Fine Mapping Coincident QTL for Multiple Traits Linked with *Gpc1* on Chromosome 6H of Barley.

Lu Yin[1], Ahmad Sallam[2], Ed Schiefelbein[1], Guillermo Velasquez[1], Karen Beaubien[1] and Kevin Smith[1]

[1]Department of Agronomy and Plant Genetics, University of Minnesota; [2]Department of Plant Pathology, University of Minnesota

SMALL GRAINS INITIATIVE
UNIVERSITY OF MINNESOTA
Minnesota Agricultural Experiment Station

Introduction

Barley with minimal kernel discoloration (KD), low deoxynivalenol accumulation, resistance to Fusarium head blight (FHB), net blotch (NB) and bacterial leaf streak (BLS), and low grain protein (GP) content (115 - 135 g/kg) are desirable for the malting and brewing industry. At the same time, it is important to improve agronomic traits such as yield, lodging, stem breakage, height and maturity for the benefits of barley growers. QTL studies have associated these traits with a region of chromosome 6H near bin 6 of barley (de la Pena et al., 1999; Canci et al., 2004; etc.). This region is also orthologous to *Gpc-B1* influencing GP in wheat (Distelfeld et al., 2008).

Some of these traits are unfavorable associated, posing a challenge for breeding. For example, the cultivar Chevron has good FHB and KD resistances, but high GP and poor agronomic traits (de la Pena et al., 1999), while the cultivar Karl has low GP but higher instance of KD. Whether genetic associations among these traits can be broken through recombination remains to be known (due to linkage instead of pleiotropy).

We used backcrossing to introgress this 6H region from Chevron into the variety Lacey to determine genetic associations among these quantitative traits. The resulting near-isogenic population (Figure 1) has Lacey background with variable introgression from Chevron at 30-100 cM.

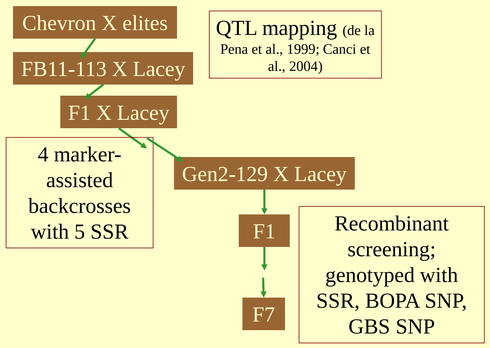

Figure 1. Gen10 mapping population developed by Chevron X elite crosses, 4 generations of backcrosses to Lacey while selecting for Chevron allele spanning this 6H QTL region, and recombinant screening through several generations of selfing.

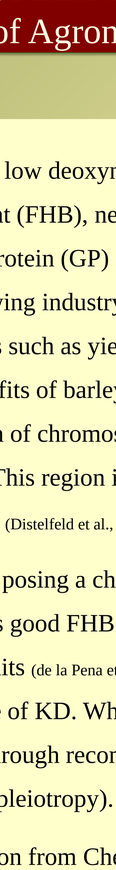

Figure 2. Graphical representation of chromosome 6H of Chevron, Lacey, FB11-113 and Gen2-129 (from left to right). Gen2-129 is a near-isogenic BC4 line to Lacey and carries a segment of Chevron spanning this 6H QTL region.

Objectives

1. Validate previous associations of the above traits to this 6H region.
2. Fine map the 6H region for the above traits.
3. Recover recombinants with favorable associations (if trait associations are 'breakable').

Methods

1. 101 recombinants of Gen10 population were phenotyped for 13 traits with RCBD at ≥ 2 field locations/greenhouse in 2015 with ≥ 2 reps.

2. Analysis of variance was conducted to determine traits having significant line effects. For traits measured at multiple locations, Levene's Test for homogeneity of variance and coefficients of variation were used to determine if we should conduct combined location ANOVA (Gomez & Gomez, 1984).

3. Correlations among traits are performed after correction for sig. rep and loc effects, if any. When traits exhibited sig. line X loc effect, we examined individual locations.

Results

Traits	Trials	Chevron	Gen2-129	Lacey	Gen10 population				
					Mean	Min	Max	p value[a]	Other sig effects[b]
Ldg	Yld_CR	9.0	6.0	2.8	3.9	1.0	9.0	ns	Rep
	Yld_StP	8.8	4.5	3.5	4.9	0.0	8.0	0.0230	Rep
Senesc	Yld_CR	16	18	18	17	15	24	< 0.001	Loc, Loc/Rep, Loc X Line
	Yld_StP	8	3	5	7	2	17		
NDVI	Yld_CR	0.42	0.51	0.56	0.50	0.34	0.78	< 0.001	Loc, Loc/Rep, Loc X Line
	Yld_StP (07/01)	0.64	0.52	0.58	0.61	0.43	0.76		
	Yld_StP (07/07)	0.43	0.29	0.31	0.39	0.20	0.62		
	NB_CR	0.27	0.22	0.27	0.24	0.16	0.48		
FHB	FHB_CR	2	11	15	13	5	26	0.00990	Loc/Rep
	FHB_StP								
BLS	BLS_CR	3	7	6	6	2	8	ns	Loc, Loc/Rep
	BLS_StP								
NB	NB_CR	2	2	4	3	1	8	< 0.001	Rep
HD	Yld_CR	29	25	23	25	18	36	< 0.001	Loc, Loc/Rep
	FHB_CR								
	FHB_StP								
Stem break	Yld_CR	100	60	0	21	0	70.00	< 0.001	
Ht	Yld_CR	102.5	98.5	95.3	98.6	86.0	110.0	< 0.001	Loc/Rep
	FHB_StP	90.5	103.0	96.0	97.3	76.0	110.0		
Yld	Yld_CR	694	1106	1271	1186	498	1758	0.00338	Loc, Loc/Rep
	Yld_StP								

Table 1. Statistical summary of lodging, senescence date (days in July), Normalized Difference Vegetation Index, FHB, BLS, NB, heading date (days in June), stem breakage, height (cm) and yield (g) of 101 Gen10 lines, its parents (Gen2-129 and Lacey) and Chevron.
[a] Line effect; [b] **S**ignificance at 0.05 level.
Row effects were detected in most of the traits but were not included in the model.

1. Senescence date, FHB, NB, stem breakage, yield showed significant line effect suggesting these traits are associated with this 6H QTL region.

2. Heading date was similar across lines except for one outlier (Gen10-093), which will be investigate further. Thus, heading date is unlikely influenced by Chevron allele at the 6H region.

3. BLS showed no significant line effect and is likely not associated with this 6H region.

4. Lodging showed slightly sig. line effect (p=0.023) in one of two environments; this was similarly found for height (p=0.019); NDVI had sig. line effect in three of four environments (not shown in Table 1). These traits might have minor QTL at the 6H region.

5. Lodging was also measured at FHB_StP and was detected as a sig. covariate for FHB score suggesting FHB and lodging might be controlled by the same locus.

	Ldg_Yld_StP	Senesc_Yld_CR	Senesc_Yld_StP	NDVI_Yld_CR	NDVI07_YldStP	NDVI_NB_CR	FHB	NB_CR	Hdng	StemBr_Yld_CR	Ht_Yld_CR	Yld
Ldg_Yld_StP	1	0.15	0.35	0.1	0.42	0.05	-0.26	0.03	-0.07	-0.09	0.08	0.28
Senesc_Yld_CR	0.15	1	0.61	0.78	0.45	-0.02	-0.08	0.02	0.18	-0.6	-0.34	0.39
Senesc_Yld_StP	0.35	0.61	1	0.57	0.87	0.01	-0.18	0.13	0.19	-0.54	-0.12	0.55
NDVI_Yld_CR	0.1	0.78	0.57	1	0.46	-0.04	0.02	-0.01	0.08	-0.68	-0.4	0.36
NDVI07_YldStP	0.42	0.45	0.87	0.46	1	-0.07	-0.13	0.09	0.09	-0.45	-0.03	0.53
NDVI_NB_CR	0.05	-0.02	0.01	-0.04	-0.07	1	-0.02	0.6	-0.08	-0.01	-0.01	-0.12
FHB	-0.26	-0.08	-0.18	0.02	-0.13	-0.02	1	0.01	-0.05	-0.16	-0.06	-0.37
NB_CR	0.03	0.02	0.13	-0.01	0.09	0.6	0.01	1	-0.16	-0.1	-0.06	0.12
Hdng	-0.07	0.18	0.19	0.08	0.09	-0.08	-0.05	-0.16	1	0.05	0.07	-0.04
StemBr_Yld_CR	-0.09	-0.6	-0.54	-0.68	-0.45	-0.01	-0.16	-0.1	0.05	1	0.37	-0.25
Ht_Yld_CR	0.08	-0.34	-0.12	-0.4	-0.03	-0.01	-0.06	-0.06	0.07	0.37	1	-0.22
Yld	0.28	0.39	0.55	0.36	0.53	-0.12	-0.37	0.12	-0.04	-0.25	-0.22	1

Figure 3. Correlation coefficients of lodging, senescence date, NDVI, FHB, BLS, NB, heading date, stem breakage, height and yield of 101 Gen10 lines after sig. rep and location effects are corrected, if any.

1. NDVI correlates highly with senescence date and is a promising high-throughput tool for measuring senescence.

2. Senescence date negatively correlates with stem breakage, while positively correlates with yield suggesting these traits might be controlled by the same locus.

3. NB and FHB correlate weakly with other traits, suggesting they might be conferred by different loci to the other traits.

Future directions

1. Use Genotype-by-sequencing (Poland et al., 2012) to generate SNP data and construct a dense genetic map for this coincident QTL region.

2. Obtain phenotypic data for kernel discoloration, deoxynivalenol, and grain protein conctent.

3. Genotypic & phenotypic data are compared to narrow down the causal region for each trait to determine genetic associations of these traits.

4. Identify recombinants with favorable combinations of resistances and yield/quality traits.

References & Acknowledgements

Canci, PC, Nduulu, LM, Muehlbauer, GJ, Dill-Macky, R, Rasmusson, DC, & Smith, KP (2004). Validation of quantitative trait loci for Fusarium head blight and kernel discoloration in barley. Molecular Breeding, 14(2), 91-104.
de la Pena, R. C., Smith, K. P., Capettini, F., Muehlbauer, G. J., Gallo-Meagher, M., Dill-Macky, R., & Rasmusson, D. C. (1999). Quantitative trait loci associated with resistance to Fusarium head blight and kernel discoloration in barley. Theoretical and Applied Genetics, 99(3-4), 561-569.
Distelfeld, A., Korol, A., Dubcovsky, J., Uauy, C., Blake, T., & Fahima, T. (2008). Colinearity between the barley grain protein content (GPC) QTL on chromosome arm 6HS and the wheat Gpc-B1 region. *Molecular Breeding*, *22*(1), 25-38.
Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & Sons.
Poland, J.A., P.J. Brown, M.E. Sorrells, and J.L. Jannink (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253.
We thank Shiaoman Chao (ARS-USDA, NDSU) for providing us GBS data, Yadong Huang, Jerome Franckowiak, Jeffrey Neyhart, Tyler Tiede and other Smith lab members for information and assistance in analysis, Dill-Macky lab, Steffenson lab, Madeleine Smith lab, other collaborators in Crookston, Elijah Rasmuson and other undergraduates for assistance in field work.