

# Quantitative trait loci of acid detergent fiber and grain chemical composition in hulled × hull-less barley population

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**Abstract** Cultivated barley is the major livestock feed grain in the Northern Plains and Northwestern United States due to the fact that its short growing season and limited rainfall limit the planting and production of corn. Starch and fiber content play a significant role in feedlot performance of animals raised on barley feed. To study the underlying genetic locations and mechanisms for these traits, a recombinant inbred line population was derived from a cross between the hulled barley cultivar Valier and a hull-less Swiss landrace line, PI370970. Valier has a high acid detergent fiber content (ADF) and low starch and protein while PI370970 contains low ADF and high starch and protein content. To detect associated QTLs, data were collected and analyzed from irrigated and rain-fed environments. A total of 30 main effect QTLs and four epistatic QTLs were identified which conditioned ADF, starch and protein content under rain-fed,

irrigated and combined analyses. These QTLs were located on chromosomes 2H, 3H, 5H, 6H and 7H. Major ADF and starch QTL were identified on chromosome 7H near the *nud* locus (the locus controlling hulled vs. hull-less caryopsis). High heritability estimates for both ADF and starch content suggest that early selection for these traits during breeding would be productive. Low ADF-QTL were independently verified in a second population in a different genetic background.

**Keywords** Barley · Fiber · Net energy available for maintenance · QTL · Starch · Hull-less

## Abbreviations

ADF	Acid detergent fiber content
ISDMD	In situ dry matter digestibility
NE <sub>m</sub>	Net energy available for maintenance
QTL	Quantitative trait loci
PS	Particle size

## Introduction

Cultivated barley, *Hordeum vulgare*, is the fourth-ranked U.S. cereal crop after corn, wheat and sorghum. More than half of the barley grown in the U.S. is used for livestock feed with the remaining stocks diverted for human food and malting purposes. Barley is the major feed source for livestock in the Northern Plains and Northwestern United States due

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to its short growing season and limited rainfall, factors that limit planting and production of corn in the same regions. Barley grain chemical composition does not differ significantly from other grains, but while it contains more total protein, higher essential amino acids levels and higher crude fiber than either corn or sorghum, it has lower starch and lipid concentrations (Kellems and Church 2002).

The hull constitutes 10–13% of the dry weight of barley (Bhatty et al. 1975). Major components of the hull include cellulose, hemicellulose, lignin and pectin (Bhatty 1993). Hull-less varieties have low fiber and high starch content compared to hulled varieties (Yang et al. 1997), as evidenced by increased digestibility and energy values for non-ruminant animals fed on hull-less barley (Beames et al. 1996). Crude fiber is the indigestible portion of the cell wall made up of lignin and fibrous carbohydrates (Perry et al. 1999). Both ADF and neutral detergent fiber (NDF) procedures are used to estimate crude fiber. ADF, the residue after refluxing with 0.5 M sulfuric acid and CTAB, comprises the crude lignin and cellulose fractions (McDonald et al. 1995). Environments and genotypes both affect barley ADF (Kong et al. 1995).

Feed efficiency in beef cattle fed barley-based diets declines as ADF content increases (Engstrom et al. 1992). Two-rowed barleys contain less ADF than six-rowed and therefore contain higher nutritional value than six-rowed varieties (Kong et al. 1995). Hull-less barley also has lower ADF than hulled barley (Kong et al. 1995; Zinn et al. 1996; Bowman et al. 2001). Lehman et al. (1995) reported that the absence of hull increased dry matter digestibility by 5.5% in a comparison among 16 hulled and six hull-less varieties. Low ADF content is desirable for ruminant feed but undesirable for non-ruminant feed and human nutrition. Rumen microorganisms produce cellulose enzyme that breaks down the chemical bonds in cellulose, making it digestible to the microbe and ruminant animal, while monogastric (nonruminant) animals do not have microbes in their single-stomach and, subsequently do not have the ability to digest fibrous content.

Feed, or non-malting, barley varieties have been developed which emphasize higher yield. To date, though, limited consideration has been given to feed quality traits. For this reason, as well as the lack of a premium for improved quality feed varieties in the marketplace, it has been difficult to develop screening parameters for feed quality-related traits.

Animal performance results from the interaction of various physical and chemical characteristics of barley. Barley feed quality is frequently measured by average daily gain, carcass grade at slaughter time, feed efficiency, digestibility and animal intake. Surber et al. (2000) used an alternative approach, predicting net energy content, feed efficiency and average daily gain of cattle fed barley using simpler chemical and physical parameters such as in situ dry matter digestibility (ISDMD), ADF, starch particle size and starch content. Adoption of these criteria, should allow progress in breeding for improved feed quality.

Ruminants, unlike non-ruminants, can survive on protein-free diets as long as they do contain a nitrogen source such as urea. This ability stems from the presence of microbes in their gastrointestinal tract, capable of synthesizing both essential and nonessential amino acids (Kellems and Church 2002; Bowman and Sowell 2002). Ruminant microorganisms benefit from the association through use of some of the amino acids for their own protein synthesis and energy requirements (Broderick et al. 1991; Russell et al. 1991). By optimizing protein supply to growing or lactating ruminants, the farmer can improve efficiency of protein utilization and feed intake (Cecava 1995).

In modern barley varieties, as in many other crops, genetic bottlenecks have resulted from focusing exclusively on selection of economic traits (Martin et al. 1991). QTL mapping and marker assisted selection methodologies have been used to successfully quantify the impact of genes affecting economic traits in barley, and should work equally well to map and analyze feed quality traits.

In order to address inbreeding depression for economic traits, several barley populations have been used in QTL detection studies including six-rowed × two-rowed (Kjaer and Jensen 1996; Jui et al. 1997; Marquez-Cedillo et al. 2000; Mesfin et al. 2003), hulled × hull-less (Heun 1992; Pecchioni et al. 1999) and feed × malt (Hayes et al. 1993). The *nud* locus controls adherent (*Nud*, hulled caryopsis) vs. non-adherent (*nud*, hull-less caryopsis) lemma and palea. The *nud* locus has been mapped to the long arm of 7H chromosome (Fedak et al. 1972; Becker and Heun 1995). Recently Taketa et al. (2008) used a positional cloning approach to clone the *nud* locus, and found that an ERF family transcription factor gene that regulates a lipid biosynthesis pathway controls the adhering hull.

Bowman et al. (2001) surveyed 1480 accessions of a spring barley core collection and identified several that had normal to high starch content, low fiber ADF and low ISDMD. One of these accessions is PI370970, a six-rowed hull-less landrace line from Switzerland. In this study, PI370970 was crossed to Valier, a two-rowed hulled barley variety, from which a population of recombinant inbred lines was developed.

Objectives of this study were to study the phenotypic variation of ADF, starch and protein content in a hulled  $\times$  hull-less, two-rowed  $\times$  six-rowed barley population, to map chromosomal locations affecting ADF, starch and protein contents, to study the genetic effects of detected QTL, and to independently confirm major QTL associated with low ADF using a second breeding population.

## Materials and methods

### Plant materials

A cross between Valier (PI610264) and a Swiss landrace line (PI370970) was made in the greenhouse and F<sub>1</sub> seeds were harvested. F<sub>2</sub> plants were advanced to the F<sub>5</sub> generation by single seed descent method (SSD) in the greenhouse. F<sub>5</sub>-derived families were grown out under field conditions. Valier is a two-rowed, hulled spring barley cultivar developed by the Montana agricultural experiment station (Blake et al. 2002). PI370970, a six-rowed hull-less line selected from the USDA barley's spring core collection, was chosen for this study based on its low ADF content, high starch, low ISDMD value and large particle size (Bowman et al. 2001).

One hundred sixteen F<sub>5</sub>-derived families, parents and check varieties were planted at the A.H. Post Research Farm (Amsterdam silt loam soil) near Bozeman, MT during 2002 and 2003 cropping seasons. Entries were grown under irrigated and rain-fed environments. At each environment, entries were arranged in a randomized complete block design with two replications. Entries were planted at a rate of approximately 30 seeds/m in single head row plots 2 m long and spaced 0.3 m apart. Plots were harvested by a Wintersteiger plot combine and cleaned using a de-awner and blower.

The confirmation population was a cross between Baronesse and PI370970 made in the greenhouse.

Baronesse is a hulled, two-rowed feed barley variety with high ADF. F<sub>1</sub> seeds were harvested and progeny advanced four generations by single seed descent in the greenhouse. F<sub>5</sub>-derived families were planted in the field at the A.H. Post Research Farm in summer 2003 with one replicate at a seeding rate of approximately 30 seeds/m in single head row plots 2 m long spaced 0.3 m apart. Entries were irrigated and harvested with a Wintersteiger plot combine.

### Chemical composition determination

Duplicate grain samples from each line were ground individually to pass through a 0.5 mm screen using an Udy-Cyclone Mill (Ft. Collins). To calculate the chemical composition of barley grains on a dry basis, dry matter content (DM) was determined following the AOAC 934.01 (Association of Official Analytical Chemists (AOAC) 1999) method. ADF content was determined following the Van Soest et al. (1991) method using an ANKOM 200 Fiber Analyzer (ANKOM Technology Crop, Fairport, NY). Starch content was determined following the Amyloglucosidase/ $\alpha$ -amylase method (McCleary et al. 1994). Protein content was determined based on N content ( $N \times 6.25$ ) using a Leco FP 528 protein analyzer (Leco Crop, St. Joseph, MI). Chemical composition from the present study and ISDMD and particle size (Abdel-Haleem et al. 2009; submitted) were used to identify net energy available for maintenance (NE<sub>m</sub>) using the following equation,  $NE_m = 1.87833 - 0.00794(\text{ISDMD}) + 0.00025097(\text{starch content})$ .

### Statistical analyses

An analysis of variance (ANOVA) was performed separately for each trait in each environment in a randomized complete block design. Analysis of data combined across environments was carried out using a split-plot design with environment as the main plot (Steel and Torrie 1980). Blocks and genotypes were treated as random effects in the combined analysis for environments (Statistical Analysis System, SAS institute 1990). Variance components were estimated using the restricted maximum likelihood (REML) method of SAS PROC MIXED.

Heritability of plot mean and family mean bases were estimated as  $h^2 = (\sigma_G^2 / (\sigma_G^2 + \sigma_{\text{GEN}}^2 + \sigma_e^2))$  and  $h^2 = \sigma_G^2 / ((\sigma_G^2 + (\sigma_{\text{GEN}}^2/e) + (\sigma_e^2/er)))$  respectively

(Nyquist 1991; Holland et al. 2003); where  $\sigma_G^2$  equaled the genetic variance among the entries,  $\sigma_{\text{GEN}}^2$  represented the variance of genetic  $\times$  environment interaction,  $\sigma_e^2$  the variance of experimental error,  $e$  the number of environments and  $r$  the number of replications. Because the data were unbalanced,  $e$  and  $r$  were computed as the harmonic mean of environments and total replications across all environments, respectively (Holland et al. 2003).

Simple correlation analysis was performed for chemical composition traits (ADF, starch and protein contents) and physical traits (ISDMD and particle size) using means over the environments and PROC CORR in SAS (statistical Analysis System, SAS institute 1990).

#### Map construction and segregation analysis

Three week old seedling leaf tissue from single  $F_5$  plant representing each RIL was harvested for DNA marker analysis. DNA isolation was performed according to the procedures of Dellaporta et al. (1983). Microsatellite primers (SSR) (Liu et al. 1996; Ramsay et al. 2000) and STS primers (Kleinhofs et al. 1993; Blake et al. 1996; Sayed-Tabatabaei et al. 1999; Kunzel et al. 2000) were screened to identify polymorphisms between parents. Fifty-two informative STS and SSR markers were selected to anchor the genome to other available barley linkage maps. The 25  $\mu$ l PCR reaction mix consisted of 2 ng of forward and reverse primers, 0.5 U of Taq DNA polymerase (Promega, Madison, WI.), 0.2 mM of each dNTPs, 1 X PCR buffer (50 mM KCL, 10 mM Tris-HCl, 1 g l<sup>-1</sup> Triton X-1000), 1.5–2.5 mM MgCl<sub>2</sub>, and 25 ng template DNA. PCR amplification consisted of an initial denaturation at 94°C for 3 min, followed by 40 step cycles as follows: denaturation at 94°C for 30 s, annealing for 30 s at 94°C, and elongation at 72°C for 20 s. A final elongation step at 72°C for 5 min was also performed. Amplification products from SSR and STS primer sets were separated on either 12% polyacrylamide or 1% agarose gels. STS loci that required digestion were sequenced using the ABI 377 automated DNA sequencer, and restriction-site allelic variants at STS loci were digested with 2 U of the appropriate restriction enzyme, and separated on a 10% polyacrylamide gel.

Amplification fragment length polymorphism (AFLP) analysis was done according to Vos et al. (1995) with modifications. A 15  $\mu$ l digestion reaction

mixture including 100 ng of genomic DNA was digested with 3 U of restriction enzymes EcoRI and HpaII for 2 h at 37°C, and the reaction terminated at 65°C for 10 min. The digested mixture was ligated with HpaII and EcoRI adapters in a 1  $\times$  ligation buffer (New England Biolabs, Boston, MA) and 1 U of T4 ligase (New England Biolabs, Boston, MA) in a total volume of 20  $\mu$ l at 37°C for 3 h. The pre-amplification reaction contained 1  $\mu$ l of ligation product in 30  $\mu$ l of 0.1 mM each dNTP, 1  $\times$  buffer (Promega) 25 nM MgCl<sub>2</sub> and 0.5 U Taq and 30 ng of HpaII and EcoRI linker primers (H0 and E0 primers) without selective bases. Selective amplification was carried out using primers having three selective nucleotides in order to reduce the complexity of the products. Combinations of 4 EcoRI + 3 primers and 8 HpaII + 3 primers were used. The selective amplification reaction mix included 1  $\mu$ l of pre-amplification product in 20  $\mu$ l of 0.1 mM dNTP, 1  $\times$  buffer (Promega), 25 nM MgCl<sub>2</sub>, 0.5 U Taq, 5 ng of an EcoRI fluorescently tagged primer and 30 ng of a HpaII primer. Thermocycling conditions were as described by Vos et al. (1995). AFLP products were separated and detected using 4% denaturing polyacrylamide gels containing 8 M urea and 1  $\times$  TBE at 250 V for 3 h on an ABI 377 automated DNA sequencer. Internal molecular weight standards (Genescan-500 ROX, Applied Biosystems) were loaded with each sample, permitting automated molecular weight estimations to be made for all AFLP fragments between 50 and 500 bases long. AFLP fragments were scored using Genographer (Benham et al. 1999). Each polymorphic AFLP was identified by the code referring to the combination of EcoRI + 3 and HpaII + 3 primers followed by the estimated molecular marker size of the DNA fragment.

Two morphological markers were scored, *Nud/nud* (hulled vs. hull-less caryopsis) and *vrs1/Vrs1* (six-rowed vs. two-rowed spike). The nomenclature of the morphological markers followed the description of Franckowiak (1997). Hordein polymorphisms were assayed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) from the mature ground single seed according to Blake et al. (1982). The B, C and D hordeins banding patterns were derived from the variation at Hor-2, Hor-1 and Hor-3 loci, respectively on chromosome 1H.

Segregation and linkage analyses was done using Joinmap 3.0 (Van Ooijen and Voorrips 2001). For each marker, a  $\chi^2$  test was conducted to identify the

markers showing segregation distortion. A minimum LOD score of 3.0 was used to assign markers to groups and Kosambi's mapping function was used to calculate the map distances.

### QTL analyses

An ANOVA was performed on all traits and environments in order to identify significant genotype and genotype  $\times$  environment (G $\times$ E) interactions. When genotype effects were significant, QTL analysis was performed using QTL Cartographer 2.5 (Wang et al. 2007). A series of 1000 permutations were run to determine the experiment-wise significant level at  $P = 0.05$  of LOD for the trait (Churchill and Doerge 1994). Composite Interval Mapping (CIM) was employed to detect QTLs and estimate the magnitude of their effects using Model 6 of the Zmapqtl program module (Jansen and Stam 1994; Zeng 1994). The genome was scanned at 2 cM intervals with the window size set at 10 cM. Cofactors were chosen using the forward-backward stepwise regression method. The percentage of phenotypic variance explained by specific QTL ( $R^2$  value) was determined at the peak of QTL position as determined by QTL cartographer. The total phenotypic variance including the all significant QTLs in the model was calculated using multiple intervals mapping (MIM) method of QTL cartographer (Kao et al. 1999). MIM was performed with the significant QTLs that identified by CIM model, and was applied to test QTL effects including additive and QTL  $\times$  QTL epistatic effects by simultaneous analysis of the QTLs in multiple regression models.

Major QTL detected for ADF in the mapping population Valier  $\times$  PI370970, were likewise studied in a Baronesse  $\times$  PI370970 population. The single marker *nud* was used within the QTL region to test the association between *nud* and ADF using Proc GLM (statistical Analysis System, SAS institute 1990), and to determine significant ( $P < 0.05$ ) effect of genotypic classes on ADF.

## Results and discussion

### Phenotypic variation

Protein, ADF and starch content of parents and RILs of the Valier  $\times$  PI370970 population are summarized

in Table 1. PI370970 had higher protein and starch and lower ADF contents than Valier across all environments. Transgressive segregation was observed for these traits. In combined environments, 41 genotypes had a lower ADF content than PI370970 and 22 genotypes had a higher ADF content than Valier (Table 1). For starch content, an equal number of positive and negative transgressive segregants were observed (Table 1). Genotype had a significant effect on protein content, the effect varied among environments, and again, both positive and negative transgressive segregants were observed. These results suggest the possibility to select for moderate/low ADF and protein content and high starch content for ruminants in crosses with traits as widely varying as in Valier  $\times$  PI370970.

It was predicted that hull-less genotypes would have less fiber and more starch contents than hulled genotypes, since the hull constitutes about 10% of the barley grain dry weight (Kong et al. 1995; Zinn et al. 1996; Yang et al. 1997; Bowman et al. 2001). The population was divided according to grain type into two subpopulations. Both were significantly different in ADF and starch contents (Table 2). Starch and ADF content means of the hull-less subpopulation were similar to PI370970, while the mean of the hulled subpopulation was similar to Valier (Table 2). Many studies have shown that two-rowed barley varieties contain more starch and less fiber than six-rowed varieties (Kong et al. 1995; Narasimhalu et al. 1995) and this may be the major reason for higher animal performance of two-rowed barley as compared to six-rowed barley (Boss and Bowman 1996; Ovenell-Roy et al. 1998).

In general, we found that two-rowed genotypes contain less ADF and more starch than six-rowed (Table 3). These differences were significant for starch content but not for ADF content. Using a doubled haploid population, Frégeau-Reid et al. (2001) reported the same results. Two-rowed genotypes contained more protein content than six rowed genotypes (Table 3). These results have been previously reported in other studies (Day and Dickson 1957; Hockett and Standridge 1976; McGuire and Hockett 1983; Marquez-Cedillo et al. 2000).

The population was sorted into four groups to study the interaction between head and grain types. For ADF, starch and protein contents, there were significant differences among these groups (Table 4).

**Table 1** Means, ranges and transgressive phenotypes of Valier, PI370970 and their RILs over rainfed, irrigated and combined environments for protein content, ADF, starch content and net energy available for maintenance (NE<sub>m</sub>)

Trait	Valier	PI370970	RILs				Means
			Positive trans <sup>a</sup>	Negative trans <sup>b</sup>	Min <sup>c</sup>	Max <sup>d</sup>	
Protein (%)							
Rainfed	16.76	20.05	22	9	15.42	22.01	18.93
Irrigated	15.35	18.37	15	4	14.69	20.49	17.20
Combined	16.06	19.21	21	6	15.35	20.92	18.12
ADF (%)							
Rainfed	5.31	2.20	32	17	1.20	7.70	3.63
Irrigated	5.70	2.90	32	25	1.22	9.79	4.13
Combined	5.50	2.55	41	22	1.35	8.65	3.89
Starch (%)							
Rainfed	50.67	53.15	18	26	40.43	57.96	49.85
Irrigated	49.06	53.87	16	45	40.31	57.87	49.93
Combined	49.87	54.33	29	29	42.14	56.44	49.90
NE <sub>m</sub> (Mcal/kg)							
Combined	2.377	2.521	2	14	2.153	2.569	2.327

<sup>a</sup> Positive transgressive genotypes with low protein content, low ADF, high starch content and high NE<sub>m</sub> than PI370970

<sup>b</sup> Negative transgressive genotypes with high protein content, high ADF, low starch content and low NE<sub>m</sub> than Valier

<sup>c</sup> Minimum value

<sup>d</sup> Maximum value

The two-rowed/hull-less and six-rowed/hull-less groups were lower in ADF and higher in starch content than other groups. Two-rowed/hull-less was not significantly different from two-rowed/hulled group; both groups were higher in yield over six-rowed (unpublished data).

Where in vivo evaluation of barley genotypes is costly, monetary considerations have limited our ability to test and understand parameters involved in animal performance variation. In order to address this issue, a combination of relatively inexpensive lab analyses such as ISDMD, ADF, starch content and particle size after dry rolling could be employed to estimate barley NE<sub>g</sub>, NE<sub>m</sub>, ADG and gain/feed (Surber et al. 2000). These measurements could predict the performance of cattle fed on high barley diets and improve selection criteria of feed quality breeding programs.

Our study indicated that NE<sub>m</sub> (net energy available for maintenance) was significantly affected by genotypes (Table 1). PI370970 was superior to Valier in NE<sub>m</sub>, and several transgressive segregants were produced (Table 1). Both head type and grain type had significant effects on NE<sub>m</sub> (Tables 2, 3 and 4).

Corn NE<sub>m</sub> is 2.33 Mcal/kg and is higher than barley (2.06 Mcal/kg) (NRC 1995). Transgressive progeny from our population had as much as 20% greater energy than corn. These results point to the potential such wide crosses represent in developing genotypes with high NE<sub>m</sub>.

ANOVA of ADF and starch content across all environments indicated that genotypes and environment had significant effects, while there was no G×E interaction for both traits (Table 5). Several studies indicated that ADF and starch content are affected by environment and genotype (Kong et al. 1995; Bowman et al. 2001; Fox et al. 2008). Molina-Cano et al. (1997) concluded that there is no G × E interaction for crude fiber and starch content, while significant line × location interaction was detected for ADF (Frègeau-Ried et al. 2001). Heritabilities were calculated based on plot-basis and family mean-basis for ADF, starch and protein contents. Heritability estimates for all environments were high for ADF content and moderate for starch content (Table 5). Molina-Cano et al. (1997) reported that the broad sense heritability of five varieties at six locations were high for ADF content (80%) and low for starch content (33%).



**Table 2** Means, ranges and standard deviations of the hulled and hull-less subpopulations of Valier × PI370970 population over combined environments for protein content, ADF content, starch content and net energy available for maintenance (NE<sub>m</sub>)

Trait	Min <sup>a</sup>	Max <sup>b</sup>	Means	σ <sup>c</sup>
Protein (%)				
Hulled	15.348	20.810	17.785**	1.271
Hull-less	15.393	20.713	18.525	1.409
ADF (%)				
Hulled	1.687	8.651	4.988**	1.261
Hull-less	1.338	6.899	2.443	1.095
Starch (%)				
Hulled	42.139	53.580	48.248**	2.794
Hull-less	47.603	56.438	52.020	2/368
NE <sub>m</sub> (Mcal/kg)				
Hulled	2.153	2.419	2.280**	0.057
Hull-less	2.223	2.569	2.398	0.074

\*, \*\* Significant of pairwise comparison between hulled and hull-less subpopulation at  $P < 0.05$  and  $P < 0.001$ , respectively

<sup>a</sup> Minimum value

<sup>b</sup> Maximum value

<sup>c</sup> Standard deviation

*ns* non significant

In general, lower heritability values were estimated using plots rather than family means. No G×E interaction and high heritability suggest that the genetic variation in this population is large and not influenced by environmental factors, and that few genes are responsible for the variation in ADF. Selection for low ADF content can begin early in breeding programs because of its high heritability. Fréreau-Reid et al. (2001) believes, however, that selection intensity for ADF should not be overly stringent early in the selection process.

The significant effect of environment, G×E interaction on protein and moderate heritability estimation suggests that protein content is a complex trait (Table 5). Moderate protein levels are required for ruminant feedlots, but high N in animal waste can result in environmental pollution. The chemical and physical nature of the diet affects both size and composition of the rumen microbial population (Kotarski et al. 1992) and can increase the nitrogen (N) that winds up in animal waste. High protein barley diets had a negative effect on starch digestibility of chicks and cocks (Almirall et al. 1995).

**Table 3** Means, ranges and standard deviations of the six-rowed and two-rowed subpopulations of Valier × PI370970 over combined environments for protein content, ADF content, starch content, net energy available for maintenance (NE<sub>m</sub>)

Trait	Min <sup>a</sup>	Max <sup>b</sup>	Means	σ <sup>c</sup>
Protein (%)				
Six-rowed	15.350	20.430	17.895*	1.312
Two-rowed	16.320	20.920	18.362	1.126
ADF (%)				
Six-rowed	1.500	8.650	3.985 <sup>ns</sup>	1.986
Two-rowed	1.340	7.150	3.757	1.446
Starch (%)				
Six-rowed	42.140	56.300	49.346*	3.461
Two-rowed	44.910	56.440	50.586	2.743
NE <sub>m</sub> (Mcal/kg)				
Six-rowed	2.230	2.570	2.362**	0.081
Two-rowed	2.150	2.460	2.295	0.075

\*, \*\* Significant of pairwise comparison between six-rowed and two-rowed subpopulation at  $P < 0.05$  and  $P < 0.001$ , respectively

<sup>a</sup> Minimum value

<sup>b</sup> Maximum value

<sup>c</sup> Standard deviation

*ns* non-significant

**Table 4** Means of the four classes subpopulations of Valier × PI370970 over combined environments for ADF, protein, starch content and net energy available for maintenance (NE<sub>m</sub>)

	Protein	ADF	Starch	NE <sub>m</sub>
Two-rowed/hulled	18.13 <sup>ab</sup>	4.42 <sup>b</sup>	49.65 <sup>b</sup>	2.246 <sup>c</sup>
Two-rowed/hull-less	18.69 <sup>a</sup>	2.47 <sup>c</sup>	52.44 <sup>a</sup>	2.350 <sup>b</sup>
Six-rowed/hulled	17.45 <sup>b</sup>	5.65 <sup>a</sup>	46.58 <sup>c</sup>	2.298 <sup>c</sup>
Six-rowed hull-less	18.36 <sup>a</sup>	2.38 <sup>c</sup>	51.97 <sup>a</sup>	2.422 <sup>a</sup>

Means followed by different letters are significantly different by LSD test at  $P < 0.001$

A simple correlation analysis was carried out to understand the relationship among starch, ADF and protein content with other feed quality traits (Table 6). A highly negative correlation was estimated between starch and ADF content (−0.773). Protein content correlated negatively with ADF and starch contents, in contrast with results reported by Fréreau-Reid et al. (2001), who detected a positive correlation between protein content and ADF, but only in a two-rowed subpopulation. These results

**Table 5** Mean squares for genotypes ( $MS_G$ ), environments ( $MS_E$ ), genotype  $\times$  environment ( $MS_{GE}$ ) and error ( $MS_{ER}$ ), genetic ( $\sigma_G^2$ ) and genotype  $\times$  environment ( $\sigma_{GE}^2$ ) variance components and heritabilities for protein, ADF and starch content at rainfed, irrigated and combined environments

Trait	$MS_G$	$MS_E$	$MS_{GE}$	$MS_{ER}$	$\sigma_G^2$	$\sigma_{GE}^2$	$h^2 \pm SE^a$	$h^2 \pm SE^b$
Protein								
Rainfed	3.73**	nd	nd	0.56	1.75	nd	75.85 $\pm$ 0.04	85.01 $\pm$ 0.03
Irrigated	2.97**	nd	nd	0.52	1.29	nd	71.70 $\pm$ 0.05	82.82 $\pm$ 0.03
Combined	5.18**	255.45**	1.29**	0.54	1.06	0.43	52.29 $\pm$ 0.06	73.13 $\pm$ 0.05
ADF								
Rainfed	5.22**	nd	nd	0.32	2.48	nd	88.56 $\pm$ 0.02	93.86 $\pm$ 0.01
Irrigated	7.75**	nd	nd	1.06	3.37	nd	75.93 $\pm$ 0.04	86.21 $\pm$ 0.04
Combined	11.98**	26.87**	0.81 <sup>ns</sup>	0.69	2.85	0.06	79.02 $\pm$ 0.03	93.20 $\pm$ 0.01
Starch								
Rainfed	22.49**	nd	nd	13.03	4.47	nd	75.93 $\pm$ 0.04	86.21 $\pm$ 0.03
Irrigated	29.95**	nd	nd	7.30	11.39	nd	60.99 $\pm$ 0.06	75.61 $\pm$ 0.04
Combined	40.75**	0.36 <sup>ns</sup>	11.47 <sup>ns</sup>	10.13	7.39	0.52	40.42 $\pm$ 0.05	71.70 $\pm$ 0.05

\*\* Significant at  $P < 0.001$ <sup>a</sup> Heritability on plot-basis<sup>b</sup> Heritability on family mean-basis

SE standard error, ns non-significant, nd not determined

**Table 6** Correlation coefficients for protein content (CP), acid detergent fiber (ADF), starch content (Sch), in situ dry matter digestibility (ISDMD), particle size (PS) and net energy available for maintenance ( $NE_m$ ) of Valier  $\times$  PI370970 population

	CP	ADF	Sch	$NE_m$
CP	–	–	–	0.172*
ADF	–0.499**	–	–	–0.644**
Sch	0.247**	–0.773**	–	0.700**
ISDMD	–0.109	0.109	0.319**	–0.453**
PS	–0.120	0.219**	–0.382**	0.222**

\* Significant at  $P < 0.05$ , \*\* Significant at  $P < 0.01$ 

suggest that selection for low ADF will increase starch content and reduce protein content.

Protein content correlated negatively at a non-significant level with ISDMD and particle size. Similarly, ADF showed a positive correlation with ISDMD. ADF was positively correlated significantly with particle size.

The  $NE_m$  correlated negatively with ADF and ISDMD, and positively with starch content and particle size (Table 6). This indicates that high starch and low ADF content will decrease non-digestible components, and will result in high energy available

for maintenance and weight gain. It further suggests that low ISDMD and large particle size will inhibit the digestive rate in the rumen and thus increase energy available to the animal.

Hunt (1996) indicated that low lignin and cellulose reduced ADF content, which in turn increased the average daily gain. A negative relationship between total protein content and ISDMD suggests a relationship between protein quantity/quality and dry matter disappearance. Starch granules in the endosperm of cereal grains are embedded to varying degrees in a protein matrix. The mode of enzymatic attack depends on particular microorganisms and grain type (McAllister et al. 1993; Yanke et al. 1993). McAllister et al. (1993) suggested that proteolytic activity per unit of bacterial biomass may be influenced more by specific amino acids than by total protein in the diet. Black (2001) indicated that the susceptibility of the protein matrix to proteases varies among barley varieties. Brennan et al. (1996) found that starch-protein binding matrix is stronger in the poor malting quality varieties more than good malting quality. While information on differences in protein matrices between barley varieties is still lacking, breeding for a highly digestible protein matrix could increase available energy from barley. Moderate to low



protein in combination with low ADF, high starch, low ISDMD and large particle size would be expected to improve the selection process for better feed barley.

### QTL analyses

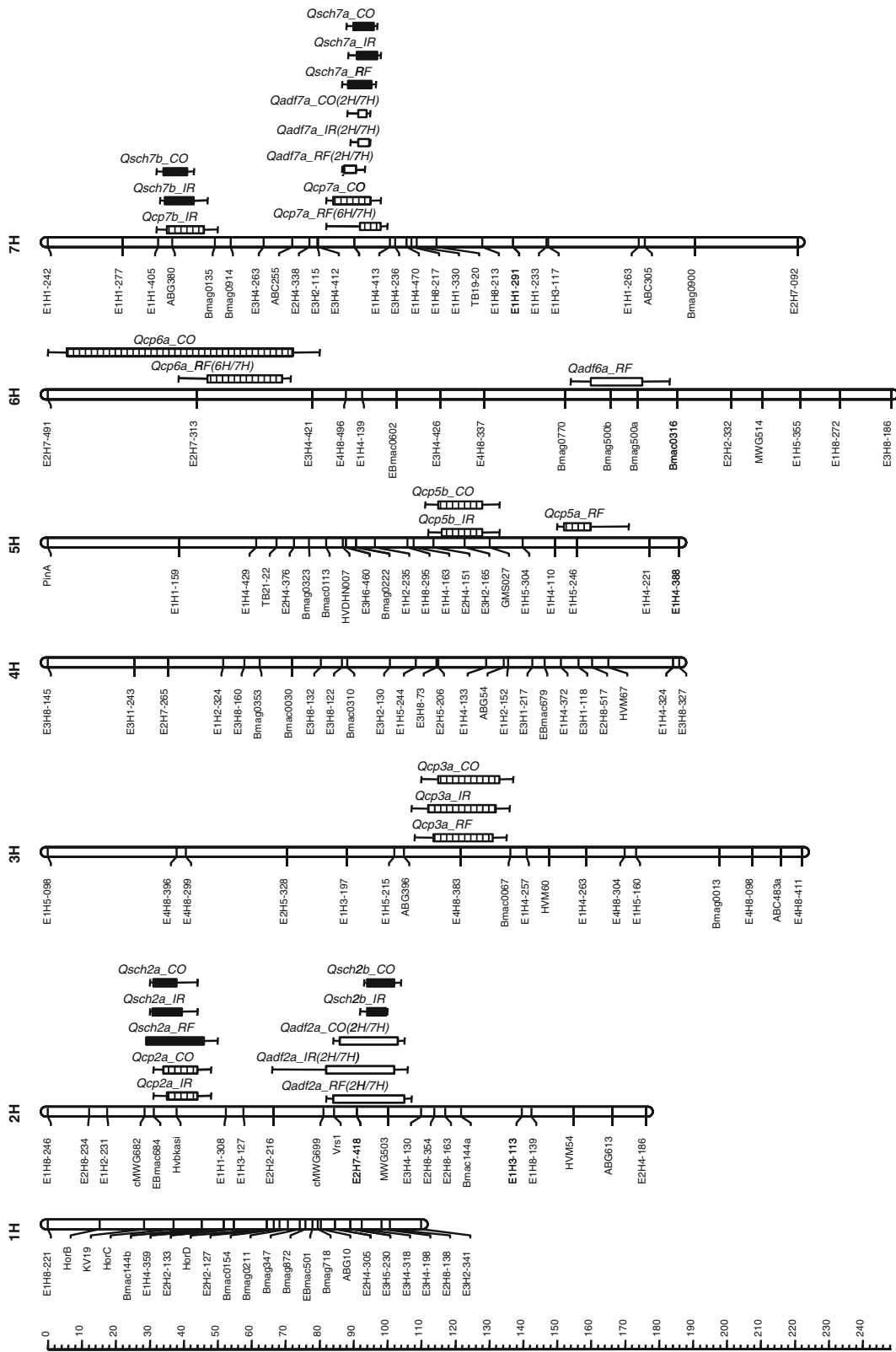
To determine chromosomal locations controlling variation in ADF, starch and protein contents, a linkage map was constructed using 32 SSR, 15 STS, three hordein proteins, 98 AFLP markers and two morphological markers (Fig. 1). Composite interval mapping (CIM) and multiple interval mapping (MIM) methods were employed to detect QTL and QTL  $\times$  QTL interactions using data generated from rain-fed, irrigated and combined data environments. A major QTL, *Qadf7a*, that flanked by *nud* locus, explained 66%, 63% and 70% of the phenotypic variation of ADF content on rain-fed, irrigated and combined environments (Table 7) respectively. *Qadf2a*, on chromosome 2H, controlled 5% of the ADF content variation (Table 7), while *Qadf6a* was detected only in rain-fed and irrigated environments. Our model explained 74–80% of ADF variation in this population. *Qadf2a* and *Qadf7a* QTLs showed additive  $\times$  additive interaction, accounting for 4% of the variation. Previous studies reported various ADF QTL on chromosomes 1H, 2H and 4H (Ullrich et al. 1996; Han et al. 2003). One QTL was located near molecular marker (MWG503) on chromosome 2H and explained 14% of the variation in a Steptoe  $\times$  Morex population (Han et al. 2003). In our study, a QTL on chromosome 2H (*Qadf2a*) flanked by MWG503 was detected. The differences in the QTL detected in our study compared with those in other reports could be related to different QTL detection methods. Another possibility is that Steptoe and Morex are hulled barley varieties, and other studies have noted the importance of the presence of the hull on ADF variation (Kong et al. 1995; Zinn et al. 1996; Bowman et al. 2001).

*Qsch7a* was the major starch QTL and explained 40% of the total phenotypic variation. *Qsch7a* was detected in rain-fed, irrigated as well as combined environments (Table 7). *Nud* flanked this QTL on chromosome 7H. An additional two QTLs, *Qsch2b* on chromosome 2H and *Qsch7b* on chromosome 7H, were detected in both irrigated and combined environments (Table 7). Total phenotypic variation for

**Fig. 1** QTL locations for acid detergent fiber (ADF) (white bar), protein (CP) (diagonal bar), starch content (SCH) (black bar) for Valier  $\times$  PI370970 population over rainfed (R), irrigated (IR) and combined environments (CO). QTL nomenclature is in the form of Q-trait-chromosome-number-position\_environment, QTLs that showed QTL  $\times$  QTL interaction are followed by chromosome numbers that showed the epistatic interaction between parentheses. Length of QTL bar indicates the LOD-1 confidence interval and QTL line is extended to LOD-2 confidence interval drops from the maximum likelihood value

starch content ranged from 49–69%. No significant QTL  $\times$  QTL interaction was detected in the present study, as compared with Frégeau-Reid et al. (2001), who detected additive  $\times$  additive epistasis for this trait. In a Steptoe  $\times$  Morex population, two QTLs controlling 21% of the total variation were identified (Ullrich et al. 1996), which might possibly have resulted from the relatively low starch content of the parents.

The overlap between *Qsch7a* and *Qadf7a* (Table 7; Fig. 1) and a highly significant correlation between starch and ADF contents suggests either the presence of tight linkage or pleiotropic effect between these traits on *nud* locus. Both head type and grain type morphology are known to affect several quantitative traits. *Nud* directly impacts grain fiber content, and therefore starch and protein percentage. This report suggests that the easiest way to improve NEm is to develop hullless feed barley varieties. The fundamental problem with this approach is that current hullless barley varieties provide higher ISDMD than their covered counterparts. This leads to bloating and acidosis. In order to deploy hullless feed barley varieties we must identify germplasm with significantly reduced ISDMD. Several protein QTLs have been detected in different populations on the seven barley chromosomes (Oziel et al. 1996; Hayes et al. 1997; Larson et al. 1996; Mather et al. 1997; Marquez-Cedillo et al. 2000). In Valier  $\times$  PI370970 population, we detected QTL associated with protein content on chromosomes 2H, 3H, 5H, 6H and 7H that explained 45–65% of the total phenotypic variation (Table 7). In combined environments, 2 major QTL on chromosomes 3H and 7H, *Qcp3a* and *Qcp7a*, accounted for 30% of total variation in protein content. *Qcp3a* was flanked with E4h8-383 on chromosome 3H, and was detected in all environments, while *Qcp7a* was flanked by the *nud* locus on 7H and was detected in the rain-fed and



**Table 7** QTLs for protein content (CP), acid detergent fiber (ADF) and starch content identified by composite interval mapping (CIM) and multiple interval mapping (MIM) for Valier × PI370970 population over rainfed, irrigated and combined environments

Trait	QTL	Chrom name	Nearest marker	QTL position <sup>a</sup>	LOD score <sup>b</sup>	Genetic effects <sup>c</sup>		$R^{2d}$	Total $R^{2e}$	Favorable allele <sup>f</sup>
						<i>a</i>	<i>eps</i>			
<i>CP (%)</i>										
Rain-fed	<i>Qcp3a</i>	3H	E4H8-383	123.51	2.51	0.603	–	15	64.1	PI370970
	<i>Qcp5a</i>	5H	E1H5-246	153.31	2.39	–0.449	–	9.7		Valier
	<i>Qcp6a</i>	6H	E2H7-313	57.91	2.98	0.548	–	14.9		PI370970
	<i>Qcp7a</i>	7H	<i>nud</i>	87.71	3.62	–0.266	–	5.8		Valier
	<i>Qcp6a</i> × <i>Qcp7a</i>					–	0.532	15.8		
Irrigated	<i>Qcp2a</i>	2H	Hvbkasi	37.91	4.03	0.471	–	10.7	44.6	PI370970
	<i>Qcp3a</i>	3H	E4H8-383	125.51	3.02	0.464	–	12.3		PI370970
	<i>Qcp5b</i>	5H	E3H2-165	122.81	2.47	0.374	–	7.5		PI370970
	<i>Qcp7b</i>	7H	ABG380	40.61	2.09	–0.427	–	12.5		Valier
Combined	<i>Qcp2a</i>	2H	Hvbkasi	37.91	3.64	0.385	–	7.1	60.6	PI370970
	<i>Qcp3a</i>	3H	E4H8-383	123.51	4.49	0.494	–	13.6		PI370970
	<i>Qcp5b</i>	5H	E3H2-165	122.81	2.83	0.37	–	6.5		PI370970
	<i>Qcp6a</i>	6H	E2H7-313	55.91	2.23	0.43	–	10.6		PI370970
	<i>Qcp7a</i>	7H	<i>nud</i>	87.71	3.94	–0.463	–	14.7	Valier	
<i>ADF (%)</i>										
Rainfed	<i>Qadf2a</i>	2H	MWG503	95.11	2.24	–0.302	–	2.9	80.2	Valier
	<i>Qadf6a</i>	6H	Bmag500b	165.81	2.63	0.265	–	4.5		PI370970
	<i>Qadf7a</i>	7H	<i>nud</i>	92.31	24.42	1.324	–	66.5		PI370970
	<i>Qadf2a</i> × <i>Qadf7a</i>						–0.267	4.5		
Irrigated	<i>Qadf2a</i>	2H	MWG503	93.11	3.6	–0.514	–	5.6	74.6	Valier
	<i>Qadf7a</i>	7H	<i>nud</i>	92.31	16.06	1.585	–	63.2		PI370970
	<i>Qadf2a</i> × <i>Qadf7a</i>					–	–0.3	4.2		
Combined	<i>Qadf2a</i>	2H	MWG503	95.11	3.03	–0.432	–	5.1	79.0	Valier
	<i>Qadf7a</i>	7H	<i>nud</i>	92.31	19.1	1.472	–	69.5		PI370970
	<i>Qadf2a</i> × <i>Qadf7a</i>					–	–0.26	3.9		
<i>Starch (%)</i>										
Rainfed	<i>Qsch2a</i>	2H	Hvbkasi	36.2	3.66	0.816	–	5.9	49.3	Valier
	<i>Qsch7a</i>	7H	<i>nud</i>	93.3	12.74	–2.288	–	44.2		PI370970
Irrigated	<i>Qsch2a</i>	2H	Hvbkasi	35.2	3.66	1.315	–	11.8	80.2	Valier
	<i>Qsch2b</i>	2H	MWG503	97.11	7.44	2.003	–	23		Valier
	<i>Qsch7b</i>	7H	ABG380	38.61	2.61	0.626	–	4.9		Valier
	<i>Qsch7a</i>	7H	<i>nud</i>	93.31	14.51	–2.626	–	39.4		PI370970
Combined	<i>Qsch2a</i>	2H	Hvbkasi	35.21	5.24	1.032	–	11.3	76.8	Valier
	<i>Qsch2b</i>	2H	MWG503	97.11	5.35	1.098	–	12.4		Valier
	<i>Qsch7b</i>	7H	ABG380	38.61	2.61	0.558	–	4.5		Valier
	<i>Qsch7a</i>	7H	<i>nud</i>	93.3	19.23	–2.266	–	47.6		PI370970

<sup>a</sup> QTL position in cM starts from the top of the designed chromosome

<sup>b</sup> LOD is the log-likelihood at QTL peak position, where LOD significance was based on 1,000 permutations and type error I with  $P < 0.05$

<sup>c</sup> Additive (*a*) and QTL × QTL epistatic (*eps*) effects were calculated following multiple intervals mapping (MIM) by simultaneous analysis of the QTLs in multiple regression models

<sup>d</sup> Proportion of phenotypic variance explained by specific QTL

<sup>e</sup> Total phenotypic variances that explained by the model

<sup>f</sup> Favorable alleles were assigned according to the traits, where lower ADF, higher starch and lower protein content are preferred for barley feed

**Table 8** Mean values for ADF for parental and genotypes classes, and percent of variations explained by *nud* loci for Baronesse × PI370970 validation population

Population	ADF (%)	SE <sup>a</sup>	Pr > F	R <sup>2b</sup>
Baronesse × PI37097	3.80	1.196	<0.0001	64.77
Baronesse ( <i>Nud</i> )	5.32	–	–	–
PI370970 ( <i>nud</i> )	2.01	–	–	–
Hulled ( <i>Nud</i> )	5.10	1.466	–	–
Hull-less ( <i>nud</i> )	1.81	0.574	–	–

<sup>a</sup> Standard deviation

<sup>b</sup> Calculated by  $R^2 \times 100$

combined environments only. Another QTL detected in irrigated and combined environments was *Qcp2a*, flanked with Hvbkasi on chromosome 2H. This QTL, which had a significant impact on grain starch and protein content, was detected in a Lewis × Clark population (See et al. 2002). Interestingly, it was adjacent to the photoperiod response gene *Ppd-H1*.

#### Confirmation of the ADF-QTL

Prior to using newly discovered QTL for breeding programs, it is important to understand three factors: (i) population structure and population size, (ii) environmental effects and G×E interactions and (iii) accurate phenotypic estimation of the trait of interest. Major QTL that control ADF and starch contents variation were detected near the *nud* locus on chromosome 7H in the Valier × PI370970 population. In order to confirm *Qafd7a* and the effect of the *nud* locus on ADF variation, it was remapped in a Baronesse × PI370970 population. Both mapping and confirmation populations were developed using PI370970 as the source for low ADF and high starch content. An ANOVA analysis of the confirmation population indicated that hull-less and hulled subpopulations were statistically distinct. Single marker analysis indicated that the *nud* locus controls about 60% of ADF variation (Table 8). Data from both the mapping and confirmation populations suggested that crossing hulled × hull-less parents allowed for highly variable expression of QTL-ADF.

In conclusion, we report here the results of QTL mapping of traits related to barley feed quality such as ADF, starch and protein contents using a recombinant inbred line population derived from a cross between high ADF and low starch contents cultivar and low

ADF and high starch contents landrace line. Our study indicated that head and grain types had a significant effect on ADF, starch, protein content and net energy available for maintenance (predicated animal performance). The two-rowed genotypes have lower ADF and higher starch content than six-rowed genotypes, and are the preferred barley for the arid Northern Plains. Two-rowed/hull-less genotypes had lower ADF and higher starch contents than two-rowed/hulled genotypes. Two major QTLs linked to the *nud* locus on chromosome 7H were found to affect ADF and starch content. These results were confirmed in a separate genetic background. Bowman et al. (2001) reported that selection of barley for low ISDMD, low ADF content, high starch content and large particle size should allow progress in breeding for improved feed quality. The presence of transgressive segregants and high/moderate heritabilities indicated that this type of cross could be employed to create an enhanced genetic base for ADF, starch, ISDMD and protein content variation. This goal might be achieved by crossing parents having widely divergent germplasm and selecting genotypes with low ADF and high starch content during early generations of breeding.

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