Plant Genetics

Fhb1 reloaded – A new player contributing to durable *Fusarium* resistance

***Fhb1* is the most effective and most widely deployed source of durable resistance against *Fusarium graminearum*, a devastating and toxin-producing fungal pathogen of wheat. Two new studies identified an atypical disease resistance gene as *Fhb1*, which will fuel a discussion over the molecular nature of this important disease resistance locus.**

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A gene encoding a histidine-rich calcium-binding protein contributes to durable FHB resistance

There is little wheat growers fear more than the sudden appearance of bleached ears in lush green wheat fields, often an unmistakable sign of Fusarium Head Blight (FHB). FHB is a devastating wheat disease caused by the fungus *Fusarium graminearum* (Fig. 1). *Fusarium* infected wheat ears produce shriveled grains with greatly reduced yield and quality. In addition, mycotoxins produced by the fungus are major concerns for human and livestock health. *Fhb1* is the most widely deployed source of genetic FHB resistance. This quantitative and broad-spectrum resistance was originally described in the Chinese wheat cultivar Sumai 3 (released in 1972) and its derivatives. Two independent studies report on the cloning of *Fhb1*[1,2]. Both studies used a map-based cloning approach to delimit *Fhb1* to a small interval on wheat chromosome arm 3BS. Functional validation showed that a histidine-rich calcium-binding protein (His) gene contributes to the *Fhb1* resistance. The His protein localizes to the nucleus, but its exact function in FHB resistance remains unknown. Although both studies agree on the gene responsible for the *Fhb1* resistance, they come to very contrasting conclusions regarding the causative allele.

In the first study, Su et al. delimited *Fhb1* to a ~300 kb interval containing 16 candidate genes[1] (Table 1). Genetic analyses in two different mapping populations and expression analyses identified *His* as the most likely candidate. Resistant wheat lines carried a large deletion in the second intron of the gene (His^R) that removed the downstream start codon. Overexpression of the putative His^R open reading frame in a susceptible wheat cultivar did not result in increased FHB resistance, but silencing and knocking out of a His^S allele present in the susceptible wheat cultivar Bobwhite did. This led Su et al. to the conclusion that His^S is a susceptibility factor and that the *Fhb1* resistance is the result of a knockout mutation caused by the large deletion.

In the second study, Li et al. fine-mapped *Fhb1* to a very small 23.8 kb physical interval using three populations comprising an impressive 11,516 plants[2]. The only gene present in this interval was *His*. Similar to Su et al., Li and colleagues identified a critical 752 bp deletion that distinguished the resistance-conferring His^R allele from His^S. Instead of proposing a gene knockout however, Li et al. report that the deletion creates a new upstream translation start codon with a functional His^R protein that is 14 amino acids longer than His^S. Transformation of a 5,164 bp genomic His^R construct including native promoter increased FHB resistance in the highly susceptible wheat line ND183. In addition, overexpression of His^R in the moderately susceptible wheat cultivar Yangmai158 also resulted in enhanced FHB resistance.

In summary, both studies identified a critical deletion in the same histidine-rich calcium-binding protein gene as the causative mutation that gave rise to the *Fhb1* resistance in wheat. While Su et al. conclude that the *Fhb1*-mediated resistance is the result of a loss-of-function mutation, Li et al. show that the same deletion resulted in a gain of function. In addition, a previous study identified a pore-forming toxin-like (*PFT*) gene, located adjacent to *His*, as a major contributor to the *Fhb1* resistance[3].

How can the seemingly contradictory results of the three papers be linked? This question has concerned the wheat community with the three groups invited to present their results in a special session at the 13th International Wheat Genetics Symposium in Tulln, Austria in May 2017. One possible explanation could be a dominant-negative effect. Hexaploid bread wheat contains two homoeologous *His* copies on chromosomes 3A (*His-3A*) and 3D (*His-3D*) that are highly similar to the *Fhb1* gene on chromosome 3B. It is possible that the His proteins form heteromultimers that are recruited by *F. graminearum* to establish infection. The existence of such susceptibility factors in wheat has been proven[4]. Silencing His^S*-3B* through RNAi or gene knockout might reduce the total amount of His protein available to form multimers, which could result in a reduction of susceptibility. The critical deletion in His^R on the other hand may result in the generation of a protein version that exerts a dominant-negative effect. The His^R protein might still be able to form multimers with His-3A and His-3D, but could block the catalytic activity or binding capacity of the complex. This could explain how both silencing of His^S and transformation of His^R could result in increased FHB resistance. A similar molecular process has been reported for the durable *Lr67* resistance gene in wheat. *Lr67* encodes a hexose transporter that confers durable resistance against multiple fungal pathogens. Two spontaneous mutations resulted in a protein variant in resistant wheat cultivars (LR67res) that exerted a dominant-negative effect through heterodimerization with homoeologous copies of the hexose transporter, resulting in abolished glucose transport[5]. Li et al. silenced His^S in the susceptible wheat line PH691 through RNAi and did not observe increased resistance. Based on these results, they conclude that the His^R protein does not provide resistance through negatively affecting the wild-type forms. However, these results directly contradict the silencing experiments performed by Su et al. The exact mode of action and nature of the *Fhb1* resistance thus needs to be determined in future experiments. While plausible functional derivation of these genes may be ascribed to dominant-negative effects it is noteworthy that the interactions may simply reflect biochemical genetic effects of the encoding interacting proteins rather than underlying causality. Bearing this in mind the divergent experimental accounts presented in the *Fhb1* studies is thus unsurprising.

What remains to be addressed, is how does *PFT* integrate into the picture? Both studies by Su et al. and Li et al. excluded *PFT* as a candidate gene based on genetic mapping and haplotype analyses. It has been shown however that plants have operon-like gene clusters, where closely linked but structurally unrelated genes are involved in the same biochemical processes[6]. It is thus possible that both *PFT* and *His* quantitatively contribute towards FHB resistance. Clearly there are ongoing lessons to be learnt from these studies, in particular when dealing with gene clusters, the extent of high resolution analysis and experimental tools commonly employed to tease apart the questions of gene '*sufficiency'* and gene '*required*' for function. Experimental methods for gene *sufficiency* based on overexpression and gene silencing are fraught with dangers of misinterpretation and therefore a timely cautionary reminder in dealing with the apparent contradictions as encountered in the FHB resistance studies. Such trappings have been evident from the recent findings on gene clusters at the broad-spectrum mildew resistance locus *Pm21* in wheat. A combination of gene overexpression and silencing studies implicated a protein kinase as PM21

until a more resolute analysis unambiguously identified a classic plant immune receptor as the causal powdery mildew broad-spectrum resistance gene[7-9].

Polyploid wheat – an evolutionary playground for durable disease resistance

An intriguing observation made in the two studies is that only one resistance-conferring *His* haplotype (*His*^{R}) has been identified, while there are several *His*^{S} haplotypes. The characteristic deletion present in *His*^{R} was not found in the homoeologous *His-3A* and *His-3D* gene copies of hexaploid wheat and a *His*^{R}-like haplotype is absent from diploid wild wheat progenitors. These findings suggest that *His*^{R} evolved after domestication through a spontaneous mutation that most likely occurred in a wheat line grown in the Yangtze Valley. A similar observation has been made for two other atypical disease resistance genes of hexaploid wheat. The *Lr67res* and *Lr34res* resistance alleles, encoding a hexose transporter and an ATP-binding cassette (ABC) transporter, respectively, also evolved as a result of sub-genome-specific mutations that spontaneously occurred after domestication[5,10]. *Lr67res*-like and *Lr34res*-like haplotypes have not been found in diploid wheat progenitors and in diploid cereals such as rice and sorghum. These findings show that the higher genome plasticity provided by the hexaploid bread wheat genome serves as a reservoir for the evolution of unique alleles that confer durable disease resistance.

The publication of the two *His* manuscripts will definitely fuel the discussion about the exact molecular nature of the *Fhb1* resistance. Further efforts are be needed to clarify the seemingly contradictory results provided by the three *Fhb1* gene cloning papers. The publication of the two *His* manuscripts is important and justified, and will allow the scientific community to critically assess the data provided in each manuscript.

Competing interests

The authors declares no competing financial interests.

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Table 1. Summary of the three *Fhb1* cloning papers.

	Rawat et al. (2016)	Su et al. (2019)	Li et al. (2019)
Source of *Fhb1* resistance	Sumai 3	Ning7840 (a Sumai 3 derivative)	Wangshuibai (a wheat cultivar from the Yangtze Valley)
Size of physical interval	~420 kb (~350 kb of Sumai 3 BAC sequences with a ~76 kb gap). The interval includes *PFT* and *His*	~300 kb, including *PFT* and *His*	23.8 kb, including *His* but excluding *PFT*
Causative gene	Pore-forming toxin-like gene (*PFT*)	Histidine-rich calcium-binding protein gene (*His*)	Histidine-rich calcium-binding protein gene (*His*)
Type of mutation	Gain-of-function	Loss-of-function	Gain-of-function
Gene validation strategies	-TILLING mutants -RNAi silencing -Haplotype analyses -Transgenic overexpression (cDNA driven by maize ubiquitin promoter)	-RNAi silencing of susceptible allele (Bobwhite) -CRSIPR mutants in susceptible cultivar (Bobwhite) -Haplotype / association analyses -Transgenic overexpression of resistant allele (cDNA controlled by the maize ubiquitin promoter)	-Transgenic expression of genomic construct driven by native promoter -Transgenic overexpression of resistant allele (cDNA controlled by maize ubiquitin promoter) -RNAi silencing in resistant and susceptible cultivars -Haplotype / association analyses
Major finding	*Fhb1* is encoded by a pore-forming toxin-like gene (*PFT*). The histidine-rich calcium-binding protein gene was ruled out for *Fhb1* candidacy based on previous studies.	*Fhb1* is a susceptibility gene. A deletion in the histidine-rich calcium-binding protein gene results in decreased susceptibility.	A deletion in a histidine-rich calcium-binding protein gene (*His*) creates a new gene variant that causes FHB resistance.

Fig. 1. Wheat ears showing symptoms of Fusarium Head Blihgt (FHB). Photograph: H. Bürstmayr, BOKU, Tulln, Austria.