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Title	QTL analysis and comparative genomics of herbage quality traits in perennial ryegrass (<i>Lolium perenne</i> L.)
Author(s)	Cogan, N. O. I.; Smith, K. F.; Yamada, T. et al.
Citation	TAG Theoretical and Applied Genetics, 110(2), 364-380 https://doi.org/10.1007/s00122-004-1848-9
Issue Date	2005
Doc URL	https://hdl.handle.net/2115/994
Rights	The original publication is available at www.springerlink.com
Type	journal article
File Information	TAG110-2.pdf



1 **QTL analysis and comparative genomics of herbage**
2 **quality traits in perennial ryegrass ([Lolium perenne](#)**
3 **L.)**

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1 **Abstract**

2 Genetic control of herbage quality variation was assessed through the use
3 of the molecular marker-based reference genetic map of perennial
4 ryegrass ([Lolium perenne](#) L.). The restriction fragment length
5 polymorphism (RFLP), amplified fragment length polymorphism (AFLP)
6 and genomic DNA-derived simple sequence repeat (SSR)-based
7 framework marker set was enhanced with RFLP loci corresponding to
8 genes for key enzymes involved in lignin biosynthesis and fructan
9 metabolism. Quality traits such as crude protein (CP) content, estimated [in](#)
10 [vivo](#) dry matter digestibility (IVVDMD), neutral detergent fibre (NDF)
11 content, estimated metabolisable energy (EstME) and water soluble
12 carbohydrate (WSC) content were measured by near infra-red reflectance
13 spectroscopy (NIRS) analysis of herbage harvests. Quantitative trait locus
14 (QTL) analysis was performed using single marker regression, simple
15 interval mapping and composite interval mapping approaches, detecting a
16 total of 42 QTLs from six different sampling experiments varying by
17 developmental stage (anthesis or vegetative growth), location or year.
18 Coincident QTLs were detected on linkage groups (LGs) 3, 5 and 7. The
19 region on LG3 was associated with variation for all measured traits across
20 various experimental datasets. The region on LG7 was associated with
21 variation for all traits except CP, and is located in the vicinity of the lignin
22 biosynthesis gene loci *xlpomt1* (caffeic acid-O-methyltransferase), *xlpccr1*
23 (cinnamoyl CoA-reductase) and *xlpssrcad2.1* (cinnamyl alcohol
24 dehydrogenase). Comparative genomics analysis of these gene classes
25 with wheat ([Triticum aestivum](#) L.) provides evidence for conservation of
26 gene order over evolutionary time and the basis for cross-specific genetic
27 information transfer. The identification of co-location between QTLs and
28 functionally-associated genetic markers is critical for the implementation of
29 marker-assisted selection programs and for linkage disequilibrium studies,
30 which will enable future improvement strategies for perennial ryegrass.

31

32 **Keywords:** *Perennial ryegrass* *Genetic map*
33 *Herbage quality* *Quantitative trait locus*
34 *Functionally-defined gene* *Lignin*

1 **Introduction**

2 The composition of cell walls, particularly the content and cross-linking of
3 lignin, is an important determinant of herbage digestibility (Buxton and
4 Russell 1988), while the biosynthesis of soluble oligosaccharides such as
5 fructans is of key importance for energy provision to the grazing animal
6 (Michell 1973; Jones and Roberts 1991). The genetic control of nutritive
7 value parameters in pasture species has been reviewed (e.g. Ulyatt 1981;
8 Stone 1994; Casler 2001), and genetic variation for specific traits has been
9 established. Digestibility is generally considered to be the most important
10 temperate grass nutritive value trait for either live-weight gain (Wheeler
11 and Corbett 1989) or dairy production (Smith et al. 1997). Deliberate
12 attempts to improve dry matter digestibility (DMD) in forage crop species
13 have led to rates of genetic gain in the range of 1 - 4.7% per annum as a
14 proportion of the initial population means (Casler 2001). Progress in
15 simultaneous improvement of yield and DMD in forage grasses has,
16 however, been variable (Wilkins and Humphreys 2003).

17 Forage quality may be directly evaluated by feeding trials, but this
18 approach is costly and limited for small quantities of herbage from
19 breeding experiments. Indirect methods of assessment include [in vitro](#)
20 digestibility with rumen liquor (Menke et al. 1979; Tilly and Terry 1963),
21 enzymatic digestion (De Boever et al. 1986) and chemical analysis of
22 cellular components (van Soest 1963). The development of near infra-red
23 reflectance spectroscopy (NIRS) analysis for prediction of forage quality
24 has facilitated rapid and non-destructive evaluation of samples from plant
25 breeding programs. NIRS has been used to develop calibrations to predict
26 a wide range of forage quality traits (Marten et al. 1984; Smith and Flinn
27 1991) including crude protein (CP) content, estimated [in vivo](#) dry matter
28 digestibility (IVVDMD), neutral detergent fibre (NDF) content (Smith and
29 Flinn 1991) and water-soluble carbohydrate (WSC) content (Smith and
30 Kearney 2000) in perennial ryegrass. NIRS estimates of DMD and related
31 nutritive value traits have been reported in a range of forage systems (e.g.
32 Carpenter and Casler 1990; Hopkins et al. 1995, Smith et al. 2004).

33 The reference genetic map for perennial ryegrass based on RFLP,
34 AFLP and SSR loci (Jones et al. 2002a,b) provides the basis for the

1 genetic dissection of phenotypic traits that vary in the mapping population.
2 QTLs for a number of traits related to vegetative and reproductive
3 morphogenesis, reproductive development and winter hardiness have
4 already been identified (Yamada et al. 2004). The framework marker set,
5 that is dominated by anonymous and non-genic genetic markers, may be
6 selectively enhanced with functionally-associated genetic markers based
7 on expressed sequences (Kurata et al. 1994; Chao et al. 1994; Schneider
8 et al. 1999; Tanksley et al. 1992). The genetic map assignment of loci
9 detected by genes associated with specific biochemical pathways permits
10 evaluation of co-location between such loci and QTLs for putatively
11 correlated traits. A functionally-associated marker-based genetic map of
12 potato (Chen et al. 2001) containing genes involved in carbohydrate
13 metabolism and transport has been used to detect co-locations with QTLs
14 for tuber starch content. Similar studies have been performed with specific
15 functionally-defined genes for traits such as disease resistance, grain
16 quality attributes, secondary metabolite biosynthesis and flowering time
17 across a range of crop species (Faris et al. 1999; Francki et al. 2004; Li et
18 al. 2004; Pflieger et al. 2001; Huh et al. 2001; Lagercrantz et al. 1996). For
19 nutritive quality traits in grass herbage, genes involved in lignin and fructan
20 metabolism provide primary candidates for analysis. Perennial ryegrass
21 cDNAs encoding enzymes involved in lignin biosynthesis (Heath et al.
22 1998; Heath et al. 2002; Lynch et al. 2002; McInnes et al. 2002) and
23 fructan metabolism (Lidgett et al. 2002; Johnson et al. 2003; Chalmers et
24 al. 2003) have been isolated and characterised. Genetic dissection of
25 herbage quality characters is consequently accessible to both anonymous
26 and functionally-associated marker systems.

27 Comparative genetic mapping in perennial ryegrass based on
28 heterologous RFLP anchor probes revealed conserved syntenic
29 relationships between the genome of perennial ryegrass and those of
30 other Poaceae species (Jones et al. 2002a). Similarities in genetic map
31 structure were particularly evident with the Triticeae cereals, such that
32 each perennial ryegrass LG showed a predominant correspondence to
33 one of the homoeologous groups of wheat and barley. The development of
34 comparative genomics analysis based on sequence comparison and

1 ortholocus prediction between Poaceae genomes has become possible
2 through the provision of large expressed sequence tag (EST) collections
3 for several species and draft genome sequences for the grass model
4 species, rice (Goff et al. 2002; Yu et al. 2002). The locations of mapped
5 functionally-defined genes in a species such as perennial ryegrass may be
6 compared to those of putative ortholoci in rice through sequence
7 alignment with map-ordered bacterial artificial chromosome (BAC) clones
8 (Chen et al. 2002). Equivalent ortholocus analysis in wheat may be
9 performed through the mapping of representative ESTs from contigs and
10 singletons to regions based on deletion bins (Endo and Gill 1996; Qi et al.
11 2003; Sorrells et al. 2003). The grasses of the Poeae tribe, including the
12 [Lolium](#) genus, are more closely allied to the cereals of the Triticeae tribe
13 within the Pooideae sub-family of the Poaceae than to the Oryzeae
14 (Soreng and Davis 1998). This close taxonomic affinity suggests that
15 comparative genomics analysis between the Poeae and the Triticeae
16 tribes may prove particularly effective for the identification of common
17 genomic structures, gene orders and orthologous QTL locations.

18 The aim of this study was to determine the genetic control of herbage
19 quality through the use of data from multiple phenotypic trials, and to
20 identify QTL-linked molecular marker loci suitable for selection
21 experiments. A number of genetically mapped lignin biosynthetic genes
22 have been evaluated for coincidence with QTL-containing regions.
23 Comparative genomics analysis with wheat has been used to explore the
24 genomic distribution and evolution of genes for lignin biosynthesis.

1 **Materials and Methods**

2

3 **Plant materials**

4 The p150/112 reference genetic mapping population was derived from a
5 pair-cross between a multiply heterozygous plant as pollinator and a
6 doubled haploid (DH) as the female parent (Bert et al. 1999; Jones et al.
7 2002a,b). The cross was generated at the Institute of Grassland and
8 Environmental Research (IGER), Aberystwyth, UK, and clonal replicates of
9 up to 183 progeny individuals and the heterozygous parent were
10 distributed to International [Lolium](#) Genome Initiative (ILGI) participant
11 laboratories for genetic and phenotypic analyses. The DH genotype
12 (DH290) did not survive and was consequently not available for
13 phenotypic analysis.

14 Clonal individual plants were grown in small pots (1/10,000 a),
15 either in glasshouses at the Yamanashi Prefectural Dairy Experiment
16 Station (YPDES), Nagasaka, Japan ((35°49' N, 138°22' E) and the
17 National Agricultural Research Centre for Hokkaido Region (NARCH),
18 Sapporo, Japan (43°00' N, 141°25' E), or in a nursery area outside the
19 glasshouse at NARCH. For the sampling of material at reproductive
20 maturity in the glasshouse, vernalisation was performed during winter by
21 setting the temperature at $7.5 \pm 2.5^{\circ}\text{C}$.

22 Samples were prepared for herbage quality analyses from
23 individual plants at six different times or locations. In 1998 and 1999,
24 samples were taken from plants grown at YPDES with a stubble height of
25 5 cm on the same June day in each year. The potted plants had previously
26 been cut back at intervals of three weeks duration during the spring. The
27 samples contained leaves with stems. For plants grown at NARCH, the
28 growth stage of the plants (vegetative or reproductive) was considered
29 during sampling. Material was collected at heading time (May or June) for
30 glasshouse-grown plants in 2002 and plants grown in the nursery from
31 April in 2002. The samples were taken from each plant at the individual
32 time of heading at the first cut of the season. Material was collected at the
33 vegetative growth stage on the same late August day in each year for
34 glasshouse-grown plants in both 2001 and 2002. The leafy plants were

1 again sampled at 5 cm stubble height. Tissue samples were placed in
2 paper bags and dried at 60°C. Dried samples were ground through the 1
3 mm screen of a cyclone mill.

4

5 **Near infra-red reflectance spectroscopy analysis**

6 The ground herbage samples were scanned using an NIRSystems Model
7 5000 scanning monochromator connected to an IBM-compatible personal
8 computer. Infracsoft International (Port Matilda, PA, USA) software was
9 used during NIRS data collection and manipulation. Absorbances were
10 measured, as $\log_{10}(1/\text{reflectance}) = \log(1/R)$, at 2 nm intervals throughout
11 the near infra-red region (1100-2500 nm). Samples were scanned twice
12 and the spectra were stored as the mean of these 2 samples.

13 NIRS spectra were transformed by a mathematical treatment
14 designated as 2,5,5,1 (Windham et al. 1989) prior to the development of
15 NIRS equations. The first number in this formula denotes that the second
16 derivative of the $\log_{10}(1/R)$ spectrum was taken, the second denotes the
17 segment gap over which the derivative was calculated, and the third and
18 fourth are the number of data points used during smoothing of the
19 spectrum (Williams 1987). Stepwise multiple linear regression (SMLR)
20 PLS techniques (Shenk and Westerhaus 1991) were then used to develop
21 NIR calibration equations for each constituent from the subset.

22

23 **Statistical analysis of data**

24 Analysis of variation was performed using GenStat for Windows, 6th
25 Edition (www.vsn-intl.com), to identify significant differences between
26 genotypes and replicate structure for all analysed traits.

27

28 **QTL analysis**

29 A framework set of genetic markers from the p150/112-based reference
30 map (Jones et al. 2002a), including the majority of the heterologous RFLP
31 loci, was combined with the perennial ryegrass SSR locus data (Jones et
32 al. 2002b) to produce a composite dataset for QTL analysis of the
33 phenotypic data. Following genetic map construction using MAPMAKER
34 3.0, a sub-set of marker loci was selected to provide even coverage of the

1 genome with marker intervals of approximately 5 cM, and consensus map
2 distances were subsequently used. Single marker regression (SMR) was
3 initially employed to identify significant variation associated with selected
4 genetic markers. Simple interval mapping (SIM: Lander and Botstein 1989,
5 Haley and Knott 1992) and composite interval mapping (CIM: Zeng, 1994)
6 methods were used to identify and confirm the presence of QTLs. All
7 analyses were performed using the QTL Cartographer 2.0 application
8 (Basten *et al.*, 1994). The maximum log-of-odds (LOD) score of
9 association between the genotype and trait data was calculated for SIM
10 and CIM, and QTL location predictions were accepted for SIM for values
11 greater than a threshold value of 2.5. Permutation analysis (1000
12 iterations) was used to establish an experiment-wise significance value at
13 the 0.05 confidence level defined as a minimum LOD threshold for each
14 trait in CIM (Churchill and Doerge 1994; Doerge and Churchill 1996). For
15 each form of interval analysis, the maximum LOD value, location of the
16 maximum LOD value on the genetic map, additive marker allele effects
17 and the proportion of phenotypic variance attributable to the QTL were
18 tabulated.

19

20 **Comparative genomics analysis**

21 Wheat ESTs related to lignin biosynthetic genes from other plant species
22 were identified by sequence annotation using the wEST-SQL database in
23 the GrainGenes resource. The nucleotide sequences were used for
24 TBLASTX analysis (version 2.2.6) through the National Center for
25 Biological Information (NCBI) facility. The chromosomal location of wheat
26 ESTs based on assignment to deletion bins (Qi *et al.* 2003) were
27 determined using the Mapped Loci query function in Graingenes-SQL
28 (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

1 **Results**

2

3 **Statistical analysis of herbage quality data**

4 For each of the measured traits, significant variation was detected
5 between members of the mapping population ($p < 0.001$) for all of the
6 experimental datasets, treated here as replicates. A large proportion of the
7 total variance was explained by the replicate structure. The replicates were
8 based upon on measurement at different stages of development and
9 growth conditions, which increases the relevance of the overall analysis
10 and conclusions, but also has impact on the replicate variance. The
11 glasshouse-grown spring harvest in 2002 was specifically compared with
12 the summer harvest in 2002, to determine the effect of the developmental
13 stage variation on the analysis. The replicate structure was significantly
14 different between the two datasets ($p < 0.001$). However, in all cases there
15 was still significant variation explained by the genotypes ($p < 0.01$). To
16 assess the replicate nature of the datasets, the two temporal replicates
17 (2001 and 2002) of summer harvests were compared. For the CP, NDF
18 and WSC traits there was significant variation between the replicates
19 ($p < 0.001$). For the EstME and IVVDMD traits, significant variation was not
20 detected between the replicates ($p = 0.18$ in both cases). In contrast, the
21 two experimental datasets from spring 2002 were analysed together, as a
22 comparative assessment of glasshouse and nursery conditions at the
23 same developmental stage. The replicate structure was again not
24 significantly different for EstME and IVVDMD ($p = 0.53$ in both cases), while
25 for the other traits there was significant variation between the replicates
26 ($p < 0.01$).

27

28 **QTL analysis of herbage quality data**

29 **Table 1**

30 **Figure 1**

31 For each of the traits significant regression was detected between trait and
32 marker data at various positions. No significant association was detected
33 between any of the traits and any marker on linkage group 6. All other

1 linkage groups displayed significant associations between markers and
2 traits (Table 1, Figure 1). Variable numbers of QTL were identified from the
3 different sampling experiments. The minimum number of QTLs detected
4 from a single dataset were from the summer harvests in 2001 and 2002,
5 with 3 QTLs in each instance, solely for the CP and NDF traits. The
6 maximum number of QTLs detected from single datasets were derived
7 from the spring 2002 nursery-grown harvest and the 1998 harvest. In
8 these instances, 11 QTLs were identified across all traits. However, QTLs
9 for CP and WSC were not detected in the dataset for the nursery-grown
10 spring harvest in 2002.

11

12 ***Crude protein (CP)***

13 A total of 7 QTLs for CP were identified from 5 of the experimental
14 datasets, with the exception of the nursery-grown spring harvest in 2002.
15 Five QTLs failed to show significance with all three analytical methods and
16 should be consequently treated with caution. The QTLs detected on LG1
17 from the summer 2002 harvest, LG3 from the summer 2001 and 1998
18 harvests and LG5 from the 1999 harvest were not significantly detected by
19 SIM. However, in all cases there was significant marker-trait association
20 using SMR, and CIM was significant for the LG1 summer 2002 harvest
21 and LG3 summer 2001 harvest QTLs. For the other 1998 and 1999-
22 derived QTLs, maximum LOD values from CIM were not significantly
23 greater than the empirically-set threshold. However, both maximum values
24 exceeded 2.0, and the location of the 1998 harvest QTL was coincident
25 with the equivalent region identified from the spring 2002 and summer
26 2001 harvests.

27 Coincident QTLs were identified on LG3 from the datasets of the
28 harvests in spring (glasshouse-grown) 2002, summer 2001 and 1998. The
29 additive effect from the spring harvest was negative, while the effects from
30 the other two harvests were positive (Table 1). The estimated percentage
31 of phenotypic variance explained by the QTLs varied from 6.5%-19.3%,
32 depending on sampling experiment and analytical method. Individual QTLs
33 were detected in a single experimental dataset on four instances (LGs 1, 2,
34 4 and 5 for harvest years 2002, 2001, 1998 and 1999 respectively).

1

2 **Estimated in vivo dry matter digestibility (IVVDMD)**

3 A total of 8 QTLs for IVVDMD were identified from 4 of the experimental
4 datasets. No significant QTLs were detected from the summer harvests in
5 2001 and 2002. Five QTLs failed to show significance with all analytical
6 methods and should be taken as indicative rather than conclusive.
7 Regions on LGs 1 and 4 were identified as significant by SMR at $p < 0.05$ in
8 the 1998 harvest dataset. The maximum LOD values for the LG1-located
9 QTL were 1.93 based on SIM and 2.35 based on CIM, although the
10 empirical threshold for CIM was 2.91, while for the LG4-located QTL the
11 maximum LOD values were 1.3 based on SIM and 3.7 based on CIM. For
12 the IVVDMD QTL from the 1999 harvest and the LG1/LG3-located QTLs
13 from the nursery-grown spring harvest from 2002, there were significant
14 associations identified by SMR and SIM, but CIM failed to identify a
15 maximum LOD value above the empirically-set threshold.

16 Coincident QTLs were identified on LG3 from the datasets of the
17 glasshouse and nursery-grown spring harvests in 2002 and the 1999
18 harvest. All additive effects were positive with maximum LOD values
19 ranging from 2.02 to 2.63 explaining 10.7 to 17.2% of the observed
20 phenotypic variance, depending on the analytical method utilised and the
21 experimental dataset (Table 1). LG7 also contained coincident QTLs from
22 both of the spring 2002 harvests. All additive effects were again positive,
23 with values ranging from 2.17 to 3.07 and explaining 10.7 to 17.2% of the
24 observed phenotypic variance, depending on the experimental dataset
25 and analytical method. Individual QTLs were detected on LGs 1 and 4 for
26 the 1998 and 1999 harvests.

27

28 **Neutral detergent fibre (NDF)**

29 A total of 13 QTLs for NDF were detected from each of the experimental
30 datasets. Nine of the QTLs failed to show significance with all analytical
31 methods. The 6 QTLs on LGs 2 and 5 were not detected by SMR (with the
32 exception of a single marker-trait association identified on LG5 from the
33 1998 harvest data) or SIM. However, CIM identified these QTL groups in
34 close repulsion linkage. The two QTLs identified on LG5 were concurrently

1 detected from the 1998 harvest and summer 2002 harvest datasets, with
2 linkage phase consistent between the two datasets. Coincident QTLs were
3 also identified on LGs 3 and 7 from the 1999 harvest and both of the
4 spring 2002 harvests. The coincident QTLs on LG7 displayed significant
5 marker and trait association through SMR. However, the maximum LOD
6 scores from SIM were close to 2.0, and the maximum LOD values under
7 CIM for both experimental datasets were c. 2.5, below the empirically set
8 LOD threshold of approximately 2.6 (Table 1). QTLs were also identified
9 on LGs 1 and 4 through significant marker-trait association, although the
10 maximum LOD scores for SMR and SIM were below the threshold value (c.
11 2.0), and CIM also failed to identify significant regions. These QTLs should
12 consequently be regarded as only indicative and treated with caution.

13

14 ***Estimated metabolisable energy (EstME)***

15 A total of 8 QTLs for EstME were detected from 4 of the experimental
16 datasets. Single QTLs on LGs 3 and 7 were identified as significant with all
17 detection methods from analysis of each of the 2002 spring harvest
18 datasets. The exception is the LG3-located QTL from the nursery-grown
19 spring harvest from 2002, which was not significantly identified by CIM. In
20 addition, an indicative coincident QTL was identified on LG3 from analysis
21 of the 1999 harvest dataset with significant marker and trait association
22 ($p < 0.01$), although maximum LOD values of 1.9 for SIM and 2.3 (with
23 threshold value of 2.9) for CIM were observed. The coincidence of this
24 QTL with those detected from other datasets gives enhanced credence to
25 a genuine effect associated with the relevant region. Individual QTLs were
26 also detected from the 1998 and nursery-grown spring 2002 harvests that
27 were not otherwise identified. The 1998 harvest data set identified QTLs
28 on LGs 1 and 4 that showed significant marker-trait association ($p < 0.05$),
29 but SIM identified maximal LOD values of only c. 1.2. For the QTL on LG4,
30 CIM identified a region of significance, but for the QTL on LG1 CIM
31 revealed a maximum LOD value of 2.3 with an empirical threshold of 2.7.
32 The region on LG1 has provided equivocal data for genetic control.

33

1 **Water soluble carbohydrate (WSC)**

2 A total of 6 QTLs for WSC were detected from datasets of the 1998, 1999
3 and glasshouse-grown spring 2002 harvests. For two of the QTLs
4 identified on LG5 from the 1998 harvest dataset, only limited supporting
5 evidence was provided by SMR and SIM. However, the two QTLs were
6 identified by CIM as linked in repulsion with additive effects of similar but
7 opposing magnitude (2.56 and -2.41 respectively). The 1999 harvest data
8 set identified QTLs on LGs1 and 7, with markers significantly associated
9 with the trait data ($p < 0.01$), but the maximum LOD values detected by SIM
10 were only 1.48 and 1.97 respectively. CIM identified the LG1 QTL as being
11 significant (maximum LOD value = 2.61 with a threshold of 2.58). However,
12 for the QTL on LG7 the LOD value was maximal at 2.39, with a threshold
13 value of 2.58. The 1998 experimental dataset also identified significant
14 marker-trait association ($p < 0.01$) with SIM maximal at a LOD value of 1.81,
15 but significant effects were identified with CIM (maximum LOD = 3.18 with
16 a threshold of 2.93). None of the QTLs were detected in coincident
17 locations.

18

19 **Co-location of herbage quality QTLs and lignin** 20 **biosynthetic gene loci**

21 **Figure 2**

22 Full-length cDNAs for the [LpCCR1](#), [LpOMT1](#) and [LpCAD2](#) lignin
23 biosynthetic genes (Heath et al. 1998; Lynch et al. 2002; McInnes et al.
24 2002) were used to detect RFLP in the p150/112 progeny set. Single
25 polymorphic loci were detected for [LpOMT1](#) using the enzyme [DraI](#) and for
26 [LpCCR1](#) using the enzyme [EcoRI](#), while three polymorphic loci were
27 detected for [LpCAD2](#) using the enzyme [EcoRI](#). The segregating loci were
28 mapped within the framework of the ILGI reference map dataset (Jones et
29 al. 2002a), detecting four loci designated [xlpomt1](#), [xlpccr1](#), [xlpcad2.1](#) and
30 [xlpcad2.3](#), respectively. The second polymorphic RFLP locus detected by
31 [LpCAD2](#) (on the basis of descending molecular size) failed to group with
32 any of the 7 LGs. The [xlpcad2.3](#) locus was located in the lower central
33 region of LG2. By contrast, the [xlpcad2.1](#), [xlpccr1](#) and [xlpomt1](#) loci were

1 closely linked within an interval of 0.9 cM on LG7, adjacent to the
2 heterologous RFLP loci xpsr154 and xpsr690. The addition of genomic
3 DNA-derived SSR markers to this framework indicates that the
4 xlpssrk14f07, xlpssrk10h-5 and xlpssrrk14b01 loci are also located within
5 this region (Figure 2), which coincides with the herbage quality QTL cluster.

6 The fructosyltransferase homologue-encoding [LpFT1](#) and [LpFT2](#)
7 genes were also assigned to the p150/112 map, detecting single genetic
8 loci in the upper distal regions of LGs 7 and 6, respectively (Lidgett et al.
9 2002; Johnson et al. 2003). However, none of the WSC QTLs identified in
10 this study co-locate with these gene loci.

11

12 **Comparative genomics of lignin biosynthetic genes in** 13 **perennial ryegrass and wheat**

14 **Table 2**

15 Wheat ESTs showing significant nucleotide similarity to annotated lignin
16 biosynthetic genes from perennial ryegrass and from other plant species
17 were identified through annotation criteria (Table 2). Significant matches to
18 each of the perennial ryegrass genes detecting LG7 loci were observed,
19 and two of the selected wheat ESTs (BE426229 and BE498785) showed
20 the most significant TBLASTX results with the [LpCAD2](#) and [LpOMT3](#)
21 genes respectively. [LpOMT1](#) and [LpOMT3](#) are very closely related at the
22 nucleotide level (Heath et al. 1998). The most significant matches for the
23 other wheat ESTs were with annotated lignin biosynthesis genes from
24 other species, either exclusively, or in addition to less significant results
25 with perennial ryegrass genes.

26 **Figure 3**

27 The chromosomal locations of the wheat ESTs that are ortholoci of
28 known OMT, CCR and CAD genes were determined based on the wheat
29 deletion bin map (Figure 3). ESTs related to each of the [LpOMT1](#),
30 [LpCCR1](#) and [LpCAD2](#) genes are located within adjacent deletion bins at
31 the distal end of chromosome 7DL. Putative ortholoci were also located in
32 distal locations on the other homoeologous group 7 chromosomes
33 ([LpCCR1](#) and [LpCAD2](#) on 7AL; [LpOMT1](#) and [LpCAD2](#) on 7BL). Each of

1 the perennial ryegrass genes also shows high sequence similarity to
2 [Oryza sativa](#) ssp. [japonica](#) rice BAC clones assigned to chromosome 8 by
3 BLASTN analysis ([LpOMT1](#): $E = 3 \times 10^{-144}$; [LpCCR1](#): $E = 8 \times E^{-145}$;
4 [LpCAD2](#): $E = 1.3 \times 10^{-150}$; J.W. Forster, unpublished data), and the
5 putative rice ortholocus of [LpCCR1](#) has been attributed to this region
6 (McInnes et al. 2002). Rice chromosome 8 is the syntenic counterpart of
7 the relevant regions of the perennial LG7 and Triticeae homoeologous 7L
8 chromosomes (Jones et al. 2002a).

9 The homoeologous group 3 chromosomes also contained putative
10 ortholoci for each perennial ryegrass gene in distal bins ([LpOMT1](#),
11 [LpCCR1](#) and [LpCAD2](#)-related loci on 3AL and 3DL; [LpCCR1](#) and
12 [LpCAD2](#)-related loci on 3BL). In addition, ESTs related to two of the three
13 gene classes were located to the distal regions of 2BS, 2DS, 6AL and 6DL,
14 and ESTs related to single gene classes were mapped to the distal
15 regions of 2AL and 6BL, as well as the interstitial regions of 5AL, 5BL and
16 5DL.

17 The distal regions of the wheat group 3L and 7L chromosomes are
18 the syntenic counterparts of the corresponding regions of perennial
19 ryegrass LGs 3 and 7, in which herbage quality QTL clusters are located.
20 Although the perennial ryegrass lignin biosynthetic genes did not detect
21 polymorphic RFLP loci on LG3, the location of OMT, CAD and CCR-
22 related wheat ESTs on 3L suggests that other members of these gene
23 families, that were not detected by RFLP analysis in the reference
24 population, may be located on this linkage group.

1 **Discussion**

2

3 **Genetic dissection of herbage quality traits**

4 A total of 42 QTLs for herbage quality traits in perennial ryegrass were
5 detected from the 6 experimental datasets. Groups of coincident QTLs
6 were identified on LGs 3, 5 and 7 and can be rationalised into 8-9 key
7 target regions for potential breeding applications. The use of various forms
8 of QTL analysis such as SMR, SIM and CIM is critical for the
9 comprehensive dissection of these datasets. Judicious comparative
10 analysis of the overall dataset by the differing approaches permitted the
11 identification of both unequivocal QTLs that are detected with high
12 significance with all methods, and indicative QTLs which should be treated
13 with caution. The IM methods were largely in agreement over QTL
14 identification. However, in several instances conflicting results have been
15 obtained for the presence of effective genomic regions, such as the QTLs
16 for IVVDMD on LGs 1 and 3 from the nursery-grown spring harvest in
17 2002 and the QTL for EstME LG4 from the 1998 harvest. The data
18 summarised in Table 1 consequently represent the QTLs that are detected
19 by all three analytical methods, those that are detected by at least one
20 method, and a small number of putative QTLs that fail significance with all
21 three methods, but closely approach the significance level with at least
22 one form of analysis.

23 Substantial groups of coincident QTLs were located on LGs 3 and 7.
24 The region on LG3 was associated with variation for all measured traits
25 across various experimental datasets. For each sampling experiment, with
26 the exception of the summer harvest data from 2002, the LG3 region was
27 identified as significant for at least one trait. A major genomic region
28 associated with herbage quality variation is defined by this analysis,
29 providing a potential target for marker-assisted selection (MAS). Similarly,
30 the cluster of coincident QTL locations on LG7 represents each of the
31 traits apart from CP. The majority of QTLs in this region were contributed
32 by the two spring harvests in 2002, but the WSC QTL from the 1999
33 dataset is also located in this region.

1 The two spring harvests from 2002 obtained consistent comparable QTL
2 locations for different traits in the regions of LG3 and LG7. A comparison
3 of the data from these two harvests provides evidence for stability of
4 genetic control between glasshouse-grown and nursery-grown samples.
5 The observed co-locations suggest that the QTLs detected by NIRS
6 analysis under controlled growth conditions may be sufficiently stable to
7 permit MAS for field-expressed performance. At the same time, variation is
8 observed in a number of genomic locations for coincidence of QTLs for the
9 same trait measured in experiments varying by season, location and year.
10 This provides preliminary evidence for QTL x E (environment) variation,
11 which has been observed in a number of detailed studies (Paterson et al.
12 1991; Lu et al. 1996; Yan et al. 1999; Yadav et al. 2003), although the
13 environmental parameters contributing to the effect are in many cases
14 unknown (Paterson et al. 2003). The presence of QTL x E interactions for
15 nutritive value traits is consistent with the known effects of environmental
16 factors such as reproductive development in grasses (Oram et al. 1974;
17 Tyler and Hayward 1982). However, genotypes of grass species have
18 been identified that consistently exhibit high nutritive value across a range
19 of environments and seasons (Casler 2001; Smith et al. 2004). The
20 relative stability of QTL effects associated with the LG3 and LG7-located
21 clusters provide the best option to overcome problems associated with
22 QTL x E in MAS applications derived from the current study.

23 Although for the NDF and WSC traits no significant correlation was
24 detected between marker and trait data using SMR, and SIM analysis did
25 not identify significant QTLs on LG5, CIM detected two QTLs in repulsion
26 on this LG for each trait from three of the experimental datasets.
27 Significant QTLs were identified for NDF from the 1998 and the summer
28 2002 harvests, and in addition WSC QTLs were detected from the 1998
29 harvest. The additive effects of the QTLs were negative and positive
30 respectively for NDF, and positive and negative respectively for WSC. A
31 similar pattern was observed for the QTLs for these traits on LG3, with the
32 additive effect opposed in direction between NDF and all other measured
33 traits at each location. These relationships are predictable due to the
34 observed negative correlation between phenotypic variation for NDF and

1 for the other traits. The digestibility of the NDF fraction of forage varies
2 between 100% (mesophyll) and 0% (xylem) in some plants (Akin 1989),
3 with the absolute value influenced by plant maturity in ryegrasses
4 (Armstrong et al. 1992), and the digestibility of the soluble component of
5 herbage is usually 100%. In consequence, any increase in the NDF
6 concentration of herbage is likely to be associated with a concomitant
7 decrease in IVVDMD. Conversely, as forage dry matter is the sum of NDF
8 and neutral detergent solubles (such as CP and WSC), any increase in
9 concentration of the soluble components of herbage that is not merely
10 associated with a change in the partitioning of dry matter between these
11 components must lead to a reduction in NDF and a corresponding
12 increase in IVVDMD.

13 Reproductive development was anticipated to influence the
14 expression of phenotypic variation for CP concentration (and potentially
15 other traits such as NDF, IVVDMD and WSC) in the mapping population.
16 This was indicated by the change in direction of effect of the additive
17 genetic component between QTLs for CP on LG3 for the spring harvests
18 in 2002 and the summer harvests in 1998 and 2001, respectively.
19 Seasonal variation for CP concentration is expected for ryegrass species
20 due to changes in plant nitrogen content associated with alterations in the
21 ratio of stems, leaf sheaths and lamina. These structures have contrasting
22 nitrogen content, and hence CP concentrations (Armstrong et al. 1992).

23

24 **Candidate gene-QTL co-location**

25 The coincident herbage quality QTLs on LG7 were assigned to a region of
26 c. 28 cM maximum length based on a decline of 2 LOD units from
27 maximum values through CIM analysis. This region is extensive at the
28 molecular level, given an average relationship between genome size (c.
29 1.6×10^9 bp haploid content: Hutchinson et al. 1979; Seal and Rees 1982)
30 and map distance (814 cM: Jones et al. 2002b) of c. 2 Mb/cM. However,
31 within this region close linkage is observed between RFLP loci detected by
32 cDNAs corresponding to three of the major classes of enzymes in the
33 pathway to monolignol biosynthesis: caffeic acid-O-methyltransferase
34 (OMT), cinnamoyl CoA-reductase (CCR) and cinnamyl alcohol

1 dehydrogenase (CAD). The maximum LOD locations for a number of the
2 QTLs coincides with the position of the lignin biosynthesis gene cluster.
3 The observation of co-location between these candidate gene loci and a
4 major QTL cluster suggests that allelic variation either in coding
5 sequences or regulatory regions (Paran and Zamir 2003) may contribute
6 to the phenotypic variation for target traits. Confirmation of this hypothesis
7 will entail more extensive analysis including association studies through
8 linkage disequilibrium (LD) mapping (Thornsberry et al. 2001; Rafalski
9 2002; Gaut and Long 2003; Flint-Garcia et al. 2003), in concert with the
10 production of phenocopies through transgenic modification such as gene
11 silencing (Vance and Vaucheret 2001). In this context, we are performing
12 single nucleotide polymorphism (SNP) development for the full-length
13 [LpCCR1](#) and [LpCAD2](#) genes, and antisense transgenic plants have been
14 generated for each of the [LpOMT1](#), [LpCCR1](#) genes. The successful
15 validation of candidate gene-based markers for components of herbage
16 digestibility would permit genotypic selection on the basis of superior allele
17 content (Sorrells and Wilson 1997) for pasture grass breeding (Forster et
18 al. 2004).

19

20 **Comparative genomics of lignin biosynthetic genes**

21 The identification of substantial macrosynteny between the genomes of
22 perennial ryegrass and the Triticeae cereals (Jones et al. 2002a) provides
23 the opportunity for comparative genomics analysis of shared traits and
24 metabolic processes, including herbage digestibility and lignification.
25 These relationships are consistent with the comparative location of
26 [LpOMT1](#), [LpCCR1](#) and [LpCAD2](#)-detected RFLP loci in the lower central
27 region of perennial ryegrass LG7 and the assignment of related wheat
28 ESTs to a distal deletion bin on 7DL, in a region of predicted conserved
29 synteny. Wheat ESTs related to [LpOMT1](#), [LpCCR1](#) and [LpCAD2](#) also
30 mapped to the distal ends of wheat chromosomes 3AL and 3DL,
31 suggesting that the locations of these genes in the wheat genome may
32 arise from ancient duplication events, with similar linear orders. However,
33 the wheat ESTs related to [LpCAD2](#) and [LpCCR1](#) differ between the group
34 3 and group 7 chromosomes, possibly due to independent gene

1 divergence following duplication. Such duplication-gene divergence
2 evolutionary events have also been observed in alignments between rice
3 chromosome 1 and wheat 3S (Francki et al. 2004). The duplication–
4 divergence hypothesis is further supported by the assignment of distinct
5 OMT-related wheat ESTs to deletion bins on each of homeologous groups
6 2, 3, 6 and 7. This suggests that each lignin biosynthesis gene class may
7 be represented in wheat by multiple diverged copies, and that members of
8 each class may be located in close association at each bin location, but
9 have not yet been mapped. A preliminary TBLASTX comparison of the
10 perennial ryegrass gene sequences against the wheat EST database has
11 identified other ESTs with significant sequence similarity that have not yet
12 been located by deletion bin mapping (data not shown). Subsequent
13 mapping of these ESTs may provide direct evidence for segmental
14 duplications of a lignin biosynthesis gene cluster during Poaceae evolution,
15 with current representatives on wheat groups 2, 3, 5, 6 and 7.

16 Due to the relatively close phylogenetic relationship between the
17 Triticeae and Poaceae grasses, perennial ryegrass may share a
18 segmental duplication pattern. The [LpOMT1](#) and [LpCAD2](#) cDNAs detected
19 small multigene families in genomic Southern hybridisation experiments
20 (Heath et al. 1998; Lynch et al. 2002), although [LpCCR1](#) revealed a lower
21 genomic complexity. As only a small proportion of the genomic loci
22 revealed RFLP in the p150/112 population, it is possible that loci other
23 than those detected on LG7 could be detected in other pedigrees, and that
24 paralogous gene variation on LG3 may contribute to the QTL effects
25 associated with this LG. The development of locus-specific SNP markers
26 for the lignin biosynthesis genes will permit specific map assignment and
27 confirm whether the existing cDNAs are derived from LG7-located loci, or
28 other related genomic locations.

29 Comparative analysis of lignin biosynthesis genes provides the
30 opportunity for detection of orthologous QTLs between species, with the
31 potential to target chromosomal regions in wheat and its relatives for
32 lignin-related traits, such as cereal residue digestibility. In this context,
33 recent research has identified a major QTL for the traits of solid stem and
34 sawfly resistance in the distal region of wheat 3BL, in a region co-

1 localising with the [LpCAD2](#) ortholocus (Cook et al. 2004). Conversely,
2 advances in physical mapping of wheat ESTs provides the basis for
3 ortholocus identification and exploitation in perennial ryegrass.

4 Comparative genetic analysis may also be extended to more distant
5 relatives of the Poaeae grasses within the Poaceae family that are used as
6 forage species, such as maize ([Zea mays](#) L.). Breeding improvement for
7 high digestibility in forage maize has been defined as an important
8 objective for animal nutrition (Lundvall et al. 1994). Fibre and lignin content
9 traits such as NDF, acid detergent fibre (ADF) and acid detergent lignin
10 (ADL) were measured by NIRS in a recombinant inbred line (RIL) maize
11 mapping family (Cardinal et al. 2003). ADF is related to EstME, and ADL is
12 negatively correlated with IVVDMD. Multiple QTLs for each trait were
13 detected, with substantial clustering on chromosomes 1, 2, 3, 5, 6, 7, 8, 9
14 and 10. Coincident locations were observed with QTLs detected in a
15 previous study (Lübberstedt et al. 1997). The conserved synteny
16 relationships between the genomes of perennial ryegrass and maize are
17 not as well understood as for the Triticeae cereals. However, LGs 3F and
18 7F in meadow fescue ([Festuca pratensis](#) Huds.), which are largely colinear
19 with their perennial ryegrass counterparts (Alm et al. 2003), correspond to
20 regions of maize chromosomes 3/8 (3F) and 6/9, 1/5 (7F) respectively.
21 Each of these chromosomes contains QTL clusters for putative
22 orthologous traits to those described in the present study. In addition,
23 several maize QTLs coincide with the location of [bm](#) (brown mid-rib)
24 mutant loci associated with lignin biosynthesis, such as the CAD-related
25 [bm1](#) locus, which maps to chromosome 5 (Baucher et al. 1998).

26

27 **Breeding implications**

28 The results of the marker-trait QTL association studies described in this
29 study provide efficient and valuable selection mechanisms for either
30 components of digestibility that are expressed throughout the growing
31 season, or traits associated with the post-reproductive decline in
32 digestibility. This targeted approach to improving nutritive value in ryegrass
33 species will prevent the need for detailed and logistically complex
34 sampling strategies that seek to negate the effects of environmental

- 1 variation. Important additional benefits will be obtained through the
- 2 breeding of cultivars to improve the late spring and early summer seasonal
- 3 deficiencies that limit forage quality in Australian pasture systems.

1 **Acknowledgments**

2 This work was supported in part by Grants-in-Aid for Scientific Research
3 (No. 14360160 to T.Y.) from the Ministry of Education, Science, Sports
4 and Culture, Japan, and the Molecular Plant Breeding Cooperative
5 Research Centre and the Department of Primary Industries, Victoria,
6 Australia. The authors thank Prof. Michael Hayward for his careful critical
7 reading of the manuscript. All experiments conducted during this study
8 comply with current Australian laws.

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1 **Table Legends**

2

3 **Table 1**

4 Summary of QTL analysis data for NIRS-calibrated herbage quality traits in the p150/112
5 reference mapping population. QTL identification was performed using the QTL
6 Cartographer software and SMR, SIM and CIM analyses were performed. QTLs
7 identified as significant with all three analytical methods are shaded in grey. The
8 criteria for inclusion of other QTLs were either significant detection with at least
9 one of the analytical methods, or the observation of maximum LOD values close
10 to, but not exceeding, the threshold values.

11

12 **Table 2**

13 Summary of sequence annotation data for lignin biosynthesis gene-related ESTs of
14 hexaploid wheat ([Triticum aestivum](#) L.) compared to putative orthologous
15 sequences from [L. perenne](#) and other species. Alignments represent percentage
16 amino acid identity over the length of the EST (in nucleotides). E values for
17 TBLASTX hits are shown in parentheses.

1

2

Figure Legends

3

4 **Figure 1**

5 Location of QTLs for NIRS-calibrated herbage quality traits on the p150/112 reference
6 genetic map of perennial ryegrass. Nomenclature of genomic DNA-derived SSR
7 (LPSSR) loci, AFLP loci and heterologous RFLP loci is as described by Jones et
8 al. (2002a,b). QTL nomenclature is adapted from McCouch et al. (1997) in the
9 form q-TRAIT-season-location-year, with details as described in footnote 1 to
10 Table 1. All QTL locations were derived from CIM analysis. All putative QTLs
11 described in Table 1 are shown, with the exception of the equivocal loci
12 qIVVDMD-98, qNDF-98, qEstME-98 and qWSC-98. Bars and lines represent 1
13 and 2 LOD unit drops from the maximum likelihood value.

14

15 **Figure 2**

16 Detailed genetic map of the lignin biosynthesis gene cluster on perennial ryegrass LG7.
17 The xlpca2.1, xlpccr1 and xlpomt1 loci were mapped within the framework of the
18 AFLP and heterologous RFLP-based map of Jones et al. (2002a). Genomic DNA-
19 derived SSR (xlpssr) loci (Jones et al. 2002b) are shown as accessory markers
20 within the target region.

21

22 **Figure 3**

23 Location of lignin biosynthesis gene-related wheat ESTs to deletion bins of hexaploid
24 wheat. The BE and BF prefixes denote EST origin, and the matching gene class
25 is shown in parenthesis following the EST number.

26

Table 1

	Trait ¹	LG	SMR	SIM				CIM based on 1000 simulations				
			P<0.01	Max LOD score	Position	a ²	R ³	LOD threshold	Max LOD score	Position	a ²	R ³
CP	qCP-su-gh-02	1	21.4-25.6 (29.4-43.9 p<0.05)	1.63	23.41	1.715	0.093	2.79	3.02	23.41	2.646	0.143
	qCP-su-gh-01	2	41-60.2	2.28	47.61	-1.146	0.159	2.83	3.19	47.61	-1.173	0.151
	qCP-sp-gh-02	3	68.9-89.7 (83.7 0.05)	2.51	70.90	-1.746	0.108	2.67	3.29	68.91	-1.920	0.121
	qCP-su-gh-01	3	(83.7-89.7 p<0.05)	1.35	87.11	0.877	0.097	2.83	2.88	87.11	1.081	0.140
	qCP-98	3	89.7-116.8	1.45	95.70	0.836	0.106	2.92	2.52	99.71	1.042	0.153
	qCP-98	4	78.6-87.2 (90.7 p<0.05) 95.7-116.7	3.06	107.11	1.124	0.193	2.92	3.62	103.11	0.971	0.136
	qCP-99	5	36.1-42.7; 52.4	1.67	52.41	2.369	0.065	2.78	2.02	46.61	2.541	0.071
	IVVDM	qIVVDM-98	1	0-13.8 p<0.05	1.31	6.01	-1.067	0.087	2.91	2.35	6.11	-1.285
qIVVDM-sp-gh-02		3	68.9-116.8	3.31	83.71	2.549	0.150	2.82	4.33	83.71	2.596	0.153
qIVVDM-sp-nu-02		1	21.4-29.4 (34.1 p<0.05) 40.5-53.9	2.23	43.91	2.435	0.107	2.80	0.78	43.91	1.572	0.030
qIVVDM-99		3	55.5-72.5	2.15	72.51	2.269	0.088	2.79	2.53	72.51	2.316	0.091
qIVVDM-sp-nu-02		3	31.5-72.5	2.58	36.31	2.632	0.114	2.80	1.22	36.31	2.022	0.044
qIVVDM-98		4	(52.1-72.3 p<0.05)	1.30	65.41	1.051	0.079	2.91	3.69	60.61	1.704	0.171
qIVVDM-sp-nu-02		7	60.3-120.6	2.45	110.51	3.078	0.172	2.80	3.02	65.91	2.534	0.113
qIVVDM-sp-gh-02		7	65.9-98.5	2.30	73.01	2.168	0.107	2.82	3.19	71.01	2.286	0.115
NDF	qNDF-sp-nu-02	1	0; 13.8-29.4; 43.9-53.9	1.93	43.91	-1.730	0.089	2.63	0.66	43.91	-0.942	0.020
	qNDF-sp-nu-02	2	-	0.34	77.81	0.710	0.015	2.63	2.70	116.81	2.814	0.110
	qNDF-sp-nu-02	2	-	0.82	131.11	-1.093	0.036	2.63	3.55	131.11	-4.053	0.118
	qNDF-99	3	68.9-72.5	2.17	72.51	-2.456	0.090	2.71	3.42	72.51	-3.129	0.133
	qNDF-sp-gh-02	3	72.5-116.8	2.93	83.71	-1.896	0.134	2.67	3.78	83.71	-1.869	0.130
	qNDF-sp-nu-02	3	22.6-72.5	2.99	36.31	-2.163	0.128	2.63	3.16	55.51	-1.902	0.100
	qNDF-sp-gh-01	4	54.3-58.6	1.90	54.31	-1.647	0.128	2.75	1.86	51.51	-2.299	0.101
	qNDF-98	5	0	0.24	6.00	0.596	0.013	2.83	4.08	0.00	-2.579	0.214
	qNDF-98	5	-	0.42	30.41	-0.703	0.019	2.83	2.03	63.31	1.692	0.090
	qNDF-su-gh-02	5	-	1.20	44.61	-1.637	0.067	2.83	3.38	51.91	-3.687	0.170
	qNDF-su-gh-02	5	-	0.17	82.71	0.673	0.011	2.83	2.87	65.31	4.570	0.224
	qNDF-sp-gh-02	7	65.9-98.5	2.00	73.01	-1.580	0.092	2.67	2.49	69.01	-1.485	0.081
	qNDF-sp-nu-02	7	88.5-98.5	1.95	88.51	-1.695	0.086	2.63	2.48	35.91	-1.705	0.087

	Trait ¹	LG	SMR	IM				CIM based on 1000 simulations				
			P<0.01	Max LOD score	Position	a ²	R ³	LOD threshold	Max LOD score	Position	a ²	R ³
EstME	qEstME-98	1	(0-13.8 p<0.05)	1.29	6.01	-0.180	0.086	2.77	2.34	6.11	-0.218	0.116
	qEstME-sp-nu-02	1	21.4-53.9	2.23	43.91	0.388	0.107	2.78	0.78	43.91	0.251	0.030
	qEstME-99	3	55.5; 68.9-72.5	1.93	72.51	0.345	0.080	2.91	2.30	72.51	0.353	0.083
	qEstME-sp-gh-02	3	68.9-116.8	3.32	83.71	0.407	0.150	2.81	4.34	83.71	0.415	0.153
	qEstME-sp-nu-02	3	31.5-44.8; 50.4-72.5	2.58	36.31	0.420	0.114	2.78	1.22	36.31	0.313	0.042
	qEstME-98	4	(52.1-72.3 p<0.05)	1.24	62.61	0.171	0.072	2.77	3.71	60.61	0.290	0.172
	qEstME-sp-gh-02	7	65.9-98.5	2.28	73.01	0.345	0.107	2.81	3.17	71.01	0.370	0.113
	qEstME-sp-nu-02	7	60.3-75.5; 88.5-120.6	2.43	110.51	0.490	0.171	2.78	3.01	65.91	0.404	0.113
WSC	qWSC-99	1	84.1	1.48	27.61	1.890	0.082	2.58	2.62	17.81	-4.963	0.089
	qWSC-98	2	56.6-65.5	1.81	58.61	1.799	0.106	2.93	3.19	58.61	2.128	0.141
	qWSC-sp-gh-02	3	55.5; 68.9-116.8	4.45	72.51	5.243	0.186	2.76	4.52	87.11	5.210	0.181
	qWSC-98	5	30.4	1.47	30.41	1.550	0.080	2.93	3.48	30.41	2.557	0.141
	qWSC-98	5	-	0.52	10.00	-0.174	0.027	2.93	2.48	60.41	-2.407	0.126
	qWSC-99	7	120.6	1.97	116.51	4.976	0.116	2.58	2.39	120.51	4.944	0.110

¹QTL nomenclature adapted from McCouch et al. (1997) in the form q-TRAIT-season-location-year. The suffixes relate to experimental datasets as follows: -sp-gh-02 = glasshouse-grown material in spring 2002; - sp-nu-02 = nursery-grown material in spring 2002; -su-gh-01 = glasshouse-grown material in summer 2001; - su-gh-02 = glasshouse-grown material in summer 2002; -98 = glasshouse –grown material from 1998; -99 = glasshouse –grown material from 1999.

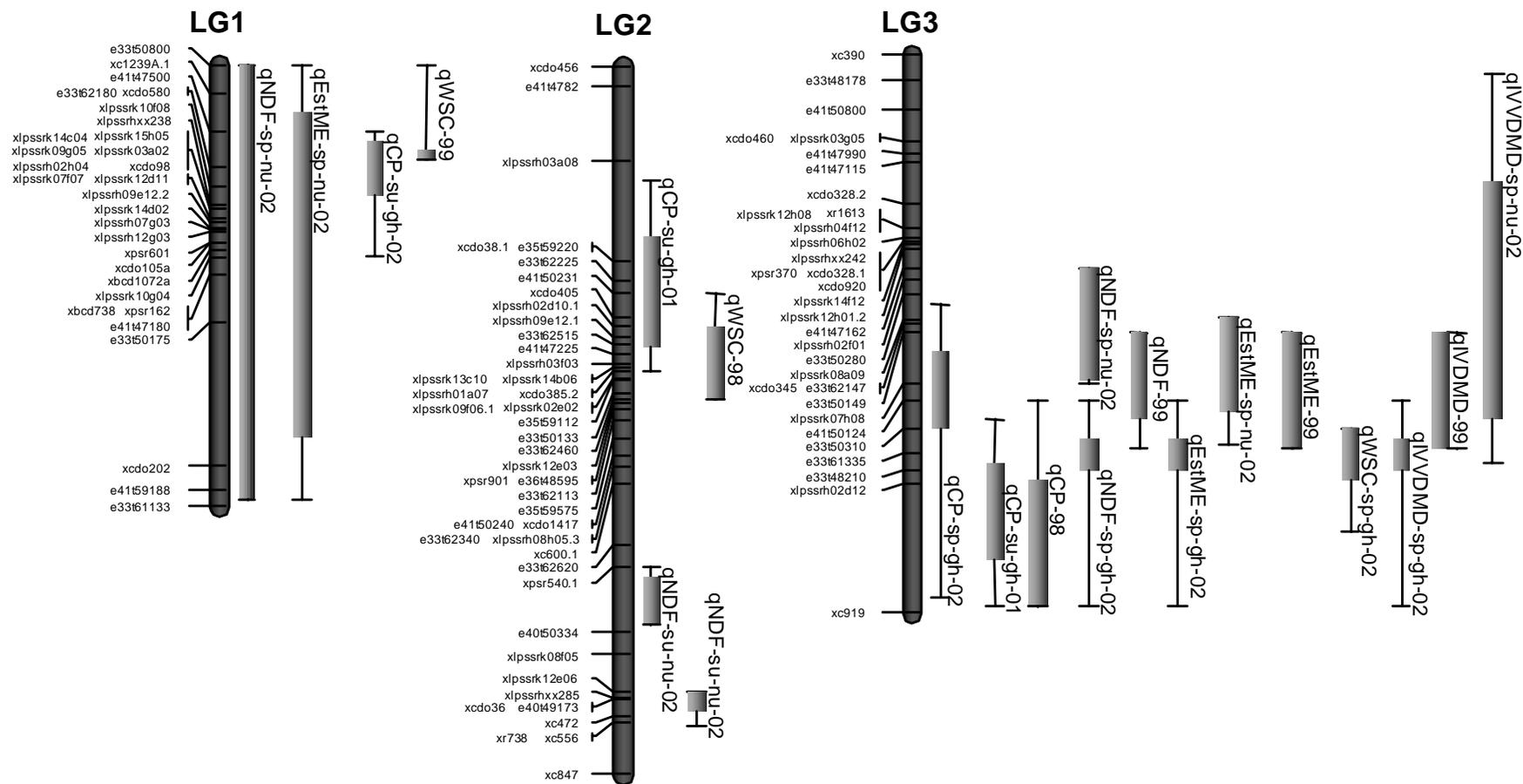
²Additive effect of substituting alternative alleles at marker locus.

³Proportion of variance explained by QTL.

Table 2

Wheat EST	EST length	TBLASTX match	Alignment	Best TBLASTX match (annotated)	Alignment
BF482769	556	L. perenne OMT1 (AF033538)	81%: 3-554 (1e-102)	T. aestivum COMT1 (AY226581)	85%: 3-554 (1e-105)
BE426229	605	L. perenne OMT3 (AF033540)	60%: 4-294 (1e-70); 60%: 277-603 (1e-70);	L. perenne OMT3 (AF033540)	60%: 4-294 (1e-70); 60%: 277-603 (1e-70);
BE498785	676	L. perenne CAD2 (AF472592)	75%: 61-393 (9e-53); 81%: 392-676 (4e-48)	L. perenne CAD2 (AF472592)	75%: 61-393 (9e-53); 81%: 392-676 (4e-48)
BE404596	566	No L. perenne hits	No L. perenne hits	S. cereale OMT (AY177404)	67%: 90-455 (2e-51)
BE406497	173	L. perenne CCR1 (AY061888)	49%: 8-172	A. thaliana CCR (AY093143)	51%: 8-172 (2e-12)
BF293181	534	L. perenne CCR1 (AF278698)	37%: 154-534 (9e-23)	A. thaliana CCR (AY093143)	49%: 22-378 (5e-35); 46%: 434-505 (5e-35)
BE443397	600	L. perenne CAD2 (AF472592)	59%: 9-320 (2e-65); 51%: 335-598 (2e-65); 41%: 600-884 (6e-8)	A. thaliana ADH (AY288079)	71%: 6-326 (3e-91); 75%: 335-598 (3e-91)
BF293156	566	No L. perenne hits	No L. perenne hits	Z. mays OMT (MZEOMT)	51%: 3-215 (4e-34); 40%: 309-383 (4e-34); 39%: 396-563 (4e-34)
BE443747	571	No L. perenne hits	No L. perenne hits	A. thaliana CCR (BT002742)	49%: 11-127 (2e-29); 40%: 245-544 (2e-29);

Figure 1



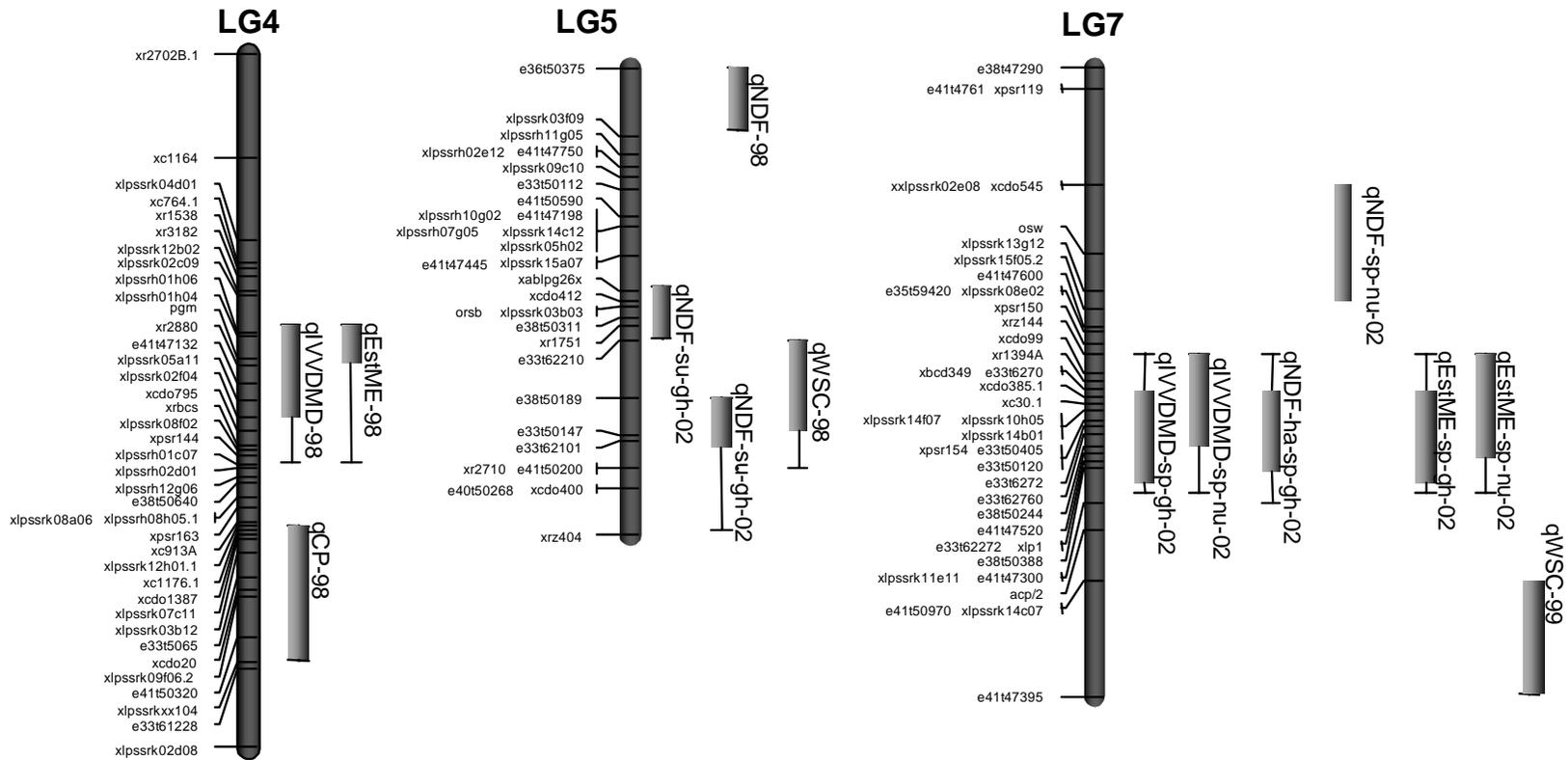


Figure 2

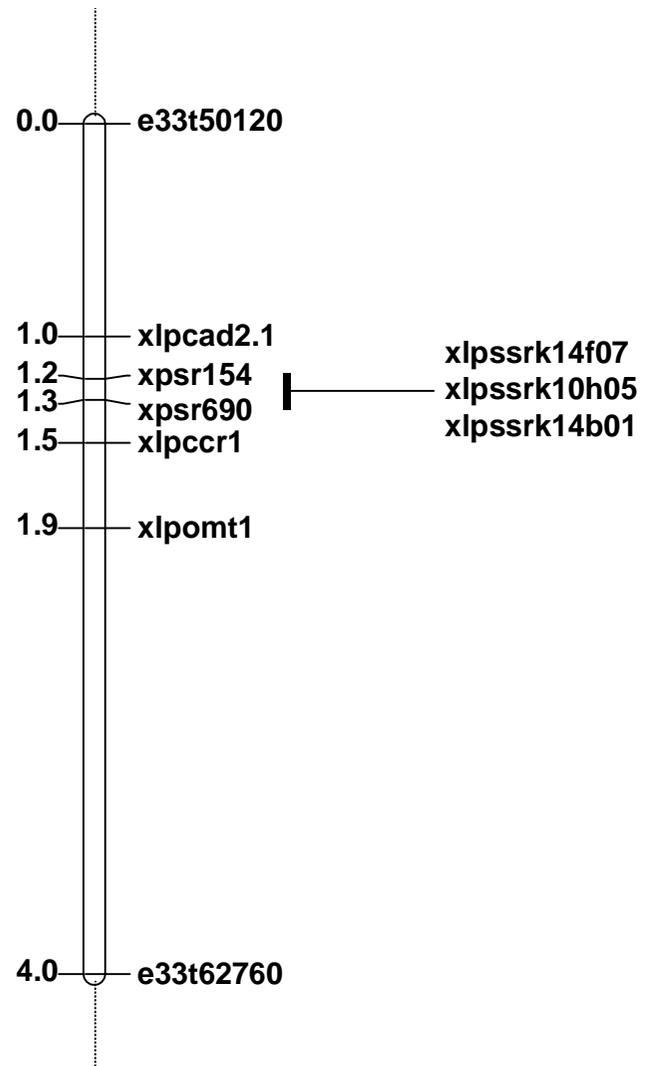


Figure 3

