

# Alien introgression for FHB resistance in wheat - challenges and strategies

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## ABSTRACT

Over one thousand accessions of wheat relatives at different ploidy levels and wheat-alien species derivatives with varied chromosome constitutions were evaluated for Fusarium head blight (FHB) resistance. FHB resistance identified from the relatives and derivatives were introgressed into adapted bread and durum wheat backgrounds using several strategies. A number of resistant introgression lines that contain minimal alien chromatin and do not have obvious linkage drag were developed. Some of the introgression lines exhibited resistance to other fungal diseases in addition to FHB. The biggest challenges of alien introgression for FHB resistance are linkage drag associated with alien chromatin and the epistatic effects of alien resistance genes with genetic backgrounds. Thus, efficient manipulation of alien chromatin and selection of proper recipient genotypes play a central role in the success of alien introgression for FHB resistance.

## INTRODUCTION

Fusarium head blight (FHB) has become a predominant fungal disease of wheat worldwide. Host resistance has been considered an economically and environmentally efficient means to manage this disease. However, progress of breeding for FHB resistance has been limited because of the lack of effective resistance to FHB and the complex inheritance of the partial resistance currently available in wheat. Limited sources of partial resistance to FHB have been identified in hexaploid wheat (*Triticum aestivum*), including the Chinese cultivar “Sumai 3” and its derivatives. Partial resistance has also been identified in tetraploid wheat species, including wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) (Oliver et al., 2007), Persian wheat (*T. turgidum* ssp. *carthlicum*), and cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*) (Oliver et al., 2008). The lack of effective FHB resistance has been a major challenge in breeding for resistance to this disease in wheat. There is an urgent need to search for novel sources of effective resistance and to efficiently deploy them in new varieties.

Relatives of wheat have proven to be a rich gene source for wheat improvement, including the genes for FHB resistance. Alien chromatin containing FHB resistance

genes has been successfully integrated into wheat genomes and conferred resistance in wheat backgrounds (Shen et al., 2004; Cai et al., 2005; Chen et al., 2005; Oliver et al., 2005). In the present study more than one thousand accessions of wheat relatives and wheat-alien species derivatives were evaluated for FHB resistance. Several hundred alien introgression lines with FHB resistance in adapted hexaploid and tetraploid wheat backgrounds were developed. Here we report the results obtained about the identification, characterization, and introgression of FHB resistance derived from the alien species and wheat-alien species derivatives in common and durum wheat.

## MATERIALS AND METHODS

We identified 74 wheat-alien species derivatives with FHB resistance comparable to the Chinese common wheat cultivar Sumai 3, the most widely used source of FHB resistance, in the previous study. The alien species involved in the development of these derivatives include *Aegilops tauschii*, *Ae. ventricosa*, *Ae. speltoides*, *Thinopyrum ponticum*, *Th. elongatum*, *Th. intermedium*, *Dasyphyrum villosa*, *Secale cereale*, *Leymous racemosus*, and oat (*Avena sativa*) (Oliver et al., 2005). These resistant derivatives were first hybridized to the adapted spring wheat cultivars Alsen, Russ, Reeder, Steele, and Glenn. The F<sub>1</sub> hybrids of these crosses were hybridized to the wheat genetic stock having the *Ph* inhibitor gene *Ph<sup>1</sup>* to induce recombination between homoeologous wheat and alien chromosomes. Fluorescent genomic *in situ* hybridization (FGISH) was employed to distinguish alien and wheat chromatin from each other using the method of Cai et al. (1998).

Five *T. turgidum* ssp. *carthlicum* (2n=4x=28, genomes AABB) and four *T. turgidum* ssp. *dicoccum* (2n=4x=28, genomes AABB) accessions identified as resistant to FHB were hybridized to the durum wheat cultivars (*T. turgidum* ssp. *durum*, 2n=4x=28, genomes AABB) Lebsack, Ben, and Mountrail in the greenhouse. Both backcrossing and doubled haploid strategies were used to introgress FHB resistance from these two tetraploid wheat species into the durum cultivars. Doubled haploids were produced using the “wheat x maize” system and colchicine treatment as described by Chu et al. (2008).

Greenhouse evaluation of Type II FHB resistance was conducted for all the materials involved in this study following the procedures of Oliver et al. (2005). The advanced introgression lines, which were identified as resistant to FHB in the greenhouse, were grown in Langdon, ND and Jianyang, China to verify their resistance under field conditions as described by Oliver et al. (2008).

## RESULTS AND DISCUSSION

The 74 wheat-alien species derivatives identified as resistant to FHB contain varied amounts of alien chromatin. Among the derivatives are wheat-alien species amphiploids and wheat-alien chromosome addition, substitution, and translocation lines (Oliver et al., 2005; 2006). These derivatives more or less exhibited undesirable traits, such as late maturity, low yield potential, undesirable quality, and tall stature, in addition to FHB resistance. Some of the derivatives also exhibited resistance to tan spot and *Stagonospora nodorum* blotch (Oliver et al., 2008). It is, however, difficult to directly utilize these derivatives in breeding for FHB resistance due to linkage drag and genetic instability. Thus, we manipulated chromosomes of the resistant derivatives to eliminate unwanted alien chromatin and deployed FHB resistance genes in adapted common wheat backgrounds following the procedure illustrated in Fig. 1.

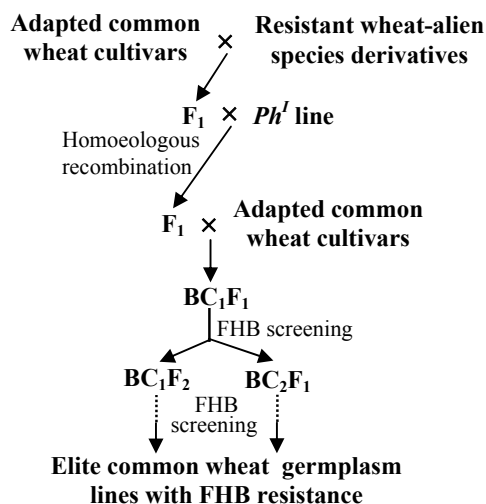


Fig. 1 Scheme of FHB resistance gene introgression in common wheat

To date, we have produced and identified 330 introgression lines with FHB resistance from two greenhouse screening seasons. Many of them exhibited resistance levels comparable to Sumai 3 under the greenhouse conditions and contain minimal alien chromatin from *Th. ponticum* ( $2n=10x=70$ ), *Th. intermedium* ( $2n=6x=42$ ), *D. villosa* ( $2n=2x=14$ ), and *Ae. ventricosa* ( $2n=4x=28$ ). Importantly, most of the introgression lines showed similar morphological characteristics to their common wheat parents. These introgression lines have been grown in the field at

Langdon and Prosper, ND and Jianyang, China to verify their resistance to FHB and to evaluate their agronomic performance under different environments.

About 120 of the introgression lines were derived from the four partial wheat-*Th. ponticum* amphiploids ( $2n=56$ ) with FHB resistance, including SS5, SS156, SS363, and SS660. SS5 and SS156 contain a complete set of wheat genomes and 14 *Th. ponticum* chromosomes (Fig. 2a), while SS660 contains 40 wheat and 16 *Th. ponticum* chromosomes and SS363 contain 40 wheat, 14 *Th. ponticum*, and 2 wheat-*Th. ponticum* translocated chromosomes (Oliver et al., 2006). Through the procedure illustrated in Fig. 1, we generated and evaluated thousands of progeny derived from the crosses in which these four amphiploids were involved. Each progeny contained various amounts of *Th. ponticum* chromatin and had different chromosome constitutions as shown in Figs. 2b-d. The Introgression line #1 and #2 contain 4 and 2 *Th. ponticum* chromosomes, respectively (Figs. 2b,c). Both were resistant to FHB, but showed low fertility, slim spikes, and late maturity. We further manipulated chromosomes in those lines to eliminate unwanted *Th. ponticum* chromatin and associated genes for the undesirable traits. Consequently, a number of germplasm lines like introgression line #3 (Fig. 2d), which exhibited FHB resistance and contained minimal *Th. ponticum* chromatin, were developed. Moreover, these lines did not show obvious linkage drag. It has been a challenge to develop alien introgression lines that can be used directly in wheat breeding, especially for traits with a complex genetic basis such as FHB resistance. However, careful manipulation of chromosomes, proper population size to recover the recombinants of interest, and efficient methods of selection, such as FGISH and molecular markers, facilitate alien introgression and the development of elite germplasm.

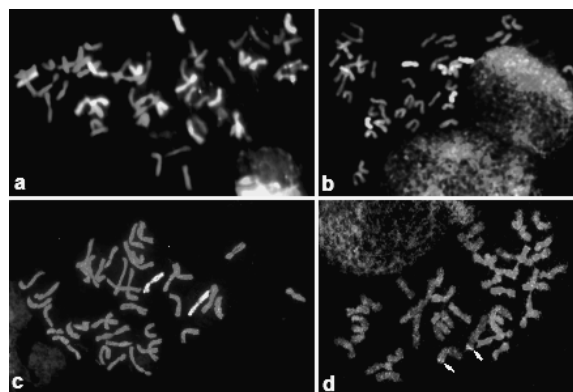


Fig. 2 FGISH patterns of mitotic chromosomes. a Partial wheat-*Th. ponticum* amphiploid 'SS5' with 42 wheat (gray) and 14 *Th. ponticum* chromosomes (white); b Introgression line #1 with 42 wheat (gray) and 4 *Th. ponticum* chromosomes (white); c Introgression line #2 with 42 wheat (gray) and 2 *Th. ponticum* chromosomes (white); d Introgression line #3 with 40 wheat (gray) and 2 wheat-*Th. ponticum* translocated chromosomes (gray and white). Arrows point to the *Th. ponticum* fragments in the translocated chromosomes.

Effective resistance to FHB has not been found in durum wheat. Introgression of FHB resistance from common wheat into durum has not been very successful (Elias Elias, personal communication). We have transferred FHB resistance from tetraploid relatives of durum wheat, which were identified as resistant to FHB (Oliver et al., 2008), to adapted durum wheat backgrounds. Since the tetraploid relatives, including Persian wheat and cultivated emmer wheat, contain the same genomes as durum wheat, i.e. “AABB”, we have been introgressing FHB resistance genes from these two tetraploids into durum genomes through the doubled haploid and backcrossing procedures.

We developed 551 doubled haploids (DH) from the crosses of the durum cultivars Lebsack, Ben, and Mountrail with the resistant Persian wheat and cultivated emmer wheat accessions. Some of the DHs showed resistance to FHB in our preliminary disease screening. These DHs have been extensively evaluated for FHB resistance under both greenhouse and field conditions. The resistant DHs will be selected to cross with the respective durum parent and new DHs will be generated from the second crosses. Again, the new DHs will be screened for FHB resistance in the greenhouse and the resistant DHs identified in the greenhouse screening will be evaluated for FHB resistance and other agronomic performance under field conditions. We anticipate developing durum germplasm lines with FHB resistance QTL derived from the tetraploid relatives and without serious linkage drag through this approach. In addition, these DHs have been used for molecular mapping of the FHB resistance QTL derived from the tetraploid relatives.

We experienced difficulties in generating DHs from some of the crosses. For those crosses, we used the backcrossing method for introgression. Under this circumstance, we selected resistant BC<sub>1</sub>F<sub>1</sub> plants and advanced generations from BC<sub>1</sub>F<sub>2</sub> to BC<sub>1</sub>F<sub>5</sub> using the single seed descent method. To date, we have generated 559 BC<sub>1</sub>F<sub>5</sub> lines. Greenhouse screening has identified resistance to FHB in some of these lines. We have been verifying resistance of these lines to FHB and evaluating their agronomic performance under field conditions.

The nature of polygenic inheritance and the strong epistasis associated with FHB resistance make alien introgression difficult. We found that some genotypes are more suitable for the expression of specific FHB resistance QTL than others. Also, a suppressor gene for FHB resistance QTL was identified in tetraploid wheat (Stack et al., 2002; Garvin et al., 2003). Therefore, proper selection of recipient genotypes could play a significant role in the efficient deployment of a favourable trait, especially for those with strong epistasis and a complex genetic basis.

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