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 x 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 *Avr-vnt1*
MP1976	Przecław/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	Zybiszów/ Poland	2020	EU41_A2	No data
MP1956	Węgrzce/Poland	2020	EU41_A2	No data
MP1942	Przecław/Poland	2020	EU37_A2	V1/V2
MP1940	Boguchwała/Poland	2020	EU37_A2	V1/V2
MP1938	Przecław/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	Czaple 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-mch1**)	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

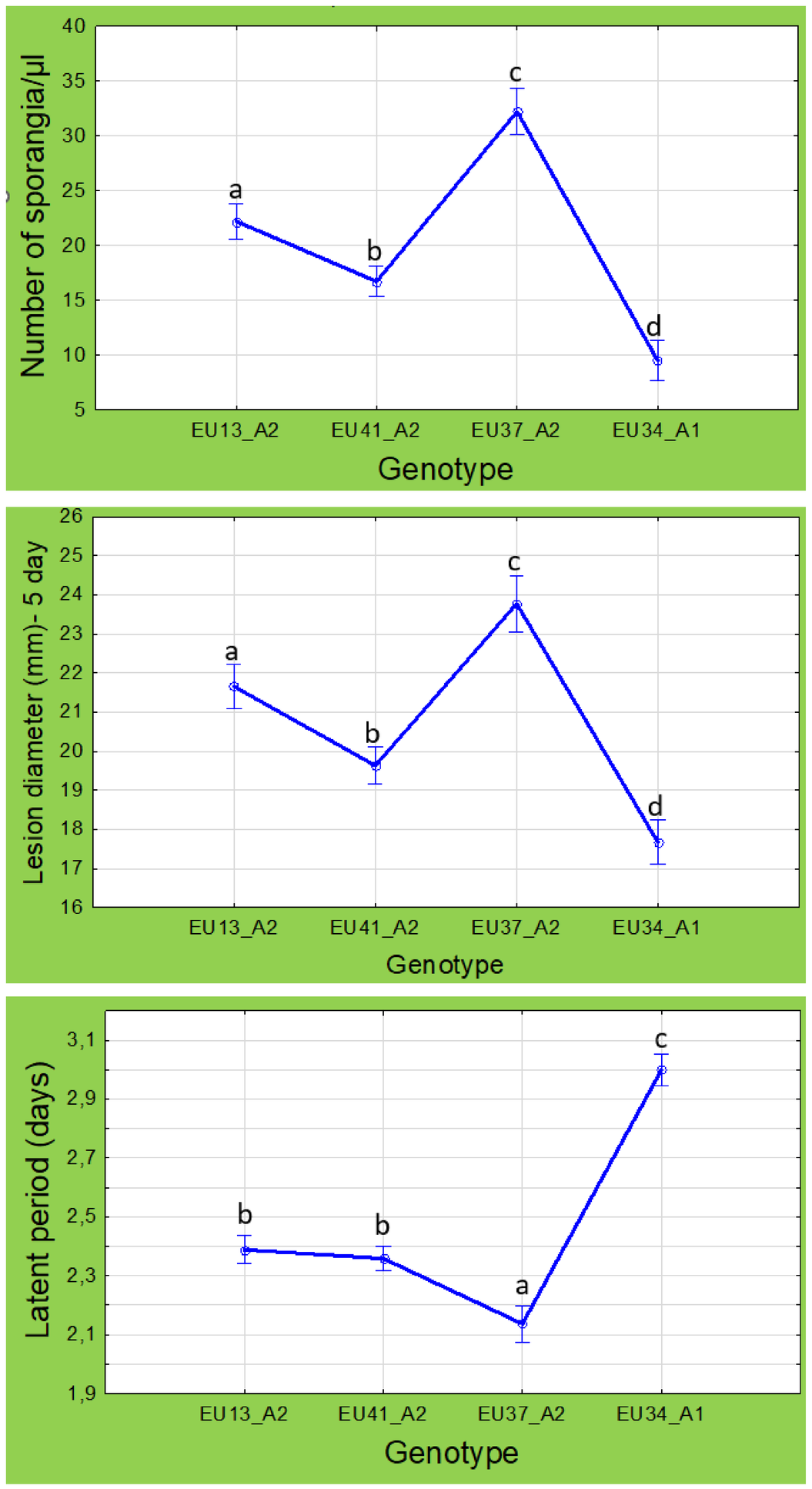

Figure 2. Aggressiveness of four *P. infestans* genotypes (EU_13A2, EU34_A1, EU37_A2 and EU41_A2) based on lesion diameter, latent period and number of sporangia produced by isolates from each genotype. Vertical bars represent 0.95 confidence intervals. The letters indicate statistically different groups, created based on ANOVA + Tukey's test.
a) Latent period (days) (Effect: F(3.163)=172.24, p=0.000)
b) Number of sporangia/µl (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 tasted for aggressiveness to ensure better representation of tested *P. infestans* genotypes.

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