Introduction

Many wild relatives have rearranged their chromosomes relative to that of wheat making gene transfer difficult, if not impossible, via recombination. Some wild species' chromosomes were found to transmit preferentially to the offspring and are called gametocidal chromosomes. The gametocidal genes in Aegilops sharonensis are exclusively transmitted to the next generation through both the male and female gametes when introduced into wheat. Thus, those traits linked with gametocidal genes are found in all offspring without the requirement for selection. Gametocidal genes induce chromosomal fragmentation, in gametes, early embryo and endosperm development. Previous studies indicate that at least two-linked genes are involved in the mechanism responsible for preferential transmission. The first, the 'breaker', induces chromosome breakage while the second, the 'inhibitor', prevents chromosome breakage.

The chromosomal region (45S) carrying the gametocidal genes from Ae. sharonensis has been translocated onto Chr 4D of Braggad wheat (introgression line IlS 8/2 and 8/9; King et al., 1996) and Chr 4B of Chinese Spring (T4B-48); Friebe et al., 2003) as shown in Fig. 1 (Grewal et al., 2017). Friebe et al. (2003) also developed a knock-out mutant of the "breaker" element Gc on Ae. sharonensis chromosome 45S (T4B-48*), which has lost the chromosome 'breaker' function, but has retained the 'inhibitor' element.

Characterisation of the gametocidal introgression from Ae. sharonensis

68 SNP Markers were developed in the gametocidal introgression between wheat and Ae. sharonensis using genome syntenic with rice and Brachypodium, publicly available wheat genomic sequence and Ae. sharonensis transcriptome sequence (Bouyoukos et al., 2013) as shown in Fig. 2.

A hybrid F1 population created by crossing the IlS 8/2 and 8/9 with Huntsman wheat was irradiated with a dose 300 Gy. 1658 M3 plants were phenotyped for spike fertility. Of the 68 markers developed, 54 were used to genotype the M3 population to reveal 16 lines carrying deletions of varying sizes in the 45S segment and order the markers further within the 45S segment as shown in Fig 3.

Synteny-based approaches to establish a putative gene order along Ae. sharonensis 45S

Among the 68 markers developed in the 45S segment, 57 orthologous genes were identified in rice, 61 in barley, and 51 in barley.

Targeted Sequencing of the 45S introgression

In order to find the mutation responsible for the fully fertile phenotype in line T4B-48*1, a comparison of the sequence of the mutant introgression was made with its non-mutant sequence from the T4B-48* line using target capture and next-generation sequencing as shown in Fig. 4. This work resulted in 18 potential candidate genes in Ae. sharonensis for the gametocidal ‘breaker’ element Gc as shown in Table 1.

RNA-seq analysis of the 45S introgression

Previous work suggests that the Gc element has the maximum effect during early embryo development 28-32 hours after pollination (King and Laurie, 1993). Thus, ovules were dissected from the following crosses, as shown in Figure 5, 30 hours after pollination and total RNA extracted for gene expression analysis.

1. CS x shL
2. As x A5
3. M48 x C54(48)B5
4. M48 x T4B-48*1

Where C54 is Chinese Spring wheat, A5 is Ae. sharonensis and M48 is CS monosome for Chr 4B and was used as the female donor. The latter when crossed with a Gc element containing line shows more chromosome breakage than normal euploid wheat. RNA-seq results have been obtained and the data is being analyzed.