

# SESSION 1

## LATE BLIGHT & EARLY BLIGHT

### **K1 Potato health globally : current status and upcoming challenges**

Didier Andrivon (INRAe – IGEPP, France)

### **O2 Influence of weather conditions and production methods on the first occurrence of *Phytophthora infestans* and *Alternaria sp.* in Austria**

Vitore Shala-Mayrhofer (Austrian Chamber of Agriculture, Austria)

### **O3 Diversity analyses of key genes involved in the interaction between potato and *Phytophthora infestans***

Jadwiga Śliwka (IHAR-PIB, Poland)

### **P1 Diversity analysis of *Rpi-ber1* and *Rpi-vnt1* genes determining broad-spectrum resistance to *Phytophthora infestans***

Paluchowska Paulina (IHAR-PIB, Poland)

### **P2 Aggressiveness test of *Phytophthora infestans* isolates with different effector alleles**

Ludwiczewska Mirella (IHAR-PIB, Poland)

### **P4 The *R2* gene is still efficient in bringing resistance to some *P. infestans* strains**

Sylvie Marhadour (inov3PT/INRAE - IGEPP, France)

### **P6 Susceptibility to potato late blight (*Phytophthora infestans*) of *Solanum tuberosum* Chilotanum group, on detach leaf assay and field conditions**

Ivette Acuña (INIA, Chile)

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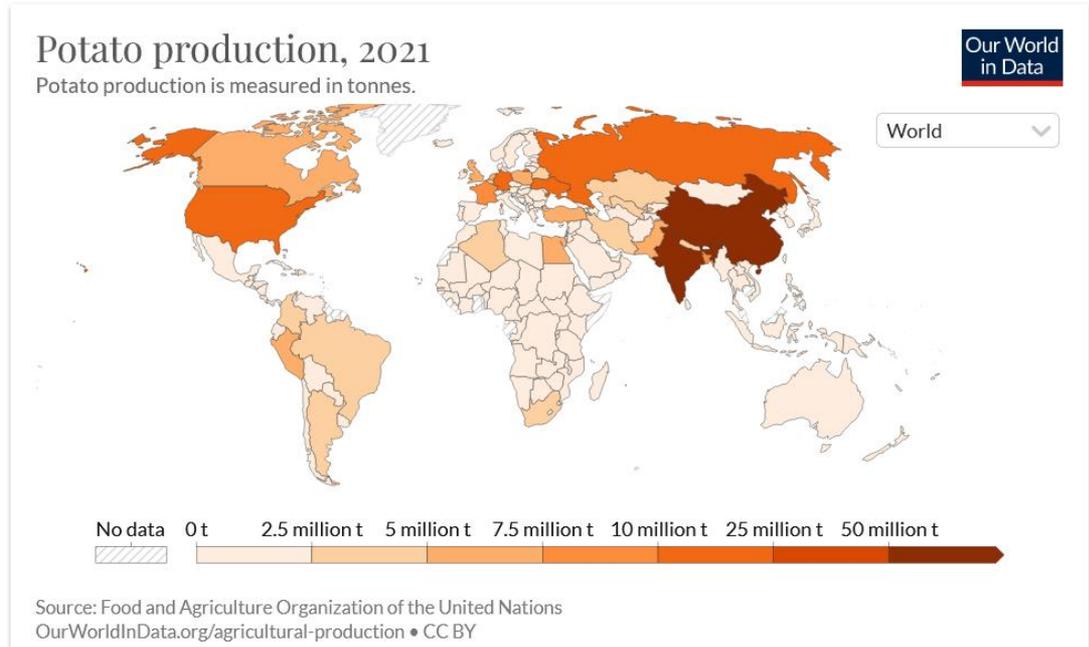
➤ **Potato health globally:**  
*current status and upcoming challenges*

Didier Andrivon

# ➤ Potato - globally

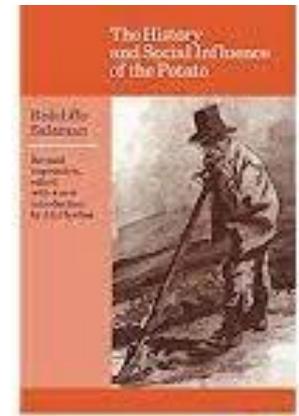
## ➤ Potato grown worldwide

- Critical for food security, mainly in Americas and Asia
- Large diversity of ecozones and production systems
- Shifting major production areas



## ➤ Multiple services provided

- Provisioning
- Regulating
- Cultural and social



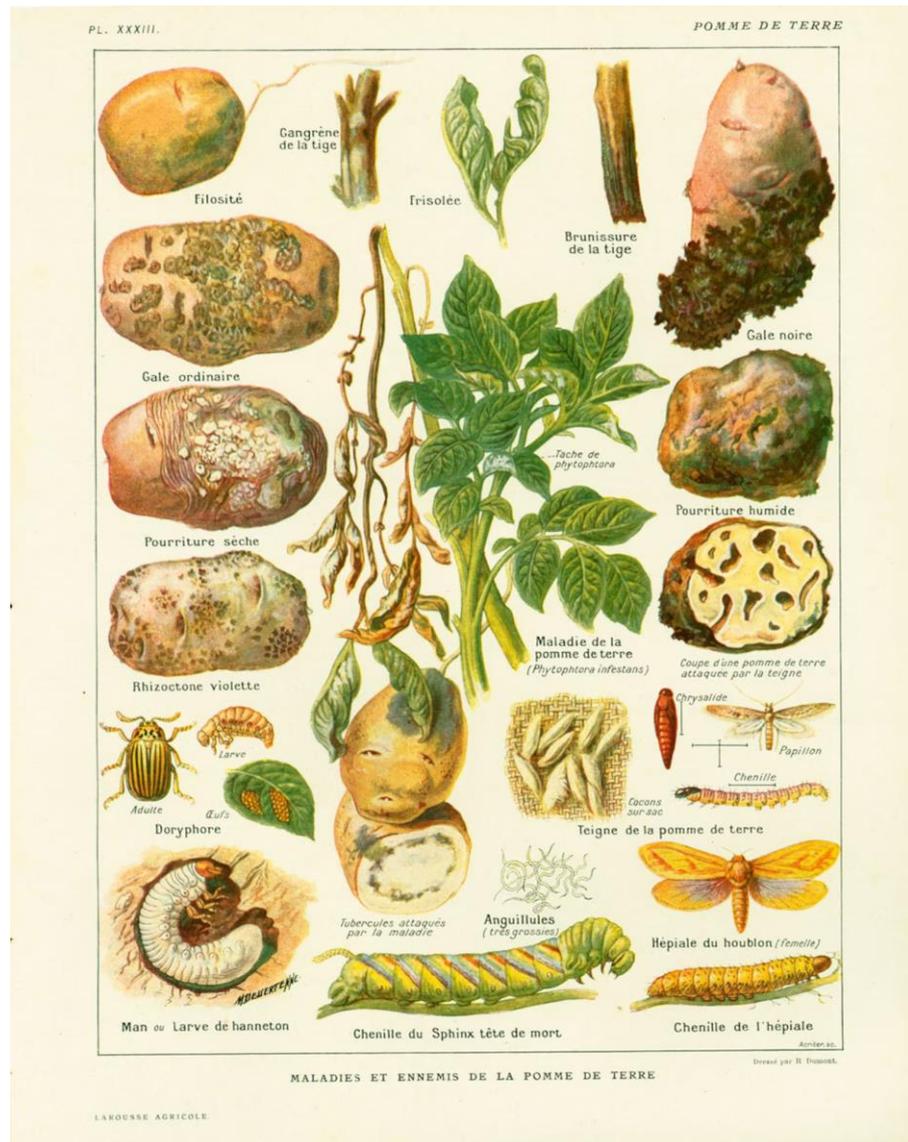
# ➤ Potato – a vulnerable crop

## ➤ Vast array of important pathogens

- Vegetative propagation
- chronic pathogens (viruses, bacteria)
- Seed borne inoculum

## ➤ Global assessment of health status and impacts missing

- The GPHA initiative



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Titre de la présentation

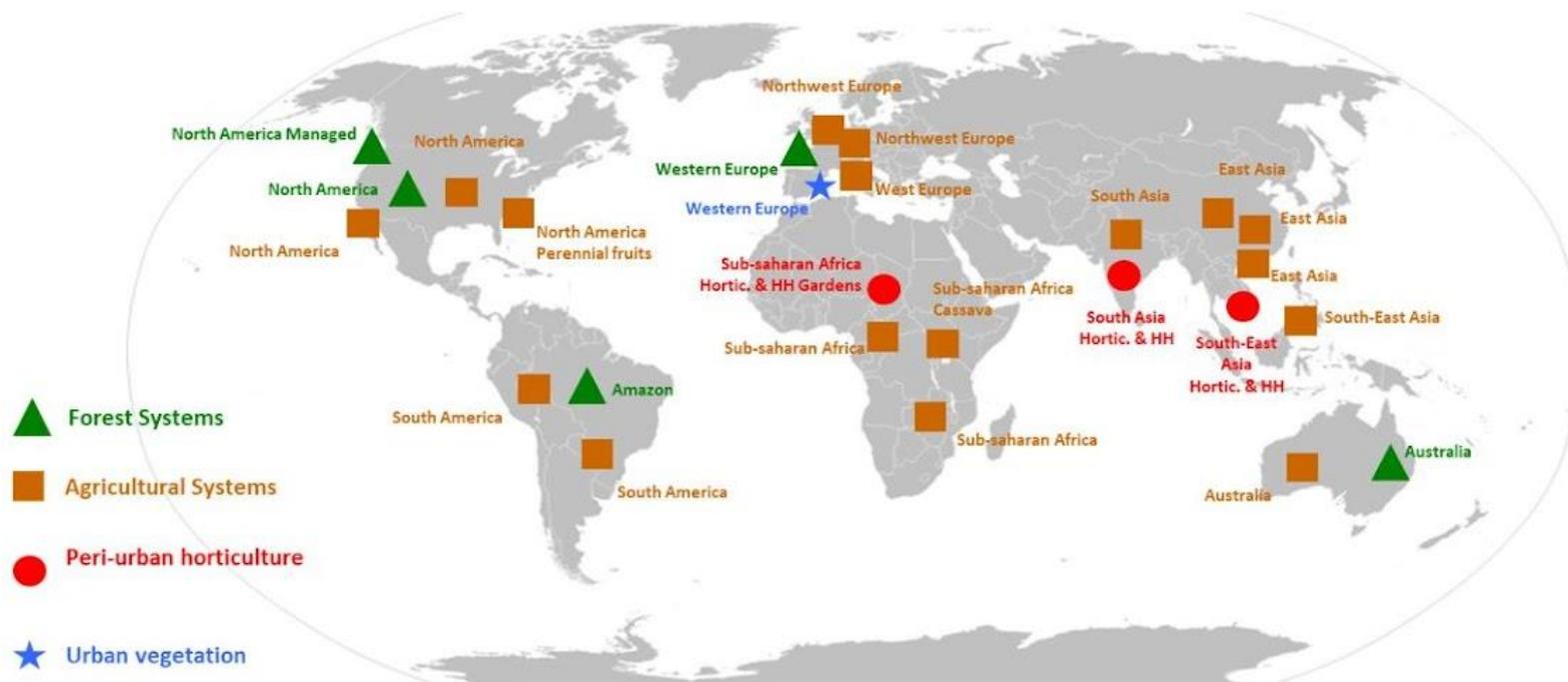
Date / information / nom de l'auteur

# ➤ The GPHA assessment – what's this?

## ➤ An ambitious ISPP project

- Started 5 yrs ago
- Covering a wide diversity of plants ( not just crops)
- Aiming at providing a global view of plant health status worldwide

## Global Plant Health Assessment: Systems and Ecoregions



# ➤ The GPHA assessment – where are we now?

## ➤ How?

- Voluntary expert contributions
- Selection of 'keystone cases'
- For each case, a report
  - Based on existing knowledge and references
  - Under a common format

## ➤ Some choices and shortcuts

- Focus on diseases
- Assessment based on ecosystem services linked to plant health
- Status over past 30 yrs and foreseeable trends

## ➤ Products and deliverables

- Reports compiled and analysed
  - International workshop, Toulouse October 2021
  - ICPP Lyon , August 2023
- An e book with all 26 reports available to date >  
[https://www.isppweb.org/about\\_gpha.asp](https://www.isppweb.org/about_gpha.asp)
- A feature paper with the main (provisional) conclusions> *Plant Disease (First Look)* -  
<https://doi.org/10.1094/PDIS-01-23-0166-FE>
- A dedicated website > <https://sites.google.com/view/global-plant-health-assessment/home>

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# ➤ The potato GPHA– what's up, doc?

## ➤ Three reports on potato

- South America
- East Asia
- Western Europe
  
- Missing areas still to be covered
  - Mediterranean basin
  - North America
  - Sub-saharan Africa

## ➤ Diverse pathogen profiles

- Late blight prevalent everywhere
- *Ralstonia* a major threat in the tropics – Asia & South America
- Tuber diseases important in Europe
  - Link to marketing and uses
  
- Viruses problematic in many developing countries
  - Seed systems are key



# ➤ The GPHA assessment - what's up, doc?

- **Current health status : decent... but artificially so and declining**

Plant System	World Eco-region	Overall state of plant health	Level of confidence in assessment: Plant health
	South America		reasonably confident
	East Asia		reasonably confident
	West Europe		very confident

- **Ecosystem services rendered**

- Primarily provisioning: fair to poor, often declining
- Regulating: seldom assessed, poor and declining
- Cultural: not assessed for want of suitable data

**Global Plant Health Assessment and Serge Savary**

**A global assessment of the state of plant health**

***Plant Disease (First Look)* - <https://doi.org/10.1094/PDIS-01-23-0166-FE>**

**INRAE**

EAPR Pathology and Pests Section meeting – Arras, 4-6 September 2023

# ➤ Challenges and impacts

- Climate change
- Globalisation of trade
- Changing pathogen complexes and diversity
- Diet transitions



# > Climate change

Farmers from the NEPG zone will globally produce 7 to 11 % less potatoes due to climate change

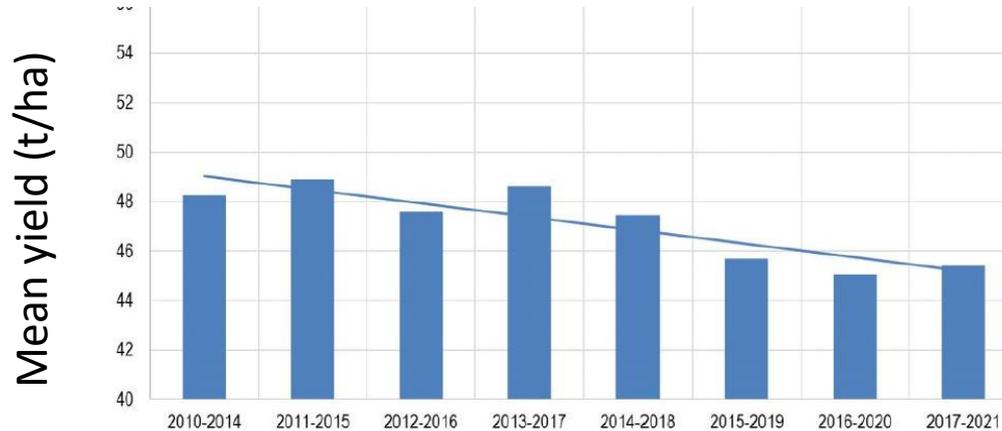
12-09-2022



During its last meeting prior to Potato Europe 2022, the NEPG estimates that global potato production in the NEPG zone (EU-04) will be down by 7 to 11 %

Source : <https://nepg.info/wp-content/uploads/2022/09/220912-NEPG-press-release-GB-final.pdf>

### NEPG yield trend, 2010-2021



source: NEPG



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# ➤ Climate change

## ➤ *Water supply*

- critical for tuber production
  - > sometimes too low > 2022
  - > sometimes too high > 2021
- Strongly correlated to temperatures

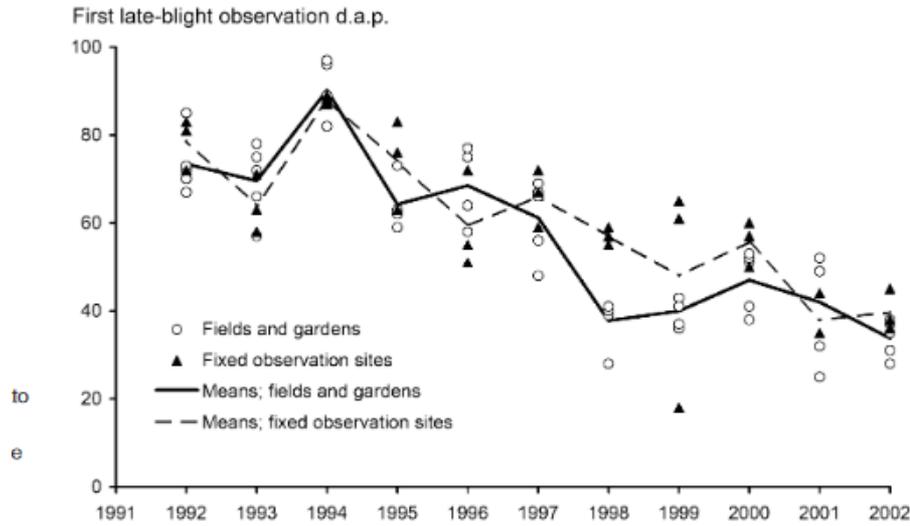
## ➤ *Impacts*

- Direct effects on tuber growth and plant physiology
  - Gross yield, tuber caliber and shape
  - Shifting production zones and calendars
- Indirect effects through
  - pathogen severity and distribution ( eg late blight)
  - Increasing production costs ( water, but also energy and sometimes pesticides)



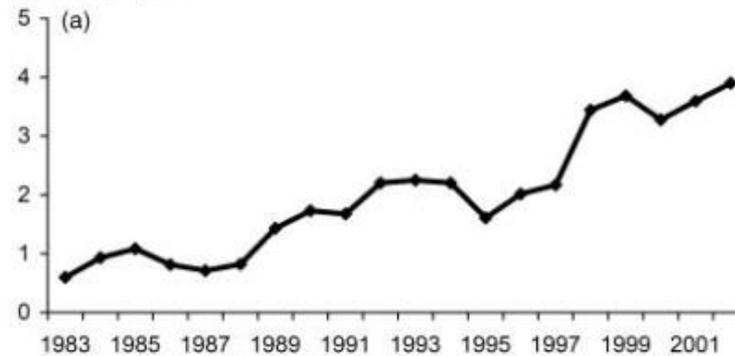
# ➤ Climate change

## ➤ Observing past impacts...



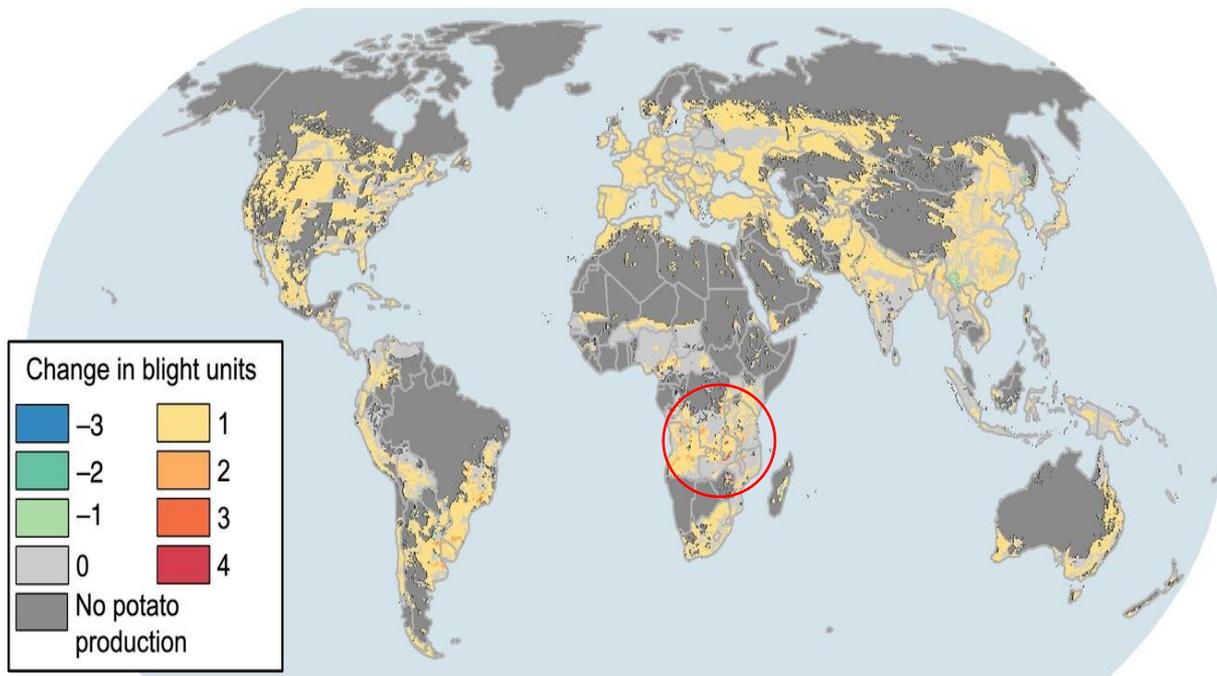
Hannukkala et al., 2007  
*Plant Pathology* 56: 167 –176  
DOI: 10.1111/j.1365-3059.2006.01451.x

Estimated number of sprays/ha



# ➤ Climate change

## ➤ ... and predicting future ones



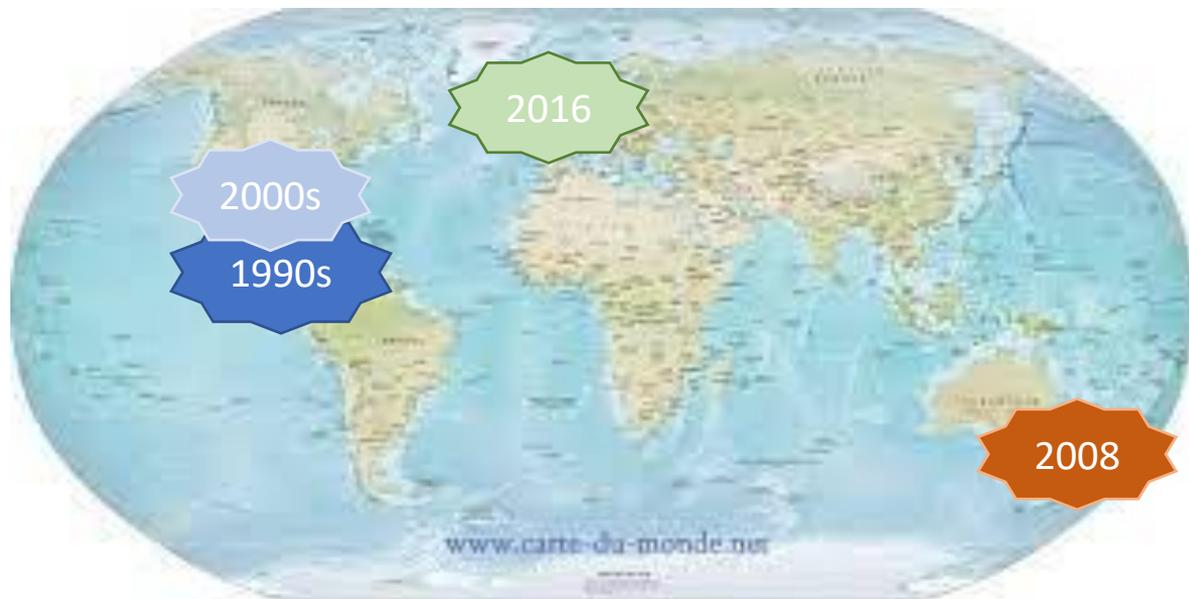
Late blight risk variation  
between 1961/1990  
and 2040-2059

Hijmans et al, 2014  
*Global Change Biology* 20: 3621-3631,  
DOI: ([10.1111/gcb.12587](https://doi.org/10.1111/gcb.12587))

# ➤ Globalisation of trade

## ➤ Large scale movement of plants... and pathogens

- ✓ New strains and pandemic distribution : *P. infestans* EU-13\_A2 & EU 2 A1
- ✓ New species – zebra chip



# ➤ Pathogens/pest complexes and diversity

## ➤ *New associations of genotypes and pathogenic species*

### ➤ Ex: Black leg complex

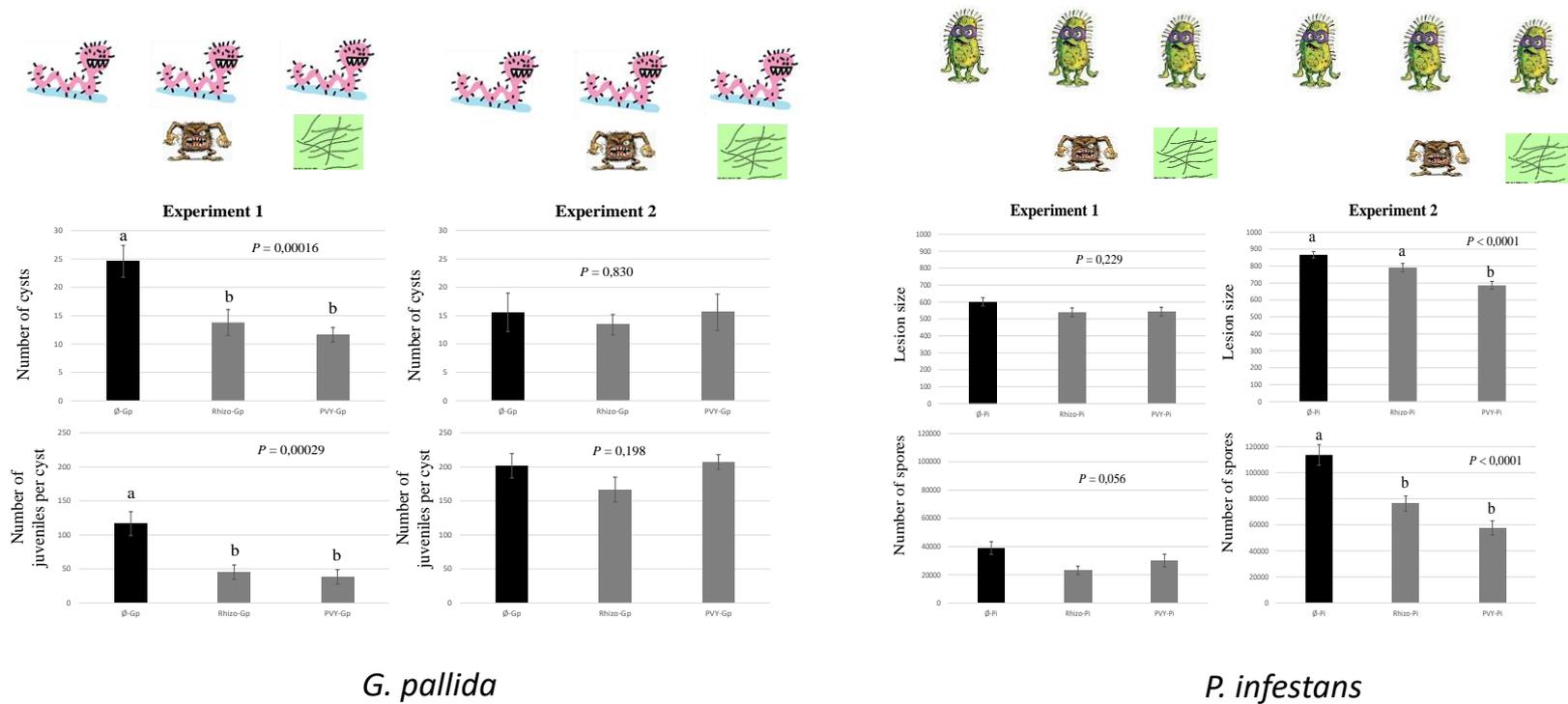
- New species detected
- Expanded range of habitats ( *P versatile*)
- Invasive behaviour ( *P parmentieri*, *D solani*)
- Antagonism and synergism

### ➤ Consequences for control

- Upgraded surveillance schemes and methods
- Ecological interactions can be used for biological control
- Attention to be paid to as yet unidentified species in collections ( *P punjabense*? Other groups yet to be named?)



## ➤ Altered host behaviour in the presence of mixed infections



**Conclusion : Mixed infections = more resistant hosts**

Next questions:

Is it always the case?

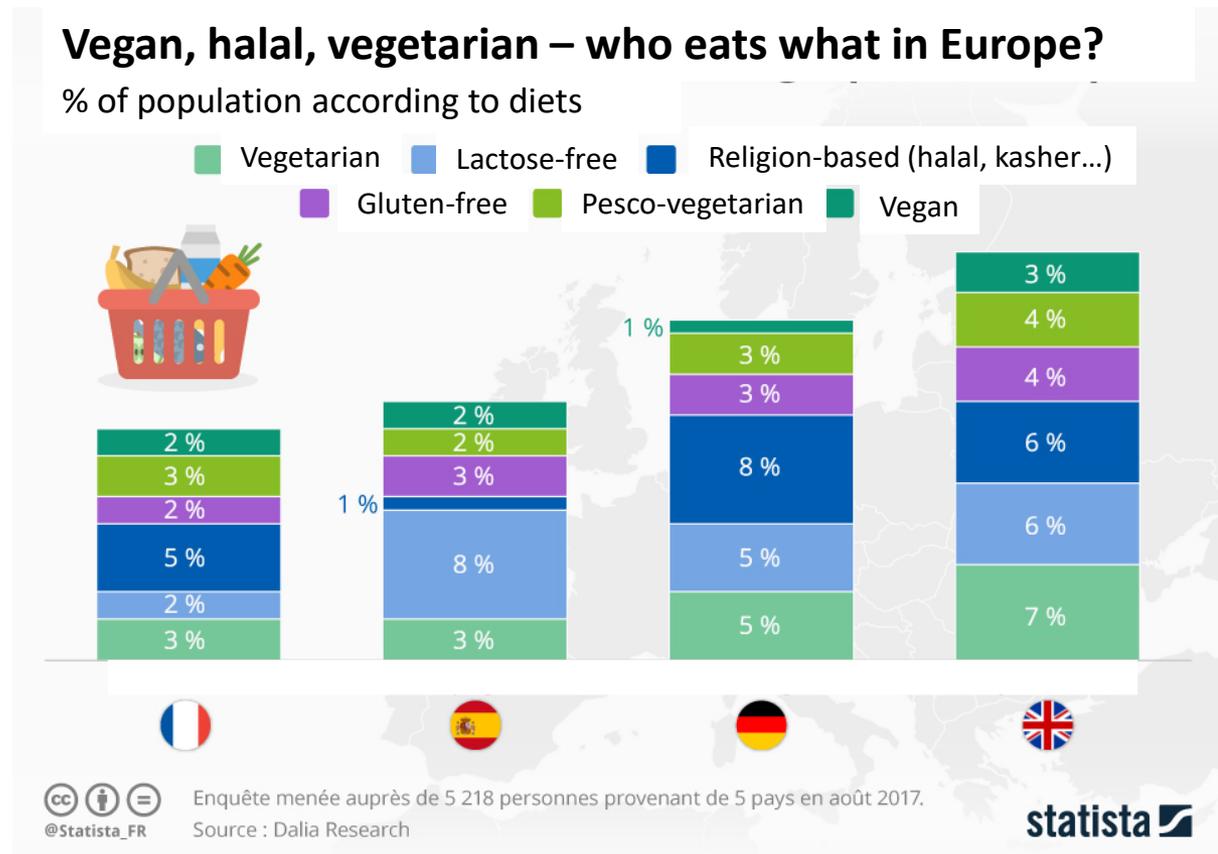
What mechanisms explain this?

Andrion et al., 2023 –  
Plant Pathology 72: 667-676

# ➤ Food regimes transitions

## ➤ Changing diets

### ➤ Vegetarian/vegan/low meat on the rise

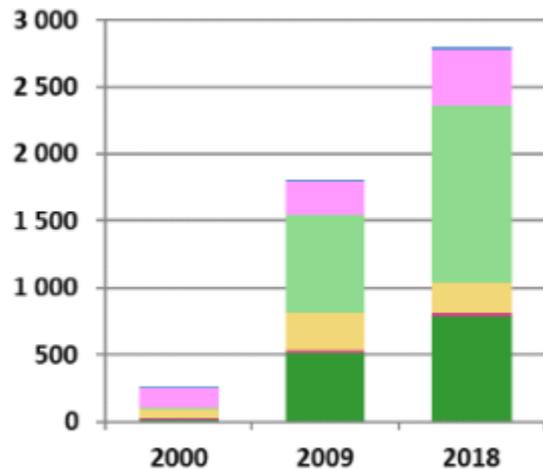


# ➤ Food regimes transitions

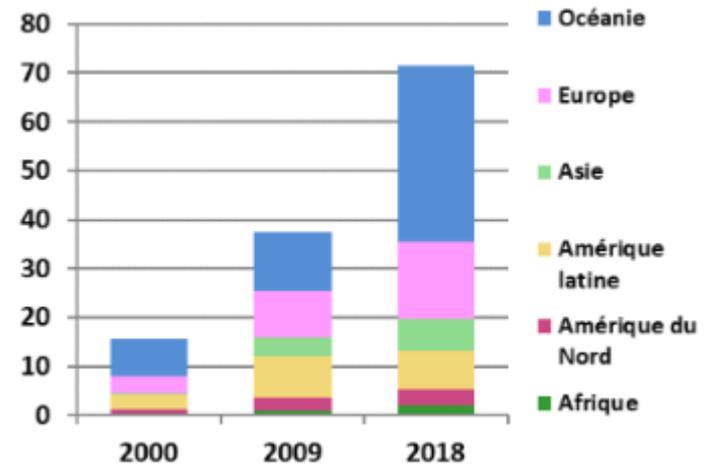
## ➤ *Changing production modes*

### ➤ Organic on the rise worldwide

**Number of organic farms ( \*1000)**



**Organic acreage ( Mio ha)**



Source: Agence Bio, 2020 –  
*L'agriculture bio dans le monde*

# ➤ Food regimes transitions

## ➤ *Consequences*

- Changes in expected production distribution

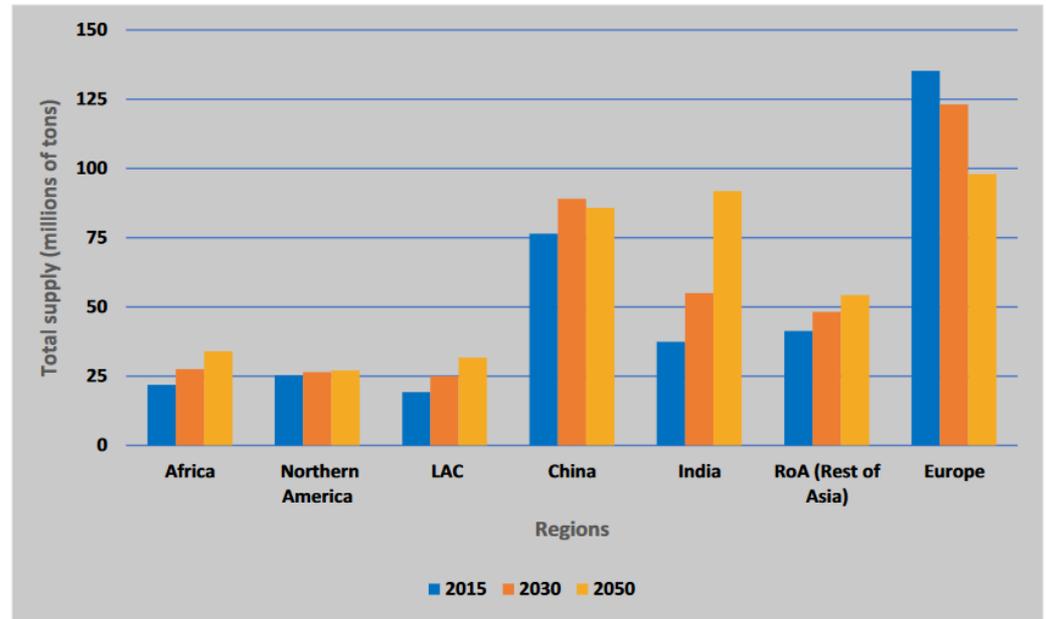


Fig. 8 The future of potato production adapted from Rosegrant et al. 2017

- Plant protection methods to change



# ➤ Some take-home messages

## ➤ **Potato health globally – decent, but fragile**

- Convincing evidence that potato health is currently managed worldwide...
- But:
  - at the expense of heavy and unsustainable use of pesticides ( fungicides, insecticides, herbicides; nematicides)
  - many different pathogen profiles according to ecoregions and cropping systems

## ➤ **Major challenges ahead**

- Climate change
  - Impacts host and pest distribution
  - Impacts also pest severity and timing
- Globalisation of trade
  - Higher genetic uniformity
  - Fast human mediated long distance dispersal of pathogen strains and species
- Pathogen diversity and complexes
  - Work to be done on ecology/interactions within pathogen complexes
- Transitions in food regimes and requests for food security
  - Will potato remain 'the famine shield' it has historically been?

## ➤ **New research directions – and opportunities**

- Time for ICHM rather than IPM?



# ➤ Meeting these challenges – where to go next in potato health research?

## ➤ Get prepared - forecasting upcoming situations

- Sensors
- Models
- Expert studies ( such as the GPHA one)

## ➤ Shifting from ‘single disease management’ to ‘integrated potato health management’

- Ecology – analyse interactions between pathogens/pests
- Agronomy - designing and assessing low pesticide potato cropping systems
  - Example : talks and posters by A Kröner and colleagues
- Social sciences:
  - take acceptability and risk management into consideration
  - Multi-actor assessment ( growers , but also downstream actors requirements)



## ➤ Thanks...

- **To the GPHA experts for the assessment of the current status of potato health**
- **To the meeting organisers for inviting me to give this talk**
- **To all of you for listening to it!**



# Influence of weather conditions and production methods on the first occurrence of *Phytophthora infestans* and *Alternaria sp.* in Austria

Shala-Mayrhofer Vitore<sup>1</sup>, Muck-Arthaber Julia<sup>2</sup>, Pachtrog-Wilfinger Vera<sup>2</sup>, Hubert Köppl<sup>3</sup>,  
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<sup>5</sup>AGRO DS Österreich, Wulkaprodersdorf, Austria

# Phytophthora infestans and Alternaria sp. on potatoes



- This fungal disease *P. infestans* is one of the most important potato diseases worldwide
- The damage is caused by a reduction in the assimilation area, which massively restricts tuber growth
- Yield reductions of 30 % are not uncommon. Humidity of over 80% and temperatures above 20 ° C represent optimal conditions for the fungus



- Potato disease *Alternaria sp.* can be found in all potato growing countries
- Symptoms are caused by two different kinds of *Alternaria*: Infestation with *Alternaria solani* (early blight), angled to round spots (up to 2 cm) with concentric rings and with *Alternaria alternata* (leaf blight) numerous small spots (up to 0.5 cm) appear on the leaves
- Optimum temperature for spore formation is 20 ° C. Spore germination only occurs at high relative humidity or when a water film is present on the leaf surface

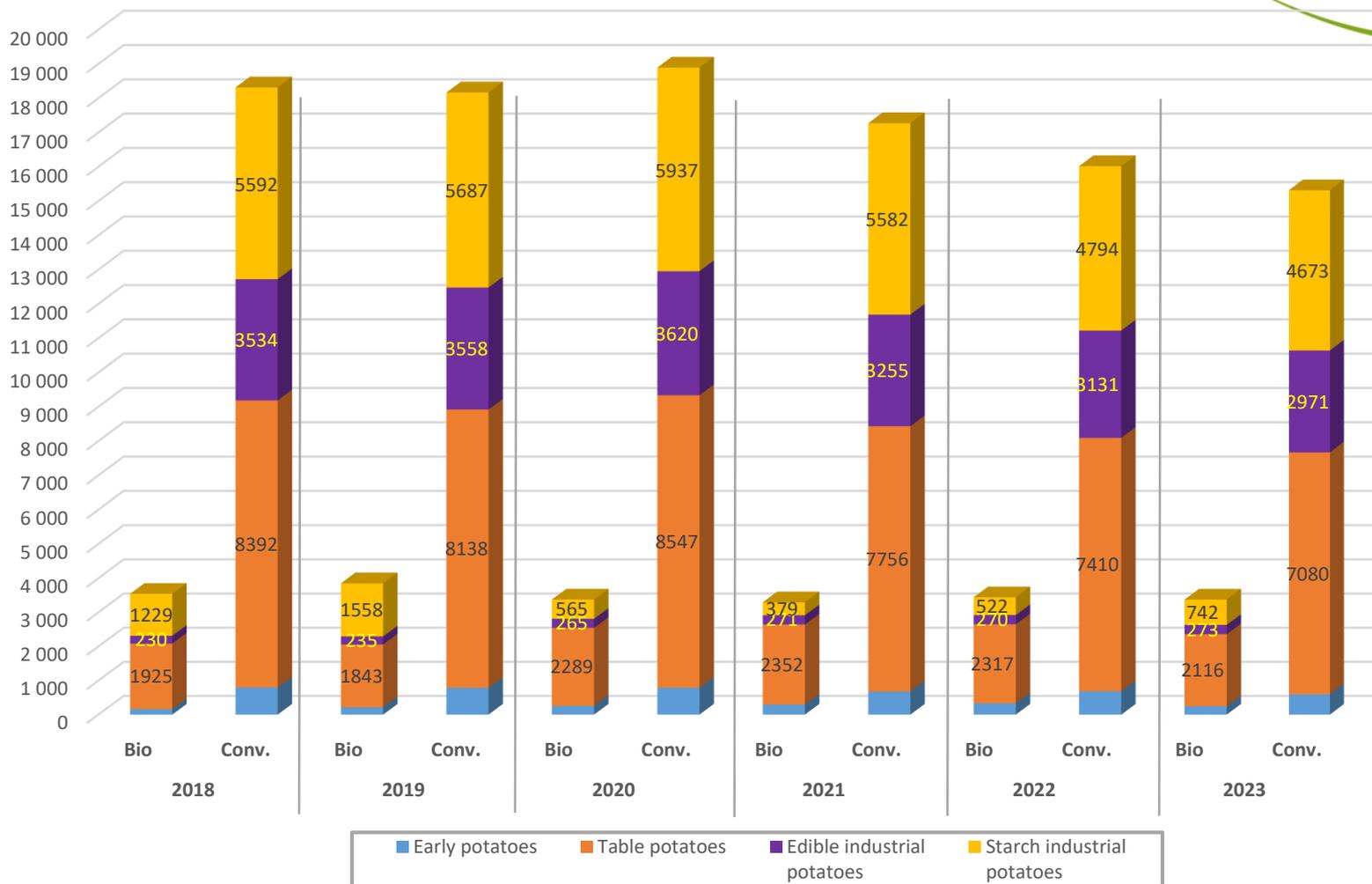


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- Damages caused by *P. infestans* and *Alternaria sp.* are also very high in Austria



# Potato acreage (ha) in Austria since 2018



Mit Unterstützung von Bund, Ländern und Europäischer Union

Bundesministerium  
Land- und Forstwirtschaft,  
Regionen und Wasserwirtschaft

LE 14-20  
Erneuerung für den Ländlichen Raum

Europäischer  
Landwirtschaftsfonds für  
die Entwicklung des  
ländlichen Raums:  
Hier investiert Europa in  
die ländlichen Gebiete.



# Austrian alert service for plant protection / potatoes



**Pflanzenschutz**  
**WARNDIENST**  
warndienst.at

LEINBERG, 2019

● KEIN BEFALL  
● MÄSSIGER BEFALL  
● STARKER BEFALL (SCHADSCHWELLE ÜBERSCHRITTEN)

Hochwertige Monitorings und Prognosen **FÜR 64** Schaderreger

Mit Unterstützung von Bund, Ländern und Europäischer Union  
 Bundesministerium Land- und Forstwirtschaft, Regionen und Wasserwirtschaft  
 LE 14-20

## Monitorings Field / Laboratory

Mehltau  
 Gelbrost  
 Braunrost  
 Septoria notorum  
 Septoria tritici  
 Microdochium nivale (Schneeschimmel)  
 Drechslere tritici-repentis (DTR) und der Schädlinge  
 Halmbruch  
 Septoria nodorum, S. tritici  
 Viruskrankheiten: BYVD, CYDV, WDV, BDV

### *P. infestans* *Alternaria sp.*

Drahtwürmer (ELATMON Projekt)  
 Agriotes brevis  
 Agriotes lineatus/proximus  
 Agriotes obscurus  
 Agriotes sordidus/rufipalpis  
 Agriotes sputator  
 Agriotes ustulatus

Gefleckter Kohltriebrüssler  
 Rapsstängelrüssler  
 Rapsglanzkäfer  
 Kohlschotenrüssler  
 Kohlschotenmücke  
 Erdflöhen  
 Zuckerrübe (ZUCKMON Projekt)  
 Schwarze Bohnenblattläuse  
 Grüne Pfirsichblattläuse  
 Erdflöhen  
 Rüssler  
 Cercopsora beticola  
 Echter Mehltau  
 Rost

Maiszünsler  
 Maiswurzelbohrer  
 Deoxynivalenol  
 Zearalenon  
 Fumonisine  
 Aflatoxine  
 Grüne Reiswanze  
 Grüner Erbsenblattlaus  
 Schwarze – Bohnenlaus  
 Nanovirennachweis  
 Baumwollkapselwurm  
 Kohlflye  
 Knoblauchgallmilbe  
 Apfelblütenstecher  
 Apfelsägewespe  
 Apfelwickler  
 Kleiner Fruchtwickler  
 Bräunlicher Obstbaumwickler  
 Fruchtschalenwickler  
 Pflaumensägewespe  
 Pflaumenwickler  
 Pfirsichwickler  
 Kirschfruchtfliege  
 Walnussfruchtfliege  
 Falscher Mehltau  
 Amerikanische Rebzikade  
 Kirschessigfliege  
 Traubenwickler  
 Varro-Milbe

## Prediction model

Acker  
 Septoria tritici  
 Septoria nodorum  
 Braunrost  
 Drechslere tritici-repentis – DTR  
 Gelbrost  
 Zwergrost  
 Echter Mehltau  
 Netzflecken  
 Ramularia  
 Rhynchosporium  
 Halmbruch

### *P. infestans*

Rapsstängelrüssler  
 Kohltriebrüssler  
 Rapsglanzkäfer  
 Kohlschotenrüssler  
 Kohlschotenmücke  
 Rapserrdflöhen  
 Obst (inkl. T-Sum)  
 Apfel-, Pflaumen-, Pfirsich- und kleiner Fruchtwickler  
 Birnblattsauger  
 Mehliges Apfelblatt-, und Apfelgrasläuse  
 Obstbaumspinnmilbe  
 Pfennigminiermotte  
 Apfelsägewespe  
 Apfelschorf  
 Feuerbrand  
 Obstbaumkrebs  
 Apfelwickler  
 Wein  
 Falscher Mehltau  
 Echter Mehltau  
 Schwarzfäule  
 Schwarzholz  
 Phänologie  
 Biene  
 Varro-Milbe

Monitoring of *P. infestans* and *Alternaria sp.* since 2017  
 Prediction model for *P. infestans* since 2016 (ISIP/ZEPP)  
 Monitoring of *Agriotes sp.* since 2019

Mit Unterstützung von Bund, Ländern und Europäischer Union

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# Austrian alert service for plant protection / potatoes



## Ikwarndienst

warndienst.at



Startseite Acker Gemüse Obst Wein Bienen PSM-Filter Erklärvideos Infobox

Getreide Mais **Kartoffel** Raps Soja Zuckerrübe PSM-Filter



**Krankheiten in Kartoffeln**  
Prognose Behandlungsbeginn  
Kraut- und Knollenfäule (*Phytophthora infestans*)



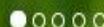
**Krankheiten in Kartoffeln**  
Prognose Folgebehandlungen  
Kraut- und Knollenfäule (*Phytophthora infestans*)



**Krankheiten in Kartoffeln**  
Monitoring  
Kraut- und Knollenfäule (*Phytophthora infestans*), *Alternaria*



**Schädlinge**  
Monitoring  
Drahtwürmer (*Agriotes sp.*) bei Kartoffeln und Mais



# Aim of monitoring



- To inform farmers on time about the occurrence and incidence of potatoe diseases (monitoring data is published online in a timely manner)
  - Evaluation of factors / parameters influencing the occurrence and spread of diseases
  - Providing information to optimize decisions on the type and timing of plant protection measures in integrated and organic production
  - Reduction of pesticide use
- Standardized data for validation / development of forecast models (e.g. *Alternaria sp.*)
- Forecasting would allow farmers and advisors to plan and take measures at the right time



# Methodology, field introduction



## ▪ *Phytophthora infestans*

An untreated window (served as control) of at least 100 m<sup>2</sup> is checked once a week (beginning of June until middle / of August)

After the first occurrence of *Phytophthora*, the frequency of infestation is recorded separately for stems and leaves.

Indication of infestation frequency (IF) = proportion of infested plants

0 = No infestation

1 = < 1 %

2 = 1 - 10 %

3 = 11 - 25 %

5 = 26 - 50 %

6 = 51 - 100 %

## ▪ *Alternaria sp.*

An untreated window (served as control) of at least 100 m<sup>2</sup> is checked once a week (beginning of June until middle / of August)

0 = No infestation.

1 = Isolated symptoms on some plants.

2 = Symptoms on the lowest leaves on the majority of plants

3 = Symptoms on the lowest leaves and isolated on the middle to upper leaves on the majority of plants

4 = Symptoms on a large part of the plants except for the upper leaves, haulm still largely green

5 = Symptoms on a large part of the plants except for the upper leaves, haulm already largely dead



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# Methodology



- Field - Monitoring: 2017-2022
- Total number of locations: up to 50 locations per each year
- Parameters that were noted during field assessments: Variety, previous crop, tillage, fertilizer, soil type, drying, date of cultivation, date of emergence.



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- Crop rotation / Intercropping:  
Spelt, Peas, Barley, carrots, clover, grain, corn, corn, rapeseed, seed, corn, silage corn, soybean, summer durum, spring barley, summer oats, summer poppy, spring wheat, triticale, winter durum, winter oilseed rape, winter rye, winter triticale, winter wheat, sorghum, sugar beet, onion, buckwheat, cress, oil currant

- Not protected from wind
- Protected from wind

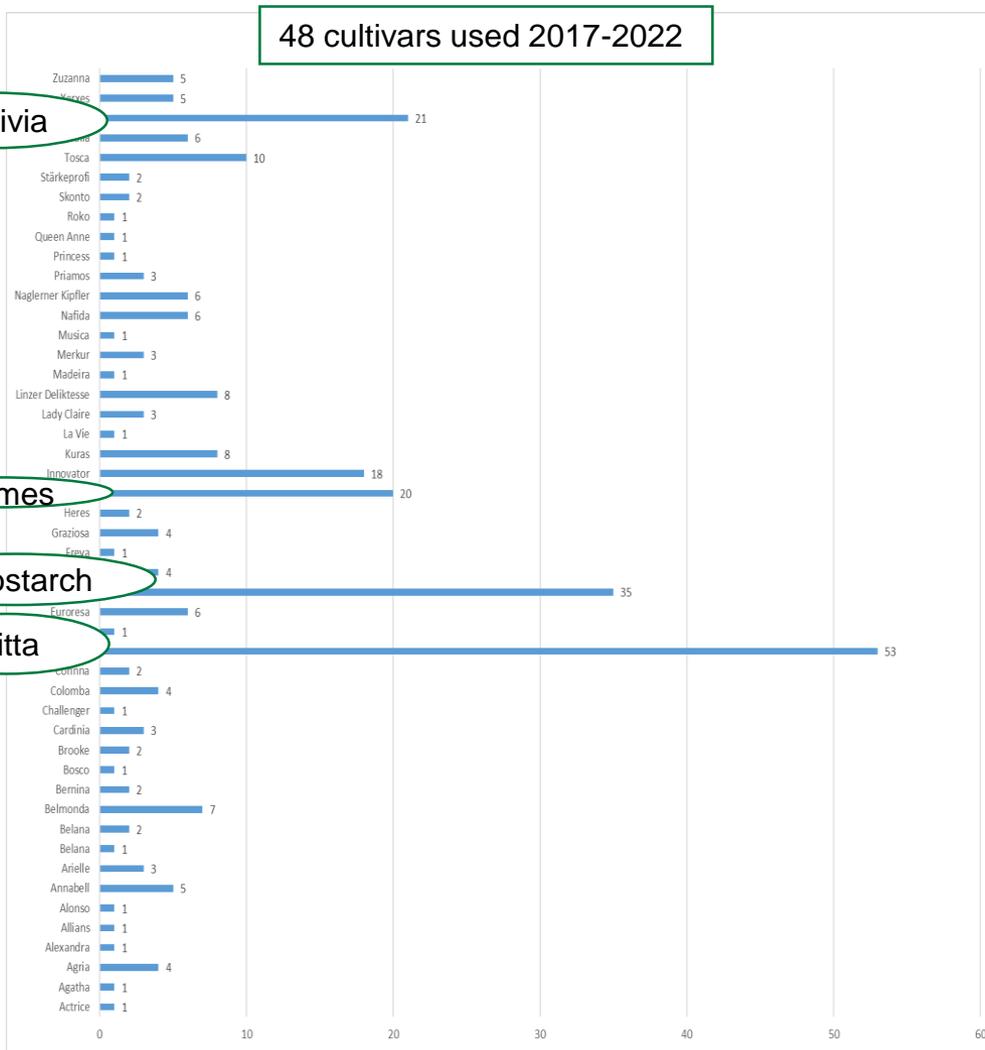
- Total number of cultivars: 48 (2017-2022)



# Methodology



48 cultivars used 2017-2022



Descriptive list of cultivars

Sorte, Züchterland	Zulassungsjahr	Eignung <sup>1)</sup>	Kootyp <sup>2)</sup>	Wuchstyp der Pflanze <sup>3)</sup>	Wuchserform der Pflanze <sup>4)</sup>	Reifzeit	Blattrollvirus	Y-Virus	Dürffleckenkrankheit	Krautfläule	Knochenfläule	Kartoffelschorf	Eisenfleckigkeit
<b>Sehr früh bis früh reife Speisesorten</b>													
Adora, NL	1995	S,F	vf	Z	ha	1	3	3	-	6	6	4	3
Agata, NL	1991	S	f	B	ha	2	4	3	6	6	5	6	2
Anuschka, D	2003	S	f	Z	a	2	2	4	5	6	4	4	-
Berber, NL	1985	S,C,F	vf	Z	ha	2	4	5	5	6	2	6	5
Donald, NL	2000	S,F,C	m	Z	ha	3	4	4	-	4	5	5	-
Erika, A	2007	S,Sa	f	Z	ha	2	3	1	-	5	4	5	-
Impala, NL	1992	S	vf	Z	ha-bw	3	4	4	-	5	5	4	2
Minerva, NL	1989	S,C	vf	Z	ha	2	4	1	5	7	5	6	3
Nöstling, A	2021	S	vf	Z	ha	3	3	3	5	5	4	3	-
Romina, A	1988	S,C,F	vf	Z	ha	3	3	5	6	8	6	5	2
<b>Früh bis mittelfrüh reife Speise- und Verarbeitungsorten</b>													
Alonso, A	2011	S	vf	Z	a	5	3	2	3	4	5	3	-
Beltina, D	1995	S,C	vf	Z	a	5	6	1	-	5	3	3	3
Bosco, A	2012	S	m	S	a	5	8	2	4	4	3	3	-
Chiara, A	2019	S	vf	Z	ha	5	4	1	4	4	3	4	-
<b>Ditta, A</b>	1988	S	f	Z	a	5	4	6	3	4	2	3	2
Evita, A	1994	S,C,F	f	Z	a	4	6	3	4	6	5	4	2
Exquisa, D	1994	S,Sa	f	Z	ha	4	2	2	-	4	6	4	4
Fontane, NL	2001	S,C,F	m	S	a-ha	5	5	5	3	5	5	4	1
Galata, A	2012	S	vf	S	a	5	7	5	4	5	4	4	-
Graziosa, A	2017	S,Sa	f	Z	ha-bw	4	4	1	3	5	3	3	-
<b>Hermes, A</b>	1972	C,S,St	m	Z	ha	4	3	6	4	5	3	3	2
Marizza, A	2012	S	vf	S	a	4	7	1	4	5	5	4	-
Martina, A	2009	S	vf	Z	ha	4	4	2	4	5	4	4	-
Meireska, A	2015	S	vf	S	a	4	3	2	4	6	5	4	-
Naglerer Kipfler, A	1955	Sa,S	f	B	ha	5	5	8	-	7	8	3	3
Pepino, A	2018	S	vf	Z	ha	4	4	3	4	5	3	3	-
Roko, A	1997	S,C	vf	Z	ha	5	5	1	3	4	3	5	3
Sokrates, A	2014	F,S,C	m	Z	a	5	4	5	3	5	4	5	-
Tosca, A	2001	S	vf	S	a-ha	5	3	5	4	5	5	4	2
<b>Valdivia, A</b>	2013	S	f	Z	a-ha	4	3	1	4	5	3	3	-
<b>Mittel bis spät reife Speise- und Verarbeitungsorten</b>													
Agria, D	1988	S,C,F	m	Z	a-ha	6	5	6	4	5	3	7	4
Asterix, NL	1991	S,F,C	m	Z	ha	6	6	5	-	5	4	3	2
Bionta, A	1992	S	vf	Z	a-ha	9	5	1	2	3	2	5	2
Diego, A	2011	F,C,S	m	Z	a	7	3	1	3	4	5	4	-
Fabiola, A	2005	S	vf	Z	ha	6	6	1	3	5	5	4	2
Herbstgold, A	2019	S	vf	Z	a	6	5	1	5	5	5	4	-
Longinus, A	2020	F,C,S	m	B	ha-bw	6	4	1	4	5	5	5	2
Meichip, A	2021	C,F,S	m	Z	ha	6	3	4	4	5	4	5	-
Siegfried, A	2019	C,F,S	m	B	a	6	5	1	4	5	3	5	-
<b>Mittel bis spät reife Stärkesorten</b>													
Bonza, D	2005	St	m	Z	bw	8	3	1	3	5	4	4	3
Jumbo, D	2004	St,C	m	B	ha	6	3	1	3	4	4	5	-
Kuras, NL	1995	St,C	sm	Z	ha	9	4	2	2	3	2	4	3
Siktus, A	2019	St	sm	Z	ha	7	3	1	4	4	4	5	-
Skonto, D	2007	St	m	Z	bw	8	3	1	4	4	4	4	3
Tomensa, D	1994	St,F,C,T	sm	B	a	5	2	3	4	5	5	5	4
Trabant, A	2013	St	sm	Z	ha	7	6	1	4	4	4	3	-
Xerxes, A	2014	St	sm	Z	a-ha	7	5	1	3	4	3	4	-

Eurostarch: Low to medium

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Bundesministerium Land- und Forstwirtschaft, Regionen und Wasserwirtschaft

LE 14-20  
Umweltprogramm für den Landwirtschaftsbereich



# Monitoring-Map 2023



## Kartoffel Monitoring 2023

Beim Kartoffel-Monitoring werden sowohl das Erstauftreten, als auch der weitere Krankheitsverlauf der Kraut- und Knollenfäule, sowie der Alternaria-Dürrefleckenkrankheit, beobachtet. Die Ergebnisse sollen dabei helfen, den aktuellen Krankheitsdruck in den Kartoffel-Anbauregionen – und damit die Notwendigkeit von Fungizidbehandlungen – besser einschätzen zu können. Ergänzend dazu stehen ein Prognosemodell für die **Erstbehandlung** sowie ein Prognosemodell für **Folgebehandlungen** gegen Kraut- und Knollenfäule zur Verfügung.

### Parameter wählen

Gesamtüberblick

Gesamtüberblick

Krautfäule

Alternaria

Alle | Burgenland | Niederösterreich | Oberösterreich

### Saison

2023

prognostizierten Infektionsdruck anzeigen

+  
-



### Monitoringdaten vom 26.05.2023 bis 23.08.2023

**7021 Drassburg, 1, Burgenland**  
Linzer Delikatess, Vorfrucht: Winterweizen, sandiger Lehm, Staulage, Anbaudatum: 17.04.2023, Aufaufdatum: 10.05.2023

2023	17.4.	21.4.	25.4.	29.4.	3.5.	7.5.	11.5.	15.5.	19.5.	23.5.	27.5.	9.6.	16.6.	28.6.	6.7.	1.8.
BBCH	-	-	-	-	-	-	-	-	-	-	30	45	55	65	69	87
Krautfäule	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Alternaria	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0

09.04.2023 -1109259- 01.08.2023

### Legende

#### Krautfäule, Alternaria

- kein Befall
- Erstauftreten
- mäßiger Befall im Bereich der Schadschwelle
- hoher Befall über der Schadschwelle
- Keine aktuellen Daten vorhanden
- Außerhalb des Erhebungszeitraums
- Anzahl der Standorte in dieser Region

#### Infektionsdruck

- sehr niedrig
- niedrig
- mittel
- hoch
- sehr hoch

#### Schadschwellen ausblenden

#### Phytophthora

- < 1% befallene Pflanzen
- 1 - 10% befallene Pflanzen
- 10 - 25% befallene Pflanzen
- 26 - 50% befallene Pflanzen
- 51 - 100% befallene Pflanzen

#### Alternaria

- auf einigen Pflanzen vereinzelt Symptome
- auf einem Großteil der Pflanzen Symptome auf den untersten Blättern
- auf einem Großteil der Pflanzen Symptome auf den untersten und vereinzelt auf den mittleren bis oberen Blättern
- auf einem Großteil der Pflanzen Symptome bis auf die oberen Blätter und Kraut noch weitgehend grün
- auf einem Großteil der Pflanzen Symptome bis auf die oberen Blätter und Kraut dadurch bereits weitgehend abgestorben



© V. Pachtrog-Wilfinger, LK NÖ

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Bundesministerium  
Land- und Forstwirtschaft,  
Regionen und Wasserwirtschaft

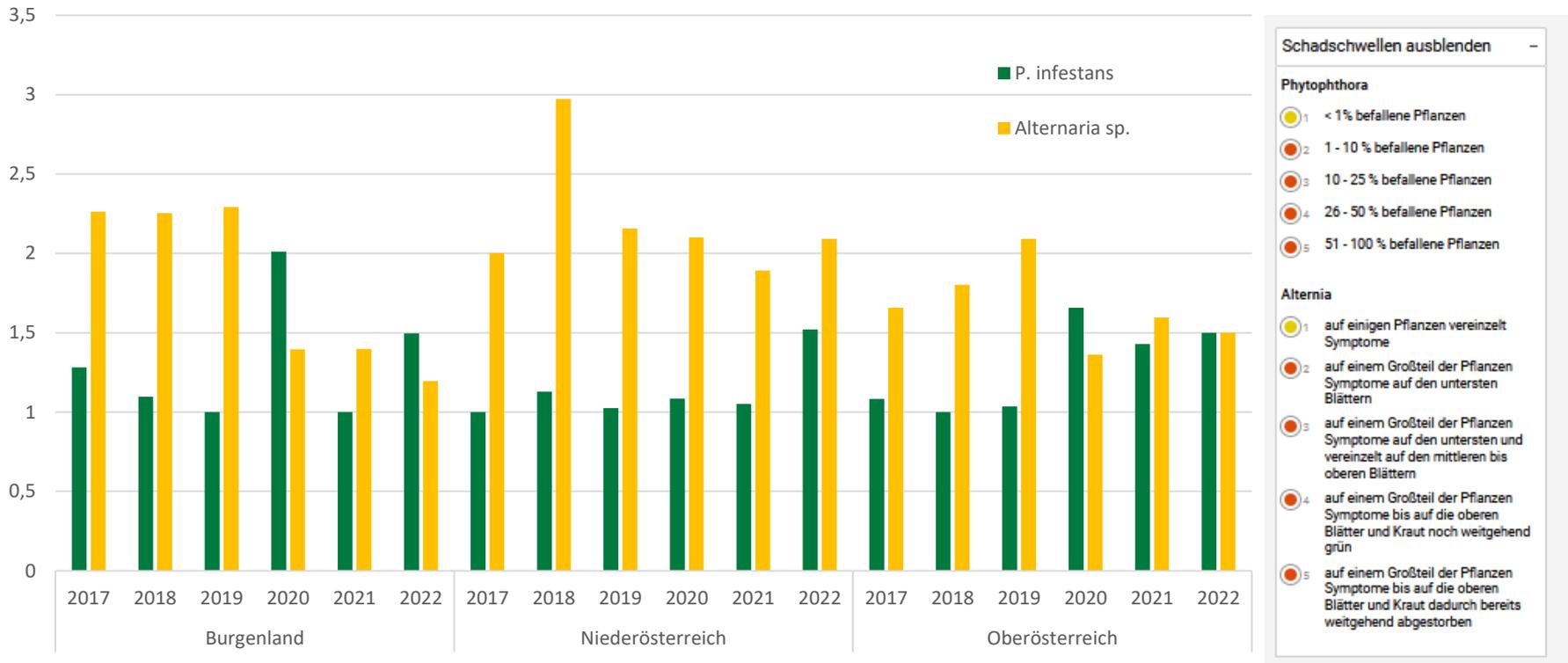
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Entwicklung für eine lebensfähige Zukunft



# Results



Disease incidence *P. infestans* and *Alternaria* sp. 2017-2022



# Results and conclusions



## ➤ Weather conditions

### ▪ ***P. infestans***

- Due to higher precipitation in Upper Austria higher infestation was observed than in Lower Austria
- In months of June and July (beginn of infection) due to heavy rainfall higher infestation of *P. infestans* was observed with regional often small scale differences

### ▪ ***Alternaria sp.***

- *A. solani* and *A. alternata* occurred more frequent in the eastern parts of Austria in 2018 where a combination of irrigation and high temperature boosted the outbreak of *Alternaria species*
- Very often sunlight on water droplets burn the upperside of the potato leaves. By this saprophytes like *Alternaria sp.* may easily penetrate the cuticula and start the infection.

- In general during the monitoring period 2017 – 2022 *Alternaria sp.* was the prevailing potato disease in Austria



# Results and conclusions



- The spread and occurrence of the disease in the following year is influenced by the potatoes remain on the cultivated area after harvesting (potatoes which remain on the cultivated area do not freeze due to mild winters (climate change) and thus lead to higher infections in the following year (more inoculum present)
- **Production methods**
- Varietal susceptibility: leaf type or stem type. Tillage, previous crop, location: waterlogged location, fertilization (farm fertilizer) higher infestations are still being analysed in detail

➤ Detailed statistics and publication of the data is planned and will follow soon



- Shala-Mayrhofer Vitore
- Muck-Arthaber Julia
- Pachtrog-Wilfinger Vera
- Hubert Köppl
- Stefan Winter
- Kurt Foltin

Project leader

Coordinator

Implementation monitoring, professional part



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Europäischer  
Landwirtschaftsfonds für  
die Entwicklung des  
ländlichen Raums:  
Hier investieren Europa in  
die ländlichen Gebiete.



# Thank you for your attention!

**DI Dr. Vitore Shala-Mayrhofer**

Alert service for plant protection | Project leader

Austrian Chamber of Agriculture

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M. +43 676 83441 8538

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[www.warndienst.at](http://www.warndienst.at)



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Europäischer  
Landwirtschaftsfonds für  
die Entwicklung des  
ländlichen Raumes  
Hier investieren Europa in  
die ländlichen Gebiete.



# DivGene

## Diversity analyses of key genes involved in interaction between potato and *Phytophthora infestans*

Paluchowska P<sup>1</sup>, Yin Z<sup>1</sup>, Lysøe E<sup>2</sup>, Rossmann SL<sup>2</sup>, Ludwiczewska M<sup>1</sup>, Janiszewska M<sup>1</sup>, Sobkowiak S<sup>1</sup>, Eikemo H<sup>2</sup>, Skogen M<sup>2</sup>, Brurberg MB<sup>2,3</sup>, Śliwka J<sup>1</sup>

<sup>1</sup> *Plant Breeding and Acclimatization Institute - National Research Institute (IHAR-PIB), Platanowa 19, Młochów, Poland*

<sup>2</sup> *Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway*

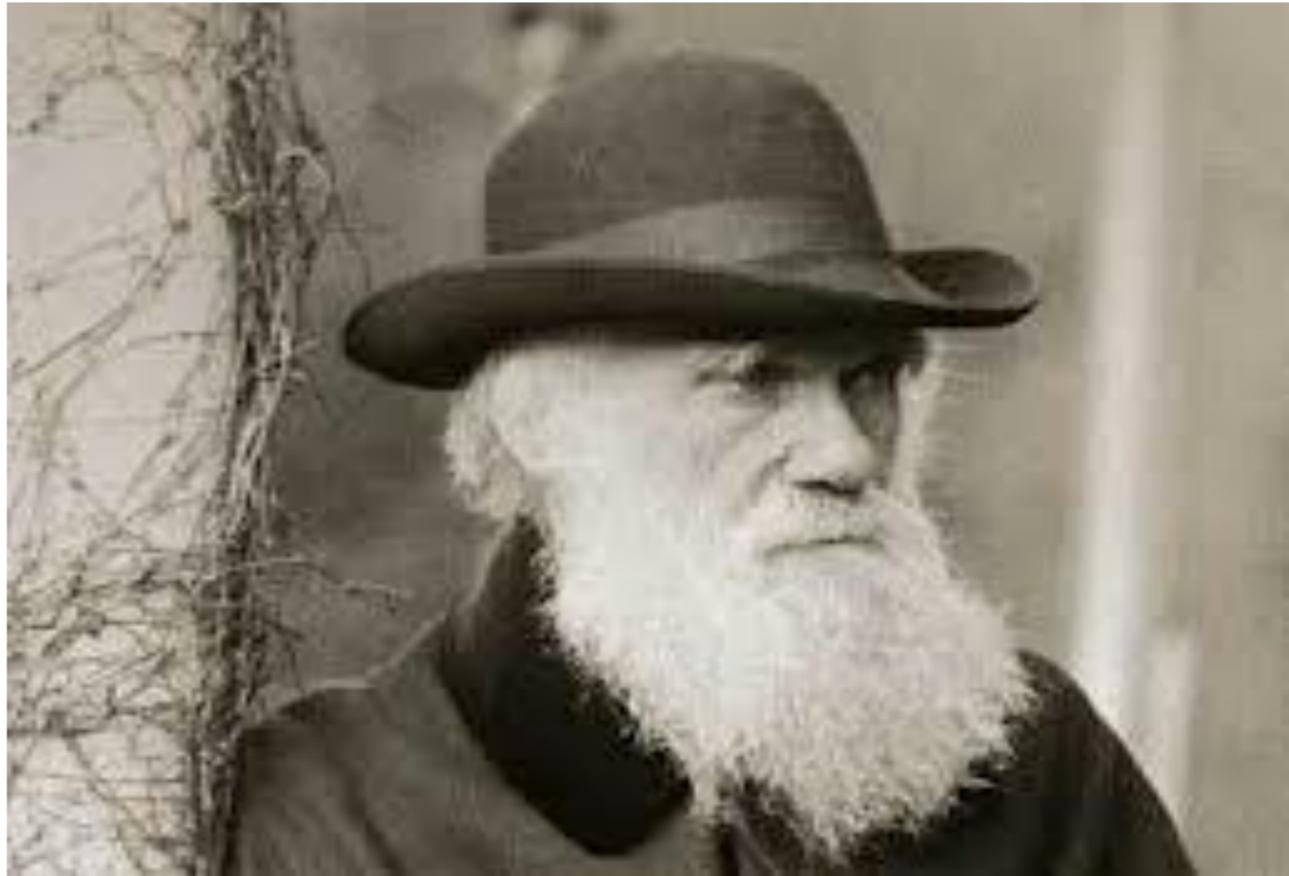
<sup>3</sup> *Norwegian University of Life Sciences (NMBU), Ås, Norway*

# Presentation overview

1. Introduction
2. Amplicon sequencing: a pilot study on *Ry<sub>sto</sub>* gene
3. Amplicon sequencing of *Rpi* (**R**esistance to **P**hytophthora *infestans*) genes
4. Amplicon sequencing of *P. infestans* effector genes
5. Exploitation of results: gene diversity and expression versus plant and pathogen phenotypes



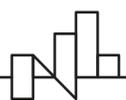
# Fighting late blight



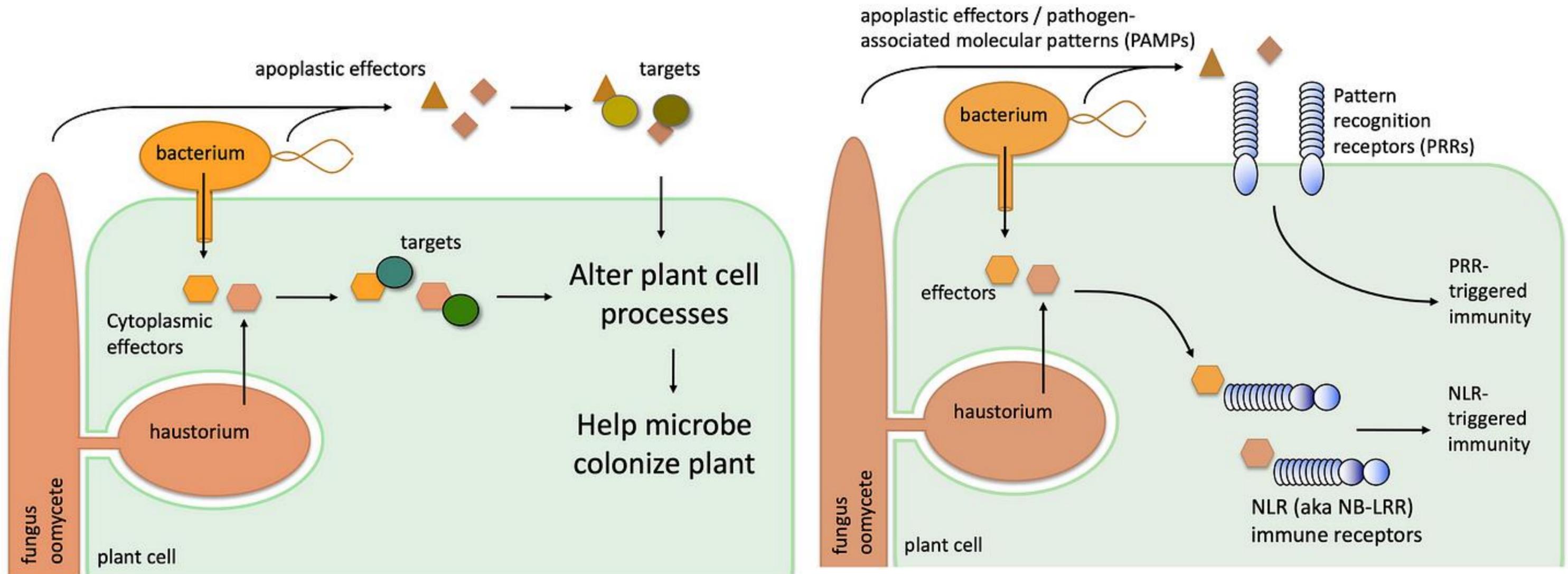
## Charles Darwin

- Tubers from Chile 1835
- 1876-1882 141 letters on potato disease
- Project with James Torbitt
- Growing potatoes from true seeds
- Selection of resistant individuals

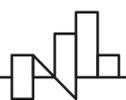
„I see *since* I wrote to you **someone has urged the necessity of sending to S. America for new seed!**” [C. R. Darwin to W. D. Fox, 1846]



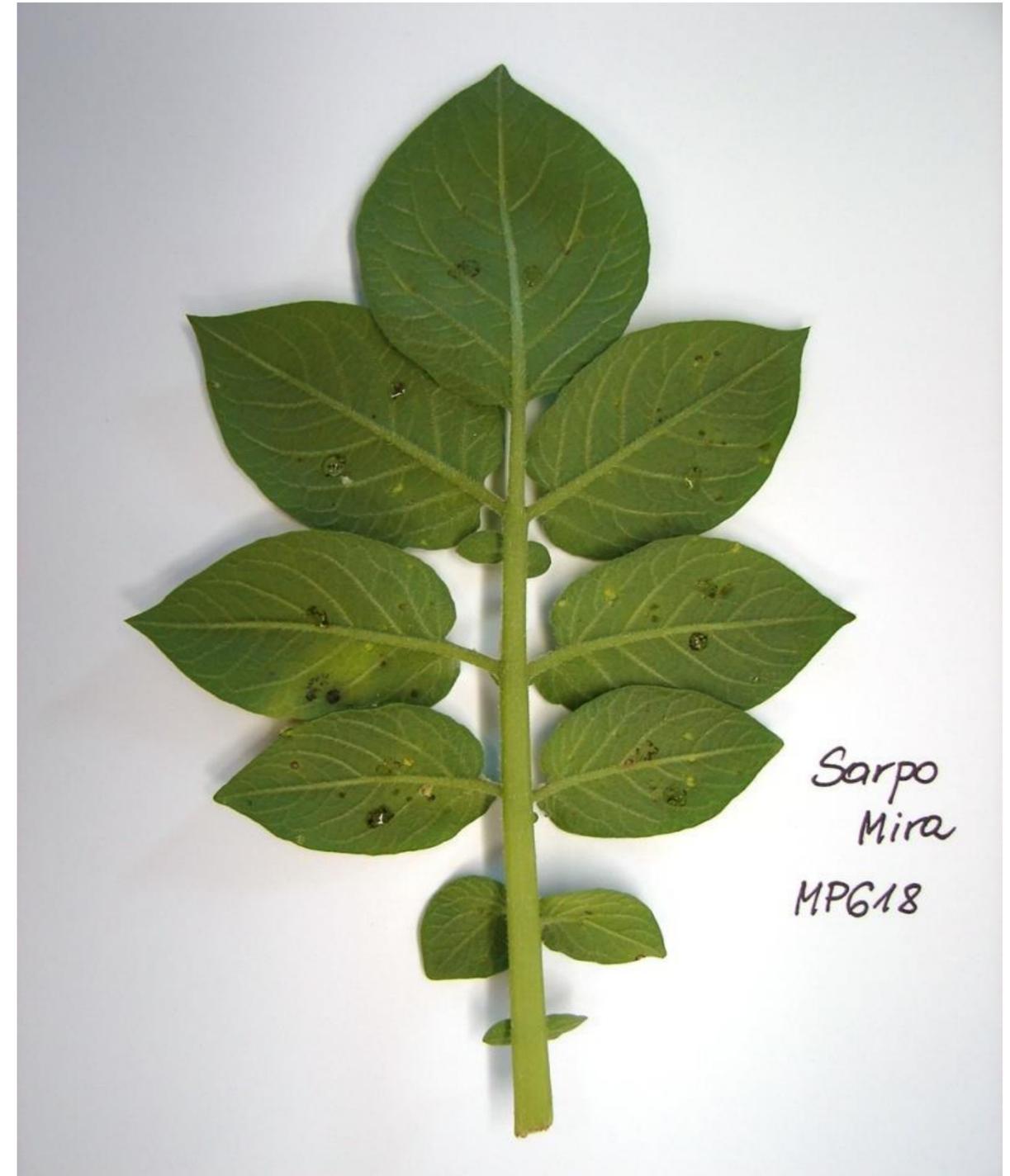
# Key genes involved in host-pathogen interaction



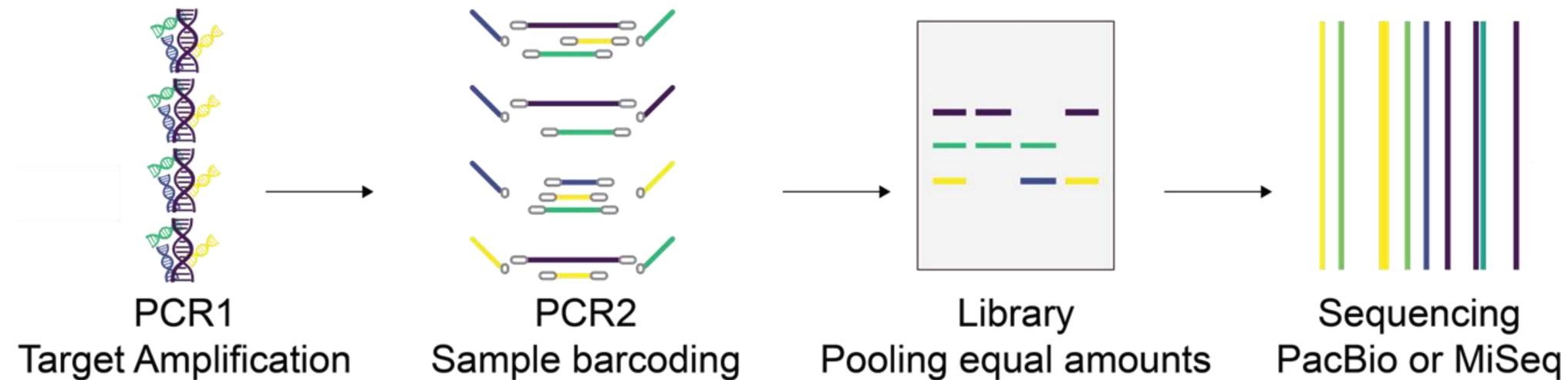
Kamoun, S. (2021). NLR receptor networks: filling the gap between evolutionary and mechanistic studies. Zenodo



# Hypersensitive reaction



# Two sequencing platforms



- + Cheaper per run
- + In-house at NIBIO
- + Easy to iterate and experiment
- Limited to max. 350-400 bp amplicons



Illumina MiSeq

<https://www.illumina.com/systems/sequencing-platforms/miseq.html>



PacBio Sequel II

<https://www.pacb.com/products-protocols/meet-the-new-sequel-ii-system/>

- + Sequencing of long amplicons
- + HiFi reads with few mistakes
- Off-site (Norwegian Sequencing center)
- Long wait times
- ca. 6x cost per read



# Amplicon sequencing – pilot study on *Ry<sub>sto</sub>* gene

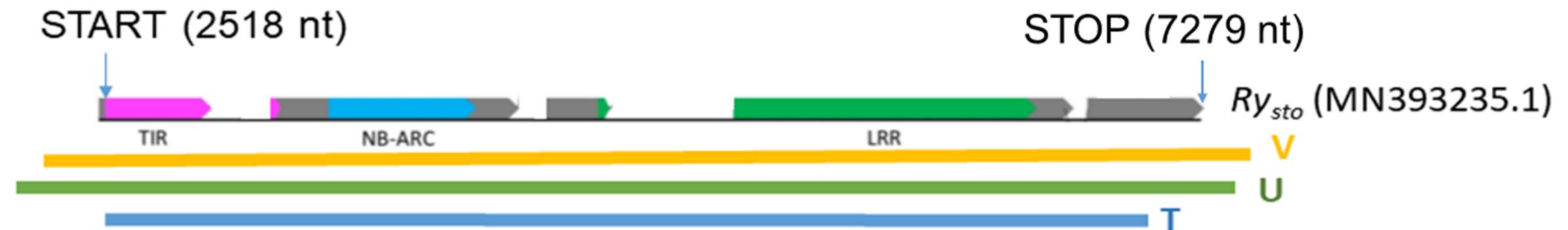


*Ry<sub>sto</sub>* from *Solanum stoloniferum*:

- Confers extreme resistance to PVY
- Mapped on Chromosome XII
- Sequenced (TIR-NB-LRR)

***Solanum stoloniferum* donor of gene *Ry<sub>sto</sub>***

<https://ics.hutton.ac.uk/germinate-cpc/#/home>



298 genotypes representing 29 accessions of 26 tuber-bearing *Solanum* species, IHAR-PIB's collection

Song et al. 2005; Flis et al. 2005;  
Grech-Baran et al. 2020



# Amplicon sequencing – pilot study on *Ry<sub>sto</sub>* gene

Results:

1. ASVs from 4430-5966 bp
2. 55 unique *Rysto-like* sequences detected in 72/298 potato genotypes
3. From 1 to 8 sequences per genotype
4. From 1 to 13 sequences per species
5. 54 new sequences from a single PacBio sequencing reaction

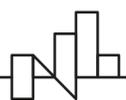
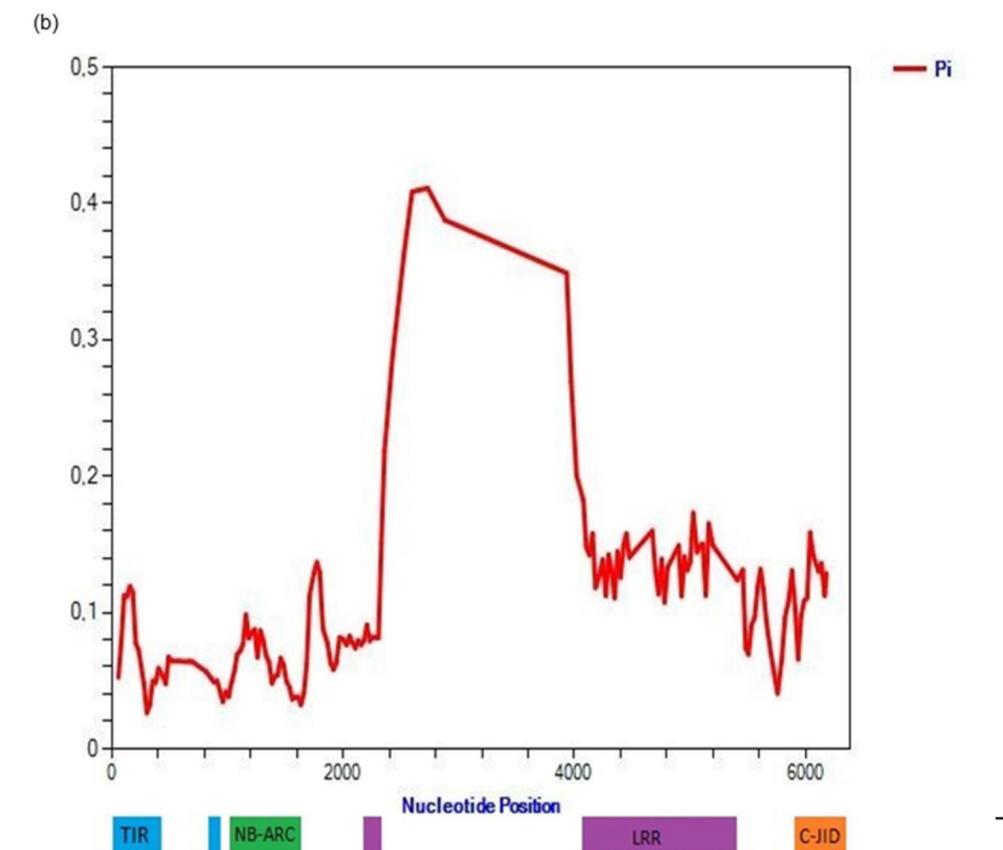
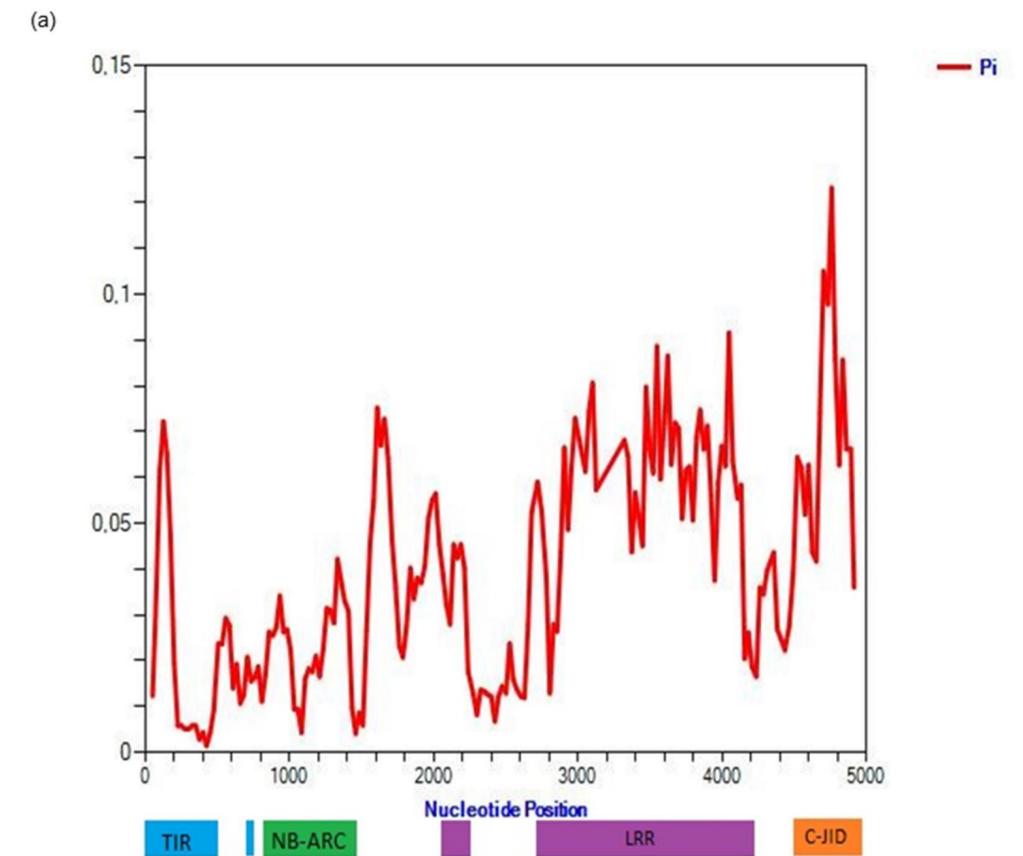
Nucleotide diversity ( $P_i$ ) of the *Ry<sub>sto</sub>* homologues

(a) 25 variants obtained with U and V primers;

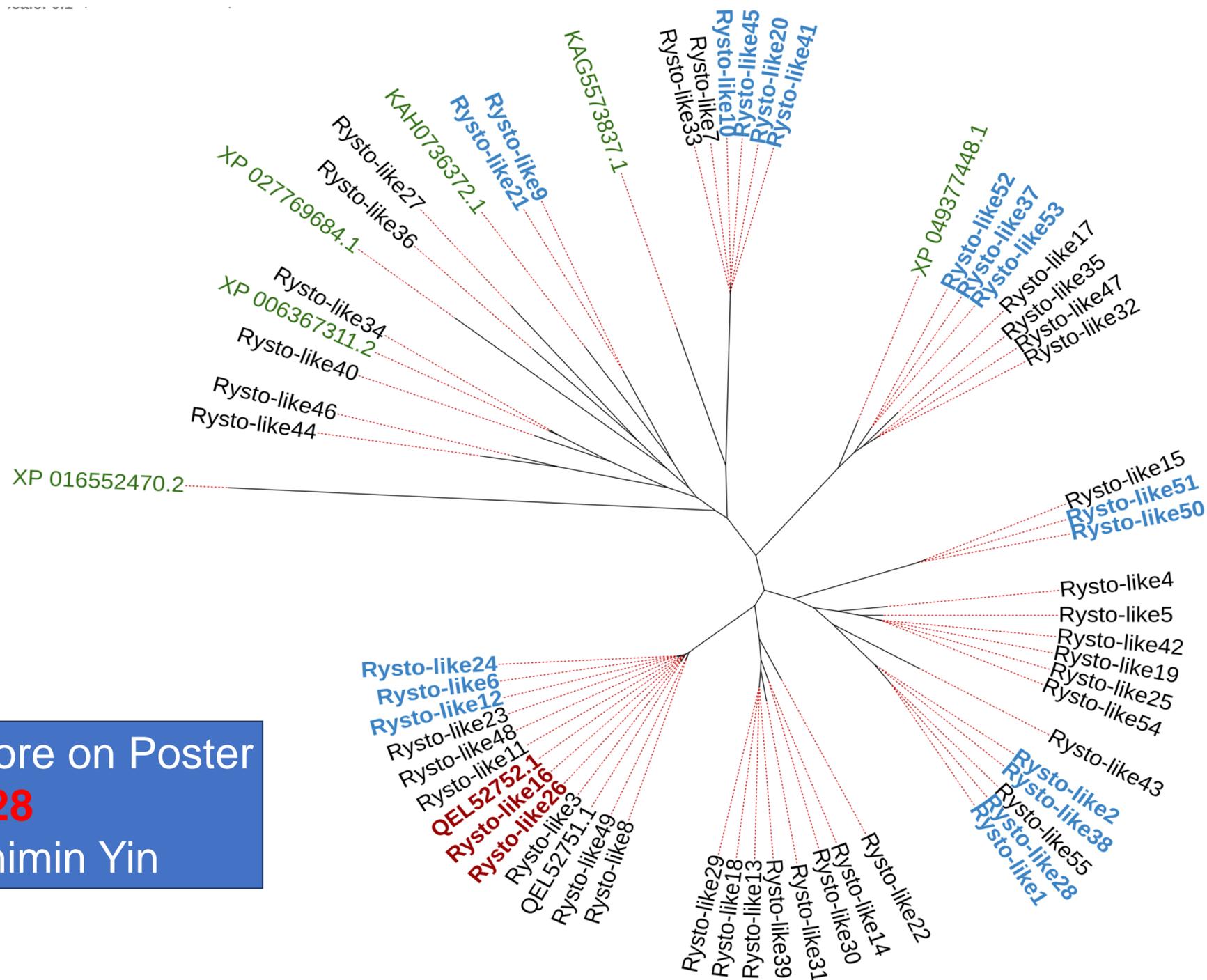
(b) 30 variants obtained with T primer (without the ATG start codon).

Domains:

TIR; blue; LRR; Purple; NB-ARC; green; (C-JID; orange).



# Amplicon sequencing – pilot study on *Ry<sub>sto</sub>* gene



Phylogenetic tree of the predicted Rysto-like protein sequences

Constructed using ClustalW and FastTree2.

**Red:** 100% identity to the Rysto protein.

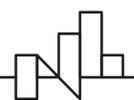
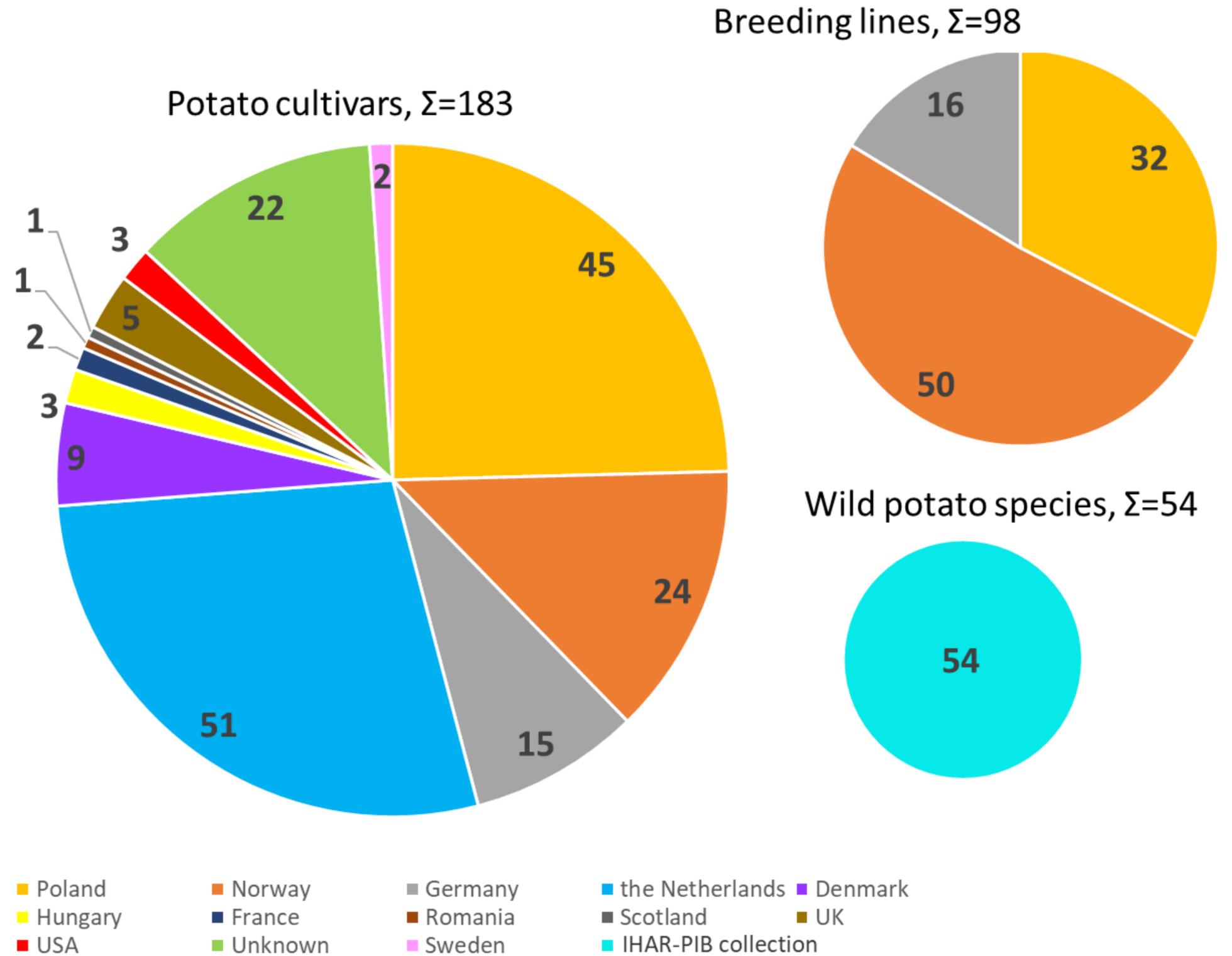
**Blue:** 100% identity to each other within a branch.

**Green:** other proteins from *Solanaceae*.

More on Poster  
**P28**  
Zhimin Yin

# Amplicon sequencing of *Rpi* genes

Plant material  
N = 335



# Amplicon sequencing of *Rpi* genes

List of target *Rpi* genes

#	Gene	Controls	Reference sequence
1	<i>R1</i>	R1, R5, R6, Bzura	AF447489
2	<i>R2</i>	R2, Bzura	FJ536346
3	<i>R3a</i>	R3, Allouete	AY849382
4	<i>R3b</i>	R3, Allouete	JF900492.1
5	<i>R8</i>	R8, Kelly, Sárpo Mira	KU530153
6	<i>R9</i>	R9	Armstrong et al. 2019
7	<i>Rpi-phu1 (Rpi-vnt1)</i>	Allouete, Gardena	FJ423044.1
8	<i>Rpi-blb1</i>	16-34/2, 16-34/5 ( <i>S. stoloniferum</i> )	AY426259.1
9	<i>Rpi-blb2</i>	Toluca	DQ122125
10	<i>Rpi-ber1</i>	16-40/1 ( <i>S. berthaultii</i> )	MW390806.1
11	<i>Rpi-chc1.1</i>	Carolus,	MW383255
	<i>Rpi-chc1.2</i>	16-40/1 ( <i>S. berthaultii</i> )	MW410797



# Amplicon sequencing of *Rpi* genes

1. Barcoded PCR products (2500 bp - ca. 4000 bp) amplified and sequenced for 7 genes, so far
2. From 2 to 14 ASVs were obtained per gene in 243 potato genotypes
3. From 1 to 5 ASVs were obtained per potato genotype
4. The highest number of ASVs per gene was obtained for *R3a* (in total 14), while the lowest number of ASVs was obtained for the *R1* gene (2)
5. Highest numbers of different *Rpi* genes were detected in potato cultivars:

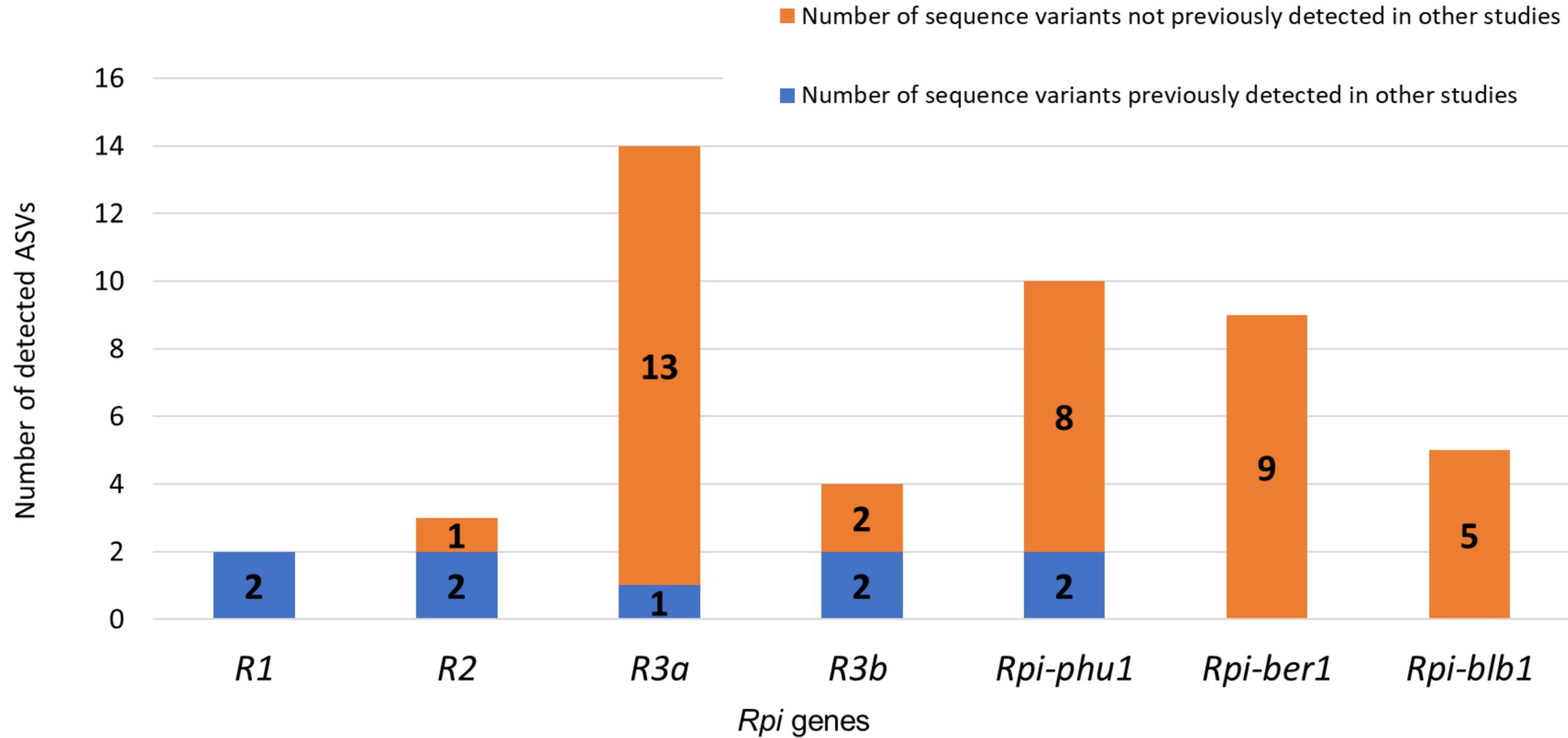
Escort: *R1*, *Rpi-abpt*<sub>T86</sub>, *R3a*, *R3b*, *R3b*<sub>G3111</sub>

Klepa: *R1*, *R2-like*, *R3a-like*, *Rpp13-like*, *R3b*<sub>G3111</sub>

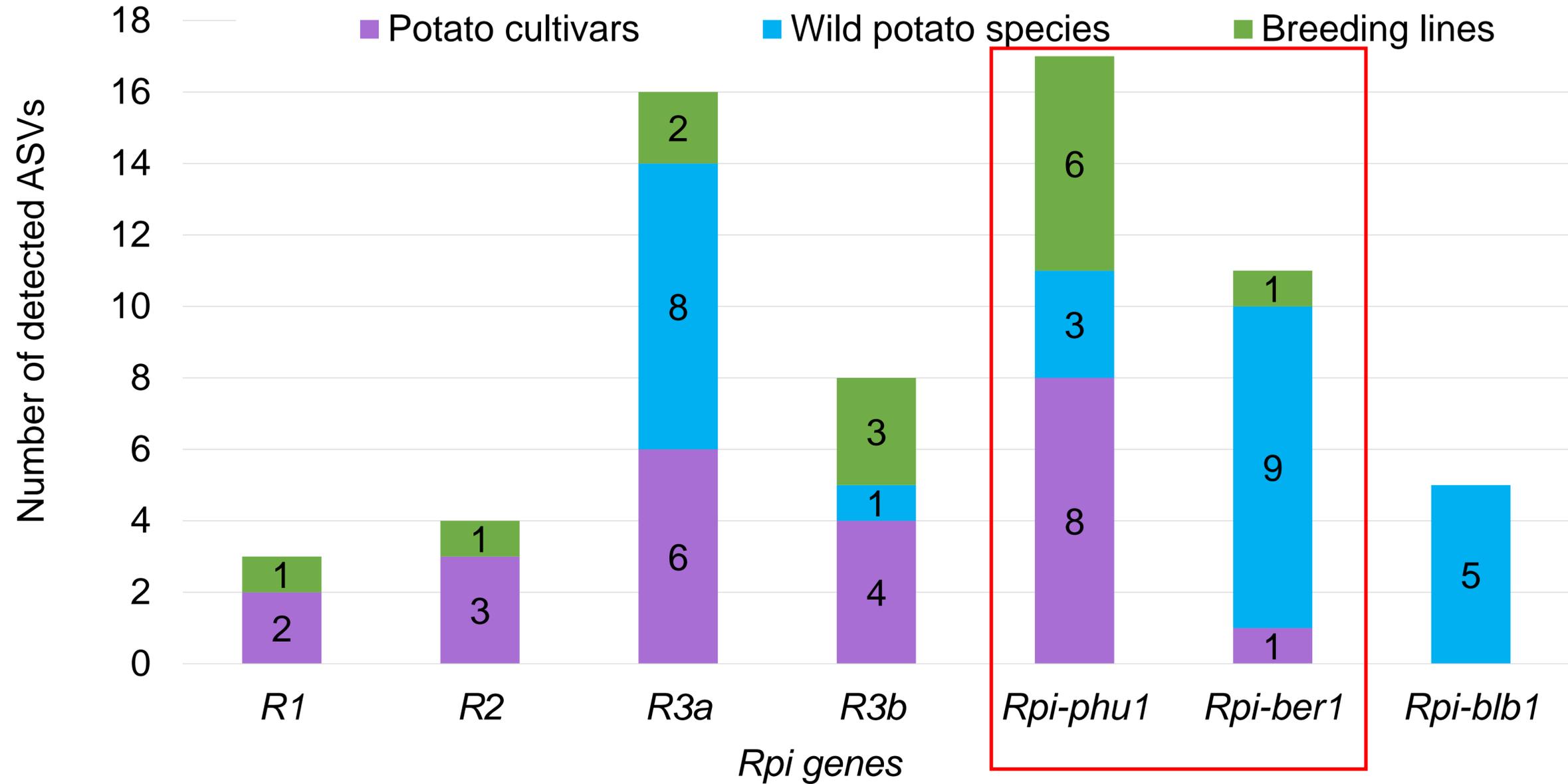
Rudawa: *Rpi-abpt*<sub>T86</sub>, *R3a*, *R3a-like*, *R3b*, *R3b*<sub>G3111</sub>



# Amplicon sequencing of *Rpi* genes



# Amplicon sequencing of *Rpi* genes

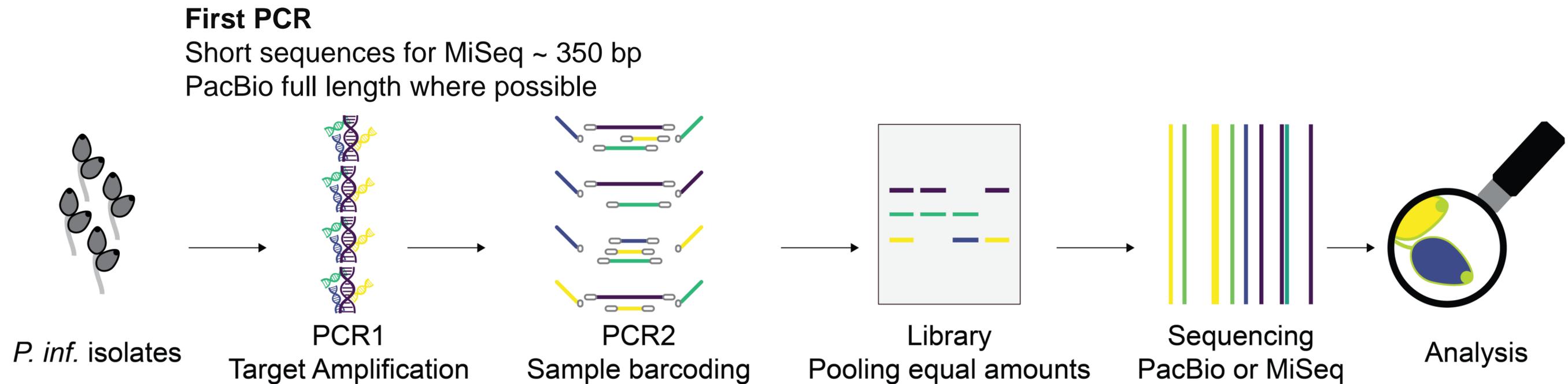


More on Poster  
**P1**  
Paulina  
Paluchowska



# Amplicon Sequencing of *P. infestans* genes (NIBIO)

2-step PCR for high flexibility



**Second PCR**  
Adding **indexes** and  
adapters



# Target genes and isolates



*P. inf.* isolates

- Ca. 400 isolates from Norway and Poland
- Some of Polish isolates belong to major clonal lineages
- Norwegian isolates largely originated from sexual populations
- Isolates from different countries

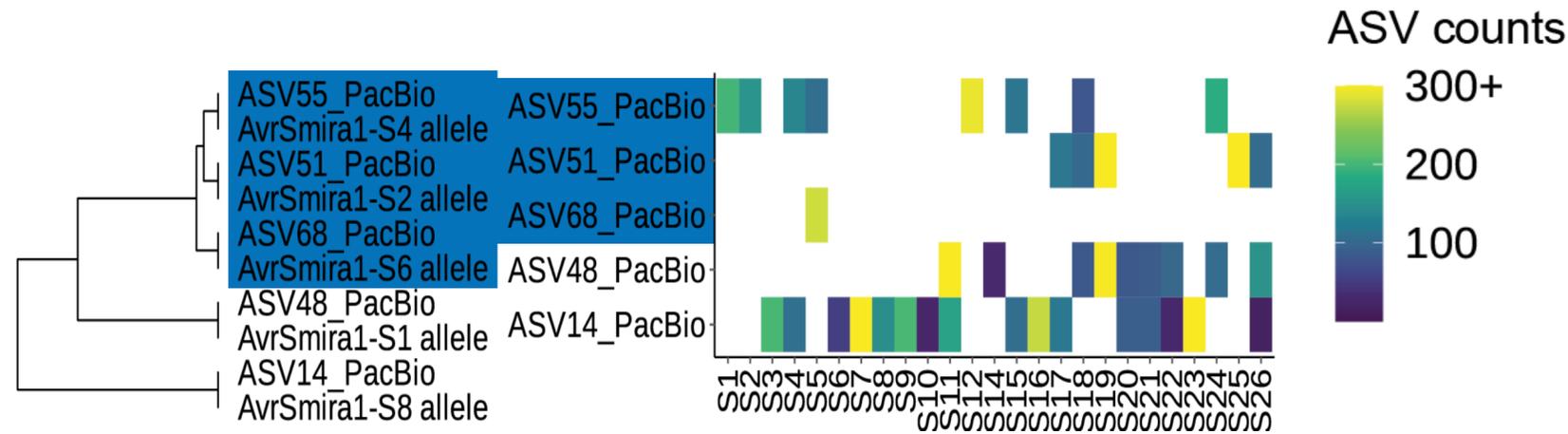


PCR1

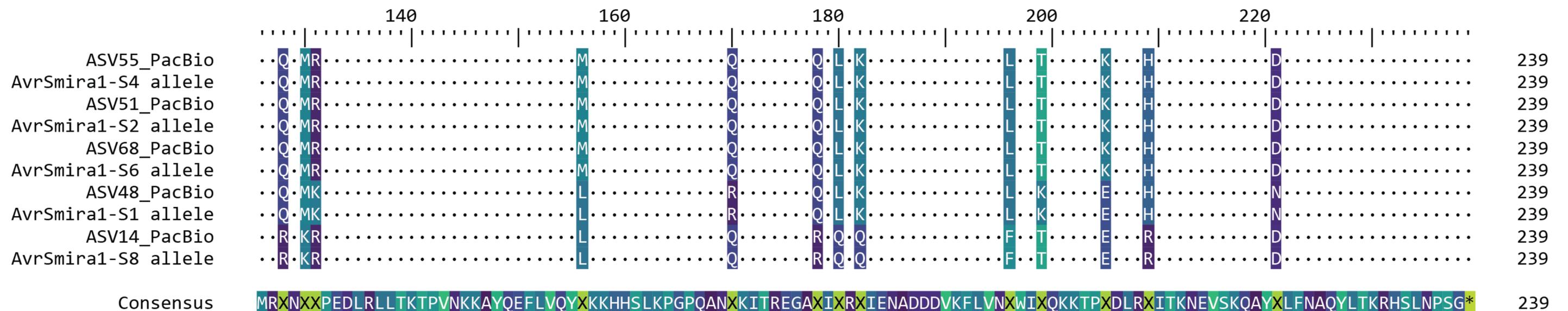
## Target Amplification

- Over 50 effector genes
- Well-studied and predicted putative effectors
- Multiple housekeeping genes

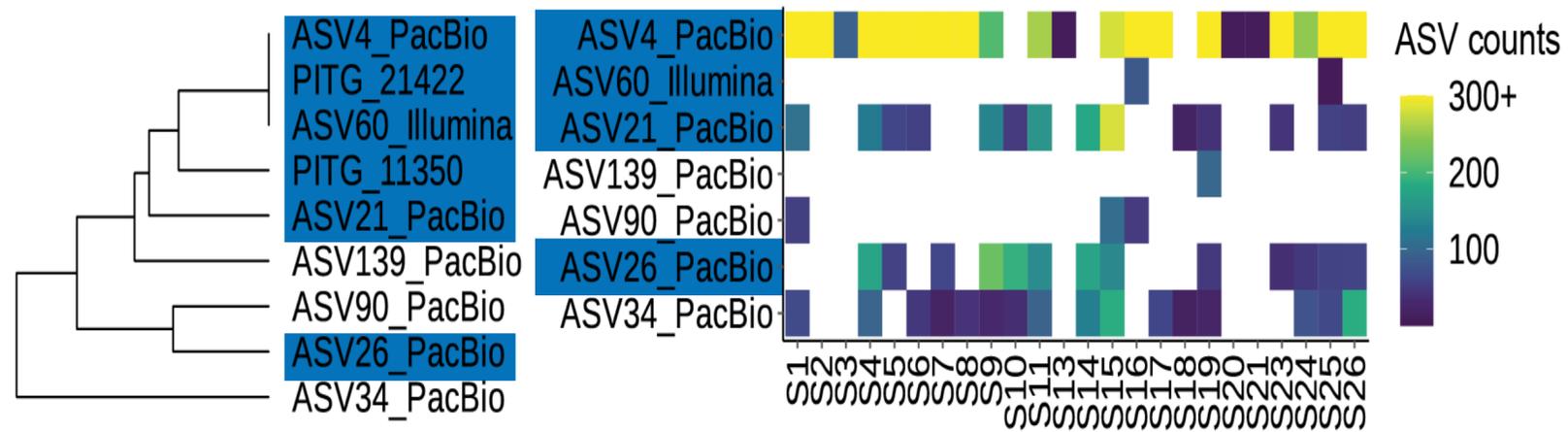
# Results: *AvrSmira1* variants in 13\_A2 strains



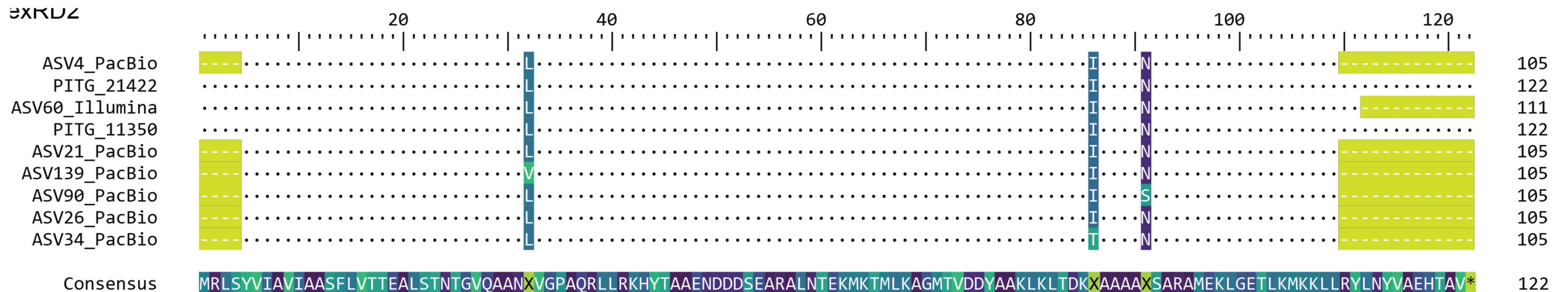
- Five *AvrSmira1* ASVs from PacBio
- All five variants are identical to known alleles
- Three ASVs code for identical proteins
- Potential triploidy for some isolates



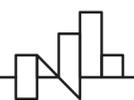
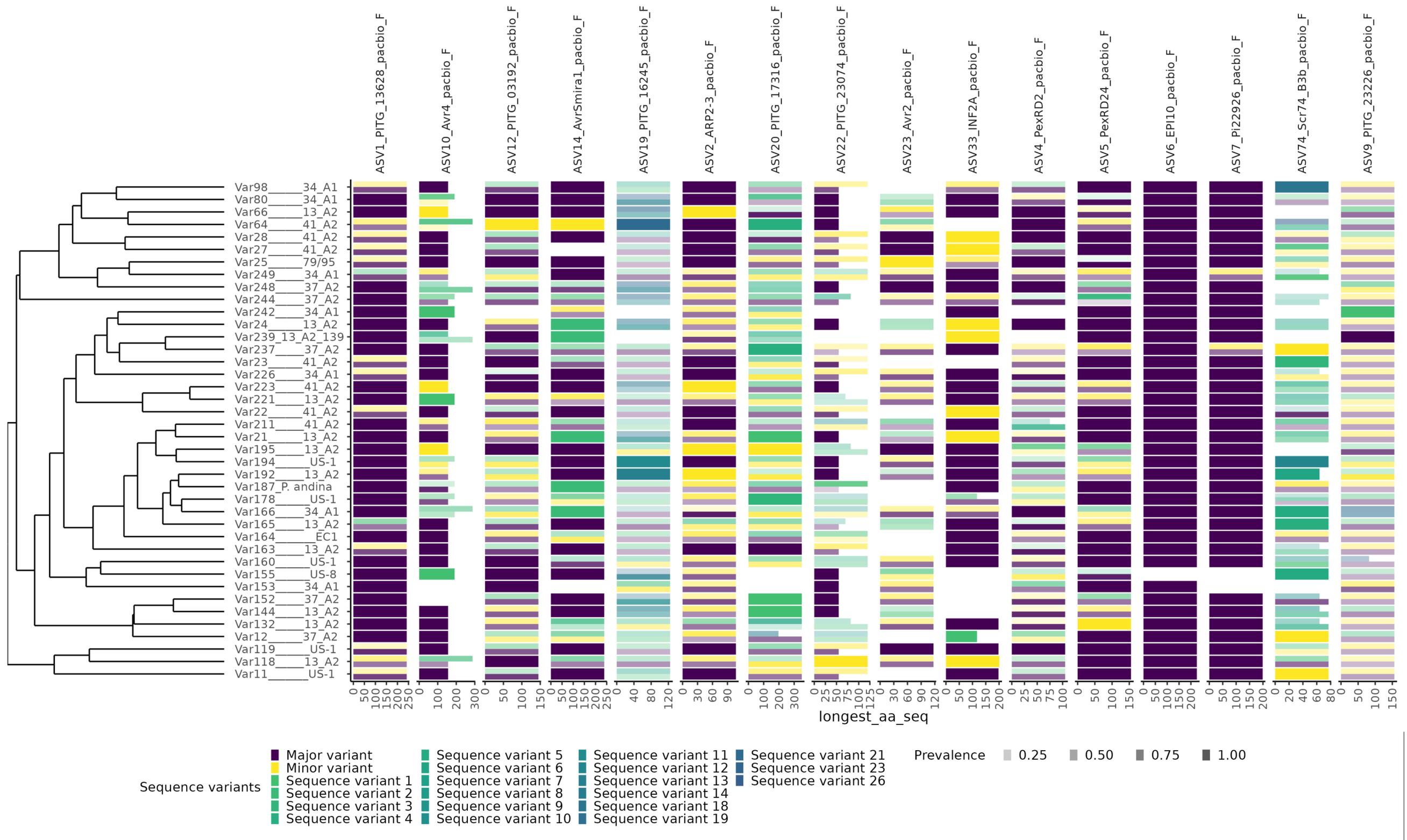
# PexRD2 variants in 13\_A2 strains



- Six *PexRD2* ASVs from PacBio, one from Illumina
- Variant captured in PacBio and Illumina was identical to known allele over sequenced length
- Three ASVs from PacBio and one from Illumina code for identical proteins over sequenced length
- AA-level variation in three positions



# And much more to discover...



# Exploitation of results: gene diversity and expression versus plant and pathogen phenotypes

## ***P. infestans* aggressiveness test**

- 5 leaflets × 3 cultivars × 19 isolates × 3 replications × 2 dates
- Measurement of latent period, lesion diameter, sporulation intensity

## **Virulence test**

- 3 leaflets × 23 cultivars × 12 isolates

## **Sequencing of effector genes**

- 12 isolates × 2 *Avr-vnt1* primer pairs - were sequenced by Sanger method
- 4 isolates - data from sequencing effector by Illumina sequencing

## **Statistics**

- ANOVA + Tukey's test (Statistica 13.0 software package)

Genotype	Number of isolates
EU13_A2	5
EU34_A1	4
EU37_A2	3
EU41_A1	7

Experiments on expression of plant and pathogen genes in progress



# *P. infestans* aggressiveness

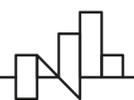
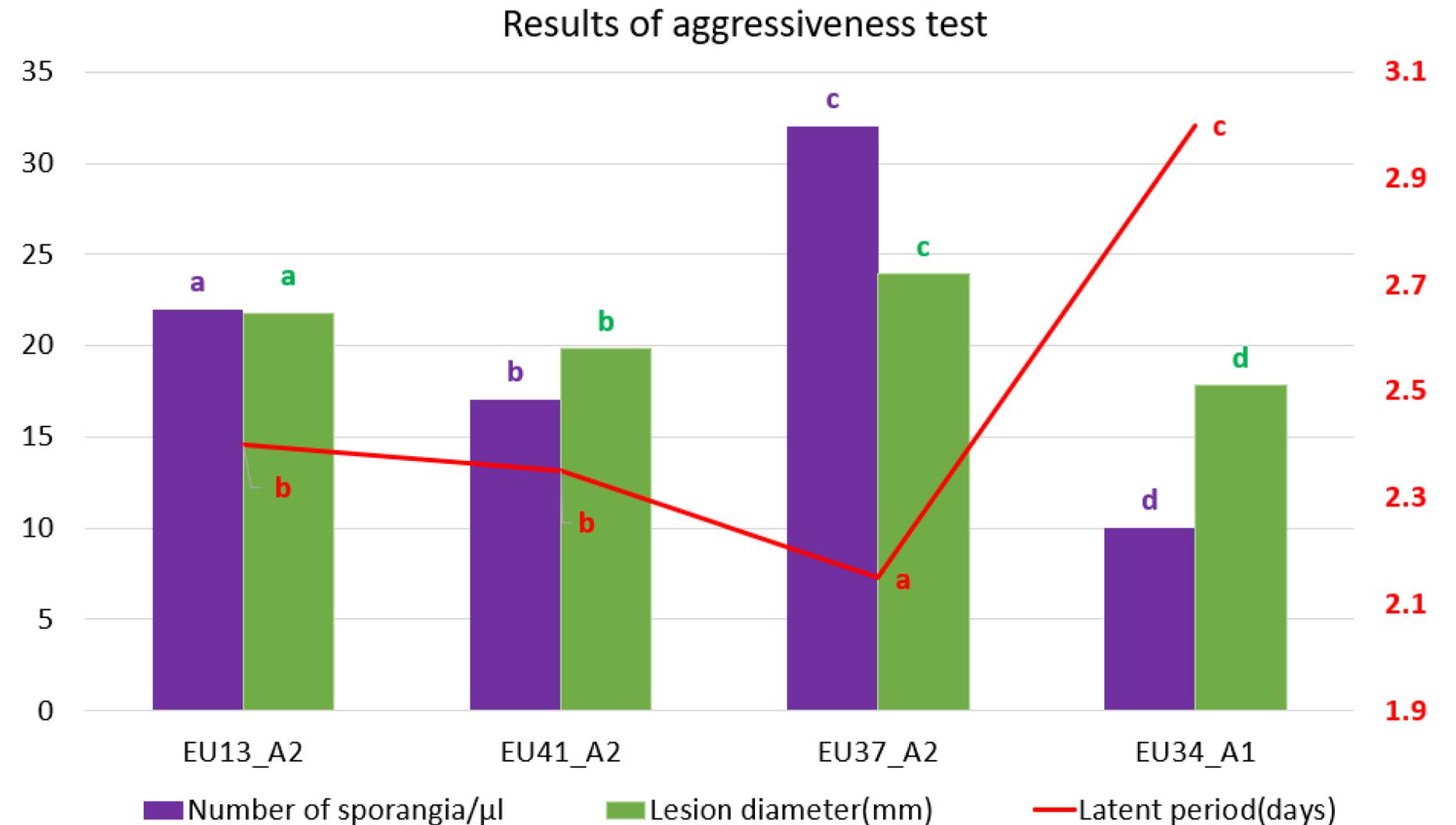
- Diversity of *Avr-vnt1* effector within genotypes
- The EU37\_A2 genotype of *P. infestans* was the most aggressive
- Isolates differed in virulence between and within SSR genotypes
- More isolates will be tested and data will be analysed in context of effector repertoire discovered by AmpSeq

More on Poster

**P2**

Mirella

Ludwiczewska





Thank you for attention

The research leading to these results has received funding from the Norwegian Financial Mechanism 2014-2021, project DivGene:  
UMO-  
2019/34/H/NZ9/00559



# Diversity analysis of *Rpi-ber1* and *Rpi-vnt1* genes determining broad-spectrum resistance to *Phytophthora infestans*

Paulina Paluchowska<sup>1</sup>, Zhimin Yin<sup>1</sup>, Erik Lysøe<sup>2</sup>, Simeon Lim Rossmann<sup>2</sup>, Mirella Ludwiczewska<sup>1</sup>, Marta Janiszewska<sup>1</sup>, May Bente Brurberg<sup>2,3</sup>, Jadwiga Śliwka<sup>1</sup>

<sup>1</sup>Plant Breeding and Acclimatization Institute - National Research Institute (IHAR-PIB) in Radzików, Młochów Division, Poland

<sup>2</sup>Norwegian Institute of Bioeconomy Research (NIBIO), Ås, Norway

<sup>3</sup>Norwegian University of Life Sciences (NMBU), Ås, Norway

*Phytophthora infestans* is an oomycete that causes the infamous potato late blight disease. Costs associated with crop losses and chemical control of late blight are estimated to be more than € 9 billion per year. Wild *Solanum* species are valuable sources of genes conferring resistance to *P. infestans* (*Rpi*). To date more than 70 *Rpi* genes have been discovered in potato and wild *Solanum* species. Many *Rpi* genes have become ineffective due to the rapidly evolving pathogen, but genes such as *Rpi-ber1* from *Solanum berthaultii* and *Rpi-vnt1* (*Rpi-phu1*) from *Solanum venturii* and from still confer resistance to many *P. infestans* strains (Figure 1).

**AIM:** Diversity analysis of *Rpi-ber1* and *Rpi-vnt1* (*Rpi-phu1*) genes in potatoes using amplicon sequencing (AmpSeq) approach.

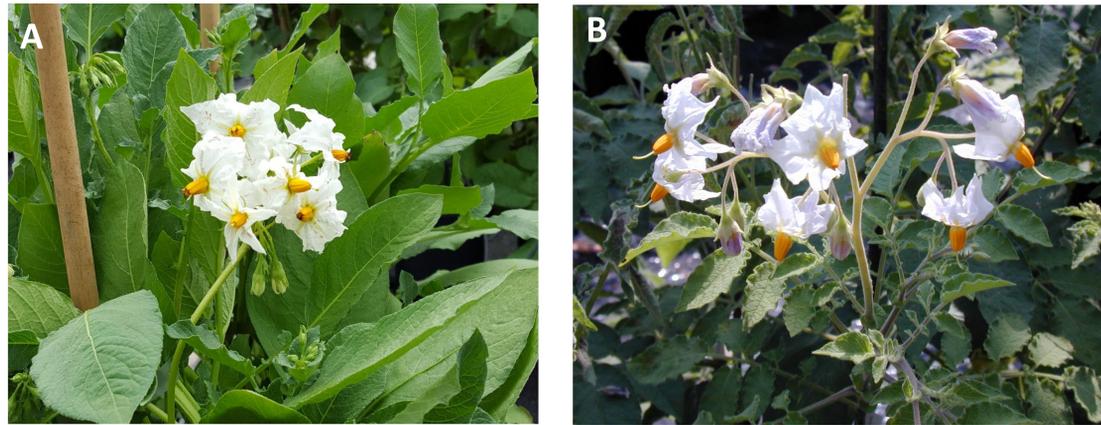
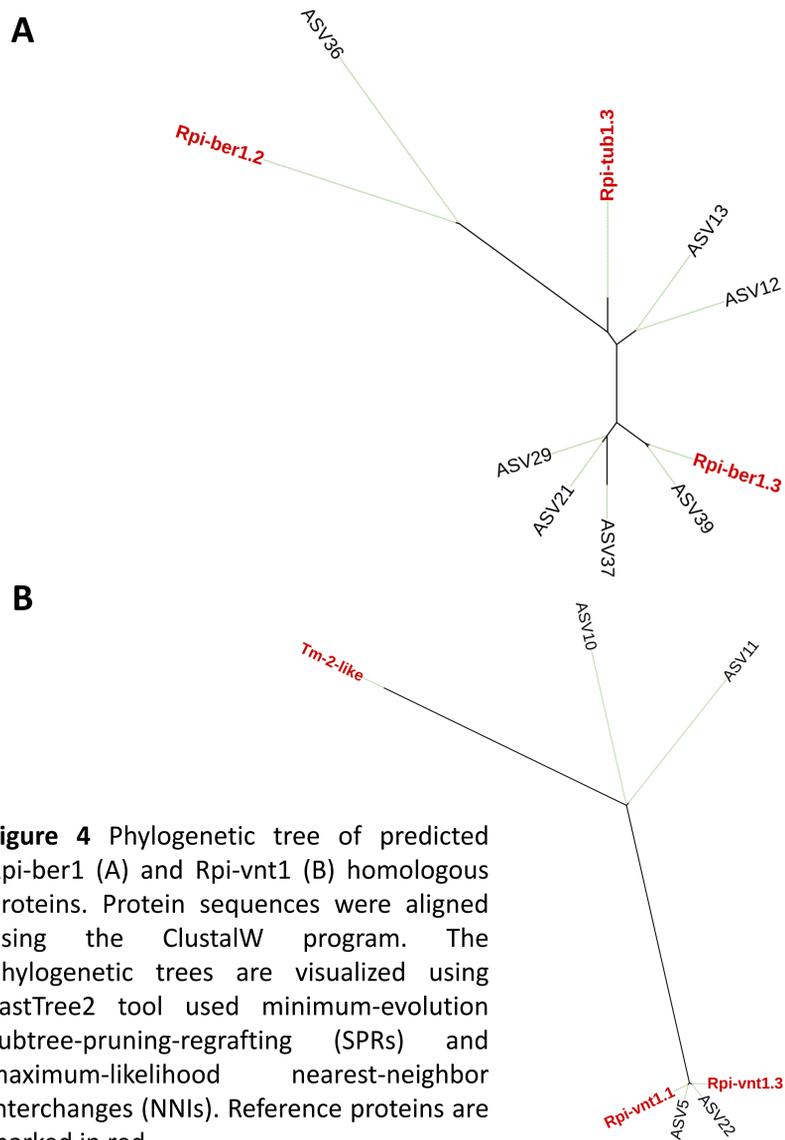


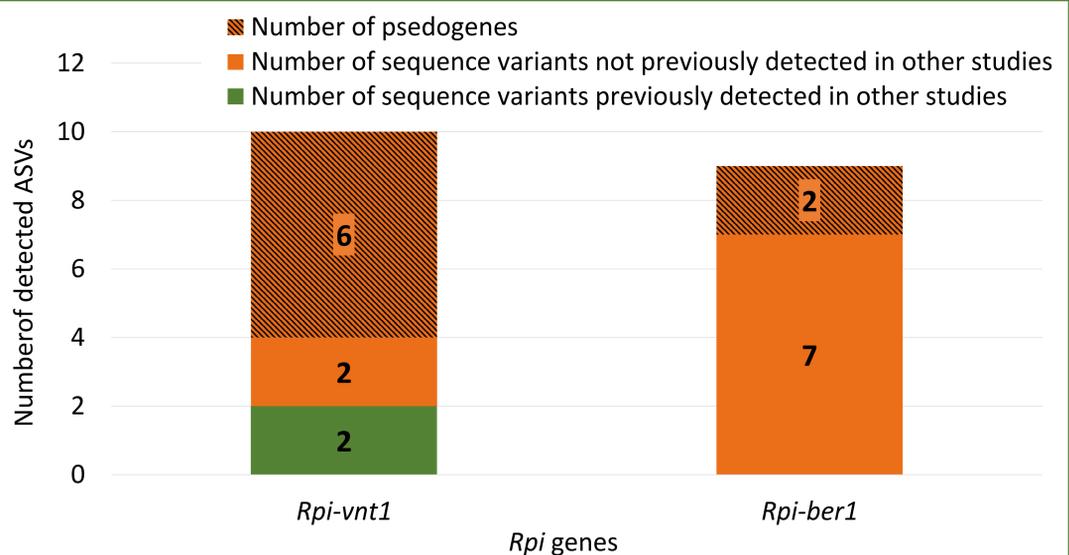
Figure 1 *Solanum venturii* (A) and *Solanum berthaultii* (B) <https://ics.hutton.ac.uk/germinate-cpc/#/home>

## MATERIALS AND METHODS:

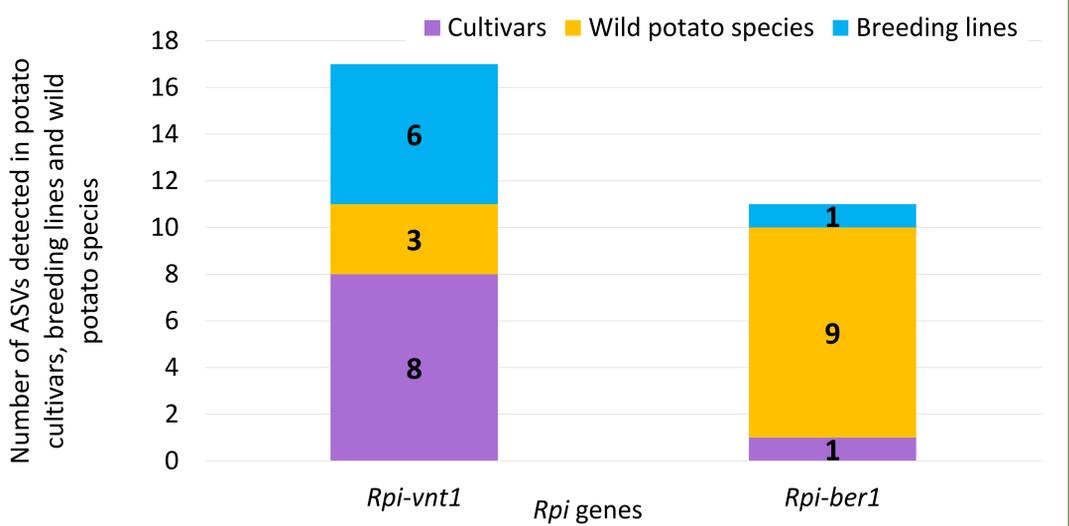
- 335 potato genotypes (183 potato cultivars grown in Poland and Norway, 98 breeding lines and 54 genotypes of wild potato species).
- Detection of *Rpi* genes fragments using 1-3 PCR primer pairs per gene.
- Amplification of entire coding regions of the *Rpi* genes and sequencing using the PacBio single-molecule real-time (SMRT) circular consensus sequencing (CCS) method.



**Figure 4** Phylogenetic tree of predicted *Rpi-ber1* (A) and *Rpi-vnt1* (B) homologous proteins. Protein sequences were aligned using the ClustalW program. The phylogenetic trees are visualized using FastTree2 tool used minimum-evolution subtree-pruning-regrafting (SPRs) and maximum-likelihood nearest-neighbor interchanges (NNIs). Reference proteins are marked in red.



**Figure 2** Number of amplicon sequence variants (ASVs) detected, using PacBio single-molecule real-time (SMRT) circular consensus sequencing (CCS) method



**Figure 3** Number of amplicon sequence variants (ASVs) detected in potato cultivars, wild potato species and breeding lines

## RESULTS & CONCLUSIONS:

- Using PCR primers, the presence of *Rpi-ber1* and *Rpi-vnt1* genes was detected in 47 and 78 potato genotypes, respectively.
- So far, full length *Rpi-ber1* and *Rpi-phu1* genes, were sequenced from 17 and 40 potato genotypes, respectively.
- For the *Rpi-vnt1*, 10 amplicon sequence variants (ASVs) were detected, of which 8 were new variants not previously detected in other studies (including 6 pseudogenes; Figure 2).
- For the *Rpi-ber1*, 9 new ASVs were detected (including 2 pseudogenes; Figure 2).
- 9 ASVs in wild potato species and 8 ASVs in potato cultivars were detected for *Rpi-ber1* and *Rpi-vnt1* genes, respectively (Figure 3).
- We confirmed the presence of the *Rpi-vnt1.1* and *Rpi-vnt1.3* in 14 potato genotypes.
- In 7 potato genotypes, 2 variants of *Rpi-vnt1* showed approx. 88% protein identity to the Tm-2-like from *S. verrucosum* and approx. 86% identity to *Rpi-vnt1* protein (Figure 4).
- Rpi-ber1* variants showed >99% identity to *Rpi-ber1.2* protein (ASV36) and >98% identity to *Rpi-ber1.3* (ASV21, 29, 37 and 39) and *Rpi-tub1.3* (ASV12 and 13) (Figure 4).
- Rpi-vnt1-like* pseudogenes are widespread (6 variants detected in more than half of the tested genotypes).
- Rpi-ber1-like* pseudogenes were detected in 11 potato genotypes.
- Rpi-ber1-like* genes were found in *S. punae*, *S. albicans*, *S. arrac-papa*, *S. sparsipilum* and *S. kurtzianum*. These wild potato species may be new sources of resistance to *P. infestans*.
- The AmpSeq strategy proved to be reliable and efficient and will allow us to obtain data on the diversity of genes crucial for the potato defence against *P. infestans*.

### REFERENCES:

Monino-Lopez D, et al. 2021. Allelic variants of the NLR protein *Rpi-ehc1* differentially recognize members of the *Phytophthora infestans* PexRD12/31 effector superfamily through the leucine-rich repeat domain. The Plant Journal 107.1: 182-197.  
Foster SJ, et al. 2009. *Rpi-vnt1.1*, a *Tm-2(2)* homolog from *Solanum venturii*, confers resistance to potato late blight. Mol Plant Microbe Interact. 22(5):589-600.

The research leading to these results has received funding from the Norwegian Financial Mechanism 2014-2021, project DivGene: UMO-2019/34/H/NZ9/00559

# Aggressiveness test of *Phytophthora infestans* isolates with different effector alleles

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**Background:** *Phytophthora infestans* (Mont) de Bary, is the causal agent of late blight and is one of the main constraints of potato and tomato production worldwide. *P. infestans* can infect leaves, stems, potato tubers, and tomato fruits. During infection the pathogen secretes effector proteins that suppress the defense system of the plant. Plants have evolved to recognize effectors, resulting in an evolutionary cycle of defense and counter-defense in plant-microbe interactions. The pathogen has a heterothallic mating system but reproduces primarily in a clonal manner where the clonal lineages have varying aggressiveness. The lineages are routinely defined by analysis of simple sequence repeat (SSR) markers.



Figure 1. Leaflets of potato cv. Craigs Royal inoculated with isolate of *P. infestans*: Six days post inoculation

**Aim:** Test aggressiveness of 19 *P. infestans* isolates representing four genotypes prevalent in Europe: EU13\_A2, EU34\_A1, EU37\_A2 and EU41\_A2.

**Materials:**

- Susceptible potato cultivars: Craigs Royal, Irys, Tarpan
- 19 *P. infestans* isolates listed in Table 1

**Methods:**

**Aggressiveness test**

- 5 leaflets × 3 cultivars × 19 isolates (Table 1) × 3 replications × 2 dates
- Measurement of latent period, lesion diameter, sporulation intensity

**Virulence test**

- 3 leaflets × 23 cultivars × 12 isolates (Table 2)

The *P. infestans* isolate was identified as virulent if symptoms (lesions, sporulation) were observed

**Sequencing of effector gene**

- 12 isolates × 2 *Avr-vnt1* primer pairs - were sequenced by Sanger method
- 4 isolates - data from sequencing effector by Illumina sequencing

**Statistics**

- ANOVA + Tukey's test (Statistica 13.0 software package)

Table 1. *P. infestans* isolates used for the tests and results of sequencing of effector gene.

Isolate	Origin	Years	Genotype	Effector <i>Avr-vnt1</i>
MP1976	Przeclaw/Poland	2021	EU13_A2	V1/V3
MP1943	Węgrzce/Poland	2016	EU13_A2	V1/V3
MP1960	Sulejów/Poland	2017	EU13_A2	V1/V3
MP1932	Węgrzce/Poland	2020	EU13_A2	V1/V2/V3
MP1995	Węgrzce/Poland	2021	EU13_A2	V1*
MP1934	Karzniczka/Poland	2020	EU41_A2	V1/V2/V3
MP1931	Karzniczka/Poland	2020	EU41_A2	V1/V2/V3
MP1935	Węgrzce/Poland	2020	EU41_A2	V1/V2/V3
MP1936	Węgrzce/Poland	2020	EU41_A2	V1/V2/V3
MP1933	Karzniczka/Poland	2020	EU41_A2	V1/V2/V3
MP2019	Zybizów/Poland	2020	EU41_A2	No data
MP1956	Węgrzce/Poland	2020	EU41_A2	No data
MP1942	Przeclaw/Poland	2020	EU37_A2	V1/V2
MP1940	Boguchwała/Poland	2020	EU37_A2	V1/V2
MP1938	Przeclaw/Poland	2020	EU37_A2	V1/V2
MP 940	Proszowice/Poland	2008	EU34_A1	V1*
MP 849	Boguchwała/Poland	2007	EU34_A1	No data
MP 938	Czapple Małe/Poland	2008	EU34_A1	V2*
MP 2076	Węgrzce/Poland	2018	EU34_A1	V1*

\* data from Illumina sequencing

Table 2. Results of virulence test. A-avirulent isolate, V-virulent isolate. Genotypes colour-coded as in Table 1.

Isolate	Black's differential set	Different cultivars								
		Bzura (homologue R2)	Sarpo Mira (Rpi-Smira1, Rpi-Smira2, R3a, R3b, R4+QTL)	Biogold (Rpi-abpt)	99-12/8 (Rpi-rmch1)	99-10/36 (Rpi-rzc1)	04-IX-21 (Rpi-phu1)	Kelly	Alouette (Rpi-vnt1.3; R3a; R3b)	Gardena (Rpi-phu1)
MP1976	1.2.3.4.5.6.7.8.10.11	V	V	V	V	A	A	V	V	V
MP1934	1.2.3.4.5.6.7.8.11	V	V	V	V	A	A	V	A	V
MP1960	1.2.3.4.5.6.7.8.9.10.11	V	V	V	V	V	A	V	V	V
MP1932	1.2.3.4.5.6.7.8.10.11	V	A	V	V	A	A	A	V	V
MP1995	1.4.6.7.10.11	A	A	A	A	A	A	A	A	A
MP1934	1.2.3.4.5.6.7.8.11	V	V	V	V	A	A	V	A	V
MP1931	1.2.3.4.5.6.7.8.10.11	V	A	V	A	A	A	V	V	A
MP1935	1.2.3.4.5.6.7.8.10.11	V	V	V	V	V	A	V	A	A
MP1936	1.2.3.4.5.6.7.8.10.11	V	A	V	V	A	A	V	V	A
MP1933	1.2.3.4.5.6.7.8.10.11	V	V	V	A	A	A	V	V	A
MP2019	1.2.3.4.5.6.7.8.10.11	V	A	V	V	A	A	V	A	V
MP1956	1.2.3.4.6.7.8.10.11	V	A	A	V	A	A	V	A	V
MP1942	1.2.3.4.5.6.7.8.10.11	V	V	V	A	A	A	V	A	A
MP1940	1.2.3.4.5.6.7.8.10.11	V	A	V	A	A	A	V	A	A
MP1938	1.2.3.4.5.6.7.10.11	V	A	V	A	A	A	A	A	A
MP940	1.3.4.6.7.11	A	A	A	V	A	A	A	A	A
MP849	1.2.3.4.5.6.7.10.11	V	A	V	V	A	A	A	A	A
MP938	1.3.4.6.7.8.10.11	V	V	V	V	A	A	A	A	A
MP2076	1.4.6.7.10.11	V	A	A	V	A	A	A	A	A

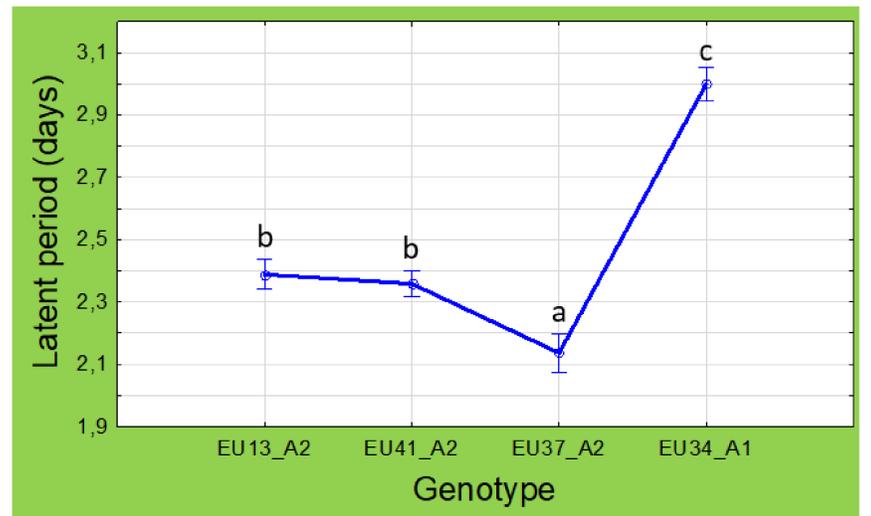
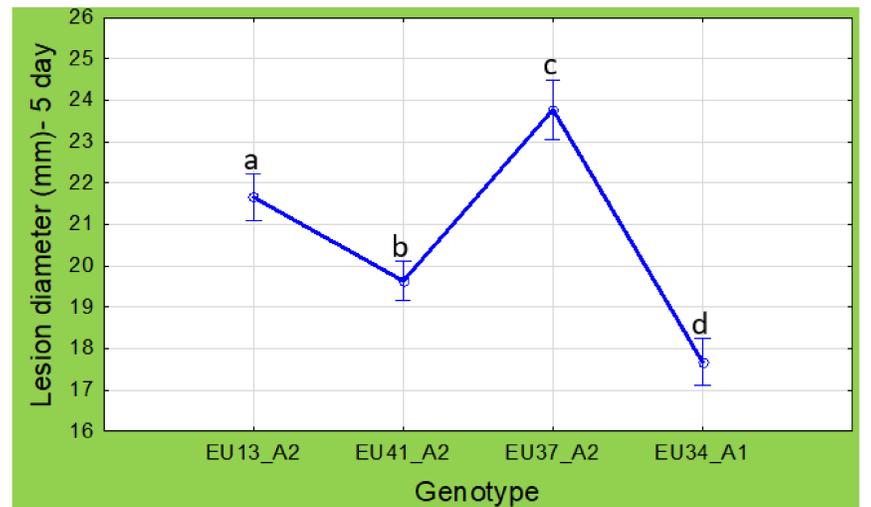
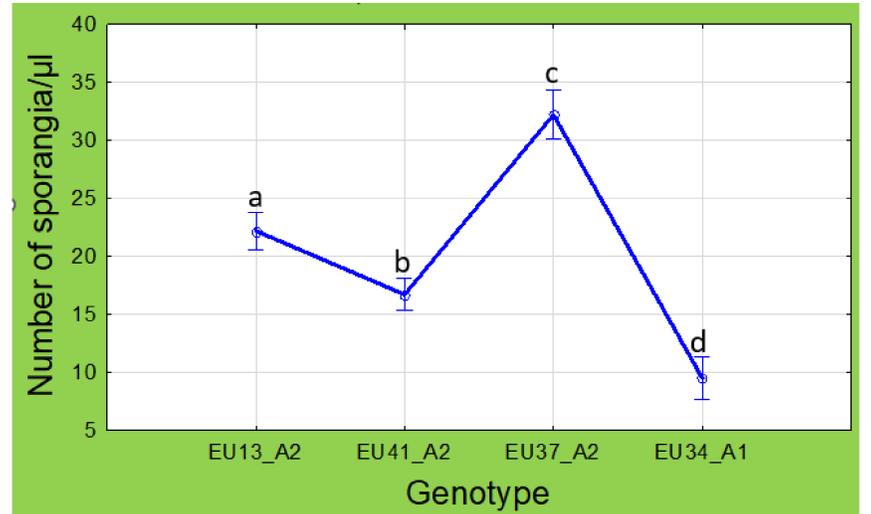


Figure 2. Aggressiveness of four *P. infestans* genotypes (EU\_13A2, EU34\_A1, EU37\_A2 and EU41\_A2) based on lesion diameter, latent period and number of sporangia produced by isolates from each genotype. Vertical bars represent 0.95 confidence intervals. The letters indicate statistically different groups, created based on ANOVA + Tukey's test.

a) Latent period (days) (Effect:  $F(3.163)=172.24$ ,  $p=0.000$ )

b) Number of sporangia/ $\mu$ l (Effect:  $F(3.953)=92.663$ ,  $p=0.000$ )

c) Lesion diameter (mm)-5 day (Effect:  $F(3.172)=66.498$ ,  $p=0.000$ )

**Results:**

**Aggressiveness (Figure 2)**

- Isolates of EU34\_A1 produced smallest amount of spores, had the longest latent period and caused smallest lesions.
  - Isolates of EU37\_A2 produced biggest spores, had the shortest latent period and caused biggest lesions.
  - There were no statistically significant differences in the latent period between isolates of EU13\_A2 and isolates of EU41\_A2 genotypes.
- Results of *Avr-vnt1* sequencing are shown in Table 1. Diversity of *Avr-vnt1* effector was noted within EU13\_A2 and EU34\_A1 genotypes.
- Isolates differed in virulence between and within SSR genotypes.

**Conclusion and future plans:**

- The EU37\_A2 genotype of *P. infestans* was the most aggressive.
- Based on ongoing sequencing of multiple effector genes from a large number of *P. infestans* isolates from Poland and Norway, we will analyse how differences in effector repertoire affect aggressiveness.
- More isolates will be tested for aggressiveness to ensure better representation of tested *P. infestans* genotypes.

The research leading to these results has received funding from the Norwegian Financial Mechanism 2014-2021, project DivGene: UMO- 2019/34/H/NZ9/00559





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# The *R2* gene is still efficient in bringing resistance to some *P. infestans* strains

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## Key messages

Using different types of phenotyping and plant materials, we showed that the presence of *R2* gene was associated with the reduction of rAUDPC, a significant decrease of the sporulation and a significant decrease of the lesion extension rate.

Although *R2* has been defeated years ago (Pilet et al 2005), its interest in resistance breeding is shown.

We think the combination of defeated and undefeated R genes in new varieties is an interesting option to increase the durability of resistance. Breeding using molecular markers is the only option to achieve this goal.

## Results in field experiments

B2 mapping family (see M&M part) was evaluated under field conditions between 2004 and 2007 (Fig 1). A high level of resistance to late blight was shown, however a strong year effect was also detected. We assumed a complex pattern of resistance factors (Marhadour et al 2013).

*R2* explained a significant part of the resistance observed during this first period of phenotyping (Table 1).

More recently (2017 to 2019), a subset of the population was reevaluated under field conditions to check the stability of the resistance factors characterized earlier. The interest of *R2* was confirmed with these new data as *R2* was associated with a significant reduction of rAUDPC (Fig 2).



Marker	rAUDPC 2004	rAUDPC 2005	rAUDPC 2006	rAUDPC 2007
<i>R2</i>	38,1%	11,7%	27,5%	43,7%
SNP marker	8,5E-17	5,0E-09	1,4E-20	9,2E-36

Table 1: *R*<sup>2</sup> and p-value of the variance analysis performed using the SNP marker for *R2* and the rAUDPC (relative area under the disease progress curve) measured each year between 2004 and 2005 under field conditions on the whole B2 population n=275 (see Marhadour et al 2013 for details about the phenotyping).

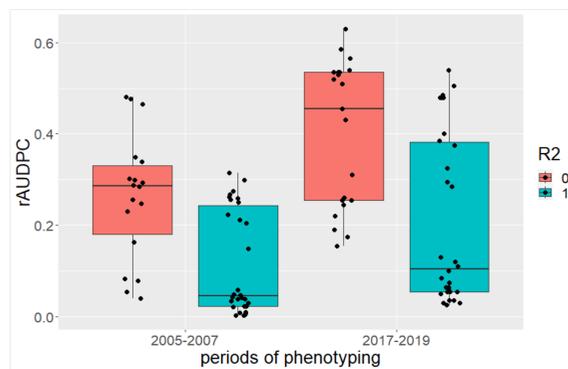


Fig 2 rAUDPC (relative area under the disease progress curve) values obtained on a subset of 15 genotypes of the B2 mapping family depending on the period of phenotyping (2005-2007 vs 2017-2019) and on the presence (1)/absence (0) of *R2*. Each genotype was repeated 3 times during the first period and twice during the second one, all the data are figured.

Fig 1: Fields experiments were performed under natural conditions of contaminations. One row of infector was planted every two rows of experimental genotypes. Disease progression was evaluated weekly using a visual scale of foliage destruction.

## Results in detached leaf assay (DLA)

As compared to field tests, DLA offers the possibility to control and calibrate the inoculum. DLA are performed on a short period (max 7 days, Fig 2) on survival organs.

Presence of the *R2* gene was associated with a reduction of the lesion extension rate evaluated using a visual necrosis scale and a reduction of the observed sporulation (Tables 2&3).



Fig 2: 17 genotypes of the B2 mapping population were inoculated using 2 strains of *P. infestans*. 4 leaflets were inoculated per genotypes and strains. The experiments were repeated twice consecutively. Scoring was performed 5 and 7 dpi using a visual necrosis scale and a binocular loupe for sporulation.

Necrosis scale	<i>R2</i> absent	<i>R2</i> present
0	5	7
1	25	32
2	37	85
3	91	40
4	90	25

Table 2: #leaflets observed in each class of the scale necrosis depending on presence of the *R2* gene. 3 series of tests and the 2 strains were grouped representing 30 genotypes ( $\chi^2=69,963$ ;  $ddl=3$ ;  $p\text{-value}=4,3 \cdot 10^{-15}$ , classes 0 and 1 were grouped for the  $\chi^2$  test).

Table 3: #leaflets observed depending on the observation of sporulation and the presence of the *R2* gene. 3 series of tests and the 2 strains were grouped representing 30 genotypes ( $\chi^2=224,77$ ;  $ddl=1$ ;  $p\text{-value}=2,2 \cdot 10^{-16}$ ).

	No sporulation observed	Sporulation observed
<i>R2</i> absent	18	230
<i>R2</i> present	157	45

## Material and Methods

Plant material: subsets of a 4x mapping population named B2 originating from a controlled cross INRA89T123. 3 x BERBER (Marhadour et al 2013) multiplied by Comité Nord/Sipre then maintained in BRC BrACysol and multiplied by UMR Igepp.

Phenotyping tests: 1) field tests in natural conditions of contaminations (oceanic climatic conditions, (Marhadour et al 2013)), 2) stem tests in greenhouse, adapted from Danan et al (2009) 3) detached leaf assays, adapted from Euroblight protocol.

Genotyping : presence of *R2* gene evaluated using the SNP marker developed by Meade et al 2020.

*Phytophthora infestans* strains: 20P50.25 (39A1) provided by R. Mabon, R. Corbière and M. Guibert (UMR Igepp) and FR20.104 (37A2) provided by P. Dewaegeneire (inov3PT)

## Results in stem test

Stem test allows to control the strains of *P. infestans*, to precisely evaluate stem necrosis (Fig 3 & 4) on a relatively long period (4 weeks) using whole plants (as compared to DLA).

Presence of the *R2* gene is associated with a significant reduction of the stem necrosis (Fig 4). The necrosis progression rate was reduced by 3,9 mm/day during the whole period ( $p < 0,0001$ ) using both strains.

Results are in line with those obtained in a panel of 288 breeding lines and varieties using 2 other strains of *P. infestans* (Marhadour et al 2022).



Fig 3: Stem test allows to measure length necrosis after inoculation at the apex (left). In the centre, resistance is shown by a short necrosis (30 dpi) whereas on the right, susceptibility is expressed (30 dpi).

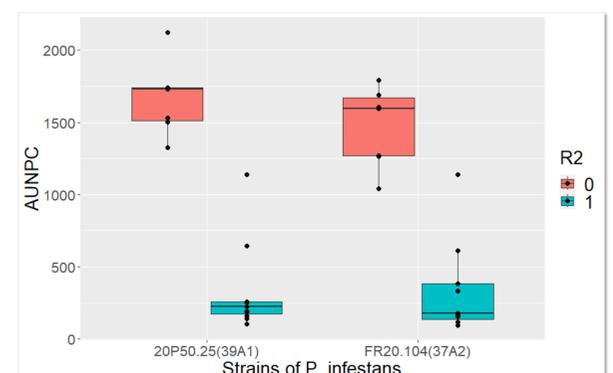


Fig 4: AUNPC (Area under the necrosis progress curve) obtained using 2 strains of *P. infestans* on 15 genotypes of the B2 mapping population depending of the presence (1) /absence (0) of the *R2* gene. Plant genotypes were repeated 4 times for each strain.

# Susceptibility to potato late blight (*Phytophthora infestans*) of *Solanum tuberosum* Chilotanum group, on detach leaf assay and field conditions

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## INTRODUCTION

Potato late blight is the most important disease of potato crop, causing production losses when environmental conditions are favorable. Varietal resistance is an important factor in the integrated management of this disease. The native potato of Chile are *Solanum tuberosum* Chilotanum group, it is of wide diversity and relevant as a genetic heritage [1].



## OBJECTIVE

The objective of this work was to evaluate the susceptibility of 10 native potato varieties used by farmers in the Chiloe archipelago, in Chile.

## METHODOLOGY

Experimental plots were established with these varieties under field conditions in 2019 to 2021 in Chiloe and Osorno, Los Lagos Region, in a randomized complete block design with 4 repetitions (Photo 1). The percentage of foliage damaged by late blight was determined and the AUDPC and rAUDPC were calculated. Additionally, native varieties were grown in greenhouse pots, leaf discs were cut from plant leaflets, placed in Petri dishes, inoculated with 10 ul of a concentration of  $2 \times 10^6$  zoospores/ml of a local isolation of *P. infestans* genotype EU-2A1 and incubated at 16 °C [2]. The necrosis and sporulation on each disc was evaluated between 24 hrs and 90 hrs post inoculation, every 6 hrs (Photo 2).



Photo 1: Aerial view of the varietal resistance evaluation plots, 2020-21 season. The difference in foliage damage is observed in the rows with susceptible plants (brown foliage) versus the most resistant to late blight (green foliage).

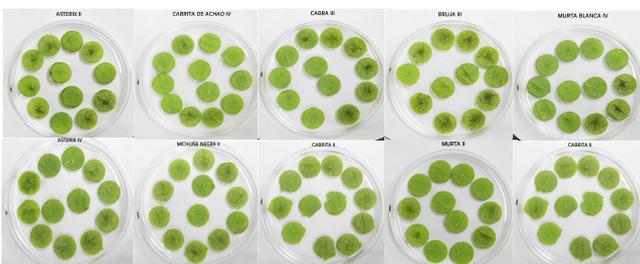


Photo 2: In vitro evaluation test for susceptibility to late blight on leaf discs. Varieties with a greater amount of sporulation and necrosis on the discs are more susceptible.

## RESULTS

The results of damage under field conditions show significant differences between them, with RAUDPC of 0.13 and 0.65. The most susceptible variety was Cabrita and the most resistant was Murta (Figure 1 and Table 1). In addition, the development of necrosis and sporulation on the leaf discs shows that Cabrita developed necrosis at 42 hours and sporulation at 54 hrs., with 80% sporulation at 90 hrs, while Murta begins with necrosis at 42 hrs and sporulation at 66 hrs with 21.6% at 90 hrs (Figure 2).

Variety	RAUDPC	Susceptibility index*
Viscocha MIP	0,45 bc	7,6
Bruja MIP	0,45 bc	7,6
Bruja	0,43 c	7,3
Cabra	0,38 c	6,5
Murta	0,13 e	2,2
Cabrita	0,57 a	9,0
Murta ojuda	0,53 ab	8,9
Cabrita de Achao	0,30 d	5,1
Michuñe negra	0,20 d	3,4
Viscocha	0,43 c	7,3
Variation coef.	11,93	
F value	21,57	*Index between 1 to 9, where 1 is very resistant and 9 is very susceptible
Probability (P<0.05)	<0,0001	

Table 1. RAUDPC for Late blight on native potato varieties and its susceptibility index.

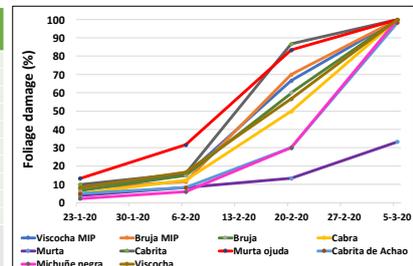


Figure 1. Late blight damage on foliage of potato plants of different native varieties under Chiloe Island conditions, Chile, 2019-20 season.

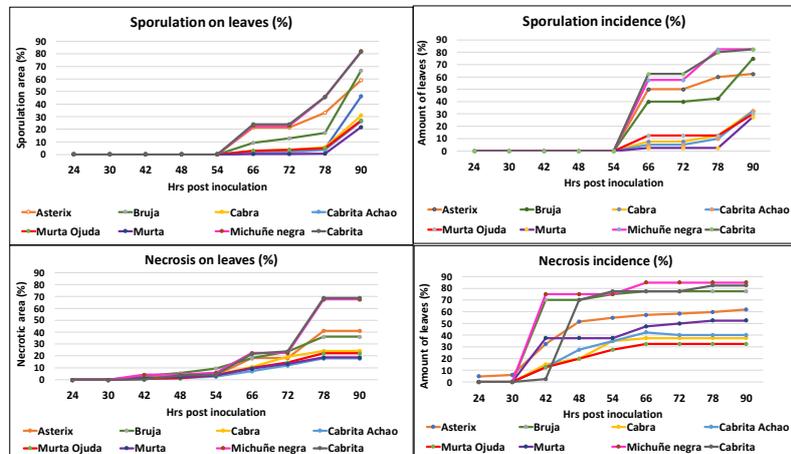


Figure 2. Severity and incidence of necrosis and sporulation in native potato leaflets inoculated with *Phytophthora infestans* under in vitro conditions.

## Comments

- There was a high correlation between field experiment and in vitro assay.
- The native varieties show high susceptibility to late blight, under both methods, being Murta the most resistant.
- The phytopathological characterization of these varieties allows us to know the genetic potential they present and their diversity.

## References

- [1] Solano, J., Mathias, M., Esnault, F., and Brabant, P. 2013. Genetic diversity among native varieties and commercial cultivars of *Solanum tuberosum* ssp. *tuberosum* L. present in Chile. Electronic Journal of Biotechnology, 16(6), 8-8. <http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/1474>
- [2] Solano, J., Acuña, I., Chauvin, J.E., and Brabant, P. 2016. In-Vitro Evaluation of Resistance to Late Blight (*Phytophthora infestans* Mont. De Bary.) in *Solanum* Accessions Native to Chile, by Inoculation of Detached Leaflets. American Journal of Plant Sciences Vol.7 No.3,581-589.