

# THE COLONISATION OF DIPLOID AND TETRAPLOID POTATO PLANTS BY *DICKEYA SOLANI* OBTAINED FROM INFECTED TUBERS



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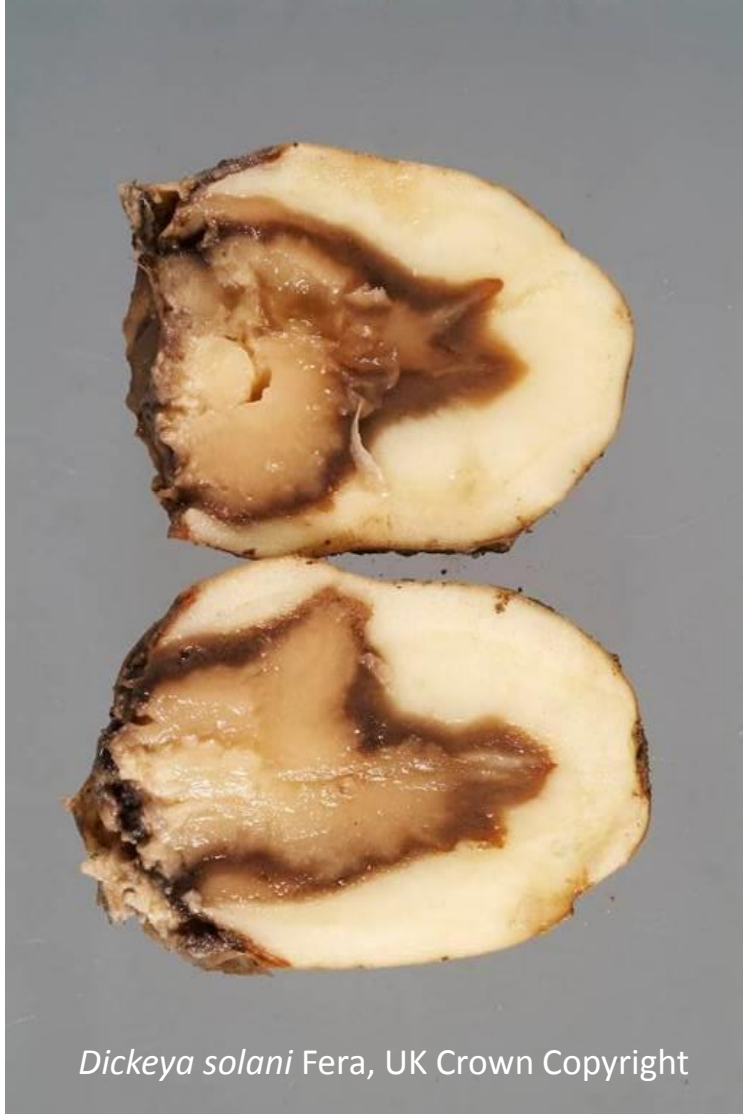
**Granted by the Ministry of Agriculture and Rural Development (IHAR-PIB 4-3-00-3-01)**

# Potato Blackleg

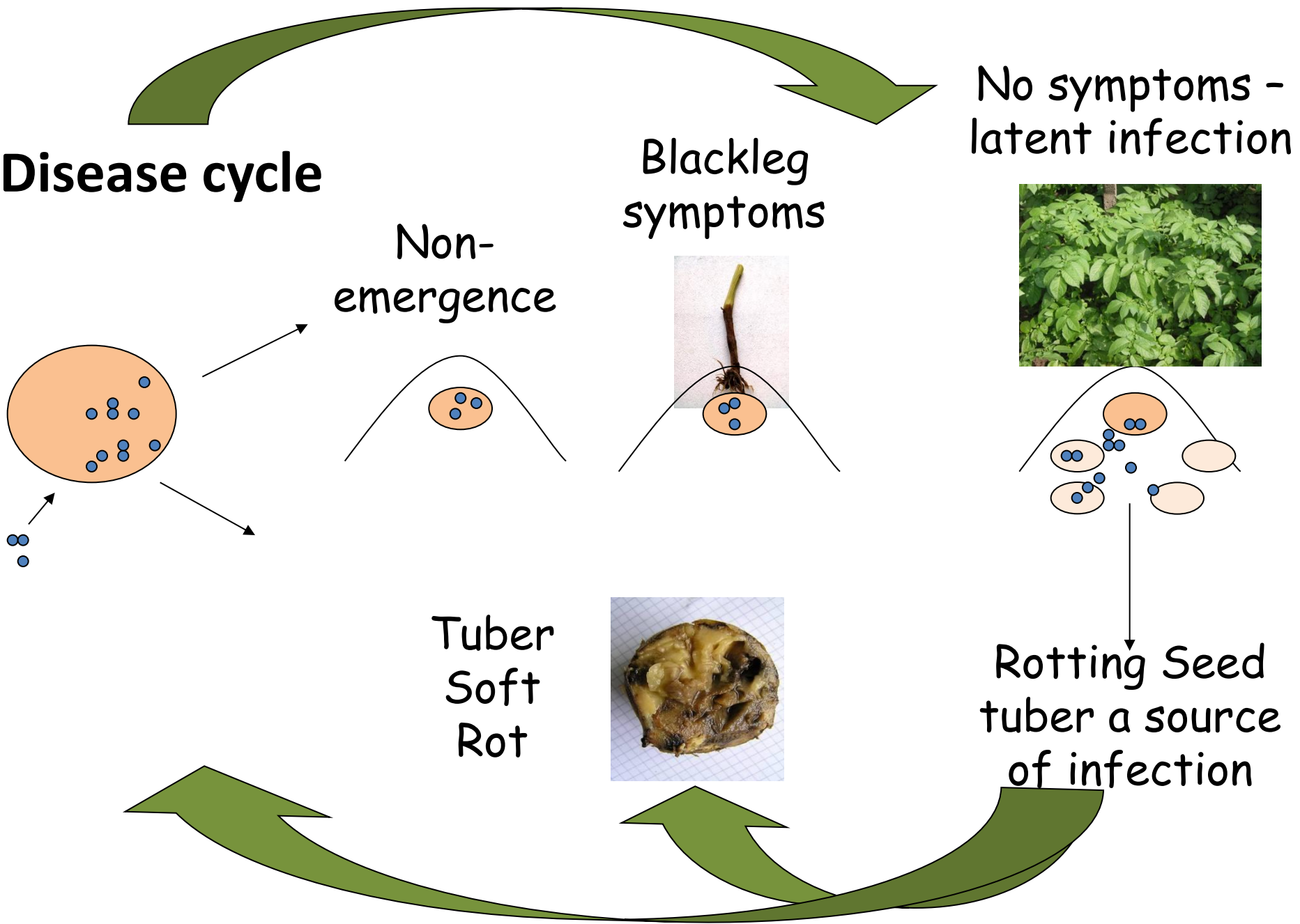




# Tuber Soft Rot



# Disease cycle



No symptoms - latent infection



Non-emergence

Blackleg symptoms



Rotting Seed tuber a source of infection

Tuber Soft Rot



# Pectinolytic bacteria pathogenic to potato in Poland

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<i>Pectobacterium atrosepticum</i>	57 % (1996-1997; Waleron et al. 2013)
<i>P. carotovorum</i> subsp. <i>carotovorum</i>	37 % (1996-1997; Waleron et al. 2013)
<i>P. wasabiae</i>	6 % (1996-1997; Waleron et al. 2013)
<i>P. carotovorum</i> subsp. <i>brasiliense</i>	1.8 % of Pcc (since 1996, Waleron et al. 2015)
<i>Dickeya solani</i>	5.0 % of SRE 2009-2013 (Potrykus et al. 2016) (N=39)
<i>D. dianthicola</i>	

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# Goals

- Characterization of resistance to *Dickeya solani* in diploid interspecific hybrids of *Solanum*.
- Are progeny tubers of the most tolerant diploids, grown from vacuum infiltrated seed tubers with *D. solani*, infected?



# Testing tuber resistance to *D. solani*



- *Dickeya solani* – IBF0099 (syn. IPO2276) (Golanowska *et al.* 2015)
- Bacterial suspension –  $10^8$  CFU/ml
- 10  $\mu$ l inoculum (hole size - 10mm x 2mm)

(Laurila *et al.* 2008; Lebecka, 2001)



# Testing tuber resistance to *D. solani*

- 26 (2x) hybrids of *Solanum*
- check cultivars: Irys and Glada

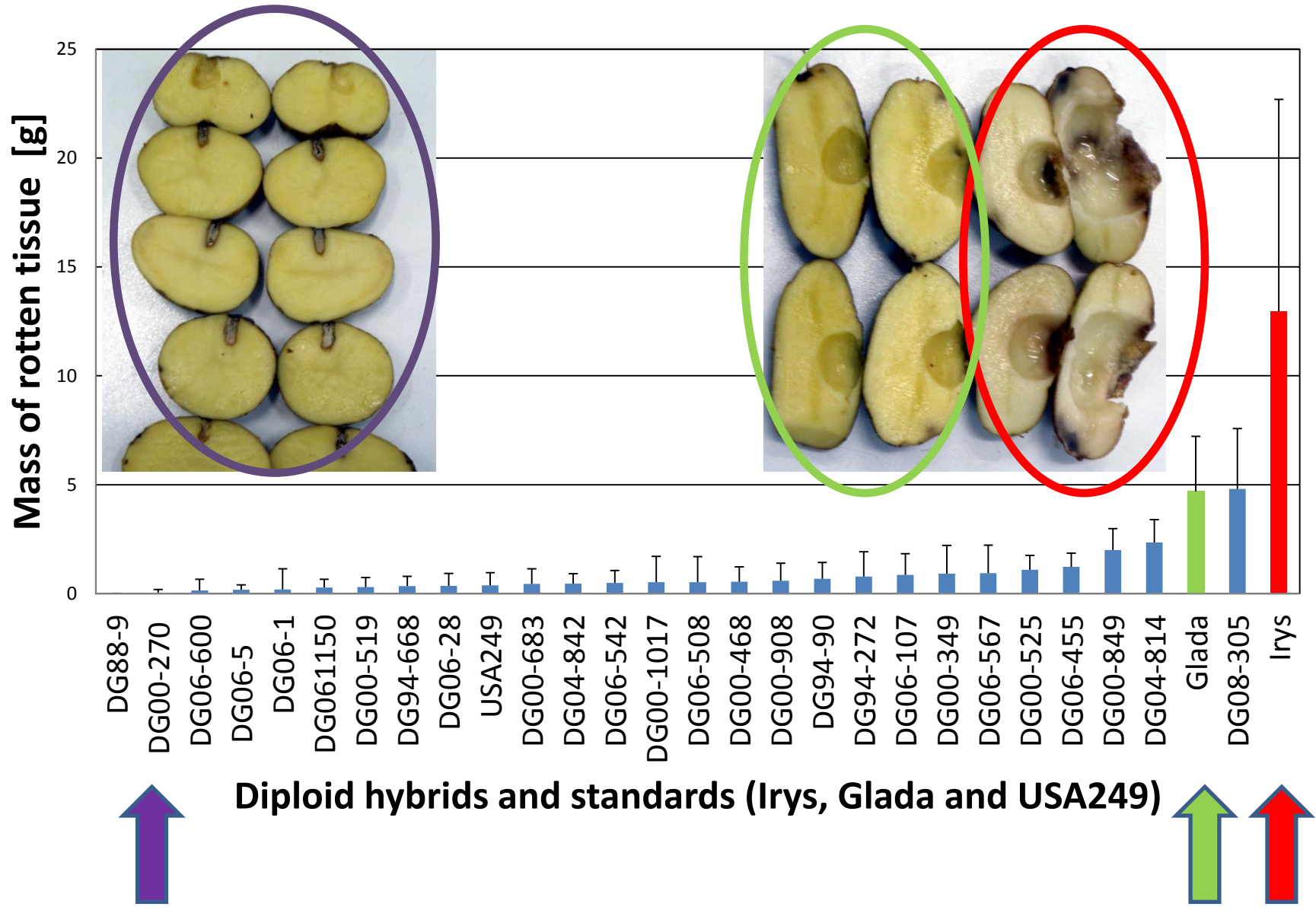


3 dates x 5 tubers x 2 replications

3 - 4 days, temperature 26 °C

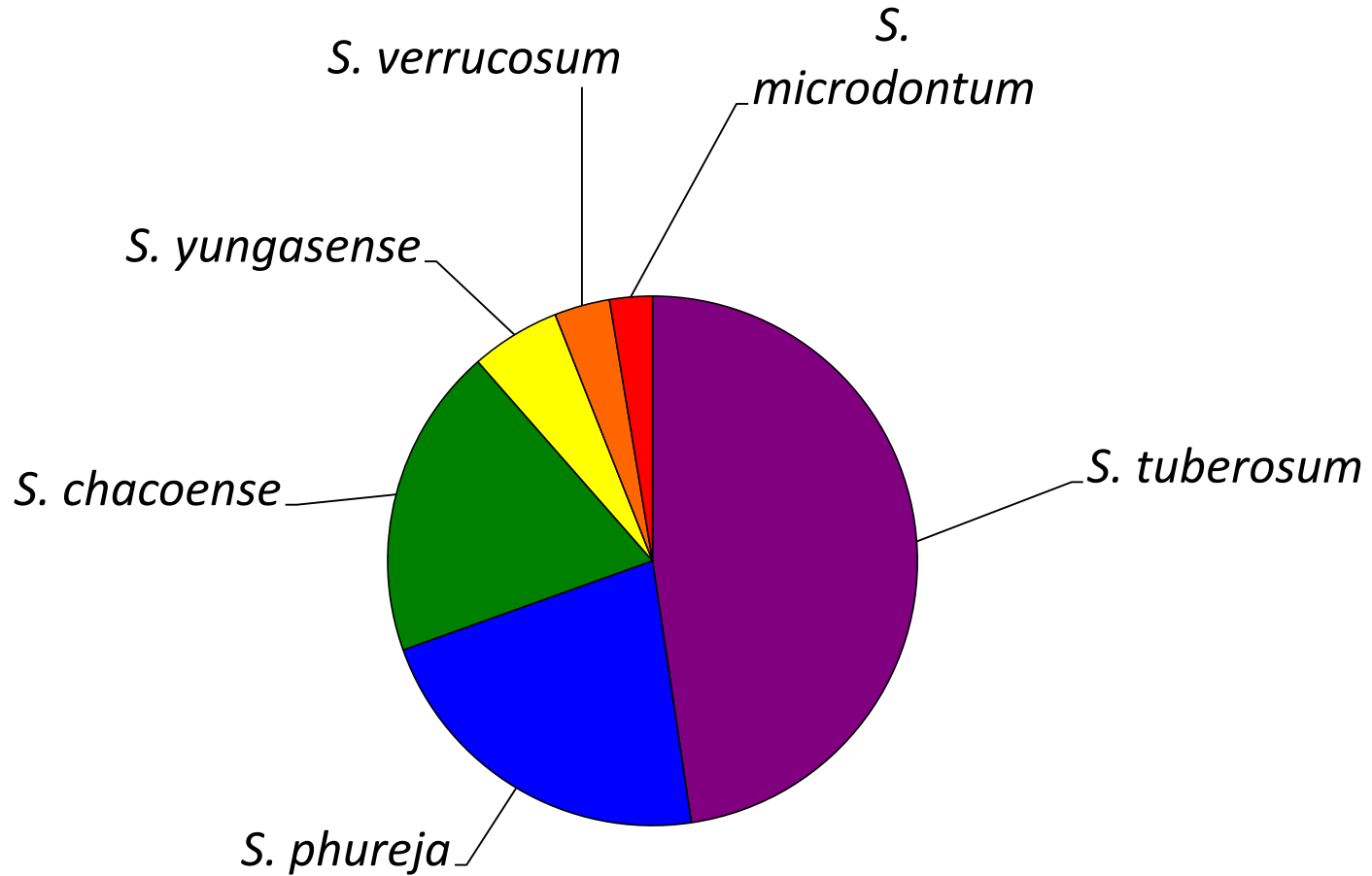


# Testing tuber resistance to *D. solani*



# The *Solanum* hybrid (2x)

DG 00-270



# Testing plant resistance to *D. solani*

## Inoculation of the base of the potato stem

- 26 (2x) hybrids of *Solanum*
- 7 cultivars
- 2 check cultivars: Irys and Glada
- 26 - 80 plants/ genotype



(the method acc. to Pietrak, 2001)

# Testing plant resistance to *D. solani*



*D. solani* -  $10^8$  CFU/ml



# Testing plant resistance to *D. solani*



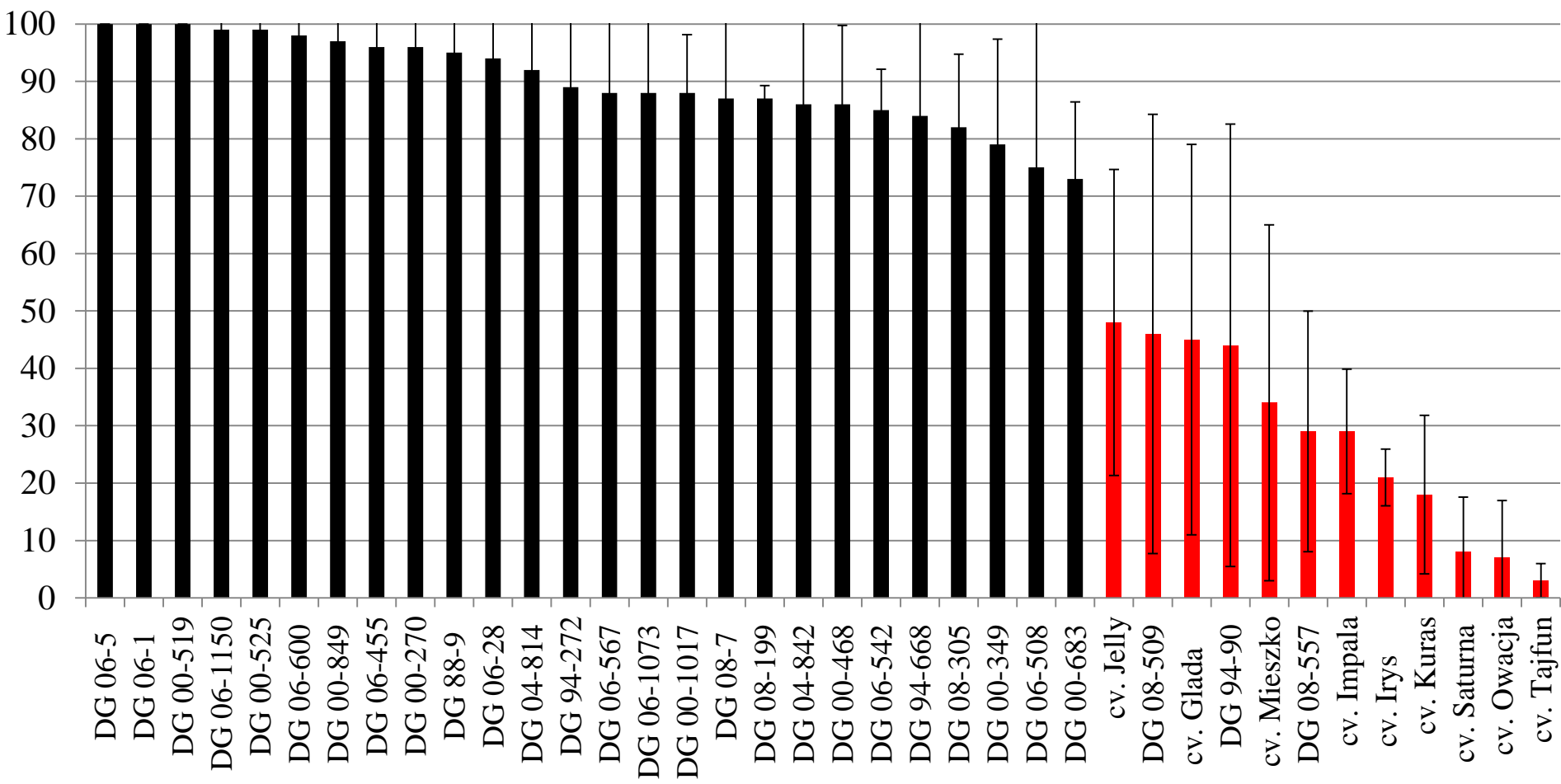
1<sup>st</sup> date – blackleg symptoms in **three days** post inoculation

# Testing plant resistance to *D. solani*



2<sup>nd</sup> date – wilting, necroses -10 days post inoculation

# Plants without symptoms of infection (%)



 – significantly different at  $P < 0.001$



# Testing plant resistance to *D. solani*

## Inoculation of seed tubers

- Cultivars: Irys, Kondor
- $10^8$  and  $10^7$  CFU/ml
- Sprouted tubers 17 h in water before inoculation, 0.1 Mpa for 20 min, allowed to dry for 2 hours
- planted in pots
- DNA extracted 2 weeks post inoculation



(the method acc. to Helias *et al.* 2000)



# Detection of *D. solani* by real time PCR

## Primers:

SOL-C - Pritchard *et al.* (2012),

ds - Van Vaerenbergh *et al.* (2012).

Bacterial DNA: 2,48 ng/ $\mu$ l (1 $\mu$ l/reaction)

DNA from plant samples: 5 ng/  $\mu$ l

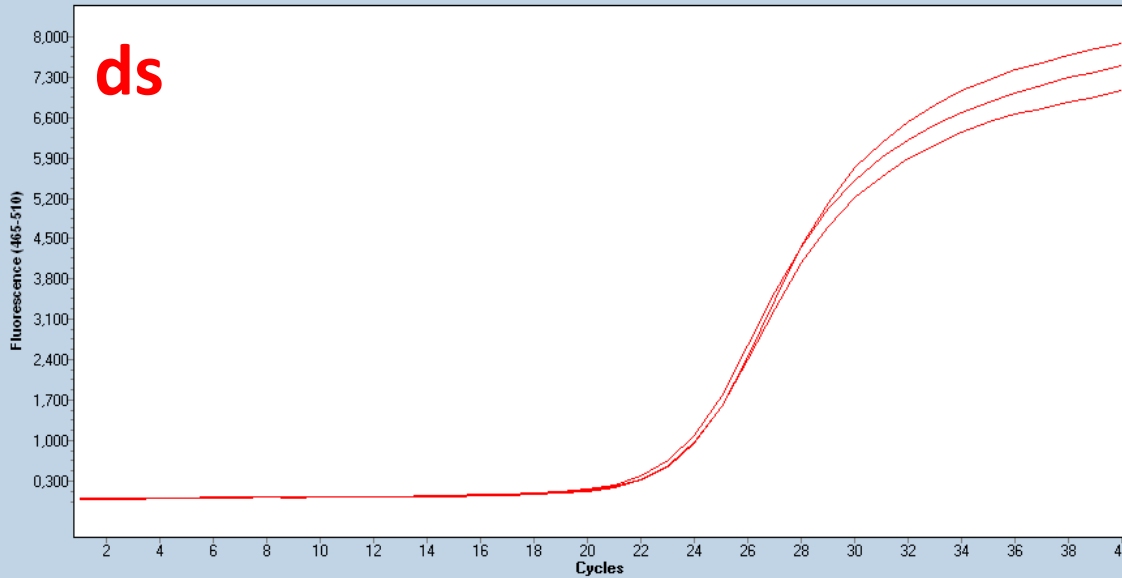
Negative control DNA from healthy plant King Edward

Reference gene:  $\beta$ -tubulin

# Detection of *D. solani* by real time PCR

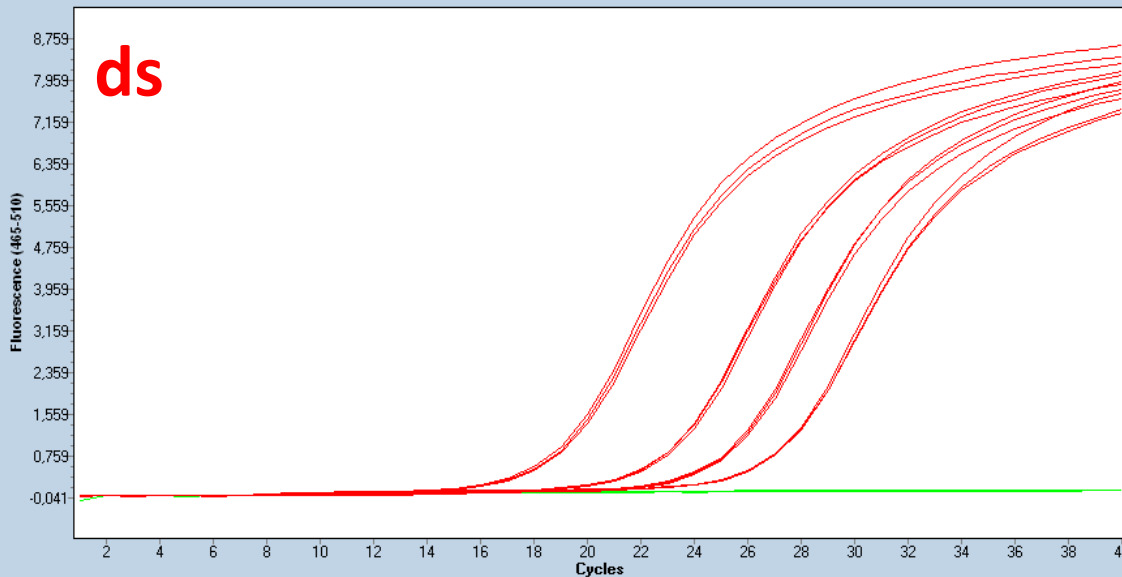
Inoculation method	Origin of DNA		Crossing point value (Cp) relative expression ( $2^{-\Delta Cq}$ ) of target sequence to the reference gene Tub					
			ds	Tub	$2^{-\Delta Cq}$	SOL-C	Tub	$2^{-\Delta Cq}$
DNA dilluted 200 x	IFB0099	Bacteria	23,2	0		23,8	0	
DNA dilluted 100 x			21,8	0		22,5	0	
Healthy	King Edward	Stem	0	22,8	0	0	22,8	0
	Irys		0	24,7	0	0	24,5	0
2 weeks PI $10^7$	Irys	Stem	22,8	22,5	0,84	23,1	22,4	0,59
	Kondor		26,9	23,0	0,07	27,4	23,1	0,05
2 weeks PI $10^8$	Irys		18,7	22,8	17,4	19,5	22,7	8,68
	Kondor		24,9	22,9	0,23	25,1	22,8	0,20
12 weeks post inoculation of the stem	DG 08-305	Roots	0	28,9	0	0	28,9	0
	DG 00-270		0	27,1	0	0	27,2	0
	DG 08-305	Tuber	0	22,6	0	0	22,6	0
	DG 00-270		0	22,7	0	0	22,7	0

Amplification Curves



*D. solani* (positive control)  
2,48 ng/ul (1 µl/reaction)

Amplification Curves



**Red from the left:**

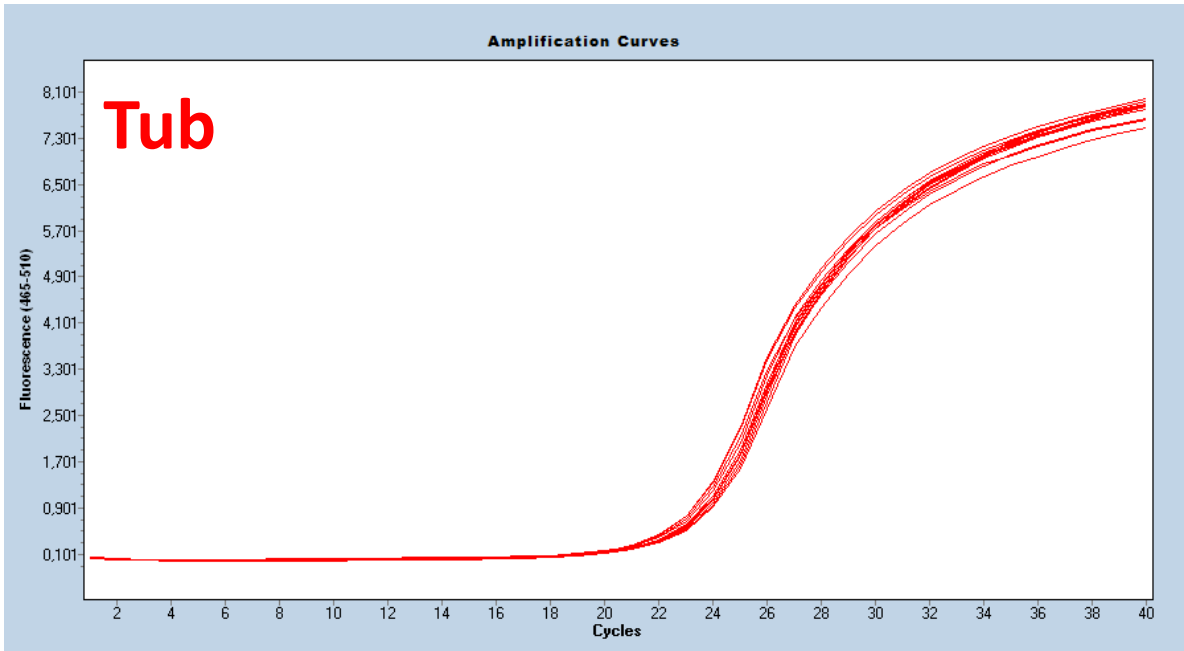
Cv. Irys  $10^8$  ++++

Cv. Irys  $10^7$  +++

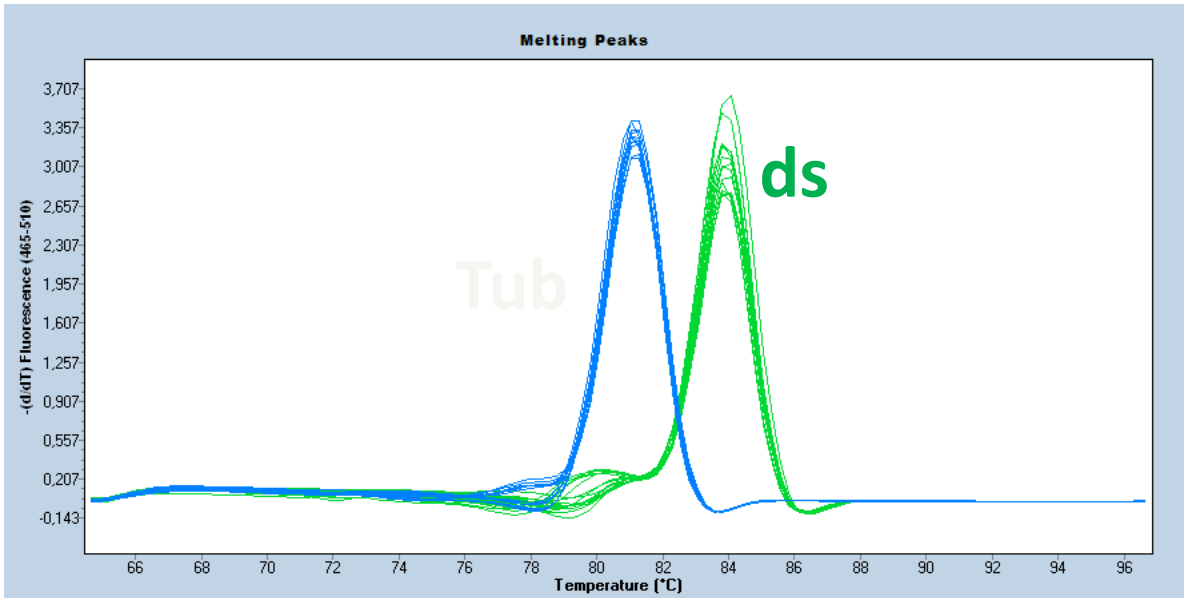
Cv. Kondor  $10^8$  ++

Cv. Kondor  $10^7$  +

**Green:** negative control cv. Irys  
healthy



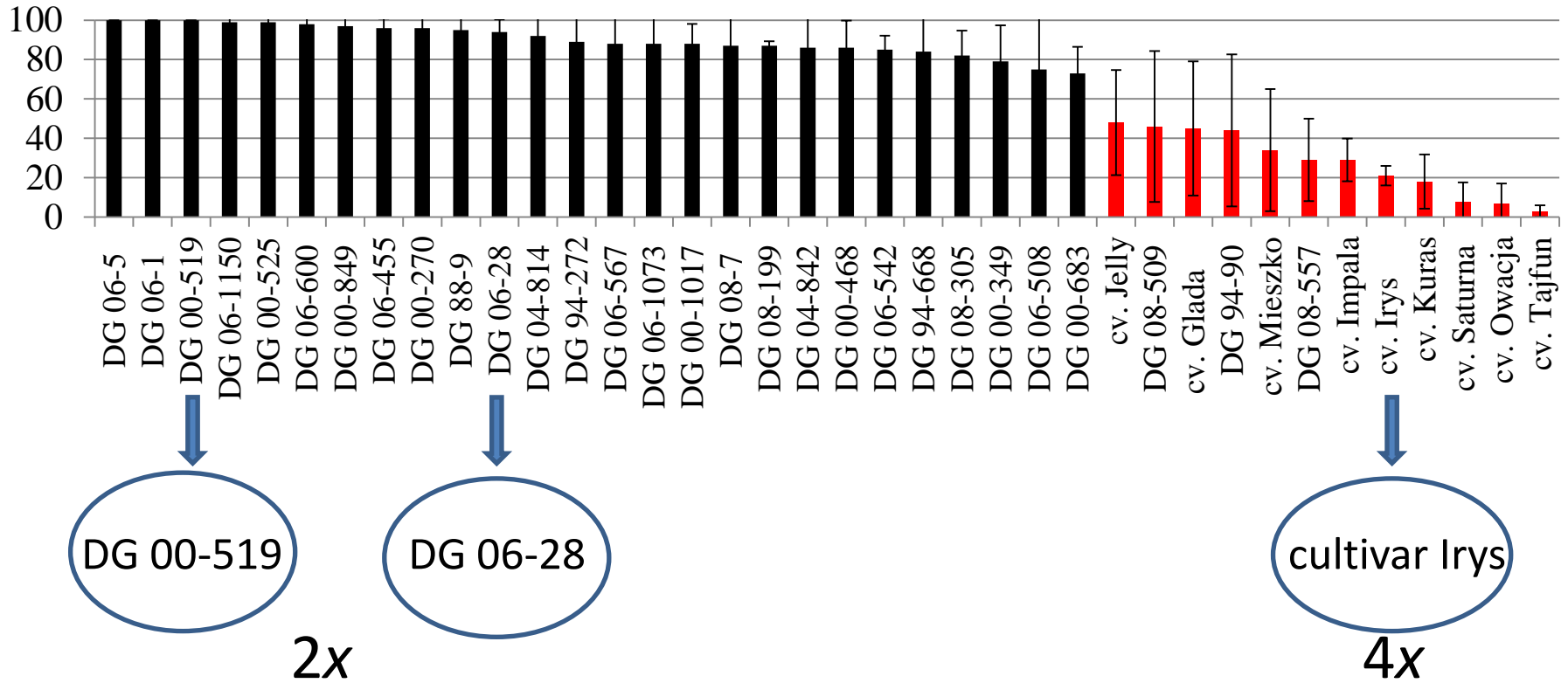
Reference gene -  $\beta$ -tubulin



**Melting curves:**  
ds **Green**  
Tub **Blue**



# Vacuum infiltration with *D. solani* of 2x and 4x potato



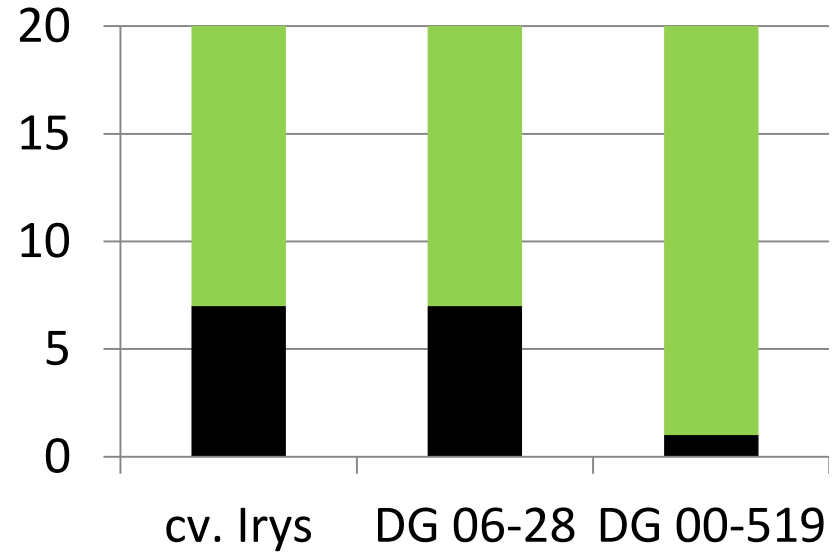
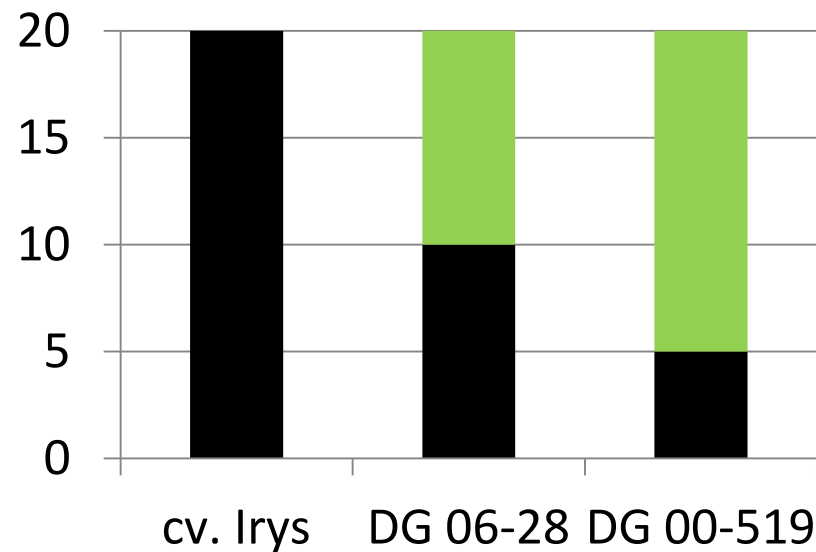
■  $10^8$  and  $10^6$  CFU/ml

# Emergence of plants

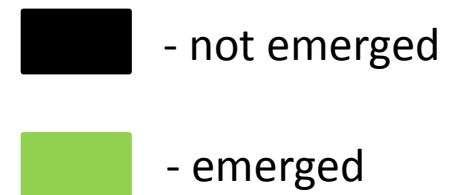
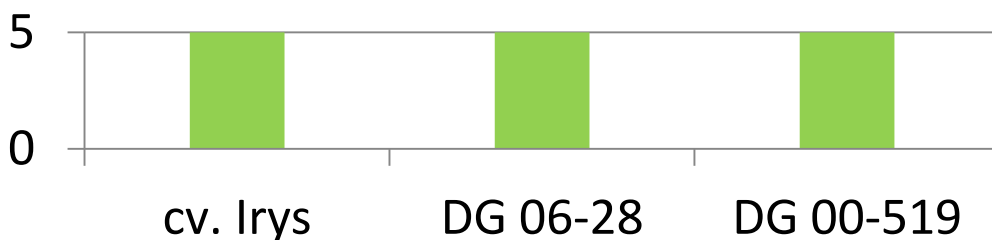
3 weeks post inoculation

$10^8$  CFU/ml

$10^6$  CFU/ml



Mock inoculated with water

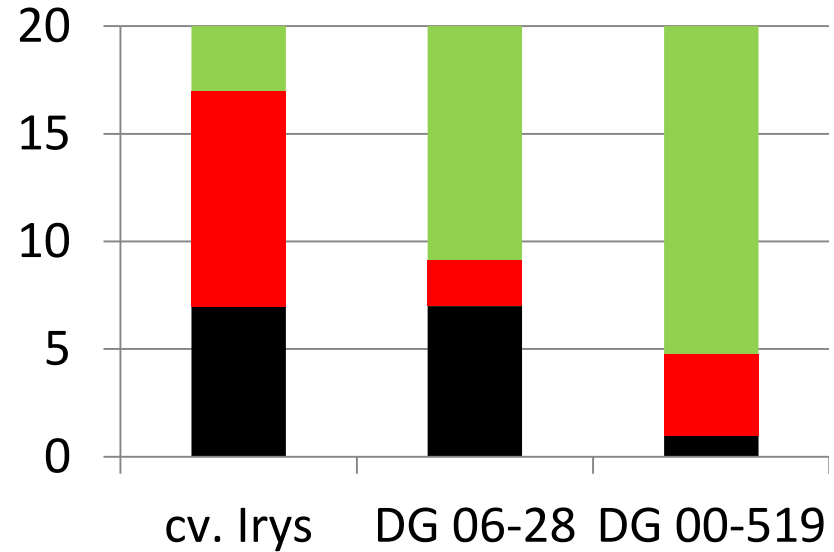
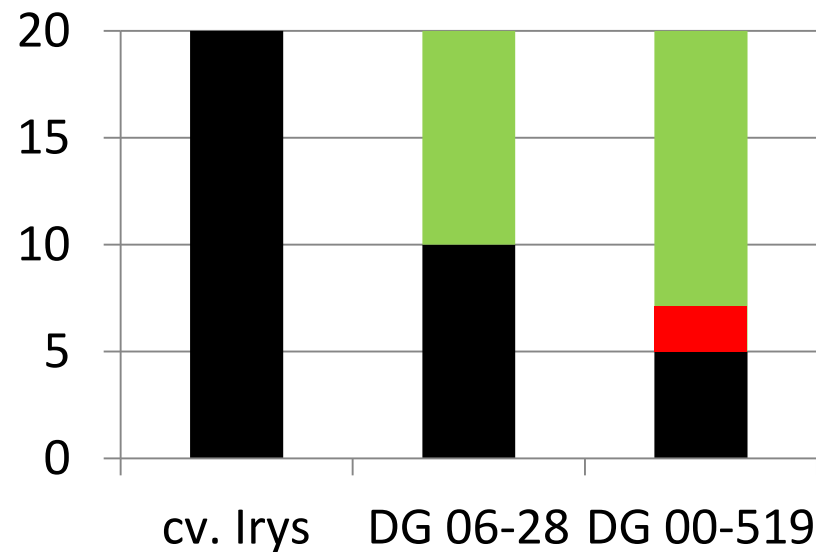


# Emergence of plants

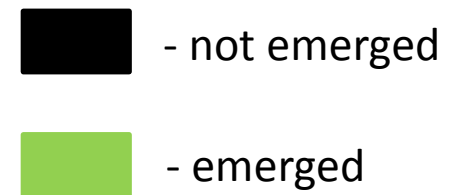
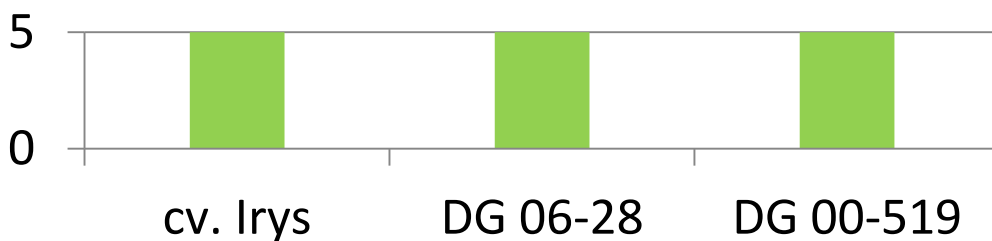
3 weeks post inoculation

$10^8$  CFU/ml

$10^6$  CFU/ml



Mock inoculated with water

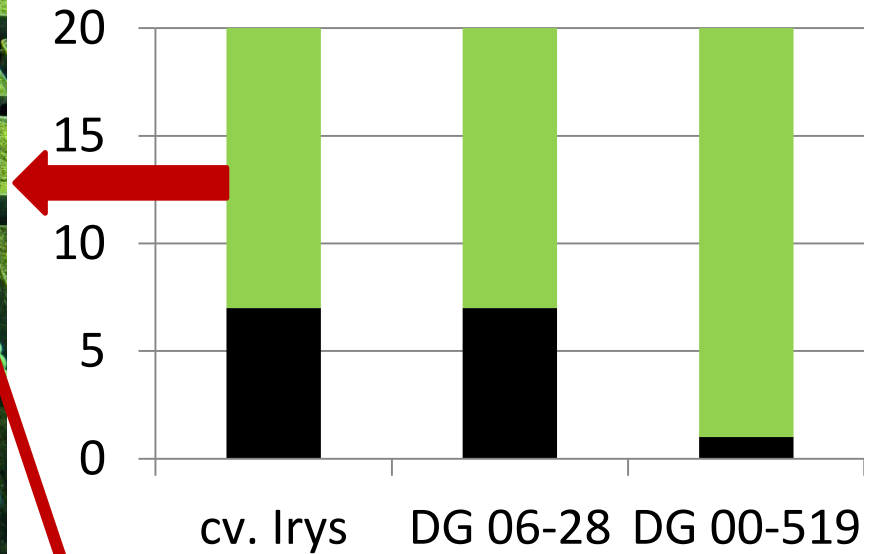


# Emergence of plants

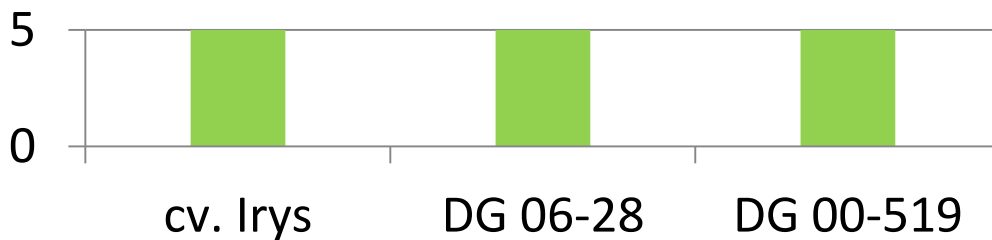
3 weeks post inoculation



$10^6$  CFU/ml



Mock inoculated with water

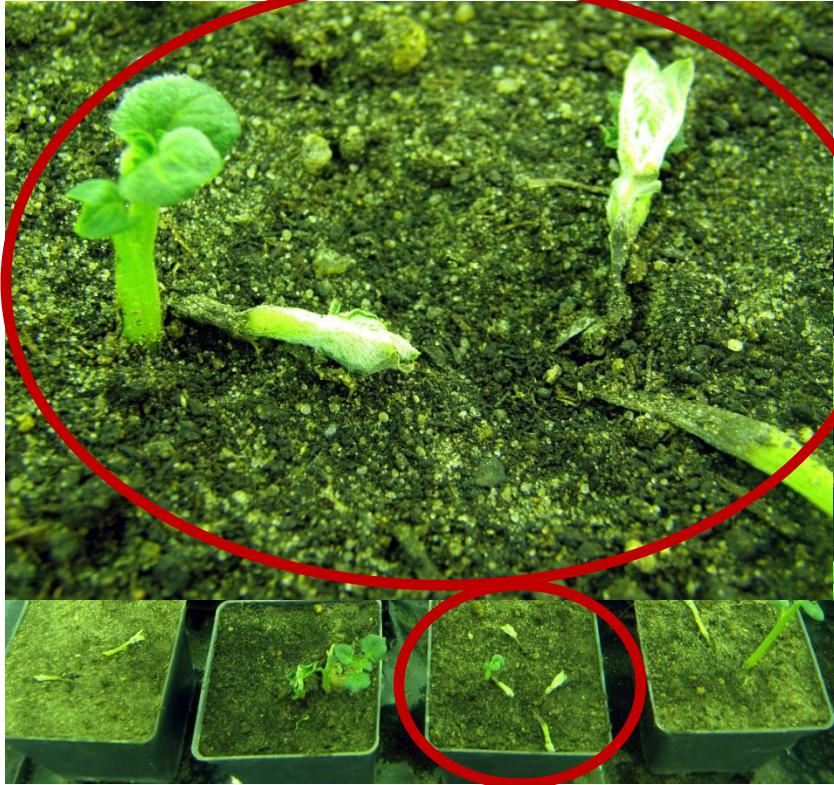


■ - not emerged  
■ - emerged

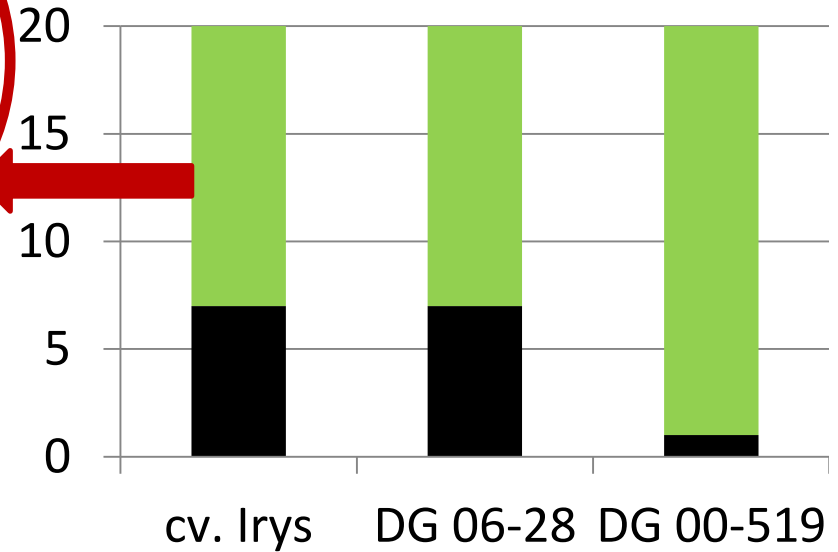


# Emergence of plants

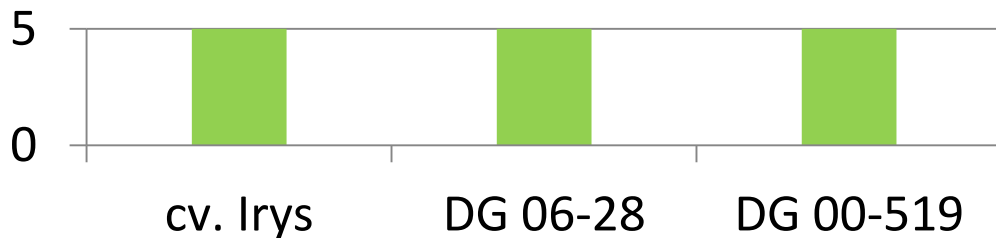
3 weeks post inoculation



$10^6$  CFU/ml



Mock inoculated with water

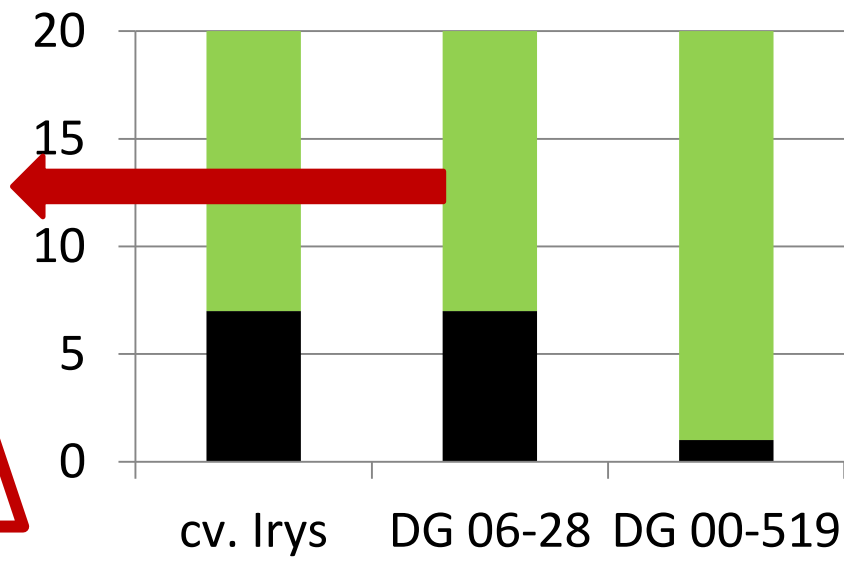


■ - not emerged  
■ - emerged

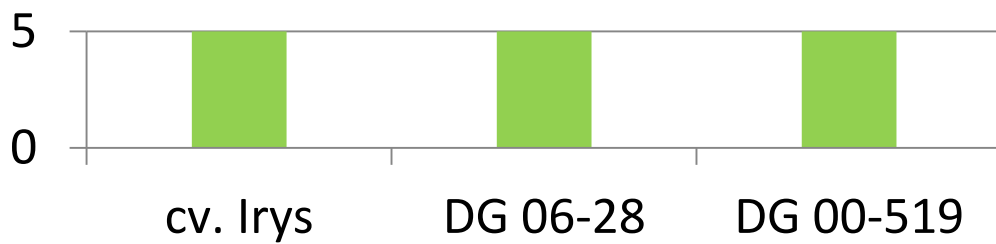
# Emergence of plants

3 weeks post inoculation

$10^6$  CFU/ml



Mock inoculated with water

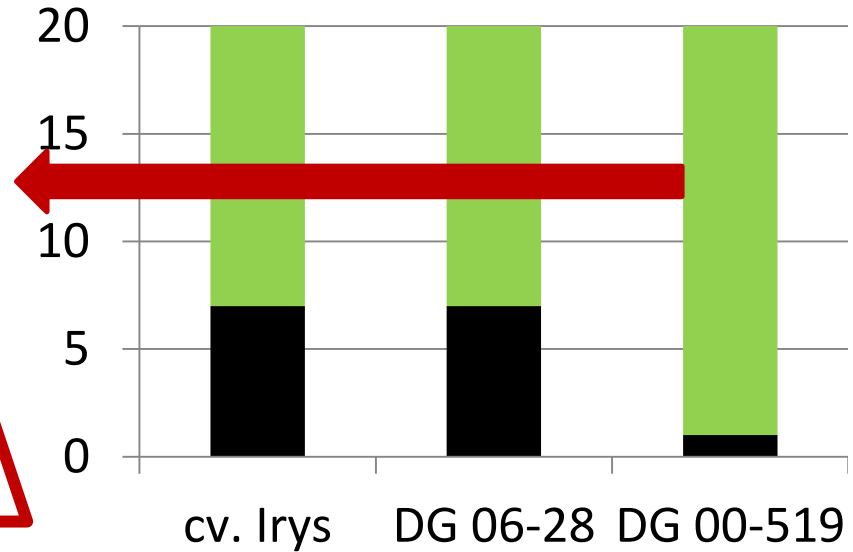


■ - not emerged  
■ - emerged

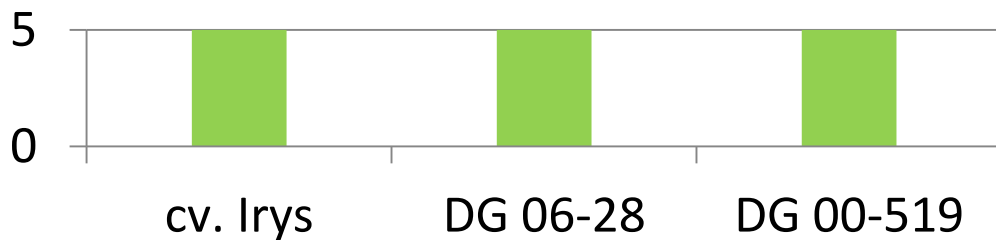
# Emergence of plants



3 weeks post inoculation

$10^6$  CFU/ml



Mock inoculated with water



-  - not emerged
-  - emerged

# CP value DG 06-28

## 30 and 60 days post inoculation (DPI)

**10<sup>6</sup> CFU/ml**

DG 06-28		30 DPI			Gel	60 DPI			Gel
		1	2	3		1	2	3	
	Stem								
1	Down	0	0	0		35	35	33	+++
	Middle	0	0	0		33	33	33	++
	Upper	0	0	0		0	0	0	
2	Down	0	0	0		0	0	0	
	Middle	35	0	0	++++	0	0	0	
	Upper	27	0	0	-----	0	0	0	
3	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
4	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	

**10<sup>8</sup> CFU/ml**

DG 06-28		30 DPI			Gel	60 DPI			Gel
		1	2	3		1	2	3	
	Stem								
1	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
2	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		34	35	0	++
3	Down	0	0	0		35	0	0	+
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
4	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	



# CP value DG 00-519

## 30 and 60 days post inoculation (DPI)

**10<sup>6</sup> CFU/ml**

DG 00-519		30 DPI			Gel	60 DPI			Gel
		1	2	3		1	2	3	
	Stem								
1	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
2	Down	0	0	0		0	0	0	
	Middle	0	0	0		35	0	0	+
	Upper	0	0	0		0	0	0	
3	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
4	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	

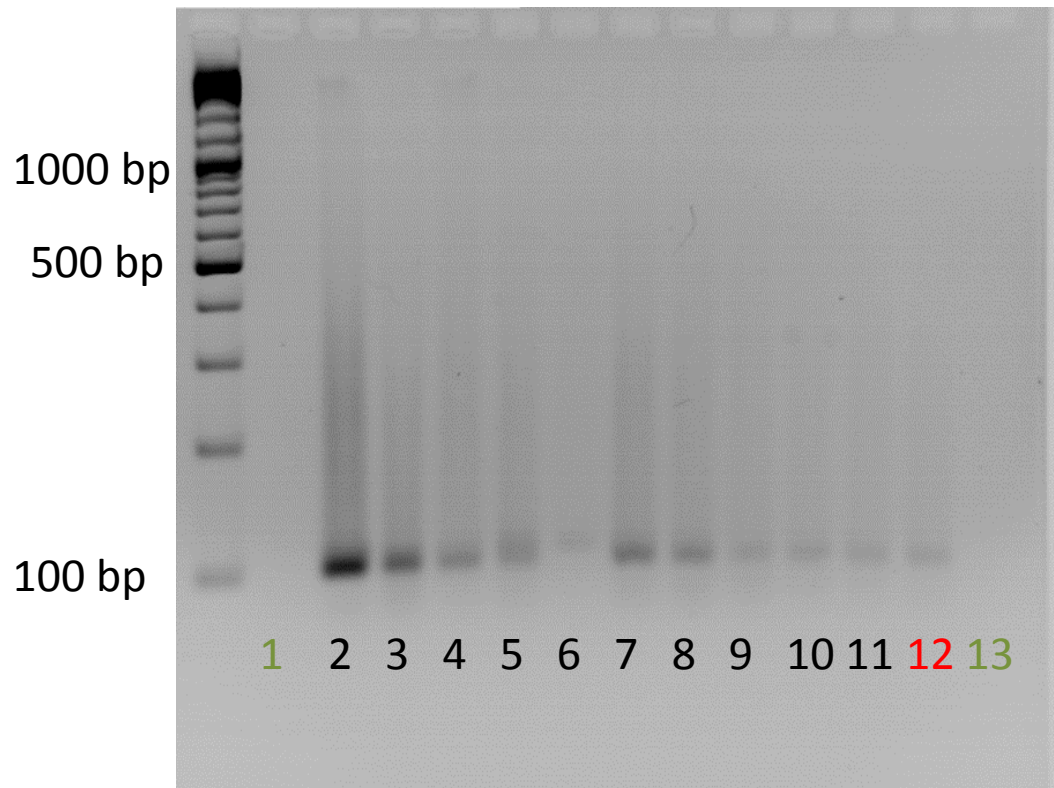
**10<sup>8</sup> CFU/ml**

DG 00-519		30 DPI			Gel	60 DPI			Gel
		1	2	3		1	2	3	
	Stem								
1	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
2	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	
3	Down	0	0	0		35	0	0	+
	Middle	0	0	0		35	0	0	+
	Upper	0	0	0		0	0	0	
4	Down	0	0	0		0	0	0	
	Middle	0	0	0		0	0	0	
	Upper	0	0	0		0	0	0	

# Confirmation of doubtful results

60 days post inoculation (DPI)

$10^8$  CFU/ml



1. **Not inoculated** DG 00-519
2. DG-00-519,  $10^8$  CFU/ml d
3. DG-00-519,  $10^8$  CFU/ml
4. DG-06-28,  $10^8$  CFU/ml
5. DG-00-519,  $10^6$  CFU/ml
6. DG-06-28,  $10^6$  CFU/ml
7. DG-06-28,  $10^8$  CFU/ml
8. Irys,  $10^6$  CFU/ml
9. Irys,  $10^6$  CFU/ml
10. DG-06-28,  $10^8$  CFU/ml
11. DG-06-28,  $10^6$  CFU/ml
12. **DS99 bacterial DNA**
13. **Not inoculated Irys**

# CP value

## 30 and 60 days post inoculation (DPI)

**10<sup>8</sup> CFU/ml**

**10<sup>6</sup> CFU/ml**

Non-emergence of cv. Irys

Cv. Irys		30 DPI				60 DPI			
					Gel				
	Stem	1	2	3		1	2	3	
1	Down	22	23	22	++++	Plant died			
	Middle	24	24	24	++++				
	Upper	31	30	31	++++				
2	Down	NT				35	0	0	++
	Middle	NT				0	0	0	
	Upper	NT				0	0	0	

# DG 06-28 - symptomless plant

60 DPI

$10^6$  CFU/ml





# DG 00-519 – symptoms of infection

60 DPI

**10<sup>6</sup> CFU/ml**



# DG 00-519 – symptoms of infection

126 days post inoculation

$10^8$  CFU/ml





# DG 06-28 – symptoms of infection

126 days post inoculation (18 weeks)

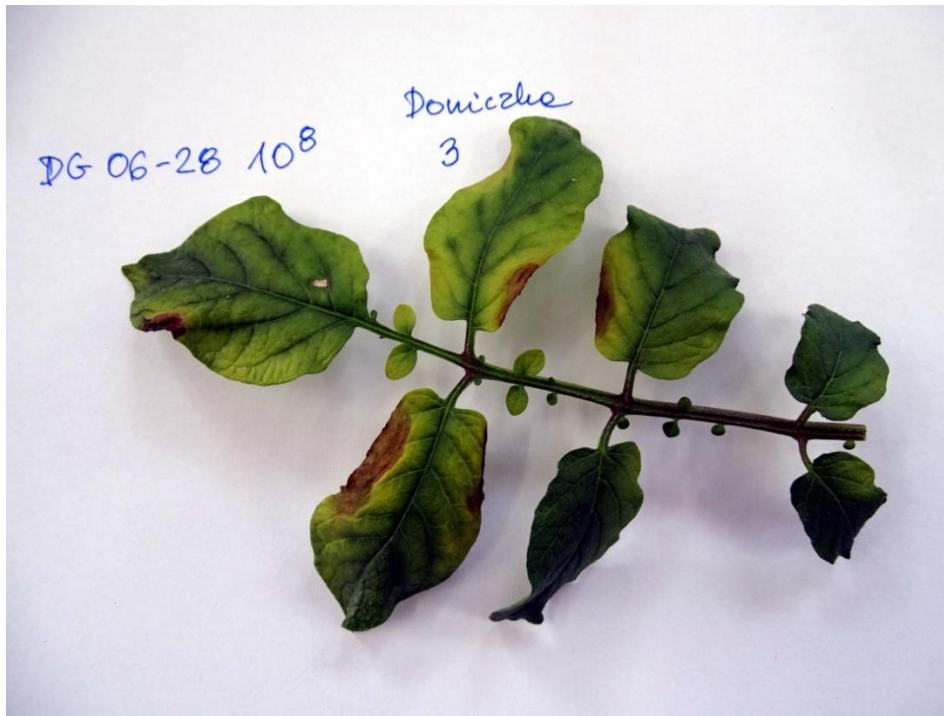


# DG 06-28 – symptoms of infection

126 days post inoculation (18 weeks)

**$10^8$  CFU/ml**

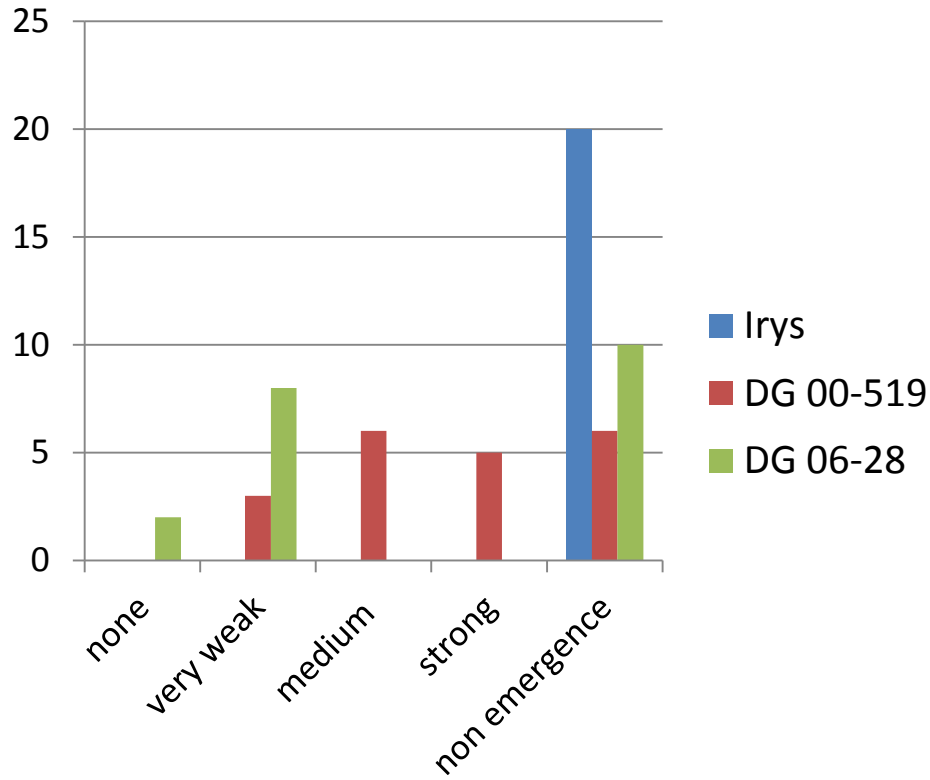
**$10^6$  CFU/ml**



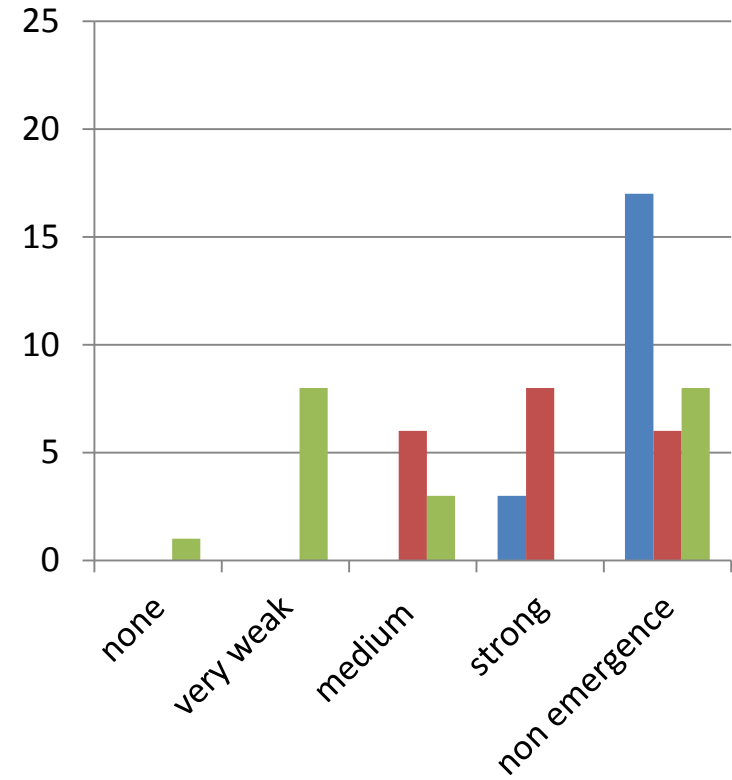
# Number of plants with symptoms of infection

126 days post inoculation (18 weeks)

$10^8$  CFU/ml



$10^6$  CFU/ml





# CP value

## 126 days post inoculation (DPI)

$10^6$

$10^8$  CFU/ml

DG 06-28

DG 00-519

Plant	Leaves	1	2	3	Gel		1	2	3	Gel
DG 06-28	1 Down	35	0	35	+++		0	35	35	+++
	Middle	0	35	0	++		0	35	0	++
	Upper	0	35	0	+		35	0	0	++
	2 Down	0	35	0			0	0	0	
	Middle	0	35	35	+++		35	35	0	++++
	Upper	35	35	35	+++		35	0	0	++++
	3 Down	0	0	0			0	0	0	
	Middle	0	0	0			0	0	0	-
	Upper	0	0	35	+		0	0	0	
DG 00-519	4 Down	35	0	0	+++		0	0	0	-
	Middle	35	0	0	+++		0	0	0	-
	Upper	35	0	35	+++		0	35	0	-
	1 Dry	0	35	0	++++		35	0	0	++
	2 Dry	0	35	35			35	0	35	++
	3 Dry	0	0	0			30	34	0	++++

# Summary

- Diploid interspecific hybrids of potato exhibit higher resistance to tuber soft rot and higher resistance/tolerance of plants to infection with *D. solani* than 4x potato cultivars.
- The progeny tubers of the most tolerant diploids, grown from vacuum infiltrated seed tubers, will be tested for latent infection with *D. solani*.



INSTYTUT HODOWLI  
I AKLIMATYZACJI ROŚLIN  
PAŃSTWOWY INSTYTUT BADAWCZY  
ODDZIAŁ W MŁOCZOWIE

THANK YOU