

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

By Corinn Sophia Small



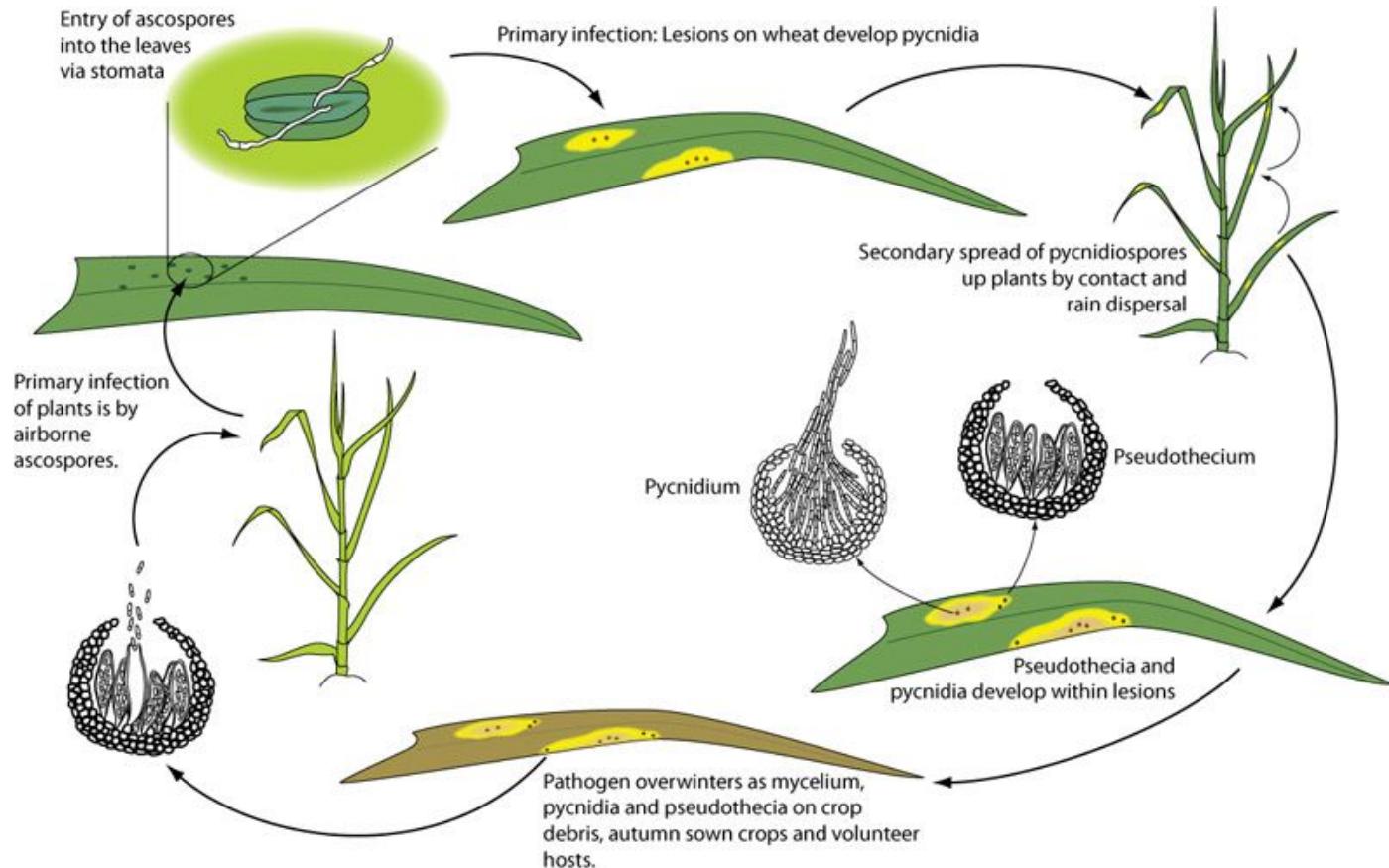
Thesis written in
Environmental Genomics group
Christian-Albrechts-University of Kiel

Septoria Tritici Blotch in wheat



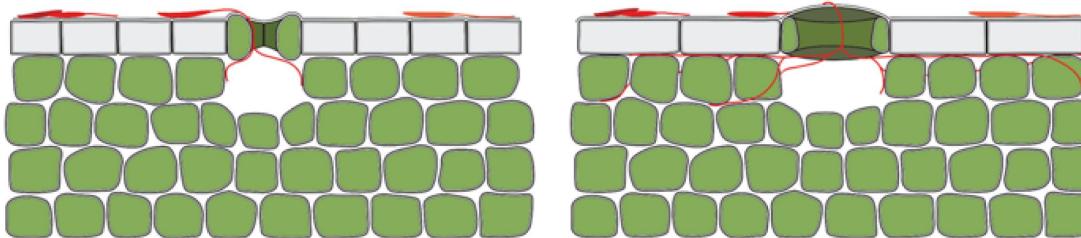
(Ponomarenko, Goodwin, and Kema, 2011); apsnets.org

Zymoseptoria tritici hemibiotrophic pathogen: complex life cycle



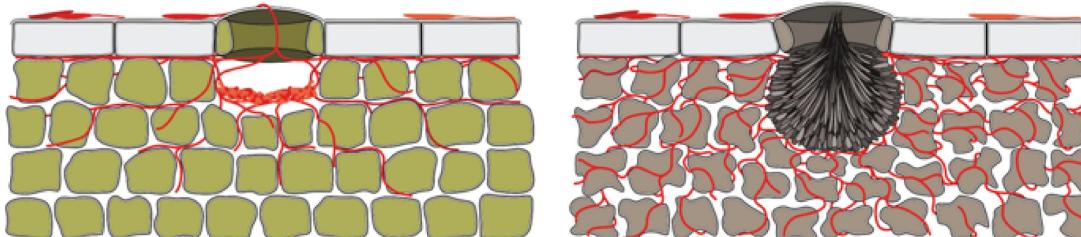
(Ponomarenko, Goodwin, and Kema, 2011)

Z. tritici colonizes live plant tissue to then induce necrosis



Stage A: Infection Establishment

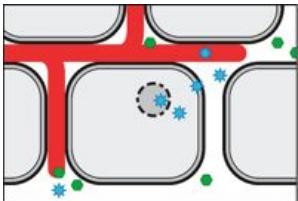
Stage B: Biotrophic Growth



Stage C: Lifestyle Transition

Stage D: Necrotrophy and Reproduction

⇨ Core infection stages require different effector profiles

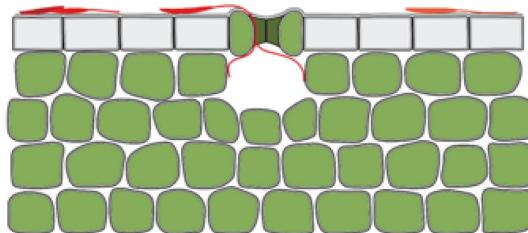


***Zymoseptoria* spp. comparative transcriptomics**

identified candidate effector genes

(Haueisen et al., 2017)

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



Stage A: Infection Establishment

Zymoseptoria spp. comparative transcriptomics

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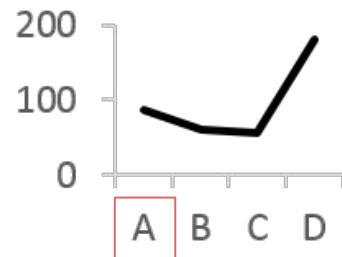
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Zt09_chr11_00287: ↑ Zt compared to Zp13 + Za17

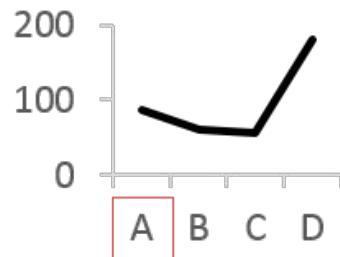


Zymoseptoria spp. comparative transcriptomics identified candidate effector genes:

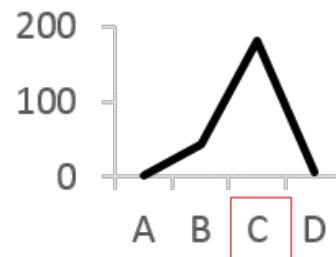
(Haueisen et al., 2017)

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Zt09_chr11_00287: ↑ Zt compared to Zp13 + Za17



Zt09_chr11_00525: ↑ Zp13 compared to Zt



Aims:

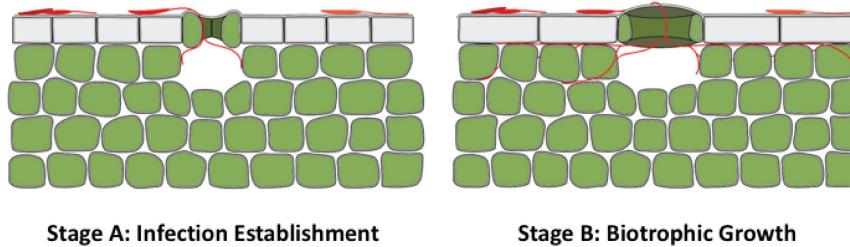
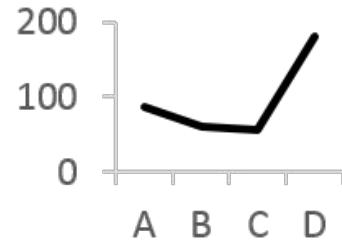
- 1) To study the functional roles of the candidate genes during wheat infection using:
 - (1) Gene deletions
 - (2) Gene over-expression

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*

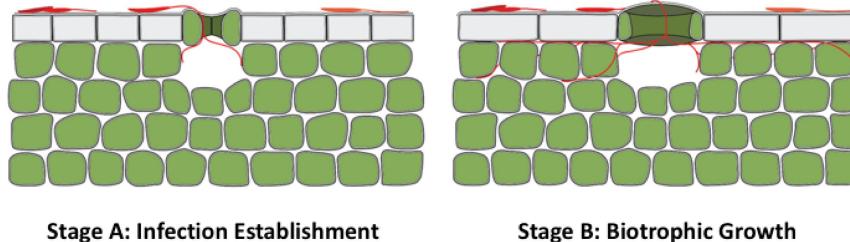
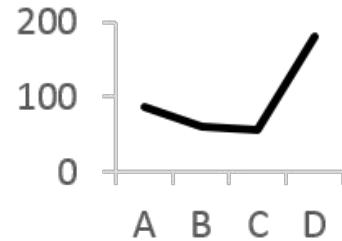
Hypotheses:

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

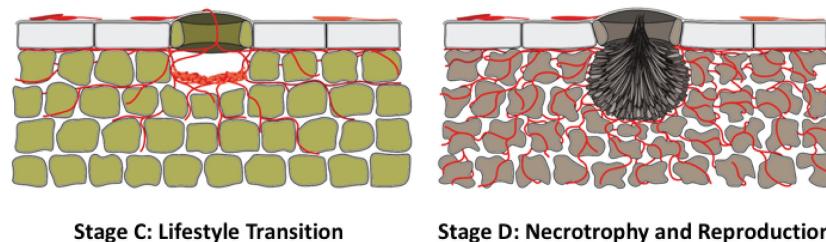
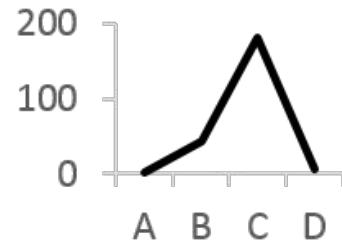


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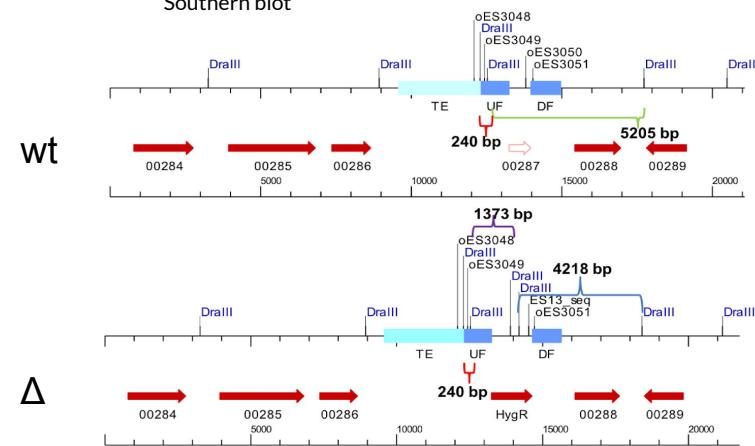
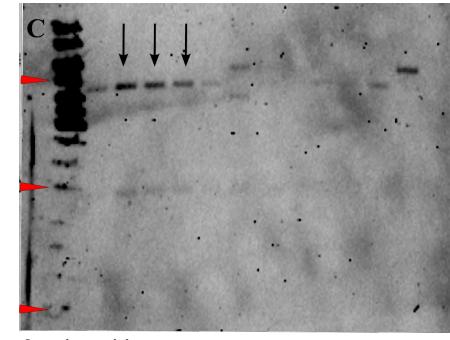
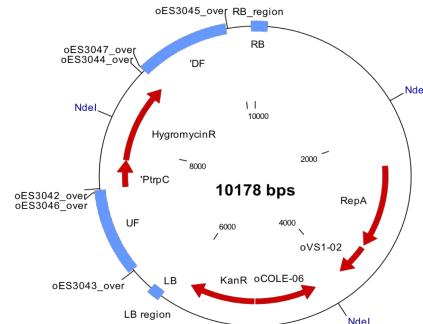
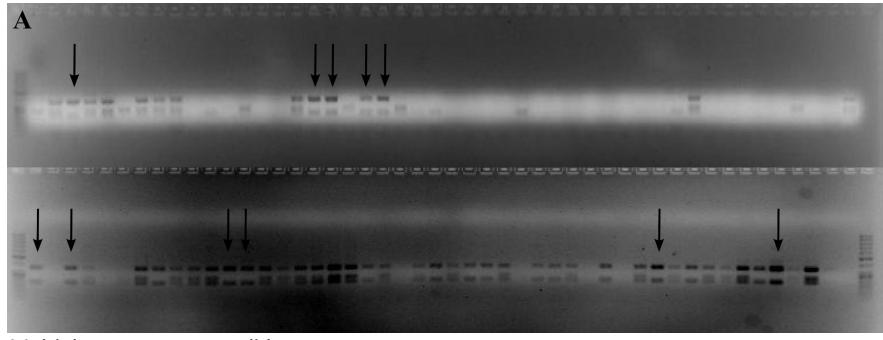
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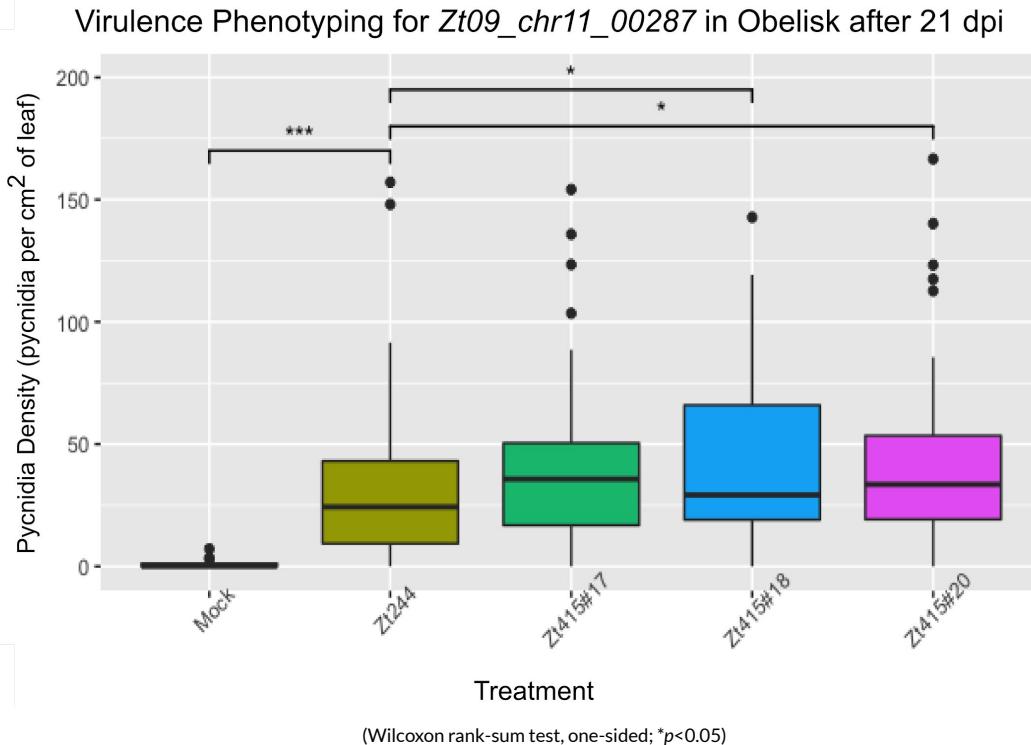
Zt09_chr11_00525 = necrotrophic effector involved in necrosis and/or pycnidia development



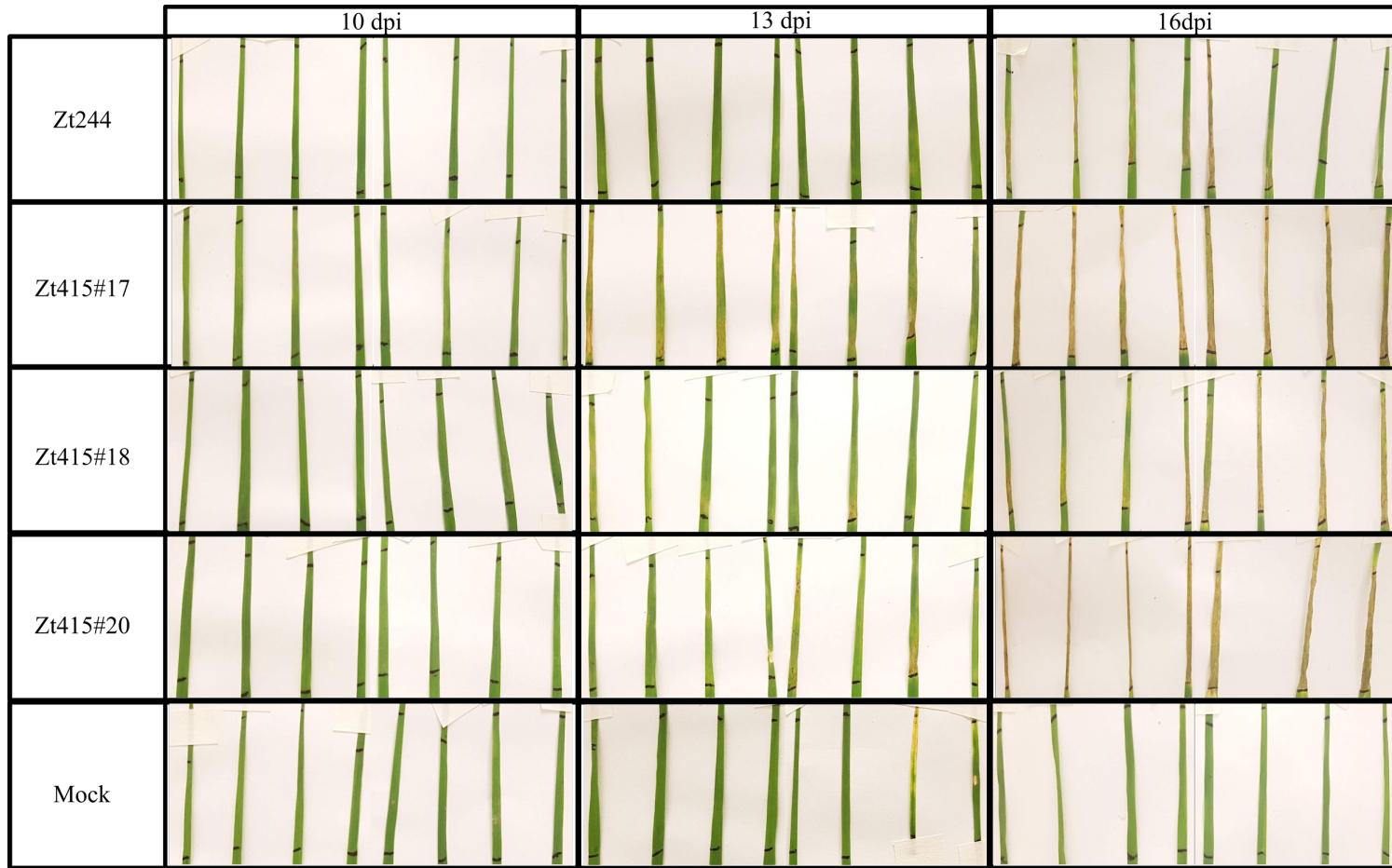
Zt415 confirmed mutant for deletion Zt09_chr11_00287



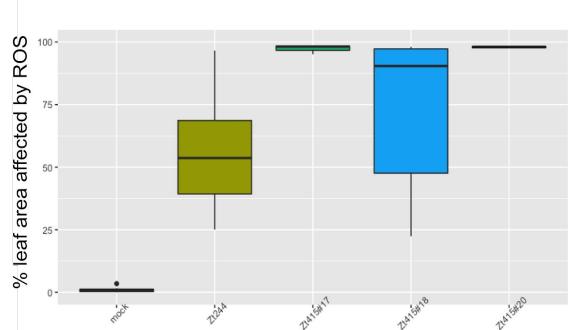
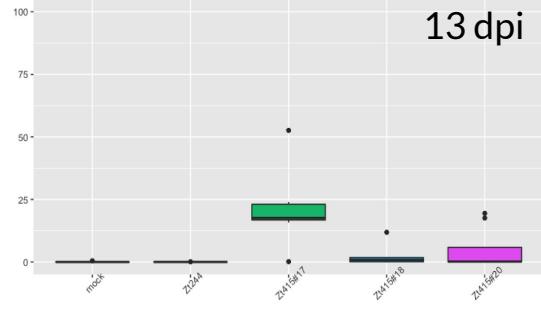
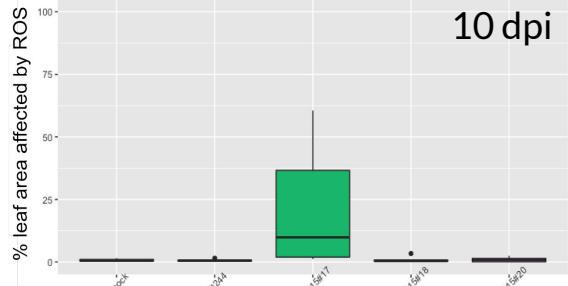
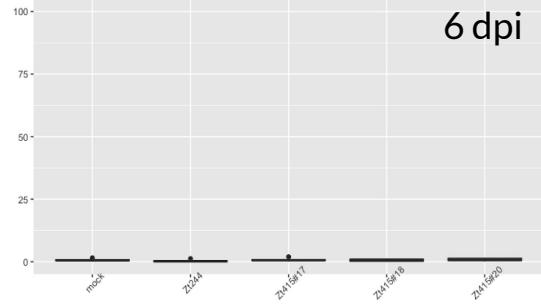
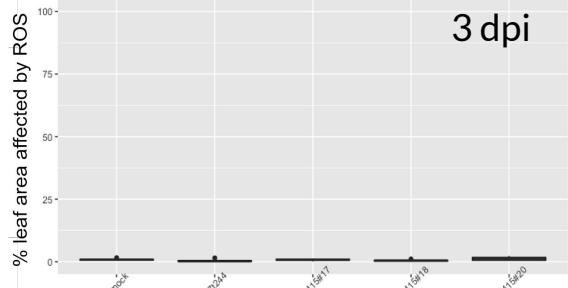
Zt415 exhibits a hypervirulent phenotype vs wildtype



Zt415 exhibits earlier necrosis development vs wildtype

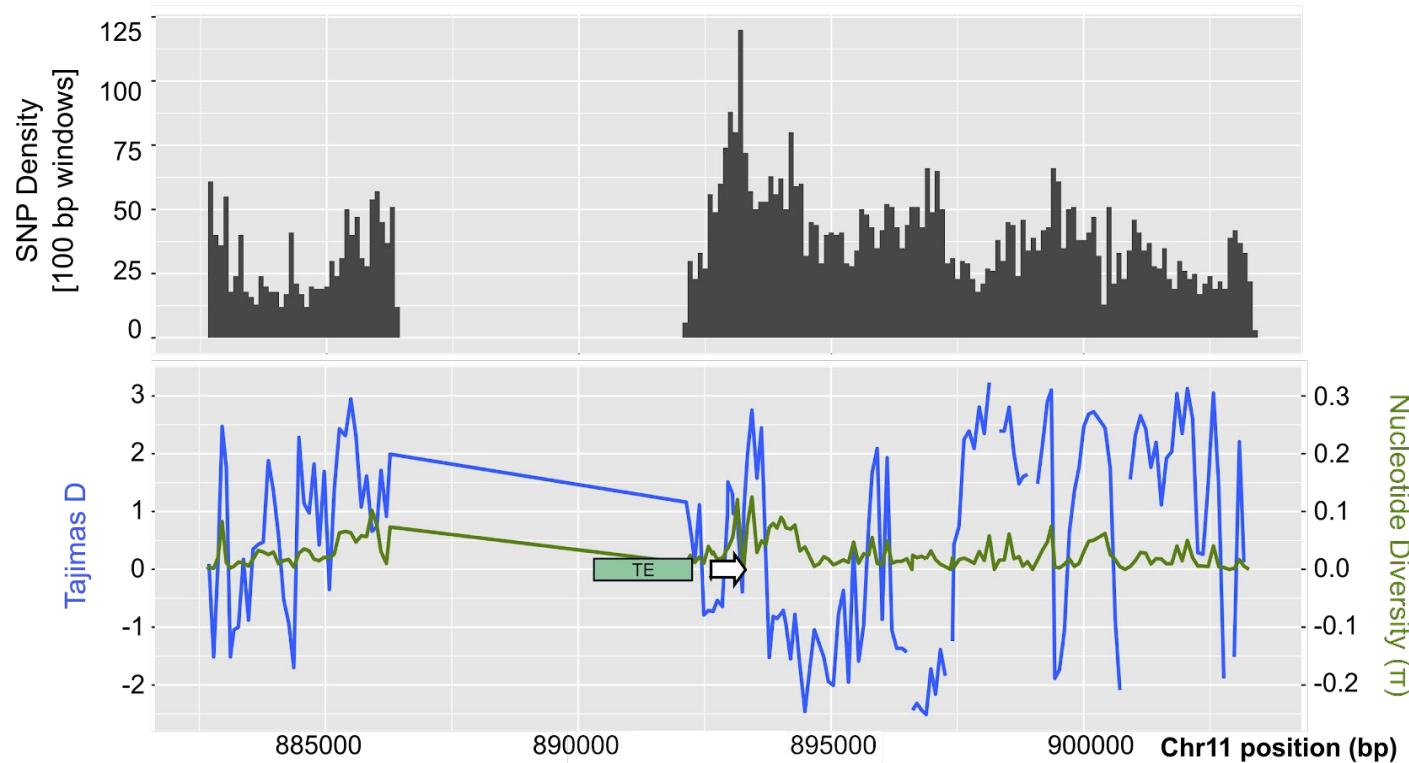


Zt415 exhibits earlier accumulation of ROS vs wildtype



Region surrounding Zt_chr11_00287 is highly variable:

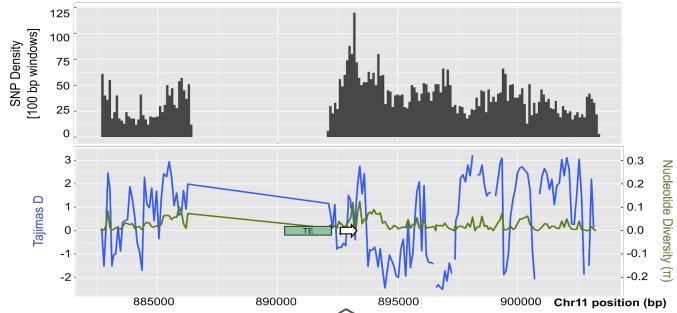
SNP density, nucleotide diversity and Tajima's D fluctuate



*Gap in plots = missing alignment data

Region surrounding *Zt_chr11_00287* is highly variable:

SNP density, nucleotide diversity and Tajima's D fluctuate



Zooming in...

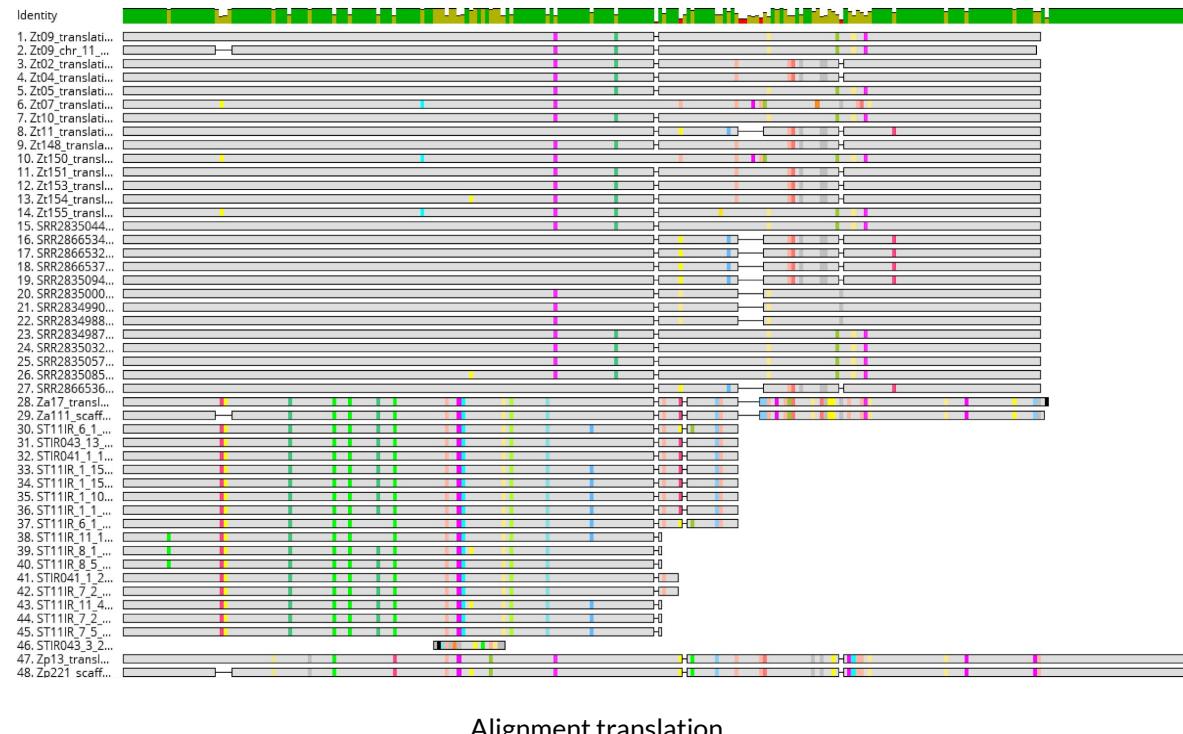
Evidence of both purifying and balancing selection occurring in *Zt_chr11_00287*



Zt09_chr11_00287 other characteristics include:

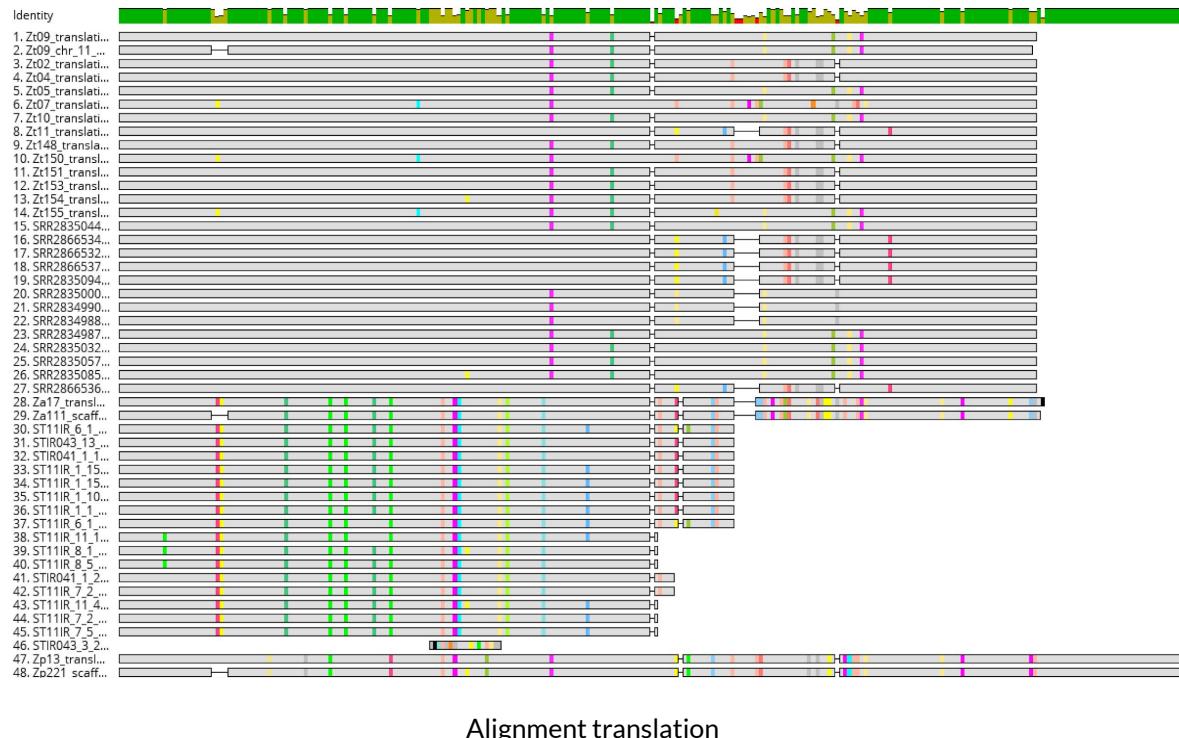
- ❖ Previous adaptive evolution:

- McDonald-Kreitmann
test= $dN/dS > pN/pS$

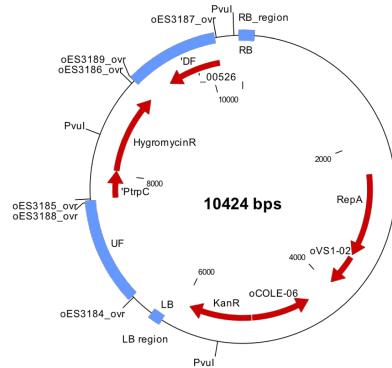
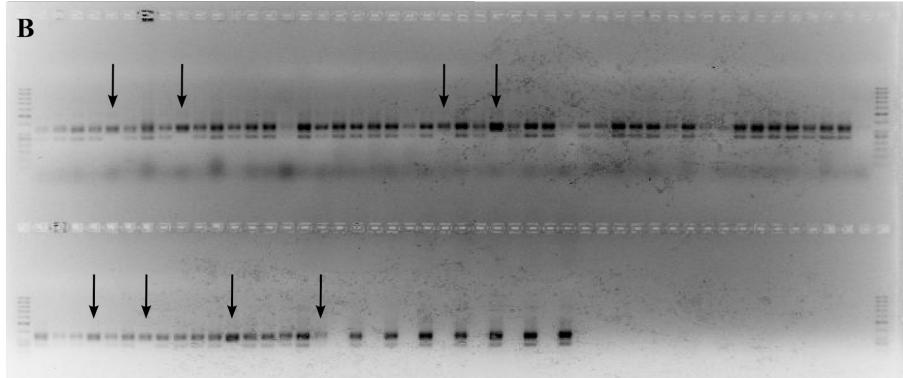


Zt09_chr11_00287 other characteristics include:

- ❖ Previous adaptive evolution:
 - McDonald-Kreitmann test= $dN/dS > pN/pS$
- ❖ Signal peptides conserved between sister species
 - High probabilities of predicted signal peptide (SignalP)
- ❖ Average $dN/dS > 1$ in *Z. tritici*
 - Adaptive evolution

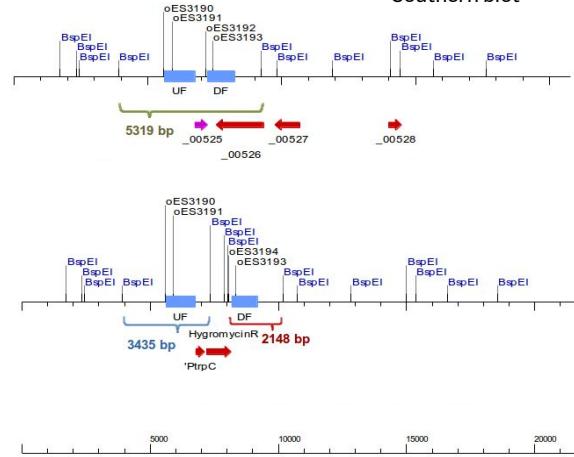
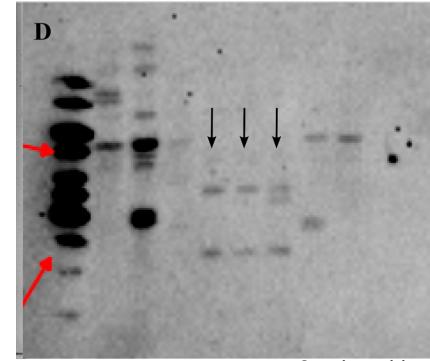


Zt419 confirmed mutant for deletion Zt09_chr11_00525



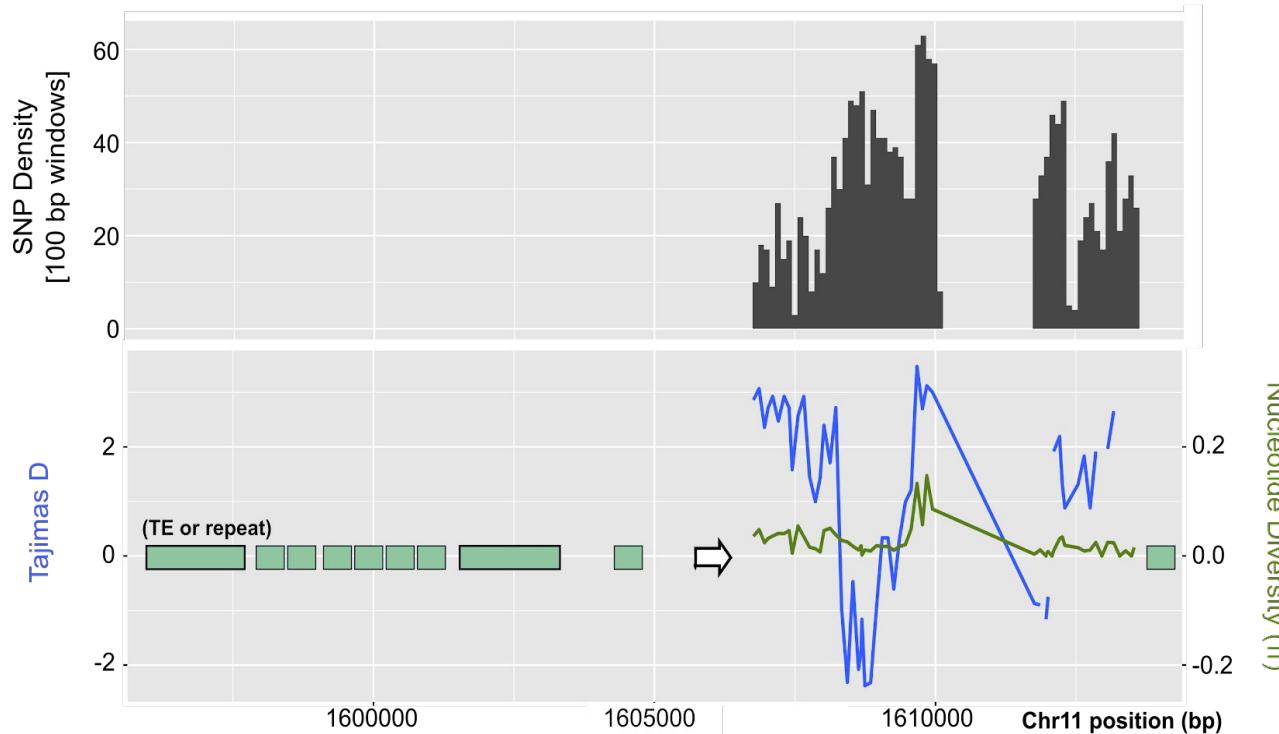
wt

Δ



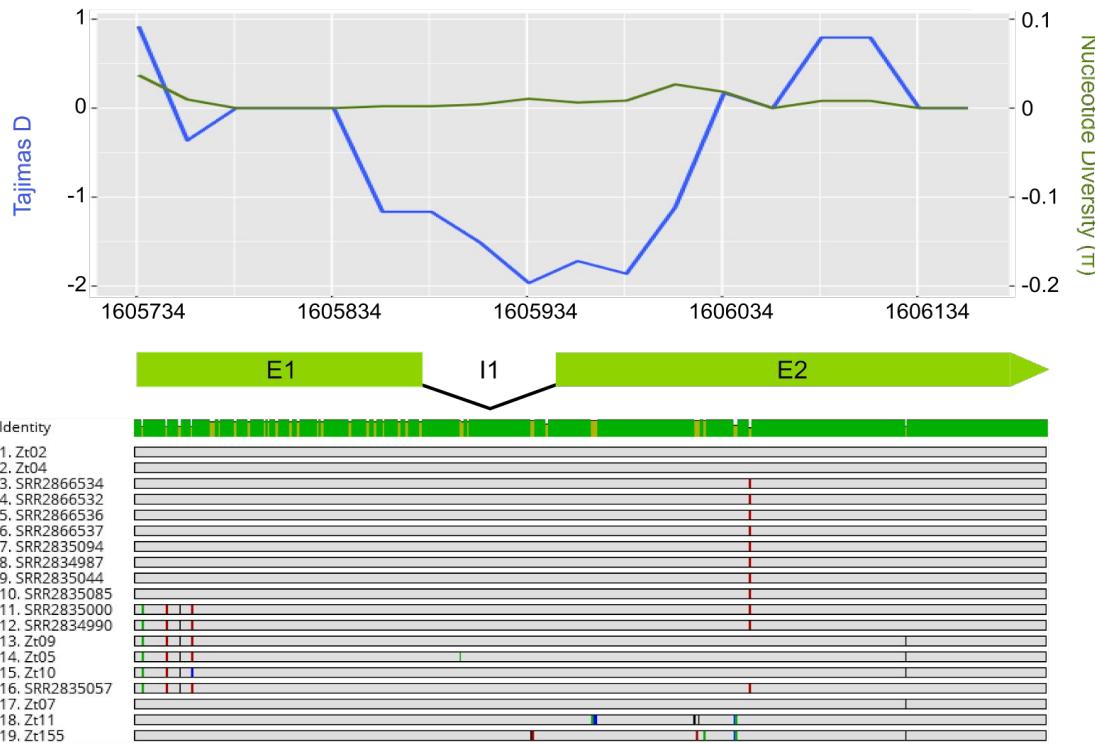
Region surrounding *Zt_chr11_00525* is also highly variable:

SNP density, nucleotide diversity and Tajima's D fluctuate; large TE cluster = drivers of variation



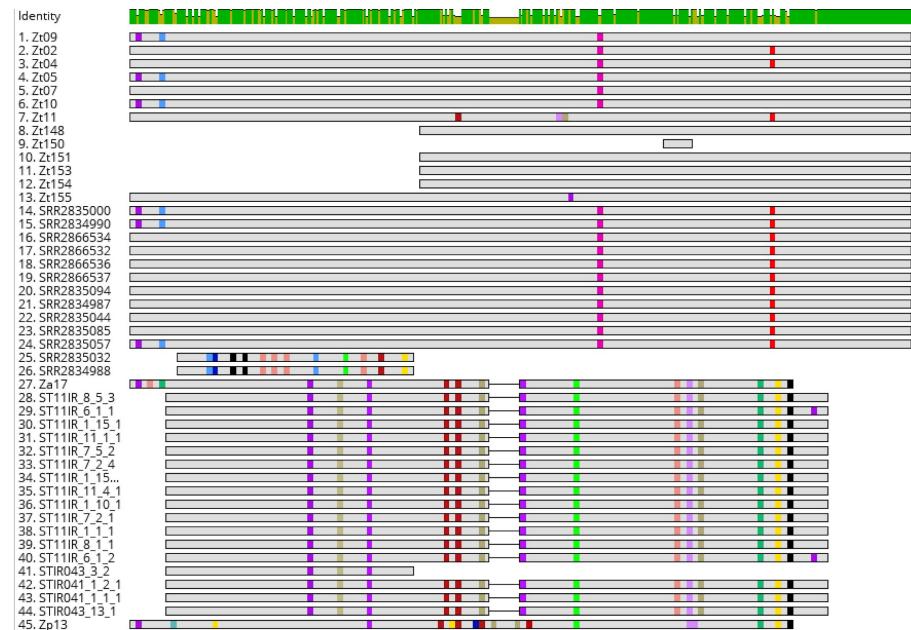
*Gap in plots = missing alignment data

Evidence of purifying selection occurring in Zt_chr11_00525



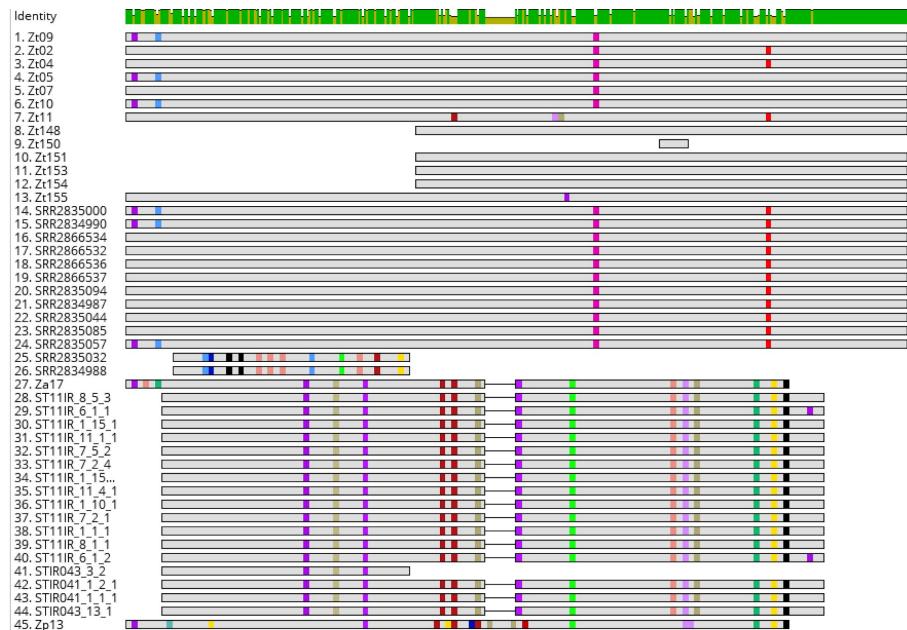
Zt09_chr11_00525 other characteristics include:

- ❖ Indications of previous purifying selection
 - (McDonald-Kreitmann test= $dN/dS < pN/pS$)

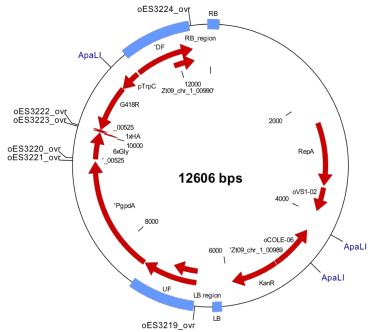


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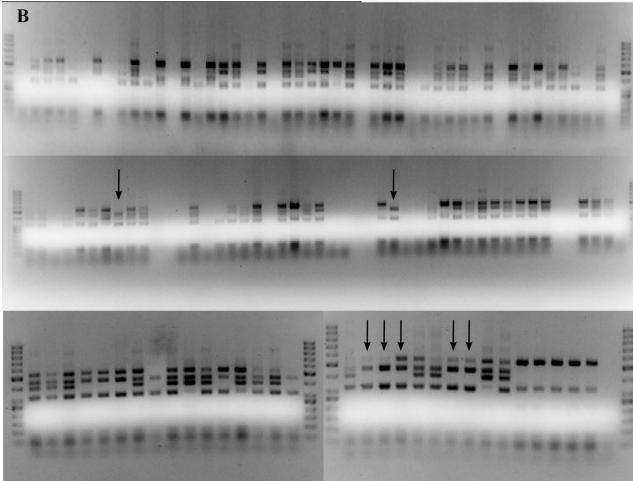
- ❖ Indications of previous purifying selection
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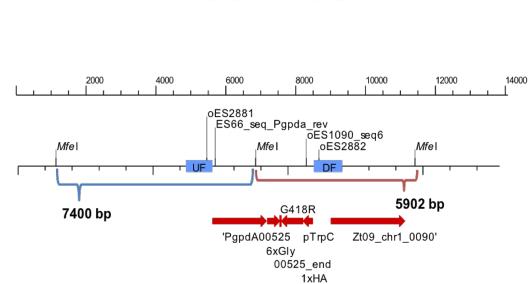
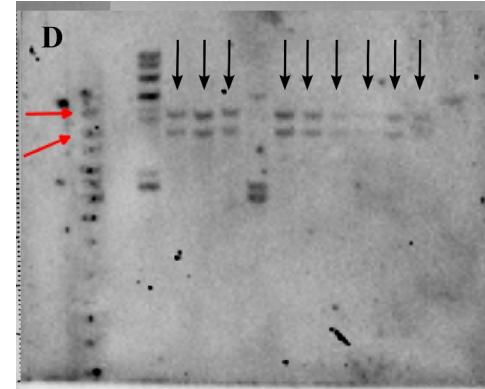
Zt421 confirmed mutant for over-expression of Zt09_chr11_00525



B



Multiplex pcr mutant candidates



Conclusions and outlook

Zt09_chr11_00287:

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Conclusions and outlook

Zt09_chr11_00287:

- ❖ Host-specific effector function not ruled out
- ❖ Evidence in support of avirulence characteristics:
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- ❖ Purifying and balancing selection rather than directional selection
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- ❖ Check deletion mutant phenotype in other cultivar backgrounds

Conclusions and outlook

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Conclusions and outlook

Zt09_chr11_00525:

- ❖ Purifying selection rather than directional selection
 - Conserved signal peptide
 - Small amount of haplotypes
- ❖ Need to functionally characterize over-expression mutant
- ❖ Check phenotypes in other cultivars and in other pathogen backgrounds

Conclusions and outlook

- ❖ Next:
 - More *in planta* phenotyping with other mutants
 - Repeat experiments
 - *In vitro* assays to check fitness differences

Thank you for your attention!

Special thanks to

Prof. Dr. Eva Stukenbrock for the opportunity to work in the Environmental genomics group!

Dr. Janine Haueisen for the fantastic supervision!

Dr. Katja Dierking for agreeing to be my second examiner!



Questions?

Supplementary slides
(didn't present slides below)

Zt89160 *in planta*

Differences seen in results
compared to Poppe *et al.*, 2015
due to:

- ❖ Different growth conditions
- ❖ Different quantification techniques

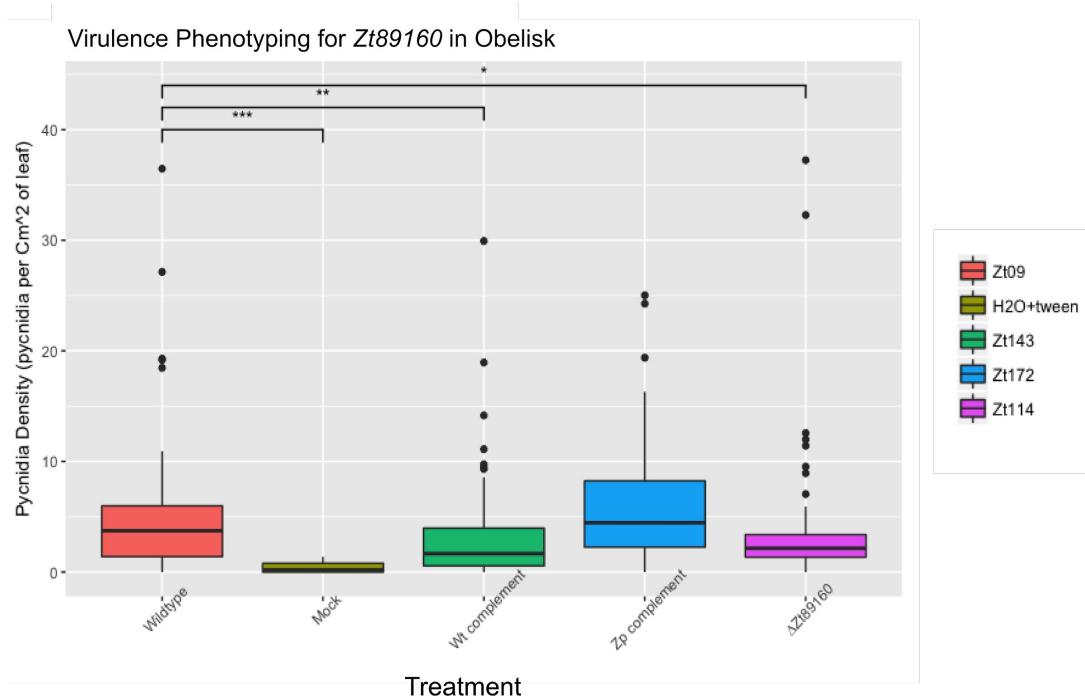
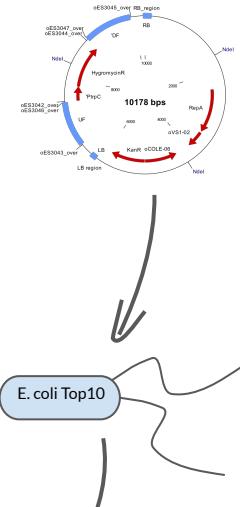
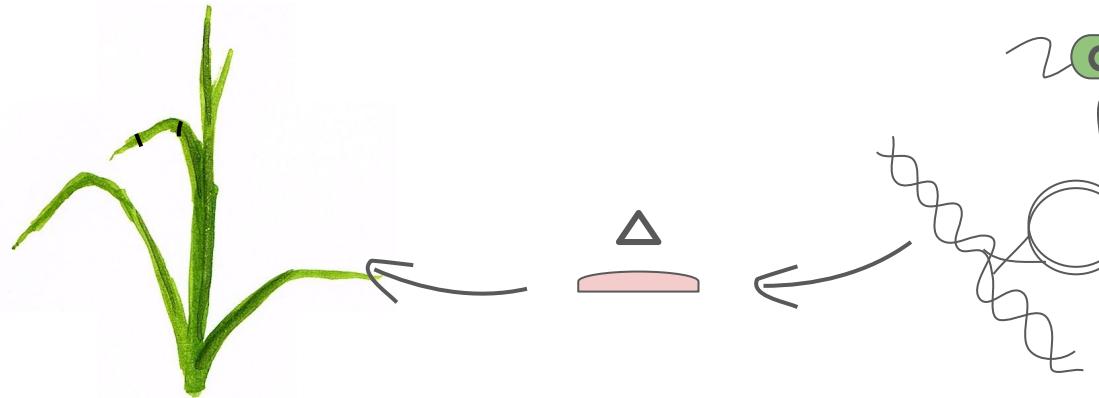


Figure 15. Virulence phenotyping for Zt114 in the wheat cultivar Obelisk. A) Pycnidia density per cm^2 of leaf. Differences in distributions tested using non-parametric Wilcoxon rank-sum test, significance (***) $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Treatments Zt114, Zt172, and Zt143: n = 30. Zt09: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

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

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

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

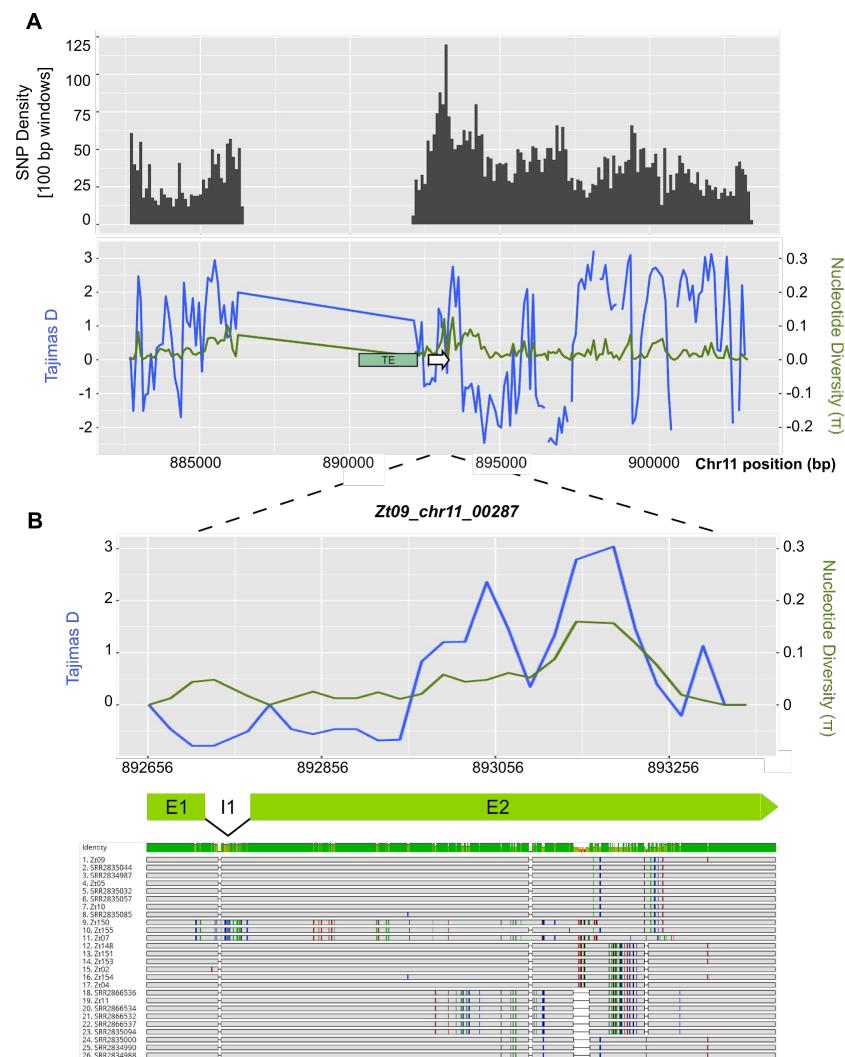
$$D = d/\sqrt{V(d)}$$

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

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

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

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



Evolutionary analysis 525

$$D = \pi - \Theta$$

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

$\pi > \Theta$, thus $\pi - \Theta = +D \rightarrow$ greater variation than expected

$\pi < \Theta$, thus $\pi - \Theta = -D \rightarrow$ less variation than expected

