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ABSTRACT

End-use quality analysis is an indispensable component of wheat breeding and genetic pipeline. However, standard protocols for end-use quality analysis are tedious and time consuming. The objective of this study was to map and tag quantitative trait loci (QTL) linked to end-use quality in hard red winter wheat. We used 90K SNP array to fingerprint 217 wheat recombinant inbred lines (RIL) derived from an elite-by-elite cross, CO960293-2/TAM 111. The population was evaluated for flour protein content using Near Infrared (NIR) spectroscopy, kernel diameter, kernel weight and dough rheology. A linear mixed model for single-trait and multi-trait QTL analysis revealed significant QTL on chromosome 1A, 1D and 2D associated with mid-line peak integral (MPI), mid-line peak time (MPT), hardness index (HI) and flour protein content (FPC). Most QTL showed significant QTL-by-trait and QTL-by-environment interactions. The QTL detected accounted up to 28.2% of the phenotypic variance in the present study. NCBI BLAST revealed that the SNP M11264 at 379.8 cM is linked to gliadin/avenin-like seed protein mRNA. Co-location of QTL linked to rheological parameters was observed on chromosome 1A and 1D suggesting possible linkage or pleiotropic effects. SNP linked to QTL for end-use quality are potential tools for marker-assisted selection in wheat.

INTRODUCTION

- The trajectory of a wheat breeding program is dictated by the both agronomic and end-use quality requirements which are intricately related to market requirements.
- The standard laboratory protocol for end use quality analysis involves kernel characterization, protein content analysis, and analysis for rheological properties.
- These protocols are tedious and time consuming and therefore relegating quality testing towards the tail end of the wheat breeding cycle when there is significant reduction in the lines to be tested.
- Moreover, during early generation the amount of seed available for extensive quality analysis is limited.
- Use of markers as a proxy for end-use quality can be a valuable tool for wheat improvement programs.
- However, this requires detection of diagnostic allelic variants linked to the trait of interest.

OBJECTIVE

- Identify and tag quantitative trait loci linked to end-use quality traits in hard red winter wheat.

EXPERIMENTAL APPROACH

- 217 F₇ RIL derived from two elite parents, CO960293-2 and TAM 111 were evaluated across 8 environments in USA
- Three environments were randomly selected for quality analysis. The environments were Bushland, TX; Etter, TX and Hays, KS
- Preliminary analysis involved single kernel characterization using single kernel characterization system SKCS 4100 (www.perten.com)
- Flour Protein content was determined using Near-Infrared spectroscopy interfaced with Simplicity Plus software v. 2.86
- Rheological and mixing properties of the dough were quantified using the Mixograph (Table 1)
- The RILs were genotyped using 90K SNP array and the data called in GenomeStudio software (www.illumina.com)
- Best linear unbiased predictors (BLUP) were computed using residual maximum likelihood
- Single trait multi-environment and multi-trait QTL model were used for QTL Analysis in GenStat (VSN 2015, Malosetti et al 2013)

RESULTS

Table 1. Mean, variance components and heritability for end-use quality

Variable name	Abbrev.	Units	σ^2_{Gen}	σ^2_{Env}	σ^2_{Res}	h^2	$\bar{x} \pm SE$
Hardness Index	HI	%	9.7	22.2	20.4	0.6	68.6 ± 0.29
Flour protein content	FPC	%	0.1	0.2	0.3	0.5	13.0 ± 0.04
Midline left time	MLT	min	1.4	1.0	0.9	0.8	3.7 ± 0.06
Midline left value	MLV	%	7.3	13.7	38.1	0.4	49.9 ± 0.42
Midline left slope	MLS	% min ⁻¹	16.3	16.3	20.1	0.7	8.5 ± 0.28
Midline left width	MLW	%	13.5	31.1	49.1	0.5	31.8 ± 1.07
Midline left integral	MLI	%TQ×min	1478.8	638.5	1191.9	0.8	122.7 ± 2.00
Midline peak time	MPT	min	1.4	1.0	0.9	0.8	4.7 ± 0.06
Midline peak value	MPV	%	12.0	34.3	38.4	0.5	54.6 ± 0.42
Midline peak width	MPW	%	5.0	5.3	30.8	0.3	24.1 ± 0.37
Midline peak integral	MPI	%TQ×min	1328.1	446.8	1319.0	0.8	175.7 ± 2.15
Midline right time	MRT	min	0.7	0.4	0.9	0.7	5.7 ± 0.06
Midline right value	MRV	%	3.3	2.3	18.7	0.3	51.9 ± 0.29
Midline right slope	MRS	% min ⁻¹	0.9	1.5	2.1	0.6	-2.8 ± 0.10
Midline right width	MRW	%	7.9	6.2	17.4	0.6	16.0 ± 0.27
Midline right integral	MRI	%TQ×min	824.4	487.7	1978.9	0.6	244.5 ± 2.79
Midline time_X value	MTXV	%	3.4	1.5	27.4	0.3	46.4 ± 0.35
Midline time_X width	MTXW	%	15.2	13.2	19.0	0.7	11.9 ± 0.27
Midline time_X integral	MTXI	%TQ×min	660.8	1061.2	1719.5	0.5	343.6 ± 2.78
Midline mixing stability	MMST	%	25.4	32.5	29.2	0.7	38.8 ± 0.81

Fig. 1. Computer generated mixogram depicting dough mixing properties for paternal and maternal parent. Variables were recorded both at midline and envelope level. In the current study, data from the midline analysis was used for QTL mapping

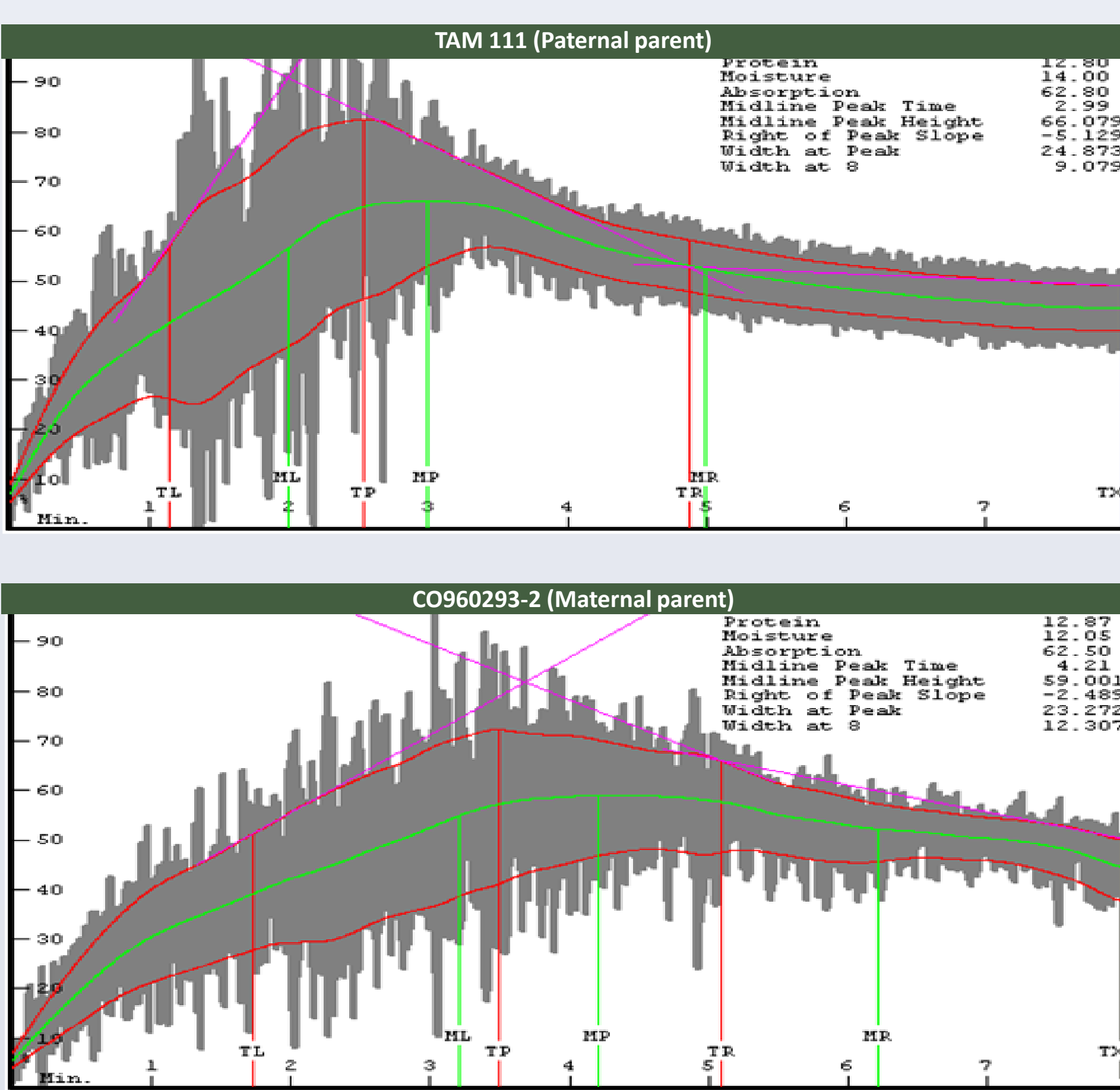


Fig. 2. Genome-wide QTL scan across the environments. The upper graph is QTL profile plot with the y-axis = threshold for declaring significance of QTL. The red horizontal represents the threshold corrected for the number of independent tests using Li and Ji (2005). The lower plot is the genome-wide heat map of significant QTL. The y-axis is the environments (traits for multi-trait model) and the x-axis represents the chromosomes. Two vertical dotted lines or a dotted and continuous line delineate a linkage group. The light blue to blue color indicates the high value allele (HVA) originates from CO960293-2 and the yellow-red color indicates the HVA originates from TAM 111

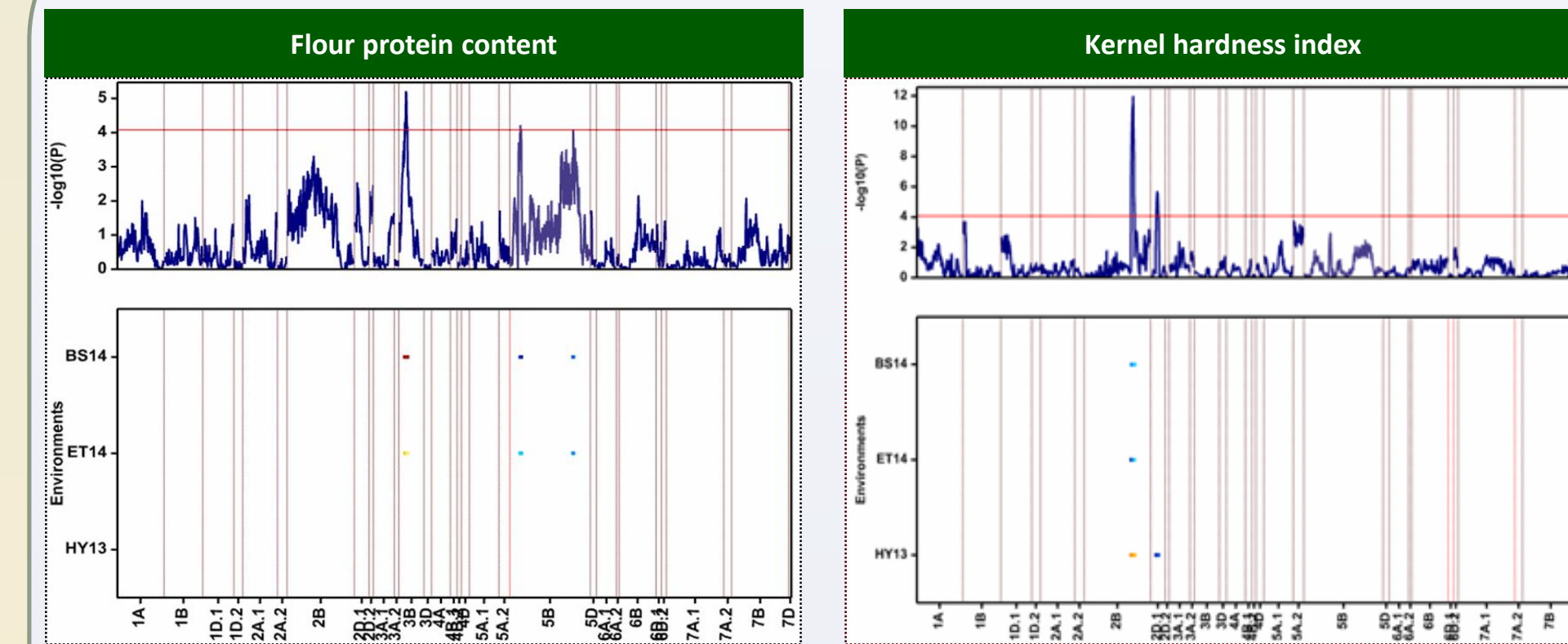
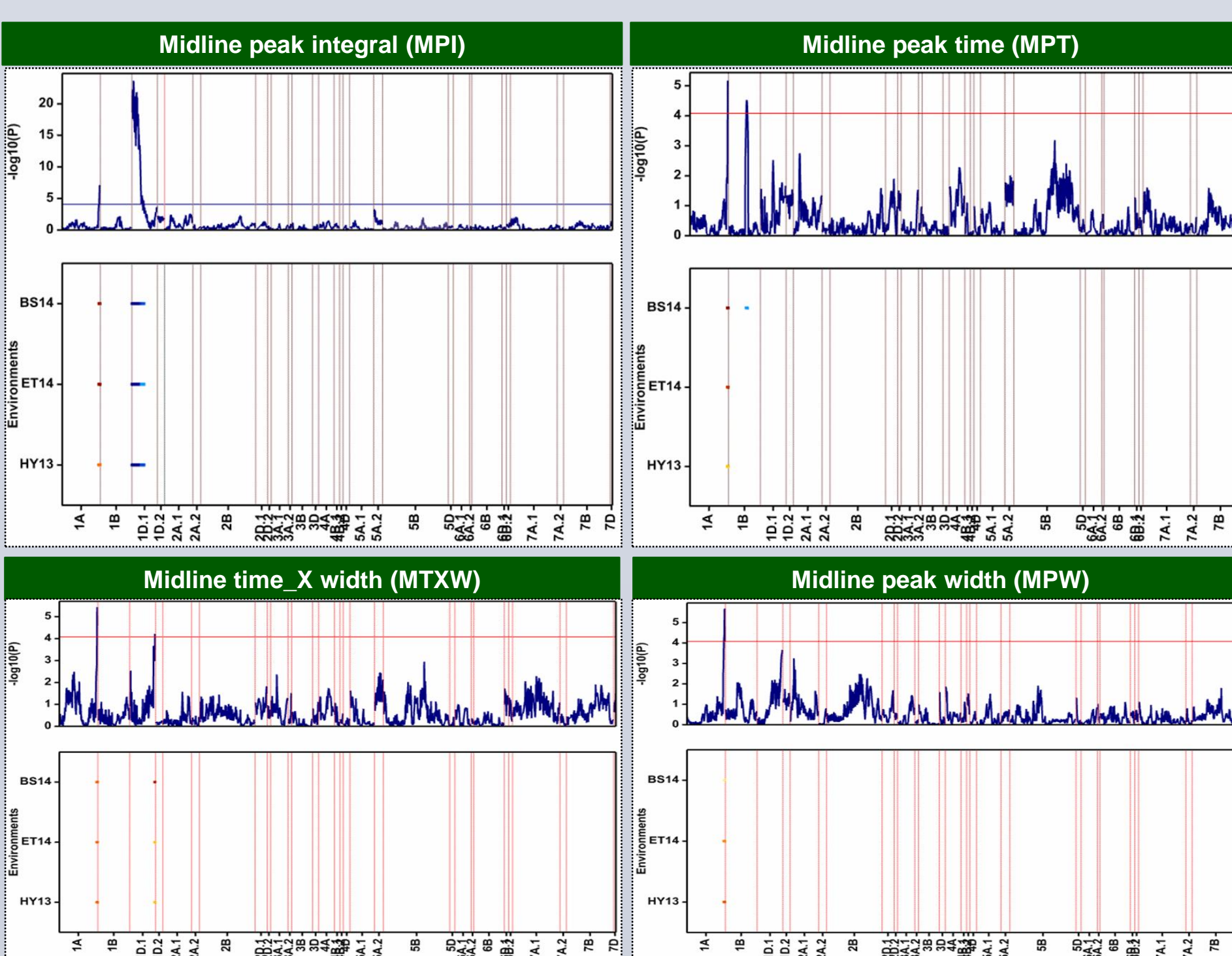
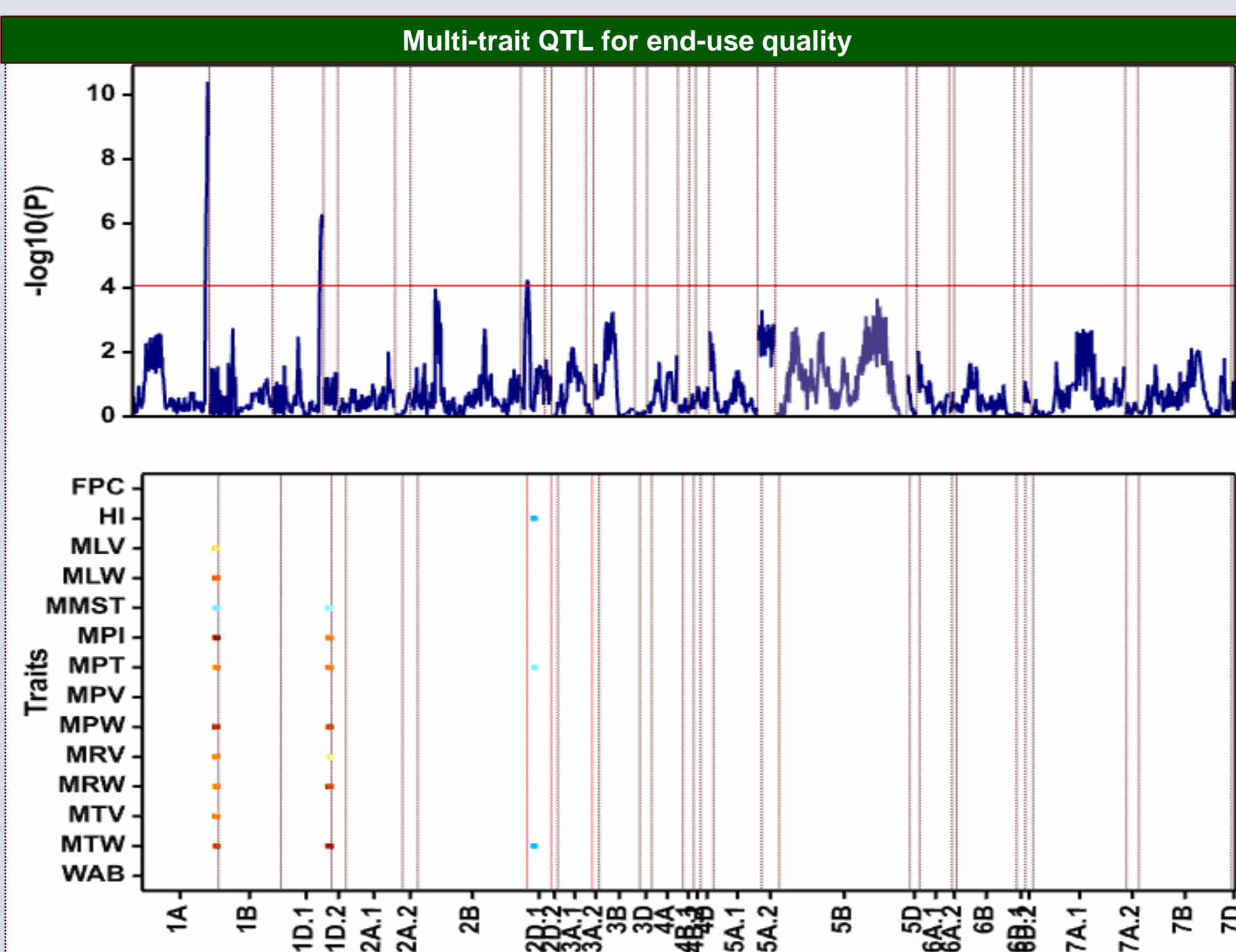


Fig. 3. Additive genetic effects for kernel characteristics and rheological properties of the dough for multi-environment QTL analysis model. Negative values indicate the high value allele (HVA) is from paternal parent (TAM 111) whereas positive values indicate HVA from the maternal parent (CO960293-2)

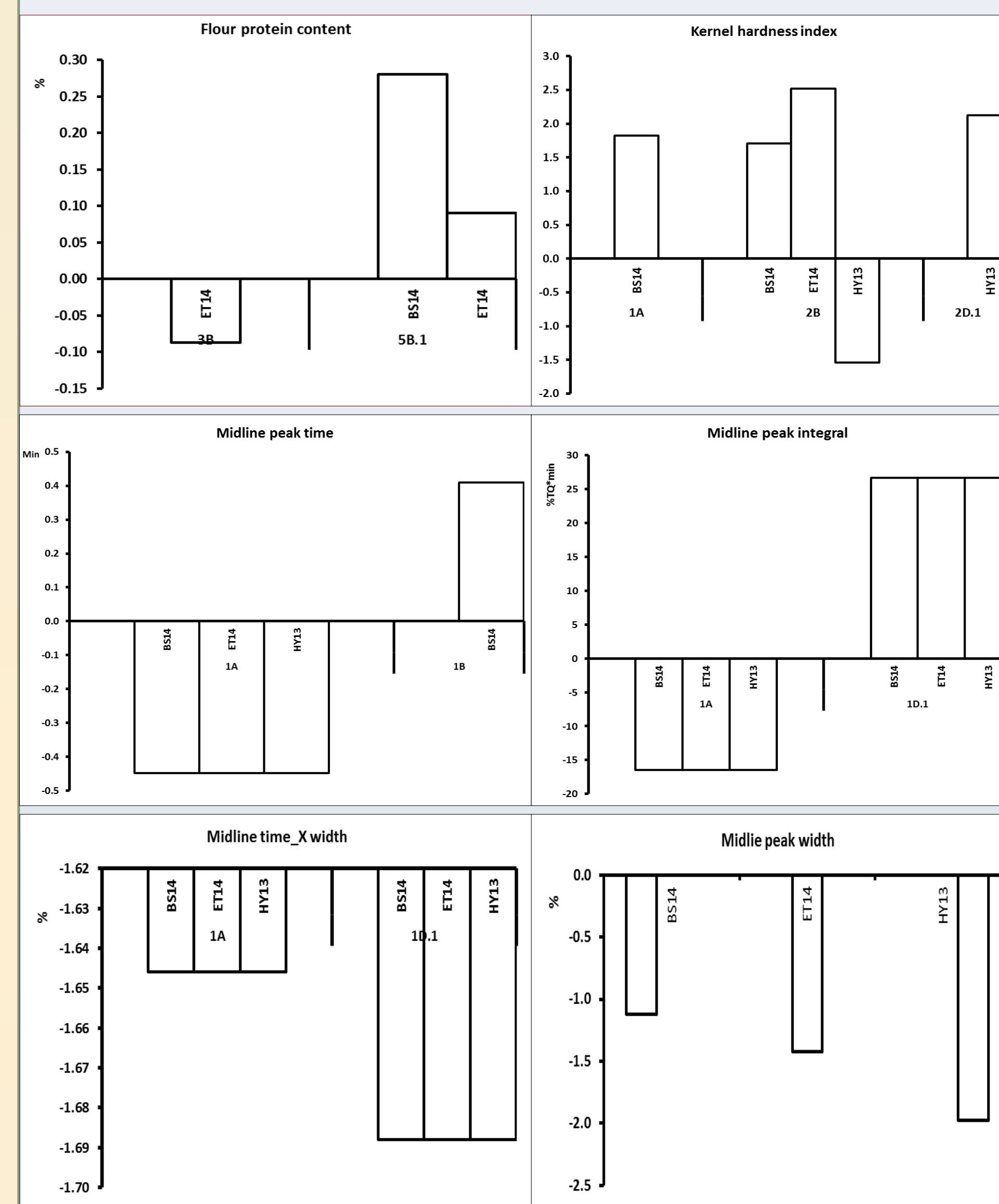


Table 2. Single trait multi-environment QTL for end-use quality

QTL name	Peak SNP	Peak Pos.	CI_LL	CI_UL	-log10(P)	Min. R ²	Max. R ²	Min. AE1	Max. AE	Trait
Qmli.tamu.1A	M7628	380.4	371.8	382.0	6.2	8.1	15.1	14.8	19.5	MLI
Qmli.tamu.1A	M12147	382.0	368.8	382.0	5.5	6.8	10.8	0.4	0.4	MLT
Qmli.tamu.1B	C2P178	178.1	161.3	194.9	4.2	9.0	9.0	0.4	0.4	MLT
Qmpi.tamu.1A	M7628	380.4	367.2	382.0	9.1	10.1	10.8	16.5	16.5	MPI
Qmpt.tamu.1D.1	M26941	12.7	8.4	17.0	21.9	26.5	28.2	26.6	26.6	MPT
Qmpt.tamu.1A	M12147	382.0	368.8	382.0	5.5	6.8	10.8	0.4	0.4	MPT
Qmpw.tamu.1B	C2P178	178.1	161.3	194.9	4.2	9.0	9.0	0.4	0.4	MPT
Qmpw.tamu.1A	M65373	379.7	364.4	382.0	5.7	3.4	9.7	1.1	2.0	MPW
Qmri.tamu.1A	M6999	357.4	346.7	368.1	5.3	3.9	12.7	10.3	15.8	MRI
Qmri.tamu.1A	M6999	357.4	330.3	382.0	4.9	4.1	6.7	0.2	0.2	MRT
Qmrv.tamu.1D.1	M9742	0.7	0.0	8.0	14.1	22.3	23.1	0.6	0.6	MRT
Qmrv.tamu.1A	M61102	376.6	361.2	382.0	5.1	3.7	9.6	0.8	1.1	MRV
Qmrv.tamu.1D	M7763	65.8	45.2	86.5	4.5	4.8	7.9	1.0	1.4	MRW
Qfpc.tamu.3B	C12P55	54.6	42.5	66.7	4.2	4.2	11.5	0.1	0.3	FPC
Qfpc.tamu.5B	M35477	82.0	70.3	93.7	5.0	4.5	11.8	0.1	0.3	FPC
Qhi.tamu.1A	M43982	292.7	282.7	302.7	5.7	13.4	13.4	1.8	1.8	HI
Qhi.tamu.2B	M3178	406.1	400.8	411.4	19.1	7.8	23.2	1.5	2.5	HI
Qhi.tamu.2D	C8P51	51.3	42.5	60.1	5.3	14.9	14.9	2.1	2.1	HI
Qkd.tamu.2B	M8143	404.3	393.1	415.5	12.5	2.8	12.2	0.01	0.03	KD
Qkd.tamu.2D	M22544	73.9	37.1	110.7	3.6	2.0	5.7	0.01	0.02	KD
Qskw.tamu.6A	M13129	128.5	120.1	136.9	8.0	10.6	15.5	0.03	0.03	KD
Qskw.tamu.2B	M8143	404.3	386.6	422.0	10.6	8.1	8.7	0.6	0.7	SKW
Qskw.tamu.6A	M13129	128.5	120.6	136.4	8.3	9.0	16.3	0.6	1.0	SKW
Qmtxv.tamu.1A	M65288	379.0	366.5	382.0	6.6	3.1	11.3	1.3	1.3	MTXV
Qmtxv.tamu.1D.1	C3P234	233.9	221.5	246.2	3.4	3.4	11.4	0.7	1.7	MTXV
Qmtxw.tamu.1A	M65373	379.7	368.4	382.0	6.0	6.3	9.6	1.6	1.6	MTXW
Qmtxw.tamu.1D.1	M65713	247.5	233.1	248.2	6.2	6.6	10.1	1.7	1.7	MTXW

AE, additive effects; Min., minimum; Max., maximum; CI, confidence interval; LL, lower limit; UL, upper limit; Pos., position; R², proportion of phenotypic variance explained by the QTL

Table 3. Multi-trait QTL for end-use quality. Negative additive effects indicates HVA from TAM 111

QTL name	Peak SNP	Peak pos.	CI_LL	CI_UL	-log10(P)	R ²	AE	Trait
Qmli.tamu.1A	M7628	380.4	371.0	382.0	6.1	14.2	-17.3	MLI
Qmli.tamu.1D.1	C3P2	2.1	0.0	5.8	13.0	32.4	-2.8	MLS
Qmli.tamu.1A	M12147	382.0	360.8	382.0	5.2	7.7	-0.4	MLT
Qmpi.tamu.1A	M7628	380.4	369.3	382.0	7.3	12.4	-15.7	MPI
Qmpt.tamu.1A	M12147	382.0	360.8	382.0	5.2	7.7	-0.4	MPT
Qmpw.tamu.1A	M65288	379.0	368.2	382.0	5.8	12.6	-1.4	MPW
Qmpw.tamu.1D.1	M3277	248.2	232.1	248.2	4.5	9.3	-1.2	MPW
Qmrv.tamu.1A	M7628	380.4	371.1	382.0	5.9	14.2	-17.3	MRI
Qmrv.tamu.1A	M78618	343.8	305.4	382.0	2.6	5.6	-0.3	MRT
Qmrv.tamu.1A	M65373	379.7	366.6	382.0	4.6	10.8	-1.1	MRV
Qmrv.tamu.1D.1	M22056	245.8	233.8	248.2	5.3	11.6	-1.4	MRW
Qmtxv.tamu.1A	C1P378	377.8	366.2	382.0	4.9	11.9	-1.3	MTXV
Qmtxw.tamu.1A	M65373	379.7	368.4	382.0	5.9	12.2	-1.7	MTXW
Qmtxw.tamu.1D	M65713	247.5	237.3	248.2	6.3	13.2	-1.8	MTXW

AE, additive effects; CI, confidence interval; LL, lower limit; UL, upper limit; Pos., position; R², proportion of phenotypic variance explained by the QTL

- The RIL showed significant differences (Table 1) with parents revealing marked differences in mixograph mixing properties (Fig. 1).
- The end-use quality variables showed moderate (0.4-0.6) to high (> 0.6) heritability except MTXV (Table 1).
- Most QTL for kernel characteristics and dough rheology were detected on chromosome 1A and 1D (Figure 1-3, Table 2 & 3).
- Midline peak time, corresponding to dough development time, mapped on 1A and 1B with an additive effect (AE) of 0.40 (Table 2).
- Midline peak integral which correspond to the amount of work input for dough development mapped on 1D with an AE of 26% (Table 2).
- The flour protein content QTL was mapped on chromosome 3B and 5B. The hardness index (HI) QTL were detected on 2B & 2D.1 (Fig. 3).
- Multi-trait QTL for end-use quality were detected on chromosome 1A, 1D and 2D.1 (Fig. 2).
- QTL co-localization was observed on chromosome 1A for MRV, MTXV, MPW, MTXW, MLI, MPI, MLT, and MPT (Table 2).
- QTL linked to MRI and MRT were also co-located on 1A but in a different position.
- Co-location of QTL was also detected for MRT and MPI on 1D.1; KD and SKW on 6A; MLT and MPT on 1B (Table 2).
- Co-location of QTL linked different end-use traits suggest linkage disequilibrium between these QTL or possible pleiotropic effects.
- The co-location was supported by the QTL multi-trait model which showed QTL on 1A and 1D linked to multiple traits (Fig. 2, Table 3).
- AE for MPI & MTXW were repeatable across environment suggesting minimal effect of QTL-by-environment interaction (QEI) (Fig. 3).
- QEI was observed for HI QTL on 2B indicated in the QTL heat map as a switch in color (HVA) from one environment to the next (Fig. 2 & 3)
- NCBI BLAST of significant SNP revealed that M11264 at 379.8 cM is linked to gliadin/avenin-like seed protein mRNA
- Other SNP were linked to predicted protein

CONCLUSIONS

Both multi-trait and single trait model revealed that genetic segments on chromosome 1A and 1D play an important role in rheological properties of the dough in wheat. All variables including MPT, MPI, MPW were mapped on 1A and 1D. FPC is linked to QTL on chromosome 3B and 5B. SNP linked to end-use quality QTL in this study could be useful in MAS. The basic local alignment search tool revealed SNP M11264 on chromosome 1A linked to gliadin/avenin-like seed protein mRNA. This SNP could be useful in MAS for end-use quality and in future genetic studies in wheat

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