QTL analysis of morphological, developmental and winter hardiness-associated traits in perennial ryegrass (*Lolium perenne* L.)

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ABSTRACT

Quantitative trait loci (QTLs) for a number of agronomically important traits of perennial ryegrass (*Lolium perenne* L.) were identified using a reference molecular marker-based genetic map. Replicated phenotypic data was obtained for a number of field-assessed morphological and developmental traits as well as the winter hardiness-associated characters of winter survival and electrical conductivity. Marker-trait association analysis was performed using a number of methods, and a high degree of congruence was observed between the respective results. QTLs were detected for morphological traits such as plant height, tiller size, leaf length, leaf width, fresh weight at harvest, plant type, spikelet number per spike and spike length, as well as the developmental traits of heading date and degree of aftermath heading. A number of traits were significantly correlated, and coincident QTL locations were identified. No significant QTLs for winter survival in the field were identified. However, a QTL for electrical conductivity corresponding to frost tolerance was located close to a heading date QTL in a region that may show conserved synteny with chromosomal regions associated with both winter hardiness and flowering time variation in cereals. The QTL analysis of multiple phenotypic traits provides the basis for marker assisted selection (MAS) of important agronomic characters, allowing genetic improvement of yield, quality and adaptation in perennial ryegrass breeding.

KEYWORDS

Lolium perenne, QTL analysis, morphological traits, heading date, winter hardiness, conserved synteny, molecular breeding.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is the most widely sown perennial forage grass in temperate regions of the world. The popularity of perennial ryegrass in pastoral agriculture is largely due to high yield of digestible nutrients combined with good tolerance to grazing and adequate seed production (Wilkins, 1991).

The morphogenesis of individual grass plants within a grazed sward plays a key role in determining herbage yield, persistence and recovery from grazing. In vegetative plants, plant morphogenesis is described by three key variables: leaf appearance rate, leaf elongation rate and leaf lifespan. The expression of each of these traits is under both genetic and environmental control (Lemaire and Chapman, 1996), and leaf development in *Lolium* has been demonstrated to be under genetic control in a number of studies (Edwards and Cooper, 1963; Rhodes, 1973; Hazard et al*.,* 1996). Structural characteristics of plants such as tiller number, leaf number and leaf size are the result of these morphogenetic traits, and their measurement in breeding programs allows a dissection of the complex herbage yield trait as well as predictions of the response to grazing.

Different ecoclimatic regions, or pastures under different grazing regimes, may provide alternative selection goals for these traits. For instance, cool season growth is an important breeding objective in climates with mild winters. Mediterranean genotypes of perennial ryegrass have more rapid rates of leaf appearance and elongation in winter than genotypes from Northern Europe and hence better cool season herbage yield (Cooper, 1964). However, this trait may be related to the onset of reproductive development (Kemp et al*.*, 1989) and be

negatively correlated with survival during harsh winters. In addition, selection for large leaves has been shown to increase yield under infrequent grazing (Rhodes and Mee, 1980; Hazard and Ghesquière, 1997). However, interactions between grazing management and optimal leaf size have been detected, with short-leaved selections being better adapted to frequent cutting (Hazard and Ghesquière, 1997).

Genetic variation in morphogenetic traits associated with reproductive development is of practical importance in perennial ryegrass breeding not only due to the potential correlation between these traits and vegetative production, but also to the association of these traits with seed yield (Elgersma, 1990a). Traits such as spike numbers, spikelets per spike and florets per spikelet have been shown to be heritable (Elgersma, 1990b).

The manipulation of these morphological and developmental traits in a breeding program can be improved by knowledge of the underlying genetic control mechanisms. The detection of QTLs associated with these traits in perennial ryegrass is consequently likely to provide breeders with enhanced possibilities for the development of highly adapted germplasm.

Perennial ryegrass is susceptible to winter stresses caused by both snow moulds and low temperatures (Jamalainen, 1974; Jönsson and Nilsson, 1985; Nakayama et al., 2001). In addition to the improvement of growth characteristics, increased winter hardiness of perennial ryegrass is an important breeding objective in northern cold climate regions. Genetic analysis of the components of winter hardiness has been carried out in cereals such as wheat (*Triticum*

aestivum L.) and barley (*Hordeum vulgare* L.) (Cahalan and Law, 1979; Brule-Babel and Fowler, 1988; Doll et al., 1989), allowing the chromosomal location of the genes controlling these characters to be determined. A similar approach may be employed for pasture grasses.

Genetic map-based analysis permits the dissection of complex phenotypes by resolving the locations of interacting and pleiotropic genetic factors. There have been relatively few reports to date of QTL analysis for agronomic traits in perennial ryegrass, due to the absence of a sufficiently well developed genetic map. An enhanced molecular marker-based genetic linkage map of perennial ryegrass has recently been constructed through the activities of the International *Lolium* Genome Initiative (ILGI) (Forster et al., 2001), using the p150/112 one-way pseudo-testcross mapping population. The current map contains 109 restriction fragment length polymorphism (RFLP) loci detected by heterologous probes from wheat, barley, oat and rice. Comparative genetic mapping has allowed the alignment of the perennial ryegrass genetic map with those of wheat, rice and oat, revealing substantial conserved synteny with the genomes of Triticeae species (Jones et al., 2002a). The p150/112 genetic map has been further enhanced by the assignment of nearly 100 polymorphic perennial ryegrass simple sequence repeat (LPSSR) loci (Jones et al., 2001) to locations on each of the seven linkage groups (Jones et al., 2002b). As a consequence, a total of more than 200 co-dominant genetic markers have been mapped in the p150/112 family, along with about 200 amplified fragment length polymorphism (AFLP) loci, permitting detailed genetic analysis of traits that vary within this

population. The SSR loci provide the means to align genetic maps between different mapping families and extrapolate QTL locations between divergent germplasm sources.

Our objective in this study was to use the enhanced genetic map of perennial ryegrass to locate QTLs for a range of agronomic phenotypic traits in the p150/112 population. The emphasis was on morphological and developmental traits associated with pasture productivity, reproductive development traits and winter hardiness characters that may influence survival and subsequent performance in cold climate environments.

MATERIALS AND METHODS

Genetic Mapping Family

The p150/112 reference genetic mapping population was derived from a pair-cross between a multiply heterozygous plant as pollinator and a doubled haploid (DH) as the female parent (Bert et al., 1999; Jones et al., 2002a). The cross was generated at the Institute of Grassland and Environmental Research (IGER), Aberystwyth, UK, and clonal replicates of up to 183 progeny individuals and the multiply heterozygous parent were distributed to ILGI participant laboratories for genotypic and phenotypic analysis. The doubled haploid genotype (DH290) did not survive and was consequently not available for phenotypic analysis.

Phenotypic Assessment of Morphological and Developmental Characters

Individual plants from the p150/112 mapping family were transplanted at Nagasaka, Japan (35^{o}49' N, 138^{o}22' E) in the field nursery of the Yamanashi Prefectural Dairy Experiment Station (YPDES) in July 1996 with five replicates of each genotype in a randomised complete block design. Morphological characters (plant height, tiller number, tiller size [diameter of the stem of the reproductive tiller], leaf length, leaf width, fresh weight at second harvest, plant type, number of spikelets per spike and spike length) and developmental characters (heading date and aftermath heading) were measured in the subsequent year. Two cutting regimes were performed in 1997, the first at the

heading date for each genotype and the second on July 14th for all genotypes. Plant height (maximum length in cm from the base to the top of the plant) and tiller number were measured in cm on 30th June 1997, while spike length, leaf length and leaf width (in cm) and tiller size (in mm) were measured at the heading date for each genotype. Leaf length and leaf width were measured using the flag leaves from single tillers, and spike length, number of spikelets per spike and tiller size were measured on the same tillers. Fresh weight at second harvest (14th July, 1997) was measured in grams, plant type was measured (on 30th June, 1997) on a scale from 1-9 with 1 being most erect and 9 being most prostrate, heading date (ear emergence) was measured in days after May 1st 1997 and the degree of aftermath heading (on 14th July 1997) was measured on a scale from 0-9 with 0 corresponding to no heads and 9 corresponding to many heads. Measurements were made on one plant per genotype from each of the five replicates for tiller number, fresh weight, plant type, plant height, heading date and aftermath heading. Measurements were made on 5 leaves from two replicates (10 leaves in total) for leaf length, leaf width, number of spikelets, spike length and tiller size at the time of heading.

Phenotypic Assessment of Winter Hardiness Characters

One plant of each p150/112 mapping family genotype was grown outside in pots at the National Agricultural Research Centre for Hokkaido Region (NARCH) located at Sapporo, Japan (43°00' N, 141°25' E). Electrical conductivity was measured on 3 leaves from each plant. Leaves were excised in 5 cm long

sections at a 10-15 cm height from the base in December 1999, and were shredded, placed into culture dishes and transferred to a temperature of -2^{o}C. After a 12 hr equilibration period, the temperature was lowered manually in 1^{o}C decrements every one hour to a final value of -6^{o}C and samples were then held for 8 hrs before being placed in microtubes. Distilled water (1.0 ml) was added to each tube, and samples were held at 5^{o}C for 12 hrs. The conductivity of the resulting solution was measured using a conductance meter. A comparative value for 100% leakage was obtained by freezing replicate samples from each genotype at -80^{o}C for 4 hrs.

Individual plants from the p150/112 mapping family were also grown at the field premises of NARCH, with four replications in replicated block design from 1999 onwards. Survival in the field at Sapporo following the winter of 1999-2000 was measured in April 2000 using a visual assessment score (from 1 to 5) of plant recovery on all 4 replicates.

Statistical Analysis

Data analyses were carried out in SAS (SAS Institute Inc.). The significance of progeny and replicate effects were analysed using general linear modelling (Proc GLM), Broad sense heritabilities were calculated according to Wricke and Weber (1986). The Shapiro-Wilk statistic (W-test) was used to assess the normality of averaged data (normal option in Proc UNIVARIATE). Spearman's rank-order correlation coefficients were determined for pair-wise comparisons of averaged trait data (spearman option in Proc CORR).

QTL Analysis

A framework set of genetic markers from the p150/112-based reference map (Jones et al., 2002a), including the majority of the heterologous RFLP loci, was combined with the perennial ryegrass SSR locus data (Jones et al., 2002b) to produce a composite dataset for QTL analysis of the phenotypic data. Following genetic map construction using MAPMAKER 3.0, a sub-set of marker loci was selected to provide even coverage of the genome with marker intervals of close to 5 cM, and consensus map distances were subsequently used. Simple linear regression (SMR) was initially employed to identify significant variation with selected genetic markers to provide approximate locations for QTLs. The log-of-odds (LOD) score of association between the genotype and trait data was calculated using interval mapping (IM) in MAPMAKER/QTL (Lander et al., 1987) with the free model of QTL effect. A minimum LOD threshold of 2.0 was selected for significance of location of the QTL for IM. Composite interval mapping (CIM: Zeng, 1994) was performed using the Windows QTL Cartographer 2.0 application (Basten et al., 1994). Permutation analysis (1000 iterations) was used to establish an experiment-wise significance value at the 0.05 confidence level defined as a minimum LOD threshold for each trait in CIM (Churchill and Doerge, 1994; Doerge and Churchill, 1996). For each form of interval analysis, the maximum LOD value associated with the most closely linked marker, the weight value associated with additive marker allele effects and the proportion of the phenotypic variance attributable to the QTL were tabulated.

RESULTS AND DISCUSSION

Analysis of phenotypic variation for agronomic traits

The distribution data for morphological and developmental traits are shown in Figures 1(A) and (B). Substantial variation is observed for the majority of these characters, with a range of 30.8 cm for plant height, 194.3 g for fresh weight at second harvest, 24 days for variation in heading date and 4 units for plant growth type from moderately erect to moderately prostrate. The corresponding data for the winter hardiness characters are shown in Figure 1(C). The average phenotypic score for the heterozygous parent was located towards one tail of the progeny distribution range for the majority of traits. The parental phenotype was very close to the progeny mean for the spike length, number of spikelets per spike and winter survival traits, providing evidence for transgressive segregation.

Significant phenotypic variation was detected between progeny individuals for all traits except for leaf width (Table 1). Broad sense heritability values for significantly variable traits ranged from 0.46 for leaf length to 0.9 for heading date. Significant replicate effects were observed for tiller number, plant type and winter survival. The distribution of averaged data deviated significantly from normality for tiller number and aftermath heading traits (skewed towards high numerical values) and for the leaf length, fresh weight, plant type, heading date and electrical conductivity traits (skewed towards low values).

Coefficients of phenotypic correlations between traits ranged from non-significant to a maximum value of 0.68 (Table 2). A number of the

morphological and developmental traits showed highly significant positive correlations, such as plant height with spike length, tiller size and leaf length, and plant type with heading date. The positive correlations between shoot growth characteristics reflect the overall developmental pattern of a large, vigorous plant. The reproductive morphogenetic trait of number of spikelets per spike is positively correlated with these characteristics, and may also reflect general plant vigour. The positive correlation between heading date and plant type has been previously described for populations such as the Australian variety ‘Kangaroo Valley’ (Shah et al., 1990), in which erect growth habit is associated with early flowering and prostrate growth habit is associated with later flowering. These effects are also consistent with the observed positive correlation in this study between later heading date and reduced plant height. However, the correlation between later flowering and decreased tiller number is not general and may be an artefact due to the complex history of the heterozygous parent in this cross. Significant negative correlation between heading date and plant height was also anticipated in this study, as each genotype was cut at the heading date and showed variable rates of regrowth at the time of data collection. However, the variation of plant type and tiller number is likely to be largely independent of the rate of regrowth. Variation for tiller number will include the large number of tillers formed during growth prior to flowering, as well as the relatively small number formed during regrowth.

There were no significant correlations between winter survival and any of the morphological or developmental traits measured.

Comparison of analytical techniques for marker-trait association

Significant associations between marker and trait data were established for the majority of traits using single marker regression (SMR), based on analysis of variance (ANOVA) at the $p < 0.01$ significance level. Interval mapping (IM) subsequently identified 17 significant QTLs (maximum LOD $\geq$ 2) for 11 of the 13 measured traits. Composite interval mapping (CIM) was also performed on the dataset following determination of empirical LOD thresholds, allowing the identification of 20 QTLs for the 11 traits (Table 3). A large proportion of QTL locations were identified by both IM and CIM. For example, for the heading date trait, genetic markers in the interval from 39.9 to 72.3 cM on linkage group (LG) 4 were significantly associated with the trait data by SMR, while IM detected a QTL with maximum LOD value of 4.0 close to the xlpssrh01h06 locus at 53.5 cM, and CIM detected a QTL with a maximum LOD value of 3.6 at 56.3 cM with an empirical LOD threshold of 2.9.

A number of discrepancies in QTL detection were identified through comparison of the different analytical techniques. In several instances, SMR analysis revealed significant marker-trait associations and IM detected QTLs above the LOD threshold, but the trait-specific threshold value determined for CIM was not exceeded. For instance, for the spike length trait, genetic markers in the interval from 25.5 to 84.1 cM on LG1 were significantly associated with the trait data, while IM detected a QTL with maximum LOD value of 4.7 close to the e33t50175 locus at 53.9 cM, but CIM detected a QTL with a maximum LOD value

of 2.7 at 53.9 cM with an empirical LOD threshold of 2.8. In this case, the identification of a significant region of the genome controlling the trait by SMR and IM and the closeness of the maximum and threshold values determined by CIM would tend to support the inference of a genuine genetic effect in this region. Other examples are less obvious: for the trait of plant height, SMR analysis revealed significant associations for markers on LG1 and IM identified a QTL with a maximum LOD value of 3.6, but the maximum LOD value determined by CIM was 1.3, considerably lower than the threshold value of 2.8. In addition, for both the leaf length and leaf width traits, only single markers were significantly associated with the respective trait data. IM identified significant QTLs with maximum LOD values of 2.1 for both traits, but no significant QTLs were identified by CIM. Clearly, QTL detection in such circumstances should be treated as indicative rather than definitive.

The different results obtained by different forms of analysis are also shown for the trait of electrical conductivity. The terminal marker on LG4 (xr2702Bb) was identified as significantly associated with the trait and IM detected a QTL with maximum LOD value of 2.0 close to this marker. However, no significant QTLs were detected on this LG by CIM. In contrast, two significant QTLs on LG6 were detected by CIM, with maximum LOD values greater than the threshold value of 2.6. No supporting evidence is available from SMR or IM analysis for these QTL. The LG6 electrical conductivity QTLs are in close linkage and in repulsion phase, as indicated by weight values of –2.99 and 3.7 respectively, as well as accounting for similar proportions of the phenotypic variance. The similar but opposing

effects of these regions may account for the failure of detection by SMR and IM. For both LGs (4 and 6), but on different criteria, the QTL effects for electrical conductivity are suggestive, but require further validation.

Genetic control of morphological and developmental traits

Between 1 and 3 QTLs were detected for each of the morphological and developmental characters with either IM or CIM (Table 3, Figure 2). QTLs for different traits were frequently located in the same chromosomal region, with coincident groups on LGs 1, 3, 4 and 5. The coincident QTLs corresponded to traits that were significantly correlated, and the directions of the QTL effects were in agreement with the sign of the correlations. For instance, coincident QTLs for plant height, tiller size, number of spikelets per spike and spike length were identified by IM on LG1, and these traits show significant positive phenotypic correlations. The QTL weight values determined by both types of mapping analysis (Table 3) were also all positive.

Coincident QTLs for traits associated with plant size have been identified in three regions, most strikingly on LGs 1 and 3. The positive weight values of the co-locating QTLs suggest two possible interpretations. Allelic variation at a single pleiotropic locus could be responsible for the concurrent increase (or decrease) of phenotypic values for the relevant traits. In barley, large QTL effects on plant height are associated with variation at the *denso* dwarfing gene (Bezant et al. 1996), which maps to 3H, the syntenic counterpart of perennial ryegrass LG3 (Jones et al., 2002a). It is possible that single underlying scale-determining loci may have been detected in this study. Alternatively, a number of *cis*-linked alleles with similar directions of effect may be present at different loci. In either case, selection for a single set of linked markers would lead to an increase for each trait.

Despite the highly significant correlations between heading date and the morphogenetic traits of plant type, tiller number and plant height, only one QTL (for plant type) co-locates with the heading date QTL. This observation supports the previous inference that plant type and tiller number are largely independent of the period of regrowth after cutting, and that the harvesting regime following the heading date did not impair the ability to detect QTLs for these traits. If the QTLs resulting in phenotypic variation for these traits had been a simple reflection of genetic factors segregating for heading date, a large number of coincident QTLs would have been expected.

The only traits in this study that reveal non-coincident QTLs were plant type (LG7) and aftermath heading (LG6). These QTLs offer the potential to select for the corresponding traits without correlated effects on other traits. Plant type is of significance in the breeding of perennial ryegrass for turf quality, which is associated with a more prostrate growth habit. The large plant type QTL on LG7 provides a good target for marker-assisted selection of growth habit. Aftermath heading is usually associated with early flowering perennial ryegrass varieties suitable for hay making, which tend to show reduced perenniality and persistence. For this reason, a high degree of aftermath heading is likely to be disfavoured as a breeding objective but could provide a target for counter-selection based on linked marker analysis.

A single QTL for heading date was observed in the current study. However, a number of QTL positions for this trait have been reported from the analysis of single mapping populations in other Poaceae species. Previous mapping studies

in perennial ryegrass have produced more complex results (Hayward et al., 1994). The absence of common markers between the population used in this and the present study prevents any inference of common location. The number of QTLs and their relative importance may vary according to the origin of the genotypes used to construct mapping families. Studies on geographical populations of *Lolium* species covering the climatic range from the Mediterranean region to northern and central Europe revealed a regular cline in flowering responses to temperature and photoperiod (Cooper, 1960). The heterozygous parent of the p150/112 mapping population was derived from a cross between eastern European (Romanian), southern European (north Italian ecotypes) and northern European ('Melle' or 'S23') genotypes, and might be expected to represent a variety of response genes. It is also likely that small QTLs for heading date have not been detected in this analysis. The single QTL for heading date on LG4 accounts for about 20% of the phenotypic variation based on IM, but the character shows a high broad sense heritability (0.90), suggesting that a substantial proportion of the heritable variation has not been attributed to specific genomic regions.

Genetic control of winter-hardiness traits

No significant QTLs were detected for winter survival. It is known that field assessment of winter survival may be confounded by experimental errors (Fowler, 1979). The electrical conductivity method, also known as the ion leakage method, has been used extensively for the evaluation of freezing tolerance through

measurement of the release of cellular electrolytes after freezing (Dexter et al., 1930; Dexter et al., 1932). Previous studies of the winter survival of barley, wheat, rye and triticale cultivars in Finland, where frost was considered to be the more important stress factor than snow (Hömmö, 1994), showed good correlations with hardening ability as assessed by the electrical conductivity method. A single QTL for electrical conductivity was detected in the upper part of LG4 by SMR and IM, but not CIM, accounting for 11.8% of the total phenotypic variation and adjacent to the heading date QTL. Late flowering and reduced freezing tolerance (as indicated by high electrical conductivity) is not significantly correlated in this cross, but the weight values of the linked QTLs were positive, suggesting that *cis*-linked alleles were causing an increase in phenotypic values of both traits. This is inconsistent with previously identified negative correlations between freezing tolerance and heading date (Humphreys and Eagles, 1988) and may be due to the complex nature of the cross.

Winter hardiness in wheat (Sutka, 1994; Galiba et al., 1995, 1997) and barley (Pan et al., 1994) is associated with QTLs on the homoeologous group 5 chromosomes in the same region as the vernalisation response genes that control heading date. In this study, winter hardiness-associated and heading date QTLs were located on LG4 in perennial ryegrass. Perennial ryegrass LG4 has been proposed to correspond predominantly to the homeologous group 4 chromosomes of the Triticeae cereals (Jones et al., 2002a). However, the upper part of LG4 has been shown to contain heterologous RFLP markers that map to the wheat homeologous group 5 chromosomes and its syntenic counterpart, rice

chromosome 3. Evolutionary translocations between group 4 and group 5 homoeologous chromosomes have been observed for several Triticeae genomes (Devos et al., 1995). A comparative map has been constructed for meadow fescue (*Festuca pratensis* Huds.) using heterologous anchor RFLPs, many of which are common with the perennial ryegrass study (Alm, 2001, 2003). A region syntenic with the portion of the Triticeae 5L chromosomes that corresponds to rice chromosome 3 is present on the upper part of meadow fescue LG4. The *Lolium* and *Festuca* genera are closely related, and the high level of recombination observed in triploid F_1 hybrids between *L. perenne* and *F. pratensis* suggests conservation of gene order (King et al., 1998). An evolutionary translocation between linkage groups corresponding to groups 4 and 5 of the consensus wheat map may have occurred before the divergence of the Poeae grasses. Alm (2001) also performed QTL analysis of frost and drought tolerance in meadow fescue and detected a small QTL for frost tolerance on LG4. This provides further evidence for the presence of genes for winter hardiness on the group 4 chromosomes of the *Lolium* and *Festuca* genera as compared to the group 5 homoeologous chromosomes of the Triticeae.

Conclusions

The analysis presented here provides an insight into the genetic control of a number of important growth and adaptation characters in current perennial ryegrass breeding programs, along with an interpretation of their interdependence in genetic and developmental terms. However, it should be

noted that although substantial replication was performed within the experimental design, the study is restricted to a particular locality and time. For this reason, it is conceivable that genotype x environment (G x E) interactions could lead to the detection of different QTL locations for equivalent traits in other studies. The establishment of the p150/112 mapping family at a number of ILGi-participant laboratories now provides the basis for such comparative QTL mapping studies.

The use of several marker-trait association analysis techniques such as SMR, IM and CIM provides the means to assess the robustness of QTL detection in a single experimental design. A high degree of congruence between the results of different methods was observed in the present study, with the majority of QTLs detected by the standard IM analysis confirmed by SMR and CIM. However, CIM detected a number of extra QTLs for several traits, and estimates of maximum LOD value with CIM showed both increases and decreases of significance compared to IM. These results have provided evidence to support the inference of real genetic effects associated with particular genomic regions, and may be used to prioritise the selection of putative marker-QTL combinations for implementation studies.

The identification of QTL locations for selected agronomic traits by molecular-marker based mapping will allow the design of appropriate marker-assisted selection strategies. This will include co-selection for desirable characters with coincident QTL locations and counter-selection to break potential unfavourable linkages between negatively correlated traits. The linked marker haplotypes associated with favourable QTL alleles in the current family are

available for marker assisted selection. The use of a reference genetic map containing readily transferable SSR and RFLP markers will permit the inference of common QTL locations for the same traits in other experimental pair-crosses, as well as comparative genetic analysis of homologous characters in other Poaceae species.

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FIGURE LEGENDS

Fig. 1

Frequency distribution bar-charts for traits associated with (A) vegetative morphogenesis, (B) reproductive morphogenesis and development and (C) winter hardiness measured in the progeny set of the p150/112 reference genetic mapping population in perennial ryegrass. Phenotypic scores have been assigned to intervals on the y=0 axis up to the maximum value indicated for each interval. The mean score of the heterozygous p150/112 parent is indicated in the relevant interval by an arrow, with the appropriate numerical value. The doubled haploid parent was not available for phenotypic analysis.

Fig. 2

Location of QTLs for morphological traits, developmental traits and electrical conductivity on the SSRP, RFLP and AFLP-based reference genetic map of perennial ryegrass, based on the results of interval mapping (IM). The maximum likelihood position of the QTL is indicated with an arrow. Bar and line lengths indicate a LOD drop of 1.0 and 2.0 respectively on either side of the maximum likelihood position.

Table 1. General statistics for phenotypic traits measured in this study

	Reps	W[a]	Skew[b]	Significance[c]		
				Progeny	Rep	H^{2d}
Plant Height (cm)	5	0.98^{ns}	-0.39	***	ns	0.74
Tiller Number	5	0.93**	-0.42	***	***	0.81
Tiller Size (mm)	2	0.97^{ns}	-0.39	***	ns	0.59
Leaf Length (cm)	2	0.74***	3.79	**	ns	0.46
Leaf Width (cm)	2	0.97^{ns}	0.03	ns	ns	0.30
Fresh Weight	5	0.96*	0.57	***	*	0.73
Plant Type (1-9)	5	0.91***	0.84	***	***	0.86
Number of Spikelets per Spike	2	0.96^{ns}	-0.69	***	ns	0.55
Spike Length (cm)	2	0.98^{ns}	-0.49	***	ns	0.85
Heading date (days after 1.05.97)	5	0.96*	0.67	***	ns	0.90
Aftermath heading (0-5)	5	0.95**	-0.41	***	ns	0.73
Electrical Conductivity	1	0.91***	1.35	-	-	-
Winter survival (0-5)	4	0.97^{ns}	0.10	***	**	0.48

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = $P > 0.05$.
[a] Normality of averaged data tested using the Shapiro-Wilk statistic.
[b] Skewness of averaged data. Negative deviations indicate skewness towards high numerical values.
[c] Significance of progeny and replicate effects analysed using general linear modeling.
[d] Broad sense heritabilities.

Table 2. Correlation coefficients between traits estimated using Spearman's rank correlation analysis

	Plant Height	Tiller Number	Tiller Size	Leaf Length	Leaf Width	Fresh Weight	Plant Type	Number of Spikelets per Spike	Spike Length	Heading Date	Aftermath Heading	Electrical Conductivity
Tiller Number	0.63***											
Tiller Size	0.45***	0.25*										
Leaf Length	0.61***	0.43***	0.55***									
Leaf Width	0.18^{ns}	0.11^{ns}	0.5***	0.39**								
Fresh Weight	0.56***	0.75***	0.34**	0.48***	0.30**							
Plant Type	-0.41***	-0.32**	0.05^{ns}	0.10^{ns}	0.17^{ns}	-0.04^{ns}						
Number of Spikelets per Spike	0.36**	0.19^{ns}	0.46***	0.36***	0.31**	0.34^{ns}	-0.19^{ns}					
Spike Length	0.68***	0.38***	0.67***	0.68***	0.35*	0.51***	-0.01^{ns}	0.63***				
Heading Date	-0.63***	-0.58***	-0.27*	-0.18^{ns}	-0.01^{ns}	-0.51***	0.60***	-0.20^{ns}	-0.33**			
Aftermath Heading	-0.04^{ns}	-0.3**	0.10^{ns}	0.15^{ns}	0.15^{ns}	-0.14^{ns}	0.07^{ns}	0.22*	0.05^{ns}	0.06^{ns}		
Electrical Conductivity	0.05^{ns}	0.15^{ns}	-0.08^{ns}	0.08^{ns}	0.08^{ns}	-0.24*	-0.07^{ns}	0.12^{ns}	0.09^{ns}	-0.03^{ns}	-0.22^{ns}	
Winter Survival	-0.02^{ns}	-0.10^{ns}	0.14^{ns}	0.03^{ns}	0.03^{ns}	0.07^{ns}	0.19^{ns}	-0.01^{ns}	0.07^{ns}	0.01^{ns}	0.14^{ns}	0.02^{ns}

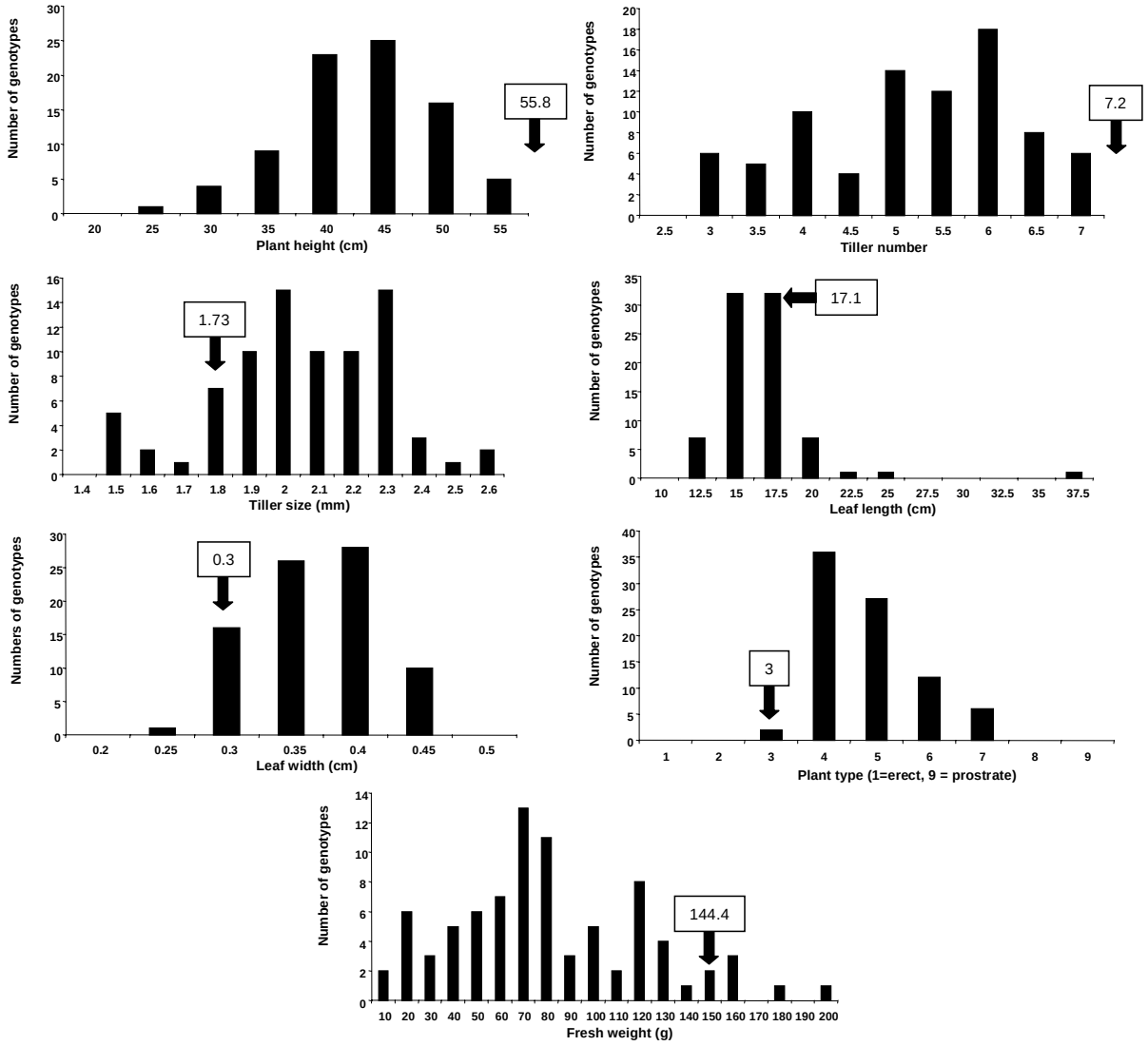
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = $P > 0.05$.

Table 3. Summary data for marker-trait association analysis in the perennial ryegrass p150/112 reference genetic mapping family

Trait	Linkage group in p150/112 reference genetic map	Single marker regression cM region pr(f) <0.01	Interval mapping			Composite interval mapping					
			Maximum LOD Value	Weight[a]	Phenotypic variance attributable to QTL (%)	LOD threshold[b]	Maximum LOD Value	Weight[a]	Phenotypic variance attributable to QTL (%)	Maximum LOD position (cM)[c]	Max po
Plant Height (cm)	1	25.6-84.1	3.6	6.0	22.0	2.8	1.3	3.65	7.5	65.9	43
	3	31.5-72.5	5.4	7.0	29.0		2.7	5.16	12.6	52.4	44
Tiller Size (mm)	1	25.6-92.3	4.8	0.28	32.7	2.7	2.6	0.20	12.0	55.9	43
	3	22.6-90.7	5.1	0.29	28.0		4.3	0.26	16.1	39.1	31
Leaf Length (cm)	5	16.6	2.1	2.1	11.6	5.5	1.0	3.26	6.1	15.3	
Leaf Width (cm)	3	39.1	2.1	0.04	11.3	2.8	2.3	0.06	10.4	39.1	31
Fresh Weight (g)	4	-				2.8	3.3	-32.50	13.2	19.8	10
	5	0-30.4	4.2	39.3	23.0		5.6	39.30	22.8	18.6	1
Plant Type (0-9)	4	52.1-112.2	3.0	0.78	16.8	2.8	4.0	1.56	13.9	52.1	51
	4	52.1-112.2	3.4	0.83	18.1		2.2	0.50	5.9	100.7	90
	7	35.9-88.5	6.5	-1.1	31.4		8.5	-1.01	26.8	59.0	4
Number of Spikelets per Spike	1	84.1-92.3	2.5	1.8	14.5	2.8	3.4	2.04	16.5	79.9	53
Spike length (cm)	1	25.6-84.1	4.7	3.8	29.9	2.8	2.7	2.70	11.8	53.9	43
	3	31.5-87.2	4.3	3.5	23.4		2.6	2.59	11.2	58.2	57
	5	0-44.6	3.3	3.0	18.8		3.6	2.68	13.8	16.6	1
Heading Date (d)	4	39.9-72.3	4.0	4.7	20.9	2.9	3.6	4.64	14.2	56.3	54
Aftermath heading (0-5)	6	61.2-69.1	2.3	-0.4	12.7	2.9	2.6	-0.42	10.7	64.9	56
Electrical conductivity	4	0	2.0	1.9	11.8	2.6	1.6	1.71	8.8	6.0	
	6	-					2.7	-2.99	11.8	50.0	4
	6	-					3.3	3.70	12.6	58.9	56

[a]Defined as the additive effect on average phenotype of substituting a single B allele for an A allele at the relevant marker locus in an AB x AA backcross mapping structure.
[b]Based on the results of permutation analysis with 1000 iterations.
[c]Based on the genetic map coordinates of Jones et al. (2002b).

Fig. 1(A)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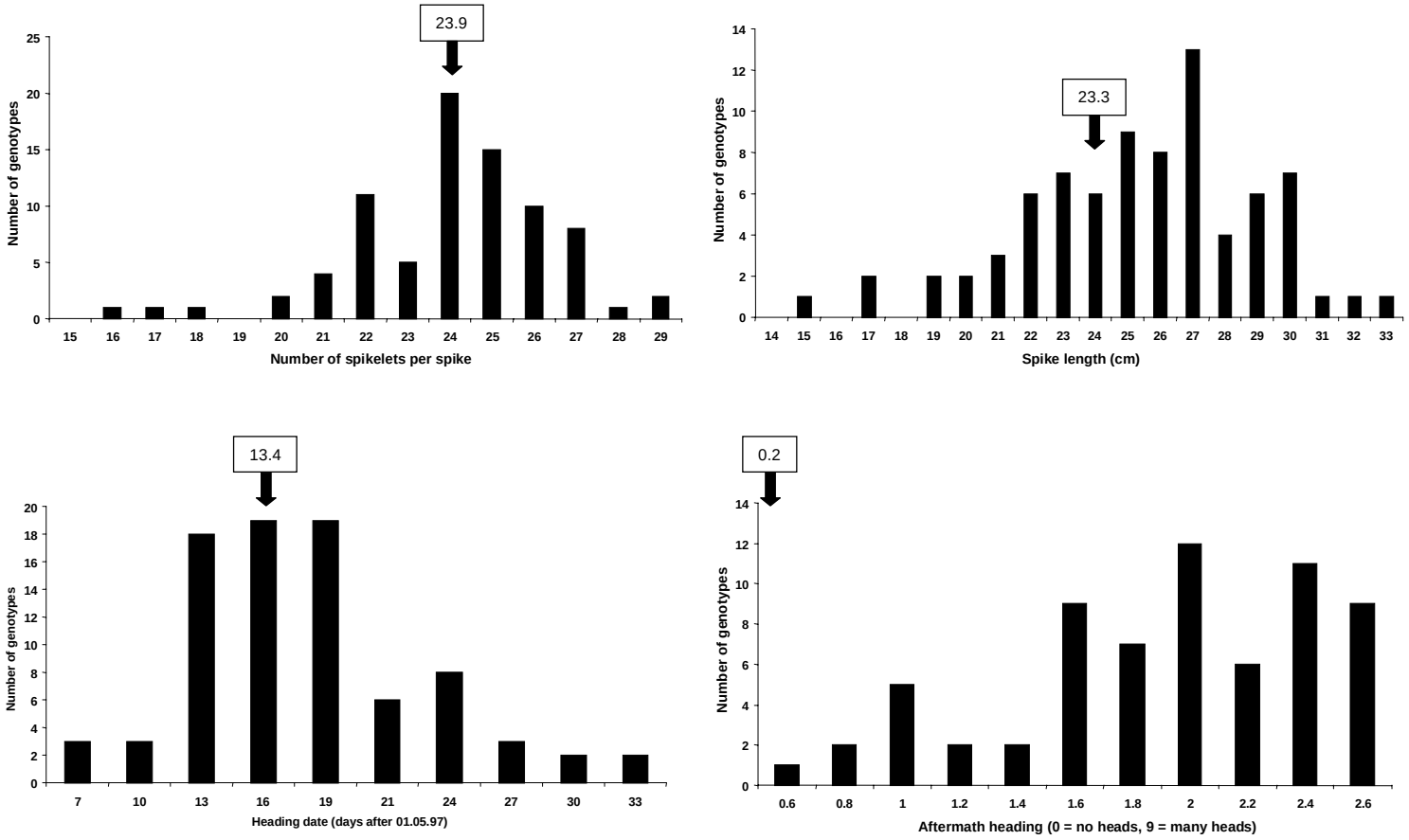
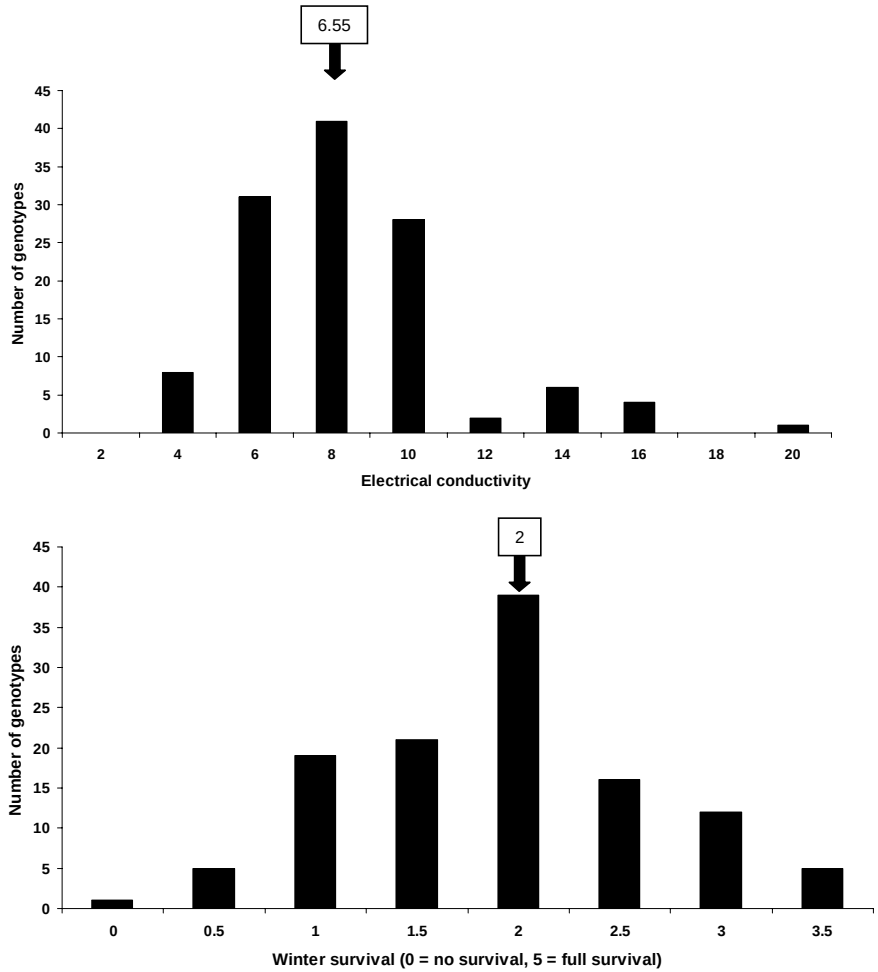
Tiller number
7.2
Tiller size (mm)
1.73
Leaf length (cm)
17.1
Numbers of genotypes
Leaf width (cm)
0.3
Plant type (1=erect, 9 = prostrate)
3
Fresh weight (g)
144.4

Fig. 1 (B)


23.9
Number of genotypes
Number of spikelets per spike
23.3
Number of genotypes
Spike length (cm)
13.4
Number of genotypes
Heading date (days after 01.05.97)
0.2
Number of genotypes
Aftermath heading (0 = no heads, 9 = many heads)


6.55
Number of genotypes
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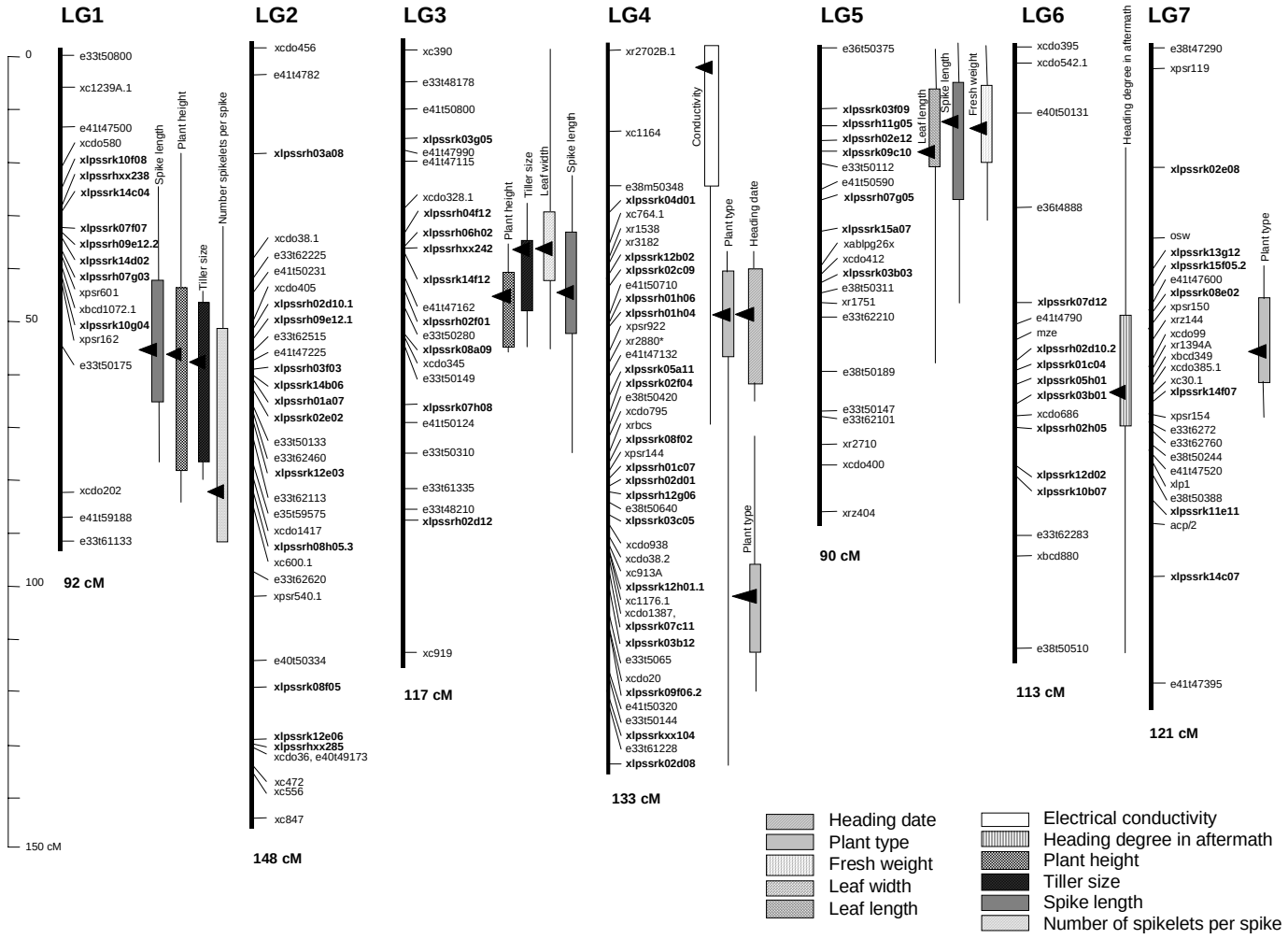
40
35
30
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20
Electrical conductivity
2
Number of genotypes
45
40
35
30
25
20
15
10
5
0
0
0.5
1
1.5
2
2.5
3
3.5
Winter survival (0 = no survival, 5 = full survival)

Fig. 1 (C)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LG2
LG3
LG4
LG5
LG6
LG7
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50
100
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xcdo580
xlpssrk10f08
xlpssrhxx238
xlpssrk14c04
xlpssrk07f07
xlpssrh09e12.2
xlpssrk14d02
xlpssrh07g03
xpsr601
xbcd1072.1
xlpssrk10g04
xpsr162
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xcdo202
e41t59188
e33t61133
Spike length
Plant height
Tiller size
Number spikelets per spike
92 cM
xcdo456
e41t4782
xlpssrh03a08
xcdo38.1
e33t62225
e41t50231
xcdo405
xlpssrh02d10.1
xlpssrh09e12.1
e33t62515
e41t47225
xlpssrh03f03
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xlpssrh01a07
xlpssrk02e02
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xc556
xc847
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xc390
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xc919
Plant height
Tiller size
Leaf width
Spike length
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xc764.1
xr1538
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xr2880*
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xrbcs
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xc913A
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xlpssrk03b12
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xcdo20
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e33t50144
xlpssrkxx104
e33t61228
xlpssrk02d08
Conductivity
Plant type
Heading date
Plant type
133 cM
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xlpssrk03f09
xlpssrh11g05
xlpssrh02e12
xlpssrk09c10
e33t50112
e41t50590
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xlpssrk15a07
xablpg26x
xcdo412
xlpssrk03b03
e38t50311
xr1751
e33t62210
e38t50189
e33t50147
e33t62101
xr2710
xcdo400
xrz404
Leaf length
Spike length
Fresh weight
90 cM
xcdo395
xcdo542.1
e40t50131
e36t4888
xlpssrk07d12
e41t4790
mze
xlpssrk02d10.2
xlpssrk01c04
xlpssrk05h01
xlpssrk03b01
xcdo686
xlpssrk02h05
xlpssrk12d02
xlpssrk10b07
e33t62283
xbcd880
e38t50510
Heading degree in aftermath
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e38t47290
xpsr119
xlpssrk02e08
osw
xlpssrk13g12
xlpssrk15f05.2
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xlpssrk08e02
xpsr150
xrz144
xcdo99
xr1394A
xbcd349
xcdo385.1
xc30.1
xlpssrk14f07
xpsr154
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e33t62760
e38t50244
e41t47520
xlp1
e38t50388
xlpssrk11e11
acp/2
xlpssrk14c07
e41t47395
Plant type
121 cM
Heading date
Plant type
Fresh weight
Leaf width
Leaf length
Electrical conductivity
Heading degree in aftermath
Plant height
Tiller size
Spike length
Number of spikelets per spike

Fig. 2