Fusarium crown rot genetic resistance in cereals - what is in the breeding pipelines?

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Take home message

- Breeding lines with enhanced resistance/tolerance to Fusarium crown rot (FCR) in adapted Australian backgrounds are available for breeding companies
- Introduced Fhb7 gene from a wheat relative showing significant yield and resistance/tolerance improvement under FCR pressure
- Additional sources of FCR resistance urgently needed to further improve Australian wheat and barley.

Background

Fusarium crown rot (FCR), mainly caused by *Fusarium pseudograminearum*, is a widespread and destructive disease impacting the production of many crop species including wheat, barley, durum, oat, and triticale. Its prevalence has increased significantly in recent years, particularly within conservation cropping systems, due to the increased frequency of cereal in rotations and stubble retention. In Australia alone, FCR in wheat causes an estimated cost of $404 million per year in lost yield. Enhancing genetic resistance in commercial varieties, coupled with appropriate crop management practices, is recognized as critical to the development of effective control measures for this economically important disease. However, the scarcity of high-quality resistance sources in cereal crops poses a challenge to successful breeding efforts.

What have we achieved?

Partial resistance to FCR has been observed in existing cultivars, wild relatives, and landraces. Despite the identification of numerous putative quantitative trait loci (QTL) associated with FCR resistance in wheat, a lingering question persists: 'why hasn't the FCR problem been resolved'. One reason is that many identified QTL are either with minor effects or only detected in a specific environment. In addition, various physiological and developmental traits, such as flower time and plant height, can influence FCR resistance, complicating the interpretation of identified QTL. Furthermore, reproducible and reliable phenotypic methods are essential for accurately mapping QTL.

To address the complexity of field trials, a high throughput and reliable seedling assay was developed by CSIRO to screen genetically diverse germplasm and detect QTL conferring FCR resistance. Using this seedling assay three major QTL were identified in wheat, one on chromosome 3B inherited from *Triticum spelta* and two QTL on chromosomes 2D and 5D inherited from EGA Wylie(b). In barley, three QTL were also detected (one from wild barley and the

other two from landrace) on chromosomes 1H, 4H and 6H. Given the aforementioned reasons, validating the effects of a given QTL is deemed essential before its incorporation into breeding programs. Consequently, we developed near isogenic lines (NILs) that are essentially identical except for the presence or absence of the resistant locus for each of these QTL. These NIL pairs were then subjected to intensive field testing to ensure the resistant locus increased resistance in the paddock. Resistant loci identified from our seedling assay showed a significant reduction in whitehead incidence in wheat and decreased stem browning, and yield loss in barley under field conditions (Figure 1) (Liu and Ogbonnaya, 2015; Zheng *et al.*, 2022).

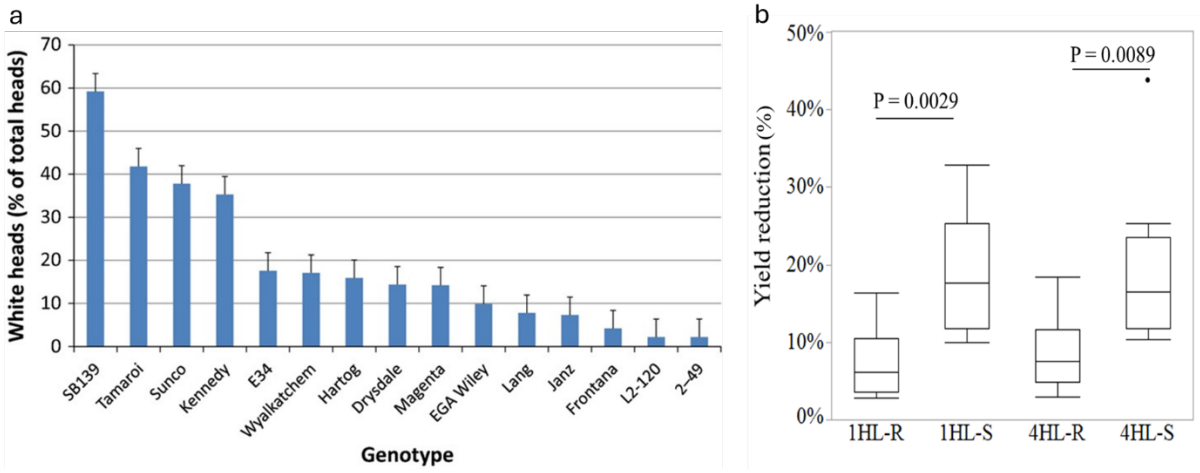

Figure 1. (a) Fusarium crown rot resistance (measured by percentage of whiteheads) of L2-120 (containing the resistant allele of 3B from CSCR6) compared to several other genotypes under field conditions (cited from Liu and Ogbonnaya 2015); (b) Box plot distribution of grain yield reduction between the isolines with (-R) or without (-S) the 1H and 4H resistant loci (cited from Zheng *et al.*, 2022).

In both wheat and barley, pyramiding all three FCR QTL into a single genetic background enhanced the resistance more strongly than the effect of a single locus or two separate loci. However, markers derived from the QTL mapping studies cannot be reliably used to tag a QTL due to limited resolution. Therefore, diagnostic markers targeting each of these resistant loci in wheat and barley were generated by analysing NIL-derived population and RNA-seq targeting multiple NIL pairs. With the assistance of the generated markers, breeding lines possessing four resistant loci (on 2B, 2D, 3B and 5D) for wheat and three resistant loci (on 1H, 4H, and 6H) for barley have been generated in adapted backgrounds and provided to commercial breeding companies.

What have we found recently?

Our current focus is on introducing the Fhb7 gene into Australian wheat and combining it with other loci already in wheat. This gene, derived from a wheat relative *Thinopyrum*, showed resistance to both Fusarium head blight and FCR. In our single-row field trials conducted in 2023 where FCR was severe, backcrossed (BC) lines with this gene had significantly higher grain yield compared to the lines without the gene in the presence of FCR. The largest difference between these two groups of lines was observed at Narrabri with the difference as high as 11.4%. Significant differences were also detected between these at both other field sites, 8.7% at Forest Hill and 7.2% at Boorowa (Figure 2). Similarly, disease severity based on whiteheads and stem browning also differed significantly, with the lines in the resistant group showing less severe symptoms.

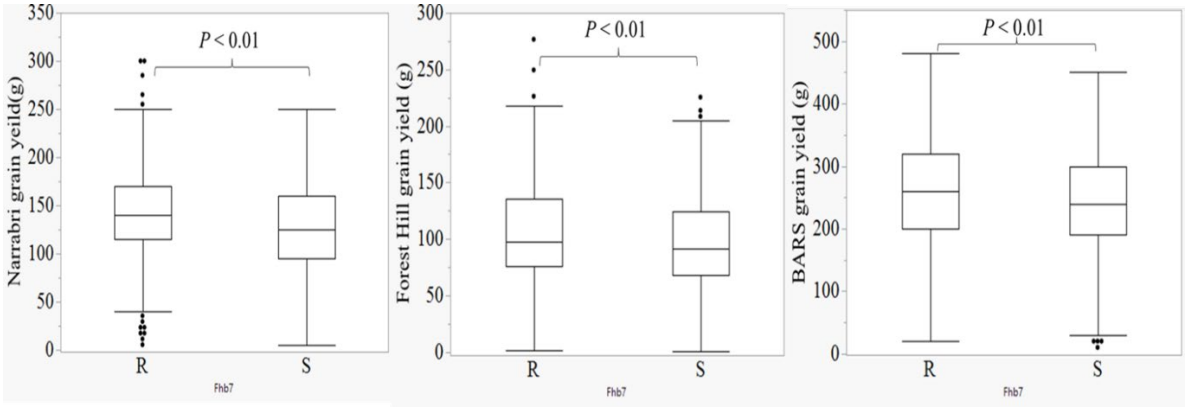

Figure 2. Comparison of grain yield from the two groups with (R) and without (S) Fhb7 genes at three sites (Narrabri, Forest Hill, Boorowa) under FCR pressure in 2023.

Breeding lines with five loci (on chromosomes 2B, 2D, 3B, 5D and 7D (Fhb7), respectively) were also assessed in 2023. The genotypes with Fhb7 and 3B locus had the highest grain yield in the presence of FCR, followed by the lines with all five resistant loci, which were significantly higher than the lines without any of these loci. Disease severity indicated the same trends. The lines without any of these resistant loci were the most susceptible to FCR infection among all the 32 combination groups. Similarly, the lines with all five resistant loci and lines with Fhb7 and 3B locus also showed the lowest disease severities in whiteheads or stem browning (Figure 3).

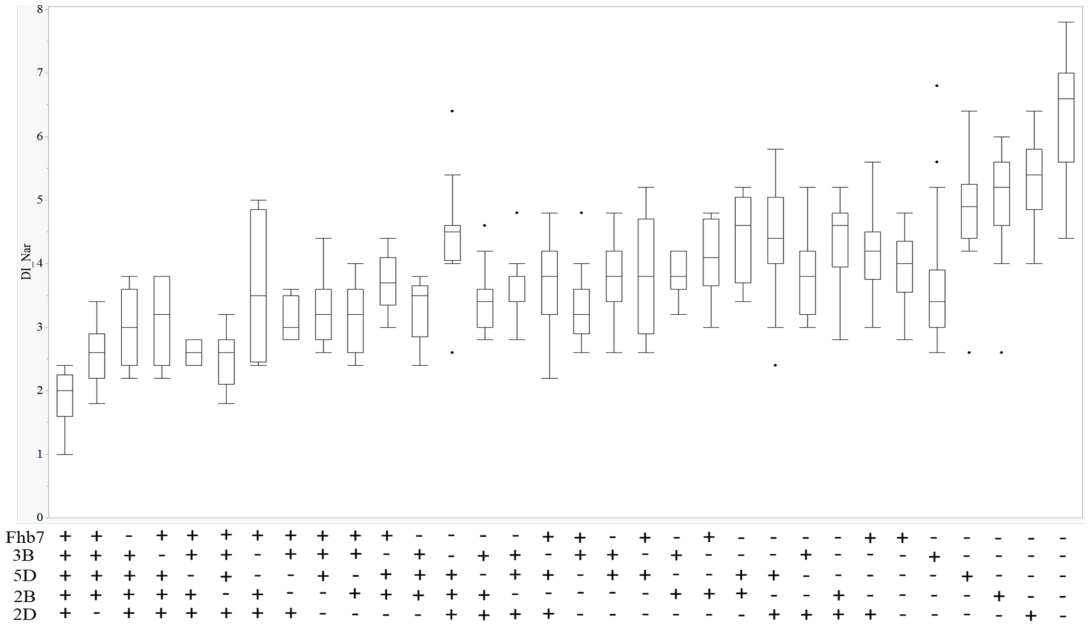

Figure 3. Box plot distribution of DI (stem browning) between the lines with various combinations of the breeding lines. Boxes indicate the 25 and 75 percentiles, respectively; the median is indicated by the solid horizontal line. '+' represents the lines presence of the resistant loci, while '-' represents the lines absence of the resistant loci.

Recently, we developed and analysed a pair of NIL targeting the 2D locus in wheat. This NIL pair showed significant differences in FCR resistance as well as drought tolerance (Su *et al.,* 2024). Analysis of the RNA-seq from this NIL pair revealed that similar regulatory frameworks were activated in coping with these two stresses.

Barley is a silent sufferer of FCR as few whiteheads occur. Results from previous studies showed that barley had more severe symptoms (stem browning and seedling death) and

accumulated higher fungal biomass compared to wheat.. Data from our field trials showed that barley suffered similar grain yield loss to that of wheat, but what caused such yield loss in barley is unknown. We showed for the first time that the reduction of fertile tiller numbers was mainly responsible for grain yield loss in barley infected by FCR (Figure 4). In addition, we recently identified a novel QTL conferring FCR resistance in barley, which was also found to be associated with drought tolerance.

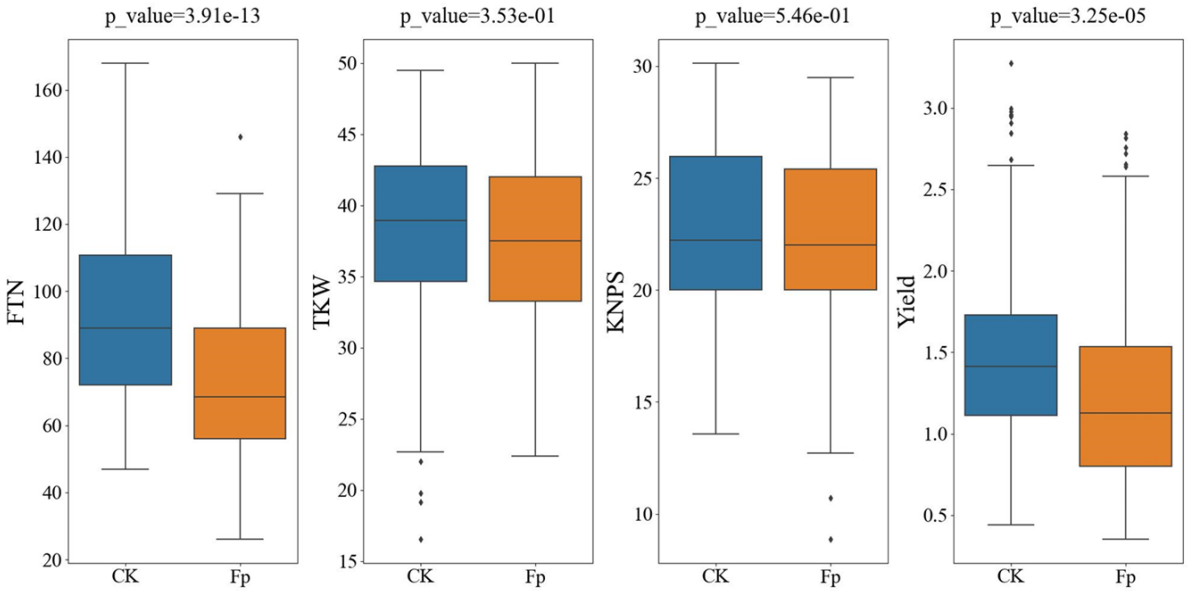

Figure 4. Box plots distributions of fertile tiller number (FTN), thousand kernel weight (TKW), kernel number per spike (KNPS), and yield between non-inoculated (CK) and *Fp*-inoculated (Fp) treatments at Narrabri, NSW and Gatton, QLD in 2019 and 2020.

What are the next steps?

Up until now, only five loci conferring FCR resistance are utilized in Australian breeding programs and three of them are derived from Australian varieties. This highlights the urgent need for additional sources of resistance, particularly those not yet present in Australian varieties, to provide breeders with a wider pool of resistance. Novel resistant sources have been identified by our team and CSIRO is actively pursuing the characterization of these novel resistance sources and incorporating them into Australian wheat/barley breeding programs.

Of all the FCR resistance reported so far, the one on chromosome 3B showed the largest effect. Working toward cloning the causal gene underlying this locus and developing perfect markers, we at CSIRO sorted and sequenced the 3B chromosome, as well as the donor genotypes itself. We have also obtained >6,000 M5 mutants from a line containing the R gene. By mapping a NIL-derived population, we have defined this locus within a 0.2 cM interval containing 14 high confident genes with SNP variations between the R and S isolines. Clearly, cloning the gene underlying the 3BL locus would advance our understanding of FCR resistance/tolerance mechanisms and minimize the damage from FCR infection.

We at CSIRO successfully cloned the first gene conferring FCR resistance in barley. Investigating the potential of transferring this resistance to wheat through gene editing represents an exciting avenue for further improving FCR resistance.

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