

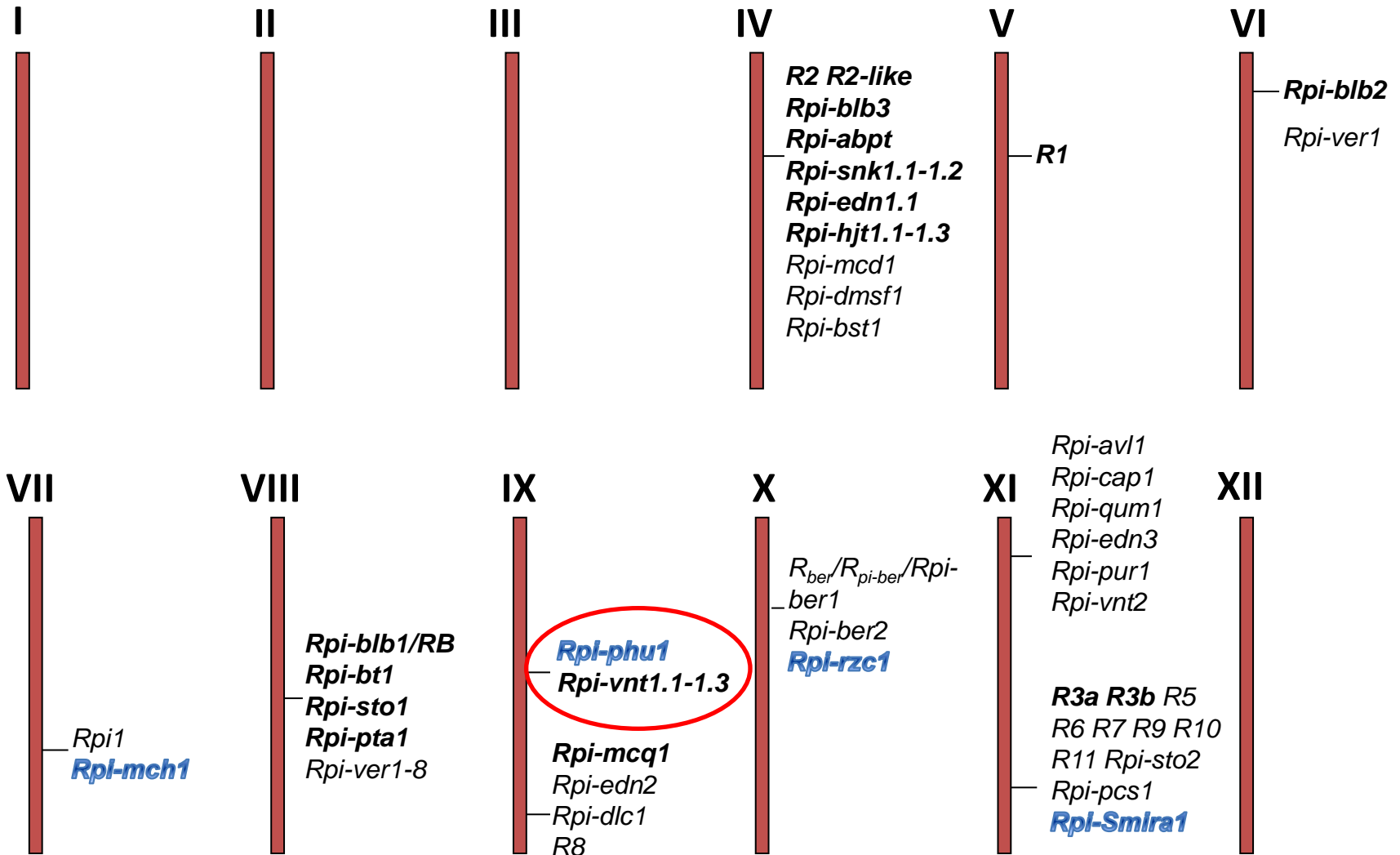


Expression profiles of the late blight resistance gene *Rpi-phu1*

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Hot spots of R genes for resistance to *P. infestans* on potato genetic map





Diploid , 2 EBN

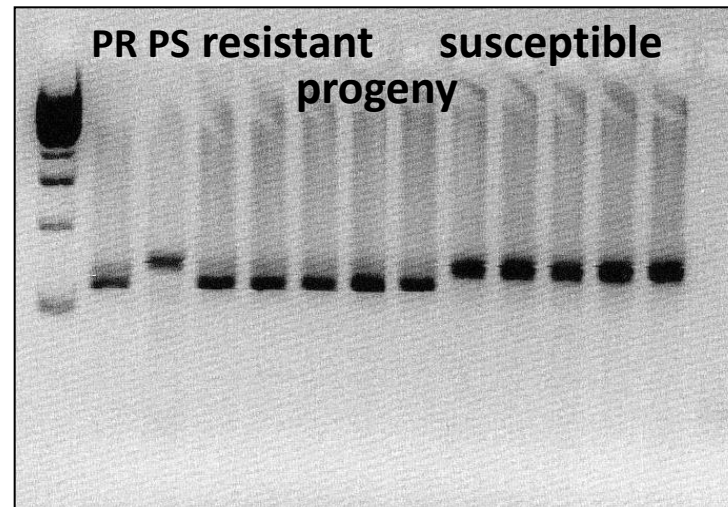
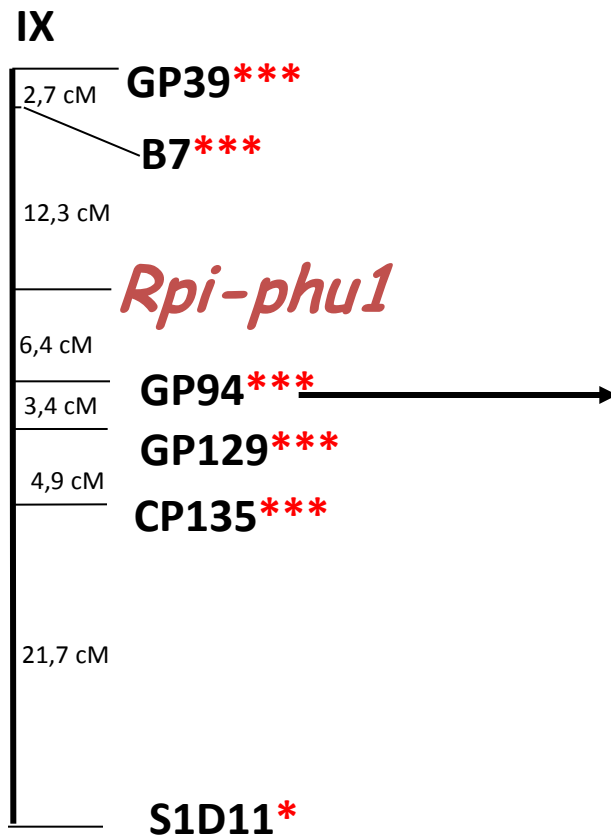


Solanum stenotomum



Solanum phureja

Identification of the *Rpi-phu1* gene on chromosome IX of potato genome



Theor Appl Genet (2006) 113:685–695
DOI 10.1007/s00122-006-0336-9

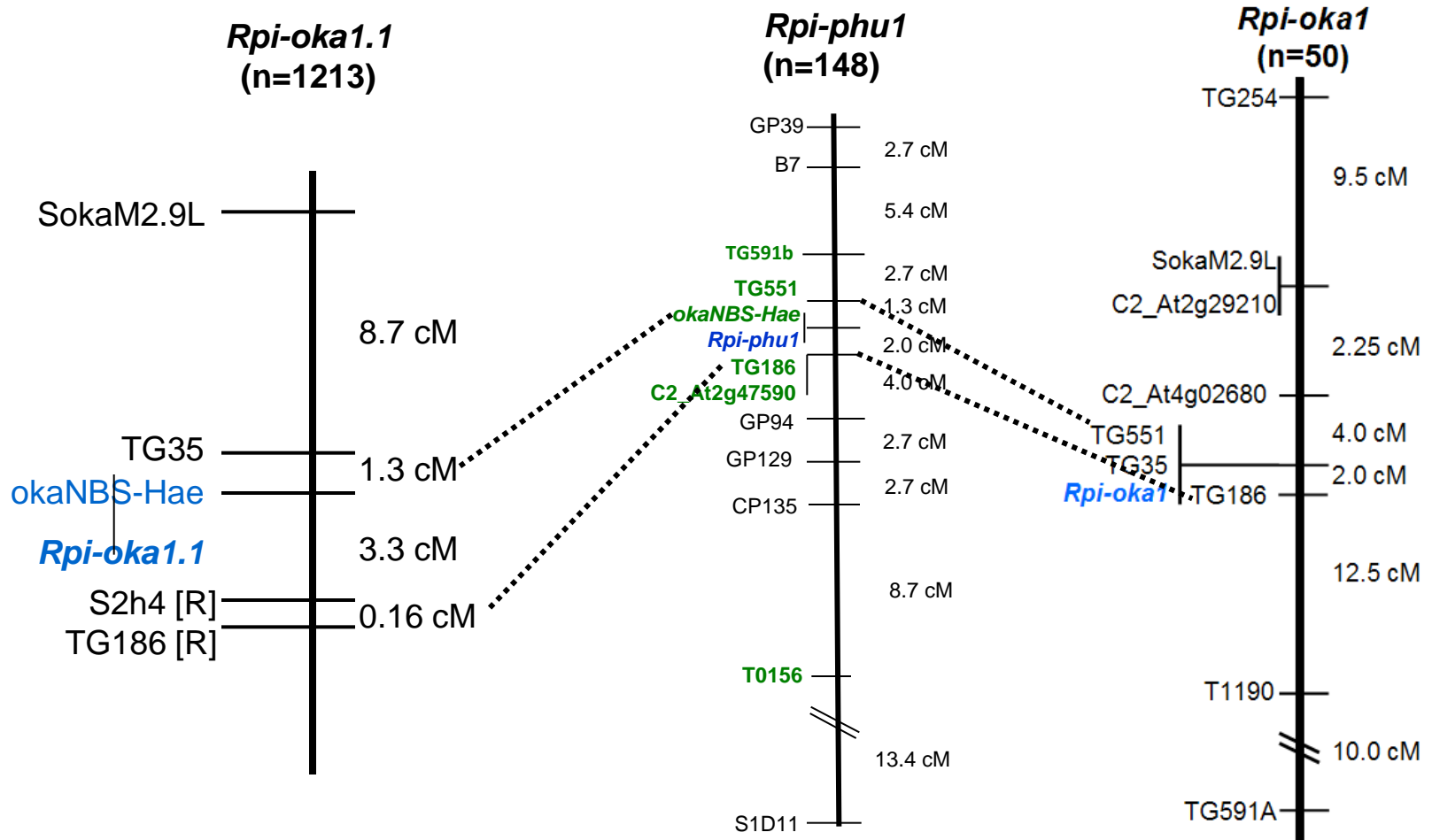
ORIGINAL PAPER

Markers significantly linked with the resistance: * - $p < 0.05$; *** - $p < 0.001$
(U Mann-Whitney test)

The novel, major locus *Rpi-phu1* for late blight resistance maps to potato chromosome IX and is not correlated with long vegetation period

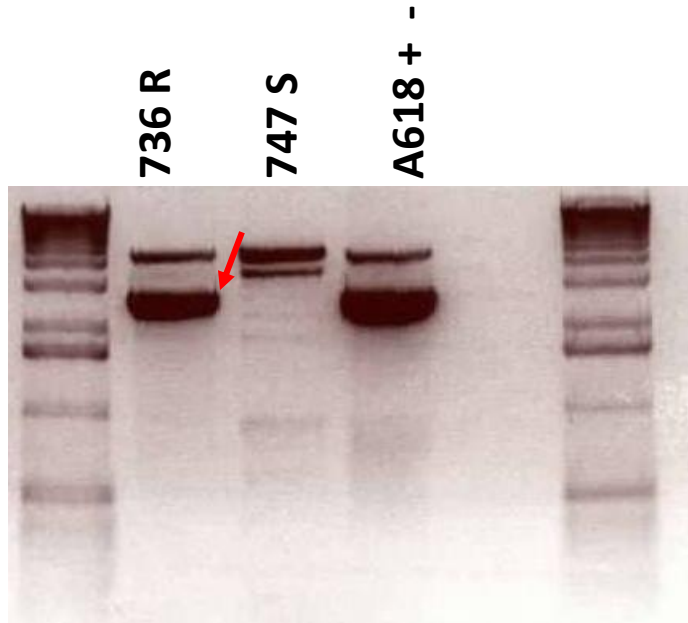
J. Śliwka · H. Jakuczun · R. Lebecka · W. Marczewski ·
C. Gebhardt · E. Zimnoch-Guzowska

THE SAINSBURY LABORATORY



S. okadae = *S. venturii*

THE SAINSBURY LABORATORY



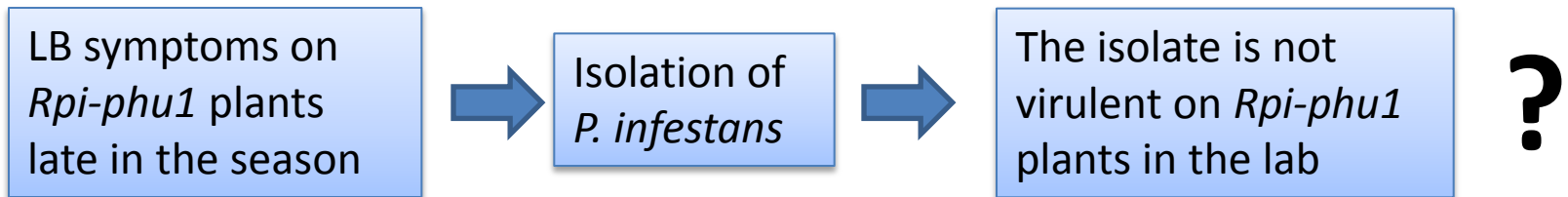
Rpi-phu1 = Rpi-vnt1.1

MPMI Vol. 22, No. 5, 2009, pp. 589–600. doi:10.1094/MPMI-22-5-0589. © 2009 The American Phytopathological Society

***Rpi-vnt1.1*, a *Tm-2²* Homolog from *Solanum venturii*, Confers Resistance to Potato Late Blight**

Simon J. Foster,¹ Tae-Ho Park,¹ Mathieu Pel,² Gianinna Brigneti,¹ Jadwiga Śliwka,³ Luke Jagger,¹
Edwin van der Vossen,² and Jonathan D. G. Jones¹

Why are we interested in expression?



Hypotheses:

1. Isolate lost virulence in vitro
2. *Rpi-phu1* gene is not (sufficiently) expressed due to:

- Genetic background
- Plant age
- Day length



Plant Pathology (2012)

Doi: 10.1111/ppa.12018

Influence of genetic background and plant age on expression of the potato late blight resistance gene *Rpi-phu1* during incompatible interactions with *Phytophthora infestans*

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Material

P. infestans

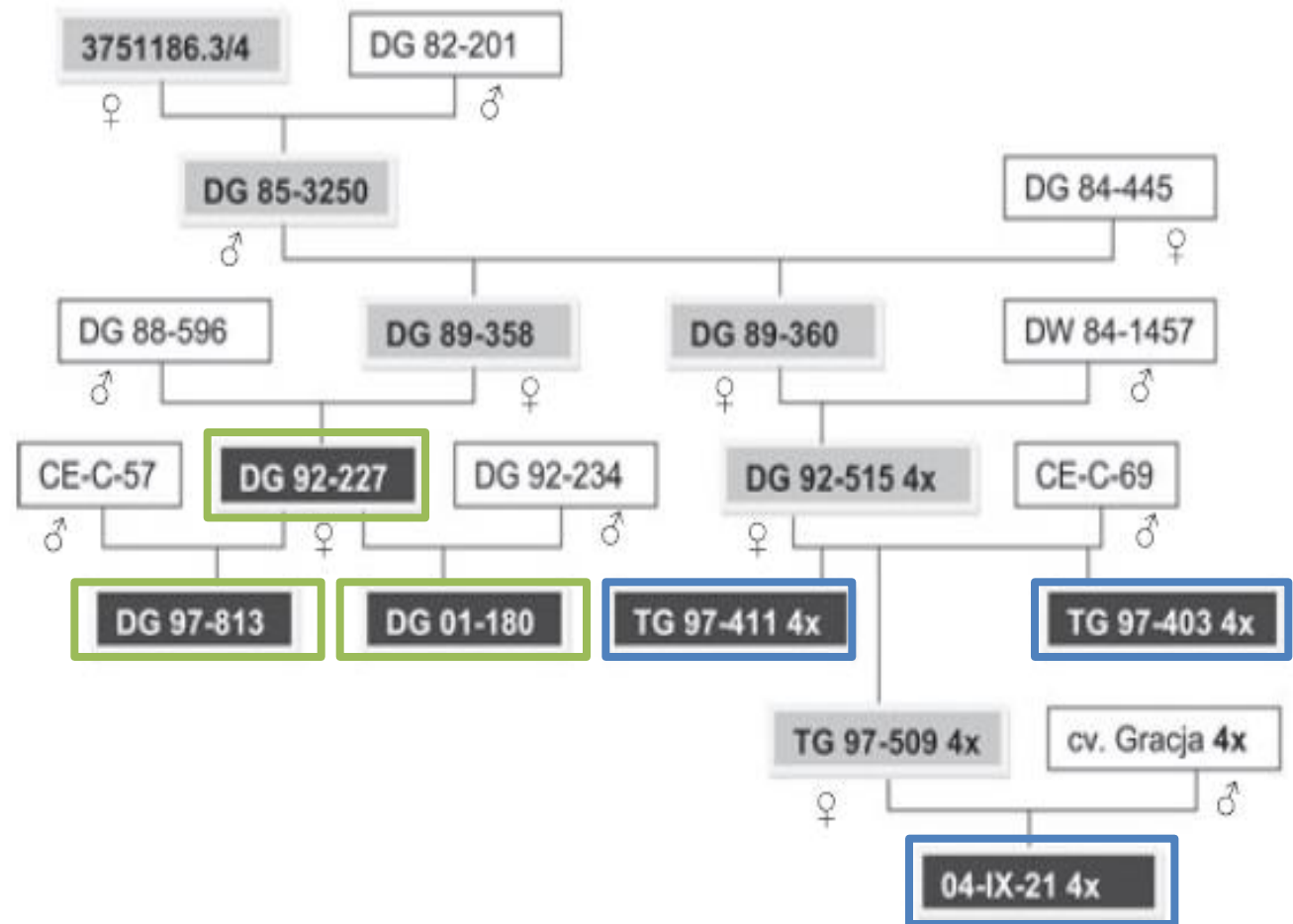
- MP324 avirulent on *Rpi-phu1* plants
- MP1162 isolated from *Rpi-phu1* plants but avirulent in lab tests
- EC1 described as virulent on *Rpi-vnt1.1* plants



Plant material

diploid (2x)

tetraploid (4x)



Methods

- Plants grown in climatic chambers
 - High relative humidity 75-90%
 - temperature
 - 16°C night
 - 18°C day
 - Sampled on 0, 1, 3 and 5th day
 - Spray inoculation



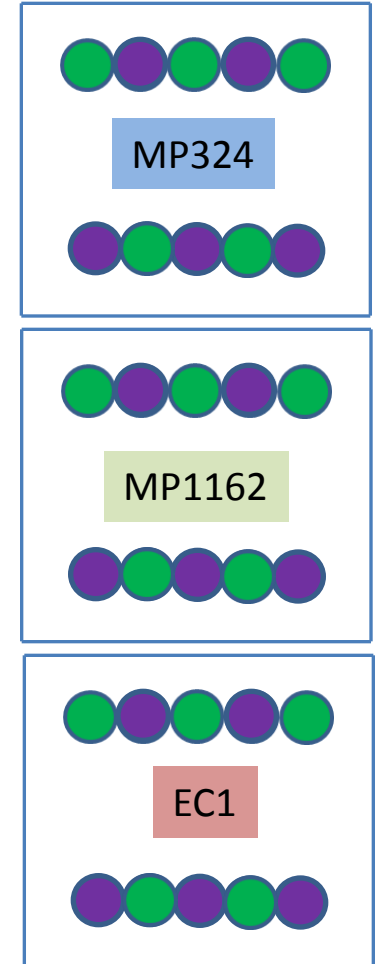
Methods

- RNA isolation
 - Spectrum Plant Total RNA Kit, Sigma
 - DNase I, Sigma
- RT-PCR
 - Maxima First Strand cDNA Synthesis Kit for RT-qPCR, Fermentas
- real-time PCR
 - SYBR Green
 - Relative expression
 - α -tubulin



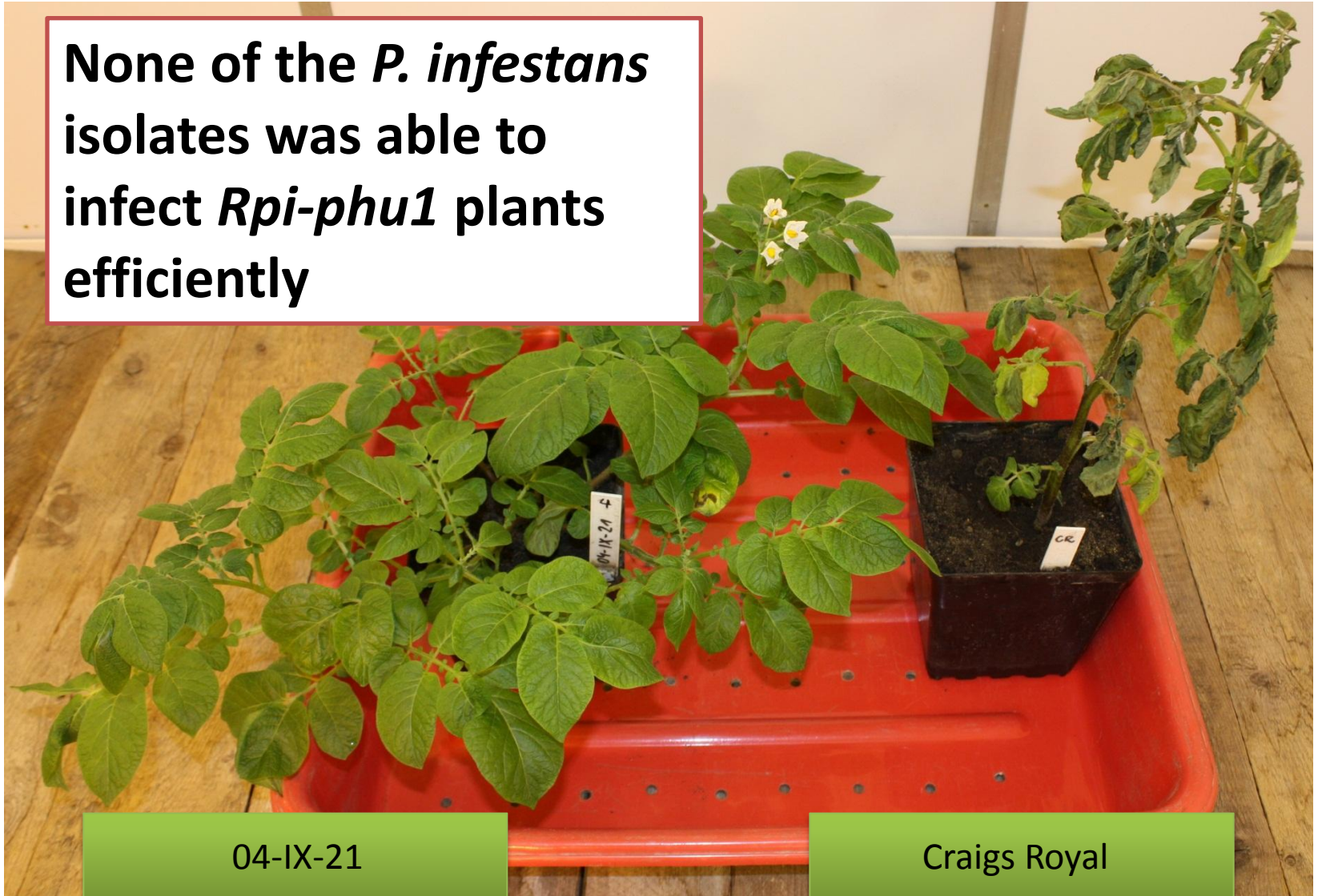
Relative expression level of *Rpi-phu1* after inoculation with different *P. infestans* isolates

- *P. infestans*
 - MP324
 - MP1162
 - EC1
- Plants
 - test: 04-IX-21 with *Rpi-phu1* gene
 - control: Craigs Royal



Infection

None of the *P. infestans* isolates was able to infect *Rpi-phu1* plants efficiently



04-IX-21

Craigs Royal

Potato genetic background

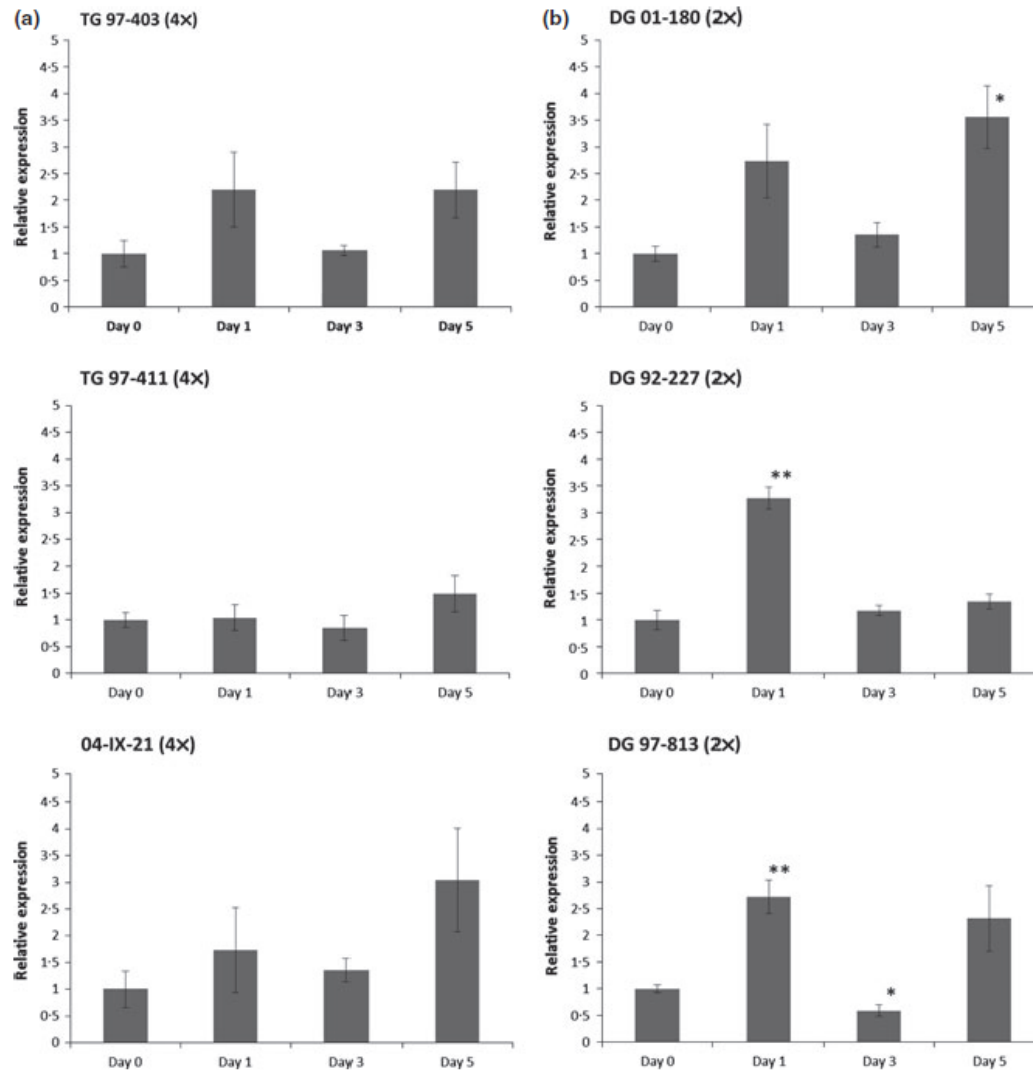


Figure 3 *Rpi-phu1* gene expression in tetraploid (left hand side) and diploid (right hand side) genotypes of potato inoculated with *Phytophthora infestans*. Tetraploid lines (4x): TG 97-403, TG 97-411 and 04-IX-21. Diploid lines (2x): DG 01-180, DG 92-227 and DG 97-813. Data are average relative expression values of five biological replicates. Leaf samples were collected on day 0 (before inoculation) and 1, 3 and 5 days post-inoculation. For each genotype, day 0 was the baseline against which all other time points were compared. Significant differences using a *t*-test for the $\Delta\Delta C_t$ values are indicated as follows: * $P < 0.05$, ** $P < 0.01$. Bars correspond to standard errors.

Potato genetic background day by day

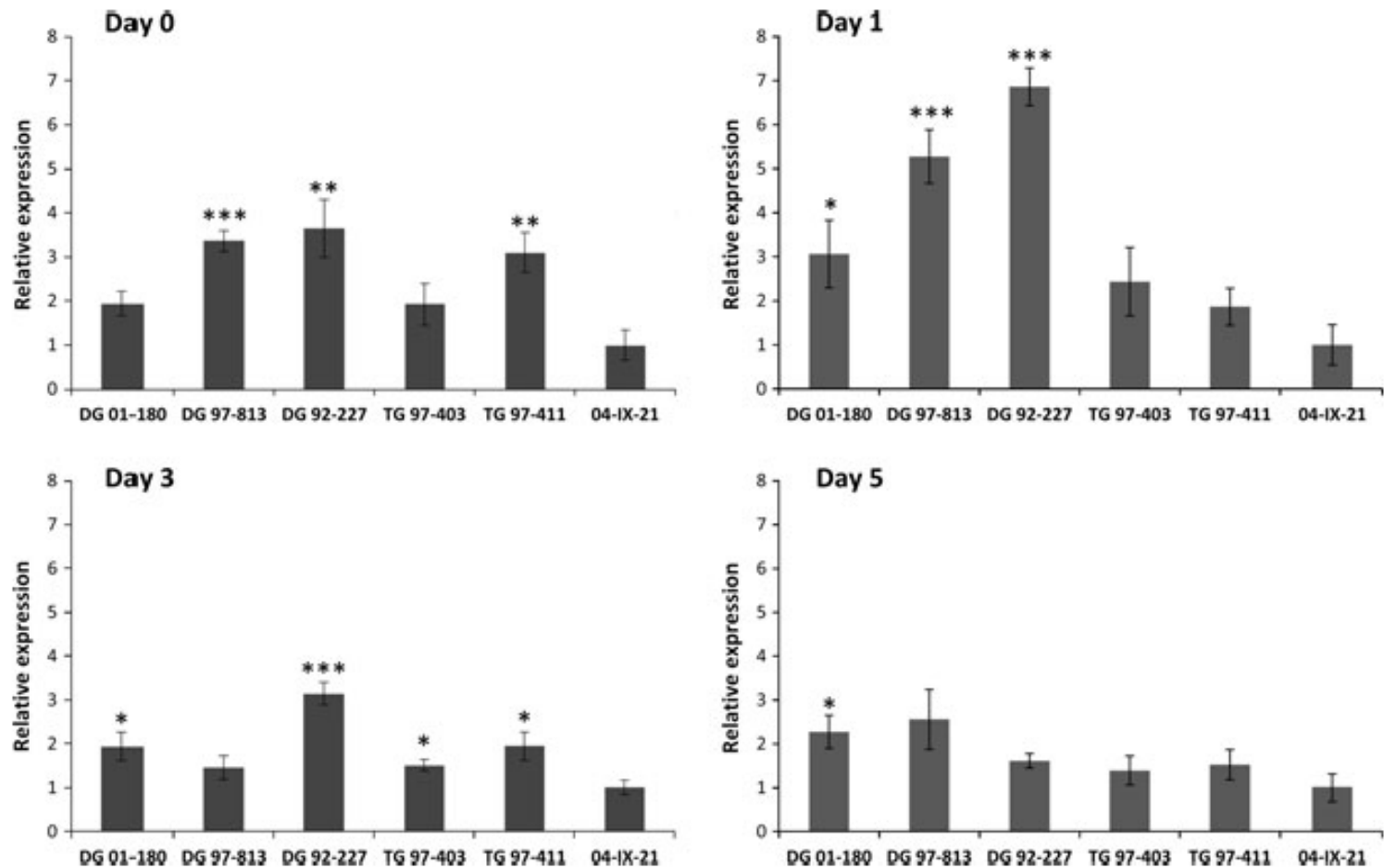


Figure 4 *Rpi-phu1* gene expression in three diploid (DG 01-180, DG 97-813, DG 92-227) and three tetraploid (TG 97-403, TG 97-411, 04-IX-21) potato lines inoculated with *Phytophthora infestans*. Data are average relative expression values of five biological replicates. Expression in genotype 04-IX-21 was the baseline against which all other genotypes were compared. Leaf samples were collected on day 0 (before inoculation) and 1, 3 and 5 days post-inoculation. Significant differences using a *t*-test for the $\Delta\Delta C_t$ values are indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Bars correspond to standard errors.

Plant material

diploid (2x)

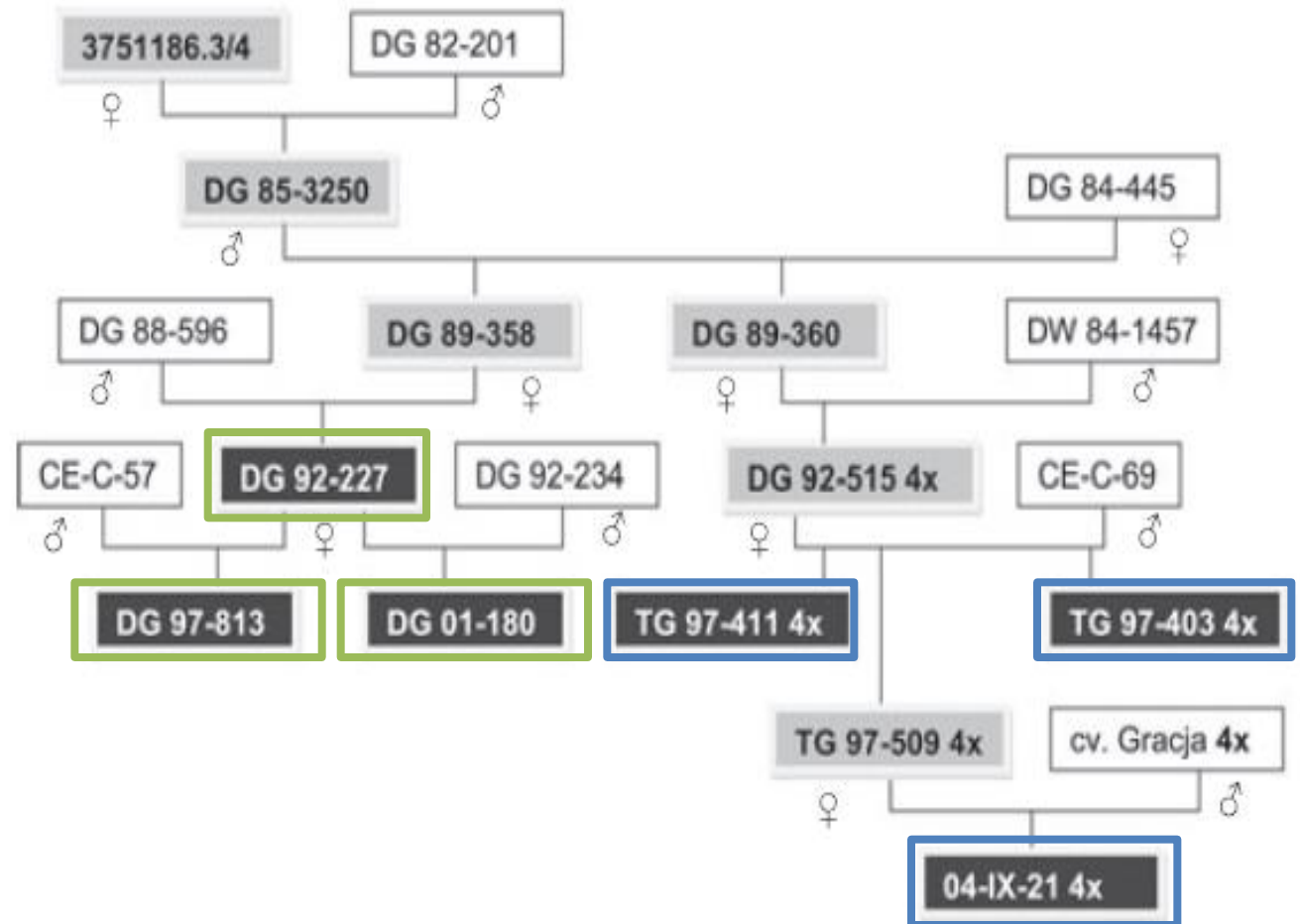
tetraploid (4x)

Donor genome

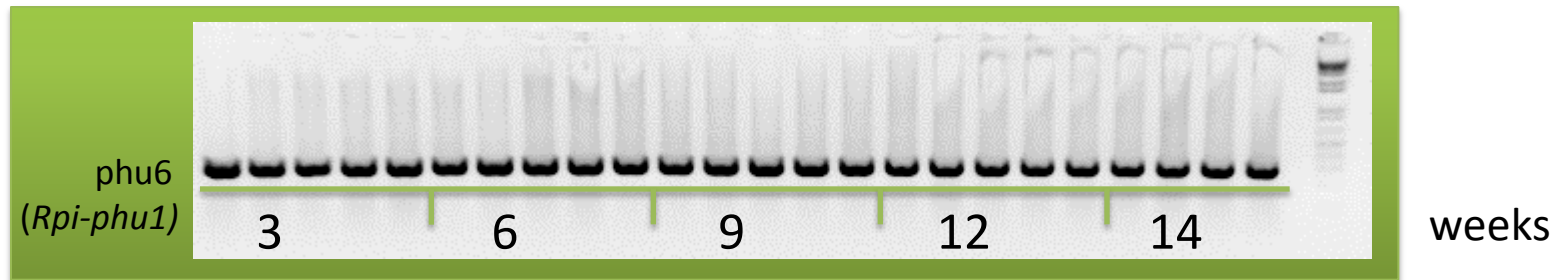
12.5%

6.25%

3.12%



Plant age and *Rpi-phu1* gene expression



- Gene *Rpi-phu1* was expressed in all plants
- All plants were resistant

Plant age

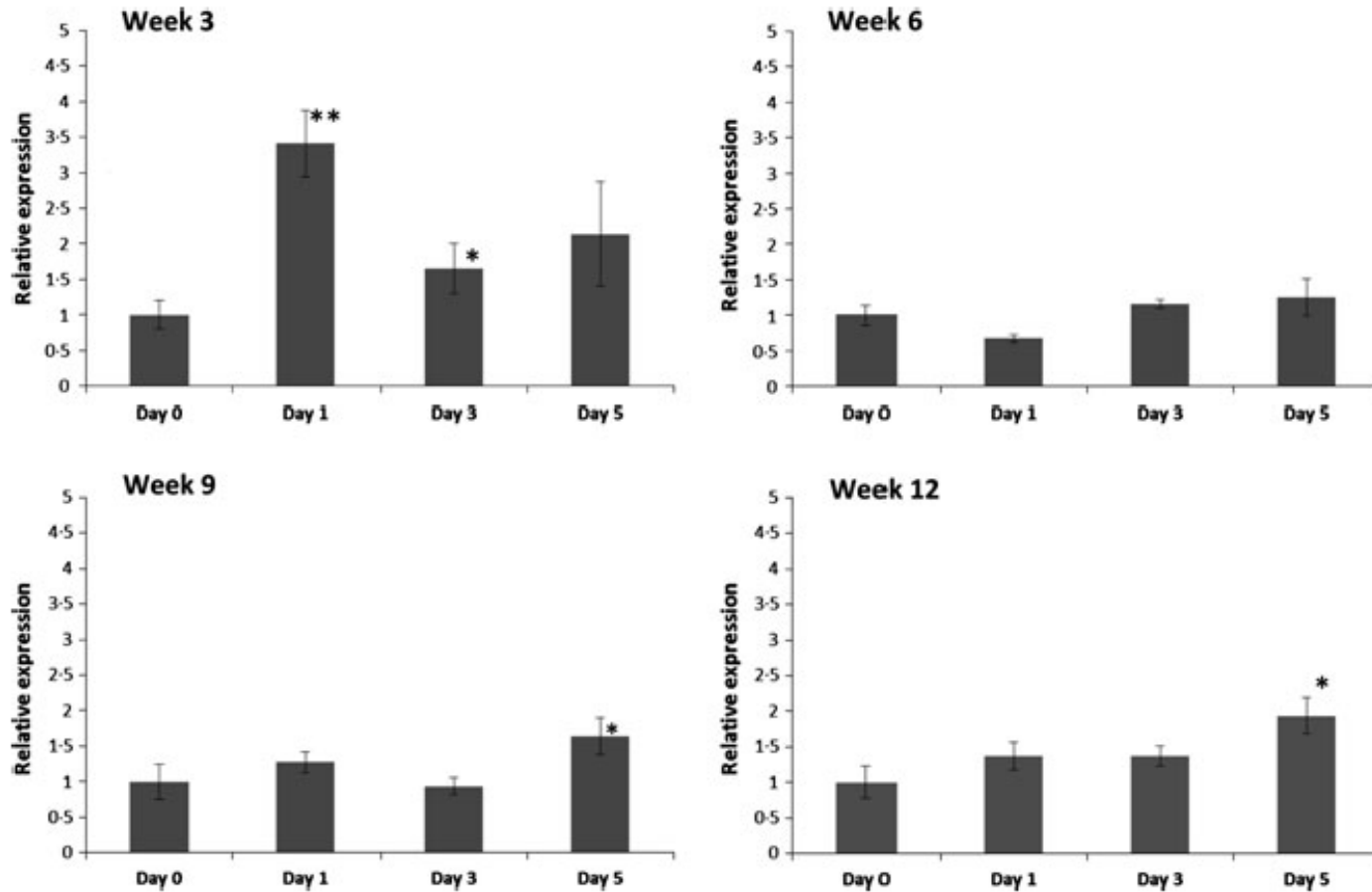


Figure 5 *Rpi-phu1* gene expression in potato line 04-IX-21 plants inoculated with *Phytophthora infestans* at different ages (3–12 weeks). Data are average relative expression values of five biological replicates. Leaf samples were collected on day 0 (before inoculation) and 1, 3 and 5 days post-inoculation. Day 0 is the baseline against which all other time points were compared. Significant differences using a *t*-test for the $\Delta\Delta C_t$ values are indicated as follows: * $P < 0.05$, ** $P < 0.01$. Bars correspond to standard errors.

Plant age, day by day

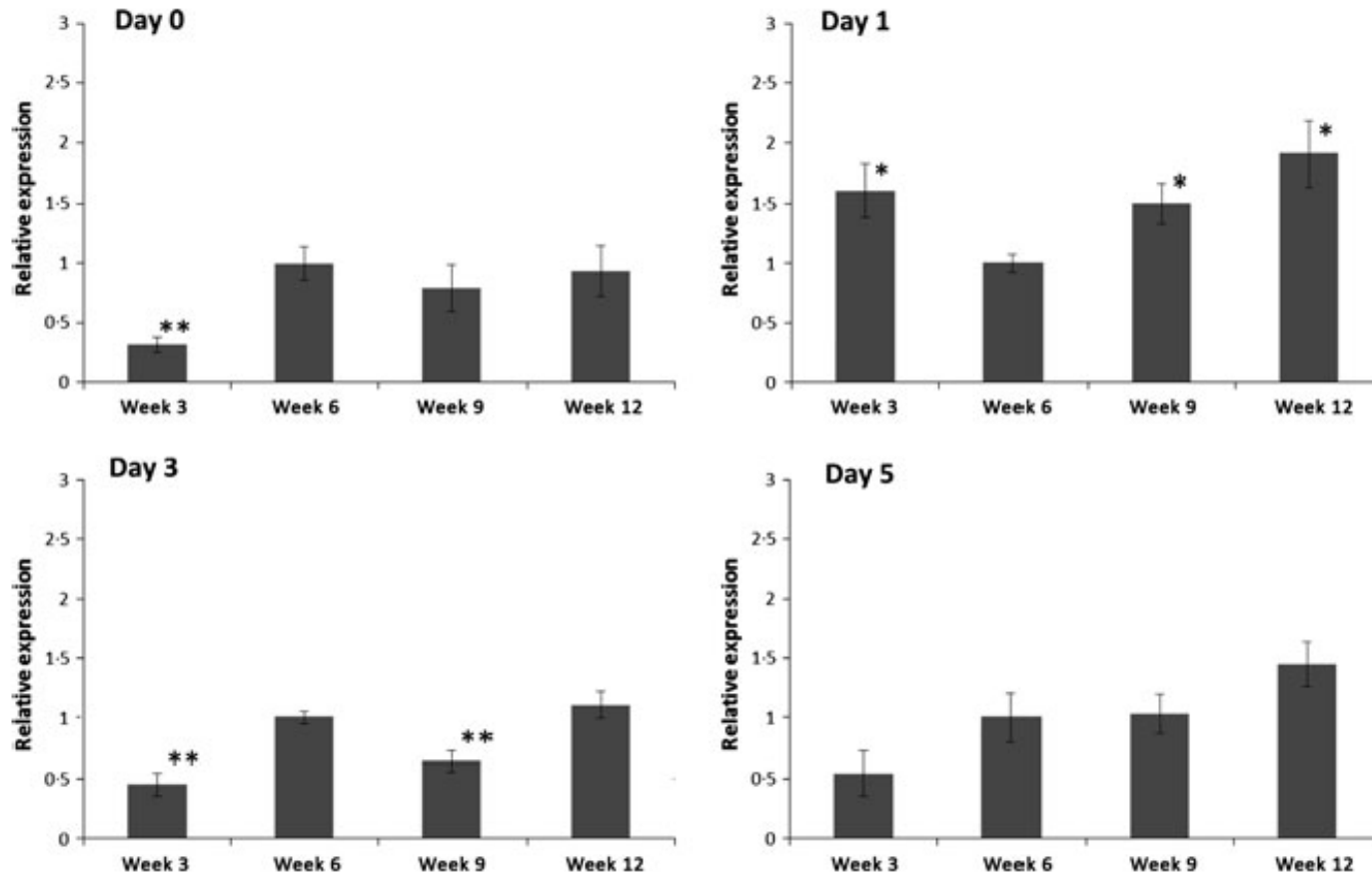
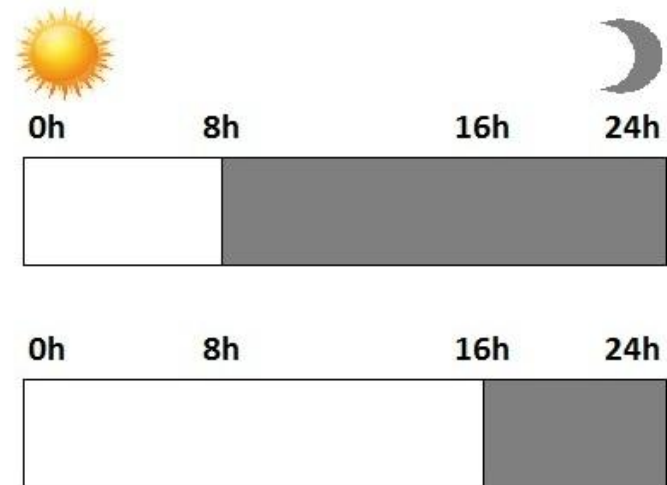


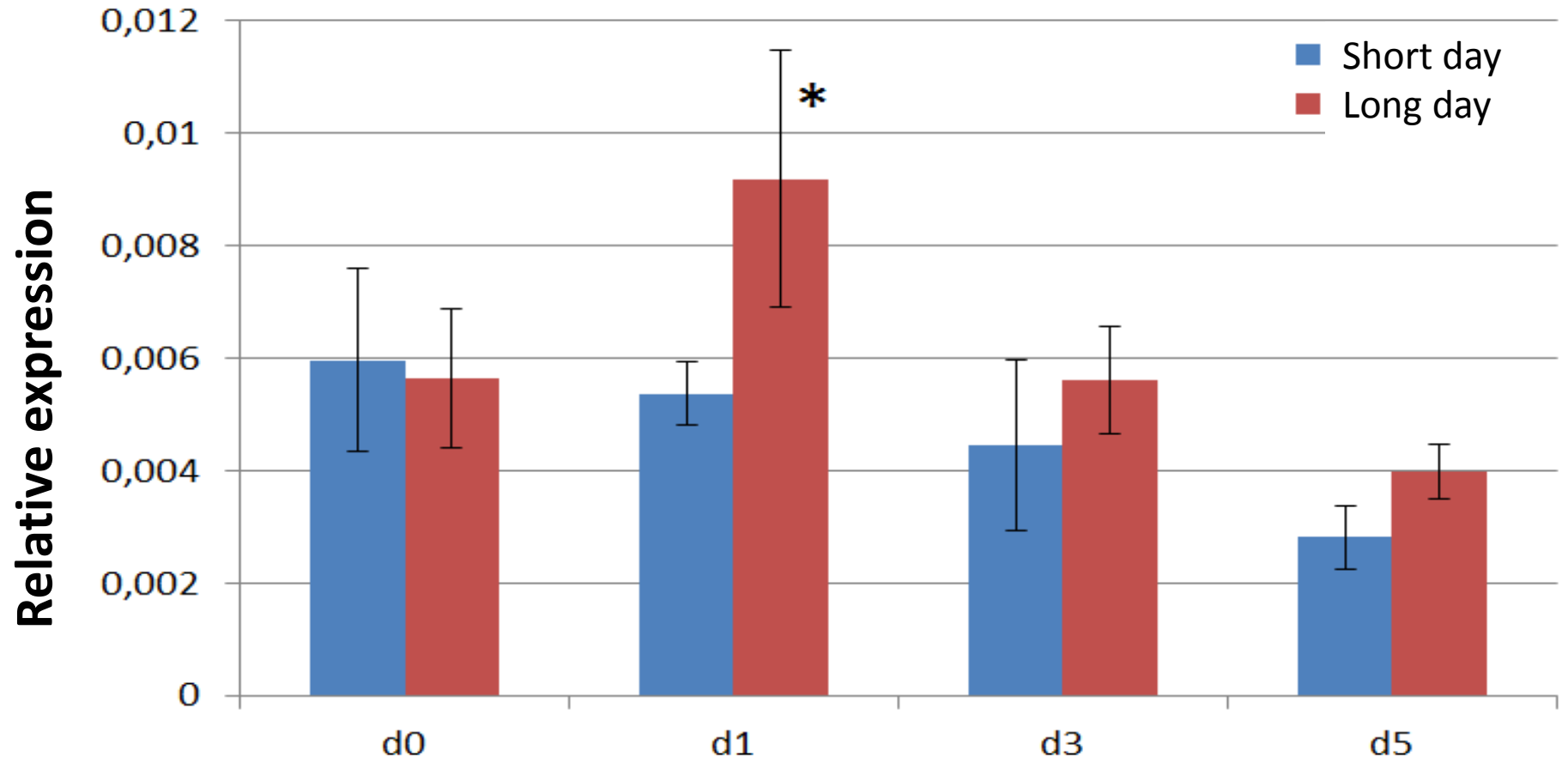
Figure 6 *Rpi-phu1* gene expression during aging of potato line 04-IX-21 plants inoculated with *Phytophthora infestans*. Data are average relative expression values of five biological replicates. Leaf samples were collected from plants after 3, 6, 9 and 12 weeks of growth at day 0 (before inoculation) and 1, 3 and 5 days post-inoculation. At every stage, expression in 6-week-old plants was the baseline against which all other ages were compared. Significant differences using a *t*-test for the $\Delta\Delta C_t$ values are indicated as follows: * $P \leq 0.05$, ** $P \leq 0.01$. Bars correspond to standard errors.

Daylength and *Rpi-phu1* gene expression

- *P. infestans*: MP324
- Plants
 - test: 04-IX-21 with *Rpi-phu1*
 - control: Craigs Royal
- Warunki
 - Short day (8h)
 - Long day (16h)



Day length and *Rpi-phu1* gene expression



Summary

- Compatible interaction is a challenge in case of *Rpi-phu1*
- *Rpi-phu1* expression is different in 2x and 4x potatoes (donor genome?) and upregulated 1 day post inoculation (2x)
- In 3-week-old plant *Rpi-phu1* expression is lower but it is enhanced after contact with pathogen

- No good explanation for the field observation so far
- Day length?
- Interaction of the above factors?
- *Avr-vnt1* may be switched off

Acknowledgements

J. Jones

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UK

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