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Evaluation of barley chromosome-3 yield QTLs in a backcross F_2 population using STS-PCR

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Abstract Chromosome 3 displayed the two largest yield QTLs in a previous study of 150 doubled haploid lines derived from a cross of Steptoe and Morex barley varieties. Low-copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps as demonstrated by the Steptoe and Morex map produced by the North American Barley Genome Mapping Project (SM NABGMP). However, this technique can be cumbersome when applied to practically oriented plant breeding programs. In the present report, we demonstrate the conversion of RFLPs to more practically useful PCR-based markers that are co-dominant and allelic to the barley chromosome-3 RFLP markers from which they derive. We have used these sequence-tagged-site (STS) PCR markers to evaluate the putative yield QTL components of the Steptoe chromosome 3 in a Morex backcross population. Headshattering, plant lodging, and yield measurements are reported from five replicated field experiments conducted under diverse growing conditions in Montana. Our study detected significant effects for all three traits in a chromosomal region that evidently corresponds to the larger of the two previously reported chromosome-3 QTLs. However, we failed to detect any yield or other effects which might be coincidental to the second largest yield QTL. The genetic effects of the yield QTL identified in our first backcross breeding population show similar magnitude, environmental interactions, and association with lodging and headshattering QTLs observed in the SM NABGMP experiments. Our study elucidates complex environmental conditioning for headshattering and plant lodging which probably underlie the variable yield effects observed under different growing conditions.

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Introduction

Southern analysis of RFLPs was used in the North American Barley Genome Mapping Project to identify the locations of numerous genes with significant effects on yield and malting characteristics (Hayes et al. 1993; Kleinhoffs et al. 1993). Thirty one QTLs for grain quality traits, including grain protein, alpha-amylase, diastic power and percent malt extract, were detected in the analysis of a doubled-haploid population derived from a cross between Steptoe and Morex varieties. Morex contributed positive alleles for 28 of the 31 QTLs detected for the value-added grain-quality traits. These genetic effects on grain quality appeared to be stable across diverse growing conditions of Northwestern United States. Conversey, Steptoe showed positive alleles at five of six grain-yield QTLs detected while Morex contributed one positive yield QTL. Many of these yield QTLs were environmentally conditioned.

The two largest QTLs with positive effects on grain yield were ascribed to Steptoe chromosome 3. The larger of these two yield QTLs coincided closely with clearly resolved, single-interval peaks for lodging and plant height (Hayes et al. 1993) and also for headshattering (unpublished data). Hayes et al. (1993) posited that plant lodging was a causal factor of the larger yield QTL on chromosome 3. However, this yield component did not explain the beneficial value of the Steptoe allele in the Montana dryland experiment where lodging was insignificant. Neither lodging nor heading-date factors could be attributed to the second largest yield QTL. Although Hayes et al. (1993) reported that the yield effects of the Steptoe chromosome 3 were consistently positive, significant QTL environment interaction terms were reported for both QTLs on chromosome 3.

Minor effects for grain protein and alpha-amylase were detected near the second largest yield QTL. However, no

other negative pleiotropic effects on value-added malting characters were apparently associated with the Steptoe chromosome-3 yield QTL. Therefore Hayes et al. (1993) postulated that the Steptoe chromosome-3 yield QTLs may have useful breeding value for developing higher-yielding malting varieties. They suggested the use of an offensive breeding strategy such as marker-assisted selection for the Steptoe chromosome-3 yield genes, and a defensive strategy, such as backcross breeding, to recover the Morex alleles at numerous other QTLs for grain-quality traits.

Low-copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps. With adequate amounts of DNA, several endonucleases, and an abundant supply of clones, sufficient polymorphic information content is generally available to construct complete RFLP linkage maps (Botstein et al. 1980) using Southern analysis. The efficiency of this technique is greatly enhanced when blots are repeatedly hybridized with different clones, each identifying one or more loci. The Southern technique has been effectively used to construct complete RFLP linkage maps in relevant crosses of many crop species (e.g. Helentjaris et al. 1986; Bonierbale et al. 1988; Heun et al. 1991; Kleinhofs et al. 1993) and to identify genes controlling many agronomical traits. However, this technique can be cumbersome when applied to practically oriented plant breeding programs. Fast-paced marker-assisted selection in large populations is inefficient using Southern analysis, especially if blots are needed for only one RFLP marker.

The DNA sequences of clones mapped using Southern analysis of RFLPs can be used to design oligonucleotide primers for PCR-based analysis (Saiki et al. 1985; Mullis and Faloona 1987). The conversion of these sequence-tagged sites into polymerase chain reaction-based markers (STS-PCR) has the potential to facilitate plant genome mapping experiments in a variety of ways (Tragoonrung et al. 1992; Talbert et al. 1994). In order to make these markers more practical tools in crop improvement, techniques must be available to develop STS-PCR markers which are informative in the germ plasm of interest. As useful tools, these STS-PCR markers must co-segregate with the RFLP locus from which they derive, and should also provide co-dominant and reliable assays.

One objective of this report is to demonstrate the development and use of six such STS-PCR markers. Our hypothesis was that RFLP clones which previously mapped to barley chromosome-3 (Kleinhoffs et al. 1993) could be efficiently converted to co-dominant PCR-based markers, allelic to the RFLP markers from which they derive, and be used effectively for plant breeding. Following that, our second objective was to use these STS-PCR markers to evaluate genetic effects and agronomic components of the putative Steptoe chromosome-3 yield QTLs. Here we investigate and describe the magnitude of effects, the genetic map resolution, and the possible environmental interactions associated with the putative yield, lodging, and head-shattering QTLs previously described for chromosome-3 (Hayes et al. 1993, and unpublished data).

Materials and methods

The polymerase chain reaction (PCR) was conducted in 50-µl volumes of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.1 mM of each dNTP, 1.5 mM $MgCl_2$, 330 nM of each primer, and 0.6 units of *Taq* polymerase. Adequate genomic DNA samples were obtained using the protocol described by Edwards et al. (1991). For thermocycling, we used the GeneAmp PCR System 9600 (Perkin Elmer Cetus, Norwalk, Conn.) with one step at 94°C for 5 min, then 33 cycles of 94°, 50° and 72°C steps of 30 seconds each, and one final step of 72°C for 5 min.

The clones used to design STS-PCR primers included four American Barley Genomic (ABG) clones (Kleinhofs et al. 1993). In addition to the barley clones shown in Table 1, STS-PCR primers were also designed using the wheat genomic (WG) and oat cDNA (CDO) clones described by Heun et al. (1991). These clones were end sequenced, near the vector insertion points, so that primer sequences could be selected using OLIGO (National Biosciences, Plymouth, Minn.). Oligonucleotide primers were synthesized using the Model 391 PCR-MATE DNA synthesizer (Applied Biosystems, Foster City, Calif.). By convention, the RFLP markers, such as ABG070, are distinguished from an amplifiable STS-PCR marker using a small-case letter "a" in front of the marker locus name (e.g., aABG070).

For all PCR primer pairs shown in Table 1, except aABG070, Steptoe and Morex amplification products were cloned and sequenced for polymorphism analysis. Barley amplification sequences, using the aCDO113 (oat) and aWG110 (wheat) primers, were also used for designing new barley STS-PCR primers specific to chromosome-3 loci. The PCR products were cloned using the Invitrogen TA Cloning Kit (San Diego, Calif.), and sequenced using the Sequenase Kit (USB, Cleveland, Ohio) by the dideoxy chain-termination method (Sanger et al. 1977). GENEPRO software (Riverside Scientific, Bainbridge Wash.) was used for sequence and restriction-site polymorphism analysis.

Polymorphisms among parents and progeny were tested for restriction-site presence/absence or ribonuclease cleavage/protection of heteroduplex PCR molecules. Endonuclease digestion of STS-PCR products are typically conducted using a several-fold excess of activity units, incubated overnight at temperatures recommended by the manufacturers. Using a ribonuclease protection assay (RPA), the Mismatch Detect kit (Ambion, Austin TX) was used to test a sequence polymorphism, at the WG110 locus.

The 50 BC_1 families were derived from 50 BC_1F_2 plants by bulking seed within families through two and three generations of self pollination. This BC_1 population was constructed using a cross of DH72 (Steptoe chromosome-3 yield QTL donor) and Morex (recurrent malting variety). DH72 is a doubled-haploid line derived from an F_1 cross of Steptoe (Muir and Nilan 1973) and Morex (Rasmusson and Wilcoxson 1979), as described by Kleinhofs et al. (1993). Morex is an industry standard of U.S. barley malting varieties, and is so named for producing "more extract" (i.e., percent malt extract). Steptoe is a feed barley that has high grain yield potential, but shows poor grain-quality characteristics (e.g., has low protein value in feed grains and has many undesirable malting traits including high levels of dormancy). The Steptoe and Morex barley varieties are grown in different regions of the United States and show very different environmental adaptations. The DH72 line was selected solely because the RFLP genotype, based on the NABGMP data set, indicated that it was fixed for Steptoe chromosome-3. Otherwise, the RFLP data and recombinational analysis suggest that DH72 is 50% Morex.

Irrigated and dryland field trials of BC_1F_4 families were conducted during 1994 at Bozeman. Irrigated and dryland Bozeman BC_1F_5 trials and a BC_1F_5 dryland experiment at Havre were conducted during 1995. These three locations in Montana represent diverse growing conditions of decreasing yield potential. Each experiment (RCB) had two replications where blocks contained 53 plots including the 50 BC_1 families plus three checks (Steptoe, Morex, and DH72). The Bozeman plots were 1.5 m^2 in 1994 and 3 m^2 in 1995 and the plots in the 1995 Havre experiment were 4.5 m^2. Grain was harvested using hand sickles and mechanical threshers in 1994 and small-plot combines in 1995.

The headshattering and lodging traits were measured using a rating scale of 0–9 by S. R. L., where 0 was least severe and 9 was most severe. A rating of 9 would indicate that essentially no seed remained after headshattering or that all plants were completely lodged. Lodging was not observed in the 1994 Bozeman dryland trial, therefore lodging measurements were not taken (see Table 3) for that experiment. In the 1994 experiments, headshattering was determined by striking three intact heads (per plot) several times, and rating grain loss. In the 1995 experiments, headshattering was rated by the observation of seed loss from plants and the ground litter within each plot. All headshattering and plant-lodging ratings were observed within several days before harvest.

MAPMAKER (Lander et al. 1987) was used to genetically map the aCDO113 and aABG070 STS-PCR markers as described below. The marker×phenotype×environment interactions were tested by ANOVA using MSUSTAT (Version 5.20, Richard E. Lund, Montana State University, Bozeman, Mont., 59717).

Results

STS-PCR RFLPs

Restriction fragment length polymorphisms (RFLPs) for the STS-PCR products of the aABG070, aABG057, aABG396, and aABG377 loci are shown in Fig. 1. The aABG070, aABG396 and aABG377 amplification products appear to show single discrete amplification products that are nearly 400 bp, or greater, and apparently identical in size between Steptoe and Morex. The Steptoe and Morex amplification products of aABG070 and aABG377 show clear restriction fragment length polymorphisms when digested with *Taq*I and *Ssp*I endonucleases respectively. A restriction fragment length polymorphism, approximately 10 bp in length, was observed between two aABG396 STS-PCR *Mnl*I digestion products. However, both of these Steptoe and Morex RFLPs for aABG396 are less than 100 bp in length and may, in practice, appear as faint bands. Primers for aABG057 will reproducibly amplify a 217-bp product that contains a *Hph*I endonuclease recognition sequence in Steptoe which allows its cleavage into 105- and 112-bp fragments. Depending on reaction conditions, several larger products may be observed using these aABG057 primers. These four STS-PCR RFLPs are co-dominant polymorphisms in progeny segregating for the Steptoe and Morex chromosome-3. The RFLPs, predicted by sequence analysis, for aABG057, aABG396 and aCDO113 (Fig. 1 and 2B) are the only confirmed restriction-site polymorphisms for these Steptoe and Morex STS-PCR amplification products. In addition to the *Ssp*I polymorphism shown for aABG377 (Fig. 1), a *Hae*III-restriction site difference also distinguishes Steptoe and Morex for this marker. The aABG70 STS-PCR amplification products were not sequenced because a preliminary screen using 26 endonucleases revealed seven RFLPs for seven different enzymes.

Wheat-barley chromosome addition lines (Islam et al. 1981) were used to identify an aCDO113 STS-PCR amplification product unique to barley chromosome-3, using primers U and R (Table 1) designed from the orginal oat CDO113 clone. A low-amplification 186-bp PCR product unique to chromosome-3 was identified (Fig. 2A). Homologous, 186-bp Steptoe and Morex amplification products were cloned and sequenced. This enabled us to design new aCDO113 primers (U2 and R3, Table 1) from the barley chromosome-3 PCR products. A 1-bp difference was observed between Steptoe and Morex sequences. This se-

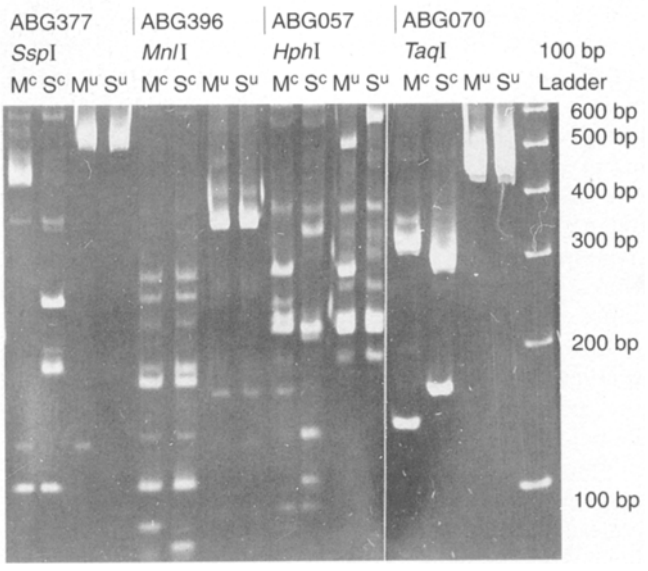

Fig. 1 Steptoe (S^c) and Morex (M^c) restriction fragment length polymorphisms at four STS-PCR loci revealed using four different endonucleases. The uncut Steptoe (S^u) and Morex (M^u) amplification products are identical in length for these four STS-PCR markers, except for three larger products unrelated to the co-dominant *Hph*I RFLP for ABG057

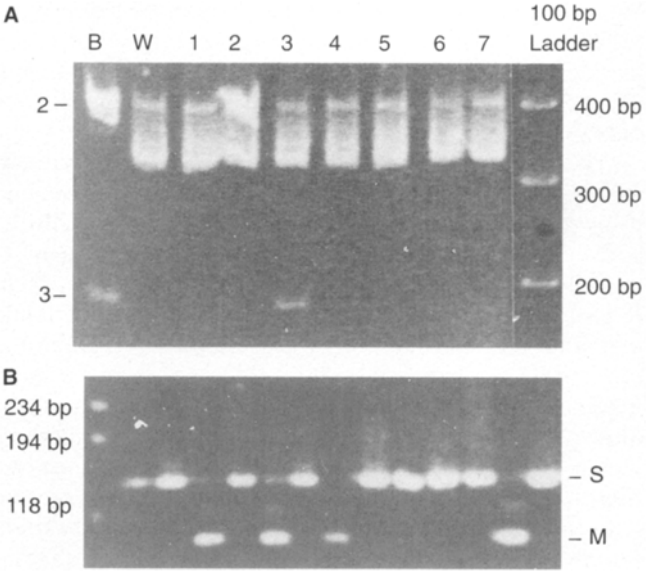

Fig. 2 **A** Products of aCDO113 STS-PCR amplification of DNA from Betzes (*B*), Chinese Spring (*W*) and the wheat barley addition lines (1–7) using primers U and R (Table 1) from the original CDO113 clone. The *numbers 2 and 3*, on the left side of the figure, indicate amplification products that are specific to the wheat-barley chromosome addition lines 2 and 3, respectively. **B** Segregation of *Bsa*MI RFLPs obtained using barley aCDO113 (U2 and R3, Table 1) primers re-designed from the Steptoe and Morex PCR products homologous to the 186-bp product amplified in wheat-barley addition line 3 (panel **A**). The letters *S and M*, on the right side of the figure, indicate the Steptoe and Morex alleles

Table 1 Description of clones, primer sequences and STS-PCR products

Clone	Insert size (bp)	Primer	Primer sequence (5′>3′)	Predicted PCR size (bp)	Observed PCR size (bp)
ABG070	600[a]	U R	ggaccaagcaaatatctcag aacacgagtttgaattttac	578[a]	430[a]
ABG057	1500[a]	U2 R2	ttataagcatagactgcggt gcacgagtgagctgagagtg	217	217
CDO113	1800[a]	U R	ttcgaagctccttctctt catgggaaacagcatagc	191	186 400
CDO113 (barley)	186	U2 R3	cattagatcaaaatgctt aggataaggcccatcgta	143	143
ABG396	500[a]	U R	gggtcacaaagacggaggag aggaaacctatgtaatcatc	388	388
ABG377	700[a]	U R	gctgctatgaggagagaacc gtgttacagccacctatttc	541[a]	520[a]
WG110	900[a]	U R	tctgatacacacctccagcg acggcgatccgtccacgagc	830	888 +others
WG110 (barley)	888	U2 R2	atacaacaggagccactaca cgaggacatcgctgagaaga	409	409
WG110 (barley)	409	U2T7	gataatacgactcactataggg +aattttctacttctc[b]	413	413
		R2SP6	agatttaggtgacactatagga +ccgggcttgggttcc[b]		

[a] Size estimated by comparison with known size standards using PAGE
[b] Underlined sequences are phage promoter sequences (see text)

quence polymorphism is recognized by the *Bsa*MI endonuclease in the Steptoe genotype, which can be easily scored as a co-dominant marker. Segregation of the aCDO113 – *Bsa*MI polymorphism, among Steptoe Morex DHLs is shown (Fig. 2B).

STS-PCR ribonuclease protection assay

Primers for the original aWG110 clone (U and R, Table 1) from wheat were first tested on the wheat-barley addition lines, so that we could clone and sequence Steptoe and Morex PCR products unique to chromosome-3. The approach was similar to that used for aCDO113 (described above). We made chromosome-3-specific primers from barley (aWG110 U2 and R2, Table 1) which amplify a single 409-bp product (data not shown). Sequence analysis revealed a single base-pair polymorphism that could not be detected using restriction endonucleases. However, we demonstrate a ribonuclease protection assay, Fig. 3, which is sensitive enough to detect a single base-pair difference. A mismatch between the Morex T7 RNA transcript and the Steptoe SP6 transcripts is completely cleaved (second lane from the left, Fig. 3). The reciprocal heteroduplex of Steptoe and Morex transcripts is not cleaved using the RNAase stock #1 (included with the Mismatch Detect kit) as shown in Fig. 3 (far left lane). However, recent experiments (data not shown) show that both reciprocal heteroduplexes of Steptoe and Morex can be cleaved using RNAase stock #3 (Mismatch Detect II kit).

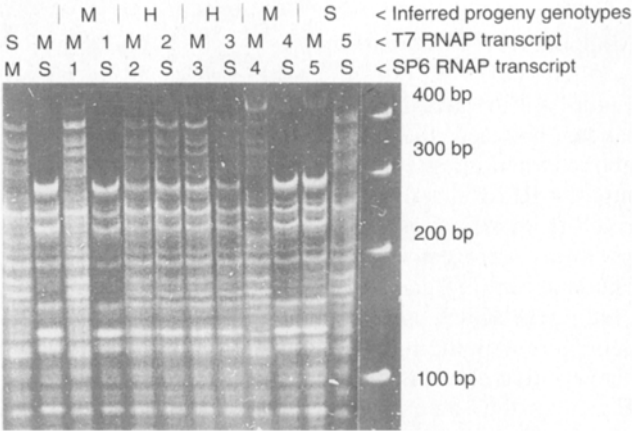

Fig. 3 Polyacrylamide-gel electrophoresis gel showing a ribonuclease protection assay for aWG110 transcription products obtained from progeny (1–5) and hybridized with Steptoe and Morex antisense transcripts. The SP6 and T7 transcripts from progeny 1–5 are each hybridized with antisense T7 and SP6 RNAP transcripts of Morex and Steptoe, respectively, as indicated. Transcription templates were PCR products generated using primers with T7 and SP6 phage promoter sequences overhanging the 5′ ends of the WG110 U2T7 and R2SP6 primers (Table 1). The inferred genotypes are indicated (*S*=Steptoe, *M*=Morex, *H*=Heterozygote) for each progeny

To genotype progeny, SP6 and T7 RNA transcripts from each offspring were hybridized with Morex and Steptoe T7 and SP6 transcripts, respectively (see Fig. 3). The SP6 transcripts of progeny homozygous for the Morex allele are protected from RNAase cleavage when hybridized to the

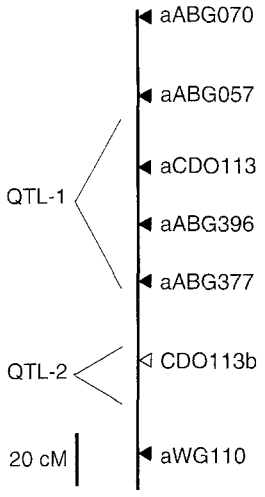

Fig. 4 Linkage map of six STS-PCR markers developed for chromosome-3 QTL evaluation (indicated by *closed triangles*). The approximate position of the largest and second largest yield QTLs reported by Hayes et al. (1993) are also indicated (QTL-1 and QTL-2, respectively). The second largest yield QTL was detected near the CDO113b RFLP locus (Kleinhoffs et al. 1993), indicated by an *open triangle*; however, our aCDO113 STS-PCR marker mapped to a different region of chromosome-3 as shown in the figure

Morex T7 transcript. The T7 transcripts of those same progeny hybridized to the Steptoe SP6 transcript are completely cleaved by RNAase. The opposite is observed for progeny homozygous for the Steptoe allele. Heterozygous progeny are partially cleaved in both hybridizations with T7 and SP6 antisense transcripts of Morex and Steptoe, respectively. In this way, the aWG110 STS-PCR primers can also be used as a co-dominant marker for progeny segregating for the Steptoe and Morex chromosome-3.

Mapping STS-PCR markers

Figure 4 illustrates the relative placement and genetic distances between the six STS-PCR markers, described above, when integrated with a NABGMP data set including 434 RFLP markers for 150 doubled-haploid lines derived from a F_1 cross of Steptoe and Morex. These map positions were determined using MAPMAKER and the Haldane centimorgan function. The STS-PCR markers, including aABG057, aABG396, aABG377 and aWG110, co-segregate with their respective chromosome-3 RFLP loci. The relative placement of these four markers, as shown in Fig. 4, is the same as that reported by Kleinhofs et al. (1993). The aABG070 STS-PCR marker also co-segregates with the original ABG070 RFLP data (Lynn Dahleen, personal communication). However, the original ABG070 RFLP locus was not included on the map of Kleinhofs et al. (1993) and was not readily available in our SM NABGMP RFLP data set. MAPMAKER analysis of the ABG070 RFLP data, using the SM NABGMP map, places this marker near the distal end of chromosome-3p (Fig. 4). It has been confirmed using the wheat-barley chromosome addition lines (Islam et al. 1981), that the aABG070 STS-PCR amplification product is unique to barley chromosome-3. It is also noteworthy that aABG070 is known to co-segregate with barley chromosome-3 telomere sequences (A. Kilian, personal communication). The only STS-PCR marker that did not co-segregate with previous RFLP data is aCDO113. The STS-PCR marker did not coincide with the CDO113b locus reported by Kleinhofs et al. (1993) but it did map to chromosome-3 (as shown in Fig. 4) using MAPMAKER and the 434-point SM NABGMP data set.

Percent phenotypic variance explained by markers

The percent phenotypic variances explained by four STS-PCR markers which showed signficant marker-by-phenotype interaction with ANOVA are shown in Table 2. The aWG110 and aABG070 markers showed no signficant association with the agronomic traits evaluated and are omitted from Table 2. Otherwise the percent variances reported in Table 2 are all significant ($P>F$ significant at less than 0.05). The segregation ratios of progeny (S:H:M) for each of the markers listed in Table 2 are as follows: aABG377 (8:28:14), aABG396 (8:28:14), aCDO113 (4:31:15), and aABG057 (15:25:20). The segregation ratio for aCDO113 shows a significant deviation from the expected 1:2:1 F_2 ratio using chi-square analysis. Since aABG396 showed the greatest interaction with yield, and is also strongly associated with headshattering and plant-lodging traits, it was selected for analysis of QTL environment interactions and the description of yield QTL breeding values (Tables 3 and 4) as reported in the following sections.

QTL-by-environment interaction and overall yield correlations

The percent variance explained by the aABG396 marker and overall yield correlations (in parentheses) are reported for each trait by experiment (Table 3). The overall severity

Table 2 Percent variance explained for three traits using chromosome-3 markers with significant phenotypic effects. Results were obtained from combined analysis of variance over five diverse growing environments

Trait	ABG377	ABG396	CDO113	ABG057
Yield	5.8%	7.4%	3.4%	2.9%
Shatter	7.9%	13.9%	5.1%	13.7%
Lodging	14.1%	13.8%	5.6%	14.3%

Table 3 Percent variance explained by the aABG396 marker for lodging, shattering, and yield traits measured over five diverse growing environments. The overall correlations of plant lodging and headshattering with grain yield are also shown in parentheses for each experiment

Environment	Bozm irr 1994	Bozm irr 1995	Bozm dry 1994	Bozm dry 1995	Havre dry 1995
Plant lodging	20.4% (NS)	15.4% (–0.22)	NS (NS)	23.3% (NS)	NS (+0.52)
Headshattering	6.4% (–0.40)	46.5% (–0.62)	12.0% (NS)	10.3% (NS)	6.1% (–0.37)
Grain yield	10.3%	36.5%	NS	5.4%	NS

NS = not significant

Table 4 Summary of the breeding value for yield of the ABG396 marker over five diverse growing locations

Environment	Bozm irr 1994	Bozm irr 1995	Bozm dry 1994	Bozm dry 1995	Havre dry 1995	Combined analysis
Yield breeding value (kg/ha)	615[a]	1135[a]	397	325[a]	247	543
Yield breeding value as a percent[b]	12.9%	23.5%	8.5%	7.3%	7.4%	12.2%
Morex kg/ha	4564	4817	4371	4355	2989	78.5
DH72 kg/ha	5962	6344	5354	5107	4704	5494
Steptoe kg/ha	5752	4967	6569	5629	4967	112.2

[a] $P>F$ significant at less than 0.05
[b] Percent of average yield for progeny fixed for the aABG396 marker allele

of plant lodging and headshattering was extremely variable across the five experiments. Virtually no lodging was observed in the 1994 Bozeman dryland experiment, and headshattering had to be rated using a fragility test because natural discrimination was not apparent (see Materials and methods). Conversely, ripening at the Havre experiment occurred so rapidly that plants appeared to be almost indiscriminately snapped, lodged, and shattered by the time that the plots could be harvested. Although the Steptoe marker allele at aABG396 showed reduced lodging at Havre, this putative effect was not significant (Table 3). Note, however, that the putative lodging effect of the ABG396 marker in the Havre experiments was convoluted by an overall positive correlation of yield and lodging (Table 3). The strongest yield effect at the ABG396 marker was observed in the 1995 Bozeman irrigated experiment (Tables 3 and 4), where discrimination of natural headshattering and head snapping was easily observed. This irrigated experiment was planted and harvested 5 and 11 days later than the 1995 Bozeman dryland experiment which showed less natural headshattering and greater overall lodging succeptibility to a windstorm which occurred in late July.

Magnitude of QTL effects

The breeding value of the Steptoe aABG396 marker allele in each environment, and combined over environments, is also shown in Table 4. For comparison across environments, the breeding values are also reported as a percent of the average yield observed for progeny fixed for the Morex ABG396 marker allele. Although the aAGB396 marker-by-environment interaction term was significant ($P>F$ significant at less than 0.05), the breeding values of the Steptoe aABG396 marker allele were consistently positive across environments. For comparison, the grain yields of the pedigree checks are also shown in Table 4. Morex was consistently the lowest-yielding parent, while Steptoe and DH72 were split across irrigated and dryland experiments. Interestingly, DH72, which is fixed for Steptoe chromosome-3, outperformed Steptoe in the irrigated environments where the breeding value of the Steptoe aABG396 marker allele was greatest.

Discussion

Our results confirm the positive effects of the Steptoe allele at the largest yield QTL on chromosome-3. The most probable location of this QTL, we believe, is very near the aABG396 and aCDO113 STS-PCR loci and is flanked by two other markers, aABG057 and aABG377. The genetic map location of this yield QTL corresponds very well to the largest yield-QTL effect detected in the SM NABGMP experiment. The breeding value of the Steptoe aABG396 marker allele was 543 kg/ha or 12.2% of progeny fixed for the Morex alleles. This is very similar to the previously reported value of 734 kg/ha which was 12.8% of the Morex genotype (Hayes et al. 1993). Similar to Hayes et al. (1993), we observed that the map position of yield QTLs is incidental with respect to plant lodging and headshattering QTLs, and that these agronomic traits are strongly conditioned by the growing environment.

This study demonstrates that headshattering is a major component of the largest yield QTL observed by Hayes et al. (1993). From Tables 3 and 4, it appears that the relative breeding value of the Steptoe chromosome-3 yield QTL is greatest where natural headshattering is most apparent. The comparison of the 1995 Bozeman irrigated and dryland experiments are especially revealing in this regard. Other than having different planting and harvest dates (as previously discussed), only one short application of water distinguished the 1995 Bozeman irrigated and dryland experiments. Yet these differences were enough to create a much better headshattering screen in the irrigated experiment, and a slightly greater lodging effect, at the ABG396 marker locus in the dryland experiment. Based on this comparison of the 1995 Bozeman experiments, it is clear that natural headshattering, associated with the ABG396 marker, can have dramatic yield effects. This shattering usually occurs during the later stages of ripening.

Interestingly, our results also show that the major yield QTL allele of Steptoe is closely associated with a morphological gene for short rachis internode length that we mapped (unpublished data) to the region between the aABG377 and aABG396 markers (Fig. 4). The rachis internode gene may be allelic to the *erectoides* (*ert-c*) dense

spike gene which maps to barley chromosome-3 (Sogaard and vonWettstein-Knowles 1987). The headshattering effect we observed could also be related to this rachis internode-length gene (Table 2). However, it is perhaps more likely that the headshattering effect is a result of the *bt2,bt* genes which are separate from the *ert-c* gene (Sogaard and vonWettstein-Knowles, 1987). If the hypothesis that *ert-c* is allelic to the rachis internode-length gene is correct, then comparative mapping suggests that *bt2,bt* would be in the region of aABG396-aABG057 (Fig. 4). Therefore, the putative location of the *bt2,bt* genes would coincide nicely with the headshattering effects described in Table 2. The genetic map position of the lodging QTL is not clearly resolved in our experiment, as it was in the Hayes et al. (1993) study, and it would be unreasonable to speculate whether the putative lodging gene(s) is allelic to any of the genes discussed above.

We have not been able to confirm the second largest yield QTL reported by Hayes et al. (1993), linked to the CDO113b locus between the aWG110 and aABG377 STS-PCR marker loci on Fig. 4. Interestingly, this putative yield QTL coincides with a yield QTL detected in a doubled-haploid population derived from a cross of European two-row spring barley genotypes (Thomas et al. 1995). The yield QTL reported by Thomas et al. (1995) mapped near the *denso* morphological gene, also between aWG110 and aABG377 on Fig. 4; however, no other agronomic QTL(s) were detected in this region which could explain the observed yield effect. The yield effects that Thomas et al. (1995) observed near aWG110 were similar in magnitude compared to the second largest yield QTL reported by Hayes et al. (1993). However, we failed to detect significant effects for yield and other agronomic effects (e.g., headshattering or lodging) using the aWG110 STS-PCR marker in our backcross experiment. It is recognized that the large recombinational gap between the aABG377 and WG110 STS-PCR loci will have diminished our ability to detect the putative QTL in this region. Adding to the problem of recombination, our BC_1F_2 derived population is one-third the size of the SM double-haploid population and lacks a comparable number of homozygous contrasts. However, the Steptoe chromosome-3 yield should become progressively more apparent in backcross populations, compared with an F_2-derived population. Indeed, we have had no problem detecting the effects of the larger of the two putative chromosome-3 QTLs. Hayes et al. (1993) observed significant QTL×environment interaction for this putative yield locus; therefore we may have failed to detect the second largest QTL effect because the environments described in this study were not conducive to its expression.

Our study demonstrates six co-dominant STS-PCR markers which provide a useful linkage map of barley chromosome-3. In our observations (data not shown) the polymorphisms which distinguish Steptoe from Morex, also differentiate Steptoe from most other spring germ plasms tested (two-row and six-row). All STS-PCR markers except aCDO113 co-segregate with the RFLP loci described in the SM NABGMP experiment. The SM NABGMP map includes the CDO113b locus in a region sparsely populated with markers, between ABG377 and WG110 (shown in Fig. 4). This result was surprising because only one chromosome-3 amplification product was observed using primers for the original CDO113 clone (Fig. 2A). The only other amplification product observed clearly belonged to wheat-barley chromosome 2 (Fig. 2A).

Additional markers for the chromosome-3 minus arm would also be useful to test the validity of the second largest yield QTL reported by Hayes et al. (1993). The latter set of STS-PCR makers would certainly be of interest for breeders and geneticists working with genotypes which may carry the yield QTL associated with *denso* (Thomas et al. 1995).

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References

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Edwards, Johnstone C, and Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19:1349

Hayes PM, BH Liu, SJ Knapp, F Chen, B Jones, TK Blake, J Franckowiak, D Rasmusson, M Sorrells, SE Ullrich, D Wesenberg, A Kleinhofs. (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley (*Hordeum vulgare*) germ plasm. Theor Appl Genet 87:392–401

Helentjaris TD, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Genetics 118:356–363

Heun M, Kennedy AE, Anderson JA, Lapitan, NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34: 437–447

Islam AKRM, Shepard KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Kleinhofs A, A Kilian, MA Saghai Maroof, RM Biyashev, P Hayes, FQ Chen, N Lapitan, A Fenwick, TK Blake, V Kanazin, E Ananiev, L Dahleen, D Kudrna, J Bollinger, SJ Knapp, B Liu, M Sorrells, M Heun, JD Franckowiak, D Hoffman, F Skadsen, BJ Steffenson (1993) A molecular, isozyme, and morphological map of the barley genome. Theor Appl Genet 86:705–712

Lander ES, Green P, Abrahamson P, Barlow J, Daly A, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Muir CE, Nilan RA (1973) Registration of Steptoe barley. Crop Sci 13:770

Mullis KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol 155:335–350

Rasmusson DC, Wilcoxson RD (1979) Registration of 'Morex' barley. Crop Sci 19:293

Sogaard B, vonWettstein-Knowles P (1987) Barley: genes and chromosomes. Carlsberg Res Commun 52:123–196

Saiki RK, Scarf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction-site analysis for diagnosis of sickle-cell anemia. Science 230:1350–1354

Sanger F, Niklen S, Coulsen AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Thomas WTB, Powell W, Waugh R, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Swanston JS Ellis RP, Hanson PR, Lance RCM (1995) Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). Theor and Appl Genet 91:1037–1047

Talbert LE, Blake NK, Chee PW, Blake TK, Magyar GM (1994) Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. Theor Appl Genet 87:789–794

Tragoonrung S, Kanazin, Hayes PM, Blake TK (1992) Sequence-tagged-site-facilitated PCR for barley genome mapping. Theor Appl Genet 84:1002–1008