Quantitative trait loci mapping for biomass yield traits in a *Lolium* inbred line derived F_2 population

U. C. M. Anhalt · J. S. Heslop-Harrison (Pat) · H. P. Piepho · S. Byrne · S. Barth

Abstract *Lolium perenne* L. (perennial ryegrass) is the principle forage grass species in temperate agriculture. Improving biomass yield still remains one of the most important aims of current forage breeding programmes. A quantitative trait locus (QTL) study investigating biomass yield traits in perennial ryegrass was carried out in greenhouse and field environments. The study is based on an F_2 population consisting of 360 individuals derived from two inbred grandparents where the F_1 has a large biomass yield phenotype. For both experimental environments co-localized QTL for biomass yield traits including fresh and dry weight and dry matter were identified on linkage groups 2, 3 and 7. A major QTL for fresh and dry weight was identified on LG 3 which explained around 30% of the phenotypic variance in the field experiment. The findings of this study are discussed with regard for their potential in research and breeding.

U. C. M. Anhalt · S. Byrne · S. Barth (✉)
Teagasc, Crops Research Centre, Oak Park, Carlow,
Ireland
e-mail: susanne.barth@teagasc.ie

U. C. M. Anhalt · J. S. Heslop-Harrison (Pat)
Department of Biology, University of Leicester, Leicester
LE1 7RH, UK

H. P. Piepho
Institute for Crop Production and Grassland Research
Bioinformatics, University of Hohenheim, Fruwirthstrasse
23, 70593 Stuttgart, Germany

Introduction

Lolium perenne L. (perennial ryegrass) is the principle forage grass species used in temperate agriculture. High digestibility and good nutritional characteristics make it particularly suitable as a forage grass (Hannaway et al. 1999). Some important targets of perennial ryegrass breeding programmes include disease resistance, nutrient use efficiency, extending the growing season, persistency and yield. It is estimated that breeding in the past 50 years has yielded 4–5% gain in dry matter yield per decade (Wilkins and Humphreys 2003). Despite this, improving yield still remains one of the most important aims of current forage breeding programmes.

Classical breeding programmes can be assisted with the use of marker assisted breeding technology (Stuber et al. 1999). However, prior to this, the knowledge of the underlying genetic control of the target trait is required. One approach to dissect the genetic basis of complex traits is quantitative trait loci (QTL) mapping. Using molecular markers tightly linked to QTL associated with forage yield can be applied in molecular breeding programmes to assist traditional breeding. Marker-assisted selection (MAS) is one approach and relies on the establishment of a linkage relationship between a marker and a character of agronomic

importance, although the success of MAS with a complex trait such as biomass presents a challenge.

QTL studies investigating biomass yield have been reported in a number of species including Arabidopsis (Lisec et al. 2008), sorghum (Lin et al. 1995), or rice (Yu et al. 1997). Some studies in ryegrass have reported QTL for biomass yield related traits of leaf width, fresh weight, and dry matter (Armstead et al. 2008; Turner et al. 2008; Yamada et al. 2004). However, to date no larger study has reported QTL with reasonable sized effects on biomass yield in perennial ryegrass. In order to perform a QTL study investigating biomass, a population with excellent segregation for biomass is required. The present study makes use of an F_2 population based on two inbred grandparents where the F_1 has a large biomass yield phenotype greater than both parents and therefore by definition, displays heterosis. The resulting heterotic effect subsequently segregates widely in the F_2 generation. A genetic linkage map has been generated for this F_2 population (Anhalt et al. 2008). The creation of F_2 populations has been limited in perennial ryegrass because of the inherent difficulty in generating highly homozygous inbred lines. This was overcome in this case by an interspecific approach. This difficulty in generating homozygous inbred lines because of self-incompatibility and inbreeding depression represent the major barriers to exploiting heterosis in *Lolium perenne*, a technique that has yielded such enormous benefits in some of the major food crops.

The objectives of the present study were to measure biomass in an F_2 mapping population, segregating widely for yield, under both field and glasshouse conditions and attempt to identify major QTL for biomass yield.

Materials and methods

Plant materials

The 'F_2 biomass' population consisting of 360 F_2 genotypes has been described elsewhere (Anhalt et al. 2008). The two parental inbred ryegrass lines, which were chosen as parents of the single F_1 generation, originated each from an inter-specific cross between *Festuca pratensis* (meadow fescue) and *L. perenne* (Connolly and Wright-Turner 1984). In the pedigree

of the maternal inbred parent the ryegrass cultivar 'S24' was included and for the paternal inbred line parent the ryegrass cultivar 'Premo'. The initial interspecific hybrid was backcrossed for several generations to the ryegrass parent and selfed for 9 or 10 generations. The maternal parent of the F_1 population was hand emasculated and was pollinated with pollen from the paternal parent. Pollinated florets were bagged in cellophane bags, individual F_1 seed was harvested, and single F_1 plants were raised and self-pollinated by bagging in cellophane pollination bags to generate independent F_2 populations. One of these independent F_2 populations was used for subsequent experiments and genetic map construction.

Experimental design

An alpha lattice design was used for both the field and greenhouse experiments. For the generation of experimental layouts, Agrobase Generation 2TM was used (Agronomix Software, Inc., Winnipeg, Manitoba, R3N 0S4, Canada). Replicated greenhouse and field experiments were designed. Each replicate comprised 45 incomplete blocks, with each block consisting of nine different genotypes and in each of the incomplete blocks either the maternal, paternal or the F_1 genotype was included. The greenhouse experiment with plants in single pots was carried out from autumn 2005 to spring 2006 in three replicates under two greenhouse environments. Two out of the three replicates were placed in a greenhouse environment with the following climatic conditions: average night and day temperatures of 11 and 19°C, respectively. A third replicate was placed in a second greenhouse environment with an average night temperature of 6°C and day temperature of 19°C. The plants for the field experiment were planted in Oak Park, Carlow (Ireland) in spring 2006 with two replicates and the experiment was carried out over the two growing seasons from autumn 2006 to autumn 2007. In the field experiment, each genotype was planted in 'mini-swards' consisting of six clonal plants.

Collection of phenotypic data

From the greenhouse experiment three harvests were carried out in December 2005, February 2006 and

April 2006. For the field experiment, four harvests were taken in August 2006, October 2006, May 2007, and August 2007. After each cut the plants were supplied with 80 kg N/ha. To reduce experimental errors during harvesting, a measuring unit was used for the cuts to achieve uniform cutting heights. The cutting height was 8 cm for the greenhouse experiment and 5 cm for the field experiment. Since the plants in the field were smaller when the first harvest was done, the cutting height in the field was lower than in the greenhouse. Plant samples were dried to constant weight in an oven at 60°C. Fresh weight (g) and dry weight (g) were measured. Dry matter (%) was calculated as (dry weight/fresh weight) × 100. The trait leaf width was measured in the greenhouse in two replicates in February 2006. Four measurements were taken on four fully expanded young leaves of each genotype. The measurements were taken in the middle of the fully expanded leaf blades using callipers. The average of these four values of each genotype was taken for further calculations.

Climatic data were collected at the Institute monitoring station in Teagasc, Oak Park, Carlow.

Data analysis

All data analyses were carried out in SAS V9.1 (SAS Institute Inc. 2004. SAS OnlineDoc® 9.1.3. Cary, NC: SAS Institute Inc.).

Data of plants, which were alive but with a too low weight to be weighed out or which were under the cutting height were analysed as zeros values.

Log transformations for dry matter in the greenhouse and field and square root transformation for fresh and dry weight in the field were computed for the non-normally distributed data. All other traits remained untransformed. Analysis of variance (ANOVA) was carried out using PROC MIXED for each of the traits and both experiments.

To calculate means for parents, F_1 and F_2 at each harvest, the treatment effect was modelled by a fixed effect with four levels (F_1, F_2 and the two parents) and a random effect for entries within F_2. The random effect was fitted only for F_2 using the method described in Piepho et al. (2006). When transformed data were analysed, standard errors for backtransformed means, which estimate medians on the original scale, were computed by the delta method.

Broad sense heritability for each trait was calculated on a plot basis for the greenhouse and field environment using the formula: $H^2 = \sigma_g^2/\sigma_p^2 = \sigma_g^2/\left(\sigma_g^2 + \sigma_e^2\right)$, where σ_g^2 represents the genetic variance, σ_p^2 the phenotypic variance and σ_e^2 the environmental/error variance.

Pearson correlations were calculated for the measured traits fresh weight, dry weight, dry matter and leaf width.

QTL analysis

The sum of the yields of all harvests per plot were used for the QTL analysis in a mixed model using the model statement R : R · IBLOCK + R · IBLOCK · PLOT (Piepho et al. 2003) with the factors: plots (PLOT) within incomplete blocks and incomplete blocks (BLOCK) nested within complete replicates (R).

The F_2 population described previously (Anhalt et al. 2008) was used for QTL detection. Briefly, the map consists of 75 markers (10 AFLP and 65 SSR marker) across seven linkage groups with a total map length of 592 cM and an average map density of 8 cm. Sixty-three percent of the markers in the F_2 population did not fit the expected Mendelian ratios and showed significant ($P < 0.05$) segregation distortion. MapQTL® Version 4.0 (Van Ooijen et al. 2002) was used for QTL detection. Interval mapping (IM) followed by multiple QTL models (MQM) mapping was used to estimate the map position, LOD score, and genotypic effect of potential QTL, expressed as percentage of phenotypic variance explained. Markers with the closest positions to LOD peak of QTL identified by IM were selected as co-factors for MQM mapping. Genome wide LOD significance thresholds were determined using permutation tests with 1,000 iterations at a 5% significance level. QTL charts were displayed with MapChart Version 2.2 (Voorrips 2002).

Estimated means of the distribution of the quantitative trait associated with each genotype were used to rate the genetic effect of each QTL at the position of the highest LOD score. Only additive and dominant effects and no epistatic genetic effect can be found in an F_2 breeding programme since there is only one single F_1 genotype existing (Liu 1998). Estimated mean of distribution of the quantitative trait are displayed using the output data of the QTL

mapping in MapQTL® Version 4.0 (Van Ooijen et al. 2002).

Results

Phenotypic data

Generally, across the greenhouse and field experiments yields were highest in the F_1 generation. Across all harvests, biomass yields were much higher in the field than in the greenhouse experiment because of the mini-swards consisting of six clonal plants compared to the greenhouse experiment with single plants. Also growth was restricted in pots and the length of time between the harvests was different. Broad sense heritabilities were calculated for the three biomass traits in the greenhouse and the field experiments (Table 1).

Biomass fresh and dry weight values (Table 2) for parental, F_1 and F_2 genotypes had a wide range. Maternal and paternal lines had lower values compared to the F_1 and F_2 line (Table 2) which could point to heterosis in the F_1 and in F_2 genotypes and inbreeding depression in the parental lines (Table 2). Dry matter did not show the same effect for F_1 and F_2 genotypes compared to the inbred lines. Parental lines had a higher range in dry matter compared to the F_1 and F_2 lines. Biomass yield values had a wider range in the field than the greenhouse. These high differences in values of the F_1 and F_2 genotypes compared to the inbred lines in the field were obtained since genotypes grew better in the field compared to their parental inbred genotypes, which could have been due to environmental influences like soil structure or water availability. The parental inbred lines performed poorly under field conditions: they either died

or were shorter than the harvested height. After establishment in the greenhouse in 2005, the plants seemed to develop better in the late winter period than in the spring period, which could be influenced by higher irradiance in springtime. In 2006, more pronounced differences were found between individual harvests in the field, whereas in 2007 the genotypes had established under field conditions with the environmental conditions being more suitable for *L. perenne*. The rainfall distribution was notably different from 2006 to 2007, with only a third of the rainfall in June, July and August 2006 compared to 2007 (average 38 mm vs. 115 mm) and the reverse pattern during September, October and November 2006–2007 (127 mm vs. 41 mm).

Pearson correlation showed that all traits were significantly correlated ($P < 0.0001$). Fresh weight and dry weight were strong positive associated ($r = 0.979$), and leaf width had a moderate positive correlation with fresh weight ($r = 0.385$) and dry weight ($r = 0.352$) in the greenhouse experiment. Negative and moderate correlations were found between dry matter and fresh weight ($r = -0.370$), dry weight ($r = -0.204$), and leaf width ($r = -0.246$) in the greenhouse experiment. In the field experiment fresh weight and dry weight had a strong positive correlation ($r = 0.996$); whereas dry matter had a moderate negative correlation with fresh weight ($r = -0.474$) and dry weight ($r = -0.411$).

Identification of biomass QTL

A permutation test identified a LOD threshold between 3.2 and 3.5 for individual LGs, at which a QTL could be declared with 95% confidence. Using MQM mapping identical positions for fresh weight and dry weight QTL were consistently detected on LG 2, 3 and 7 (Table 3; Fig. 1) with one additional QTL for fresh and dry weight in the field on LG 5, which could not be found in the greenhouse experiment. The explained variance for the trait fresh weight at the QTL positions was 28% in the greenhouse and 50% for the field experiment and dry weight of 29% in the greenhouse and 53% for the field experiment.

Dry matter QTL were detected on LG 2, 3 and 7 in the field experiment (Table 3; Fig. 1). These were the only dry matter QTL detected in this study. Dry matter QTL appear to be in the same positions to other biomass QTL. Dry matter had an overall

Table 1 Broad sense heritabilities (H^2) on a plot basis for the traits fresh weight, dry weight, dry matter, and leaf width in the greenhouse and the field experiment, respectively

Trait	H^2 greenhouse experiment	H^2 field experiment
Fresh weight	0.4310	0.7393
Dry weight	0.4331	0.7406
Dry matter	0.2379	0.3799
Leaf width	0.3755	N/A

N/A Not applicable

Table 2 Median estimates of biomass yield and dry matter (±standard error, computed from log-transformed scale by delta method) for the three greenhouse and three field harvests averaged across the replicates: Average (Av) of fresh weight (g), dry weight (g), dry matter (%) of parental (PFa paternal, PM maternal), F$_1$ and F$_2$ lines

	Fresh weight (g)				Dry weight (g)				Dry matter (%)			
	Av PM	Av PFa	Av F$_1$	Av F$_2$	Av PM	Av PFa	Av F$_1$	Av F$_2$	Av PM	Av PFa	Av F$_1$	Av F$_2$
Greenhouse												
Harvest 1	1.9 ± 0.8	2.4 ± 0.7	7.4 ± 0.4	6.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	20.3 ± 1.0	21.0 ± 0.9	18.2 ± 0.4	15.0 ± 0.1
Harvest 2	1.0 ± 0.5	1.0 ± 0.6	9.0 ± 0.4	6.5 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	1.8 ± 0.1	1.2 ± 0.0	22.4 ± 0.7	22.9 ± 0.8	20.9 ± 0.5	19.5 ± 0.2
Harvest 3	4.9 ± 1.0	6.2 ± 1.0	10.9 ± 1.5	8.0 ± 0.3	1.0 ± 0.2	1.3 ± 0.2	2.5 ± 0.1	2.0 ± 0.0	23.3 ± 0.8	21.1 ± 0.7	21.7 ± 0.6	20.7 ± 0.2
Field												
Harvest 1	0.0 ± 0.0	0.2 ± 0.1	27.9 ± 15.7	20.5 ± 5.3	0.0 ± 0.0	0.1 ± 0.0	9.5 ± 3.1	7.2 ± 1.1	36.7 ± 4.0	36.8 ± 2.0	34.2 ± 0.7	35.7 ± 0.3
Harvest 2	0.0 ± 0.0	0.3 ± 0.3	64.3 ± 56.0	45.1 ± 14.7	0.0 ± 0.0	0.1 ± 0.0	14.2 ± 5.7	10.7 ± 1.6	N/A	24.3 ± 4.5	22.5 ± 1.0	24.8 ± 0.4
Harvest 3	0.2 ± 0.2	0.1 ± 0.1	174.9 ± 143.4	94.0 ± 47.4	0.1 ± 0.0	0.0 ± 0.0	46.5 ± 19.2	25.1 ± 6.3	29.7 ± 0.8	26.8 ± 1.4	26.6 ± 0.3	26.9 ± 0.2
Harvest 4	0.9 ± 0.8	0.5 ± 0.5	135.7 ± 112.4	97.4 ± 40.9	0.3 ± 0.1	0.2 ± 0.1	38.0 ± 16.4	26.9 ± 5.7	31.1 ± 1.1	28.7 ± 1.4	28.0 ± 0.4	28.2 ± 0.2

Greenhouse harvest dates: harvest 1 (December 2005), harvest 2 (February 2006), harvest 3 (April 2006). *Field harvest dates*: harvest 1 (August 2006), harvest 2 (October 2006), harvest 3 (May 2007), harvest 4 (August 2007)

N/A No data displayed because of too many missing values

explained variance of 21% for the field experiment. Leaf width was only calculated for the greenhouse experiment and QTL were identified on LG 3 and 4 (Table 3; Fig. 1). The total variance explained by these QTL was 15%.

The QTL with the largest effect for biomass yield were identified on LG 3 and were detected using both glasshouse and field experiment data. The QTL for dry weight accounted for 15% and 33% of the phenotypic variance in the glasshouse and field experiments, respectively. When looking at the allelic contribution of parental lines at QTL positions, it was noticeable that the maternal allele ('a') was dominant (Table 3). This appears to be true for all major QTL identified on LG 3. However the QTL for biomass yield on LG 2 showed additive gene action, where the yield of the heterozygote ('ab') was greater than either of the homozygotes.

Discussion

There have been previous QTL studies in *L. perenne* that have looked at biomass traits (Armstead et al. 2008; Turner et al. 2008; Yamada et al. 2004). However, this manuscript represents the first large scale biomass study in perennial ryegrass reporting QTL for both fresh and dry weight. Our study took advantage of an F$_2$ population derived from selfing an F$_1$ plant displaying considerable heterosis. This population exhibited significant segregation for biomass yield, making it the ideal population in which to study the underlying genetic components of the trait.

All biomass yield related values in the present study were highest for fresh and dry weight in the F$_1$ generation compared to the parental genotypes which is in accordance to the heterosis theory (Becker 1993). Decreasing heterosis values were observed over the harvest times for the field experiment, which can be explained by the much better growth over time and faster development of the F$_1$ and F$_2$ genotypes compared to the parental genotypes. Dry weather conditions in 2007 did not affect the performance of the F$_1$ and F$_2$ genotypes as much as the parental genotypes in the field, supporting the hypothesis that hybrids show increased yield stability. Similar findings were reported before in wheat hybrids (Bruns and Peterson 1998) where enhanced stability and improved agronomic traits were found in hybrids

Table 3 QTL for the greenhouse and field experiment for the traits fresh weight, dry weight, dry matter and leaf width as determined by multiple QTL mapping (MQM): Positions on linkage groups in cM, maximum LOD scores, percent of the phenotypic variation explained by the quantitative trait loci (QTL), position of QTL peak in cM on genetic map, marker selected as co-factor for MQM mapping, marker interval of QTL position

	LG	LOD threshold	LOD	% Explained variance	cM	Marker interval	Co-factor marker	Mean value (grams) for each allele class at the LOD peak of the QTL		
								aa	ab	bb
Fresh weight										
Greenhouse	2	3.2	4.3	6.5	52.9	G04_059–G04_030	M15185	17.1	20.3	15.6
Field	2	3.3	8.2	10.0	52.9	rv1269–M15185	G04_059	8.6	13.1	12.3
Greenhouse	3	3.2	8.2	14.7	12.4	EagcMcta–230–LPSSRK14F12	rv1133	20.2	22.4	11.6
Field	3	3.3	15.3	29.5	12.4	rv1133–LPSSRK14F12	rv1133	13.9	15.0	4.0
	5	3.3	4.0	3.7	53.6	rv1139–rv1188	–	11.8	11.8	9.2
Greenhouse	7	3.2	5.0	6.8	46.0	G04_002–B3A3	rv1411	18.7	19.9	15.0
Field	7	3.3	5.6	6.6	46.0	G04_002–B3A3	rv1411	11.5	12.8	9.3
Dry weight										
Greenhouse	2	3.3	3.4	5.1	52.9	G04_059–G04_030	M15185	3.2	3.7	3.0
Field	2	3.3	7.7	9.1	52.9	rv1269–M15185	G04_059	4.7	6.9	6.4
Greenhouse	3	3.3	8.2	14.6	12.4	EagcMcta–230–LPSSRK14F12	rv1133	3.7	4.2	2.3
Field	3	3.3	17.3	32.6	12.4	rv1133–LPSSRK14F12	rv1133	7.4	7.9	2.2
	5	3.3	3.9	3.6	53.6	rv1139–rv1188	–	6.3	6.2	5.0
Greenhouse	7	3.3	6.7	9.1	46.0	G04_002–B3A3	rv1411	3.6	3.7	2.8
Field	7	3.3	6.8	7.8	46.0	G04_002–B3A3	rv1411	6.2	6.7	4.8
Dry matter										
Field	2	3.2	3.8	6.6	52.9	G04_059–G04_030	M15185	3.4	3.3	3.3
	3	3.2	3.3	8.2	12.4	rv1133–LPSSRK14F12	rv1133	3.3	3.3	3.4
	7	3.2	4.2	6.0	39.5	EacaMcac–213–rv141	G04_002	3.4	3.4	3.3
Leaf width										
Greenhouse	3	3.5	6.8	7.7	27.3	B1A2–EacaMctc-100	B1A2	2.6	2.6	2.2
	4	3.5	6.0	6.9	14.1	DLF25–rv0380	G04_072	2.6	2.5	2.3

Genetic effects of QTL as estimated from means data for QTL mapping

aa Maternal inherited allele, *ab* maternal, *bb* paternal inherited allele

compared to pure line plants. It is likely that much of the heterosis in the F_1 generation is due to the increased heterozygosity and the loss of homozygous recessive alleles that may have accumulated during inbreeding of the parental genotypes. The accumulation of homozygous recessive alleles during inbreeding is the principle reason for observed 'inbreeding depression'.

Negative heterosis was observed for % dry matter and could be explained by increased cell sizes of the F_1 and F_2 plants compared to the parental lines. The cells may have been bigger and contained more water in F_1 and F_2 genotypes, which was removed by drying. This is supported by the results of the correlation analysis where dry matter content and leaf width were negatively correlated. Another explanation could be that in the F_1 and F_2 genotypes more water was stored than in the parental plants, which would explain why dry matter is increasing when leaf width is decreasing. Similar findings were reported for maize (Hoecker et al. 2006) in primary roots where the cortical cells were expanding in the hybrid plants. This would be also in agreement with another study in maize were a more rapid growth rate of leaf blades in hybrids was explained by increased cell number or enlargement of the cells (Uchimiya and Takahashi 1973). Dry matter is an important trait in plant development and ecology because it is
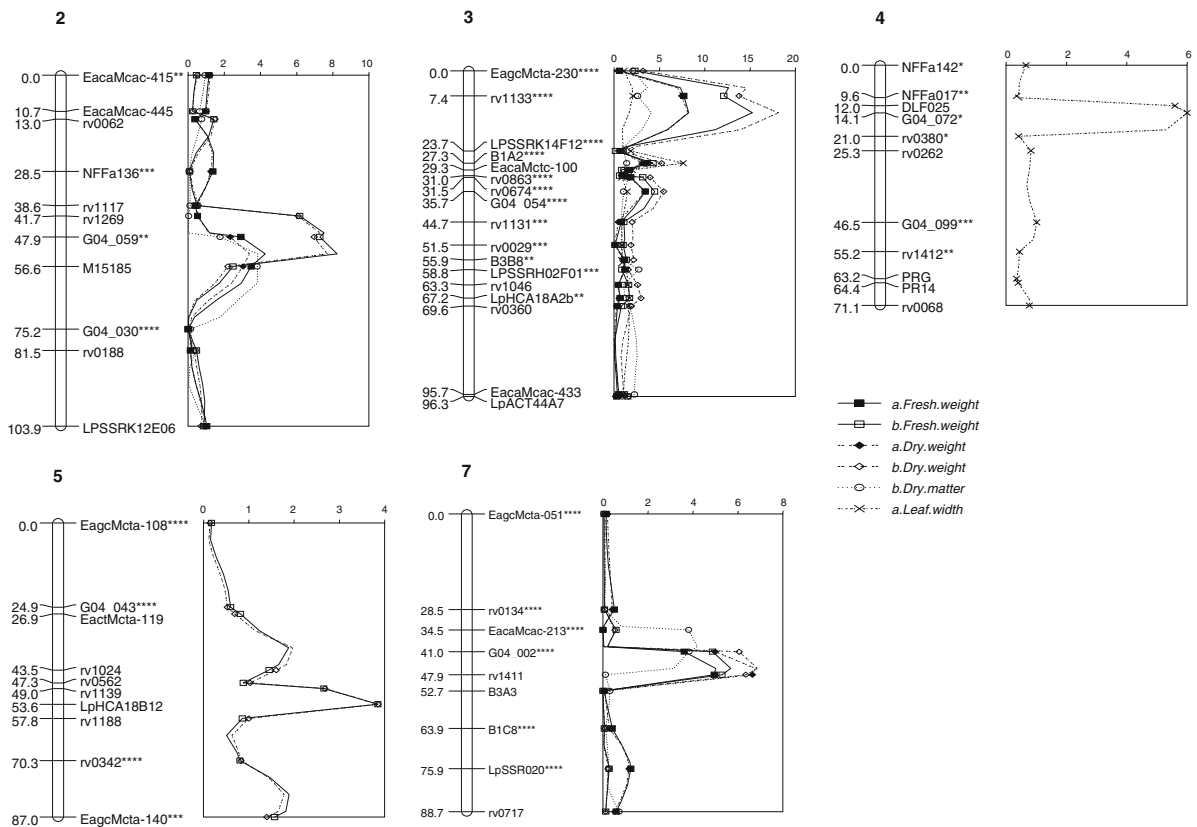
Fig. 1 Linkage groups (LGs) 2, 3, 4, 5 and 7 of the 'F₂ biomass' population genetic map (Anhalt et al. 2008). Distances are given in cM. *Asterisks* indicate segregation distortion (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). LOD scans for the traits fresh weight, dry weight, dry matter, and leaf width in the greenhouse ((a) and *filled shapes* and the field (b) and *open shapes*) are shown at each QTL position. QTL were calculated by MQM mapping in MapQTL V. 4.0 and are displayed with MapChart V. 2.2

directly connected with the growth rate and ecological behaviour of the plant.

QTL analysis was performed using phenotypic data from both a field and a glasshouse experiment. Three major QTL regions were identified on LG 2, 3 and 7 (Table 3; Fig. 1). This is the first report of QTL for fresh and dry weight on these three linkage groups in perennial ryegrass. These regions contained co-localizing QTL for both fresh weight and dry weight. In addition these QTL were identified for both the field and glasshouse experiments. Co-localizing QTL for fresh weight and dry weight are not surprising considering the significant correlation between both traits. Additional QTL for fresh weight and dry weight were detected on LG 5 in the field experiment. Overall more variation was explained in the field experiment, which could be because of more suitable environmental conditions for the genotypes. The

QTL for both fresh and dry weight on LG 3 explained the largest proportion of the phenotypic variance.

When examining the allelic contribution of parental lines to the major QTL on LG 2 and LG 3 differences were observed. The QTL on LG 3 appeared to be controlled by dominant gene action with the maternal parent contributing the allele for increased biomass. The QTL on LG 3 was controlled by additive gene action and the heterozygote had higher fresh and dry weights than either homozygote. Biomass yield is a highly complex trait and QTL for fresh and dry weight represent yield related QTL but they may also represent basic physiological processes that have an impact on yield. An example of this would be maturity or heading date. In this study heading date could not be measured in parallel to biomass yield due to the cutting regime. A lack of common markers between genetic linkage maps makes it difficult to superimpose

previous heading date QTL studies onto the present linkage map.

There have only been three previous QTL studies reporting on traits analysed in this study. Armstead et al. (2008) identified QTL for leaf width on LG 7 using the F₂ 'WSC' population. Turner et al. (2008) reported on fresh weight QTL on LG 1, 4, and 5 and autumn dry matter QTL on LG 3. Yamada et al. (2004) identified fresh weight QTL on LG 5 and leaf width QTL on LG 3 in the 'ILGI' population. However, a lack of common markers between various linkage maps makes it difficult to predict whether or not they represent the same QTL region. A large-scale QTL study on herbage quality traits has previously been performed in the perennial ryegrass p150/112 population (Cogan et al. 2005). The traits measured were crude protein, neutral detergent fibre, water soluble carbohydrate, in vitro dry matter digestibility and estimated metabolisable energy. Interestingly, a large cluster of QTL for all traits measured was detected on LG 3. The use of common markers to link the maps together would be useful to determine the relationship between the relatively large effect QTL for biomass yield detected on LG 3 in this study and the QTL for herbage quality parameters identified by Cogan et al. (2005).

There is controversy surrounding the use of spaced plants for studies of biomass. Forage yield measured in spaced plants is considered as a poor predictor of yield (Hayward and Vivero 1984). However, there are practical limitations to performing QTL studies in swards. In this study we created mini swards in the field comprised of six clonal replicates. We also performed a glasshouse study using single plants. It is worth noting that similar results were obtained from experiments using artificial mini swards in the field and single plants in the greenhouse.

The presented study is based on a reliable dataset for biomass yield heterosis and has identified consistent QTL regions for biomass yield over environments and replications. In summary, with glasshouse data we accounted for fresh weight 28%, for dry weight 29%, and for leaf width 15% of the phenotypic variation, respectively. With the field data we accounted for 50% of the phenotypic variance for fresh weight, 53% for dry weight, and 21% for dry matter. The development of cultivars with increased biomass yield is still a target trait of breeding programmes. Some of the identified QTL are therefore very promising for the identification of candidate genes for heterotic biomass yield after future detailed fine mapping efforts.

References

Anhalt UCM, Heslop-Harrison P, Byrne S, Guillard A, Barth S (2008) Segregation distortion in *Lolium*: evidence for genetic effects. Theor Appl Genet 117:297–306. doi:10.1007/s00122-008-0774-7

Armstead IP, Turner LB, Marshall AH, Humphreys MO, King IP, Thorogood D (2008) Identifying genetic components controlling fertility in the outcrossing grass species perennial ryegrass (*Lolium perenne*) by quantitative genetics. New Phytol 178(3):559–571. doi:10.1111/j.1469-8137.2008.02413.x

Becker H (1993) Pflanzenzüchtung. Eugen Ulmer Verlag, Stuttgart, 150 pp

Bruns R, Peterson CJ (1998) Yield and stability factors associated with hybrid wheat. Euphytica 100:1–5. doi:10.1023/A:1018364801101

Cogan NOI, Smith KF, Yamada T, Francki MG, Vecchies AC, Jones ES, Spangenberg GC, Foster JW (2005) QTL analysis and comparative genomics of herbage quality traits in perennial ryegrass (*Lolium perenne* L). Theor Appl Genet 110:364–380. doi:10.1007/s00122-004-1848-9

Connolly V, Wright-Turner R (1984) Induction of cytoplasmic male-sterility into ryegrass (*Lolium perenne*). Theor Appl Genet 68:449–453. doi:10.1007/BF00254817

Hannaway D, Fransen S, Cropper J, Teel M, Chaney M, Griggs T, Halse R, Hart J, Cheeke P, Hansen D, Klinger R, Lane W (1999) Perennial ryegrass (*Lolium perenne* L.). A Pacific Northwest Extension Publication, vol. PNW 502. Oregon State University, Washington State University, University of Idaho

Hayward MS, Vivero JL (1984) Selection for yield in *Lolium perenne*. II. Performance of spaced plant selections under competitive conditions. Euphytica 33:787–800

Hoecker N, Keller B, Piepho HP, Hochholdinger F (2006) Manifestation of heterosis during early maize (*Zea mays* L.) root development. Theor Appl Genet 112:421–429. doi:10.1007/s00122-005-0139-4

Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the *Poaceae*, in reference to an interspecific sorghum population. Genetics 141:391–411

Lisec J, Meyer RC, Steinfath M, Redestig H, Becher M, Witucka-Wall H, Fiehn O, Törjék O, Selbig J, Altmann T, Willmitzer L (2008) Identification of metabolic and biomass QTL in *Arabidopsis thaliana* in a parallel analysis of

RIL and IL population. Plant J 53:960–972. doi:10.1111/j.1365-313X.2007.03383.x

Liu HL (1998) Statistical genomics: linkage, mapping, and QTL analysis. CRC Press, Boca Raton

Piepho HP, Büchse A, Emrich K (2003) A hitchhiker's guide to mixed models for randomized experiments. J Agron Crop Sci 189:310–322. doi:10.1046/j.1439-037X.2003.00049.x

Piepho HP, Williams ER, Fleck M (2006) A note on the analysis of designed experiments with complex treatment structure. HortScience 41:446–452

Stuber CW, Polacco M, Senior ML (1999) Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci 39:1571–1583

Turner LB, Cairns AJ, Armstead IP, Thomas H, Humphreys MW, Humphreys MO (2008) Does fructan have a functional role in physiological traits? Investigation by quantitative trait locus mapping. New Phytol 179:765–775. doi:10.1111/j.1469-8137.2008.02495.x

Uchimiya H, Takahashi N (1973) Kinetics of heterosis in growth of the leaf blade in *Zea mays* L. Ann Bot (Lond) 37:147–152

Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2002) Map QTL 4.0: software for the calculation of QTL positions on genetic maps. Plant Research International, Wageningen

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93(1):77–78. doi:10.1093/jhered/93.1.77

Wilkins PW, Humphreys MO (2003) Progress in breeding perennial forage grasses for temperate agriculture. J Agric Sci 140:129–150. doi:10.1017/S0021859603003058

Yamada T, Jones ES, Cogan NOI, Vecchies AC, Nomura T, Hisano H, Shimamoto Y, Smith KF, Hayward MD, Forster JW (2004) QTL analysis of morphological, developmental, and winter hardiness-associated traits in perennial ryegrass. Crop Sci 44:925–935

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang QA, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231. doi:10.1073/pnas.94.17.9226