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Markers polymorphic among malting barley (*Hordeum vulgare* L.) cultivars of a narrow gene pool associated with key QTLs

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Abstract Barley used for malting is a fine-tuned organism, and it requires breeding within narrow gene pools for realistic cultivar enhancement. Significant phenotypic advance within such narrow gene pools has been achieved and the necessary genetic variability for breeding progress has been documented, but it was not well understood. This study was conducted to further characterize detectable genetic variability present within a select set of four closely related malting barley cultivars using three types of molecular markers: RFLP, PCR-RAPD and AFLP. The markers that identified polymorphism among the select malting cultivars tended to link with each other and to map in chromosomal regions associated with quantitative trait loci (QTLs) for agronomic and malting quality traits that differed among the four cultivars. Although RFLPs identified the least amount of polymorphism, the differences detected by the RFLPs best fit the chronology of the cultivars. These results indicate that a large amount of the genetic variability necessary for cultivar improvement may have originally been present in the breeding gene pool, but does not rule out *de novo* variation. Study of the populations from crosses within this narrow germplasm is needed to further elucidate the basis of the phenotypic variability found among these select barley cultivars.

Keywords DNA markers · Barley · *Hordeum* · Mapping · QTLs

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Introduction

Barley (*Hordeum vulgare* L.) is a major cereal grain grown for malt, the primary ingredient of beer. The enhancement of malting barley cultivars is a complex process which involves the improvement of as many as 22 malting quality traits such as malt extract yield, kernel plumpness, enzyme activity for starch modification and percent grain protein, along with many agronomic traits including grain yield, lodging resistance and seed shattering (Rasmusson and Phillips 1997). Although the complexity of malting barley cultivar improvement has led barley breeders to work within narrow gene pools, significant gains have been realized, indicating that sufficient genetic variability is present in such pools (Wych and Rasmusson 1983; Horsley et al. 1995).

A goal of the molecular mapping activity in barley is to locate quantitative trait loci (QTLs) for map-based cloning or to find associated markers for molecular marker-assisted selection (MMAS) to supplement ongoing breeding programs. QTLs have been defined for agronomic traits such as days to heading, plant height, lodging, days to maturity, seed yield, 100-seed weight, test weight, winter hardiness and seed dormancy, as well as for malting quality traits such as grain protein, alpha-amylase activity, diastatic power, fine-coarse extract difference, soluble protein and beta-glucan (summarized by Hayes et al. 2001). Knowledge of the distribution of the QTLs for these traits and their associated markers may help barley breeders more-efficiently assemble new gene combinations for the improvement of barley cultivars. Markers can also be used to assess patterns of genetic variation among cultivars, as Dahleen (1997) assessed RFLP differences among 28 North American barley cultivars and found low amounts of polymorphism among parental cultivars within breeding programs. The polymorphisms observed tended to associate with agronomic and malting QTLs. These observations were similar to those reported by Hayes et al. (1997) with AFLP markers.

The objectives of the present study were to determine if markers distinguishing closely related six-rowed mal-

ting barley cultivars were associated with QTLs controlling agronomic and malting quality traits, and to compare the findings with actual performance data of the cultivars. This study was unique in that it compared and combined the findings of three different marker types, RFLP, PCR-RAPD and AFLP, which, in combination, covered more than 80% of the barley consensus map. The marker results also were compared with coefficients of parentage between cultivar pairs. This information will be useful to those planning marker-assisted selection procedures for the enhancement of six-rowed malting barley.

Table 1 Pedigree and year of registration of six-rowed malting barley cultivars studied for molecular marker polymorphisms

Cultivar	Pedigree	Year of registration notice
Morex	Cree/Bonanza	1979
Robust	Morex/Manker	1983
Excel	Cree/Bonanza//Manker/3/2*Robust	1991
Stander	Excel//Robust/Bumper	1993

Table 2 Keygene N.V. primer numbers and selective primer extensions used in 12 different combinations for AFLP analysis of barley cultivars in Table 1. These primer combinations were recommended for barley genome analysis by Life Technologies (Gibco BRL, Gaithersburg, Md., U.S.A.)

*Eco*RI selective sequences	*Mse*I selective sequences	Primer combinations used
E32: AAC	M47: CAA	E32:M47 E32:M48[a]
E33: AAG	M48: CAC	E32:M49 E32:M50[a]
E35: ACA	M49: CAG	E32:M60 E32:M61[a]
E36: ACC	M50: CAT	E32: M47 E35:M47
E37: ACG	M60: CTC	E36:M47 E37:M47[a]
E41: AGG	M61: CTG	E33:M47[a] E41:M47

[a] Analyzed with LiCor infrared instrumentation instead of conventional isotopic detection

Materials and methods

The RFLP experiments were conducted at Fargo, North Dakota, while the PCR-RAPD and AFLP portions of this study were conducted at Aberdeen, Idaho. The RFLP probes were chosen to provide a representative coverage of the barley genome. The six-rowed malting barley cultivars in this study were developed by the Minnesota Agricultural Experimental Station, St. Paul, Minnesota (Table 1). These cultivars were selected for study because they are from a narrow gene pool, yet the cultivars have been continually enhanced for agronomic and malting quality traits. Seed for the portion of the study conducted at Fargo were obtained from locations listed in Dahleen (1997). Seed for the experiments conducted at Aberdeen were obtained from Dr. Berne Jones of the USDA-ARS Cereal Crops Research Unit, Madison, Wisconsin.

PCR-RAPD procedures followed that given in Hoffman and Bregitzer (1996). PCR-RAPD markers were initially assigned to marker intervals on the Steptoe/Morex map using the technique of Mgonja et al. (1994) as revised by Franckowiak and Dahleen (1996), and the PCR-RAPD interval assignments were reported in Dahleen et al. (1997).

The AFLP primer combinations used and their respective Keygene N.V. identification numbers are given in Table 2. These primer combinations were selected from a list recommended for barley genome analysis by Life Technologies (Gibco BRL, Gaithersburg, Md., U.S.A.). There was no overlap of the primer pairs surveyed here and those reported by Becker et al. (1995), Hayes et al. (1997) or Powell et al. (1997). The AFLP technique for the primer pairs not marked with a superscript "a" in Table 2 was carried out using an AFLP Analysis System-I kit and the accompanying instruction manual purchased from Life Technologies (Gibco BRL, Gaithersburg, Md., U.S.A.), and was also outlined in Hoffman et al. (2000). AFLP using primer pairs marked with a superscript "a" in Table 2 was conducted differently. Instead of labeling *Eco*RI primers with ^{33}P-dATP, an infra-red dye was attached to *Eco*RI primers that were custom-made by the LI-COR Corp. (Lincoln, Neb., U.S.A.). Agronomic and malting quality QTL data and placements were obtained from Wentz (2000) and Hayes et al. (2001). Malting quality data for the four malting barley cultivars were gathered from the 1992 and 1993 Mississippi Valley Uniform Regional Barley Nurseries. Agronomic performance data were obtained from the 1994 Mississippi Valley Uniform Regional Barley Nursery.

Coefficients-of-similarity (COS) per marker type were calculated between all six possible pairs of the four cultivars using the NT-SYS-PC program, version 1.8 (Rohlf 1993). Coefficients-of-parentage (COP) between each cultivar pair were calculated using the KIN software package (Tinker and Mather 1993). One-hundred percent homozygosity within cultivars was assumed for the COP calculations.

Table 3 Frequencies of marker polymorphisms among four barley cultivars of a narrow genetic base. Numbers in front of the slash (/) represent the number of polymorphic markers, and numbers following the slash are the total number of markers surveyed as to marker type and chromosome. Numbers below each fraction are the frequencies of polymorphism

Chromosome	Marker type			All markers	Approx. marker coverage (cM)	Polymorphisms per cM
	RFLP	PCR-RAPD	AFLP			
1 (7H)	1/22	1/11	3/20	5/53	184	0.03
	0.04	0.09	0.15	0.09		
2 (2H)	3/24	1/8	3/17	7/49	169	0.04
	0.13	0.13	0.18	0.14		
3 (3H)	0/17	1/7	1/21	2/45	176	0.01
	0.00	0.25	0.05	0.04		
4 (4H)	3/15	6/7	4/11	13/33	125	0.10
	0.20	0.86	0.25	0.39		
5 (1H)	0/12	0/4	1/13	1/29	148	0.01
	0.00	0.00	0.08	0.03		
6 (6H)	2/10	2/6	4/14	8/30	138	0.06
	0.20	0.17	0.29	0.27		
7 (5H)	4/17	3/8	3/10	10/35	200	0.05
	0.23	0.37	0.30	0.29		
Overall genome	13/117	14/51	19/106	46/274	1,140	0.04
	0.11	0.27	0.18	0.17		

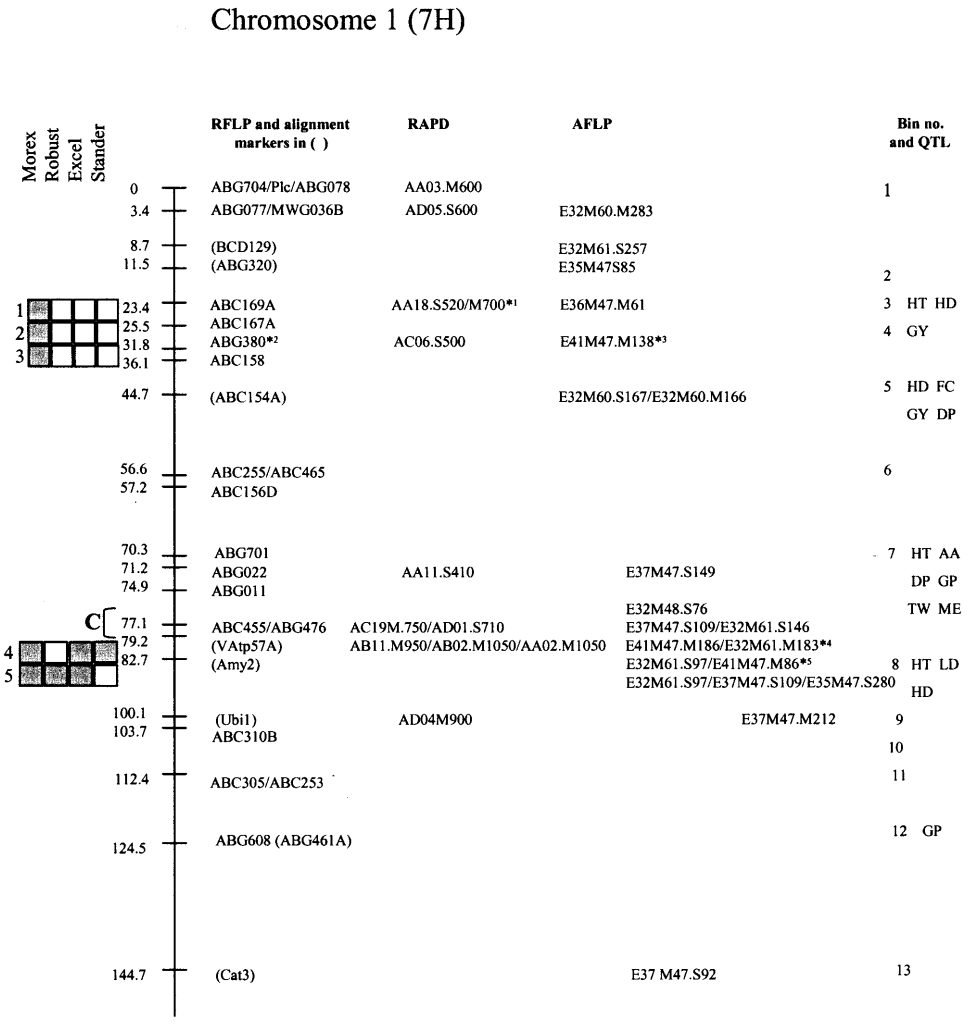


Fig. 1 Map positions of discriminating markers (*) on barley chromosome 1 (7H) and associated QTLs from a summary of QTL studies (Wentz 2000 and Hayes et al. 2001). Centromeric regions are marked with a *left bracket and a "C"*, to the left of each chromosome drawing; symbols under the heading Morex, Robust, Excel and Stander that are *shaded differently* indicate polymorphism at the corresponding numbered locus. Key to the QTL abbreviations is as follows: *AA*, alpha-amylase; *BG*, beta-glucan; *DP*, diastatic power; *GP*, grain protein; *ME*, malt extract; *FC*, fine-coarse difference; *KB*, Kohlbach index; *HD*, heading date; *HT*, plant height; *GY*, grain yield; and *LD*, lodging

Table 4 Coefficients-of-similarity (COS) between the six possible cultivar pairs for each marker type and marker types combined. Coefficients-of-parentage (COP) are included for comparison

Item	COS of marker type			Marker types combined	COP
	RFLP	PCR-RAPD	AFLP	COS	
Morex-Robust	0.94	0.82	0.86	0.88	0.61
Morex-Excel	0.91	0.78	0.90	0.88	0.65
Morex-Stander	0.90	0.88	0.90	0.90	0.48
Robust-Excel	0.95	0.96	0.94	0.96	0.87
Robust-Stander	0.93	0.78	0.91	0.91	0.59
Excel-Stander	0.97	0.78	0.93	0.92	0.60
Means	0.93	0.84	0.91	0.91	0.63

Results

The barley genetic linkage map has been divided into 100 regions called bins, each 10–20 cM in length. The 274 molecular markers we tested covered 83 of the 100 bins. Forty six (16.8%) of the surveyed markers identified a polymorphism between at least one pair of the four cultivars (Table 3). The polymorphisms were detected in 13 RFLP, 14 PCR-RAPD and 19 AFLP loci, and fit into 29 of the 83 bins. The RAPD and AFLP procedures detected a higher frequency of polymorphism as compared to RFLPs. The highest number of polymorphic loci was detected on chromosome 4 followed by chromosomes 6 and 7. Fewer polymorphisms were detected on chromosomes 1 and 2 and even fewer polymorphisms were apparent on chromosomes 3 and 5. This result was reflected in the number of polymorphisms detected per Kosambi centiMorgans (cM).

Greater amounts of marker polymorphism were found when Morex was compared with the later-released cultivars (Table 4). The least amount of marker polymorphism was observed in the Robust-Excel comparison. These trends were reflected in the COP values. Of the three marker types, the more conservative RFLP markers

Fig. 2 As in Fig. 1, but for barley chromosome 2 (2H)

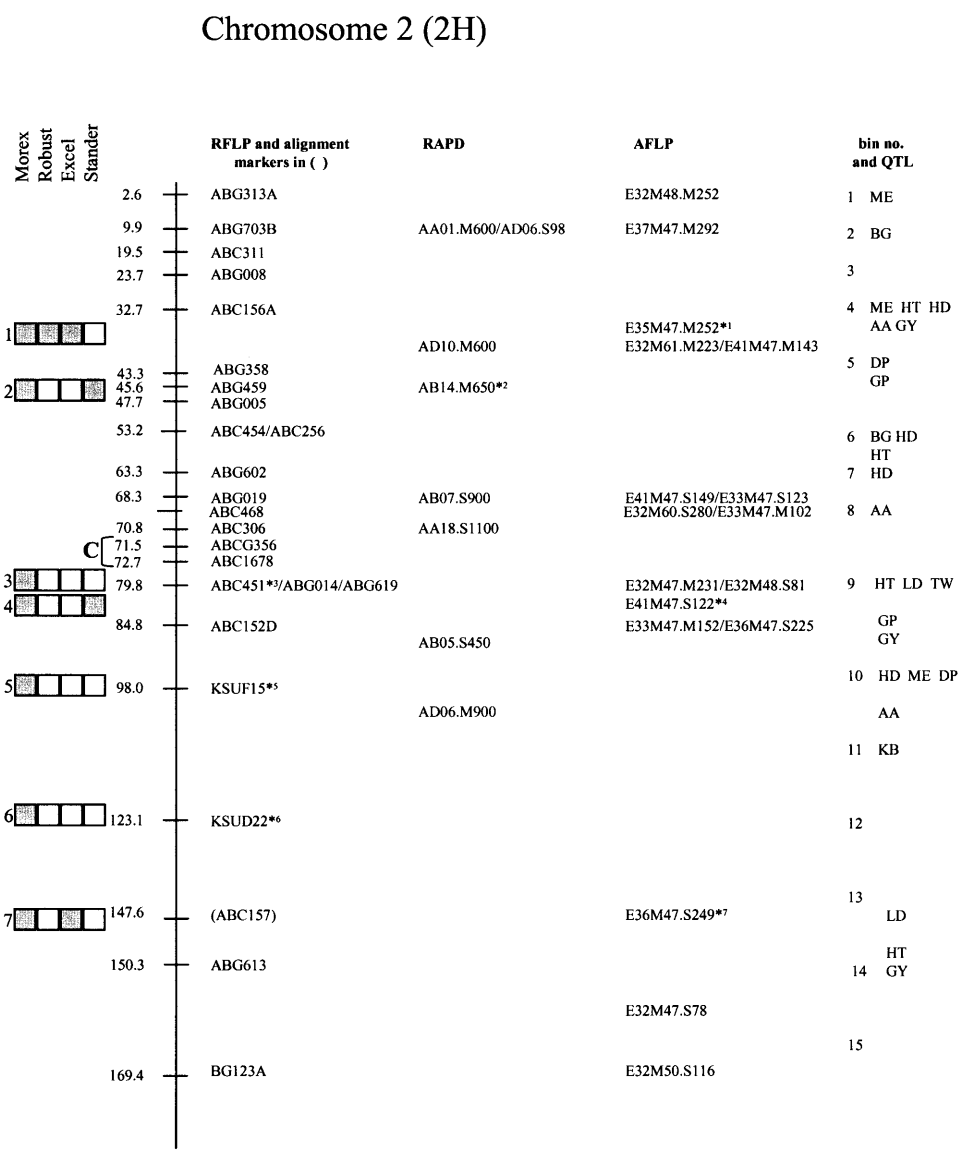


Table 5 Agronomic data for four malting barley cultivars evaluated in the Mississippi Valley Regional Nursery, 1994 report. Data are means over 3 years and four locations

Cultivar	Yield (kg/ha)	Test weight (kg/hl)	Height (cm)	Lodging (%)	Heading date[a]
Morex	4013.4	59.0	86.2	34.7	20.1
Robust	4436.4	60.9	84.6	22.5	21.4
Excel	4677.0	59.7	79.5	20.4	21.3
Stander	4683.6	60.4	78.5	22.0	21.8

[a] Days after May 31

best fit the chronology of the cultivars (Table 1) and the pair-wise COPs (Table 4).

The map locations indicated that the markers that could discriminate between any pair of cultivars in Table 1 were not evenly distributed throughout the barley genome (Figs. 1, 2, 3, 4, 5, 6, 7). The markers identifying polymorphism formed linkage groups on six of the seven barley chromosomes. Twenty one of the 29 chromosome bins that contained polymorphic markers were clustered into eight groups of consecutive bins. The other eight polymorphic loci were in non-consecutive bins, like those on chromosome 3 (Fig. 3).

The largest linkage group was the 13 markers that mapped near the centromere on the long arm of chromosome 4 (Fig. 4). Several agronomic and quality trait QTLs have been detected in this region. The polymorphism patterns among the cultivars (left margin, Fig. 4) fit well with QTL regions (Fig. 4) and trait data (Tables 5, 6). Regions that have the Morex-unique alleles are associated with differences in lodging, grain yield, malt extract or heading date. The two regions with patterns unique to Stander are associated with differences in plant height and heading date. The markers that paired Morex with Stander and Robust with Excel mapped to a

Fig. 3 As in Fig. 1, but for barley chromosome 3 (3H)

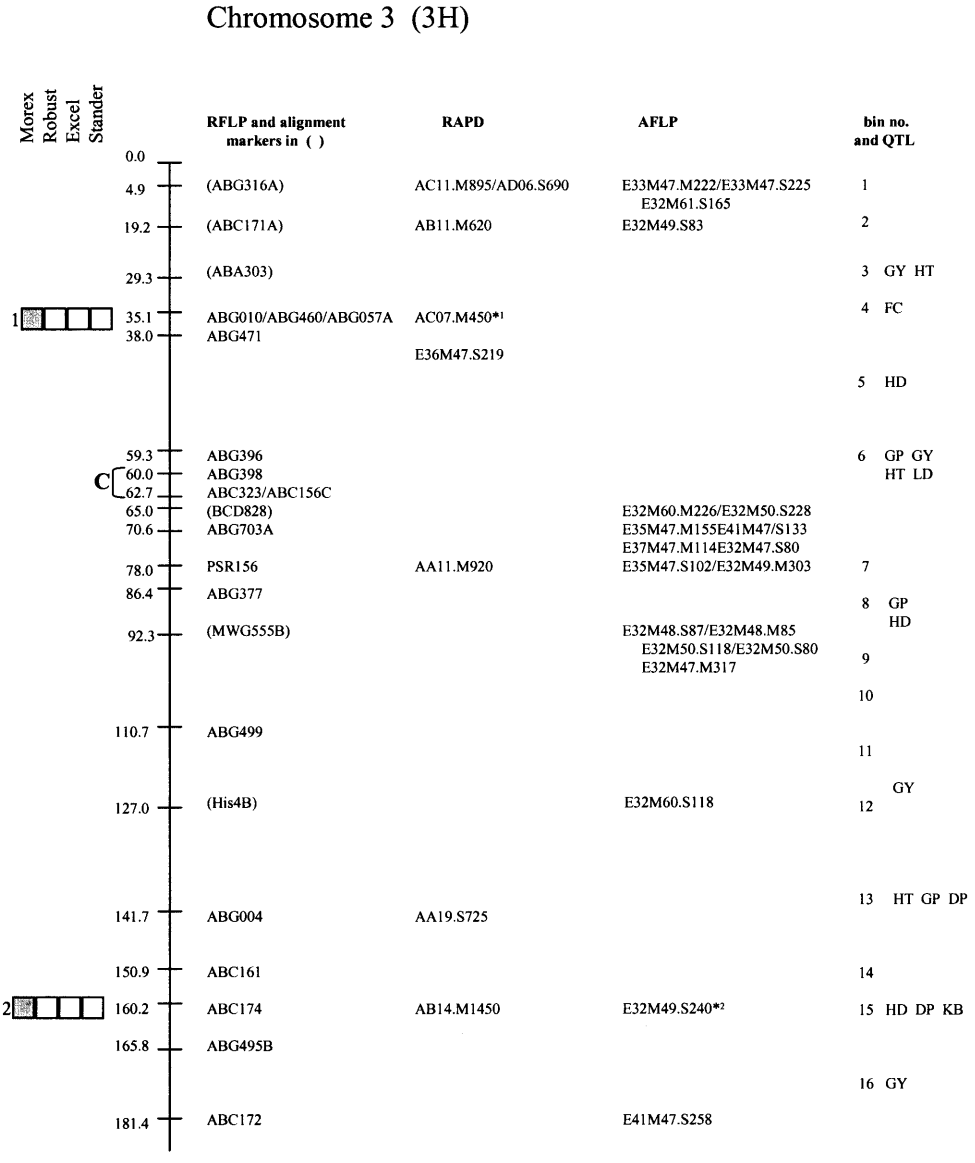


Table 6 Malting quality data for four malting barley cultivars evaluated in the 1992 and 1993 Mississippi Valley Regional Nursery. Data are means of seven locations in 1992 and four locations in 1993

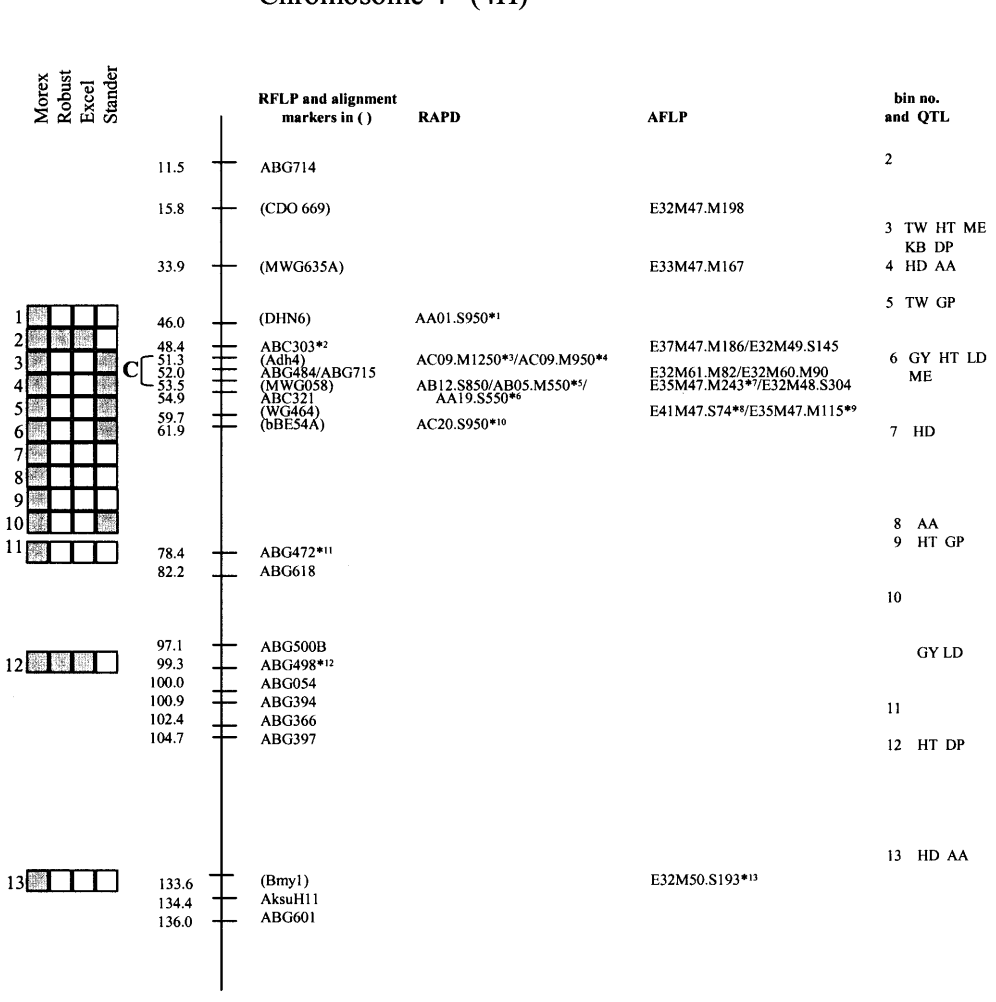
Cultivar	Malt extract (%)	Grain protein (%)	Alpha-amylase (20 deg. Units)	Diastatic power (deg.)	Fine-coarse difference (%)	Kolbach index (%)	Beta-glucan (%)
Morex	77.7	14.5	45.3	144	2.68	40.2	0.6
Robust	78.6	13.5	34.1	130	3.81	37.7	0.8
Excel	79.2	12.7	41.4	122	2.91	40.8	0.8
Stander	79.5	12.9	43.0	131	2.63	42.2	0.8

region associated with alpha-amylase activity and diastatic power, and these traits varied similarly with the marker polymorphisms (Table 6).

The results on chromosome 6 (Fig. 6) showed a linkage group of eight markers detecting polymorphism on both chromosome arms proximal to the centromere. The polymorphism patterns in the left margin fit trait differences and QTLs inferred in the centromeric region of chromosome 6 were associated with grain yield, heading date, alpha-amylase activity, malt extract and diastatic power.

The distribution of the markers detecting polymorphisms on chromosome 7 (Fig. 7) was unlike that of chromosomes 4 and 6. Instead of mapping near the centromere, the ten polymorphic markers mapped distally to the centromere on the short arm, and proximally and dis-

Fig. 4 As in Fig. 1, but for barley chromosome 4 (4H)



tally to the centromere on the long arm. The three distal markers on the short arm mapped near a predetermined QTL for plant height. Again, the polymorphism patterns fit well with the differences in plant height among the three cultivars. The polymorphic marker closest to the centromere overlapped with several QTLs. Of these, alpha-amylase activity best fit the polymorphism pattern and trait expression. The two next distal markers on the long arm of chromosome 7 coincided with QTLs for the Kohlbach index and plant height. The four most-distal polymeric markers mapped to a region associating with four QTLs (Fig. 7). The polymorphism patterns align with trait differences for two of these, heading date and malt extract.

The five markers on chromosome 1 (Fig. 1) detecting polymorphism formed two small linkage groups, one on the short arm distal to the centromere, and the other on the long arm, proximal to the centromere. The distal group was associated with QTLs for heading date, grain yield, plant height and fine-coarse difference. The polymorphism pattern unique to Morex matched trait differences for all of the above except for the fine-coarse difference. The centromeric group was associated with grain yield and grain protein QTLs, but was linked to QTLs for alpha-amylase, diastatic power, grain protein, heading date and malt extract. The Robust-unique pattern best fit the expression of alpha-amylase (Table 6) while the Stander-unique pattern best matched the expression of grain yield (Table 5).

Seven markers that detected polymorphisms were linked or loosely linked along the entire length of chromosome 2 (Fig. 2) except in the telomeric regions. This pattern was consistent with the distribution of many QTLs on this chromosome. Trait differences for heading date, malt extract, the Kohlbach index and grain yield generally followed the polymorphism patterns of the cultivars, except that of the most-distal AFLP marker which paired Morex with Excel and Robust with Stander (Fig. 2).

The two markers detecting polymorphism on chromosome 3 (Fig. 3) were located on each arm. The one on the short arm overlapped with QTLs for grain protein and yield, and the polymorphism pattern agreed with cultivar variation for both traits. The other marker matched polymorphism pattern and trait expression with QTLs for heading date and grain yield. The sole AFLP

Fig. 5 As in Fig. 1, but for barley chromosome 5 (1H)

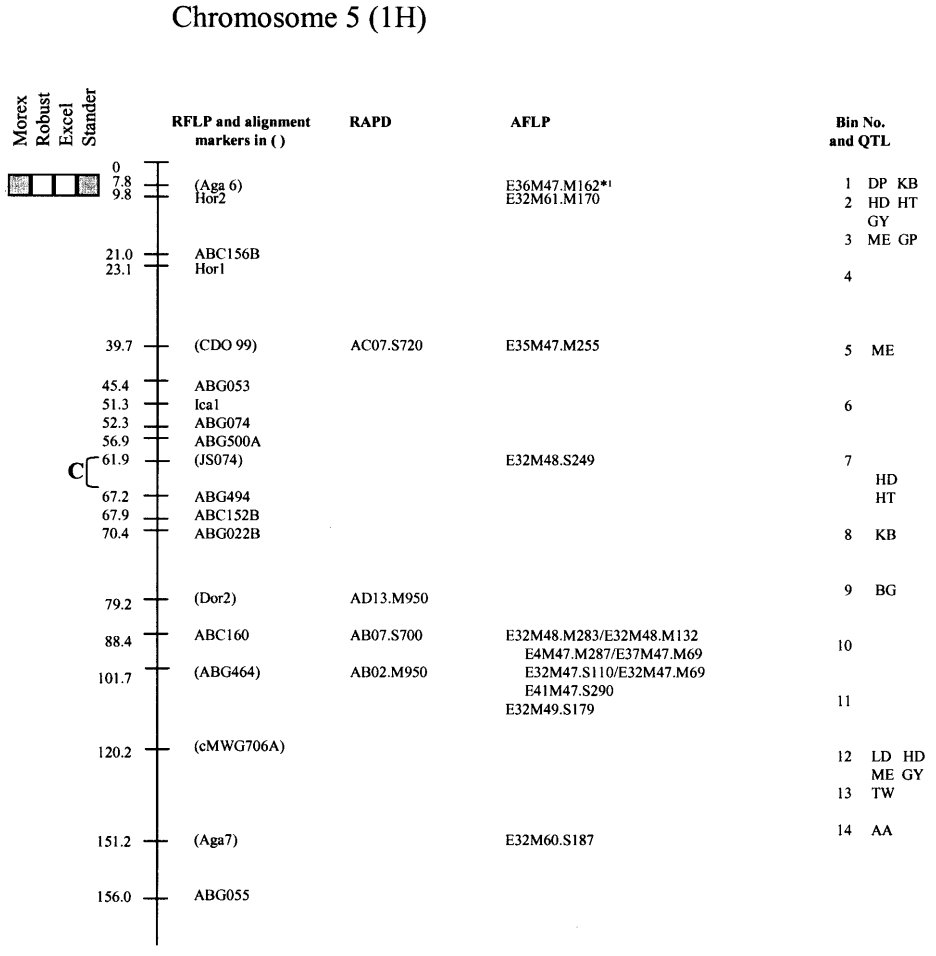


Table 7 Total QTL regions per chromosome for each trait and the number of QTLs in regions that showed polymorphism between the four cultivars

Trait	Chromosome							Total
	1	2	3	4	5	6	7	
Grain yield	2/1	3/3	3[a]/0	2/2	2/0	2/1	3/2	17/9
Test weight	1/0	1/1	0/0	2/1	1/0	2/0	4/2	11/4
Plant height	3/2	4/1	3/1	4/2	2/1	2/1	3/3	21/11
Lodging	1/1	2/2	1/0	2/2	0[a]/0	1/1	2/1	9/7
Head date	3/2	3/1	3/2	3/2	3/0	4/1	4/2	23/10
Malt extract	1/0	2[a]/1	0/0	1[a]/1	3/0	1/1	4/2	12/5
Grain protein	2/0	2/1	3/0	2/2	1/0	0/0	3/1	13/4
Alpha-amylase	1/0	2/2	0/0	3/2	1/0	1/1	3/3	11/8
Diastatic power	2/1	2/1	2/1	1[a]/1	1/1	2/1	3/2	13/8
Fine-coarse difference	1/0	0/0	1/1	0/0	0/0	0/0	3/1	5/2
Kohlbach index	0/0	1/0	1/1	1/0	1/0	0/0	3/2	7/3
Beta-glucan	0/0	2/1	0/0	0/0	1/0	0/0	0/0	3/1

[a] An additional QTL was located on this chromosome in a region not covered by the RFLP, AFLP or RAPD markers tested in this study

marker detecting polymorphism on chromosome 5 (Fig. 5) was on the distal short arm. This corresponded with QTLs for heading date, plant height, alpha-amylase activity, diastatic power, malt extract, grain protein and the Kolbach index. The polymorphism pattern best matched the cultivar differences for alpha-amylase.

The number of QTLs identified in different mapping studies (Hayes et al. 2001) varied for the 12 traits examined in this study (Table 7). The markers we tested covered all but five of the 150 QTL locations listed for the traits in Hayes et al. (2001). Approximately half (72/145) of these QTLs were in regions where at least

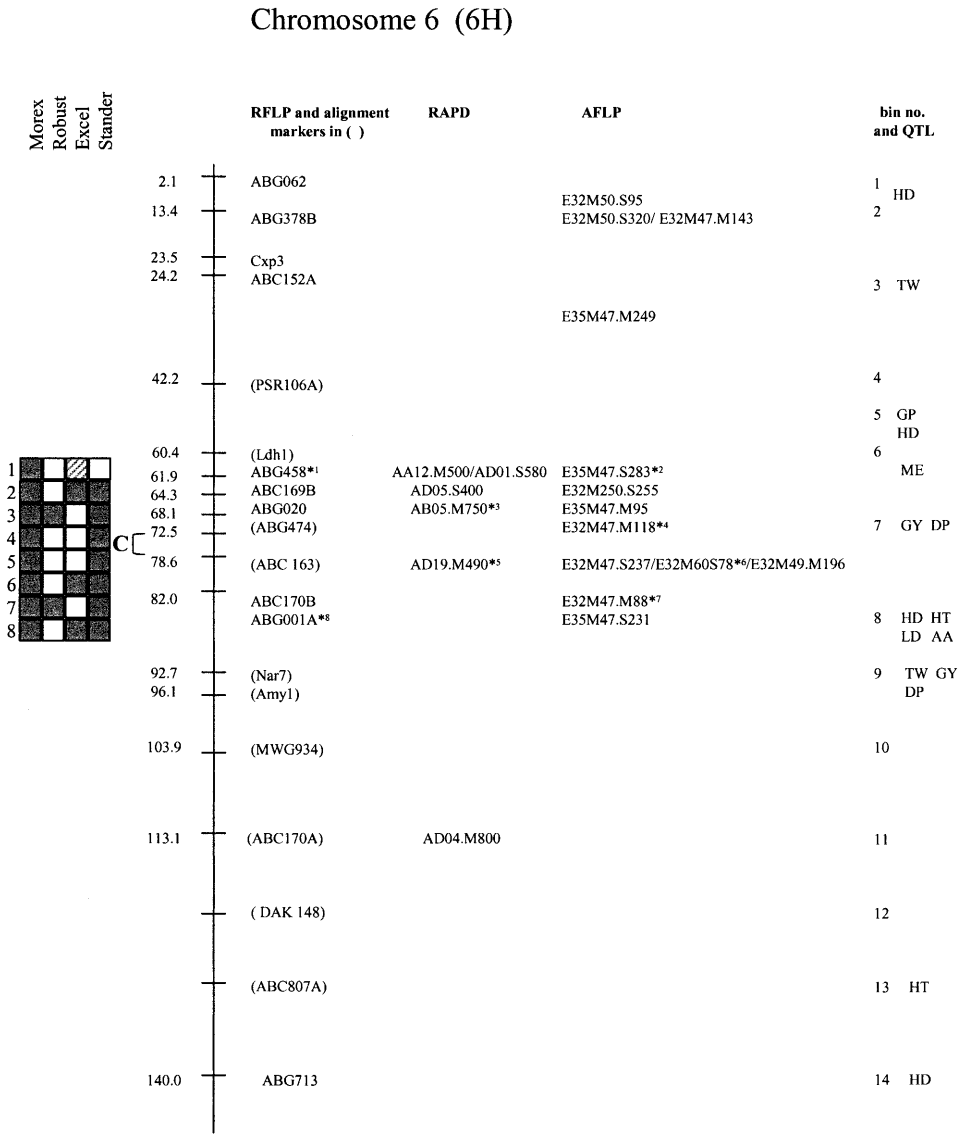


Fig. 6 As in Fig. 1, but for barley chromosome 6 (6H)

one marker identified polymorphisms between the four cultivars. QTL locations were mostly fixed in the four cultivars for some traits, such as test weight, grain protein and beta-glucan, with only one-third or fewer of the QTL regions showing polymorphism. Approximately 40% of the QTLs for heading date, malt extract, fine-coarse difference and the Kohlbach index were polymorphic between the four cultivars. Yield and height alleles were fixed at half of the associated QTLs. Other traits, such as lodging, alpha amylase and diastatic power, were still highly variable. These results show that QTLs for many traits were fixed for the desirable alleles.

Discussion

The barley cultivars used in this study are the result of breeding in a very narrow gene pool. Even though Morex, Robust, Excel, and Stander are closely related (Table 1), they still exhibit many differences for agronomic and malting quality traits (Tables 5–6). Our results show that polymorphic genomic regions identified by molecular markers in this narrow gene pool tend to be associated with inferred agronomic and malting quality QTLs of trait phenotypes that differed among the four cultivars. The presence of different patterns among the four cultivars in short genetic distances (see Fig. 4, centromeric region) indicates that progress has been made in selecting desired allele combinations through traditional breeding methods.

Approximately 17% (46/274) of the markers surveyed detected a large number (16 by linkage; 35 by linkage and crossover) of chromosomal regions. A study by Hayes et al. (1997) looked at AFLP polymorphism among Morex, Excel and Stander covering 33 of the 100 chromosome bins. They found nine AFLP markers that were distinguished among these cultivars compared to 12 found in this study, with Robust excluded. Only one of these appear to match a polymorphic genomic region found in this study based on the polymorphism patterns

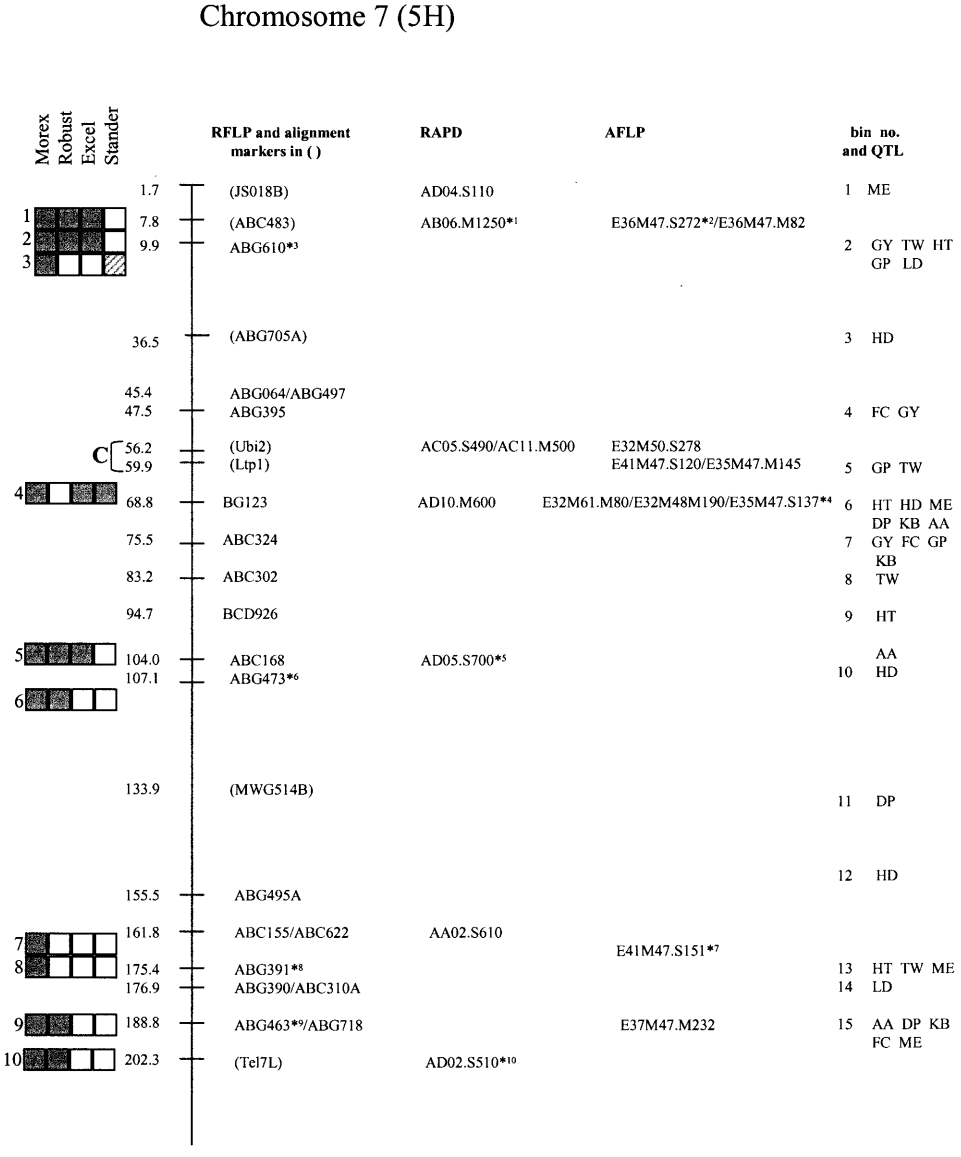


Fig. 7 As in Fig. 1, but for barley chromosome 7 (5H)

of three cultivars, a region on chromosome 6 (6H) identified by the RAPD marker AB05.M750, where Excel differed from the other cultivars. The other chromosome regions differing between Morex, Excel and Stander were newly identified in this study. The differences in AFLP results between this and the Hayes study may be due to the use of different AFLP primer pairs, and the different chromosome bins surveyed by the AFLP markers.

There was a higher frequency of polymorphism among the four cultivars detected by PCR-based procedures as compared to RFLP. Because the RFLP procedure can generate multiple alleles, one might expect the RFLP procedure to identify more polymorphisms. However, roughly one-third of the RFLP clones used in this study were from cDNA libraries. This portion of the RFLP probes, and possibly some of the genomic clones, hybridized to conserved coding regions while a larger portion of the PCR-based primers, especially RAPDs, could detect less-conserved flanking regions.

The level of marker polymorphism (average cultivar-pair marker COS of 0.91) among these four closely related cultivars was consistent with the pedigrees and high COPs of the four cultivars (average COP of 0.63). In comparison, Martin et al. (1991) found similar mean COPs of 0.57 within their most-related clusters of barley cultivars and mean COPs of 0.02 or less between groups of highly unrelated six- and two-rowed cultivars from similar time eras. Even with a narrow gene pool, large genetic gains can be obtained in a breeding program. Rasmusson and Phillips (1997) hypothesized a number of genetic mechanisms to explain these large gains, including recombination and epistasis uncovering existing genetic variation and *de novo* generation of allelic variation. Further study of crosses within a narrow germplasm could be used to elucidate the underlying basis of the phenotypic variability found among this select group of six-rowed malting barley cultivars. Data for agronomic traits (Table 5) and malting quality (Table 6) are avail-

able for the four cultivars under the auspices of the regional nursery trials. In examining information in these tables, and keeping in mind the chronology of the cultivars (Table 1), it is apparent that adjustments to traits have been made in the germplasm over time. For example, the agronomic data show a trend for increased yield and heading date and reduced height. Test weight and lodging showed both increases and decreases over time. The malting quality data show similar trends in that malt extract has been increased a few percent while seed protein has been lowered. Alpha-amylase activity was initially lowered in Robust and then restored to levels comparable to that of the malting quality standard of Morex by the release of Stander. Diastatic power (a measure of starch degradation), fine-coarse extract difference and the Kohlbach index showed both increases and decreases over the time of cultivar development. Beta-glucan showed an initial increase from Morex to Robust and then was held constant. These data are reflected in what was observed in the association of cultivar-discriminating markers with QTLs controlling these traits. QTLs are often clustered, so a discriminating marker could be located in a region associated with a number of traits. For example, the AFLP marker E36M47.M162 on chromosome 5 was in a region associated with plant height, heading data, malt extract, grain protein, diastatic power and the Kohlbach index. The marker data indicated that Morex and Stander showed one banding pattern while Robust and Excel showed a different pattern. Only the data for alpha-amylase matches the cultivar differences for marker phenotype, so it is likely that this AFLP band is associated with alpha-amylase differences between Morex, Robust, Excel and Stander. A direct test for these associations would require the creation of a mapping population from each pair of cultivars and extensive agronomic and quality testing of each population.

This information should be useful to further investigate and map these candidate regions of key QTLs and to further test candidate regions for marker-assisted selection procedures. This method of identifying candidate QTL regions, by surveying polymorphism at the DNA level among cultivars from a narrow germplasm base, would serve as a starting point for crops without complete molecular-marker maps. This study also identifies some PCR-based markers that could be used in future marker-assisted selection procedures that would serve as non-radioactive alternatives to RFLP markers, which usually require the use of high-energy radioisotopes to detect RFLP fragments in large-genome sized organisms such as barley.

It was noted that QTLs for test weight, grain protein, and beta glucan mostly were not associated with marker polymorphism, and this led us to infer that these regions are genetically fixed among the four cultivars. This corresponded to the small differences among the cultivars reported in Tables 5 and 6. If barley breeders want to further alter such traits with fixed QTL regions, they will need to carefully introduce new sources of variability into their crossing program. It appears that for some of the traits, like lodging and yield, there still is sufficient genetic variability within the studied germplasm pool to make additional gains.

In summary, three types of DNA markers were tested for polymorphism among four cultivars comprising a narrow genetic base. The markers found to discriminate between at least one cultivar pair tended to map in regions associated with agronomic and malting quality QTLs mapped in previous studies. For most markers, quantitative traits that followed the marker polymorphism patterns could be identified, although one marker did not fit the data for the 12 traits evaluated. This chromosome region is most likely associated with other quantitative traits that differ among the four cultivars. The tendency of discriminating markers to associate with QTLs directly involved in malting barley enhancement indicates that genetic variability exists in this narrow six-rowed malting barley germplasm base. Considering the polymorphism patterns identified by the molecular markers, it is likely that both pre-existing genetic variation and *de novo* variation were responsible for gains in agronomic performance and malting quality in this narrow breeding germplasm. Studies of segregating populations from crosses within this narrow germplasm could further elucidate the underlying basis of the phenotypic variability found among this select group of six-rowed malting barley cultivars and in similar barley breeding pools.

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