Disease resistance

Title: A new player in race-specific resistance
One sentence summary: The map-based cloning of *Stb6* identified wall-associated kinases as important players in race-specific disease resistance in wheat

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Fungal pathogens cause serious economic losses in crop production. *Zymoseptoria tritici* causes Septoria tritici blotch (STB) in wheat and is one of the most devastating and adaptive fungal pathogens in temperate climates (Figure 1). So far, none of the 21 genetically defined major STB resistance genes has been molecularly isolated. In a publication in Nature Genetics, Saintenac and colleagues now describe the molecular identification of the first STB resistance gene, *Stb6,* through map-based cloning and they show that it encodes a wall-associated receptor kinase (WAK)-like protein[1].

The *Stb6* gene is one of the most widely used sources for STB resistance in wheat breeding. The widespread distribution of *Stb6* in the wheat gene pool turned out to be a huge advantage for its molecular isolation because the authors could make use of various genomic resources that have recently been generated for the large and complex bread wheat genome. For example, the wheat landrace Chinese Spring, which has been chosen to generate the wheat reference genome sequence, carries *Stb6*. Hence, by making use of a recently published Chinese Spring whole genome assembly the authors were able to rapidly identify candidate genes spanning the *Stb6* region. *Stb6* is also present in Cadenza, a wheat cultivar for which the entire protein coding sequences have been sequenced from 1,200 ethyl methanesulfonate (EMS)-treated mutant plants[2]. This enabled the rapid validation of candidate genes in the *Stb6* interval. In combination with additional gene validation strategies the authors confirmed that the *TaWAKL4* gene was *Stb6*. In contrast to most race-specific resistance genes identified in plants so far, which encode intracellular nucleotide binding site-leucine-rich repeat (NLR) receptors[3], *Stb6* encodes a wall-associated kinase protein. An analysis of 98 accessions with broad genetic background revealed a high sequence conservation of the gene in bread wheat, with a predominating single resistance haplotype. The Stb6 protein is possibly located to the plasma membrane, connected to the cell wall and consists of a putative extracellular galacturonan-binding domain (GUB_WAK), an intracellular non-RD kinase and a concanavalin A-like domain. Surprisingly, it is lacking any of the extracellular subdomains which are found in all other characterized WAKs.

In the last few years *WAK* genes have emerged as important players in cereal disease resistance. Examples include the rice *Xa4* bacterial blight resistance gene[4] and the two maize wall-associated kinases *qHSR1*[5] and *Htn1*[6] conferring partial, quantitative resistance to head smut and northern corn leaf blight, respectively. In contrast to *qHSR1* and *Htn1* the resistance conferred by *Stb6* is race-specific and is only effective against pathogen strains expressing the *AvrStb6* virulence effector. The recent molecular cloning of *AvrStb6*[7,8] now allows for the first time to study the gene-for-gene interaction between a WAK and a pathogen effector.

The current dogma that is based on research done in the model plant *Arabidopsis thaliana* suggests that WAK proteins perceive oligogalacturonide-based ligands[9]. However, *AvrStb6* encodes a small cysteine-rich protein. Whether AvrStb6 is directly perceived by Stb6 needs to be determined. Initial yeast-2-hybrid experiments performed by Saintenac et al. failed to detect direct interaction. It is therefore possible that Stb6 interacts with AvrStb6 in a larger complex that also contains oligogalacturonides. Alternatively, the apoplastic localization of Stb6 and AvrStb6 *in planta* might have prevented a successful outcome of the yeast interaction experiment. Interestingly, it has earlier been shown that the wheat WAK receptor encoded by the *Snn1* susceptibility gene directly binds the proteinacous SnTox1 toxin produced by the fungal pathogen *Parastagonospora nodorum*[10]. The molecular nature of Stb6 and AvrStb6 provides further support for the hypothesis that WAKs can perceive protein ligands. However, it remains to be seen if this is generally true for WAKs involved in cereal disease resistance.

The cloning of *Stb6* and *AvrStb6* raises a number of questions on the molecular mechanism of this WAK-effector interaction. Analysis of mutants as well as gene diversity revealed that the kinase domain of Stb6 is essential for its function. A single amino acid change differentiated the resistant from the susceptible form of Stb6 and the inactivation of the kinase activity is the likely cause of loss of resistance. Unfortunately, none of the identified loss-of-function mutations in Stb6 contained a missense mutation in the extracellular domain. Therefore, there is no molecular information yet on the critical sites within the extracellular domain of Stb6 that possibly interact with the ligand. It is intriguing that *Stb6* is a semi-dominant gene (similar to some NLR resistance genes) where gene dosage translates into a phenotypic difference. This suggests a short signal transduction chain which is unexpected for a receptor-like kinase.

The findings by Saintenac et al. represent an important advance towards a better understanding of the agronomically important race-specific resistance. The knowledge on the AvrStb6 protein recognized by Stb6 now allows to study recognition specificity and to deduce molecular interactions. It will be interesting to see if transient expression systems successfully used for NLR-Avr interactions also reproduce the Stb6-AvrStb6 resistance reaction which would simplify structure-function studies. After all, specificity of NLR-based resistance is not yet completely understood even after more than 20 years of intensive research efforts. A better understanding of the Stb6-AvrStb6 interaction could possibly be used in structure-guided protein engineering to generated Stb6 versions with increased broad-spectrum specificity. The discovery of WAK-based resistance in cereal crops demonstrates the need and rewards of working in complex crop genomes to identify biologically novel mechanisms. Finally, the successful identification of *Stb6* was greatly supported by novel genomic resources in wheat which are accelerating the molecular analysis of wheat traits. Given the observation that cereal genomes contain hundreds of *WAK* genes with still unknown functions, it is likely that other disease resistance genes against *Z. tritici* and possibly other pathogens also encode WAK-like proteins.

a) b)

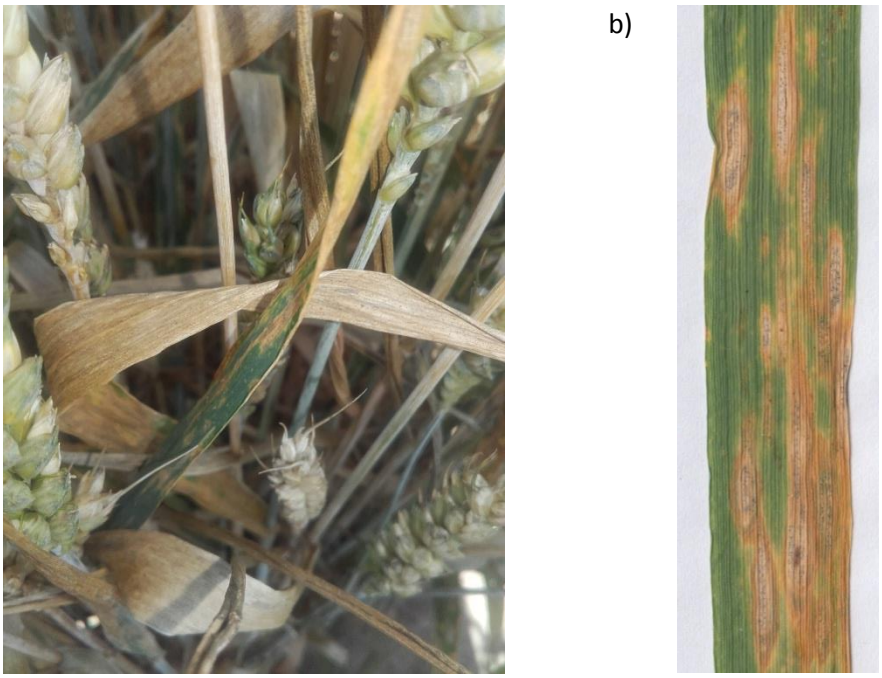

Figure 1: Disease symptoms of Septoria tritici blotch caused by the pathogen *Zymoseptoria tritici*. (a) Disease in a wheat field of a susceptible genotype in the absence of fungicide treatment. (b) Disease symptoms on a leave of wheat plant grown in the glasshouse after artificial infection. Photographs: P. Karisto and A. Mikaberidze, ETH Zürich.

Competing interests

The authors declare no competing financial interests.

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References

1. Saintenac, C. *et al.* Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria t*ritici. *Nat Genet* (2018).

2. Krasileva, K.V. *et al.* Uncovering hidden variation in polyploid wheat. *Proc Natl Acad Sci U S A* **114**, E913-E921 (2017).
3. Kourelis, J. & van der Hoorn, R.A.L. Defended to the nines: 25 years of resistance gene cloning identifies nine mechanisms for R protein function. *Plant Cell* (2018).
4. Hu, K. *et al.* Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement. *Nat Plants* **3**, 17009 (2017).
5. Zuo, W. *et al.* A maize wall-associated kinase confers quantitative resistance to head smut. *Nat Genet* **47**, 151-157 (2015).
6. Hurni, S. *et al.* The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proc Natl Acad Sci U S A* **112**, 8780-8785 (2015).
7. Zhong, Z.M. *et al.* A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene. *New Phytologist* **214**, 619-631 (2017).
8. Kema, G.H.J. *et al.* Stress and sexual reproduction affect the dynamics of the wheat pathogen effector AvrStb6 and strobilurin resistance. *Nat Genet* (2018).
9. Brutus, A., Sicilia, F., Macone, A., Cervone, F. & De Lorenzo, G. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proc Natl Acad Sci U S A* **107**, 9452-9457 (2010).
10. Shi, G. *et al.* The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease. *Science Advances* **2**, e1600822 (2016).