



# Segregation for Avirulence/Virulence in *Puccinia striiformis* Based on an Experimental Genetic System Using *Berberis vulgaris*



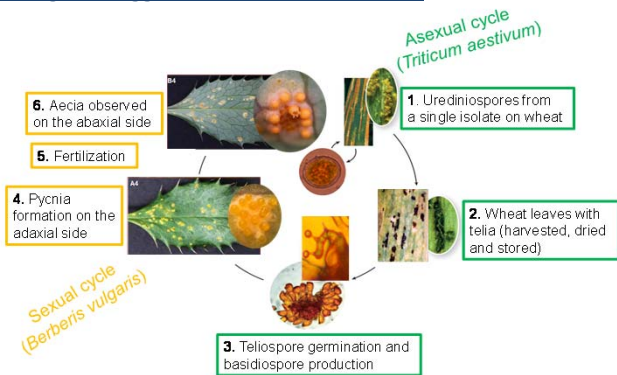
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## Objectives

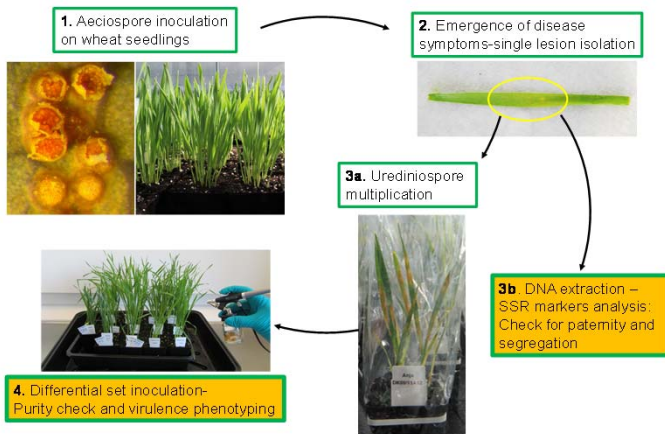
- To establish a system for sexual reproduction for *P. striiformis* by using *B. vulgaris* as alternate host (Rodriguez-Algaba et al., 2014)
- To use this system to obtain S1 progeny isolates by selfing a parental isolate
- To apply single sequence repeat (SSR) markers in sexual recombination studies of *P. striiformis* for paternity confirmation of the S1 progenies
- To analyze the inheritance of avirulence/virulence in the S1 generation of progeny isolates

## Materials and Methods

### 1. Selfing of an aggressive *P. striiformis* isolate:



### 2. Aeciospore recovery, SSR analysis and Virulence Phenotyping:



## Results

### 2. Segregation for avirulence/virulence:

- All S1 progenies were virulent for 14 of 15 host genes where the parental isolate was virulent, indicating that the parental isolate was homozygous virulent for these loci (Table 2)
- No segregation was observed for 5 of 6 genes for which the parental isolate was avirulent, suggesting that the parental isolate was homozygous avirulent for these loci (Table 2)

Table 2. Virulence phenotype of parental isolate (DK09/11) and 16 S1 progeny isolates (A2 to A19)

| Isolates                         | Virulence loci |   |   |   |   |   |   |                |   |    |    |      |    |    |    |    |    |    |     |     |     |
|----------------------------------|----------------|---|---|---|---|---|---|----------------|---|----|----|------|----|----|----|----|----|----|-----|-----|-----|
|                                  | 1              | 2 | 3 | 4 | 5 | 6 | 7 | 8              | 9 | 10 | 15 | 17   | 24 | 25 | 27 | 32 | Sd | Su | Sp  | AvS | Amb |
| DK09/11, A3, A4, A6, A7, A9, A12 | 1              | 2 | 3 | 4 | - | 6 | 7 | - <sup>a</sup> | 9 | -  | -  | (17) | -  | 25 | -  | 32 | Sd | Su | Sp  | AvS | Amb |
| A2, A10, A13, A14, A16, A18, A19 | 1              | 2 | 3 | 4 | - | 6 | 7 | - <sup>a</sup> | 9 | -  | -  | -    | 25 | -  | 32 | Sd | Su | Sp | AvS | Amb |     |
| A5, A8, A15                      | 1              | 2 | 3 | 4 | - | 6 | 7 | - <sup>b</sup> | 9 | -  | -  | -    | 25 | -  | 32 | Sd | Su | Sp | AvS | Amb |     |

<sup>a</sup> Yr8-avirulence revealed by infection type 0 (no chlorosis)  
<sup>b</sup> Yr8-avirulence revealed by infection type 1-2 (significant chlorotic flecks)

- Segregation was observed with respect to Yr8 where the parental isolate gave infection type (IT) 0 (Fig. 1B)
- Among the S1 progenies, IT 0 and IT 1-2 (Fig. 1C) developed on both Yr8 host genotypes, suggesting that the parental isolate was heterozygous for two different Avr8 alleles resulting in different, but clearly avirulent phenotypes

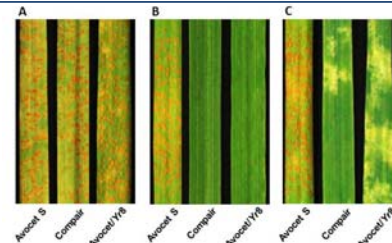


Fig. 1. Observed infection types (IT) on a 0-9 scale for reference and progeny isolates on Yr8 resistant wheat cultivars, i.e. Compare and Avocet8 and Avocet S as a susceptible control. A: reference isolate displaying a compatible interaction for Yr8 (IT 0). B: parental isolate displaying an incompatible interaction (IT 1-2) on the Yr8 resistant cultivars. C: A5 progeny isolate displaying an incompatible interaction (IT 1-2) on the Yr8 resistant cultivars.

- Segregation was also observed with respect to virulence for Yr17 where the parental isolate gave IT 5-6 (Fig. 2B)
- Among the S1 progenies, two distinct phenotypes, IT 2-4 (Fig. 2C) and 5-6, were observed on the Yr17 genotypes, the latter being similar to the parental isolate (Fig. 2B)
- This unusual segregation pattern could be explained by the presence of a heterozygous modifier gene in the parental isolate influencing the phenotypic expression of avirulence

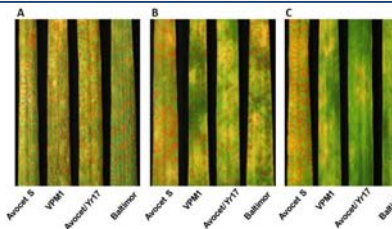


Fig. 2. Observed infection types (IT) on a 0-9 scale for reference and progeny isolates on Yr17 resistant wheat cultivars, i.e. YrM1, Avocet Yr17 and Baltmor. Avocet S is included as a susceptible control. A: reference isolate displaying a compatible interaction (IT 7-8). B: parental isolate displaying IT 6 on the Yr17 resistant cultivars. C: A5 progeny isolate displaying an incompatible interaction (IT 2-3) on the Yr17 resistant cultivars

## Results

### 1. Segregation of SSR markers:

- Genotyping with 42 SSR markers confirmed the parental origin of the progeny isolates
- Of the 42 analyzed loci, 24 were homozygous and 15 were heterozygous in the parental isolate
- Fourteen loci revealed segregation in the progenies resulting in 11 new multilocus genotypes (MLGs) (Table 1)

Table 1. Sizes of alleles at heterozygous SSR loci in parental isolate (DK09/11) and segregation of these in selfing progenies (A2 to A19) of *P. striiformis*. Sizes of alleles are given in bp. Abbreviations: a1 - allele 1, a2-allele 2, MLG-multilocus genotype, n.d.-not detected.

| MLG                | Isolate(s) | SSR loci |      |       |       |       |       |      |        |        |        |        |        |        |        |        | No. of segregated markers |     |     |      |      |     |     |     |     |     |     |      |      |    |
|--------------------|------------|----------|------|-------|-------|-------|-------|------|--------|--------|--------|--------|--------|--------|--------|--------|---------------------------|-----|-----|------|------|-----|-----|-----|-----|-----|-----|------|------|----|
|                    |            | RJN6     | RJN8 | RJN11 | RJN13 | RJN20 | RJN24 | WU12 | SUNP10 | SUNP12 | SUNP13 | SUNP14 | SUNP15 | SUNP16 | SUNP17 | SUNP18 |                           |     |     |      |      |     |     |     |     |     |     |      |      |    |
| DK09/11            | 315        | 318      | 307  | 316   | 176   | 182   | 147   | 150  | 284    | 287    | 284    | 286    | 332    | 309    | 345    | 421    | 203                       | 206 | 498 | 502  | 188  | 203 | 336 | 341 | 315 | 321 | 352 | 364  | -    |    |
| A2, A19            | 315        | 318      | 307  | 316   | 176   | 182   | 147   | 150  | 284    | 287    | 284    | 286    | 332    | 309    | 345    | 421    | 203                       | 206 | 498 | 502  | 188  | 203 | 336 | 341 | 315 | 321 | 352 | 364  | 4    |    |
| A8                 | 315        | 318      | 316  | 176   | 182   | 147   | 147   | 150  | 284    | 287    | 284    | 286    | 332    | 309    | 345    | 421    | 203                       | 206 | 498 | 498  | 188  | 203 | 336 | 341 | 321 | 352 | 364 | 5    | 5    |    |
| A3, A4             | 315        | 318      | 307  | 316   | 176   | 182   | 147   | 147  | 150    | 284    | 284    | 286    | 332    | 309    | 345    | 394    | 421                       | 203 | 206 | 498  | 498  | 188 | 203 | 336 | 336 | 315 | 321 | 352  | 364  | 6  |
| A7                 | 315        | 318      | 316  | 176   | 182   | 147   | 150   | 284  | 284    | 286    | 286    | 286    | 332    | 309    | 345    | 394    | 421                       | 203 | 206 | 498  | 498  | 188 | 203 | 341 | 341 | 315 | 321 | 352  | 364  | 6  |
| A5                 | 315        | 315      | 307  | 307   | 176   | 182   | 147   | 150  | 284    | 284    | 284    | 286    | 332    | 309    | 309    | 394    | 421                       | 203 | 206 | 498  | 498  | 188 | 188 | 336 | 336 | 315 | 321 | 352  | 364  | 8  |
| A6                 | 318        | 318      | 316  | 176   | 176   | 147   | 150   | 284  | 287    | 284    | 286    | 286    | 332    | 345    | 345    | n.d.   | n.d.                      | 206 | 206 | n.d. | n.d. | 188 | 188 | 336 | 336 | 315 | 321 | n.d. | n.d. | 8  |
| A10, A14, A15, A18 | 315        | 315      | 307  | 307   | 176   | 176   | 147   | 150  | 284    | 284    | 284    | 286    | 332    | 309    | 309    | 394    | 421                       | 203 | 206 | 498  | 498  | 188 | 188 | 336 | 336 | 315 | 321 | 352  | 364  | 9  |
| A12                | 315        | 318      | 316  | 176   | 176   | 150   | 150   | 284  | 284    | 284    | 286    | 286    | 332    | 345    | 345    | 394    | 394                       | 206 | 206 | 498  | 502  | 188 | 188 | 336 | 341 | 315 | 321 | 352  | 364  | 10 |
| A13                | 318        | 318      | 307  | 307   | 182   | 182   | 147   | 147  | 284    | 284    | 284    | 286    | 332    | 309    | 309    | 394    | 421                       | 203 | 206 | 498  | 498  | 188 | 203 | 341 | 341 | 315 | 321 | 352  | 364  | 10 |
| A9                 | 315        | 318      | 316  | 176   | 176   | 150   | 150   | 284  | 284    | 284    | 286    | 286    | 332    | 345    | 345    | 394    | 394                       | 206 | 206 | 498  | 502  | 188 | 188 | 336 | 341 | 315 | 321 | 352  | 364  | 11 |
| A11, A5            | 315        | 318      | 316  | 176   | 176   | 150   | 150   | 284  | 284    | 284    | 286    | 286    | 332    | 345    | 345    | 394    | 394                       | 206 | 206 | n.d. | n.d. | 188 | 188 | 341 | 341 | 315 | 321 | 352  | 364  | 12 |

## Conclusions

- A successful establishment of a system for genetic analysis of *P. striiformis*, using *B. vulgaris* as alternate host for completion of its sexual cycle was reported
- Recombination of alleles at heterozygous SSR loci confirmed sexual reproduction and validated that putative progeny isolates were indeed derived from the parental isolate
- By means of a selfing, it was possible to study the genetics of virulence/avirulence and to reveal the genotype of the parental isolate with regards to specific Avr genes
- Segregation for host-pathogen interaction specificity observed in two avirulence loci, i.e. Yr8, Yr17
- In order to resolve the genetics of Yr17 locus, additional progeny isolates are being produced from the parental isolate, and selfings of additional *P. striiformis* isolates diverging in level of aggressiveness are in progress

## Acknowledgements

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References:

Rodriguez-Algaba, J., Walter, S., Sørensen, C. K., Hovmøller, M. S. & Justesen, A. F. Sexual structures and recombination of the wheat rust fungus *Puccinia striiformis* on *Berberis vulgaris*. *Fungal Genetics and Biology* 70, 77-85 (2014).

