

Functional characterization and evolutionary analysis of effector candidate genes in the wheat pathogen *Zymoseptoria tritici*

By Corinn Sophia Small



Thesis written in
Environmental Genomics group
Christian-Albrechts-University of Kiel

Septoria Tritici Blotch in wheat

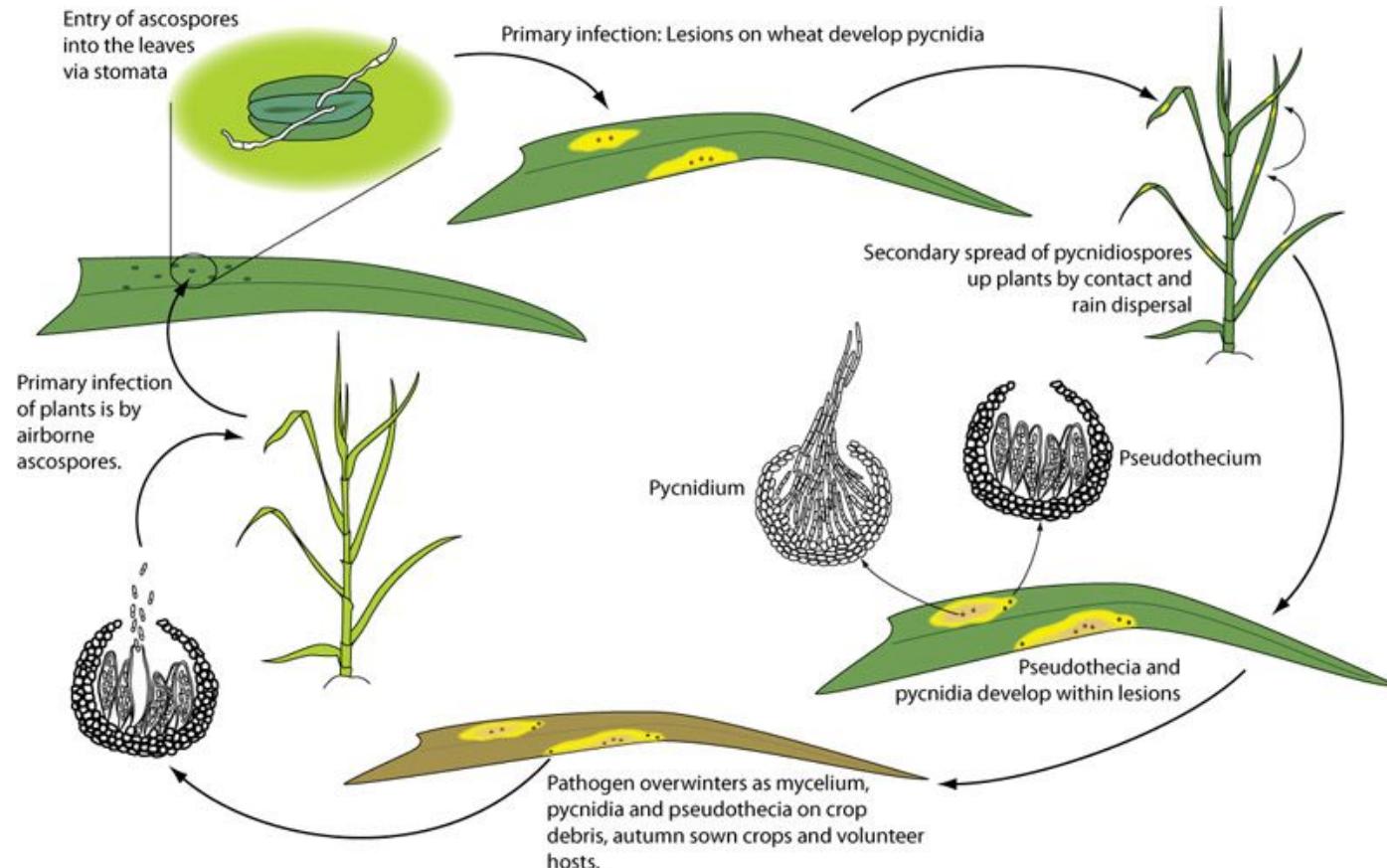


(Ponomarenko, Goodwin, and Kema, 2011); apsnets.org

The title here fits better to the next slide

Zymoseptoria tritici colonizes live plant tissue to then induce necrosis

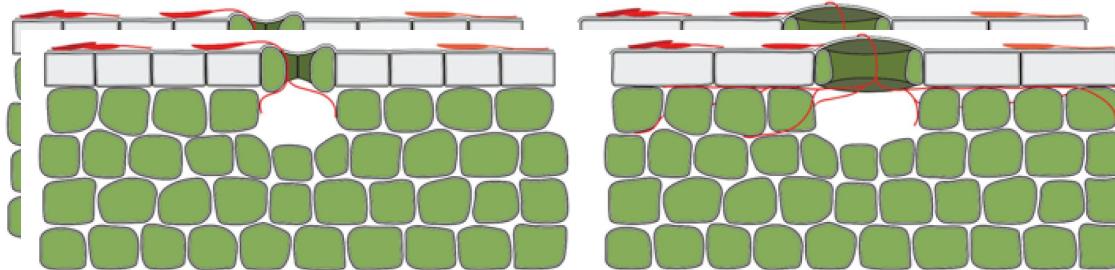
Alternative title: *Zymoseptoria tritici* - hemibiotroph wheat pathogen with complex life cycle



Zymoseptoria tritici core infection stages require different effector profiles

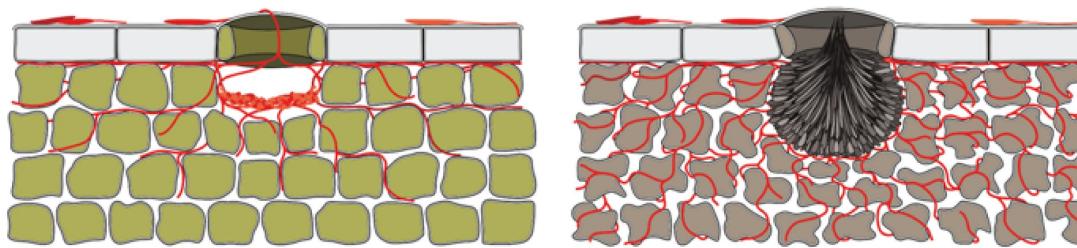
Use title from previous slide here and use the current title as “kind of conclusion”

Figure two times?



Stage A: Infection Establishment

Stage B: Biotrophic Growth



Stage C: Lifestyle Transition

Stage D: Necrotrophy and Reproduction

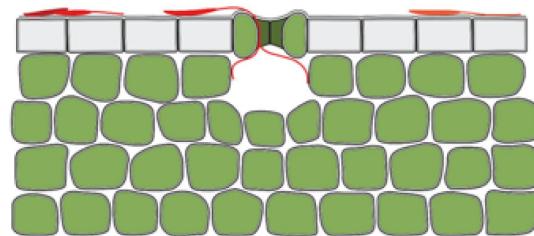
Move conclusion here, maybe animation?



***Zymoseptoria spp.* comparative transcriptomics identified candidate effector genes**

(Haueisen et al., 2017)

- compared sister species during infection Stage A: found several differentially expressed genes



Stage A: Infection Establishment

Maybe show here again the comic of stage A?

Zymoseptoria spp. comparative transcriptomics identified candidate effector genes:

(Haueisen et al., 2017)

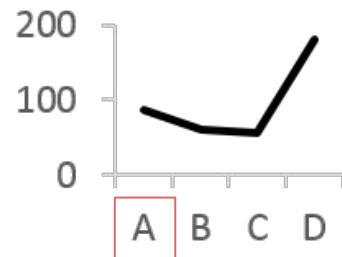
- compared sister species during infection Stage A: found several differentially expressed genes
- That had distinct expression profiles in Z. tritici corresponding to core stages

Zymoseptoria spp. comparative transcriptomics identified candidate effector genes:

(Haueisen et al., 2017)

- compared sister species during infection Stage A: found several differentially expressed genes
- That had distinct expression profiles in *Z. tritici* corresponding to core stages

Zt09_chr11_00287: ↑ Zt compared to Zp13 + Za17



That's nice!

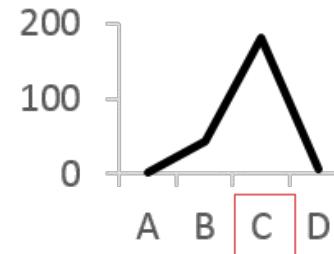
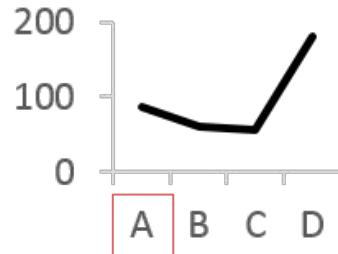
Zymoseptoria spp. comparative transcriptomics identified candidate effector genes:

(Haueisen et al., 2017)

- compared sister species during infection Stage A: found several differentially expressed genes
- That had distinct expression profiles in *Z. tritici* corresponding to core stages

Zt09_chr11_00287: ↑ Zt compared to Zp13 + Za17

Just make sure that it's still clear that
the differential expression was at stage A
- No need to change the slide, only during talk
Zt09_chr11_00525: ↑ Zp13 compared to Zt



Aims:

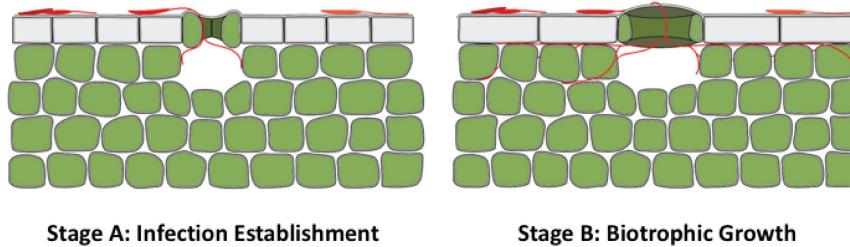
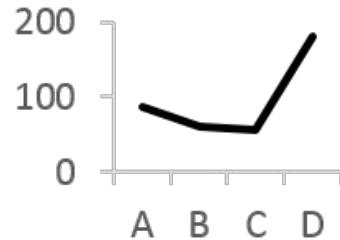
- 1) To study the functional roles of the candidate genes during wheat infection using:
 - (1) Gene deletions
 - (2) Gene over-expression

Aims:

- 1) To study the functional roles of the candidate genes during wheat infection using:
 - (1) Gene deletions
 - (2) Gene over-expression
- 2) To analyze the genetic diversity of the genes +/- 10 kb using population data:
 - (1) Within *Z. tritici*
 - (2) Between *Z. tritici* and *Z. ardabiliae*

Hypotheses:

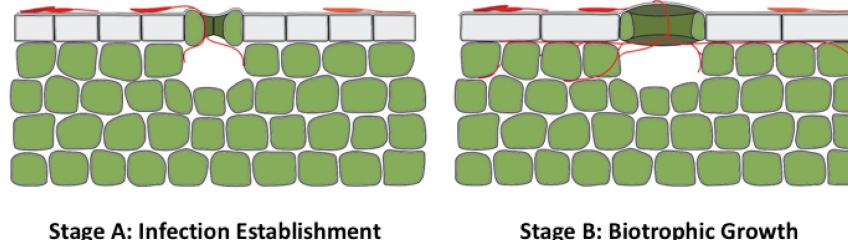
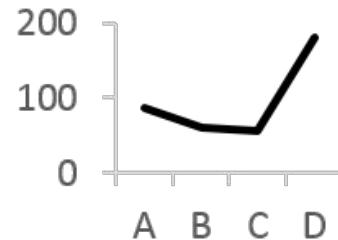
Zt09_chr11_00287 = host-specific effector involved in facilitating infection and/or growth



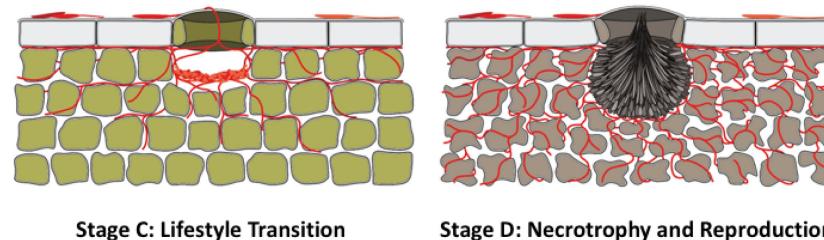
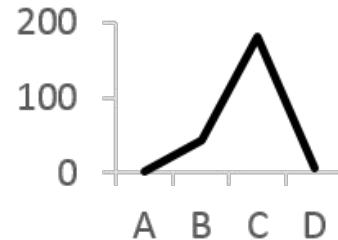
That's very good!!

Hypotheses:

Zt09_chr11_00287 = host-specific effector involved in facilitating infection and/or growth

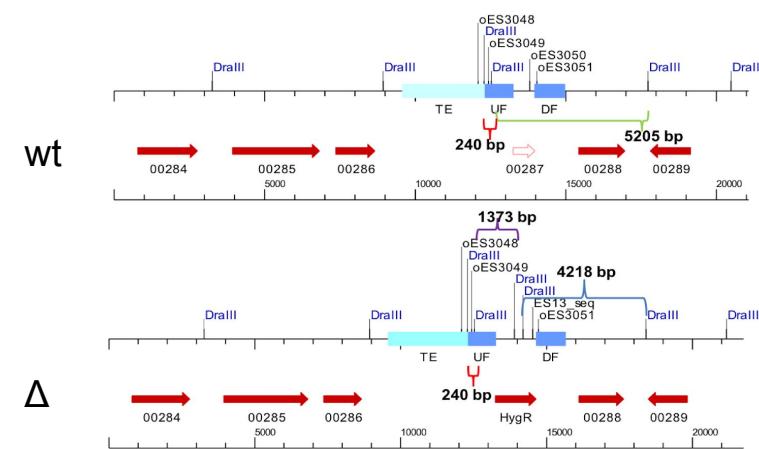
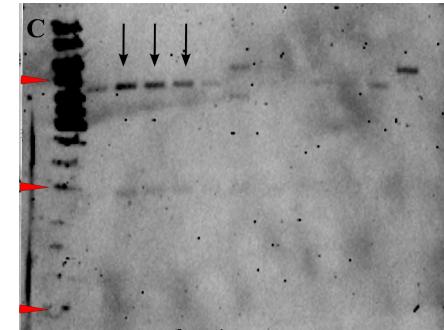
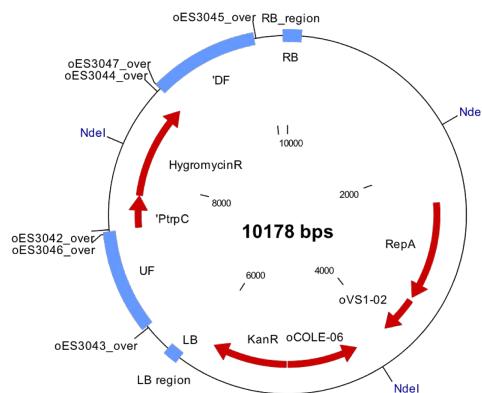
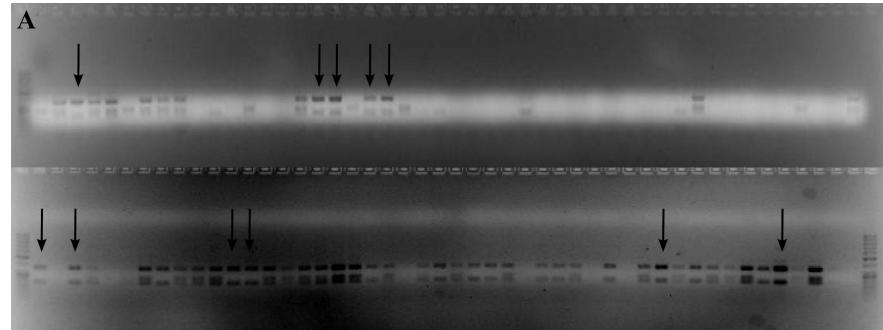


Zt09_chr11_00525 = necrotrophic effector involved in necrosis and/or pycnidia development

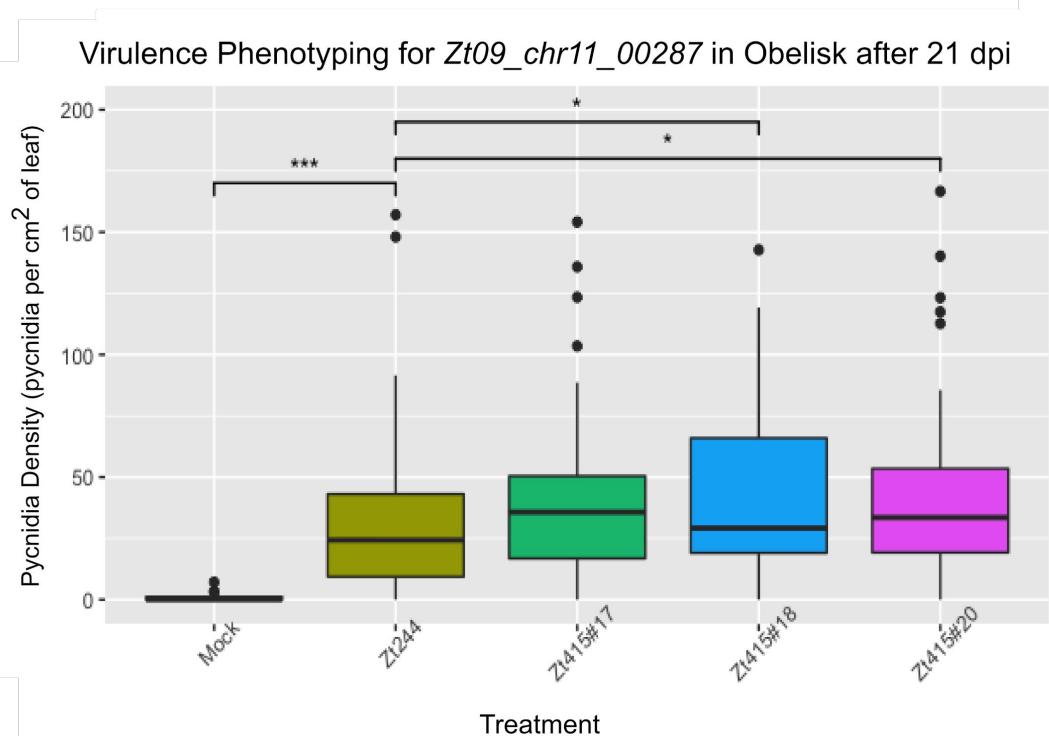


It's possible people ask what fragment sizes where expected for correct mutants - if you find time, add it on the slide.

Zt415 confirmed mutant for deletion Zt09_chr11_00287

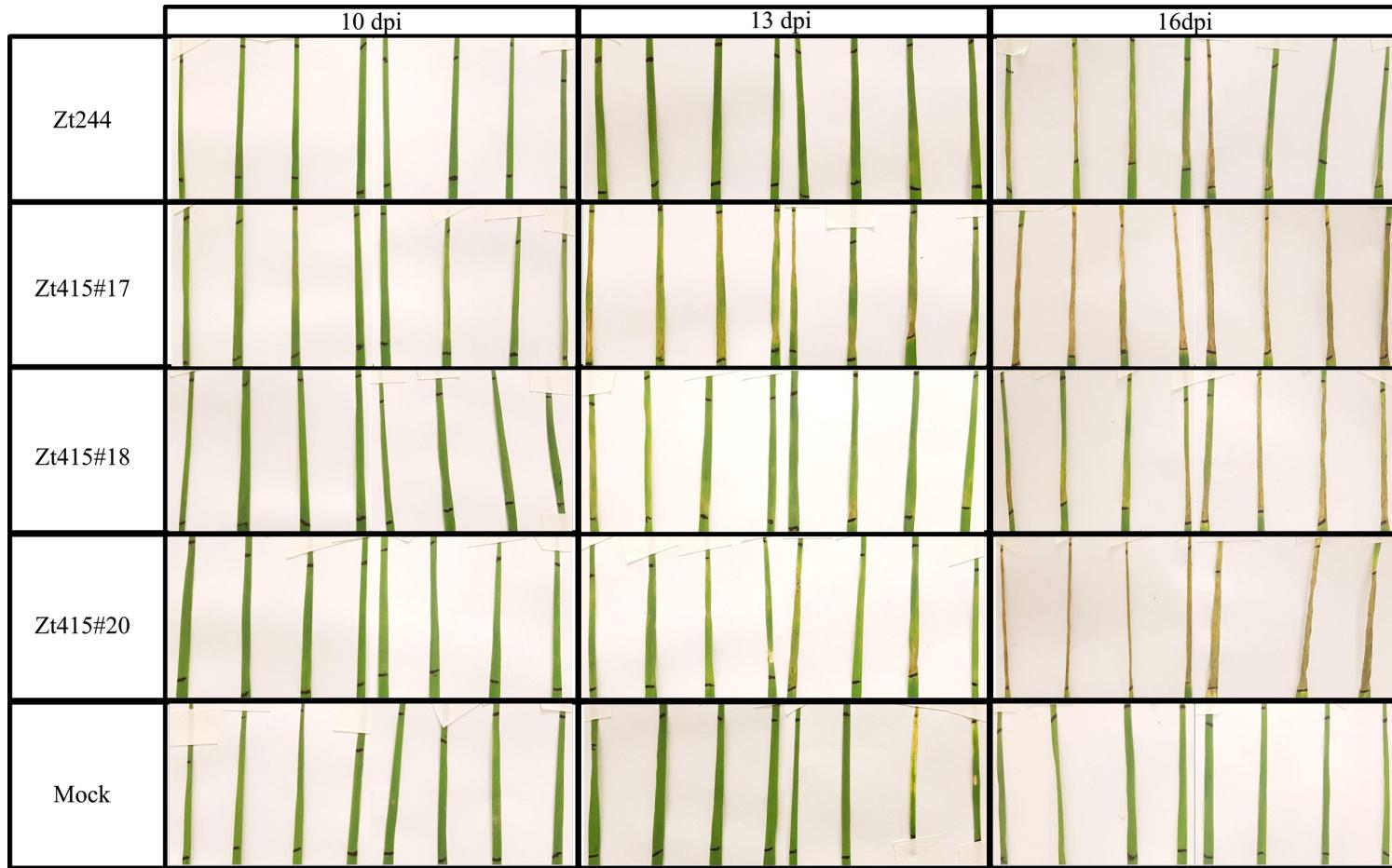


Zt415 exhibits a hypervirulent phenotype vs wildtype

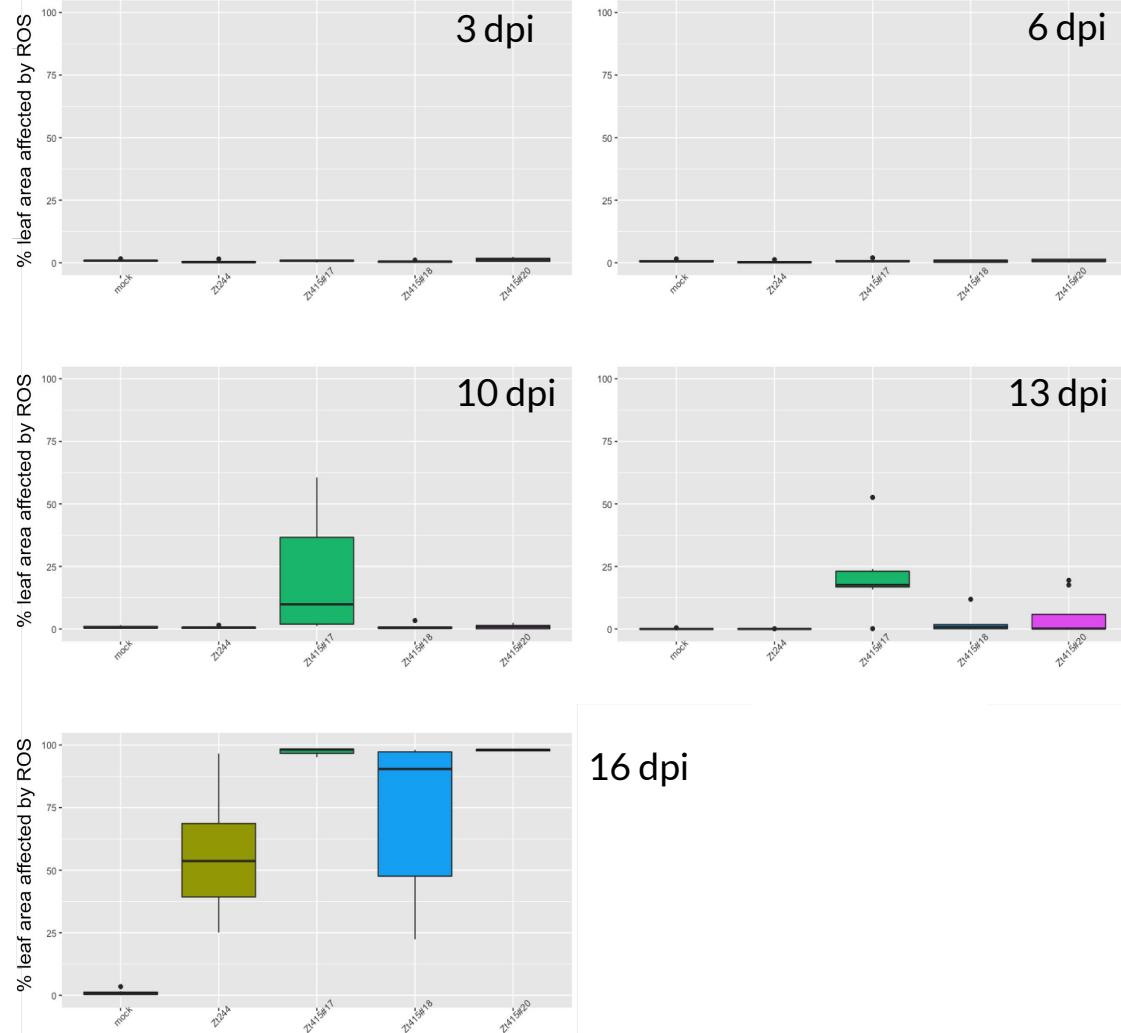


You have space to show the image (infected leaf) from slide 2 again - to remind/show people what trait you compare

Zt415 exhibits earlier necrosis development vs wildtype



Zt415 exhibits earlier accumulation of ROS

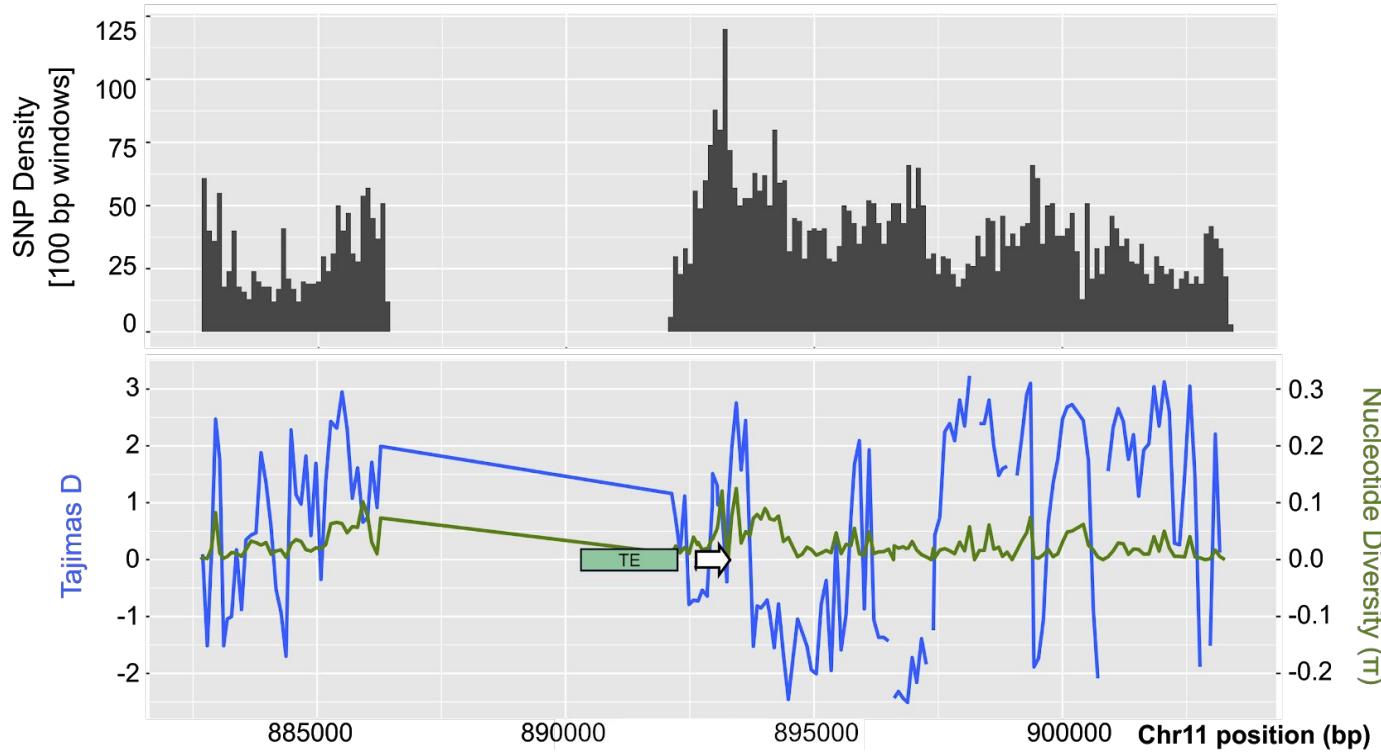


It looks good till this point. Much much better than earlier.
Nice work. It'll be fine.
Don't forget acknowledgments - thank Eva for the opportunity ...
and her and Katja for reviewing your thesis.

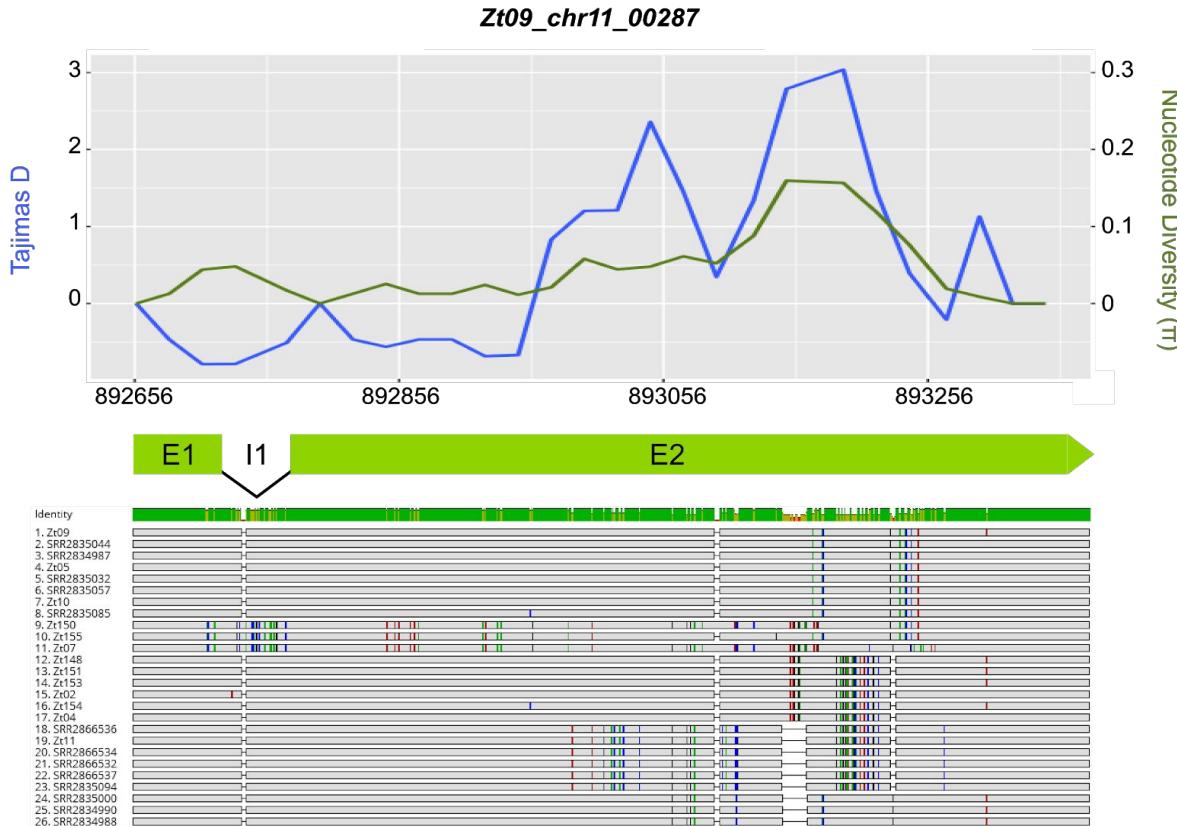
Results

Virulence phenotyping:

Region surrounding *Zt_chr11_00287* shows high levels of nucleotide variation



Zt_chr11_00287 is evolving under both purifying



Alignment translated

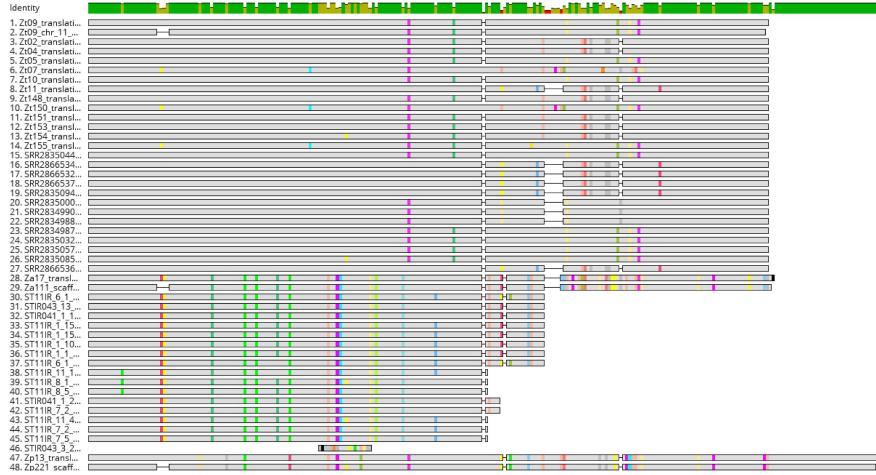


Figure 24. Translation of spliced Zt09_chr11_00287 alignment in *Z. tritici*, *Z. ardabiliiae*, and *Z. psuedotritici* isolates. Homologous sequences were aligned using MUSCLE (EMBL-EBI) and translated using the reference frame. Amino acid changes from non-synonymous mutations are highlighted. Alignment indicates more phenotypic variation between species than within; changes are seen at particular domains. Deletions within the Australian *Z. tritici* subgroup and in *Z. ardabiliiae* may have occurred. Insertions may have led to the extension of the *Z. pseudotritici* peptide sequence.

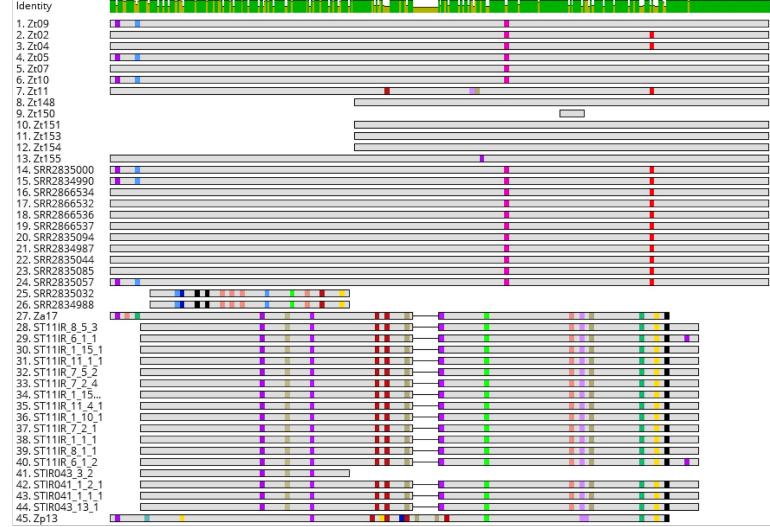


Figure 25. Translation of spliced Zt09_chr11_00525 alignment in *Z. tritici*, *Z. ardabiliiae*, and *Z. psuedotritici* isolates. Homologous sequences were aligned using MUSCLE (EMBL-EBI) and translated using the reference frame. Amino acid changes from non-synonymous mutations are highlighted. Alignment indicates more phenotypic variation between species than within, however there are a few phenotypic changes between species subgroups.

5.3 The role of the host specific candidate effector gene *Zt09_chr11_00287*

Avirulent?

the McDonald-Kreitmann test for *Zt09_chr11_00287* in *Z. tritici* and *Z. ardabiliiae* isolates resulted in the comparison of $dN/dS > pN/pS$,

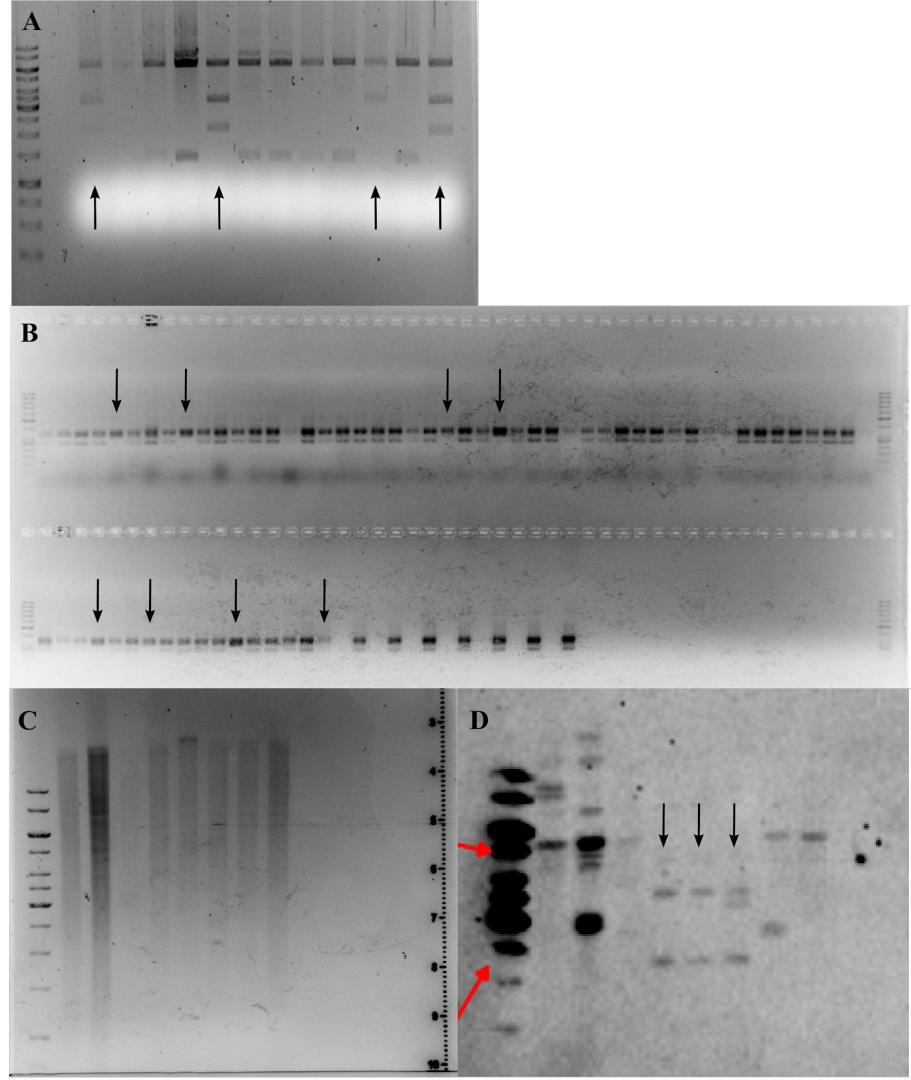
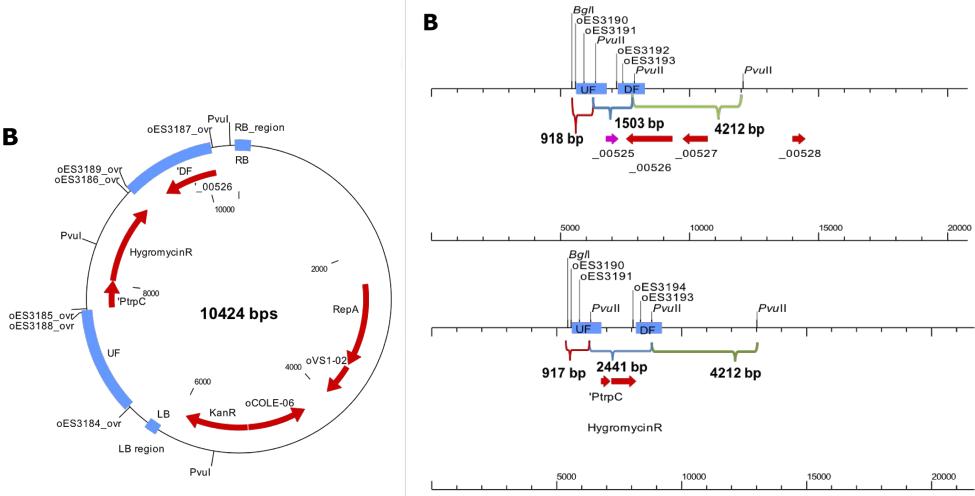
the potential role of the necrotrophic/avirulence effector candidate *Zt09_chr11_00525*

A McDonald-Kreitmann test showed dN/dS to be less than pN/pS (**dN/dS= 0.4 ; pN/pS= 1.85**), suggesting that previous orthologs of *Zt09_chr11_00525* have also evolved under purifying selection, rather than directional.

1. Plasmid design, gibson assembly & generation of *Z. tritici* mutants

a. Confirmed strains for:

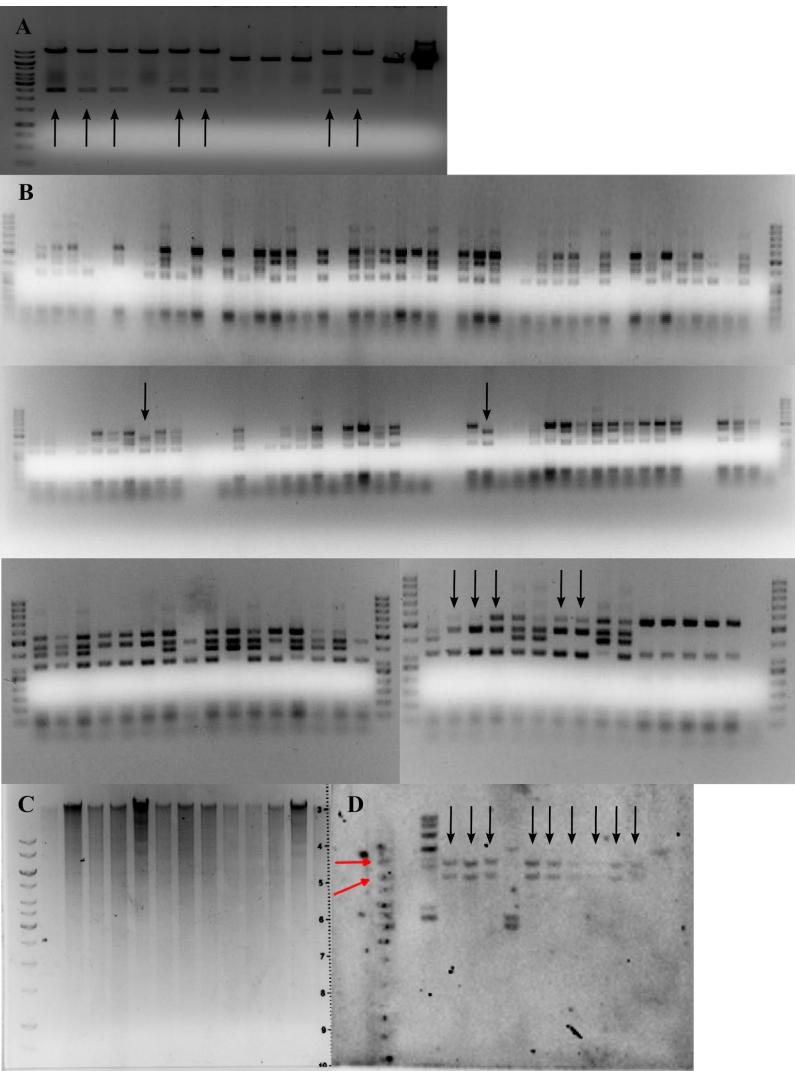
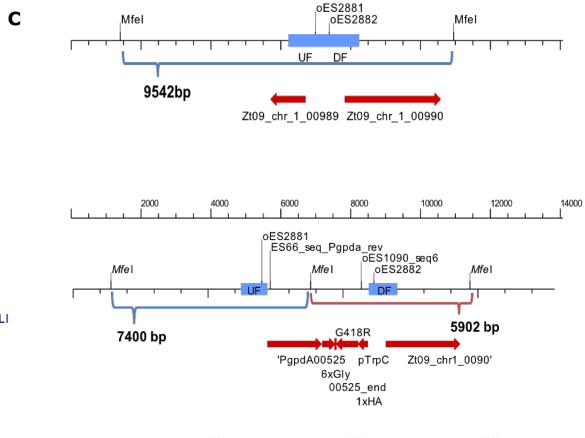
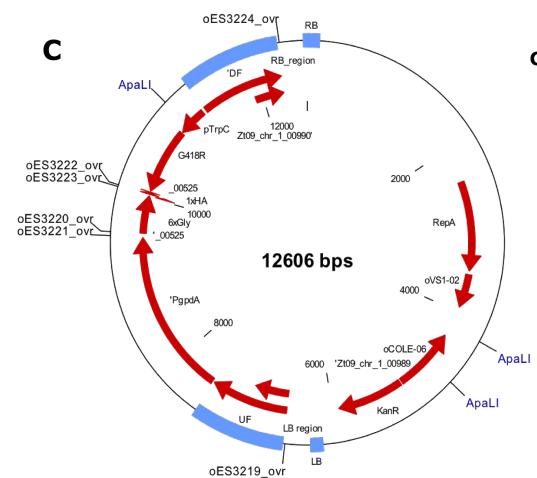
- i. Zt244 x pES218 = Zt415
- ii. Zt244 x pES234 = Zt419



1. Plasmid design, gibson assembly & generation of *Z. tritici* mutants

a. Confirmed strains for:

- i. Zt244 x pES218 = Zt415
- ii. Zt244 x pES234 = Zt419
- iii. Zt244 x pES235 = Zt421



3. Evolutionary analysis of genomic regions (gene +/- 10kb):

a. 00525 & 00287

i. 5 separate alignments of various sizes spanning
each region (adjacent/ distant from each other)

Feedback?

I'm a fun
guy!



*Did you
say
fungi!?*



2. Deletion mutants

a. *In planta* (Obelisk) virulence phenotyping

i. Zt114 (Poppe *et al.*, 2015)

1. Pycnidia quantification (finished)

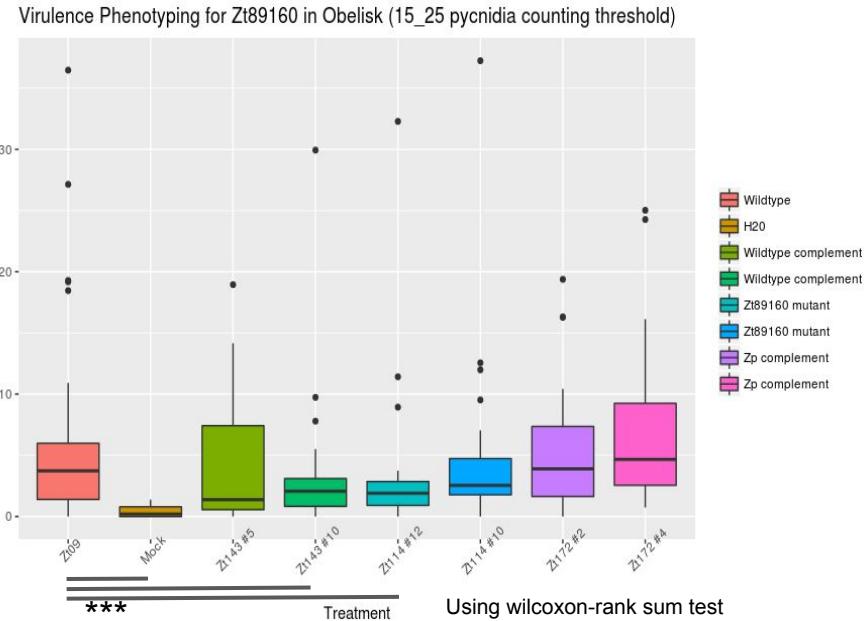
a. Differences seen in results

compared to Poppe due to:

i. Dif growth conditions

ii. Quantification

thresholds?



Zt89160 *in planta*

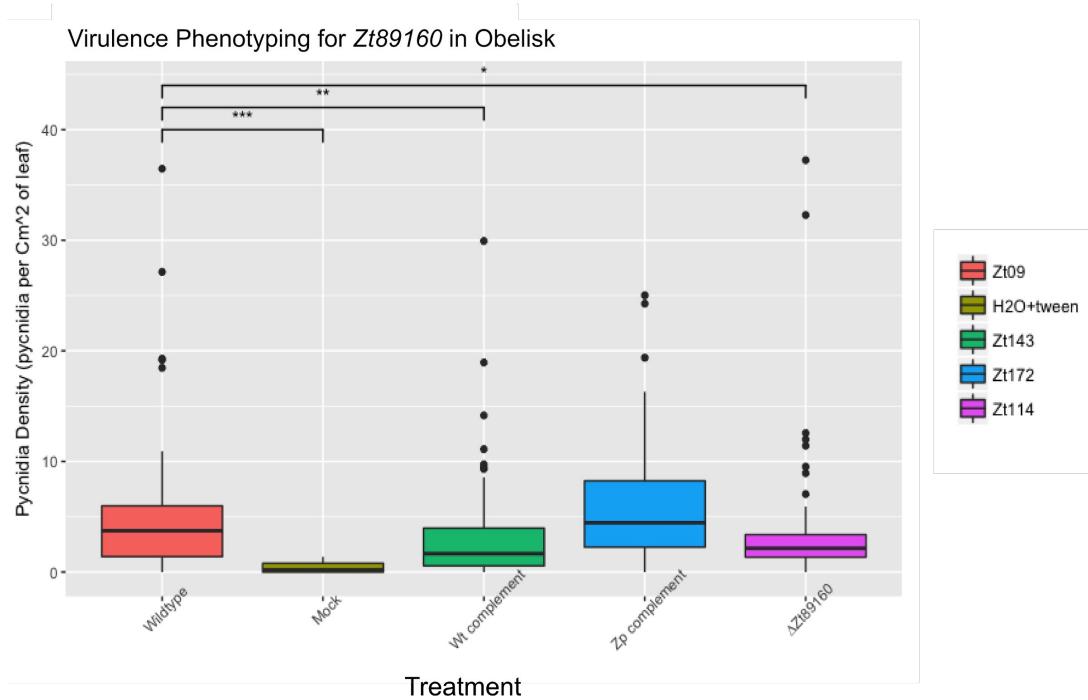
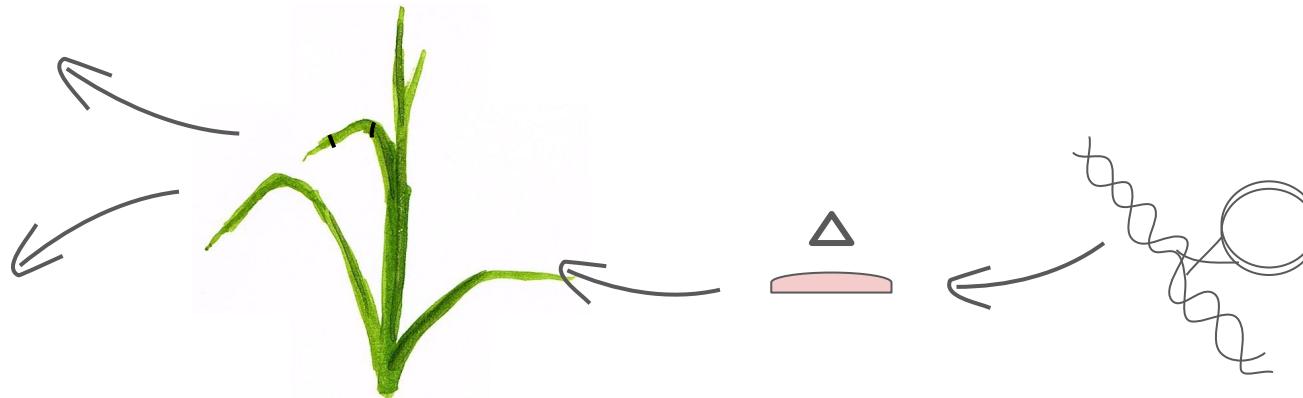


Figure 15. Virulence phenotyping for Zt114 in the wheat cultivar Obelisk. A) Pycnidia density per cm^2 of leaf. Differences in distributions tested using non-parametric Wilcoxon rank-sum test, significance (**p < 0.001, **p < 0.01, *p < 0.05). Treatments Zt114, Zt172, and Zt143: n = 30. Zt09: n = 60. Mock: n = 12.

Methods: In planta experiments

1. Plasmid design, gibson assembly & generation of *Z. tritici* mutants
2. Deletion mutants
 - a. *In planta* (Obelisk) virulence phenotyping
 - i. Pycnidia density
 - ii. ROS accumulation
 - iii. Necrosis development



Evolutionary analysis 287

$$\pi = \Theta = (S/a_1) = 2n\mu$$

$$a_1 = \sum_{i=1}^{n-1} 1/i, n = \text{sample size}$$

$$D = \pi - \Theta = 0$$

$$D = d/\sqrt{V(d)}$$

$$d = \pi - (S/a_1)$$

$$a_1 = \sum_{i=1}^{n-1} 1/i, n = \text{sample size}$$

$$\pi > \Theta, \text{ thus } \pi - \Theta = +D$$

$$\pi < \Theta, \text{ thus } \pi - \Theta = -D$$

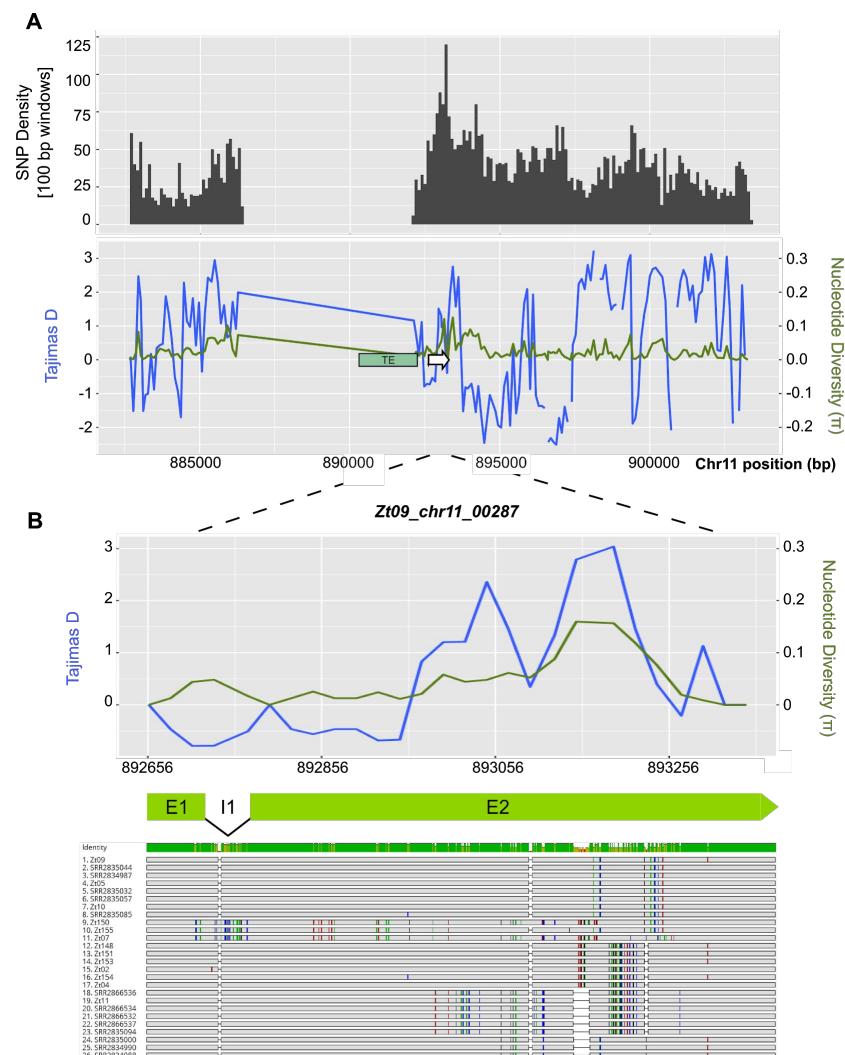


Figure 28. Screenshot of the analysis of Zt09_00287, a transposable element insertion in the Zt09 genome.

Evolutionary analysis 525

$D = \pi - \Theta = 0 \rightarrow$ neutral evolution

$\pi > \Theta$, thus $\pi - \Theta = +D \rightarrow$ balancing selection

$\pi < \Theta$, thus $\pi - \Theta = -D \rightarrow$ directional or purifying selection

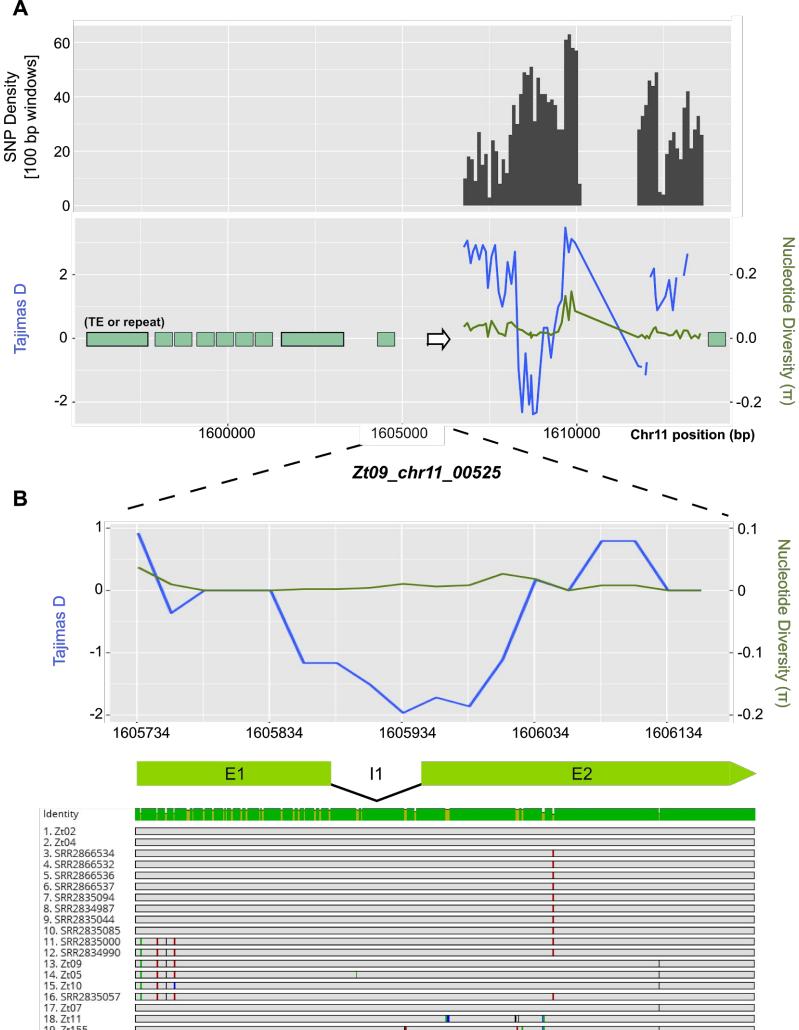


Figure 23. Sliding window analysis of the genomic region encompasses *Zt09_chr11_00525* and nucleotide variation in *Z. tritici* (A; n=26; B; n=19). A) SNP density, nucleotide diversity and Tajima's D are plotted along the corresponding