Dissecting the genomic architecture of *Puccinia graminis* f. sp. *tritici* – wheat interaction

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Outbreaks in 1916, 1935, 1953 in North America resulted in significant yield losses

Stem rust is caused by *Puccinia graminis* f. sp. *tritici*

Origin and quick proliferation of new highly virulent Ug99 group race of *Puccinia graminis* showed the importance of understanding the mechanisms of wheat-pathogen interaction to fight wheat diseases

Stem rust resistance genes classified based on their effectiveness against Ug99 stem rust race

Singh et al. 2015, Phytopathology

The majority of cloned resistance genes are NBS-LRR proteins that are important part of plant’s innate immune system responsible for recognizing pathogens

Singh et al. 2015, Phytopathology

Understanding of the fungal targets recognized by wheat opens new possibilities for designing resistant varieties

RPSS (NLR) recognize *Pseudomonas syringae* effector AvrPphB (protease) through the host’s PBS1 protein that is cleaved by AvrPphB.

By replacing a short AvrPphB’s recognition motif in PBS1 to motifs recognized by other pathogen-secreted proteases, it was possible to design “a trap” activated by other pathogens.

(Kim et al., 2016, Science)
Sr35 gene found in wild ancestor of wheat confers near immunity against Ug99 race

Sr35 gene (McIntosh, 1984)
Location: 3AL
Origin: T. monococcum

Sainenac et al., 2013

Positional cloning and functional validation showed that Sr35 encodes NBS-LRR proteins

Mapping and cloning Sr35 gene

Functional validation

Transgenic wheat with Sr35 is resistant
EMS mutants of Sr35 are susceptible

Sainenac et al., 2013

The fungal side: hunting for fungal protein effector interacting with the Sr35 by sequencing mutant strains of rust

Mutagenize stem rust spores with EMS

Mutant spores are virulent on Sr35 suggesting that effector interacting with the Sr35 is knocked out

Wheat with the Sr35 gene

Next-generation sequencing of all mutant strains (KSU IGF)

Only one candidate gene with multiple independent knock-outs of coding sequence was identified

Gene has signal peptide for secretion outside of the fungus
Multiples knock-out mutations in Sr35 virulent strains

In natural populations of stem rust AvrSr35 candidate gene from the Sr35 virulent strains (Group V) cluster separately from the Sr35 avirulent strains (Group A)

Ability of Sr35 to recognize this effector and trigger cell death was shown by infiltrating tobacco leaves with both Sr35 and effector

Efficiency Sr35
Tobacco leaves
Cell death

0 HAI
48 HAI
X - mutation disrupts interaction
Search for Avr genes and cognate R genes is labor-intensive and expensive process. There is a need in developing genetic and genomic resources to facilitate detection of Avr-R gene pairs.
Stem rust isolates show broad range of phenotypic responses on the known Sr genes suggesting the possibility of mapping Avr gene by GWAM in the panel of sequenced rust isolates

Conclusions
1. EMS mutagenesis of rust isolates combined with NGS is powerful method for detecting and validating Avr genes
2. Resource including diversity data for large panels of wheat and rust isolates combined phenotyping is powerful tool for mapping R-Avr gene pairs

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