Molecular mapping of three recombined versions of the *Lr56/Yr38* translocation in wheat

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Introduction

- Linked leaf rust (*Lr56*) and stripe (*Yr38*) resistance genes were derived from *Aegilops sharonensis* (Hackel) Maire et Weiler (Marais et al., 2006) (Fig. 1a-c).
- In a spontaneous event, an almost entire alien chromosome was translocated to chromosome arm 6AL of wheat.
- Due to its size, the full length translocation was not used in breeding programs.
- Following allosyndetic pairing induction in the absence of *Ph1*, three of the resistant recombinants that were obtained (*Lr56-39*, *Lr56-157*, and *Lr56-175*) had greatly reduced amounts of alien chromatin (Marais et al., 2010).
- The alien segments in the three recombinants appeared to be comparatively small; however, their chromosome locations and relative sizes remained unknown.

Objectives

- To determine the alien insert size in each recombinant through physical mapping (diversity array (DArT) and simple sequence repeat (SSR) markers) and genomic *in situ* hybridization (GISH).
- To also genetically map each recombinant using single nucleotide polymorphism (SNP) markers and three F_2 mapping populations.

Materials and Methods

GISH analysis: The original translocation (0352-4 with pedigree: *Ae. sharonensis*-174/ 9*CS// 3* W84-17/3/ CS/4/ W84-17) and three allosyndetic recombinants (*Lr56-39*, *Lr56-157* & *Lr56 -175*) were used for GISH following the methodology of Cai et al.,1996.

Marker analyses (SSR & DArT)

- Twenty eight mapped SSR markers (11 on 6AS & 17 on 6AL) and 756 DArT markers were used to characterize two panels of genotypes.
- The SSR panel included Chinese Spring (CS), the CS group 6 nulli-tetrasomic lines, CS ditelosomic 6AL, W84-17, *Ae. sharonensis*-174, the full length translocation and three allosyndetic recombinants.
- The DArT panel (CS, W84-17, *Ae. sharonensis*-174 and the four translocation lines) was analyzed as part of a larger group of 94 translocation lines.

SNP mapping

- F_2 and F_3 populations of each of three crosses involving recombinants *Lr56*-39, *Lr56*-157 & *Lr56*-175, respectively, were screened with mixed inoculum of *Puccinia triticina* pathotypes TDBG and MFPS. For each population 90 F_2 plants were classified as homozygous resistant, heterozygous resistant, and susceptible.
- The 90 F_2 plus F_1, 'Thatcher', W84-17, and CS controls for each population were genotyped using Illumina's Infinium wheat 9K SNP Assay (Cavanaugh et al., 2013).
- A linkage map was constructed for each population using Mapdisto 1.7.7.1.1 with default parameters.

Results and Discussion

- Preliminary results confirmed that the full length translocation consists of primarily *Ae. sharonensis* chromatin (Fig. 1d,e). However, the presence of wheat chromatin at the 6AL telomeric end of the translocation needs further confirmation, and attempts to derive clearer pictures are being continued.
- Evidence was found of the presence of small alien chromatin inserts at the end of a chromosome arm (Fig. 1f) in the three recombinants, but this needs to be confirmed by studying larger numbers of cells.

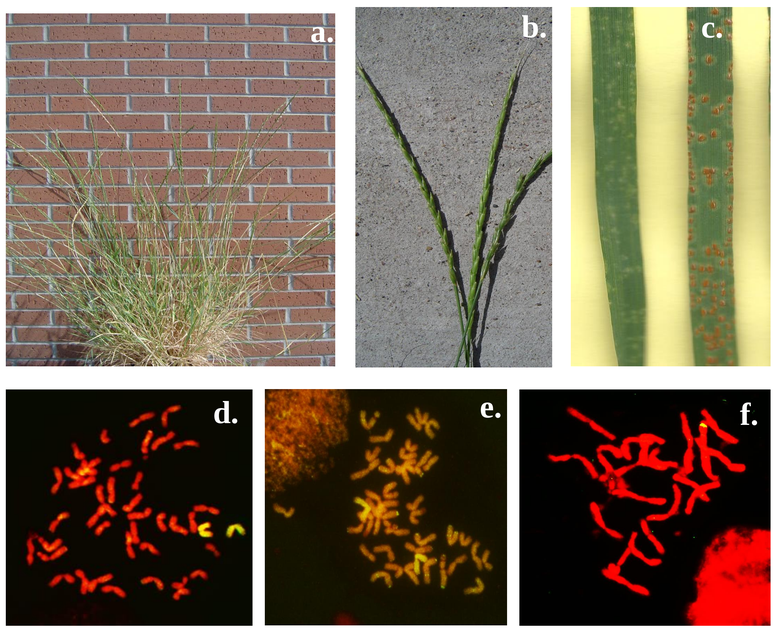

Fig. 1. (a, b) *Aegilops sharonensis*; (c) leaf rust resistant infection type of *Lr56* (left) and a susceptible infection type (right), (d,e) the full length *Lr56* translocation, (f) reduced alien chromatin in a shortened recombinant.

Genetic Mapping

- The three genetic maps (Fig. 2.) were anchored to chromosome arm 6AS using SSR markers as well as a SNP consensus map produced by Cavanagh et al. (2013).
- The three recombinants mapped to the same terminal region on 6AS and distally to the SNP locus IWA5416.
- Ninety three SNPs markers on chromosome 6A were mapped and localized to the same positions as in the consensus map of Cavanagh et al. (2013).
- The total lengths of the linkage groups for *Lr56*-39, *Lr56*-157, and *Lr56*-175 were 108cM, 157cM, and 154cM, respectively

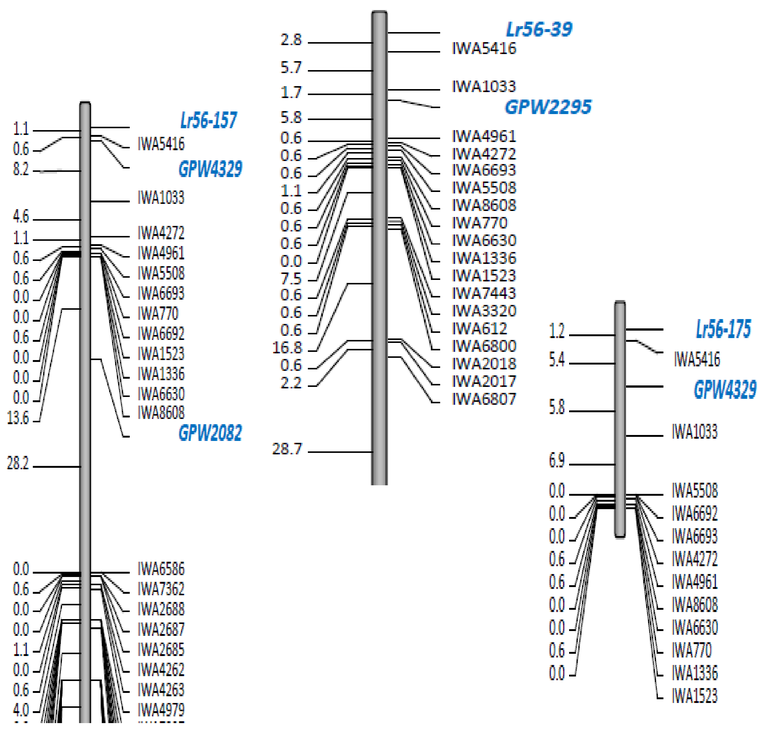

Fig. 2. Genetic maps of recombinants *Lr56*-39, -157 and -175, respectively.

Marker Analyses

- The 28 wheat-specific microsatellite loci failed to detect alien chromatin on 6A (Table 1).
- Twenty six wheat-specific DArT markers occurred within the full length translocated region. Twenty five markers were amplified in the three recombinants, yet one (previously mapped to 6A by Triticarte) was absent in all three and therefore appears to be located within the reduced alien regions. However, the location of this locus relative to the wheat SSR loci in Table 1 is unknown.
- Thus, the SSR and DArT physical mapping results were inconclusive, yet suggested that if the recombinants still occur on 6A, they could be comparatively small and located outside of the mapped region.

Table 1. Presence (1) or absence (0) of chromosome 6A microsatellite loci in the *Lr56*-full length translocation and three allosyndetic recombinants.

Locus	Deletion bin1	CSN6AT6B	CSDT6AL	Ae sharonensis-174	Lr56 Full length	Lr56-39	Lr56-157	Lr56-175
Xgpw4329	SIII	0	0	0	0	1	1	1
Xgpw2295	SIII	0	0	0	0	1	1	1
Xgwm459	SIII	0	0	0	0	1	1	1
Xgpw3087	SIII	0	0	0	0	1	1	1
Xgpw3041	SIII	0	0	0	0	1	1	1
Xgpw2082	SII	0	0	0	0	1	1	1
Xgpw7592	SII	0	0	0	0	1	1	1
Xgpw3101	SII	0	0	0	0	1	1	1
Xcfd190	SII				0	1	1	1
Xgpw4257	SII	0	0	0	0	1	1	1
Xbarc171	u	0	0	0	0	1	1	1
CENTROMERE								
Xgpw3029	LI	0	1	0	0	1	1	1
Xgpw5032	LI	0	1	0	0	1	1	1
Xwmc163	LI	0	1	1	0	1	1	1
Xgpw8068	LI	0	1	0	0	1	1	1
Xgpw7649	LI	0	1	0	0	1	1	1
Xgwm570	LII	0	1	0	0	1	1	1
Xgwm169	LII	0	1	0	0	1	1	1
Xgwm617	LII	0	1	0	0	1	1	1
Xgwm427	LII	0	1	0	0	1	1	1
Xcfa2214	u	0	1	0	0	1	1	1
Xwmc621	u	0	1	0	0	1	1	1
Xbarc1165	u	0	1	0	0	1	1	1
Xgwm334	u	0	1	0	0	1	1	1
Xwmc206	u	0	1		0	1	1	1
Xgwm256	u	0			0	1	1	1
Xcfa2153	u	0	1		0	1	1	1
Xgpw2344	LII	0	1	0	1	1	1	1
Xwmc580	u	0	1	0	1	1	1	1
Xbarc104	u	0	1	0	1	1	1	1
Xwmc59	u	0			1	1	1	1
Xgpw7388	LII	0	1	0	1	1	1	1

[1] Deletion bins defined by Sourdille et al (2014). Symbols used for the 6AS deletion bins are SI = C-6AS1-0.35, SII = 6AS1-0.35-0.65 and SIII = 6AS5-0.65-1.00; whereas the 6AL bins are indicated as LI = C-6AL4-0.55, LII = 6AL4-0.55-0.90 and LIII = 6AL8-0.90-1.00. Loci that were not mapped are indicated as "u".

Summary

- The three recombinants appear to have similar size and are located towards the 6AS telomere. The data did not reveal which is the shortest.
- Preliminary GISH results showed the presence of small regions of intercalary alien chromatin in the recombinants which is being confirmed.
- The recombined segments have normal Mendelian transmission and do not appear to have any phenotypic effects apart from the resistance. It is not known whether *Yr38* has been retained within the shortened segments.

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