

INTRODUCTION

Pre-harvest sprouting (PHS) of wheat refers to germination of wheat grain in physiologically matured spikes before harvest when prolonged wet weather occurs. PHS mainly results from early breakage of seed dormancy. PHS significantly reduces grain yield and end-use quality. Breeding PHS-resistant cultivars is the most economic method to reduce the economic losses. In general, red wheat is more resistant to PHS than white wheat. However, white wheat is the preferred wheat class for millers and many end-use products. The hard white wheat cultivar, Danby, developed by Kansas State University, has shown good PHS resistance. However, the quantitative trait loci (QTLs) controlling the PHS resistance in Danby are still unknown.

OBJECTIVES

- Construct a high density genetic linkage map using genotyping-by-sequencing (GBS) and SSR markers
- Identify PHS resistance QTLs in Danby
- Develop user-friendly markers for marker-assisted breeding to improve PHS resistance in white wheat

MATERIALS AND METHODS

- A doubled haploid (DH) population with 211 lines derived from a cross of Danby/Tiger, where Tiger is a PHS-susceptible cultivar (Fig. 1)

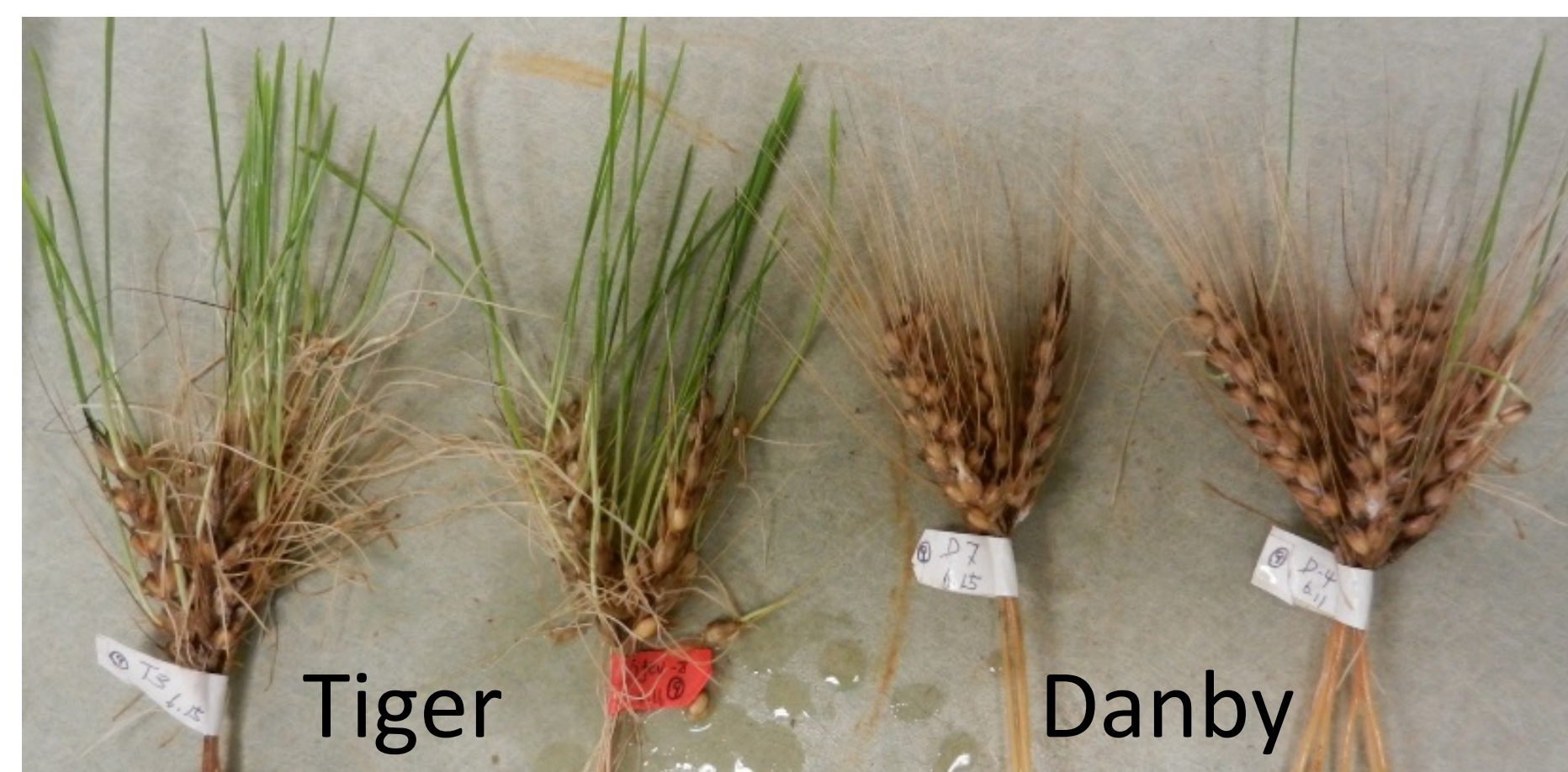


Figure 1. Two parents of the DH population used for QTL mapping. Tiger is susceptible and Danby is resistant to pre-harvest sprouting.

- A set of 46 elite breeding lines developed from Kansas-Hays wheat breeding program were used to validate new SNP marker.
- DH lines and their parents were grown in the greenhouse in 2014 and 2015 at Kansas State University, Manhattan, KS, and in the field in 2015 at Hays, KS, with two replications per experiment.
- PHS was assessed by incubating 5 spikes per line in a misting chamber and counting proportion of sprouted kernels over total kernels from the 5 spikes 7 d after incubation.
- GBS was conducted to generate single nucleotide polymorphism (SNP) markers.
- Genetic linkage map was constructed using JoinMap.
- Composite interval mapping (CIM) was performed to identify significant QTLs using WinQTLCart 2.5.

RESULTS

- GBS generated 2600 SNP markers with < 20% missing data.
- A linkage map was constructed with 1811 SNP markers and 13 SSR markers covering 34 linkage groups at 1476 cM.
- Among mapped markers, 721 (40%) are mapped on A genome, 648 (35%) on B genome and 455 (25%) on D genome (Fig. 2).

RESULTS

- Frequency distribution of PHS in the DH population shows two peaks in 2014 greenhouse experiment and skewed toward the resistant parent in 2015 field and 2015 greenhouse experiments (Fig. 3).
- A major QTL for PHS resistance was mapped on chromosome 3AS in all the three environments and explained from 20% to 44% of the phenotypic variance (Fig. 4).
- Several minor QTLs were also detected on chromosomes 2AS, 3B, 4AL, 5A and 6DS in different environments (Table 1).
- For the major QTL on 3A, SNPs in the coding region of *TaPHS1* gene reported by Liu *et al.* (2013) was not polymorphic between the parents.
- The SNP in the promoter region of *MFT-3A* gene reported by Nakamura *et al.* (2011) was polymorphic between two parents.
- A KBioscience Competitive Allele-Specific PCR (KASP) marker, KASP_MFT, was developed for this SNP (*MFT-3A*).

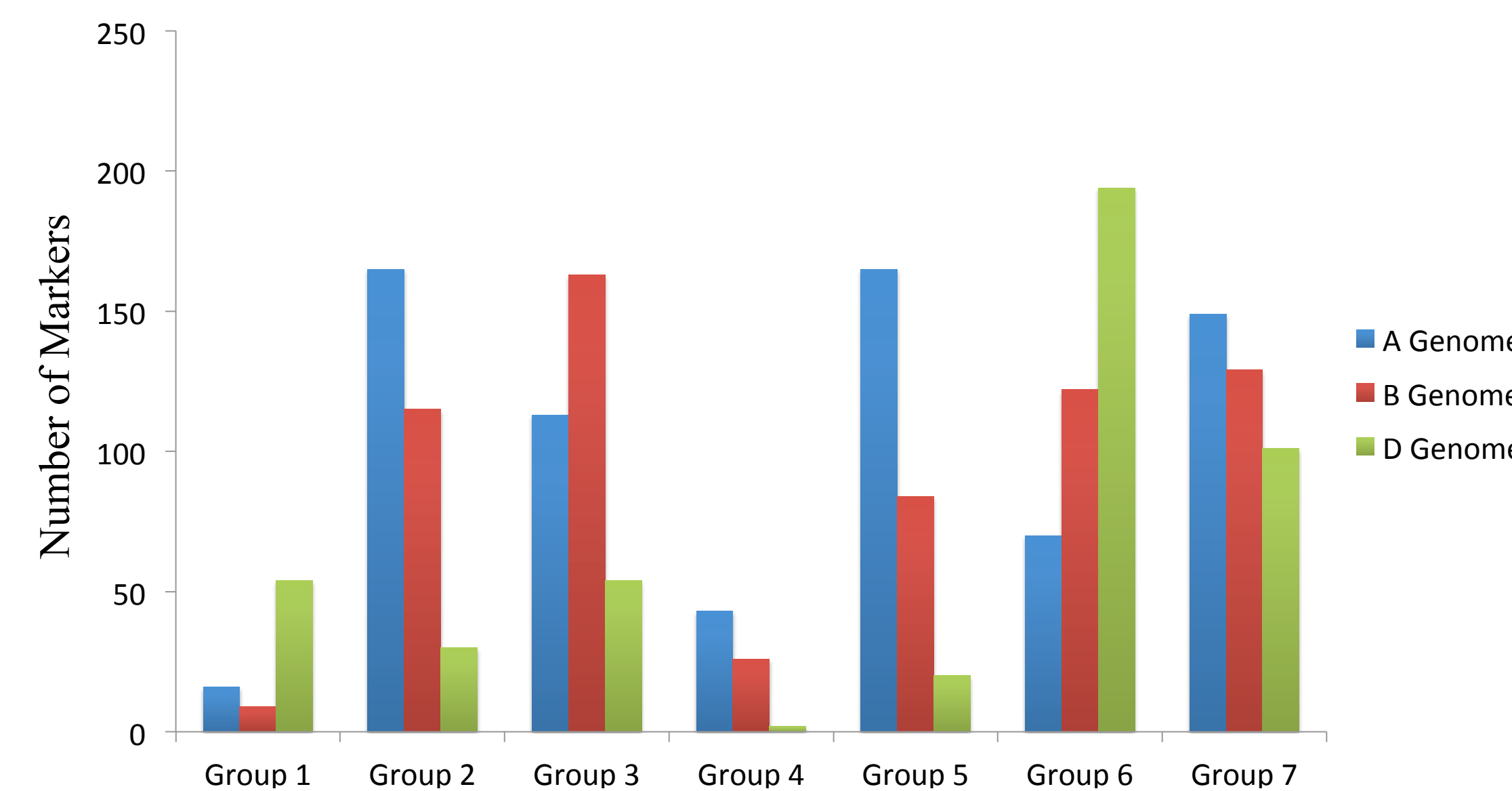


Figure 2. Distribution of GBS-SNP markers on wheat genomes

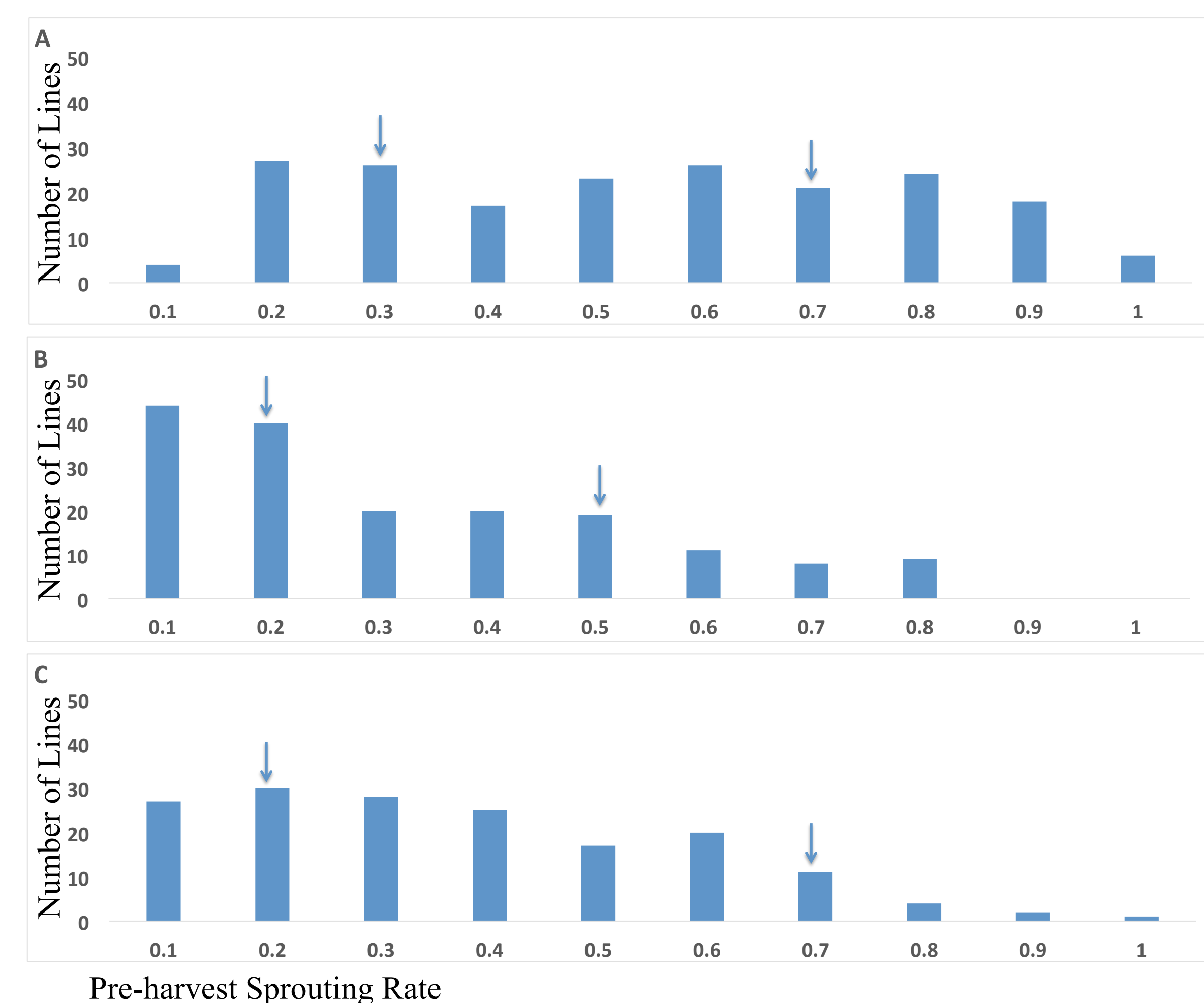


Figure 3. Frequency distributions of pre-harvest sprouting (PHS) rate in the DH population. The PHS rate is from zero to one based on proportion of sprouted kernels over total kernels from the 5 spikes after 7 d incubation and averaged from the two replications for each experiment. The blue arrows represent PHS rate of the parents. (A) PHS rate from 2014 greenhouse experiment. (B) PHS rate from 2015 field experiment. (C) PHS rate from 2015 greenhouse experiment.

RESULTS

- Marker KASP_MFT was mapped under the peak of 3A QTL (Fig. 4) and explained from 25% to 49% of the phenotypic variation.
- This SNP marker was screened for a set of 46 elite breeding lines developed from Kansas-Hays wheat breeding program and there was significant difference in PHS between two allele types: 0.45 for Danby allele type and 0.91 for Tiger allele type.

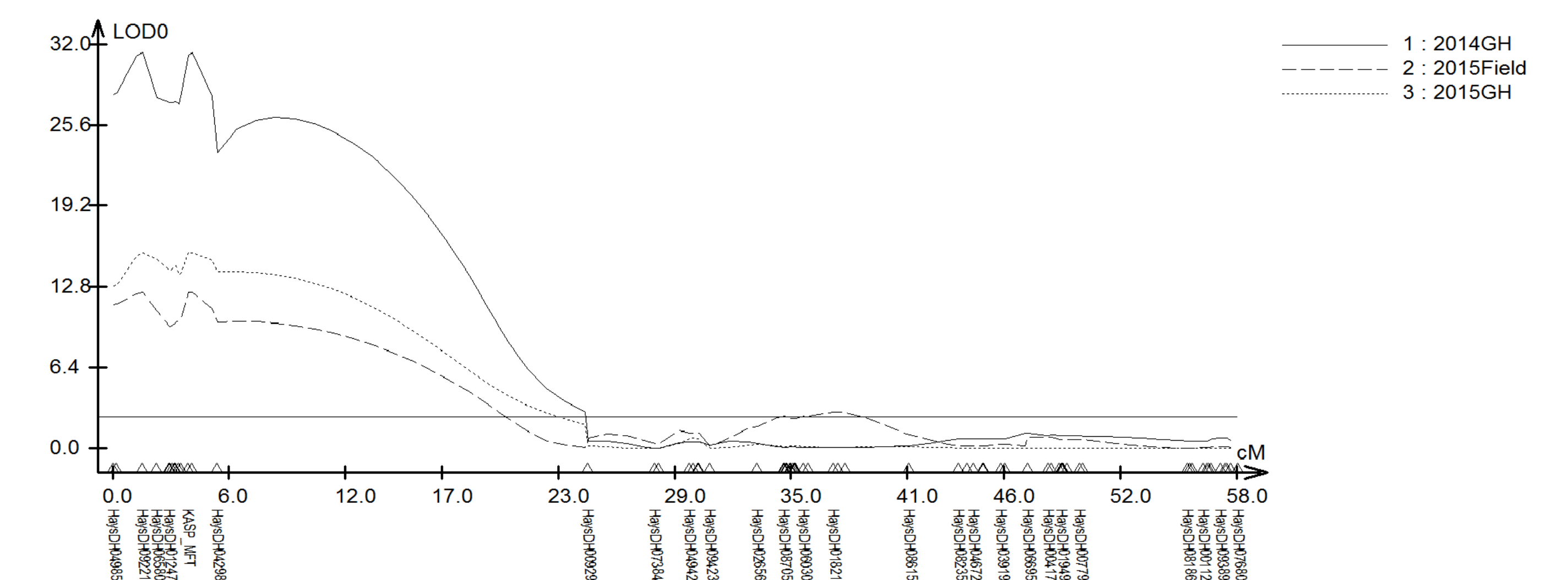


Figure 4. Composite interval mapping of a QTL for pre-harvest sprouting resistance on chromosome 3A in three different environments. GH represents greenhouse experiment.

Table 1. List of PHS resistance QTLs detected in three environments.

Environment	Chromosome	Loc. (cM)	LOD	PVE
2014GH	3A	4.11	31.40	44%
2014GH	5A	14.35	6.85	6%
2014GH	3B	3.69	3.21	3%
2015Field	3A	4.11	12.39	20%
2015Field	2AS	115.5	9.10	13%
2015Field	3B	9.11	3.56	5%
2015GH	3A	4.11	15.56	27%
2015GH	4AL	47.73	3.54	5%
2015GH	6DS	4.55	3.38	4%
2015GH	5A	14.35	3.30	4%

*PVE: Percentage of phenotypic variance explained by QTL.

SUMMARY

- A high resolution genetic map constructed with GBS-SNP markers is powerful for detecting QTLs of important agronomic traits.
- The QTL on 3A was stable across environments and mapped in the same position as *TaPHS1* gene located, however, this QTL may be a different allele from that in RioBlanco as reported by Liu *et al.* (2013).
- A KASP marker (KASP_MFT) was developed based on a SNP in promoter region and it explained the largest portion of phenotypic variance for PHS resistance in the mapping population.
- This KASP marker was validated in a set of wheat breeding lines and it can be useful for marker-assisted selection for PHS resistance from Danby.

REFERENCES

- Liu, S. B., et al. (2013). "Cloning and Characterization of a Critical Regulator for Preharvest Sprouting in Wheat." *Genetics* 195(1): 263-273.
 Nakamura, S., et al. (2011). "A Wheat Homolog of MOTHER OF FT AND TFL1 Acts in the Regulation of Germination." *Plant Cell* 23(9): 3215-3229.

ACKNOWLEDGEMENTS

This project was partially supported by Kansas Wheat Alliance and USDA NIFA Hatch project KS509. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.