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QTL Analysis of Forage Quality Traits in Barley (*Hordeum vulgare* L.)

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Despite the importance of barley as an animal feed, its forage quality has usually been neglected in breeding programs. In order to map the genomic regions, which modify barley forage quality, a population of 72 F_1-derived doubled haploid lines (DH) from the cross "Steptoe/Morex" and their two parents were sown in Karaj and Zabol provinces of Iran, in each under a randomized complete block arrangement with two replications. Forage samples were oven-dried and ground and dry matter digestibility (DMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), crude fiber (CF), crude protein (CP), water-soluble carbohydrates and ash content were measured by NIRS. Analysis of variance showed that genotype, environment and genotype–environment interaction have significant effects on almost all studied traits. Several QTLs were resolved for each studied trait in both environments. Highest LOD scores were obtained for CF, ADF and DMD on chromosome 2H and for ash and CP on chromosomes 3H and 5H, respectively. QTLs for NDF were present on all chromosomes except 4H and 7H. QTL × environment interaction and the specificity of the QTLs are discussed.

Keywords: QTL, barley, forage quality, doubled haploids, stability

Introduction

Barley (*Hordeum vulgare* L.), the fourth most produced cereal worldwide, is mainly used for animal feed, human food and malting. Traditionally, the main objectives of barley breeding programs are to develop cultivars with high grain yield and malt quality. However, forage quality is an important selection criterion, which is usually neglected in barley breeding programs.

Based on nutritive parameters, forage should have optimum dry matter concentration for proper fermentation after ensiling, high digestibility and conversion efficiency to maximize intake, and high protein content to reduce requirements of supplemental protein

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(Coleman and Moore 2003). Therefore, the quality of forage is influenced by several chemical factors including dry matter digestibility (DMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), crude fiber (CF), crude protein (CP), water-soluble carbohydrate (WSC) and ash content (Lübberstedt et al. 1997; Smith et al. 1997; Casler 2001; Cardinal et al. 2003; Coleman and Moore 2003; Han et al. 2003; Cogan et al. 2005).

Direct evaluation of forage quality through feeding experiments is costly and needs substantial quantities of breeding materials. Indirect assessment methods, including *in vitro* fiber digestibility, enzymatic digestion (DeBoever et al. 1986; Mould 2003; Tessema and Baars 2004) and chemical analysis of cellular components (Jung 1997) are preferred. These methods are slow and laborious, especially in case of multiple measurements for large samples. In turn, Near-Infrared Reflectance Spectroscopy (NIRS) which offers an inexpensive, rapid, and accurate technique for simultaneous evaluation of several forage-quality traits has become a routine method in many forage species (Roberts et al. 2004; Mentink et al. 2006).

Conventional breeding methods still remain a successful approach for improving the forage quality traits. Direct phenotypic selection for high digestibility has been successful in several forage crop species; however, measurement methods for some traits like fiber, lignin and proteins are still time consuming and expensive (Casler 2001). Most forage quality traits are quantitative in nature and controlled by several mild-effect genes, the so-called QTLs. QTL analysis has been undertaken extensively in barley for different important traits such as grain yield (Thomas et al. 1998; Peighambari et al. 2005), malting quality (Han et al. 1997; Molina-Cano et al. 1997; Borem et al. 1999; Beecher et al. 2001), disease resistance (Chen et al. 1994; Steffenson et al. 1996) and environmental adaptation (Mickelson et al. 2003). However, only few studies have been devoted to feed quality traits. Han et al. (2003) performed a QTL analysis only for grain acid detergent fiber (ADF) and Abdel-Haleem et al. (2004) mapped QTLs of feed quality-related traits such as grain ADF, grain starch, cracked grain particle size and cracked grain *in situ* dry matter digestibility.

The main objectives of this study are: mapping of QTLs controlling forage quality traits in doubled haploid lines from the cross "Steptoe × Morex" and investigation of their stability over different climatic conditions.

Materials and Methods

Plant material

Seventy-two doubled haploid (DH) barley lines from the cross "Steptoe" (CI15229) × "Morex" (CI15773) together with both parents, were used to determine forage quality. The DHs were developed through a modified *Hordeum bulbosum* technique (Chen and Hayes 1989) by the Oregon State University Barley Breeding Program and were kindly provided by Dr. Patrick M. Hayes (Department of Crop and Soil Science, Oregon State University, Corvallis, OR 973314501, USA).

Measurements

The 72 DHs as well as their parents were planted in two locations; the Research Farm of the Faculty of Agronomy and Animal Science of the University of Tehran in Karaj and the Research Farm of the Agricultural Research Station of Zabol in Zahak, under irrigated conditions. The experimental design was a randomized complete block with two replications. Each plot consisted of four rows, 3 m long with a row spacing of 25 cm. Forage samples were taken at dough stage. A 0.5 m clip sample from two middle rows was cut at stubble height and dried at 70 °C for 48 h. Dried samples were ground through the 0.1 mm screen of a cyclone mill and scanned using a Near-Infrared Reflectance Spectroscopy (NIRS, Informatics 8600) with 6–20 wavelengths ranging from 500 to 2400 nm. Details of the methodology and calibrations of NIR are given by Jafari et al. (2003).

Measurements were made on eight forage quality characteristics, namely dry matter digestibility (DMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), crude fiber (CF), crude protein (CP), water-soluble carbohydrates (WSC) and ash content. Analysis of variance was performed on the combined data from both locations using PROC GLM procedure in SAS (SAS Institute 1992).

Mapping of QTLs

For the current DH population, a molecular marker linkage map (Kleinhofs et al. 1993; Hayes et al. 1993) has been developed by the North American Barley Genome Mapping Project (currently at http://barleygenomics.wsu.edu/). This map comprises 327 markers with an average inter-marker distance of 3.75 cM (Kleinhofs et al. 1993; Hayes et al. 1993). QTL analysis was conducted separately for each trait in each environment using Windows QTL Cartographer 2.5 (Wang et al. 2007). For each trait, a series of 1000 permutations were run to determine the experiment-wise significance level, expressed as a LOD value, equivalent to $P = 0.05$ (Churchill and Doerge 1994). Composite interval mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects (Jansen and Stam 1994; Zeng 1994). The genome was scanned at 2 cM intervals and window size was set at 10 cM. Background markers were selected using stepwise regression. Confidence intervals of 95% were calculated by 1000-bootstrap re-sampling (Lebreton et al. 1998), as proposed by the Windows QTL Cartographer 2.5.

Results

Analysis of variance showed that genotype had a highly significant ($P < 0.01$) effect on all of the studied traits (Table 1). The simple effect of location and the genotype-location interaction were also significant ($P < 0.05$) for almost all studied traits. The difference between parental mean and the mean of doubled haploids was not significant, except for DMD, ADF and CF (Table 2). Transgressive segregation in both directions was significant for DMD, ADF, NDF, ADL and WSC (Table 2).

Table 1. Combined analysis of variance for eight barley forage quality traits in 72 'Steptoe'/'Morex' doubled haploid lines and two parents

S.O.V.	D.F.	Mean squares							
		DMD	ADF	NDF	ADL	CF	CP	WSC	Ash
L	1	123.27*	63.27*	366.77*	10.24*	20.75^{ns}	45.71*	125.31*	42.11*
B(L)	2	1.85	3.24	8.21	0.05	2.25	2.08	6.44	0.96
G	73	49.27**	75.54**	104.33**	0.70**	46.67**	8.84**	36.95**	2.51**
G×L	73	10.40*	18.25*	27.38*	0.16*	8.50^{ns}	3.13*	7.56*	0.45^{ns}
E	146	6.34	11.16	17.01	0.10	7.90	1.94	4.67	0.34

* and **, significant at 0.05 and 0.01 probability level, respectively; ns, non-significant.
DMD, dry matter digestibility; ADF, acid detergent fiber; NDF, neutral detergent fiber; ADL, acid detergent lignin; CF, crude fiber; CP, crude protein; WSC, water-soluble carbohydrate.

Table 2. Simple statistics and genetic gain of eight barley forage quality traits in a population of 72 'Steptoe/Morex' DHs and two parents

Item	DMD	ADF	NDF	ADL	CF	CP	WSC	Ash
'Steptoe' (P_1)	62.17	33.43	52.17	3.87	29.91	8.83	15.45	6.50
'Morex' (P_2)	65.78	28.48	50.06	3.79	25.11	10.22	15.73	7.36
P_1–P_2	–3.61**	4.95**	2.10^{ns}	0.08^{ns}	4.79**	–1.40^{ns}	–0.28^{ns}	–0.86**
[a]MP = (P1+P2)/2	63.98	30.95	51.11	3.83	27.51	9.53	15.59	6.93
[b]Min_{DHs}	57.85	25.68	43.36	3.23	23.63	7.91	11.97	5.25
[c]Max_{DHs}	67.93	38.82	60.76	4.61	32.62	11.77	18.16	7.57
[d]X_{DHs}	61.80	33.73	54.06	4.03	29.27	9.63	14.91	6.51
X_{DHs}–MP	–2.17*	2.78*	2.94^{ns}	0.21^{ns}	1.76*	0.10^{ns}	–0.69^{ns}	–0.42^{ns}
[e]UGG = | MaxDH–[f]BP |	2.15*	5.39**	8.59**	0.74**	2.71**	1.55^{ns}	2.43**	0.21^{ns}
[g]DGG = | MinDH–[h]LP |	4.59**	2.8*	6.7**	0.56**	1.48^{ns}	0.92^{ns}	3.48**	1.25**

* and **, significant at 0.05 and 0.01 probability level; ns, non-significant.
DMD, digestible dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber; ADL, acid detergent lignin; CF, crude fiber; CP, crude protein; WSC, water-soluble carbohydrate.
[a]Parental mean (mid-parent); [b]DH with minimum trait value; [c]DH with maximum trait value; [d]Mean of doubled haploids; [e]upward genetic gain; [f]parent with higher trait value; [g]downward genetic gain; [h]parent with lower trait value

From one to five QTLs were detected for each of the eight forage quality traits, with phenotypic values (R^2) between 6.5 to 26% (Table 3). In total, fifty-three QTLs were identified for the traits, forty-two of them were in common between Zabol and Karaj locations (hereafter presented as 21 common QTLs), five QTLs were found only in Karaj and the other 6 QTLs were specific to Zabol.

QTL models explained about 50.04 and 48.74% of total variation of DMD in Karaj and Zabol, respectively (Table 3). Three QTLs, which controlled DMD, were detected in both locations on chromosomes 2H, 3H and 5H (Fig. 1). Zabol and Karaj each had a specific QTL for DMD on 1H. There were four common QTLs on chromosomes 1H, 2H, 3H and 5H for ADF content. For NDF, two common QTLs were found on 1H and 3H. A specific QTL on 2H and two specific ones on 5H and 6H were found in Karaj and Zabol, respectively. Three common QTLs were detected for ADL and just one specific in Zabol experiment. Chromosome 3H carries two QTLs for ADL. These two QTLs are far apart and also

Table 3. Quantitative trait loci (QTL) for forage quality traits in a 'Steptoe/Morex' doubled haploids population in Karaj and Zabol

Trait	QTL name	Chromosome name	Nearest marker	QTL position[a]	LOD score[b]		Additive effect		[C]R^2	
					Karaj	Zabol	Karaj	Zabol	Karaj	Zabol
DMD	*Qdmd1Hsnk*	1H	Pcr2	63.1	2.51	–	0.63	–	6.92	–
	Qdmd1Hsnz	1H	His3B	100.0	–	2.63	–	0.66	–	7.87
	Qdmd2Hsn	2H	Adh8	62.0	6.22	5.00	1.04	1.13	20.37	21.05
	Qdmd3Hsn	3H	ABC171	24.3	3.56	3.96	–0.78	–0.91	11.09	13.49
	Qdmd5Hsn	5H	ABG705	36.7	6.66	4.27	1.00	0.94	18.58	14.20
ADF	*Qadf1Hsn*	1H	KsuF2A	59.0	3.78	3.00	–1.19	–1.05	12.01	9.47
	Qadf2Hsn	2H	Pox	52.6	4.65	5.96	–1.21	–1.48	14.39	19.61
	Qadf3Hsn	3H	ABC171	22.3	2.63	2.68	0.92	1.02	8.40	9.22
	Qadf5Hsn	5H	ABG705	36.7	4.70	4.45	–1.25	–1.29	15.75	14.81
NDF	*Qndf1Hsn*	1H	lca1	43.0	3.83	3.82	–1.45	–1.55	15.23	15.28
	Qndf2Hsnk	2H	ABG008	24.5	2.53	–	–0.84	–	7.36	–
	Qndf3Hsn	3H	ABC171	24.3	4.21	4.21	1.52	1.63	17.65	17.80
	Qndf5Hsnz	5H	ABG473	121.2	–	2.54	–	–0.13	–	6.44
	Qndf6Hsnz	6H	PSR167	1.5	–	2.94	–	–0.92	–	8.43
ADL	*Qadl1Hsn*	1H	ABG053	41.5	3.42	3.42	–0.11	–0.11	13.89	13.86
	Qadl3Hasn	3H	ABC171	24.3	3.29	3.28	0.10	0.11	12.82	12.78
	Qadl3Hbsn	3H	His4B	145.6	3.23	3.25	–0.15	–0.15	14.13	14.24
	Qadl6Hsnz	6H	PSR167	1.4	–	2.54	–	–0.08	–	8.58
CF	*Qcf1Hsn*	1H	KsuF2A	59.0	3.27	3.36	–0.69	–0.75	9.68	9.94
	Qcf2Hsn	2H	Pox	52.6	6.80	6.88	–1.03	–1.11	22.67	22.90
	Qcf3Hsn	3H	ABC171	24.3	3.04	3.08	0.67	0.72	9.34	9.48
	Qcf5Hsn	5H	ABG705	36.7	3.49	3.36	–0.72	–0.75	10.70	10.20
CP	*Qcp1Hsnk*	1H	KsuF2A	59.0	2.99	–	–0.40	–	10.64	–
	Qcp2Hsnz	2H	Pox	52.6	–	2.55	–	–0.57	–	9.57
	Qcp5Hsn	5H	WG364	103.8	4.82	5.20	0.32	0.36	17.95	19.77
	Qcp6Hsn	6H	ABR331	48.6	3.19	3.37	0.25	0.28	11.29	12.10
WSC	*Qwsc2Hsnz*	2H	ABG358	43.3	–	2.51	–	0.62	–	6.97
	Qwsc3Hsn	3H	ABC171	22.3	4.27	3.58	–0.68	–0.67	18.87	16.50
	Qwsc5Hsnk	5H	ABG705	36.7	2.59	–	0.53	–	7.36	–
Ash	*Qash1Hsnk*	1H	BCD351C	62.3	2.56	–	0.10	–	9.65	–
	Qash3Hsn	3H	MWG902	172.8	5.55	5.53	–0.33	–0.32	26.03	25.99
	Qash4Hsn	4H	cMWG652B	124.0	2.66	2.61	–0.17	–0.16	11.25	11.03

[a]QTL position expressed in cM, from origin of the linkage group (end of short arm). [b]Peak value of the LOD. [c]Percentage of phenotypic variance explained by the QTL. DMD, digestible dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber; ADL, acid detergent lignin; CF, crude fiber; CP, crude protein; WSC, water-soluble carbohydrate.

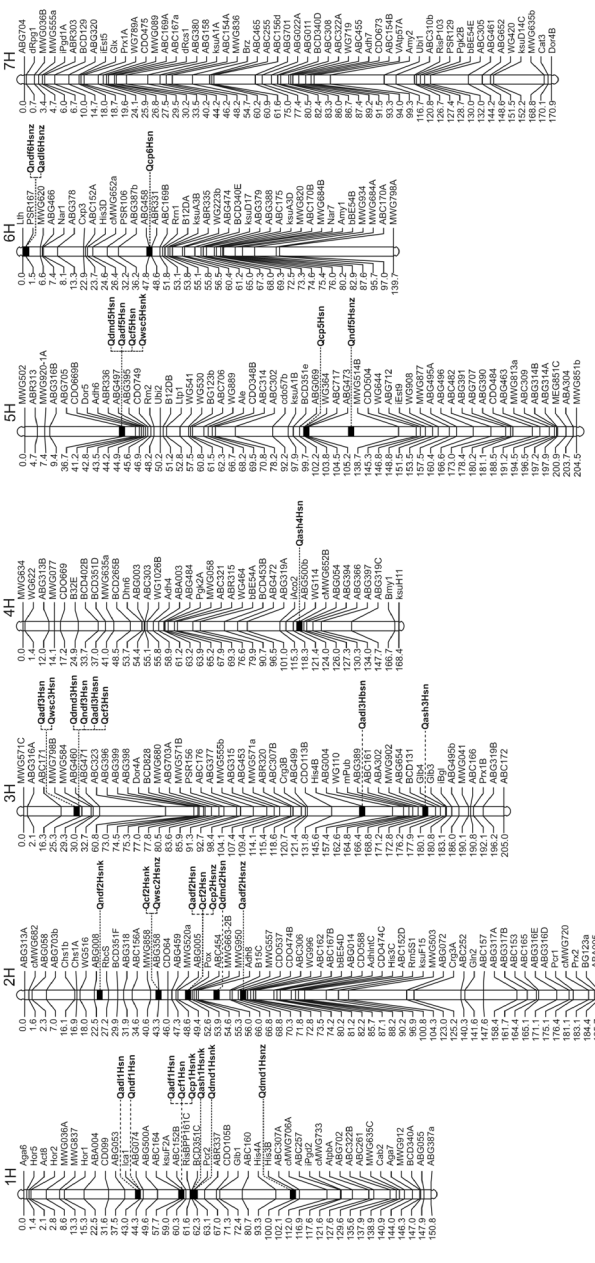

Figure 1. QTLs map of forage quality attributes, based on data from 72 doubled haploids from a cross between Steptoe and Morex, identified by composite interval mapping in Karaj and Zabol. Distances are in cM (calculated using the Kosambi mapping function)

they have different additive signs. Positive allele for *"Qadl3Hasn"* comes from "Steptoe" and for *"Qadl3Hbsn"* it comes from "Morex". Among the four QTLs detected for CF, the most prominent one is a common QTL with about 23% of phenotypic variance (R^2) in both Karaj and Zabol experiments. Positive alleles for these QTLs, except for the QTL on 3H, come from "Morex" (Table 3, Fig. 1).

Two common QTLs controlling variation in CP concentration were found on chromosomes 5H and 6H whereas two specific ones were found on 1H and 2H. A common QTL was mapped to 3H for WSC, and two environment-specific ones were mapped to 2H and 5H. A stable and quite strong QTL controlling ash was detected on 3H, while 1H and 4H each carried one specific QTL. In this study, the QTL for DMD ("*Qdmd3Hsn"*) at the right hand of marker ABC171 on 3H , coincided with QTLs for ADF, NDF, ADL, CF and WSC (Table 3, Fig. 1). QTLs *"Qadf2Hsn"* for ADF and *"Qcf2Hsn"* for CF were also co-localized. The same aspect was also observed for location-specific QTLs in Karaj.

Discussion

In our experiment the difference between the mean of DH lines and the mean of parents was not significant, therefore it is suggested that the 72 DHs should be representatives of the total possible DHs from the cross "Steptoe × Morex" (Table 2). The existence of genetic diversity among parents is a necessary measure before QTL mapping which is proved either by a significant difference between them or through transgressive segregation among progenies. The former is a sign of isodirectional allele distribution and the latter implies the dispersion of positive alleles between the two parents. In this study both cases have been documented. Similar variations between the two parents and also within the "Steptoe × Morex" DHs population, as well as transgressive segregation for forage quality characteristics have been previously reported (Bregitzer and Campbell 2001; Han et al. 2003; Abdel-Haleem et al. 2004; Peighambari et al. 2005).

QTL analysis resulted in detection of several genomic loci with potential effects on forage quality (Table 3). Number of QTLs for each trait ranged from 5 for DMD and NDF to 3 for WSC and ash. Abdel-Haleem et al. (2004) reported that QTLs for DMD were located on chromosomes 1H, 2H, 3H and 6H. They reported that the most important DMD-QTL was laid beside "Vrs1" gene, which impacts plant and seed development and suggested that this gene may have a pleiotropic effect on forage quality. In current study, 1H, 2H and 3H also harboured DMD-QTLs, and *Qdmd2Hsn* on 2H was the most important one.

Crude protein content QTLs were found in 1H, 2H, 5H and 6H, and the most prominent one was located on 5H. Previous studies have detected several grain protein QTLs on all of the seven barley chromosomes using different populations (Oziel et al. 1996; Mather et al. 1997; Marquez-Cedillo et al. 2000). Abdel-Haleem et al. (2004) detected QTLs associated with grain protein content on 1H, 2H, 3H and 4H chromosomes.

We have identified QTLs for ADF on 1H, 2H, 3H and 5H whereas Abdel-Haleem et al. (2004) reported four QTLs for this character on chromosomes 2H, 3H, 4H and 6H. Other

studies have identified several QTLs controlling grain ADF content on 1H, 2H and 4H chromosomes in "Steptoe × Morex" population (Ullrich et al. 1996; Han et al. 2003).

As QTLs potentially include large number of genes, identifying the gene actually underlying each QTL would be a necessary but cumbersome task. Previous studies have presented some candidate genes; however, very few genes have been discovered to have a direct and significant effect on forage quality traits. Casler (2001) in a study on perennial ryegrass (*Lolium perenne* L.) suggested that the WSC-QTL might be the phosphoglucose isomerase (PGI) gene.

Based on the results of current study, and also previous reports, chromosomes 2H and 3H are the most important ones in controlling forage quality. Therefore, further studies with emphasis on these chromosomes would enhance the resolution of several forage quality QTLs. There have been several cases of overlapping QTLs for different traits. These QTL co-locations, which explain phenotypic correlations among different traits, could be either because of a linkage between two genes or a pleiotropy effect of one gene. The QTL for DMD *("Qdmd3Hsn")* at the right hand of marker ABC171 on 3H coincided with QTLs for ADF, NDF, ADL, CF and WSC (Table 3, Fig. 1). QTLs *"Qadf2Hsn"* for ADF and *"Qcf2Hsn"* for CF were also co-localized. The same aspect was also observed for location-specific QTLs. In Karaj, five QTLs for ash content, CP, ADF, DMD and CF were co-located on 1H at position about 60 cM. Peighambari et al. (2005) reported also several co-locations for different traits, for example, QTLs for kernel weight and spikes per plant were located in the same region on 1H.

Consistency of QTLs across different environmental conditions is an important component for MAS. Forage quality traits usually show trivial or non-significant "genotype–interaction" effect, although examples of severe "genotype–environment" interaction also exist (Casler 2001). An important portion of forage quality QTLs that were detected in this study appears to be quite stable between locations; there is a promising scope for marker-assisted selection of these traits. In pervious studies, the utility of marker-assisted selection for traits such as yield and quality in barley has been demonstrated (Zhu et al. 1999; Ayoub et al. 2003).

In this study we have focused on digestibility, protein, carbohydrate, mineral and fiber component traits, which are supposed to be associated with forage quality. A total of 21 common and 11 location-specific QTLs were found for the eight studied traits. The high number of common QTLs and the stability of their effects can increase the efficiency of marker-assisted selection for forage quality traits in barley. Although the detected regions need to be mapped more precisely, the information obtained should help in marker-assisted selection.

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