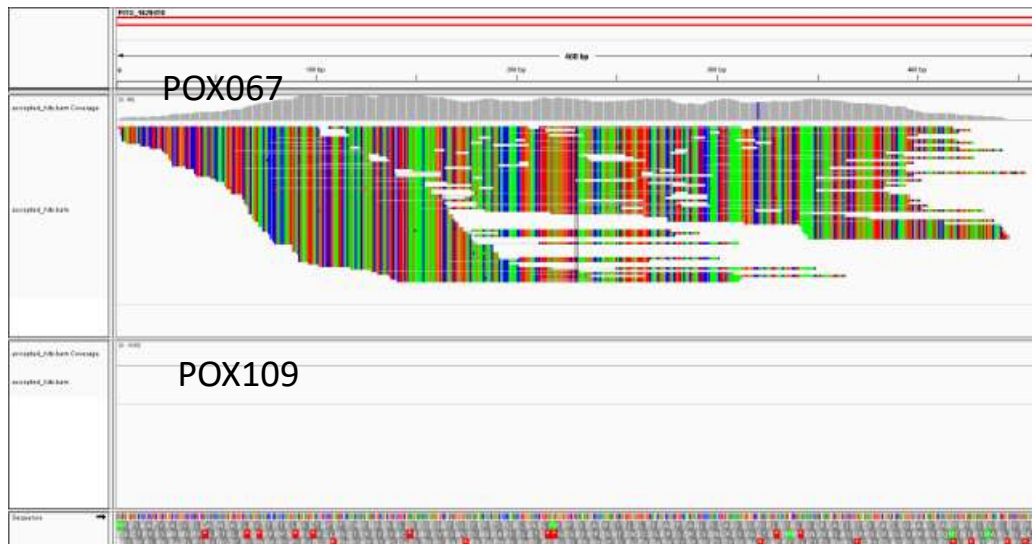
Desiree [RB] tests

Isolate	EC-1 Class I, II	US-1 Class II, III
Event	POX - 67	PPU-103
Desiree[RB] 56	3	6
Desiree[RB] 68	8	25
Desiree[RB] 70	0	15
Desiree	90	96
Yungay	100	93
LBr-40	14	18
ROC	0	0



Avrvnt1 is silenced also in Peruvian isolates

Isolate	host	Virulence on <i>S. demissum</i> R-gene differential set
POX067	"Yungay"	R1, 2, 3, 4, 5, 6, 7, 10, 11
POX109	"LBr-40"	R1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11



....but silencing is reversible

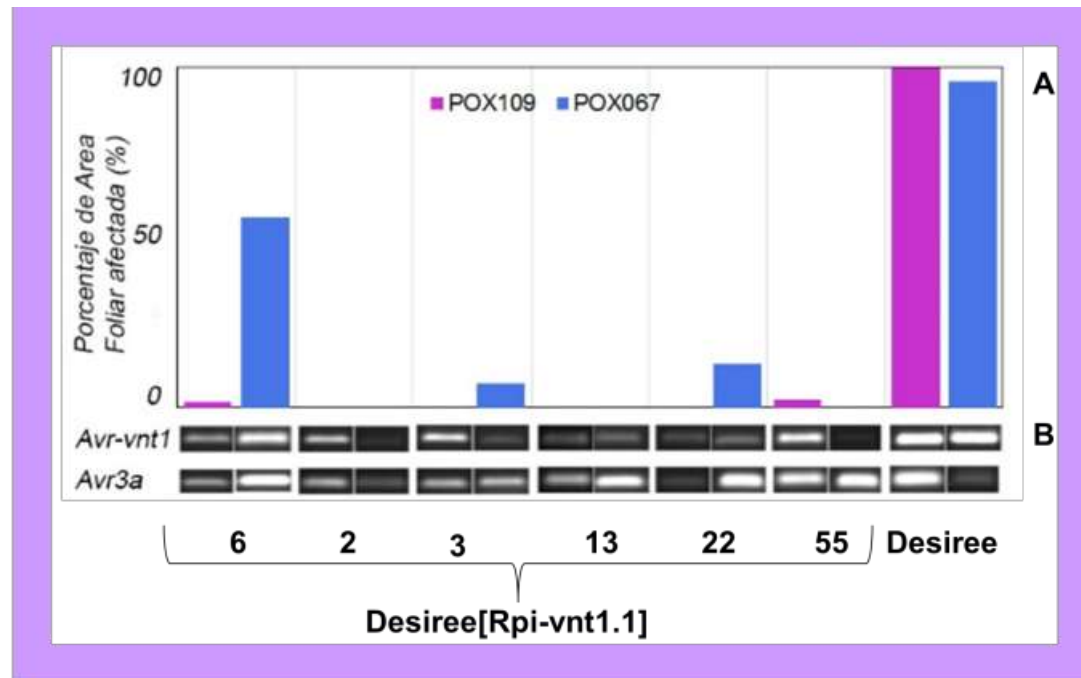
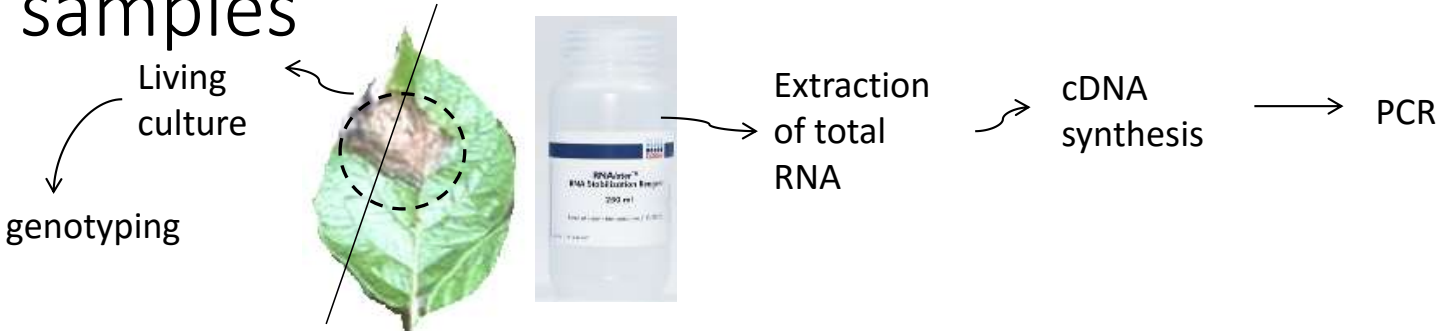


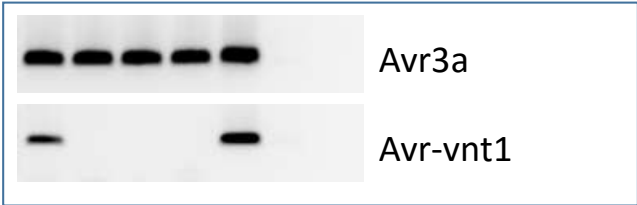
Figura 3. Nivel de Resistencia expresado en % de área foliar afectada por síntomas de tizón tardío (A) y expresión del gen de avirulencia *Avr-vnt1* y *Avr3a* en los eventos transgénicos y sin transgén infectados con POX109 (izquierda) y POX067 (derecha) de cada evento (B) a las 120 hpi.

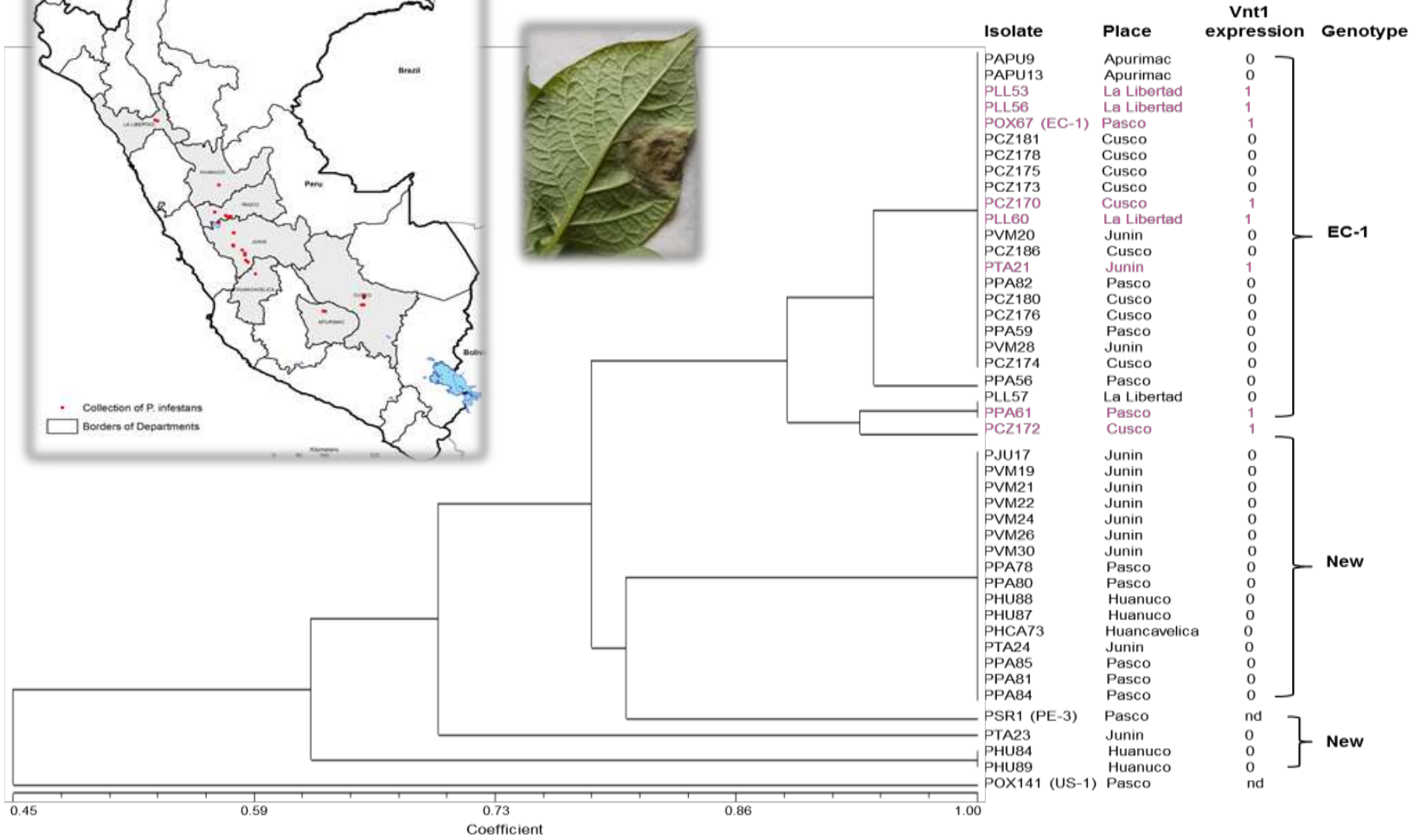
Test for *Avrvnt1* expression in field samples



primers	Target gene	purpose
F:GTCATTGCCACCTACTTC R: ATCATCTTGTCCTCAGC	Elongation factor Ef2	Positive control for <i>P. infestans</i>
F: ATGTGGCTGCGTTGACGGAGA R: TGAGCCCCAGGTGCATCAGGTA	Avr3a	Positive control for biotrophic state
F: CGAAGTTGACGGCTCCTG R: GGCTCGCTTGAACAAATCC	Avr-vnt1	test

PCR-Touchdown (T°melting: 61°C)		
94°C	4 min	1 cycle
94°C	30 s	10 cycles
70°C to 61°C	30 s	each cycle reduces 1°C
72°C	15 s	
94°C	30 s	20 cycles
60°C	30 s	
72°C	15 s	
72°C	5 min	1 cycle
4°C	End	





Rpi-blb2 - Avr-blb2 interaction

- Avrblb2 effector of *P. infestans* involved in interfering with host plant defense (Oh et al. 2009; Bozkurt et al. 2011).
- crucial role of one amino acid in the AVR protein for the recognition specificity by the Rpi-blb2 gene (Oh et al. 2009).

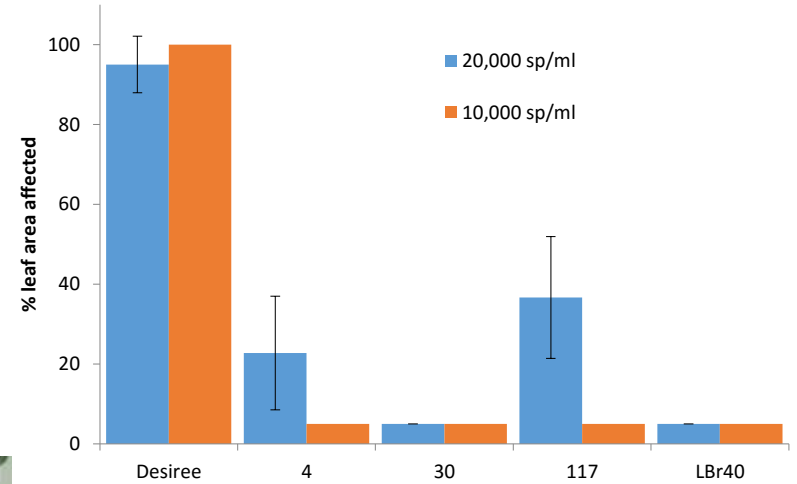
Adaptive evolution of *Phytophthora infestans* on Desiree-*Rpi-blb2* potato

	First experiment				Second experiment			
Inoculation date	05Feb2015		11Feb2015		23Feb2015		02Mar2015	
	Adaptive	Control	Adaptive	Control	Adaptive	Control	Adaptive	Control
Sporangia/ml	20,000 ^a	3,000 ^a	5,000 ^b	3,000 ^a	20,000 ^a	3,000 ^a	10,000 ^c	3,000 ^a
D[blb2]117	3	3	2	3	3	2	4	2
D[blb2]30	3	3	9	3	1	1	1	2
D[blb2]4	31	3	2	3	27	2	4	3
Desiree	3	2	2	2	2	2	2	2
LBr40	3	2	2	2	2	2	2	2

Adaptive evolution of *Phytophthora infestans* on Desiree-*Rpi-blb2* potato



23-Feb inoculated with 20K sporangia from fresh culture, 2-Mar inoculated with 10K sporangia from D[blb2]4



Summary

- RB infecting strains:
 - In low frequency in Peru
 - Have low level of aggressiveness
- Vnt1 infecting strains:
 - High frequency in Peru
- Blb2 infecting strains
 - Not yet discovered

new country-wide sampling
extending to cultivated potato and
alternative Solanaceous hosts

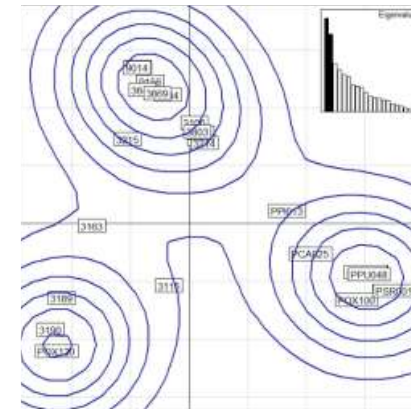


Objetivos específicos:



Mapeo de las áreas de cultivo de la papa con presencia de *P. infestans* y *R. solanacearum*

Determinar la estructura poblacional de *P. infestans* y *R. solanacearum* en las áreas muestreadas



Objetivos específicos:

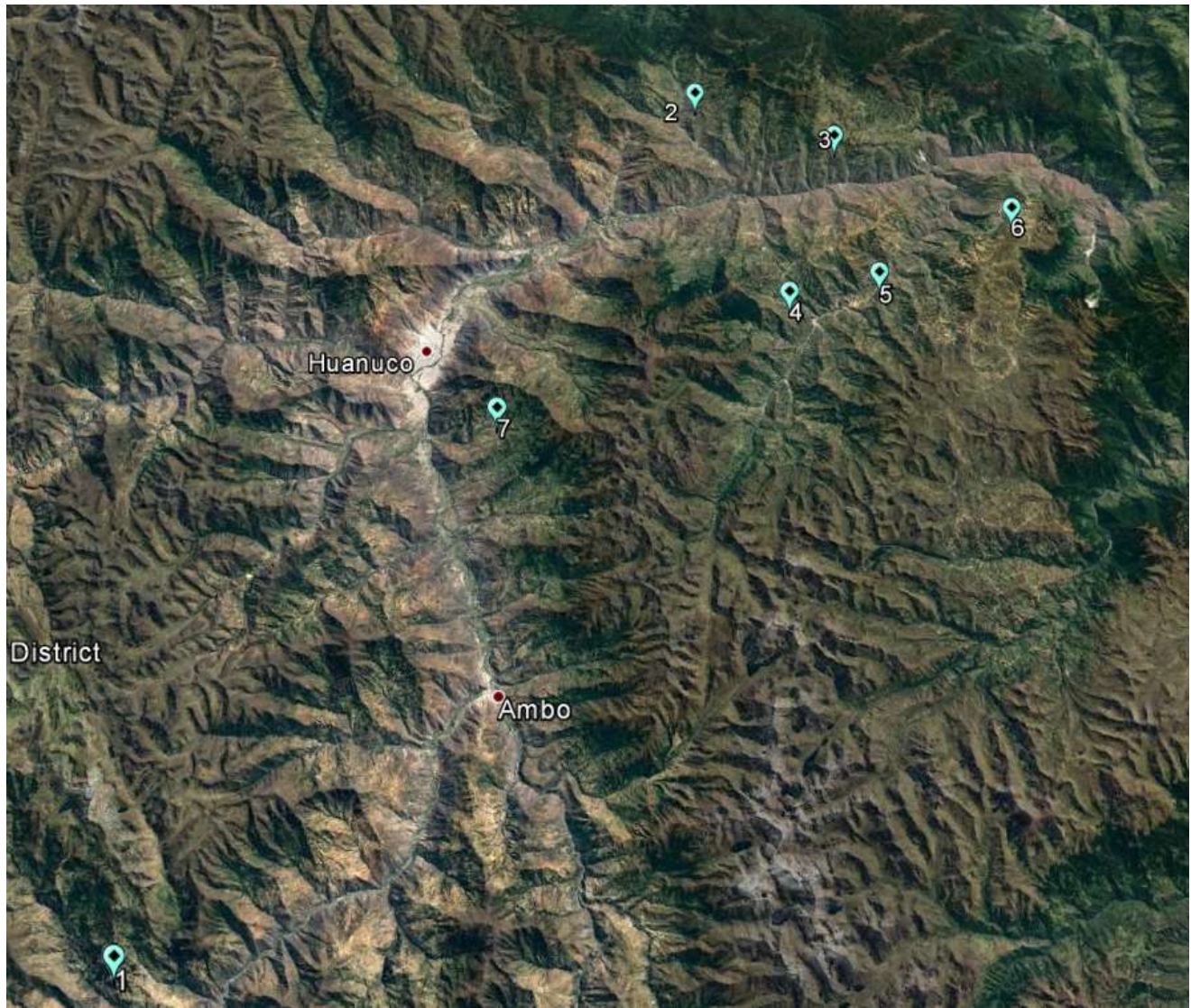
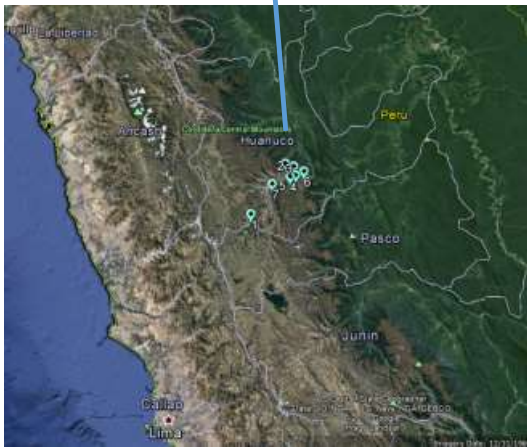


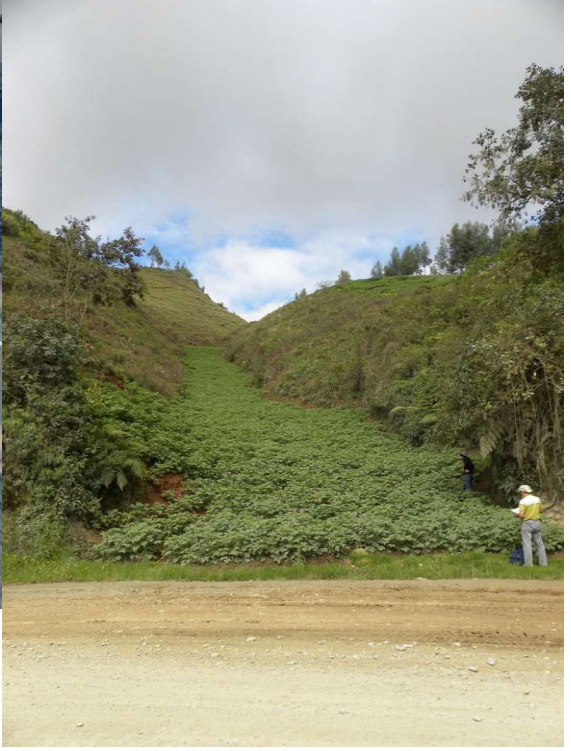
Capacitar a profesionales de instituciones nacionales en técnicas de colección, aislamiento, caracterización, mantenimiento de colecciones y bases de datos.

Identificación de especies cultivadas y/o silvestres que actúan como hospedantes alternos de *P. infestans*.











Nicandra physalodes (L.)
Gaertn.



Salpichroa tristis Miers



Salpichroa aff. diffusa Miers



Physalis peruviana L.

LISTA DE ESPECIES

- Acnistus arborescens (L.) Schltld.
- Browallia americana
- Brugmansia sanguinea (Ruiz & Pav.) D. Don
- Capsicum pubescens Ruiz & Pav.
- Cestrum auriculatum
- Cestrum falcatum
- Cestrum sp.
- Cestrum tomentosum L. f.
- Lochroma grandiflorum Benth.
- Lochroma umbellatum
- Jaltomata dentata
- Jaltomata hererae
- Jaltomata incahuasina Mione & Leiva, S.
- Jaltomata propinqua
- Jaltomata sinuosa (Miers) Mione
- Lycianthes acutifolia Ruiz & Pav.
- Lycianthes lycioides
- Lycianthes sp.
- Nicandra physalodes (L.) Gaertn.
- Physalis peruviana L.
- Salpichroa aff. diffusa Miers
- Salpichroa tristis Miers
- Solanum acaule
- Solanum albicans
- Solanum americanum
- Solanum betaceum Cav.
- Solanum caripense Dunal
- Solanum chrysotrichum
- Solanum corneliomulleri
- Solanum furcatum
- Solanum grandidentatum Phil.
- Solanum nitidum
- Solanum nutans Ruiz & Pav.
- Solanum ochranthum Dunal
- Solanum polytrichostylum
- Solanum rubicaule S.R. Stern
- Solanum trachycarpum Bitter & Sodiro
- Solanum zahlbruckneri



...tosum L. f.



Cestrum sp.



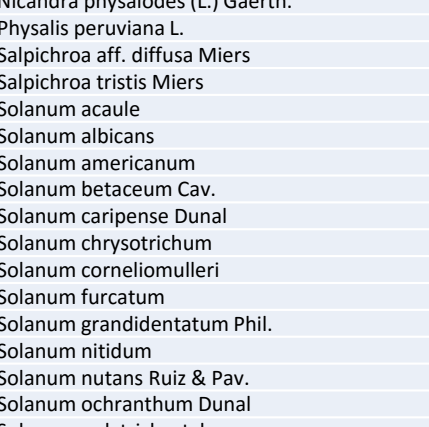
Acnistus arborescens (L.)
Schltld



Browallia americana L.



Lochroma u...
Hu...



...na grandiflorum
Benth.