Theor Appl Genet (2011) 123:369–382
DOI 10.1007/s00122-011-1590-z

ORIGINAL PAPER

QTL analyses and comparative genetic mapping of frost tolerance, winter survival and drought tolerance in meadow fescue (*Festuca pratensis* Huds.)

Vibeke Alm · Carlos S. Busso · Åshild Ergon · Heidi Rudi · Arild Larsen · Michael W. Humphreys · Odd Arne Rognli

Received: 4 August 2010 / Accepted: 31 March 2011 / Published online: 20 April 2011

Abstract Quantitative trait loci (QTLs) for frost and drought tolerance, and winter survival in the field, were mapped in meadow fescue (*Festuca pratensis* Huds.) and compared with corresponding traits in *Triticeae* and rice to study co-location with putatively orthologous QTLs and known abiotic stress tolerance genes. The genomes of grass species are highly macrosyntenic; however, the *Festuca/Lolium* and *Triticeae* homoeologous chromosomes 4 and 5 show major structural differences that is especially interesting in comparative genomics of frost tolerance. The locations of two frost tolerance/winter survival QTLs on *Festuca* chromosome 5F correspond most likely to the *Fr-A1* and *Fr-A2* loci on wheat homoeologous group 5A chromosomes. A QTL for long-term drought tolerance on chromosome 3F (syntenic with rice 1) support evidence from introgression of *Festuca* genome segments onto homoeologous *Lolium* chromosomes (3L) that this genome region is an excellent source of tolerance towards drought stress. The coincident location of several stress tolerance QTL in *Festuca* with QTL and genes in *Triticeae* species, notably dehydrins, CBF transcription factors and vernalisation response genes indicate the action of structural or regulatory genes conserved across evolutionarily distant species.

Communicated by G. Bryan.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1590-z) contains supplementary material, which is available to authorized users.

V. Alm · C. S. Busso · Å. Ergon · H. Rudi · O. A. Rognli (✉)
Department of Plant and Environmental Sciences,
Norwegian University of Life Sciences, 1432 Ås, Norway
e-mail: odd-arne.rognli@umb.no

V. Alm
Department of Research Administration,
University of Oslo, PO Box 1072, 0316 Oslo, Norway

C. S. Busso
Department of Otolaryngology, Stanley S Scott Cancer Center,
Louisiana State University Health Sciences Center,
New Orleans, LA, USA

A. Larsen
Graminor AS, Torggården, 8049 Bodø, Norway

M. W. Humphreys
Institute of Biological, Environmental and Rural Sciences
(IBERS), Aberystwyth University, Gogerddan,
Aberystwyth, Ceredigion SY23 3EB, UK

Abbreviations

QTL	Quantitative trait loci
Ft	Frost tolerance
Ws	Winter survival
Dtm	Moderate drought tolerance
Dts	Severe drought tolerance
Gs	Degree of green leaves after severe drought
Ts	Percentage of live tillers after severe drought
IM	Interval mapping
MQM	Multiple QTL mapping

Introduction

Abiotic stress tolerance like freezing tolerance, drought tolerance and winter survival are of major importance in determining persistency and yield of crops like forage grasses and winter cereals, certainly in cool temperate environments. Several studies have established that major genes, or gene clusters, involved in the control of frost and drought tolerance are located on a region of the long arm of Triticeae group 5 chromosomes. Traits like winter hardiness

(Hayes et al. 1993; Pan et al. 1994), vernalisation response and frost tolerance (Ft) (Sutka and Snape 1989; Galiba et al. 1995; Laurie et al. 1995), cold- and drought-induced abscisic acid (ABA) production (Galiba et al. 1993; Quarrie et al. 1997), and osmotic stress tolerance (Galiba et al. 1992), have all been mapped to this region. Cold acclimation or hardening of plants at low, non-freezing temperatures often increase their freezing tolerance. The C-repeat binding factor (*CBF*) genes are key regulators of the expression of cold regulated (*COR*) genes and their structure seems to be evolutionarily conserved among diverse plant lineages. The *CBF* transcription factors recognise the cis-acting C-repeat/dehydration responsive element (CRT/DRE) element in the regulatory regions of *COR* genes (Stockinger et al. 1997). Twenty *CBF* genes have been identified in barley (*Hordeum vulgare*), of which 11 are found in two tight tandem clusters on the long arm of chromosome 5H in the same region as the *Fr-H2* frost tolerance locus (Skinner et al. 2006; Francia et al. 2007). An orthologous genomic region in *Triticum monococcum* contains similar *CBF* gene clusters located at the *Fr-A*^{m}*2* frost tolerance QTL (Vágújfalvi et al. 2003; Miller et al. 2006). In *Lolium perenne* Tamura and Yamada (2007) mapped four *LpCBF* genes in a short interval on *Lolium* LG5 that is most likely syntenic with regions on *Triticeae* group 5 chromosomes. Studies of the organisation of the *CBF* cluster in barley and wheat have shown that the number of *CBF* genes at the *Fr-H2/Fr-A1* locus may vary among cultivars with winter forms having a higher copy number of some *CBFs* (Francia et al. 2007; Knox et al. 2010). The co-segregation of the *CBF* gene clusters with the barley *Fr-H2* and wheat *Fr-A*^{m}*2* frost tolerance loci, their role in cold acclimation (Stockinger et al. 1997), and the association of transcript levels of *CBF* genes with frost tolerance loci (Vágújfalvi et al. 2005) makes them obvious candidates for one of the two major frost tolerance QTLs on Triticeae group 5 chromosomes. In addition, Triticeae group 1, 2, and 7 chromosomes have genes and QTLs for photoperiodic response (Welsh et al. 1973; Law et al. 1978; Scarth and Law 1983; Scarth and Law 1984; Hayes et al. 1993; Pan et al. 1994; Laurie et al. 1995; Bezant et al. 1996; Sourdille et al. 2000) influencing adaptation and winter survival.

Winter survival is a very complex trait. Physical and physiological stresses during winter vary with climate and geographic location, and may involve acquisition and mobilisation of reserves under low temperatures, short photoperiods and combinations of desiccation, water logging, ice-encasement, anoxia, and snow cover. However, it is generally accepted that freezing tolerance is the single component that explains most of the variation in winter survival (Pulli et al. 1996). Drought stress is also highly complex involving lack of available water, high transpiration rates, supra-optimal temperatures, photo-oxidation, mineral deficiency and hard soil. The relative importance of these factors varies with location and year. Temperate grasslands of Northern Europe must be able to grow and yield during dry conditions, which limit the utility of summer dormant growth behaviour. Summer dormancy is triggered by the onset of drought and is a characteristic of many Mediterranean grass ecotypes (Humphreys et al. 1997). The physiological and adaptive mechanisms for drought resistance in grasses have been reviewed by Humphreys et al. (2005) and their relative importance attributed according to climate. The overall importance of rooting traits has been especially emphasised (Davies 2007; Durand et al. 2007). Development of molecular markers linked to chromosomal regions and/or genes that confer increased stress tolerance could lead to more efficient marker-assisted selection (MAS) procedures, and circumvent the need for costly and laborious field or laboratory tests in these crops.

Meadow fescue (*Festuca pratensis* Huds.) is one of the most important forage grass species in the northern temperate regions (Rognli et al. 2010). Grass species of the genus *Festuca* manifest higher levels of general stress tolerance than the widely cultivated ryegrasses (*Lolium* spp.); for example, tall fescue and meadow fescue show enhanced drought and cold tolerance, respectively. Since fescues and ryegrasses are closely related evolutionarily, they can be hybridised with fair ease, and this makes it possible to combine the superior forage quality of ryegrass species with the high persistency and stress tolerance of fescues in interspecific hybrids (*Festulolium*) (Thomas and Humphreys 1991). The first successful transfer of stress tolerance was the introgression of drought tolerance from tall fescue into Italian ryegrass (*Lolium multiflorum*) reported by Humphreys and Thomas (1993), which was the precursor for other successful programmes that incorporated genes from fescue species into ryegrass for a range of important agronomic traits (Humphreys et al. 2006).

The comparative linkage map of meadow fescue shows that its genome is highly syntenic with the genomes of *Lolium*, oat and the Triticeae species (Alm et al. 2003). In this study, we mapped QTLs for winter survival, frost and drought tolerance in meadow fescue, and investigated whether the QTLs co-locate with putatively orthologous QTLs or genes in Triticeae genomes and with known abiotic stress tolerance genes.

Materials and methods

QTL mapping family

The population used for QTL mapping consisted of 138 F_1 plants from a pair-cross between the Yugoslavian genotype

B14/16 (female) and the Norwegian genotype HF2/7 (male). Details of the mapping family and the linkage map that has been used for QTL mapping can be found in (Alm et al. 2003).

Freezing test

Clones of the parents and 138 progenies were divided into single tillers (ramets), transplanted into trays of 50 ramets each, raised in a greenhouse at 20/12°C under natural daylight supplemented with extra light (irradiance 110 µmol m^{-2} s^{-1}) for 12 h per day for 4–6 weeks until they had developed 3–5 tillers. The plants were then placed at 10°C/11 h photoperiod for 1 week, and subsequently cold acclimated for 2 weeks at 1°C/16 h photoperiod and 110 µmol m^{-2} s^{-1} irradiance in Conviron growth chambers. After hardening, the trays were transferred to specially designed home-freezers kept at 0°C. The freezing test was initiated by lowering the temperature to −2°C and keeping it constant for 12 h. Subsequently, the temperature was lowered by 1°C h^{-1} to the predetermined freezing temperature ranging from −8 to −12°C. The plants were kept at the final freezing temperature for 24 h, and then the temperature was raised to 0°C at a rate of 1°C h^{-1} (Pulli et al. 1996). Following thawing, the plants were cut to about 2 cm height and placed in the greenhouse for re-growth. Freezing tolerance (Ft) was scored as re-growth after 10 and 20 days, according to a scale from 0 (dead) to 9 (without damage) (Larsen 1978). Three experiments, each with ten replicates of each clone, were conducted. Each replicate consisted of three trays, and the plants were arranged within each tray according to an incomplete block design. The clones were randomised within blocks (trays), and blocks were randomised within replicates. Mean values of the adjusted means of the two experiments with the lowest CV% (26 and 39%) were used in the QTL analyses. Factorial analysis of variance was performed using AGROBASE (Agronomix Software Inc. 1998).

Winter survival in the field

Clonal ramets of the two parents and the mapping family were established in a field experiment at Ås, Akershus (59°40′N, 10°48′E), in the spring of 1998 using a randomised complete block design with three replicates. The late autumn (Oct–Dec) of 2000 was characterised by higher precipitation and milder temperatures than normal, with sudden severe frost occurring in December. The winter had long periods of extreme cold as well as large fluctuations in temperature (see Supplementary Table S1). Thus, large variation in winter survival were evident in the field in the spring of 2001, and winter survival (Ws) was scored just after commencement of re-growth using a scale from 0 (completely dead) to 9 (no visible injury). The mean values of the three replicates were used in ANOVA and QTL analyses of winter survival.

Drought test

Drought tests were performed at the University of Aberystwyth, Institute of Biological, Environmental and Rural Sciences (IBERS) according to well-established procedures (e.g. Thomas and Evans 1989; Humphreys et al. 2005). The parents and 80 randomly selected progenies were divided into four equal sized ramets, each comprising 6–8 tillers of equivalent size and age. Four replicates of the population were subsequently established in a glasshouse in four polythene-lined brick-built bins 1.3 m × 0.95 m and 0.9 m deep, containing 10 cm bottom layer of gravel beneath a 70 cm layer of sterilised soil (silty loam of the Rheidol series), and an upper 10 cm layer of soil-based potting compost. The mapping family was surrounded in each bin with two rows of barrier plants comprising unrelated meadow fescue plants. Clones in each bin were watered regularly until all had successfully established. Prior to the start of the drought treatments, all genotypes were defoliated to 4 cm (the standard tiller height used at IBERS to simulate grazing) and fertiliser applied throughout at 30 g m^{-2} of 20N:10 P:10 K % of weight. Two drought treatments representing moderate drought (about 2 months without water) and severe drought (about 5 months without water) were applied. Plants in all four bins were cut back to 4 cm after a 1-month drought. Following 2 months of drought treatment, fresh weights of herbage were taken and moderate drought tolerance (Dtm) was calculated as herbage fresh weight divided by herbage fresh weight under a normal water regime. After about 5 months of drought, the plants were cut back again to 4 cm, fresh weights were taken from surviving plants and severe drought tolerance (Dts) was calculated in the same way as Dtm. Clones in each bin were scored for the presence of green foliage at the end of the 5 months drought period (Gs, green score: 0 = dead, 10 = all green). Plants in all four bins were re-watered thoroughly and maintained under regular irrigation for a further 1 month. Surviving plants in all four bins were then cut back again to 4 cm and re-growth measured during the recovery period [drought recovery (Dr) mg]. The percentage of live tillers was scored in each bin amongst the genotypes whose leaves had re-grown following 5 months of drought [tiller survival (Ts)].

QTL analyses

QTL analyses were performed on the mean values of each trait and a subset of loci from the linkage map of the B14/16 × HF2/7 mapping family reported by Alm et al.

(2003). A total number of 156 out of 466 marker loci on the combined maternal × paternal maps were used for QTL analyses, giving an average spacing of 4.2 cM between markers throughout the genome. QTL analyses were carried out in two stages, first by interval mapping (IM) and then by multiple QTL mapping (MQM) using the MapQTL version 4.0 software (van Ooijen and Maliepaard 1996; van Ooijen et al. 2000). For traits with distribution deviating severely from the normal distribution, i.e. severe drought (Shapiro–Wilk test $W = 0.84$***) and tiller survival ($W = 0.91$***), Kruskal–Wallis tests were used in addition to IM. MQM analyses were performed with a set of co-factors for each trait selected using the automatic co-factor selection procedure of MapQTL. The markers (except for *Xibf507* on 1F) used as co-factors in the MQM analyses are printed in italics in Table 2. "Maternal", "paternal", and "maternal × paternal interaction" effects, as described by Knott et al. (1997), were estimated and tested for the fully informative peak markers by means of orthogonal contrasts using the PROC GLM procedure of SAS (SAS 2002). Fully informative markers are markers that segregate in both parents (type abxcd). LOD significance thresholds were determined by permutation tests (Churchill and Doerge 1994) using the MapQTL software. The same LOD thresholds for declaring QTL were used both in IM and MQM (Hackett 2002), and markers with peak LOD scores were chosen as the map positions of putative QTL. QTL were named following common practice, e.g. QWs1F_1 indicates the first QTL for winter survival (Ws) detected on chromosome 1F. QTL maps were drawn using MapChart (Voorrips 2002).

Cloning and mapping of candidate genes

F. pratensis orthologs of the genes *CBF6*, *IRI1*, *LOS2* and *PHYC* were cloned and mapped in the 'B14/16 × HF2/7' mapping population; *FpCBF6* and *FpIRI1* on 5F, *FpLOS2* on 7F and *FpPHYC* on 4F (Fig. 1). Two of these genes, *FpIRI1* and *FpLOS2*, have been shown to be induced by cold acclimation (Rudi et al. 2011). For detailed information about the cloning and mapping of these genes see Supplemental note 1.

Results

Trait performances

The mean values, ranges and differences between parental and F_1 means are presented in Table 1. Ft, Ws, Dtm and Gs were normally or near normally distributed, while Dts, Ts and recovery from Dr deviated significantly from normality (data not shown). Transformations ($\log_{10}$ and square root) did not improve normality, and the original data were therefore used in the QTL analyses. ANOVA showed that there was highly significant genotypic variation within the F_1 family for Ft, Ws, Dtm, Dts, and Ts ($P \leq 0.001$), and significant variation for Gs ($P \leq 0.05$), while the variation for Dr was not significant. The parents had significantly different mean values for Ft, Ws and Gs, while the F_1 mean was significantly different from at least one of the parents for all traits (Table 1). Positive transgressive segregations were observed for all traits and high parent heterosis evident for Ft, Dts, and Ts. Both parents had low freezing tolerance (2.81 for B14/16 and 3.87 for HF2/7) but the variation in the F_1 was large (2.83–5.97, mean 4.48, Table 1). However, the parents showed very different winter survival with average scores of 1.0 for B14/16 and 7.0 for HF2/7, and with a progeny mean value of 4.97.

QTLs for freezing tolerance

Three major QTLs for frost tolerance were detected on 5F and 6F (*QFt5F-1*, *QFt5F-2* and *QFt6F*) and two suggestive QTLs, *QFt4F* and *QFt7F* on 4F and 7F, respectively. The LOD scores of these QTLs ranged from 2.72 to 9.35, and individually they explained from 6.5 to 26.8% of the genotypic variation. The full MQM model with co-factors *Xibf507* (1F), *Xrz141b* (5F) and *Xcdo678b* (6F) explained 50.7% of the variation. The most significant QTL for frost tolerance was *QFt5F-2* with *Xrz141b* as the peak marker. The 2-LOD confidence interval for this QTL is 6.7 cM when estimated with IM, and only 1.2 cM when estimated with MQM with the gene *FpCBF6* located within this interval (Fig. 1). Tests of the effect of the QTL alleles using the fully informative marker *Xcdo590*, which is located only 0.6 cM from the peak of *QFt5F-2*, showed that this QTL was caused by a significant negative effect (−0.59**) from the maternal parent and a highly positive effect (0.92***) from the paternal parent (Table 2). The second QTL on 5F, *QFt5F-1* was only detected by interval mapping, and is located 20.4 cM proximal to *QFt5F-2*. The ice-renucleation inhibition gene *FpIRI1* (EU684537) maps 1.2 cM distal to *QFt5F-1*. The effects of the QTL alleles could not be tested for the peak marker of this QTL since it was not fully informative. The two QTLs on 5F are most probably distinct since the 2-LOD confidence intervals for IM on either side of the peak markers do not overlap (Fig. 1). *QFt4F* on 4F was detected both with IM and MQM, but the peak marker was different in the two mapping methods (*Xwg644* with IM and *Xwg114* with MQM). However, these markers, which have both been associated with frost tolerance in cereals (Fig. 1), are only 3.4 cM apart, and the QTL detected by the two mapping methods are most probably identical as the confidence intervals are overlapping. Using the fully informative marker *Xwg114* to test the QTL effects

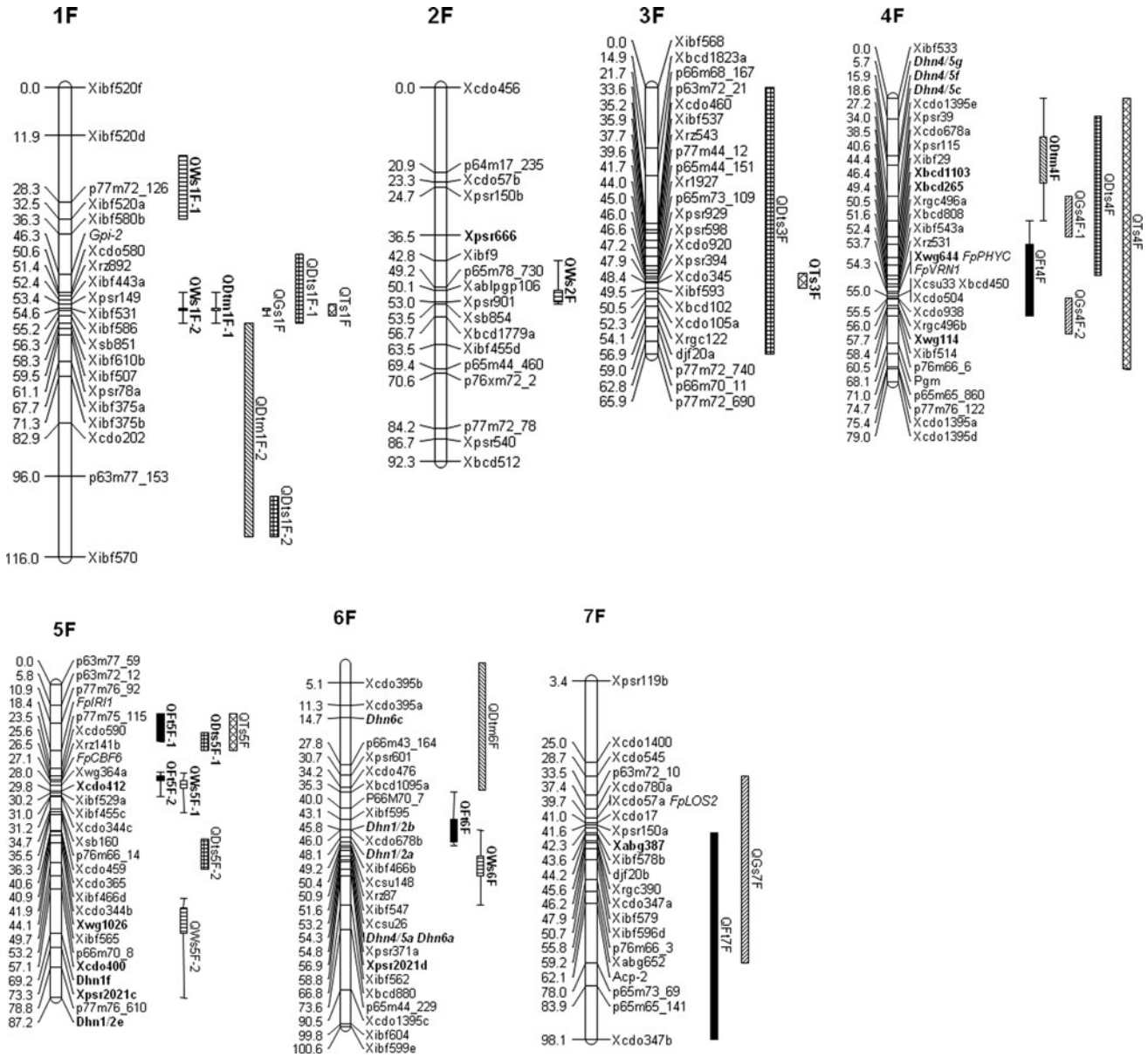

Fig. 1 *Festuca* linkage groups with positions of QTL for frost and drought tolerance, and winter survival. Thin lines indicate 2-LOD support interval for QTL detected by interval mapping, thick boxes indicate the same for QTL detected by MQM mapping. QTL printed in bold are major QTL with significance levels above the chromosome-wide LOD threshold. Markers printed in bold have been associated with QTL or genes for low-temperature tolerance in Triticeae species

Table 1 Mean values (standard deviation), ranges and tests of means for parents and the F_1 mapping population 'B14/16 × HF2/7'

Trait	P_1 (B14/16) ($n = 4$)[A]	P_2 (HF2/7) ($n = 4$)[A]	Mean of parents	F_1 ($n = 138$ or 80)[A]	Range
Frost tolerance (Ft)	2.81[a] (0.60)	3.87[b] (0.60)	3.34	4.48[c] (0.59)	2.83–5.97
Winter survival (Ws)	1.00[b] (1.00)	7.00[a] (1.73)	4.00	4.97[a] (1.28)	1.00–7.67
Moderate drought tolerance; 2 months (Dtm)	0.063[ab] (0.039)	0.031[b] (0.033)	0.047	0.144[a] (0.086)	0.018–0.521
Severe drought tolerance; 5 months (Dts)	0.008[b] (0.007)	0.0[b]	0.004	0.020[a] (0.016)	0–0.089
Recovery from drought (Dr)	13.50[a] (27.0)	0.0[a]	6.75	40.55[a] (59.25)	0–367.50
Green score (Gs)	2.00[a] (1.41)	0.0[b]	1.00	3.28[a] (1.83)	0–7.75
Tiller survival (Ts)	0.006[b] (0.012)	0.0[b]	0.003	0.059[a] (0.072)	0–0.492

[A] Means with different letters are significantly different at minimum $P < 0.05$

Table 2 QTLs detected for frost tolerance (Ft), winter survival (Ws), moderate drought (Dtm—2 months drought) and green score (Gs) using interval mapping and multiple QTL mapping (MQM) analyses

Trait	QTL[A]	LG	Interval mapping				MQM				Marker effects[C]		
			LOD	Marker peak	Position (cM)	Variance explained (%)	LOD	Marker peak[B]	Position (cM)	Variance explained (%)	Maternal	Paternal	M × P
Ft	QFt4F	4	2.60	Xwg644	54.3	8.6	2.72	Xwg114[†]	57.7	6.5	−0.50*	0.28 [ns]	−0.35 [ns]
	QFt5F-1	5	6.04	P77M76_92	10.9	24.2					(0.50***)		
	QFt5F-2	5	8.70	Xrz141b	26.5	26.8	9.35	*Xrz141b*	26.5	21.3	−0.59**	0.92***	0.37 [ns]
	QFt6F	6	3.58	Xcdo678b	46.0	15.4	4.54	*Xcdo678b*	46.0	14.1	0.30 [ns]	0.50*	−0.68**
	QFt7F	7					3.83	Xcdo347a	46.2	8.6		(0.20*)	
Ws	**QWs1F-1**	1					4.42	Xibf520d[†]-P77M72_126	21.9	14.2		(0.50*)	
	QWs1F-2	1	3.93	Xibf443a	52.4	12.9	4.04	*Xibf586*	55.2	7.7	−1.56***	−0.06 [ns]	0.85*
	QWs2F	2	6.40	Xpsr901	53.0	19.4	6.11	*Xpsr901*	53.0	10.3		(1.10***)	
	QWs5F-1	5	2.90	Xwg364a	28.0	9.3	5.58	*Xwg364a*	28.0	11.3	−1.99***	−0.42 [ns]	−0.01 [ns]
	QWs5F-2	5	2.80	Dhn1F	69.2	10.8	3.21	*Dhn1f*	69.2	7.5		(0.50*)	
	QWs6F	6	4.89	Xpsr2021d	56.9	17.0	4.88	*Xpsr2021d*	56.9	8.9	1.50***	1.34***	−0.15 [ns]
Dtm	**QDtm1F-1**	1	4.21	Xsb851 M	56.3	23.0	4.26	*Xibf586*[†]	55.2	14.2	−0.13***	0.00 [ns]	0.05 [ns]
	QDtm1F-2	1	3.28	Xibf375b-Xcdo202[†]	76.3	23.8					−0.12**	−0.07 [ns]	0.03 [ns]
	QDtm4F	4	3.64	Dhn4/5c	18.6	32.1	4.40	*Dhn4/5c*	18.6	25.7	(0.07***)		
	QDtm6F	6	2.38	Dhn6c	14.7	13.7					(0.06**)		
Gs	QGs1F	1	3.10	Xibf586	55.2	17.5	2.71	*Xibf586*	55.2	12.8	−2.09**	1.5*	−0.58 [ns]
	QGs4F-1	4	3.63	Xpsr39	34.0	30.6					(1.02*)		
	QGs4F-2	4	3.11	Xibf514	58.4	16.5	2.72	*Xibf514*	58.4	11.8	2.59**	1.07 [ns]	1.26 [ns]
	QGs7F	7					3.56	Acp2-P65M73_69[†]	67.1	17.8	(0.97*)		

[A] QTLs printed in bold are major QTLs, with LOD scores above the genome-wide significance thresholds. QTLs printed in normal font are suggestive QTLs with significance levels above the chromosome-wide LOD thresholds; [B]markers in *italics* have been used as co-factors in MQM; [C]maternal, paternal and maternal × paternal interaction effects for fully informative peak markers (ab × cd) are significant at * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$, [ns] not significant. For markers segregating aa × ab, ab × aa or ab × ab, the values are enclosed in parentheses and indicate differences between the means of genotype classes in the parent that segregates for the marker, i.e. female parent for abxaa, male parent for aaxab and maternal × paternal for abxab markers; [†]markers used to test effects in cases where the marker peak is in large intervals without markers or there are different peak markers for IM and MQM

showed that this QTL was due to a significant negative effect (−0.50*) from the maternal parent. The QTL *QFt6F* was detected by both mapping methods. The QTL explained about 15% of the genetic variation for frost tolerance, and was due to a significant positive effect (0.50*) from the paternal parent and significant negative interaction effects (−0.68**) indicating deviation of the four QTL alleles from additivity. *QFt7F* on 7F was only detected using MQM, and even if this was mapped using MQM, the confidence intervals of this marker cover as much as 44% of the chromosome (Fig. 1).

QTLs for winter survival in the field

Six QTLs were detected for winter survival, on chromosome 1F (*QWs1F-1* and *QWs1F-2*), 2F (*QWs2F*), 5F (*QWs5F-1* and *QWs5F-2*) and 6F (*QWs6F*) (Table 2; Fig. 1). Five of these were major QTLs with LOD scores ranging from 4.04 to 6.11 (MQM) while *QWs5F-2* was a suggestive QTL (LOD 3.21). Individually they explained from 7.5 to 19.4% of the genotypic variation, while the full MQM model with co-factors *Xibf586* (1F), *Xpsr901* (2F), *Xwg364a* and *Dhn1f* (5F) and *Xpsr2021d* (6F) explained 55.7% of the variation. The most significant QTL was *QWs2F*. The peak marker for *QWs2F* was PSR901, and the QTL effect is due to a large difference of 1.10 in score ($P \leq 0.001$) between the marker alleles segregating in the paternal (Norwegian) parent. PSR901 is segregating only in the paternal parent (aaxab) and thus the paternal allele b has a large positive effect on winter survival Despite the relatively large IM confidence interval of *QWs5F*-1, the second QTL on chromosome 5F, *QWs5F-2* with peak marker *Dhn1f*, does not overlap and there are probably two QTLs for winter survival on chromosome 5F. The two QTLs *QWs1F-1* and *QWs1F-2* are positioned 30–33 cM apart, have relatively narrow confidence intervals (especially *QWs1F-1*) and should therefore be regarded as two separate QTLs. Different peak markers were detected for this QTL with the two QTL mapping methods; however, the marker peaks are only 2.8 cM apart and within the confidence interval of IM (Table 2; Fig. 1). *QWs6F* is a major QTL with high LOD scores (around 4.9) with both mapping methods. The closest fully informative marker PSR371a, which is located 2.1 cM proximal to the peak marker PSR2021d, was used to estimate the QTL effects. A peculiar observation for this QTL is that whilst both the maternal and the paternal effects on this QTL were large and positive, the other two major QTLs (*QWs1F2* and *QWs5F-1*), whose effects could be estimated using fully informative markers, were caused by very large negative maternal effects (Table 2). *QWs1F-2* was in addition associated with a significant positive (0.85, $P \leq 0.05$) maternal × paternal interaction effect, which indicates the presence of dominance at this locus.

QTLs for drought tolerance

Four QTLs were detected for each of the traits moderate drought (2 months without water) tolerance (Dtm), and Gs recorded following a severe drought stress of 5 months without water. Of these, *QDtm1F-1* and *QDtm4F* were major QTLs (Table 2; 3). Six QTLs were detected for Dts of which *QDts5F-1* was a major QTL with a LOD score of 5.77 (Table 3). For tiller survival (Ts) recorded at the end of the 5 months drought treatment, four QTLs were detected with *QTs3F* as a major QTL with a high LOD score (8.39), while *QTs5F* was only detected using IM. Despite absence of significant associations with markers in this region of 5F using the Kruskal–Wallis test, the putative QTL was included as its position overlapped with the position of *QDts5F-1* and the two traits are clearly related (Fig. 1). The phenotypic correlation between Ts and Dts was 0.65 ($P \leq 0.001$). All four QTLs for Ts are either located at the same peak markers, i.e. *Xibf586* on 1F, *Xpsr39* on 4F and P77M76_92 on 5F, or within the same LOD confidence interval (*QTs3F*) as the QTLs for Dts (Table 3). All drought traits were positively correlated, with the highest correlation observed between Dtm and Dts (0.69, $P \leq 0.001$). The correlations between Dtm and Gs, and Dtm and Ts were lower, 0.47 ($P \leq 0.001$) and 0.44 ($P \leq 0.001$), respectively, supporting evidence that high growth rates in water limited conditions were not conducive to genotype survival following prolonged drought stress. The correlations between Gs and Ts, and Gs and Dts were both high (0.65, $P \leq 0.001$) as they represent different measures of tolerance assessed following severe drought. Based on these correlations we expected that QTLs for drought tolerance traits would map on top of each other or close in the same chromosomal region. This was indeed the case, and was most pronounced on chromosome 1F and 4F, with QTLs for all drought tolerance traits located in a fairly narrow interval around the peak marker *Xibf586* on 1F, and on the proximal end of chromosome 4F (Fig. 1). QTLs for Dts were found on all chromosomes except 2F and 7F. However, distinct QTLs for Dts were found on 3F (*QDts3F*) and on 5F (*Qdts5F-1* and *QDts5F-2*), and a QTL for moderate drought (*QDtm6F*) located to the proximal end of chromosome 6F where the dehydrin gene *Dhn6* was the peak marker (Fig. 1). In addition, a specific QTL for Gs green score was detected on 7F. *QDts3F* extended over the entire chromosome with the Kruskal–Wallis test indicating that 20 of the 24 markers on this chromosome were associated significantly with the trait at $P \leq 0.001$. For all QTLs that could be estimated using fully informative markers (Tables 2, 3), except for *QGs4F-2*, there were significant negative effects associated with QTL alleles from the maternal (Yugoslavian) parent and either non-significant or positive effects (*QGs1F* and *QTs1F*) associated with the

Table 3 QTLs detected for severe drought tolerance (Dts—5 months drought) and tiller survival (Ts) using interval mapping and Kruskal–Wallis test

Trait	QTL[a]	LG	Interval mapping				Kruskal–Wallis test[b]		Marker effects[c]		
			LOD	Marker peak	Position (cM)	Variance explained (%)	*K**	*df*	Maternal	Paternal	M × P
Dts	QDts1F-1	1	3.36	Xibf586	55.2	21.3	11.21**	3	−0.020**	0.008 ns	−0.003 ns
	QDts1F-2	1	3.27	P63M77_153†-Xibf570	106.0	47.0	5.60**	1	(0.008*)		
	QDts3F	3	3.37	Xpsr929	46.0	22.1	11.56****	1		(0.013***)	
	QDts4F	4	2.52	Xpsr39	34.0	18.1	3.74*	1	(0.009*)		
	QDts5F-1	5	5.77	P77M76_92-P77M75_115†	15.9	61.0	9.42****	1	(0.009**)		
	QDts5F-2	5	3.26	Xibf565	49.7	17.2	14.62****	3	−0.023**	−0.003 ns	0.014*
Ts	QTs1F	1	4.95	Xibf586	55.2	26.0	20.32*****	3	−0.09***	0.06**	−0.04 ns
	QTs3F	3	8.39	Xpsr394	47.9	56.7	5.65**	1		(0.05**)	
	QTs4F	4	2.65	Xpsr39	34.0	16.7	6.28**	1	(0.04**)		
	QTs5F	5	4.42	P77M76_92	10.9	54.9	ns	1			

[a] QTLs printed in bold are major QTLs, with LOD scores above the genome-wide significance thresholds. QTLs printed in normal font are suggestive QTLs with significance levels above the chromosome-wide LOD thresholds; [b]Kruskal–Wallis test statistics *K** significant at * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$ and ***** = $P \leq 0.00001$; [c]maternal, paternal and maternal × paternal interaction effects for fully informative peak markers (ab × cd) are significant at * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$. For markers segregating aaxab, abxaa or abxab, the values are enclosed in parentheses and indicate differences between the means of genotype classes in the parent that segregates for the marker, i.e. maternal for abxaa, paternal for aaxab and maternal × paternal for abxab markers; †markers used to test effects in cases where the marker peak is in large intervals without markers or there are different peak markers for IM and MQM

QTL alleles from the paternal (Norwegian) parent. For *QGs4F-2* the maternal effect was very large and positive (2.59, $P \leq 0.01$), while *QGs1F* had a similar large negative maternal effect (2.09, $P \leq 0.01$), and a smaller positive paternal effect (1.5, $P \leq 0.05$). The mean values for the drought traits were not significantly different between the two parents, except for Gs, for which the maternal parent was better (Table 1). Dominance, i.e. maternal × paternal interaction effects, was generally non-significant, except for *QTs1F* (Table 3).

Discussion

Comparative mapping of frost tolerance and winter survival QTLs in *F. pratensis* with Triticeae chromosomes

Chromosome 4 in fescues and ryegrasses contains one single segment with orthology to rice chromosome 3 and this segment is distributed over terminal regions of homoeologous group 4 and 5 chromosomes in all *Triticeae* genomes (Alm et al. 2003; Sim et al. 2005). A comparative map of markers and genes associated with frost tolerance on *Festuca* chromosomes 4 and 5 and the corresponding Triticeae group 5 chromosomes is presented in Fig. 2. QTLs on *Triticeae* group 5 chromosomes have been demonstrated to play major roles in frost tolerance, i.e. *Fr1* (*Fr-A1*) (Sutka and Snape 1989; Galiba et al. 1995), *Fr2 (Fr-D1)* (Snape et al. 1997), *Fr-B1* (Toth et al. 2003), and *Fr-A2 (Fr-A^{m}2)* (Vágújfalvi et al. 2003) in wheat, and winter hardiness and low-temperature tolerance QTLs, *Fr-H1* and *Fr-H2*, on barley 5H (Hayes et al. 1993; Francia et al. 2004). The vernalisation response gene *TaVRN1* is located close to the marker *Xwg644* on wheat 5A (Yan et al. 2003) and *FpVRN1,* an orthologue of *TaVRN1*, was isolated and shown to co-segregate with *Xwg644* (Ergon et al. 2006), at a similar position as the *LpVRN1* orthologue in *Lolium perenne* (Jensen et al. 2005). We find that *FpVRN1* on 4F is co-located with a small frost tolerance QTL (*QFt4F*) at marker *Xwg644*, while two major QTLs for frost tolerance are present on meadow fescue chromosome 5F. These findings are intriguing since, as mentioned above, several studies have found that the Triticeae group 5 chromosomes contain two frost tolerance loci, one of them linked closely to the *VRN1* locus. Based on the comparative map (Fig. 2) we propose that the distal QTL for winter survival on 5F (*QWs5F-2*) corresponds to the wheat *Fr-A1/Fr-H1* locus, and that the proximal frost tolerance/winter survival QTL (*QFt5F-2/QWs5F-1*) correspond to the *Fr-H2/Fr-A^{m}2* locus. Early studies in wheat found that the *VRN1* gene and the *Fr-A1* QTL were not closely linked; *Fr-A1* mapped 2 cM proximal to *Xwg644* on wheat 5A (Galiba et al. 1995; Sutka et al. 1999). However, it has been difficult to separate the effect of vernalisation from frost tolerance in the Triticeae. The current understanding is that the *Fr-A1* QTL in wheat is a pleiotropic effect of vernalisation as proposed by Limin and Fowler (2002); recently supported by studies using wheat lines with large deletions encompassing the

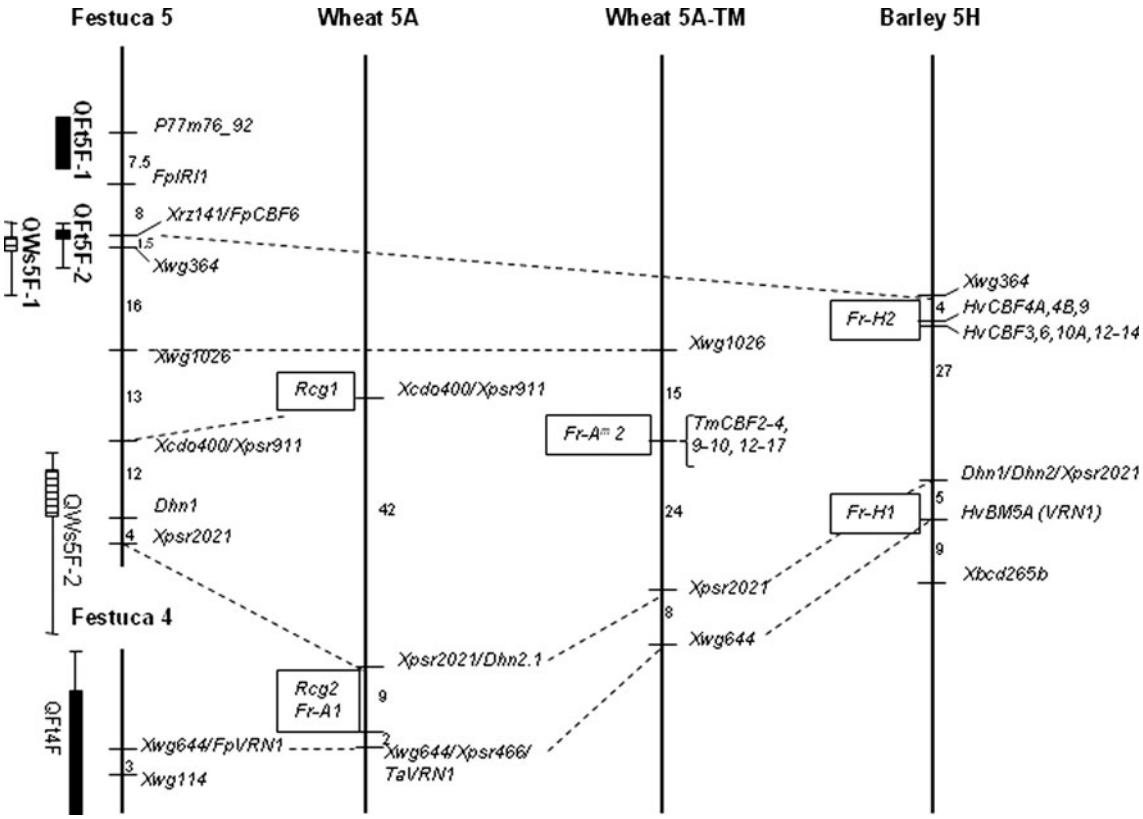

Fig. 2 A comparative map of *Festuca* chromosomes 4 and 5 with the homoeologous Triticeae group 5 chromosomes. The Triticeae group 5 maps and positions of QTLs and regulatory genes have been compiled from the following sources: Sutka and Snape 1989; Pan et al. 1994; Sutka et al. 1999; Vágújfalvi et al. 2000; Sarma et al. 2000; Choi et al. 2002; Vágújfalvi et al. 2003; Francia et al. 2004, Skinner et al. 2006; and Miller et al. 2006

complete *VRN-1* gene and several flanking genes (Dhillon et al. 2010). The interaction between the vernalisation and cold acclimation regulatory gene networks are thought to operate by extending the cold acclimation period and increasing the frost tolerance by delaying the induction of *VRN1* and thereby the transition to the reproductive stage (Galiba et al. 2009).

The structural difference between chromosomes 4 and 5 of *Festuca* and *Triticeae* makes it possible to separate the effects of vernalisation and frost tolerance/winter survival (Fig. 2). The *FpVRN1*gene on 4F co-located with a unique frost tolerance QTL (*QFt4F*), most likely a pleiotropic effect of vernalisation, while on 5F the winter survival QTL (*QWs5F*-2) is likely orthologous to *Fr-A1* on wheat 5A. Thus, there might indeed be a unique frost tolerance QTL on *Triticeae* group 5 chromosomes closely linked to *VRN1*, which is not caused by vernalisation. The close association of *QWs5F-2* with *Dhn1* and *Xpsr2021* (Figs. 1, 2), lends further support to our hypothesis that this QTL is orthologous to the low-temperature QTL found in the *VRN1/Fr-A1* region of *Triticeae* which has been linked to *Dhn1* and *Dhn2* in several studies (see Cattivelli et al. 2002).

We demonstrate that a *CBF* gene, *FpCBF6,* is co-located with the major frost tolerance/winter survival QTLs *QFt5F-2/QWs5F-1* on 5F, and maps only 0.9 cM from marker *Xwg364*, the peak marker of *QWs5F-1*. *CBF* genes are key regulators of early transcriptional response during cold acclimation (Vogel et al. 2005). In barley and hexaploid wheat, *CBF6* belongs to a cluster of many *CBF* genes closely linked to the marker *Xwg364* which co-localises with the *Fr-H2/Fr-A1* QTL (Skinner et al. 2006; Tondelli et al. 2006). A comprehensive association study of European cultivated barley, landraces and wild accessions found two SNPs in *HvCbf14,* a gene closely linked to *HvCBF6,* and one SNP in *Vrn-H1* to be statistically associated with frost tolerance (Fricano et al. 2009). One SNP in *HvCBF6* was nearly significant, and high LD was observed between SNPs in *HvCbf14* and *HvCbf6* (Fricano et al. 2009). Results from mapping of Ft and Ws in an annual (*L. multiflorum*) × perennial (*L. perenne*) ryegrass interspecific hybrid population also support the presence of a major QTL for Ft/Ws in the *Fr-H2/Fr-A2* syntenic region (Xiong et al. 2007). Taken together these results indicate that genetic regulation of frost tolerance is largely conserved among the temperate grasses.

The second Ft QTL (*QFt5F-1*) at the proximal end of 5F has no corresponding Ft QTL in cereals. The ice-renucleation inhibition gene *FpIRI1* maps 1.5 cM distal to the confidence interval of *QFt5F-1*, and is a candidate gene for this QTL. It belongs to a multi-gene family of ice re-crystallisation inhibition protein (IRIP) coding genes (Sandve et al. 2008) and is upregulated in meadow fescue during cold acclimation (Rudi et al. 2011). IRIPs have recently been shown to provide improved freezing tolerance in transgenic *A. thaliana*, which do not express these proteins endogenously in nature (Zhang et al. 2010). Since this QTL overlaps with a major QTL (*QDts5F-1*) for tolerance towards severe drought (Fig. 1), it might also be related to dehydration stress tolerance. This notion is supported by recent evidence that an introgression from the drought resistant species *Festuca glaucescens* into *L. multiflorum* at this chromosome 5 location led to enhanced drought tolerance (Ghesquiere et al. 2010). A *Festuca pratensis*-derived recessive stay-green mutant allele (*sid-1*) also co-locates to this 5F genomic region (Armstead et al. 2006) and may have relevance in tolerance to abiotic stresses. Dehydrins *Dhn4/5, Dhn6* and *Dhn1/2* all map in the region of the Ft and Ws QTLs (*QFt6F* and *Qws6F*) on chromosome 6F. In hexaploid wheat, the cold-inducible genes *Wcs120* and *wcor410* are located on wheat group 6 chromosomes (Limin et al. 1997; Danyluk et al. 1998). Choi et al. (1999) proposed that the WCS120 proteins are orthologous to the major cold-induced *Dhn5* gene located on 6HL in barley. Further evidence for genetic regulation of frost tolerance by genes on chromosome 6 was the mapping of a QTL for COR14b accumulation and two *HvCBF* loci on 6H by Francia et al. (2004). Börner et al. (2002) mapped a winter survival QTL on wheat 6AS about 30 cM distal to the marker *Xcdo476*. In the current *Festuca* population the overlapping Ft and Ws QTLs on 6F (*QFt6F* and *QWs6F*) are located 11.6 and 22.7 cM distal to *Xcdo476*, respectively, and could be orthologous to this winter survival QTL on wheat 6AS.

Malatrasi et al. (2002) mapped *Srg6*, a putative transcription factor induced by dehydration, on the short arm of barley 7H. Based on inference using the barley consensus maps (http://barleygenomics.wsu.edu) we find that *QFt7F* maps in a position orthologous to *Srg6*, while *QGs7F* is more proximal. In addition, a single gene for osmotic adjustment potential was mapped on wheat 7S (Morgan and Tan 1996), and in orthologous regions in rice (Lilley et al. 1996; Zhang et al. 2001) and barley (Teulat et al. 1998). This indicates that an osmotic adjustment determinant could be conserved across evolutionarily distant grass genomes.

Comparative mapping of drought tolerance QTLs in *F. pratensis* with Triticeae and rice chromosomes

Whilst major QTLs for growth under moderate and severe drought overlapped on 1F and 4F, distinct QTLs could be found for growth under severe drought on 3F and 5F and for growth under moderate drought on 6F (Fig. 1). Chromosome 3F is syntenic with rice chromosome 1 along its entire length (King et al. 2007). This chromosome is potentially a major source of drought tolerance in *Festuca* species having the QTL *QDts3* along its entire length associated with tolerance to severe drought stress. QTLs on rice chromosome 1 include ones involved in several drought adaptive traits like rooting and drought induced traits like osmotic adjustment (Kamoshita et al. 2008) Detailed studies by Turner et al. (2008) demonstrated clearly that 3F of *F. pratensis* carries QTLs similar to those identified in syntenic chromosomal regions in rice. Root/shoot ratio and root dry weight in rice mapped at marker CDO920 that is only 1.2 cM from the peak marker of the QTL for growth during severe drought (*QDts3F*) in the current study. It is thus possible that *QDts3F* may be associated with the root development under drought stress. Moreover, the importance of increased root growth and elevated osmotic stress tolerance derived from *Festuca* chromosome 3 alleles in *Festuca* × *Lolium* hybrids support a role of chromosome 3 in drought tolerance (Humphreys et al. 1997; Turner et al. 2008; Humphreys and Pasakinskiene 1996; Humphreys and Thomas 1993; Humphreys et al. 2005). Recently, *Festuca* 3F sequences associated with a salinity tolerance QTL were demonstrated to also contribute to increased salinity tolerance in *Festuca* × *Lolium* hybrids (Latorre 2010; Ul Haq et al. 2010). This salinity tolerance QTL is associated closely to the marker peak for the QTL for tolerance to severe drought *QDts3F* (*Xpsr929*) in the current mapping population and implies that genes for regulation of plant water status in addition to improved root growth underlie the function of this major drought tolerance QTL on 3F.

Whilst evidence for the importance of 3F in drought tolerance/plant vigour is conclusive, other chromosome loci have significant effects. Hemamalini et al. (2000) mapped a QTL for drought tolerance on rice chromosome 12 distal to the marker CDO344. This is consistent with the situation in *F. pratensis* where two QTLs for tolerance to severe drought map close to the marker CDO344, which has duplicated loci on 5F (Fig. 1). Root penetration ability has been mapped on rice chromosomes 1, 2 and 5 (syntenic with regions of 3F, 4F and 1F, respectively), root length and thickness on rice 5, and QTLs for all drought tolerance traits are present on all these *Festuca* chromosomes. In addition to rice chromosome 1, QTLs for root length have been mapped on rice chromosomes 2 and 9 (Hemamalini et al. 2000). These chromosomes are syntenic with regions on Fescue chromosomes, 6F and 5F, respectively, where several QTL for drought tolerance traits were present.

QTLs for drought tolerance traits were mapped in a *Lolium perenne* (a close relative of *F. pratensis*) population

and found to cluster on *Lolium* chromosomes 1 (L1) and 5 (L5) (Turner et al. 2008). Drought tolerance QTLs were also observed on homoeologous *F. pratensis* chromosomes in the current study, where especially for the moderate drought stress QTL *QDtm1F*, the use of common markers would indicate that the QTLs might well co-locate. In *Lolium* the site at the end of linkage group L1 was considered as a potential major site for genes controlling drought tolerance.

Winter survival can be explained by coincident locations of stress tolerance QTLs

Mapping of tolerance to several abiotic stresses in the same population makes it possible to estimate relative effects of the component traits Ft and Dt on the major trait Ws. Co-location of Ws QTLs with Dt QTLs indicate these to be due to drought, e.g. *QWs1F-2* which maps at the same position as QTLs for all drought tolerance traits, while co-location with Ft QTLs indicate freezing tolerance as the main factor, e.g. *QWs5F-1* and *QWs6F* (Fig. 1). However, there are also several examples of QTLs for different abiotic stresses not being co-located, e.g. *QDts3F* on 3F has no co-locating QTL related to Ws, nor is any drought tolerance QTL found at the same location as *QFt7F* on 7F. Ws QTLs not co-located with any component stress factors are most likely due to genes affecting seasonal adaptation, e.g. photoperiodic sensitivity. The Yugoslavian parent suffered severe winterkill while the Norwegian parent survived very well in the field (Table 1). The poor survival of the Yugoslavian genotype might be explained by its adaptation to photoperiods at lower latitudes. Ecotypes from lower latitudes will not induce hardening sufficiently early in the autumn when grown at higher latitudes. Therefore, it is likely that a part of the genetic variation for winter survival in this mapping family is due to segregation at loci involved in photoperiodic sensitivity. Thus the major QTL for winter survival on 2F (*QWs2F*), which is the single QTL that explains most of the variation (19.4%), might be due to photoperiodic sensitivity gene(s). Sourdille et al. (2000) mapped a QTL for photoperiodism on the short arm of wheat group 2 chromosomes and the major photoperiodic sensitivity gene *Ppd-H1* mapped 4.1 cM distal to the marker *Xpsr666* (Laurie et al. 1995). It has been cloned and shown to be a pseudo-response regulator (*PRR*) gene involved in the circadian clock function (Turner et al. 2005). *QWs2F* maps 16.5 cM distal to the same marker. Another possibility is that the QTL may overlie the cor14b locus, which Vágújfalvi et al. (2000) mapped on the long arm of chromosome $2A^m$ of *Triticum monococcum*, 10 cM from the centromere. Since *QWs2F* maps at a median position on the linkage group, this could be the orthologous position of the *cor14b* gene in *Festuca*. It has been demonstrated that *cor14b* is expressed at higher threshold temperatures in frost-tolerant than in frost-sensitive cultivars of barley (Crosatti et al. 1995; Crosatti et al. 1996), and that the development of frost tolerance follows closely the accumulation of COR-proteins in wheat (Fowler et al. 1996) and barley (Giorni et al. 1999). We attempted cloning and mapping of a *Festuca* homologue of *Hvcor14b* but this failed.

Concluding remarks

The coincident location of several of the QTL in *F. pratensis* with QTL and genes mapped in other grasses, notably the Triticeae species, indicate the action of structural or regulatory genes that are conserved across evolutionarily distant species. For orphan species like meadow fescue and perennial ryegrass, this is encouraging since valuable information about potential candidate genes can be drawn from the more advanced genomic research in the cereals. It is also clear that genome research on stress tolerance in the perennial forage grasses can be relevant for the cereal research community, as the discussion of the major frost tolerance loci on the homoeologous group 5 chromosomes has shown.

Acknowledgments We gratefully acknowledge Hanne Henriksen, Øyvind Jørgensen and Torleiv Veum, The Norwegian University of Life Sciences, and Britta From, Graminor AS, for excellent technical assistance. We would like also to thank Dr. Harry Thomas, IBERS (formerly the Institute of Grassland and Environmental Research), for indispensable help with the drought experiment, and Simen R. Sandve for valuable comments on the manuscript. This investigation was supported by the EU-projects 'European Gramineae Mapping Project' (EGRAM—contract no. BI04-CT97-2220) and 'Sustainable Grasslands Withstanding Environmental Stress' (SAGES—contract no. QLK5-CT-2000-00764), and Grants no. 110733/112 and 110732/130 from the Research Council of Norway.

References

Agromomix Software Inc. (1998) AGROBASE™. User's Guide and Command Reference. Winnipeg, Manitoba, Canada, pp 378

Alm V, Fang C, Busso CS, Devos KM, Vollan K, Grieg Z, Rognli OA (2003) A linkage map of meadow fescue (*Festuca pratensis* Huds.) and comparative mapping with other Poaceae species. Theor Appl Genet 108:25–40

Armstead I, Donnison I, Aubry S, Harper J, Hortensteiner S, James C, Mani J, Moffet M, Ougham H, Roberts L, Thomas A, Weeden N, Thomas H, King I (2006) From crop to model to crop: Identifying the genetic basis of the staygreen mutation in the *Lolium/Festuca* forage and amenity grasses. New Phytol 172:592–597

Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey M (1996) Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross. Heredity 77:64–73

Börner A, Schumann E, Furste A, Coster H, Leithold B, Roder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105:921–936

Cattivelli L, Baldi P, Crosatti C, Di Fonzo N, Faccioli P, Grossi M, Mastrangelo AM, Pecchioni N, Stanca AM (2002) Chromosome regions and stress-related sequences involved in resistance to abiotic stress in Triticeae. Plant Mol Biol 48:649–665

Choi DW, Zhu B, Close TJ (1999) The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 dhn genes of cv Dicktoo. Theor Appl Genet 98:1234–1247

Choi DW, Rodriguez EM, Close TJ (2002) Barley *Cbf3* gene identification, expression pattern, and map location. Plant Physiol 129:1781–1787

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Crosatti C, Soncini C, Stanca AM, Cattivelli L (1995) The accumulation of a cold-regulated chloroplastic protein is light-dependent. Planta 196:458–463

Crosatti C, Nevo E, Stanca AM, Cattivelli L (1996) Genetic analysis of the accumulation of cor14 proteins in wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare*) barley. Theor Appl Genet 93:975–981

Danyluk J, Perron A, Houde M, Limin A, Fowler B, Benhamou N, Sarhan F (1998) Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. Plant Cell 10:623–638

Davies WJ (2007) Responses of plant growth and functioning to changes in water supply in a changing climate. In: James IL, Morison MDM (ed) Plant growth and climate change, pp 96–117

Dhillon T, Pearce S, Stockinger E, Distelfeld A, Li C, Knox AK, Vashegyi I, Vágújfalvi A, Galiba G, Dubcovsky J (2010) Regulation of freezing tolerance and flowering in temperate cereals: the VRN-1 connection. Plant Physiol. doi:10.1104/pp.110.159079

Durand JL, Bariac T, Ghesquiere M, Biron P, Richard P, Humphreys M, Zwierzykovski Z (2007) Ranking of the depth of water extraction by individual grass plants, using natural o-18 isotope abundance. Environ Exp Bot 60:137–144

Ergon A, Fang C, Jørgensen O, Aamlid TS, Rognli OA (2006) Quantitative trait loci controlling vernalisation requirement, heading time and number of panicles in meadow fescue (*Festuca pratensis* Huds.). Theor Appl Genet 112:232–242

Fowler DB, Chauvin LP, Limin AE, Sarhan F (1996) The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. Theor Appl Genet 93:554–559

Francia E, Rizza F, Cattivelli L, Stanca AM, Galiba G, Toth B, Hayes PM, Skinner JS, Pecchioni N (2004) Two loci on chromosome 5H determine low-temperature tolerance in a 'Nure' (winter) × 'Tremois' (spring) barley map. Theor Appl Genet 108:670–680

Francia E, Barabaschi D, Tondelli A, Laido G, Rizza F, Stanca AM, Busconi M, Fogher C, Stockinger EJ, Pecchioni N (2007) Fine mapping of a Hv*CBF* gene cluster at the frost resistance locus *Fr-H2* in barley. Theor Appl Genet 115:1083–1091

Fricano A, Rizza F, Faccioli P, Pagani D, Pavan P, Stella A, Rossini L, Piffanelli P, Cattivelli L (2009) Genetic variants of *Hvcbf14* are statistically associated with frost tolerance in a European germplasm collection of *Hordeum vulgare*. Theor Appl Genet 119:1335–1348

Galiba G, Simonsarkadi L, Kocsy G, Salgo A, Sutka J (1992) Possible chromosomal location of genes determining the osmoregulation of wheat. Theor Appl Genet 85:415–418

Galiba G, Kocsy G, Kaursawhney R, Sutka J, Galston AW (1993) Chromosomal localization of osmotic and salt stress-induced differential alterations in polyamine content in wheat. Plant Sci 92:203–211

Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW (1995) RFLP mapping of the vernalization (*Vrn1*) and frost-resistance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179

Galiba G, Vágújfalvi A, Li CX, Soltesz A, Dubcovsky J (2009) Regulatory genes involved in the determination of frost tolerance in temperate cereals. Plant Sci 176:12–19

Ghesquiere M, Humphreys MW, Zwierzykowski Z (2010) Festulolium. In: Boller B, Posselt UK, Vernonesi F (eds) Fodder crops and amenity grasses. *Handbook of Plant Breeding* vol 5. Springer Science + Business Media, New York

Giorni E, Crosatti C, Baldi P, Grossi M, Mare C, Stanca AM, Cattivelli L (1999) Cold-regulated gene expression during winter in frost tolerant and frost susceptible barley cultivars grown under field conditions. Euphytica 106:149–157

Hackett CA (2002) Statistical methods for QTL mapping in cereals. Plant Mol Biol 48:585–599

Hayes PM, Blake T, Chen THH, Tragoonrung S, Chen F, Pan A, Liu B (1993) Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome-7 associated with components of winterhardiness. Genome 36:66–71

Hemamalini GS, Shashidhar HE, Hittalmani S (2000) Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). Euphytica 112:69–78

Humphreys MW, Pasakinskiene I (1996) Chromosome painting to locate genes for drought resistance transferred from *Festuca arundinacea* into *Lolium multiflorum*. Heredity 77:530–534

Humphreys MW, Thomas H (1993) Improved drought resistance in introgression lines derived from *Lolium multiflorum* × *Festuca arundinacea* hybrids. Plant Breed 111:155–161

Humphreys M, Thomas HM, Harper J, Morgan G, James A, GhamariZare A, Thomas H (1997) Dissecting drought- and cold-tolerance traits in the *Lolium-Festuca* complex by introgression mapping. New Phytol 137:55–60

Humphreys J, Harper JA, Armstead IP, Humphreys MW (2005) Introgression-mapping of genes for drought resistance transferred from *Festuca arundinacea* var. *glaucescens* into *Lolium multiflorum*. Theor Appl Genet 110:579–587

Humphreys MW, Yadav RS, Cairns AJ, Turner LB, Humphreys J, Skøt L (2006) A changing climate for grassland research. New Phytol 169:9–26

Jensen LB, Andersen JR, Frei U, Xing YZ, Taylor C, Holm PB, Lubberstedt TL (2005) QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat *VRN1*. Theor Appl Genet 110:527–536

Kamoshita A, Babu RC, Boopathi NM, Fukai S (2008) Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crops Res 109:1–23

King J, Armstead IP, Donnison SI, Roberts LA, Harper JA, Skot K, Elborough K, King IP (2007) Comparative analyses between *Lolium*/*Festuca* introgression lines and rice reveal the major fraction of functionally annotated gene models is located in recombination-poor/very recombination-poor regions of the genome. Genetics 177:597–606

Knott SA, Neale DB, Sewell MM, Haley CS (1997) Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor Appl Genet 94:810–820

Knox AK, Dhillon T, Cheng HM, Tondelli A, Pecchioni N, Stockinger EJ (2010) *CBF* gene copy number variation at *Frost Resistance-2* is associated with levels of freezing tolerance in temperate-climate cereals. Theor Appl Genet 121:21–35

Larsen A (1978) Freezing tolerance in grasses—methods for testing in controlled environments. Meld Norg LandbrHogsk 57(23):2–56

Latorre EM (2010) Characterisation of novel *Lolium multiflorum* germplasm for abiotic stress tolerance. Aberystwyth University, Aberystwyth

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of 5 major genes and 8 quantitative trait loci controlling flowering

time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585
Law CN, Sutka J, Worland AJ (1978) Genetic study of day-length response in wheat. Heredity 41:185–191
Lilley JM, Ludlow MM, McCouch SR, OToole JC (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice. J Exp Bot 47:1427–1436
Limin AE, Fowler DB (2002) Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the vernalization locus *Vrn-A1* in wheat (*Triticum aestivum* L. em Thell). Ann Bot 89:579–585
Limin AE, Danyluk J, Chauvin LP, Fowler DB, Sarhan F (1997) Chromosome mapping of low-temperature induced *wcs120* family genes and regulation of cold-tolerance expression in wheat. Mol Gen Genet 253:720–727
Malatrasi M, Close TJ, Marmiroli N (2002) Identification and mapping of a putative stress response regulator gene in barley. Plant Mol Biol 50:143–152
Miller AK, Galiba G, Dubcovsky J (2006) A cluster of 11 *CBF* transcription factors is located at the frost tolerance locus *Fr-A^{m}2* in *Triticum monococcum*. Mol Genet Genomics 275:193–203
Morgan JM, Tan MK (1996) Chromosomal location of a wheat osmoregulation gene using RFLP analysis. Aust J Plant Physiol 23:803–806
Pan A, Hayes PM, Chen F, Chen THH, Blake T, Wright S, Karsai I, Bedo Z (1994) Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89:900–910
Pulli S, Hjortsholm K, Larsen A, Gudleifsson B, Larsson S, Kristiansson B, Hömmö L, Tronsmo AM, Ruuth P, Kristensson C (1996) Development and evaluation of laboratory testing methods for winterhardiness breeding. Publications vol 32. Nordic Gene Bank, Alnarp
Quarrie SA, Laurie DA, Zhu JH, Lebreton C, Semikhodskii A, Steed A, Witsenboer H, Calestani C (1997) QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. Plant Mol Biol 35:155–165
Rognli OA, Saha MC, Bhamidimarri S, van der Hejden S (2010) Fescues. In: Boller B et al. (eds.), Fodder crops and amenity grasses, handbook of plant breeding 5, Springer Science + Business Media, New York, pp 261–292, doi: 10.1007/978-1-4419-0760-8_11
Rudi H, Sandve SR, Opseth LM, Larsen A, Rognli OA (2011) Identification of candidate genes important for frost tolerance in *Festuca pratensis* Huds. by transcriptional profiling. Plant Sci 180:78–85
Sandve SR, Rudi H, Asp T, Rognli OA (2008) Tracking the evolution of a cold stress associated gene family in cold tolerant grasses. BMC Evol Biol 8:245
Sarma RN, Fish L, Gill BS, Snape JW (2000) Physical characterization of the homoeologous Group 5 chromosomes of wheat in terms of rice linkage blocks, and physical mapping of some important genes. Genome 43:191–198
SAS (2002) SAS/SAS user's guide: statistics. Version 9.1. SAS Institute Inc., Cary, NC
Scarth R, Law CN (1983) The location of the photoperiod gene, *Ppd2* and an additional genetic-factor for ear-emergence time on chromosome 2B of wheat. Heredity 51:607–619
Scarth R, Law CN (1984) The control of the day-length response in wheat by the group 2 chromosomes. Plant Breed 92:140–150
Sim S, Chang T, Curley J, Warnke SE, Barker RE, Jung G (2005) Chromosomal rearrangements differentiating the ryegrass genome from the Triticeae, oat, and rice genomes using common heterologous RFLP probes. Theor Appl Genet 110:1011–1019
Skinner J, Szucs P, von Zitzewitz J, Marquez-Cedillo L, Filichkin T, Stockinger EJ, Thomashow MF, Chen THH, Hayes PM (2006) Mapping of barley homologs to genes that regulate low temperature tolerance in *Arabidopsis*. Theor Appl Genet 112:832–842
Snape JW, Semikhodskii A, Fish L, Sarma RN, Quarrie SA, Galiba G, Sutka J (1997) Mapping of frost toleranc loci in wheat an comparative mapping with other cereals. Acta Agron Hung 45:265–270
Sourdille P, Snape JW, Cadalen T, Charmet G, Nakata N, Bernard S, Bernard M (2000) Detection of QTLs for heading time and photoperiod response in wheat using a doubled-haploid population. Genome 43:487–494
Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA 94:1035–1040
Sutka J, Snape JW (1989) Location of a gene for frost resistance on chromosome 5A of wheat. Euphytica 42:41–44
Sutka J, Galiba G, Vágújfalvi A, Gill BS, Snape JW (1999) Physical mapping of the *Vrn-A1* and *Fr1* genes on chromosome 5A of wheat using deletion lines. Theor Appl Genet 99:199–202
Tamura K, Yamada T (2007) A perennial ryegrass *CBF* gene cluster is located in a region predicted by conserved synteny between poaceae species. Theor Appl Genet 114:273–283
Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). Theor Appl Genet 96:688–698
Thomas H, Evans C (1989) Effects of divergent selection for osmotic adjustment on water relations and growth of plants of *Lolium perenne*. Ann Bot 64:581–587
Thomas H, Humphreys MO (1991) Progress and potential of interspecific hybrids of *Lolium* and *Festuca*. J Agric Sci 117:1–8
Tondelli A, Francia E, Barabaschi D, Aprile A, Skinner JS, Stockinger EJ, Stanca AM, Pecchioni N (2006) Mapping regulatory genes as candidates for cold and drought stress tolerance in barley. Theor Appl Genet 112:445–454
Toth B, Galiba G, Feher E, Sutka J, Snape JW (2003) Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor Appl Genet 107:509–514
Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. Science 310:1031–1034
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
Ul Haq T, Gorham J, Akhtar J, Steele KA (2010) Dynamic QTL for stress components on chromosome 1 of rice. Func Plant Biol (in press)
Vágújfalvi A, Crosatti C, Galiba G, Dubcovsky J, Cattivelli L (2000) Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the Cor14b gene in frost-tolerant and frost-sensitive genotypes. Mol General Genet 263:194–200
Vágújfalvi A, Galiba G, Cattivelli L, Dubcovsky J (2003) The cold-regulated transcriptional activator *Cbf3* is linked to the frost tolerance locus *Fr-A2* on wheat chromosome 5A. Mol Genet Genom 269:60–67
Vágújfalvi A, Aprile A, Miller A, Dubcovsky J, Delugu G, Galiba G, Cattivelli L (2005) The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost resistance in wheat. Mol Genet Genom 274:506–514
van Ooijen JW, Maliepaard C (1996) MapQTL version 3.0: Software for the calculation of QTL position on genetic maps. CPRO-DLO, Wageningen
van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2000) MapQTL™ version 4.0: user friendly power in QTL mapping. Adden-

dum to the manual of version 3.0. Plant Research International, Wageningen

Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF (2005) Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis. Plant J 41:195–211

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93:77–78

Welsh JR, Keim DL, Pirasteh B, Ricarhds RD (1973) Genetic control of photoperiod response in wheat. In: Sears ER, Sears LMS (eds), Proceeding of the 4th International Wheat Genetics Symposium. University of Missouri, Columbia, USA, pp 879-884

Xiong YW, Fei SZ, Arora R, Brummer EC, Barker RE, Jung GW, Warnke SE (2007) Identification of quantitative trait loci controlling winter hardiness in an annual × perennial ryegrass interspecific hybrid population. Mol Breed 19:125–136

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: Comparative mapping within and across species. Theor Appl Genet 103:19–29

Zhang C, Fei S-z, Arora R, Hannapel D (2010) Ice recrystallization inhibition proteins of perennial ryegrass enhance freezing tolerance. Planta 232:155–164