Fine Mapping of a Quantitative Trait Loci for Wheat Resistance to Pre-harvest Sprouting Using Genotyping-by-Sequencing (GBS)

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INTRODUCTION

Wheat pre-harvest sprouting (PHS), germination of physiologically matured grains in a spike before harvesting, can cause significant reduction in wheat end-use quality and thus in grain sale price. Short or no seed dormancy (SD) has been considered as the major component of PHS. A quantitative trait locus (QTL) for SD and PHS resistance has been previously mapped on wheat chromosome 4AL, but markers linked to the QTL are too far from the QTL, thus not very useful for marker-assisted breeding. Genotyping-by-Sequencing (GBS) using next generation sequencing technology is very effective in SNP identification and high resolution genetic map construction, and thus facilitates QTL fine mapping.

OBJECTIVES

A) Fine map the 4A QTL for both PHS resistance and SD.
B) Develop closely linked molecular markers to the QTL for marker-assisted selection in wheat breeding programs.

MATERIALS AND METHODS

➢Plant materials include a bi-parental population of 155 F6 recombinant inbred lines (RILs) derived from Totoumai A x Siyang 936 by single-seed decent, and a natural population consisting of 205 U.S., 120 Chinese, 26 Japanese and 3 Korean wheat accessions.

➢Genomic DNA was extracted using the CTAB method, digested with *HF-PstI* and *MspI,* ligated with barcoded adaptors and Y common adaptor, then pooled together, and amplified by PCR (Poland *et al*. 2012). The PCR product was sequenced on an Ion Proton system (Life Technologies Inc.).

➢Physiologically matured wheat spikes were dried for 10 days in a greenhouse and enclosed in the moist chamber at 22℃ to evaluate sprouting rate in a spike. For SD test, 50 hand-threshed kernels from each line were germinated in a petri dish, and a weighted germination index was calculated. All phenotypes were evaluated in a greenhouse at Kansas State University, Manhattan, KS in 2005 and 2006.

➢JoinMap ver. 4.0 was used for linkage map construction and WinQTL Cartgrapher 2.5 was used for QTL analysis.

➢GBS markers mapped to the QTL region were converted to KASP SNPs and analyzed in both RIL and natural populations to verify the genotypic data generated by GBS and to eliminate missing data.

RESULTS

➢The frequency of PHS resistance and SD in the RIL population showed a continuous distribution (Fig. 1).

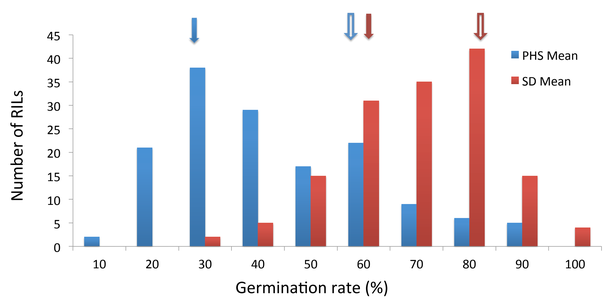

Figure 1. Frequency distributions of the mean germination rate and weighted germination index over the 2005 and 2006 experiments for PHS resistance and SD, respectively. The solid arrow represents Tutoumai A and the empty arrow represents Siyang 936.

RESULTS

➢GBS mapping identified two SNPs, *GBS212432* and *GBS109947*, in the 4A QTL region, and delimitated the QTL to a 2.9 cM interval (Fig. 2).

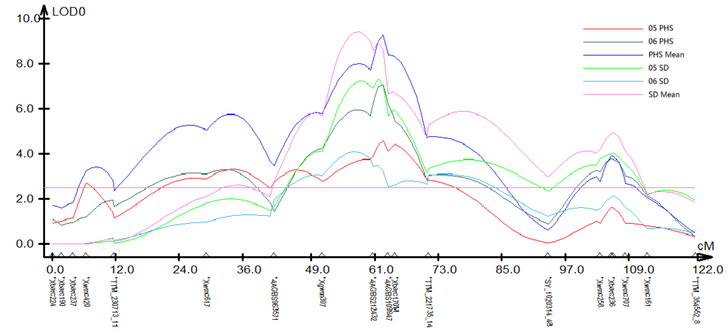

Figure 2. Interval mapping of QTLs for long period of SD and PHS resistance on chromosome 4A with SSRs and SNPs using phenotypic data from 2005 and 2006. The line parallel to the X-axis is the threshold line for the significant LOD value of 2.24 ($P < 0.05$). Genetic distances are shown in centiMorgan (cM).

➢The QTL on chromosome 4A explained 26 % phenotypic variances for both mean PHS resistance and SD (Table 1).

➢Both markers *Xbarc170* and *GBS109947* showed the largest effect on PHS resistance and long SD among all markers tested in both experiments (Table 1). However, combined analysis of *GBS212432* and *GBS109947* showed a larger effects on both traits measured than either markers (Table 1), thus QTL was located at a 2.91 cM interval between *GBS212432* and *GBS109947*.

Table 1. Closely linked markers, LOD values, and coefficients of determination (R^2) of the QTL on chromosome 4AL based on the phenotypic data of the recombinant inbred lines (RILs) derived from Tutoumai A x Siyang 936 collected in 2005 and 2006 experiments

Closely linked markers	position	2005 exp		2006 exp		Mean	
		LOD	R^2	LOD	R^2	LOD	R^2
PHS							
Xgwm397	51.04	2.80	0.08	4.28	0.13	5.79	0.16
GBS212432	60.52	3.87	0.11	6.25	0.18	8.18	0.22
GBS109947/GBS212432	62.53	4.61	0.14	7.09	0.21	9.27	0.26
GBS109947	63.43	4.13	0.12	6.23	0.18	8.40	0.23
Xbarc170	64.78	4.45	0.13	5.45	0.16	8.32	0.23
SD							
Xgwm397	51.04	4.14	0.12	3.07	0.09	5.70	0.16
GBS212432	60.52	6.94	0.19	3.46	0.10	8.60	0.23
GBS109947/GBS212432	61.53	7.32	0.22	3.48	0.11	8.98	0.26
GBS109947	63.43	5.69	0.16	2.53	0.08	6.67	0.19
Xbarc170	64.78	5.91	0.17	2.61	0.08	6.64	0.19

➢Both *GBS109947* and *GBS212432* were converted into KASP SNP markers and analyzed in a set of wheat natural population, and SNP *GBS212432* showed high diversity, but SNP *GBS109947* had a rare allele that may not be polymorphic in most of cultivars (Fig. 3).

➢Because *Xbarc170* showed a similar effect as *GBS109947* (Table 1&2) and *GBS109947* had a rare allele, *GBS212432* and *Xbarc170* can be used as franking markers for the 4A QTL in marker-assisted breeding.

RESULTS

Table 2. Difference (Dif) in PHS and SD ratings as reflected by a percentage of germinated seeds between resistance (R) and susceptible (S) alleles of two SNPs and two SSRs linked to the PHS resistance QTL on chromosome 4A.

		PHS			SD		
Locus	Genotype	2005	2006	PHS Mean	2005	2006	SD Mean
GBS109947	S	39.88	53.28	56.82	71.20	69.89	57.12
GBS109947	R	22.51	33.99	45.56	54.59	59.86	41.64
GBS109947	**Dif**	**17.37**	**19.29**	**11.27**	**16.61**	**10.04**	**15.48**
Xbarc170	S	39.88	52.87	57.29	71.70	69.93	57.33
Xbarc170	R	22.49	34.98	46.11	54.59	59.87	41.63
Xbarc170	**Dif**	**17.39**	**17.89**	**11.18**	**17.12**	**10.06**	**15.70**
GBS212432	S	38.51	52.55	57.13	71.61	70.10	56.96
GBS212432	R	23.10	34.19	45.04	53.57	58.89	41.10
GBS212432	**Dif**	**15.40**	**18.36**	**12.09**	**18.04**	**11.21**	**15.86**
Xgwm397	S	38.93	52.71	56.78	70.85	70.18	56.92
Xgwm397	R	25.19	36.73	47.02	56.89	60.13	43.72
Xgwm397	**Dif**	**13.74**	**15.98**	**9.75**	**13.95**	**10.06**	**13.20**

Summary

➢GBS can be effectively used to identify new SNP markers for QTL mapping and cloning.

➢A major QTL for PHS resistance and long SD was delimitated to a 2.9 cM interval between SNPs *GBS212432* and *GBS109947.*

➢*GBS212432* and *Xbarc170* can be used as flanking markers in marker-assisted breeding to select for 4A PHS resistance QTL.

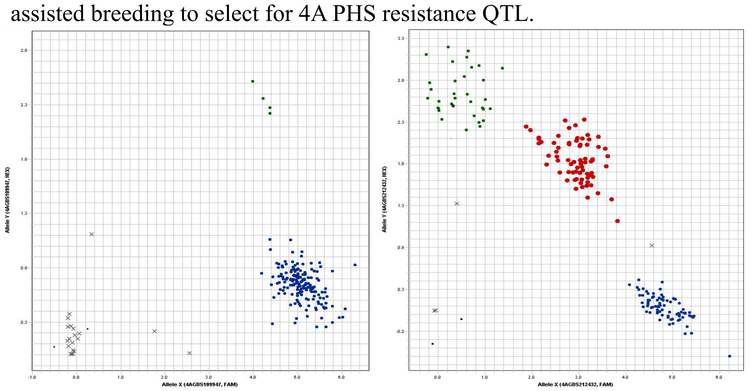

Figure 3. KASP assay profiles for *GBS109943* and *GBS212432,* two SNP tightly linked to PHS resistance QTL in 4A. The KASP SNPs were evaluated in a subset of the natural population. Blue color shows the Tutoumai A allele, green color shows the Siyang 936 allele, and the red color is the genotype of either parents. Black cross represents undetermined genotypes due to poor PCR amplification and black dots are water controls.

MAJOR REFERENCE

Poland JA *et al*. (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PloS One 7: e32253

ACKNOWLEDGEMENT

This project was partly supported by the National Research Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA National Institute of Food and Agriculture. 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.