

Differential proteome analysis, in potato tubers wound-inoculated with bacteria *Dickeya solani*, in selected cultivars with different level of resistance.



Lebecka R<sup>1</sup>,  
Dębski J<sup>2</sup>., Kistowski M<sup>2</sup>, Murawska Z<sup>1</sup>, Szajko K<sup>1</sup>, Marczewski W<sup>1</sup>

<sup>1</sup> Plant Breeding and Acclimatization Institute – National Research Institute,  
Młochów Research Center, Platanowa Str. 19, 05-831 Młochów, Poland

<sup>2</sup> Institute of Biochemistry and Biophysics, Polish Academy of Sciences,  
Mass Spectrometry Laboratory



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# Outline

- Introduction,
- Materials and methods,
- Results



# Padova Botanical Garden

Potato was grown here in 1591

(the first complete list of gardens plants by Giacomo Cortuso had 1170 entries)



By A. Tosini - G Agostini "dis. in. pictura" - lithographed by "Kiev"? in Venice

# Introduction

Blackleg



Soft rot





# Introduction

The large economic losses:

- the downgrading of seed potato plantations,
- the decrease in potato yields,
- loss of yield during storage.

*Pectobacterium atrosepticum*

*P. carotovorum* subsp. *carotovorum*

*P. parmentieri* (formerly *wasabiae*)

*P. carotovorum* subsp. *brasiliense*

*P. polaris*

*Dickeya solani*

*D. dianthicola*

*D. dadantii*

*D. zeae*



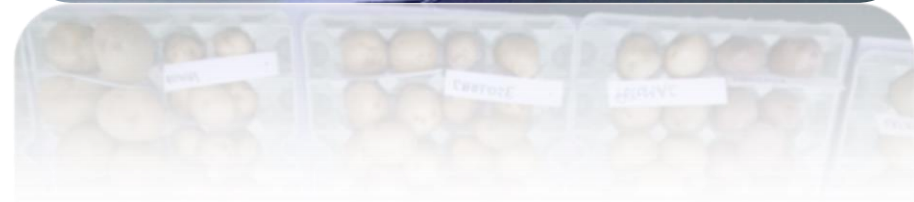
# Plant material

## 5 potato cultivars:

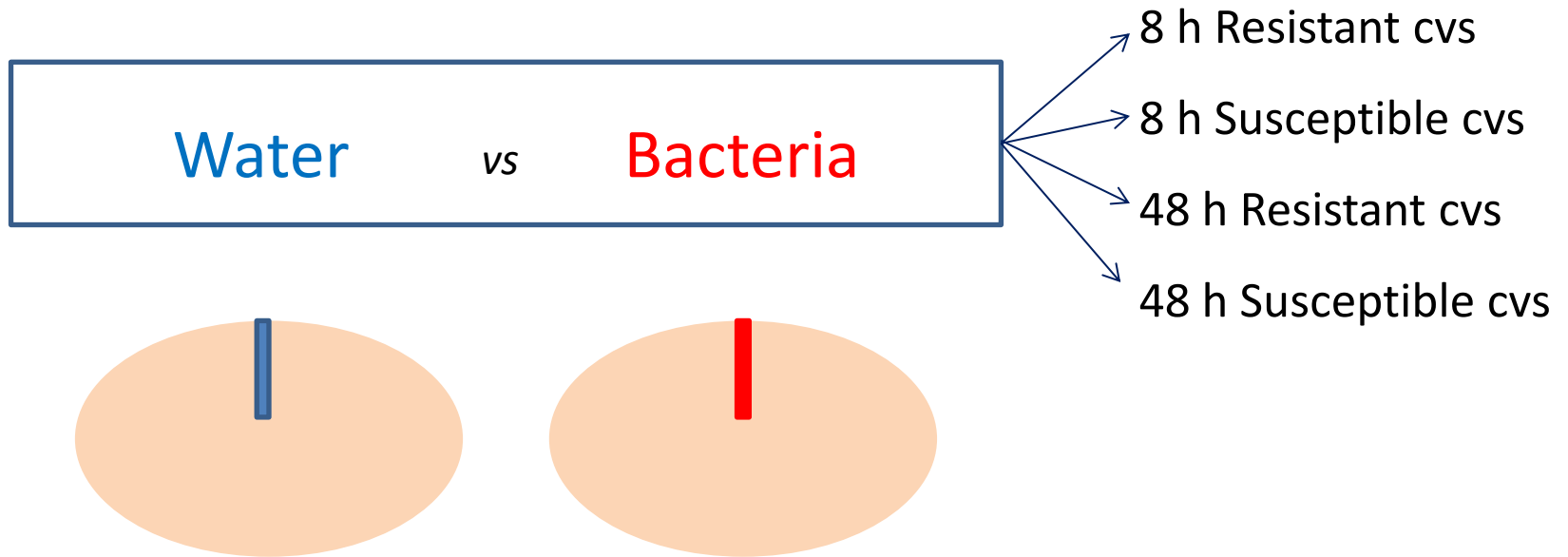
- 3 originated from cv Katahdin,
- cv Katahdin,
- susceptible cv Irys.

2 diploid clones:

- DG 00-270,
- DG 08-305

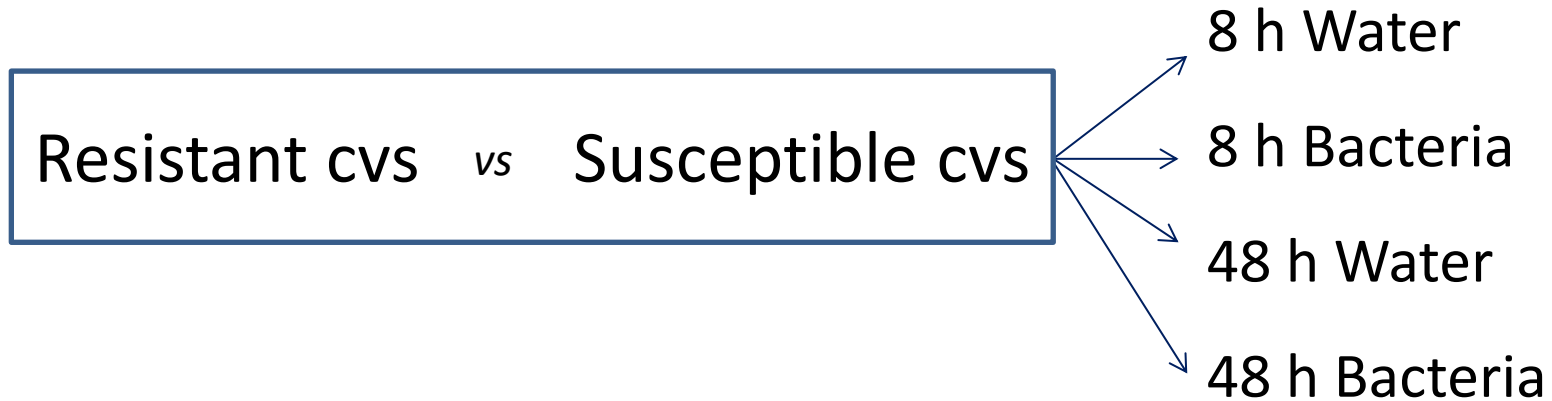


# Objectives



- ❑ Differences in proteomes of potato tubers, after wound-inoculation with bacteria in comparison with mock-inoculation

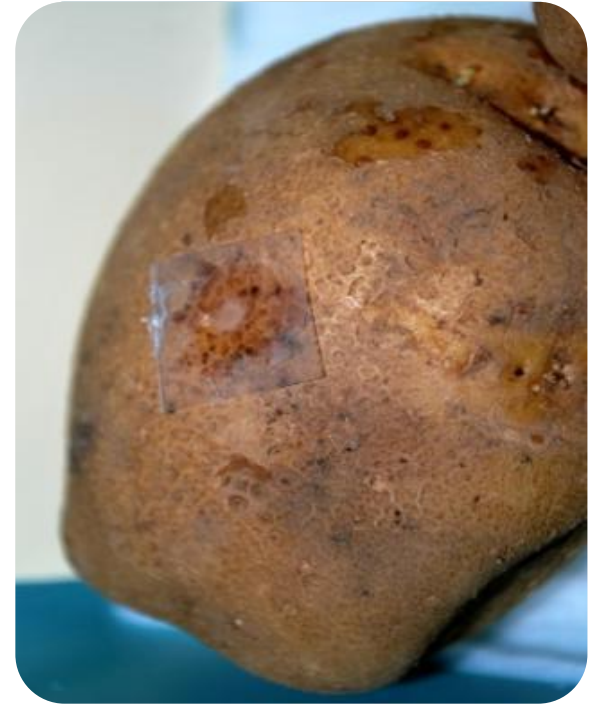
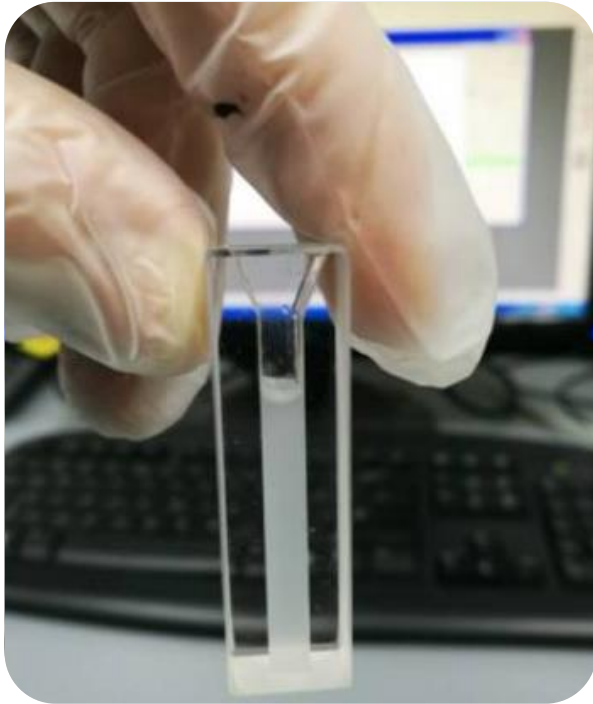
# Objectives



- ❑ Differences in proteomes of potato tubers between two groups of potato cultivars



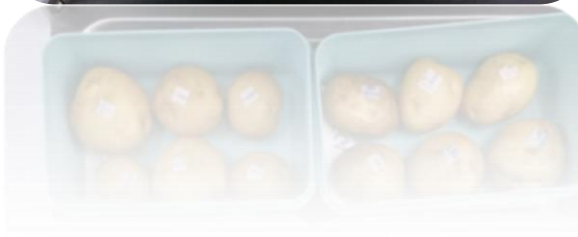
# Methods: wound-induced potato tubers



10  $\mu$ L of inoculum  $10^9$  CFU/mL

Methods: incubation at T 26°C for three days

58 tubers of each cultivar (five exp. in three years)



Resistant

Humalda

Bea



Susceptible

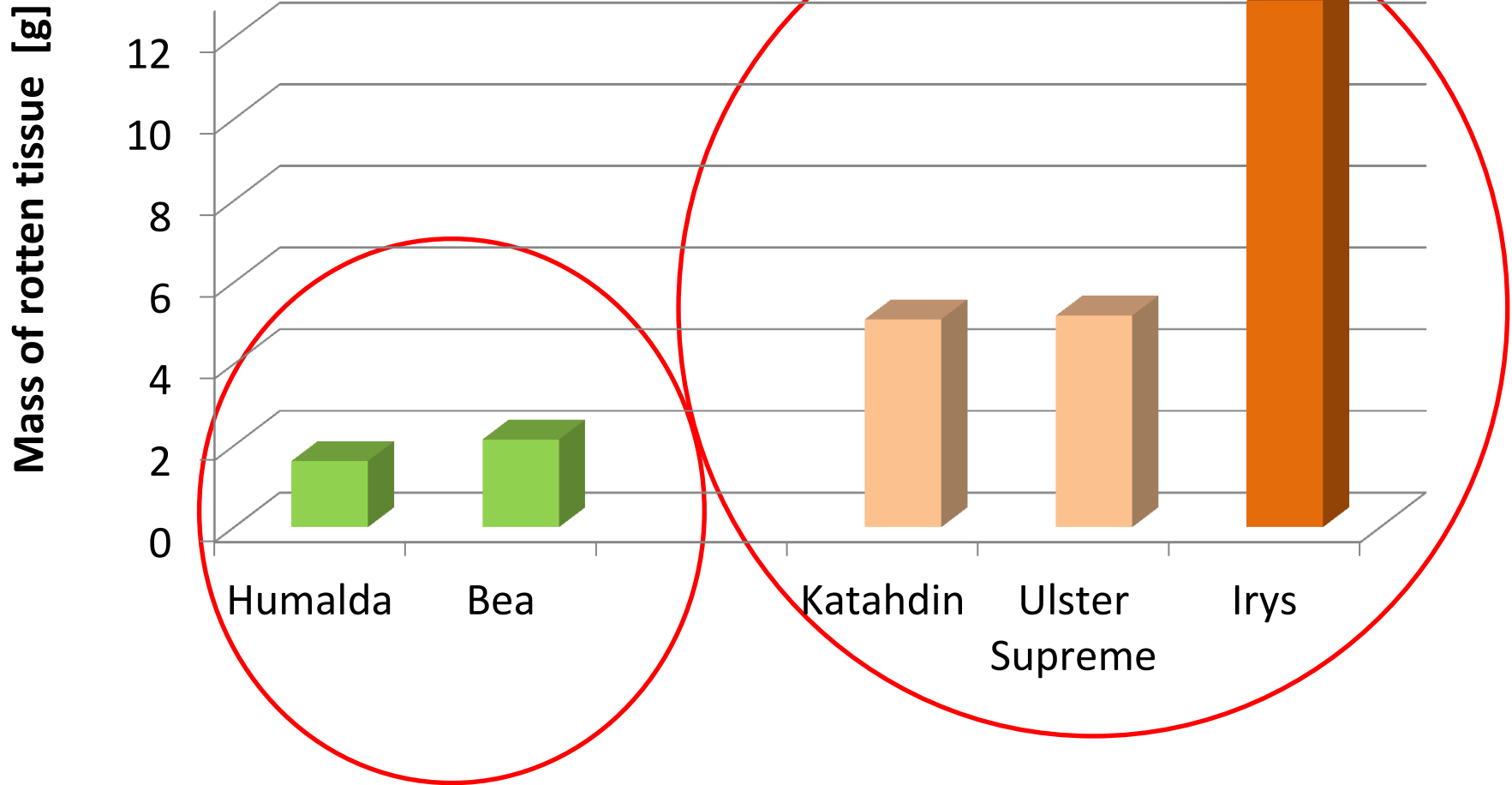
Katahdin

Ulster Supreme

Irys



# Three-year-mean of tuber rot after inoculation of potato cvs with bacteria *Dickeya solani*





# The highest differences in reaction

- Humalda



- Irys



Resistance of potato tubers to bacteria causing soft rot –  
complex polygenic inheritance



# Experimental design

```
graph TD; A[Experimental design] --> B["8 h<br/>(2017)"]; A --> C["48 h<br/>(2016)"]; B --> D[March]; B --> E[April]; C --> F[March]; C --> G[April]; D --> H[4 W]; D --> I[4 B]; E --> J[4 W]; E --> K[4 B]; F --> L[2 W]; F --> M[2 B]; G --> N[4 W]; G --> O[4 B];
```

8 h  
(2017)

48 h  
(2016)

March

April

March

April

4 W

4 B

4 W

4 B

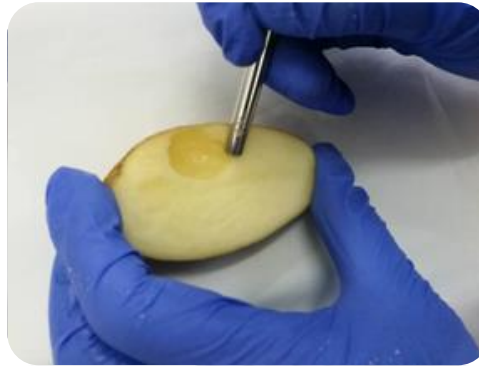
2 W

2 B

4 W

4 B

# Collection of samples



## Liofilization



## Identification of proteins by LC-MS/MS

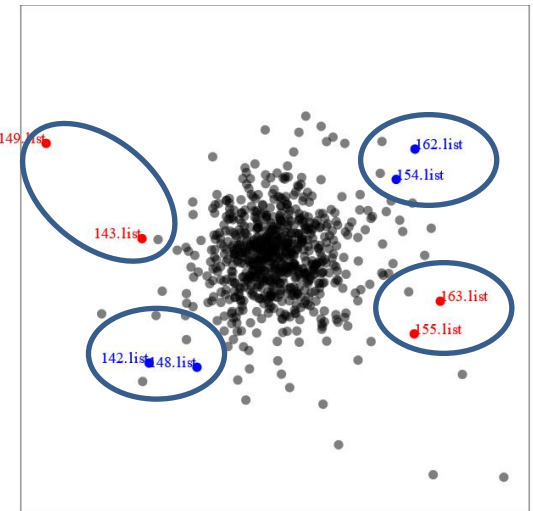
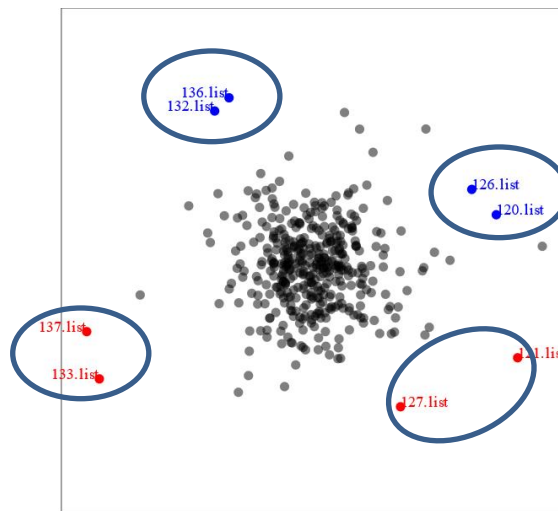
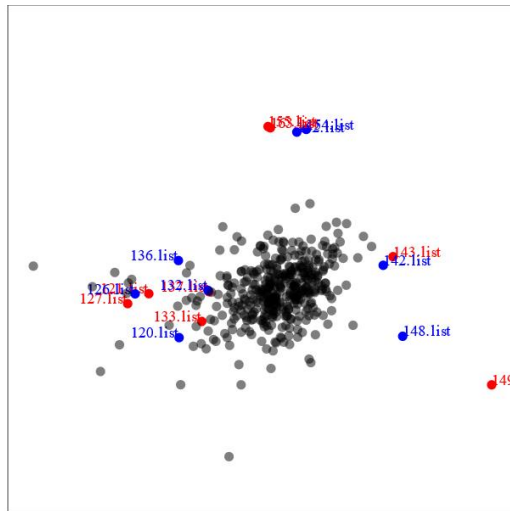
(Nano-Acquity LC system and Q-Exactive mass spectrometer)

# Humalda 8h

Two dates

21.03.2017

26.04.2017

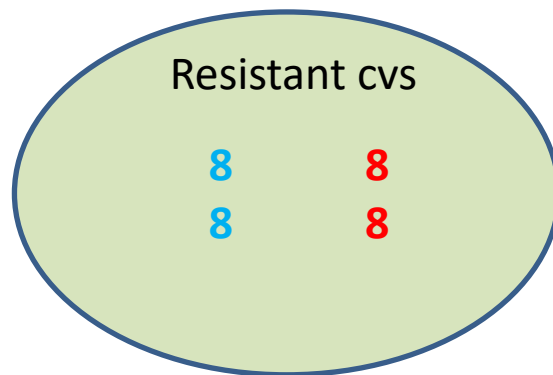


# Comparison Water - Bacteria

Cultivar	8 h		48 h	
	Water	Dsol	Water	Dsol
Bea	8	8	2	2
Humalda	8	8	6	6
Katahdin	8	8	6	6
Ulster Supreme	8	8	6	6
Irys	8	8	6	6

# Comparison Water - Bacteria

Cultivar	8 h		48 h	
	Water	Dsol	Water	Dsol
Bea			2	2
Humalda			6	6
Katahdin	8	8	6	6
Ulster Supreme	8	8	6	6
Irys	8	8	6	6

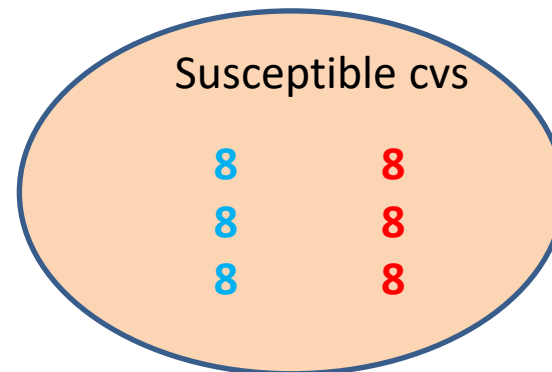
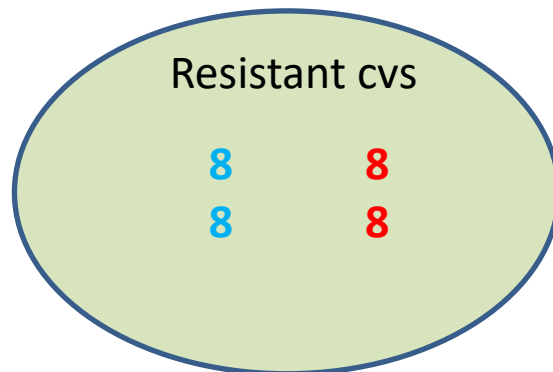


**No significant differences 8 h post inoculation**



# Comparison Water - Bacteria

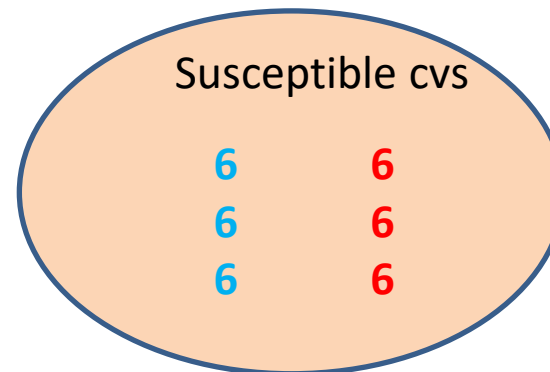
Cultivar	8 h		48 h	
	Water	Dsol	Water	Dsol
Bea			2	2
Humalda			6	6
Katahdin			6	6
Ulster Supreme			6	6
Irys			6	6



**No significant differences 8 h post inoculation**

# Comparison Water - Bacteria

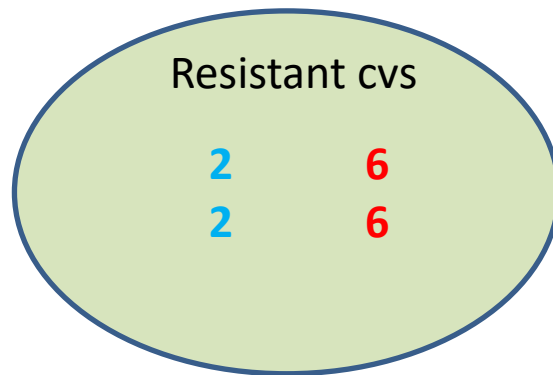
Cultivar	8 h		48 h	
	Water	Dsol	Water	Dsol
Bea	8	8	2	2
Humalda	8	8	6	6
Katahdin	8	8		
Ulster Supreme	8	8		
Irys	8	8		



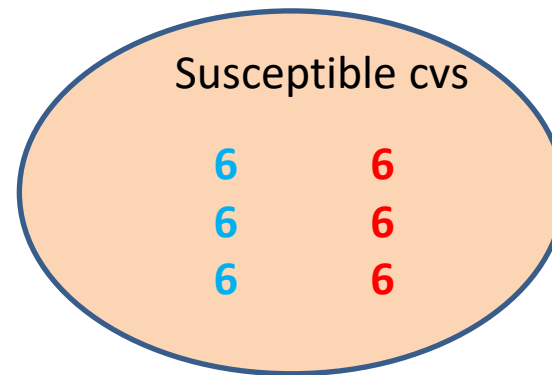
**No significant differences  
48 h post inoculation**

# Comparison Water - Bacteria

Cultivar	8 h		48 h	
	Water	Dsol	Water	Dsol
Bea	8	8		
Humalda	8	8		
Katahdin	8	8		
Ulster Supreme	8	8		
Irys	8	8		



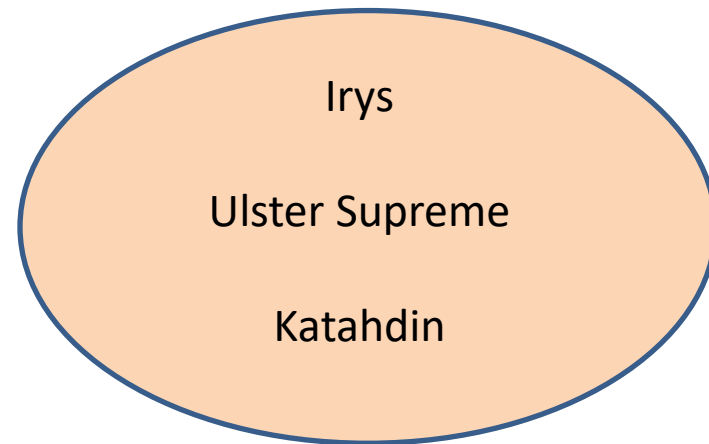
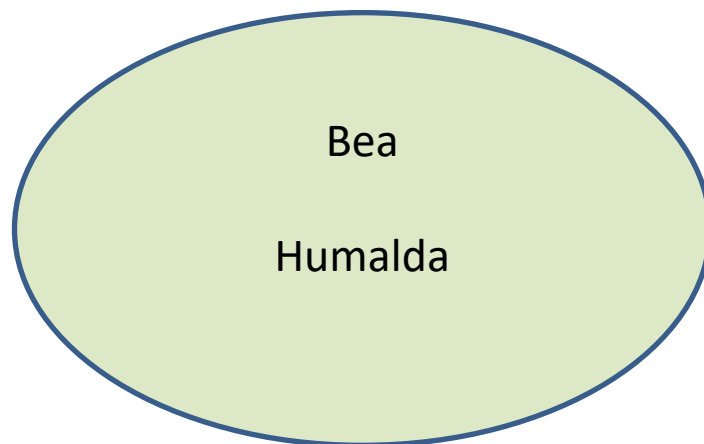
**Peroxidase**  
fold change 2.33 (P=0.0025)



**No significant differences**  
48 h post inoculation

# Comparison **Resistant** – **Susceptible** cultivars

Cultivar	8 h		48 h	
	Water	Dsol	Water	Dsol
Bea	8	8	2	2
Humalda	8	8	6	6
Katahdin	8	8	6	6
Ulster Supreme	8	8	6	6
Irys	8	8	6	6



# Higher expression of proteins in resistant potato cultivars

Category	Protein	8h		48h	
		Bacteria	Water	Bacteria	Water
Stress and defence	Proteinase inhibitor type-2	***	*	**	***
	Probable inactive patatin-03-Kuras 1	***	**	**	***
	Thiamine thiazole synthase	**	NS		
	Aspartic protease inhibitor 7			*	NS
Translation, protein processing and degradation	Patatin-05; Patatin-03; Patatin-07	***	**		
	Endopeptidase inhibitor activity (M1AMY2)			***	**
	Chymotrypsin inhibitor I, A, B and C subunits	*	*	NS	*
	Proteinase inhibitor PTI	***	NS		
	Serine protease inhibitor	NS	*	*	**
	Aspartic protease inhibitor 3; 4; 5; 6; 8; 10			*	NS
	Similar to endoplasmin			*	NS
Photosynthesis, primary metabolism	Similar to polyphenol oxidase F, catechol oxidase B			**	NS

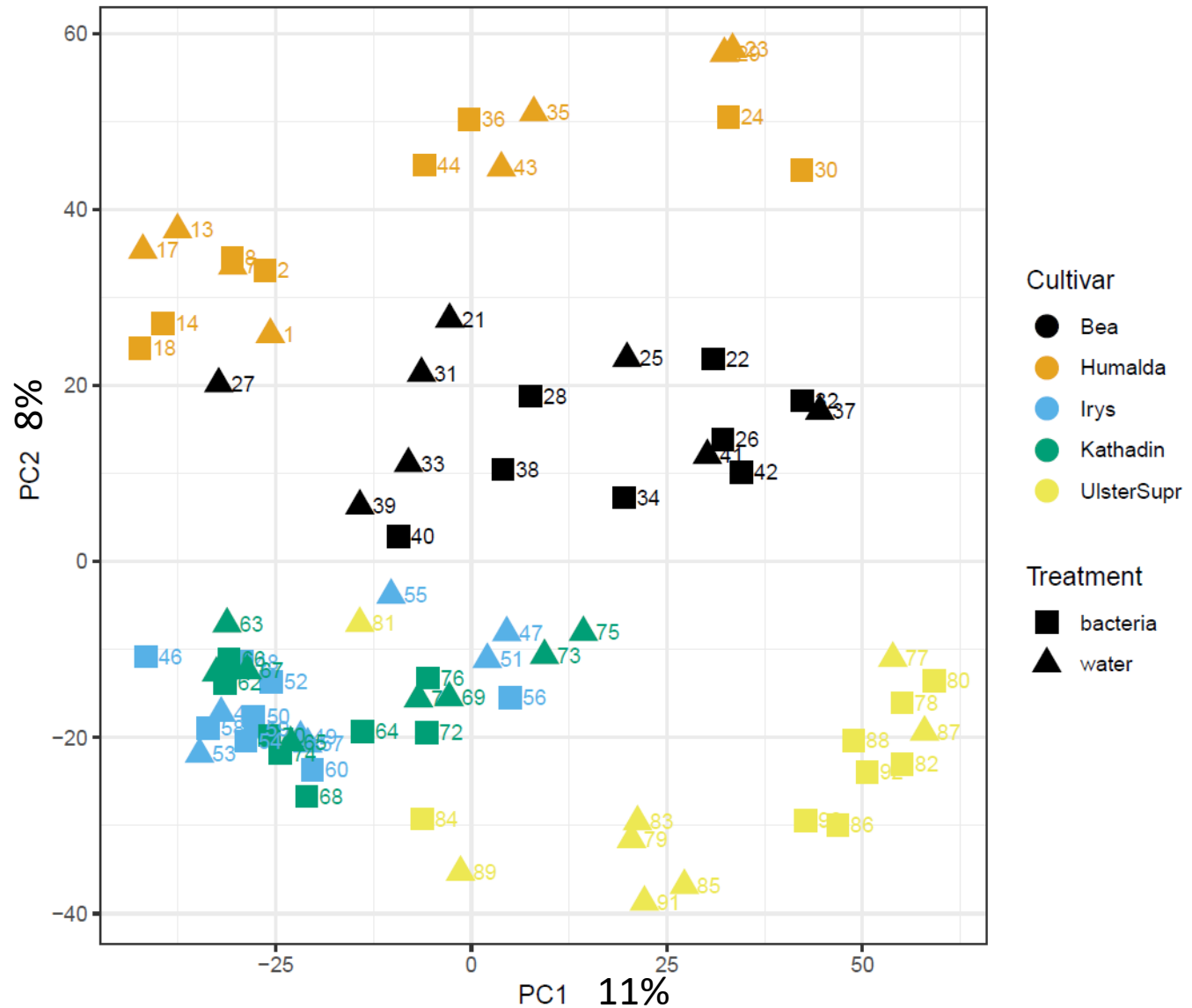
\*  $P \leq 0.1$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$



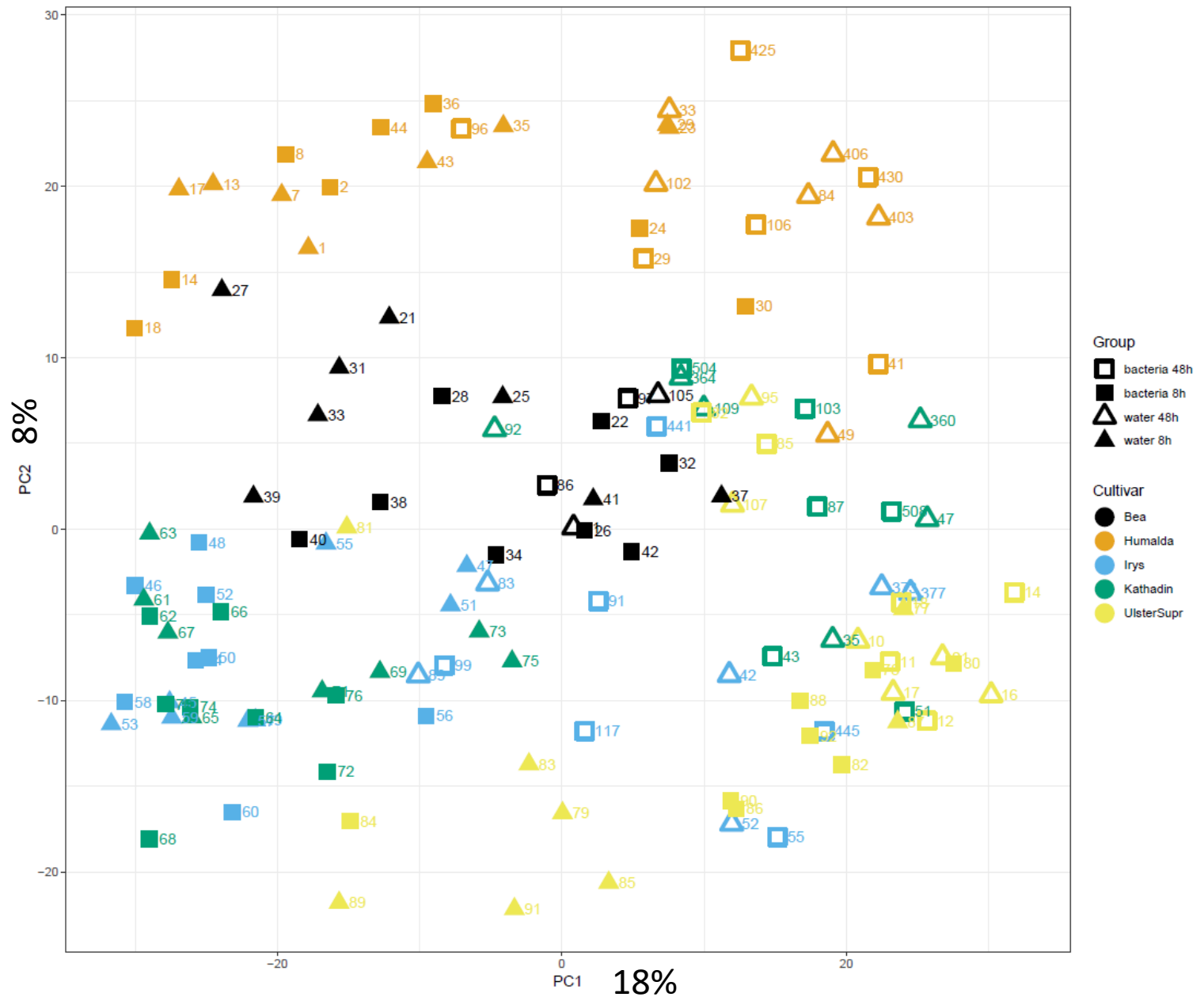
# Higher expression of proteins in susceptible potato cultivars 48 h post inoculation

Category	Protein	Bacteria	Water
Stress and defence	Patatin (16; group A2; 01; -1-Kuras 2)	***	NS
	14-3-3 protein	**	NS
	Similar to superoxide dismutase	NS	*
	Cysteine proteinase inhibitor1; 5;3	NS	*
	Chaperone protein ClpB3	NS	*
Amino acids metabolism	Similar to LL-diaminopimelate aminotransferase	*	NS
Photosynthesis, primary metabolism	Sucrose synthase	*	NS
	Similar to: induced stolen tip protein TUB8	***	NS
	4-alpha glucanotransferase	**	NS
	Similar to alpha glucan water dikinase	***	NS
	Similar to pyruvate decarboxylase 2	NS	**
Endomembrane transport	Similar to plasma membrane -associated cation-binding protein 1	*	NS

# Principal Component Analysis



# Principal Component Analysis 8 and 48 hours



8 h

# Thiamine thiazole synthase

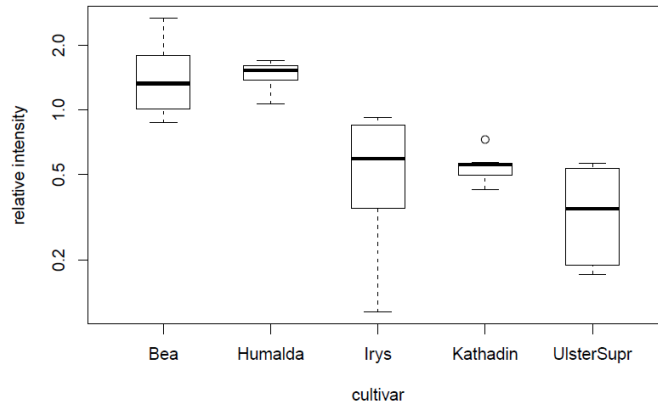
R : S

Water 2.28 ns

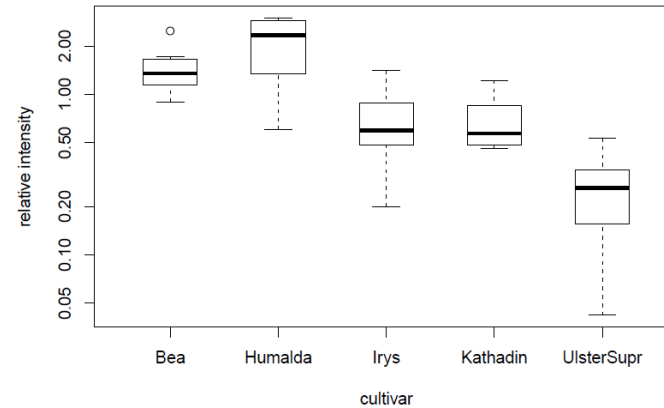
R : S

Bacteria 4.60\*\*

M1BNZ3: Thiamine thiazole synthase, chloroplastic (water, 8h)

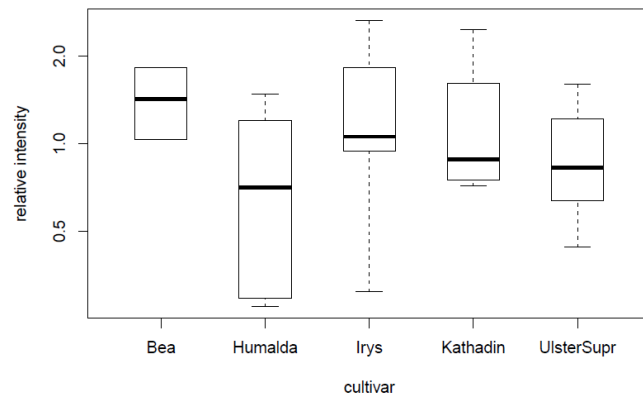


M1BNZ3: Thiamine thiazole synthase, chloroplastic (bacteria, 8h)

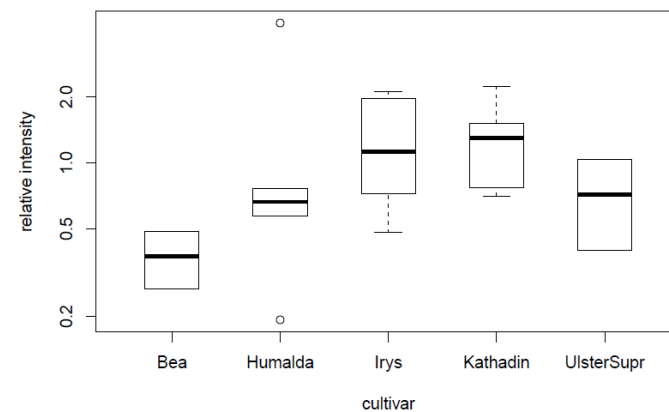


48 h

M1BNZ3: Thiamine thiazole synthase, chloroplastic (water, 48h)



M1BNZ3: Thiamine thiazole synthase, chloroplastic (bacteria, 48h)



Stress and defense

8 h

## Proteinase inhibitor PTI

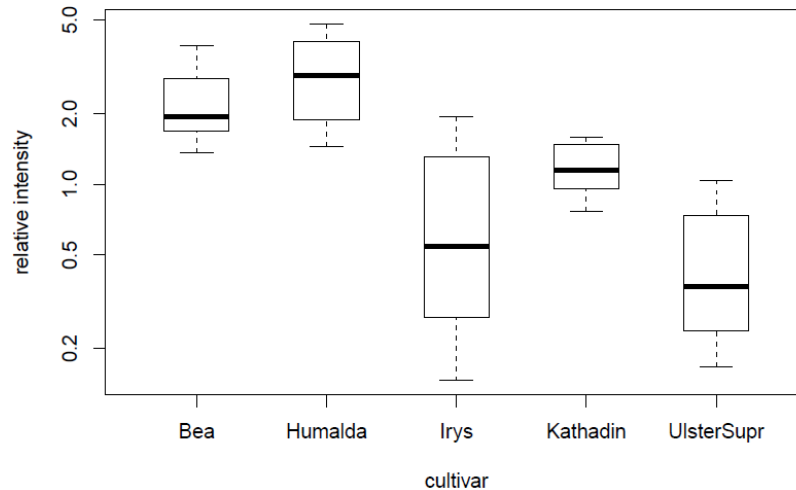
R : S

Water 2.72 ns

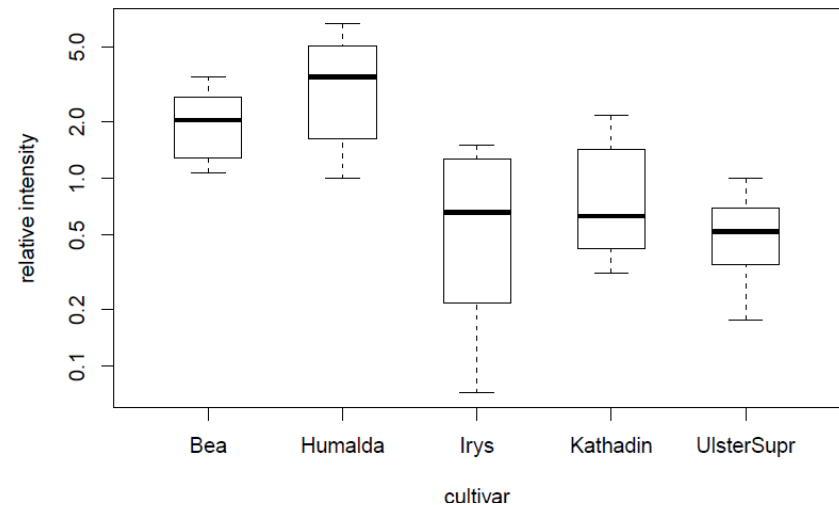
R : S

Bacteria 4.07\*\*\*

P01079: Proteinase inhibitor PTI (water, 8h)



P01079: Proteinase inhibitor PTI (bacteria, 8h)



Translation, protein processing, and protein degradation

# Higher expression of proteins in resistant diploid clone of potato

		Bacteria		Water	
Stress and defense	Metallocarboxypeptidase inhibitor	274,4	***	165.5	***
	Probable inactive patatin-03-Kuras 1	3.0	***	4.3	***
	Proteinase inhibitor type-2	15.3	***	17.7	***
	Similar to: pathogenesis related protein STH-2	1.9	***	1.6	*
	Glutaredoxin	2.3	**	3.9	
	Wound-induced proteinase inhibitor	13.3	*	29.8	**
	Similar to: Polyphenol oxidase B	3.43	*		***
	17-3 kDa class II heat shock protein	3.61	*	5.07	**
	Superoxide dismutase			2.21	**
	Patatin	6.82	*	9.56	*



DG 00-270



DG 08-305

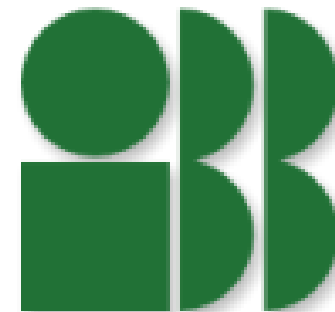
## Summary

- There are differences in proteins produced by resistant and susceptible potato cultivars 8 h and 48 h post inoculation.
- The differences observed during an early phase of infection might play significant role in inhibition of disease development.
- Most of proteins differentially expressed in resistant and susceptible potato cultivars are common in mock- and wound- inoculated tubers.





## Acknowledgments



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