Theor Appl Genet (2006) 112: 232–242
DOI 10.1007/s00122-005-0115-z

ORIGINAL PAPER

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Quantitative trait loci controlling vernalisation requirement, heading time and number of panicles in meadow fescue (*Festuca pratensis* Huds.)

Received: 1 July 2005 / Accepted: 14 September 2005 / Published online: 19 October 2005

Abstract The "BF14/16×HF2/7" mapping population of meadow fescue (*Festuca pratensis* Huds.) was characterised for number of panicles produced by non-vernalised plants in the field, vernalisation requirement (number of weeks at 6°C and 8 h photoperiod), as well as days to heading, number of panicles and proportion of shoots heading after a 12 weeks vernalisation treatment. Quantitative trait loci (QTLs) were identified and compared to QTLs and genes related to the induction of flowering in cereals and grasses. A region on chromosome 1F affected days to heading and the proportion of shoots heading. Chromosome 4F appeared to have several genes with a strong effect on vernalisation requirement. The strongest effects were located in the proximal end of 4F and may correspond to the earliness per se (eps) QTL eps6L.2 in barley and a heading time QTL in perennial ryegrass. A part of the meadow fescue orthologue of *VRN1* was sequenced and mapped to another region of 4F that also had a strong effect on vernalisation requirement. The proximal end of chromosome 5F had QTLs for days to heading and proportion of heading shoots. Syntenic regions in wheat and barley contain eps-loci. A QTL for number of panicles in the field and a QTL for proportion of heading shoots were present on chromosome 6. A region on 7F affected the variation in number of panicles among plants without a vernalisation requirement, and is syntenic to regions in perennial ryegrass, barley and rice containing orthologues of *Arabidopsis thaliana CO*.

Communicated by T. Lübberstedt

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Introduction

At a certain point in time the apical meristem starts to produce reproductive structures instead of leaves. The timing of this transition is largely controlled by environmental cues like temperature and day length. This ensures that flowering takes place at the optimal time for successful seed production.

Meadow fescue (*Festuca pratensis* Huds.) is an important fodder crop species in the northern temperate regions. It is known to have a dual induction requirement for flowering (Cooper and Calder 1964; Bean 1970; Heide 1988, 1994). First, primary induction (vernalisation) occurs in response to a certain period of low temperature. Short days interact with low temperature to stimulate primary induction. When plants are exposed to long days after primary induction, further development of the shoot apex is stimulated (secondary induction).

In the model plant *Arabidopsis thaliana* several signalling pathways appear to control the induction of flowering, the long-day pathway, the vernalisation pathway, and the autonomous pathway being the most studied (reviewed by Putterill et al. 2004). In spite of differences in flowering behaviour and the long time span since the divergence of monocots and dicots, *A. thaliana* and rice share some of the components of photoperiod sensitivity leading to the induction of flowering (Putterill et al. 2004). *HEADING DATE 6* (*HD6*) in rice encodes a homologue of the α subunit of *CASEIN KINASE 2* (*CK2*), a regulator of circadian

clock activity and flowering time in *A. thaliana*. *A. thaliana CONSTANS* (*CO*) and its rice homologue *HEADING DATE 1* (*HD1*) are regulated by photoperiod and by the circadian clock, and in turn regulate the expression of *A. thaliana FLOWERING LOCUS T* (*FT*) and its rice homologue *HEADING DATE 3a* (*HD3a*), which both promote flowering. So far, genes involved in the vernalisation pathway or the autonomous pathway in *A. thaliana* have not been identified in monocots.

In the Triticeae, a number of genes controlling flowering time have been identified and mapped (reviewed by Snape et al. 2001). These genes fall into three groups, namely those controlling vernalisation response, photoperiod response (during secondary induction), and those that are largely independent of the vernalisation and the photoperiod pathways, the so-called earliness per se (eps) genes. There is an extensive conservation of gene order within the Triticeae (Moore et al. 1995; Devos and Gale 1997) and between Triticeae and the less studied *Festuca* and *Lolium* species (Jones et al. 2002; Alm et al. 2003; Sim et al. 2005). *Festuca* and *Lolium* can therefore be expected to share much of the genes and mechanisms behind the induction of flowering with the Triticeae. Several genes controlling flowering time are found at orthologous positions in different Triticeae genomes (Snape et al. 2001). These include the Vrn-1 series on group 5 chromosomes of wheat, barley and rye, the Vrn-2 series on chromosome 4 of barley and chromosome 5 of *Triticum monococcum*, the Ppd-1 series on group 2 chromosomes of wheat and barley and the Eps-2 series on group 2 chromosomes of wheat and barley. Candidates for genes of the Vrn-1 and Vrn-2 series have been cloned and sequenced. Vrn-A^m1 (*VRN1*/*AP1*) was cloned from *T. monococcum* using a map-based approach (Yan et al. 2003). Putative orthologues of *VRN1*/*AP1* have been sequenced from hexaploid wheat (Danyluk et al. 2003; Murai et al. 2003), barley (Schmitz et al. 2000), perennial ryegrass (Petersen et al. 2004), *L. temulentum* (Gocal et al. 2001) and rice (Kyozuka et al. 2000). *ZCCT1*, a candidate for Vrn-A^m2, has been identified in *T. monococcum* and barley (Yan et al. 2004; Dubcovsky et al. 2005), and a model has been proposed where *VRN1*/*AP1*, a promoter of flowering, is repressed by *VRN2*/*ZCCT1*, which in turn is down-regulated by vernalisation (Yan et al. 2003). Wheat, barley and perennial ryegrass have genes with similarity to genes acting in the photoperiodic pathway in rice and *A. thaliana*. Sequence similarity, map locations and complementation of HD1 function in transgenic rice indicate that barley, wheat, perennial ryegrass and meadow fescue orthologues of *HD1*/*CO* exist (Griffiths et al. 2003; Nemoto et al. 2003; Martin et al. 2004; Armstead et al. 2005). Wheat *CK2a* encodes the alpha unit of casein kinase II and maps at the orthologous position of rice *HD6* (Kato et al. 2002). *CK2a* is tightly linked to *VRN1*/*AP1* in wheat, and it is therefore difficult to separate the effects of these two genes.

In both rice and cereals, the timing of the transition from the vegetative to the reproductive stage is the most commonly used measure of inductive requirements. However, the proportion of meristems undergoing transition and the number of flowers produced per panicle are equally important quantitative aspects of flower induction (Heide 1988).

The aim of the present study was to identify quantitative trait loci (QTLs) controlling the induction of flowering in meadow fescue and to relate these QTLs to relevant genes and QTLs identified in orthologous chromosomal regions of other grass and cereal species.

Materials and methods

Plant material

A full-sib family consisting of 137 progenies from a cross between a genotype [B14/16(♀)] from the Yugoslavian population B14 and a genotype [HF2/7(♂)] from the Norwegian population HF2 was used in this study. The family has previously been genotyped with a variety of molecular markers, including cereal anchor probes. These data formed the basis for the construction of a genetic linkage map (Alm et al. 2003).

Phenotyping

Non-vernalised ramets were transplanted into field trials in the spring of 1998 at two locations in southern Norway; Norderås, Ås in Akershus (59°40′N, 10°51′E, 100 m asl.), and Landvik, Grimstad in Aust-Agder (58°19′N, 8°30′E, 6 m asl.). The field experiments were designed as randomised complete blocks with three replicates and are described by Fang et al. (2004). The sum of panicles produced per plant during the season at Norderås (NPL) and Landvik (NPL) was recorded. In the beginning of April 2002, clonally maintained plants (mother plants) were divided into three ramets consisting of four to six tillers, planted in peat soil in 7.5-cm pots and grown for 3 weeks in the greenhouse (pre-growth). The pots were then transferred to a growth chamber and vernalised for 12 weeks at 6°C and 8 h photoperiod with a light intensity of 250 μmol/m^2 s (400 W HQI-BT bulbs, Osram). Similarly, other sets of mother plants were divided, pre-grown and vernalised for 9, 6 and 0 weeks, respectively. This was timed so that the vernalisation ended on the same date for all treatments (July 15). The plants were then transferred to the greenhouse for the secondary induction and grown there for 11 weeks. The average temperatures in the greenhouse were 18°C (day)/15°C (night) at the beginning of the experiment, but increased gradually up to approximately 24°C (day)/19°C (night) at the time of transfer of the vernalised plants to the greenhouse. These temperatures were maintained for the rest of the experiment. The photoperiod of the natural light increased from 13 h up to 19 h at the end of June and then decreased to 11 h at the end of the

experiment in September. Additional light was given (400 W HQI-BT bulbs, Osram) to ensure a photoperiod of minimum 16 h. Plants were fertilised once every third week with a solution of 300 g/l calcium nitrate and 400 g/l Superba Rød (7(N) – 4(P) – 21(K), L.O.G., Oslo, Norway) diluted to a conductivity of 1.5 mSiemens. The pots were organised into three randomised replicate blocks, the vernalisation treatments being sub-blocks within each block.

The number of days from the start of secondary induction to the emergence of the first panicle through the leaf sheath of the flag leaf was recorded for each pot (days to heading—DTH). A value of zero was given to pots where this had occurred during pre-growth or vernalisation. At the end of the experiment (11 weeks after transfer to secondary induction), the number of panicles (NP) and number of tillers in each pot were recorded. The proportion of heading shoots (PHS) was calculated as NP divided by the number of tillers. Vernalisation requirement (VR) was determined by the number of weeks of vernalisation required for at least one of the three parallel pots to head. Each genotype was given a score between 1 and 5 (1: 0 weeks, 2: 0–6 weeks, 3: 6–9 weeks, 4: 9–12 weeks and 5: >12 weeks vernalisation requirement).

Data analysis

The analysis of variance and calculation of Pearson correlation coefficients were performed in the statistical program package SAS 8.2 (SAS 1987), using PROC GLM and PROC CORR. Maternal, paternal and maternal × paternal interaction effects as described by Knott et al. (1997) were estimated and tested for full-informative markers (ab×cd) by means of orthogonal contrasts using PROC GLM of SAS.

QTL analyses were based on the mapping data described by Alm et al. (2003). In cases where several markers had identical map positions, only one, preferably a cereal anchor probe or full-informative (ab×cd) marker, was selected. A total of 156 markers were maintained, and these had an average spacing of 4.2 cM. Kruskal-Wallis (KW) analysis, interval mapping (IM) and multiple QTL mapping (MQM) were performed using the MapQTL 4.0 software (van Ooijen and Maliepaard 1996; van Ooijen et al. 2000). IM and MQM were only performed on traits that had an approximate normal distribution. LOD threshold levels for significant QTLs were defined for each trait and linkage group by permutation tests (Churchill and Doerge 1994) using the MapQTL software. MQM started with the QTLs identified by IM. For each QTL, one closely linked marker was used as a cofactor in the MQM model. When previously defined QTLs lost their significance or new QTLs were revealed, the MQM process was repeated until there was one significant QTL for each cofactor in the model. Before each round of MQM, the less influential cofactors were eliminated using the automatic cofactor selection option. Map positions were defined by the peak LOD score obtained in MQM and confidence intervals were defined by the peak ± 2 LOD.

Amplification of *VRN1* and characterisation of a polymorphism in *VRN1*

A segment of a homologue of *VRN1* was PCR-amplified using the primers lpVRN1_19for and lpVRN1_344rev (Jensen et al. 2005). PCR reactions (20 µl) containing 0.4 µM primers, 200 µM dNTPs, 0.05 U/µl Expand High Fidelity^{PLUS} Enzyme Blend (Roche Diagnostics GmbH, Germany), 1x reaction buffer and 50 ng genomic DNA were set up for the two parents and amplified in a Mastercycler ep (Eppendorf AG, Hamburg, Germany) using the following cycling parameters: 5 min denaturation at 94°C followed by 30 amplification cycles of 30 s at 94°C, 30 s at 50°C and 30 s at 72°C and a final extension for 5 min at 72°C. PCR products were purified with the QIAquick PCR Purification kit (Qiagen) and sequenced using the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 3100 Genetic Analyser (Applied Biosystems). For the genotyping of the mapping population, non-purified PCR products (generated as above) were digested by adding 5 U BsmAI (NEB), NEB buffer 3 and water to a volume of 40 µl and incubating at 55°C for 1.5 h. Restriction products were separated on a 1.5% agarose gel, and individuals were recognised as homozygous (a single band corresponding to the uncut PCR product) or heterozygous (three bands).

Results

Phenotyping

The NP produced in the year of planting was significantly higher at Landvik than at Norderås (Table 1). There were significant differences between genotypes at both locations. HF2/7 flowered at both locations while BF14/16 did not flower at any of the locations. Seventy-four of the 137 genotypes flowered at Norderås, while 79 flowered at Landvik. Of these there were 68 genotypes that flowered at both locations. Genotype means for NP at the two locations were highly correlated (Table 2). Thus, although there was a significant interaction between location and genotype on NP, genotypes that were able to flower without vernalisation generally did so at both locations.

HF2/7 and 78 of the genotypes were able to head independently of vernalisation in the greenhouse experiment. Out of these 78 genotypes, 70 also headed without vernalisation in the field experiment. Two of the genotypes required 0–6 weeks of vernalisation and 8 required 6–9 weeks, while 44 genotypes as well as BF14/

Table 1 Mean and range of traits related to flower induction in the 'B14/16 × HF2/7' full-sib family of meadow fescue observed in field experiments with non-vernalised plants and in a greenhouse experiment with different vernalisation treatments

Experiment	Trait	Parents		F1[a]	
		HF2/7	BF14/16	Mean	Range
Field	NPN[b]	1.0	0	3.4	0–20
	NPL[c]	10.3	0	12.8	0–76.3
	NPNH[d]	1.0	–	6.3	0.3–20
	NPLH[e]	10.3	–	22.9	0.3–76.3
Greenhouse	VR[f]	1	4.0	2.23	1–5
	DTH12[g]	27.3	34.3	28.9	18.7–46.7
	NP12[h]	7.0	4.7	4.2	0–12
	PHS12[i]	14	10	15	0–38

[a] $n = 137$
[b] Number of panicles per plant (NP) at Norderås (N)
[c] NP at Landvik (L)
[d] NP for the sub-group of genotypes that headed at N
[e] NP for the sub-group of genotypes that headed at L
[f] Vernalisation requirement (1: < 0 weeks, 2: 0–6 weeks, 3: 6–9 weeks, 4: 9–12 weeks and 5 > 12 weeks at 6°C and 8 h photoperiod)
[g] Days to heading after 12 weeks of vernalisation
[h] NP in the 12 week vernalisation treatment
[i] Proportion of heading shoots (%) in the 12 week vernalisation treatment

16 required 9–12 weeks of vernalisation. Five genotypes did not head; they presumably required more than 12 weeks of vernalisation. The intensity of heading was generally low after 0, 6 and 9 weeks of vernalisation and higher after 12 weeks of vernalisation (average NP among heading genotypes = 0.9, 1.6, 1.3 and 4.4, respectively). DTH was calculated only when all the three parallel plants headed. This was the case for 21, 29, 21 and 98 genotypes in the 0, 6, 9 and 12 weeks of vernalisation treatments, respectively. The parents had intermediate values for NP, proportion of heading shoots (PHS) and DTH, but HF2/7 was earlier and produced more panicles than BF14/16 (Table 1).

Correlations between traits recorded in the 12-week vernalisation treatment and in the field experiments are shown in Table 2. NP12 and PHS12 were correlated with each other and negatively correlated with DTH12. There was some correlation between VR and DTH12, NP12 and PHS12. VR was moderately correlated with NP in the field, while DTH12, NP12 and PHS12 were only weakly correlated with NP in the field.

QTL analysis

The main variation in the number of panicles at Norderås (NPN) and Landvik (NPL) was due to heading/no heading. The variation in the number of panicles among genotypes that headed could be due to genes different from those controlling heading/no heading. Therefore, the number of panicles among heading plants (non-heading plants excluded), termed NPNH and NPLH, was analysed in addition to NPN and NPL. The traits in the greenhouse experiment analysed for QTLs were VR, DTH12, NP12 and PHS12. DTH, NP and PHS for the shorter vernalisation treatments were not analysed due to the limited number of genotypes flowering in these treatments.

The results from KW analyses showed that many markers covering most of chromosome 4F were very strongly associated with NPN, NPL, NPLH, NPNH and VR, the proximal region of the chromosome having the strongest effects (Table 3, Fig. 1). Analyses of parental effects by orthogonal contrasts showed that there were strong maternal effects, and no paternal effects, of all full-informative markers on chromosome 4. These results were supported by results from separate KW analyses on the parental maps, showing that maternal alleles were associated with phenotypic variation, whereas paternal alleles were not (results not shown). In addition to the markers on chromosome 4, there were strong associations between marker *Dhn6c* at position 9.6 cM on 6F and NPN, NPL and NPLH. Markers *Xcdo17* (41.0 cM), *Xdjf20b* (44.2 cM) and *Xibf596d* (50.7 cM) on 7F were associated with NPNH or NPLH, but not with NPN or NPL. *Xcdo17* had significant paternal and maternal × paternal interaction effects on NPNH.

Kruskal-Wallis analysis indicated that DTH12 and PHS12 were associated with the same markers around 50 cM on 1F and with markers in an interval between 29 and 40 cM on 5F, and that NP12 was associated with *Xcdo678a* at 38.5 cM on 4F (data not shown). The presence of QTLs in these regions was confirmed by IM and MQM (Table 4, Fig. 1). Both IM and MQM detected two QTLs affecting DTH12. They were located at 41.3 cM between *Xibf580b* and *Gpi-2* on 1F (QDth1F-1) and at 31.0 cM (*Xcdo412*) on 5F (QDth5F-1). In the MQM model they had LOD scores of 4.8 and 3.6, and explained 22 and 12% of the phenotypic variation,

Table 2 Pearsons correlation coefficients between traits

Trait	Trait[a]						
	NPN	NPL	NPNH	NPLH	VR	DTH12	NP12
NPL	0.90***						
NPNH	1.00***	0.81***					
NPLH	0.82***	1.00***	0.79***				
VR	−0.55***	−0.56***	−0.23*	−0.34**			
DTH12	−0.34***	−0.36***	NS	−0.34**	0.43***		
NP12	0.22*	0.26**	NS	NS	−0.37***	−0.30**	
PHS12	0.23**	0.25**	NS	NS	−0.37***	−0.63***	0.54***

NS Not significant
[a] For a description of traits, see footnote in Table 1.
***$P < 0.001$,
**$0.001 < P < 0.01$,
*$0.01 < P < 0.05$

Table 3 Quantitative trait loci detected for number of panicles produced in the year of planting (no vernalisation) and vernalisation requirement as measured in the greenhouse experiment

LG	Position (cM)	Marker	df[b]	Trait[a]															
				NPN				NPL				NPNH				NPLH				VR			
				K[c]	Marker effect[d]			*K*	Marker effect			*K*	Marker effect			*K*	Marker effect			*K*	Marker effect		
					M	*P*	*M*×*P*		*M*	*P*	*M*×*P*		*M*	*P*	*M*×*P*		*M*	*P*	*M*×*P*		*M*	*P*	*M*×*P*
4	15.9	*Dhn4/5f*	1	45.2				45.7								16.4				18.2			
4	18.6	*Dhn4/5c*	1	57.2				57.1				9.0				14.1				23.7			
4	34.0	*Xpsr39*	1	57.3				57.0								13.2				30.2			
4	38.5	*Xcdo678a*	3	43.3	8.7	NS	NS	46.9	36.8	NS	NS									20.5	2.4	NS	NS
4	40.6	*Xpsr115*	3	50.6	9.8	NS	NS	54.1	39.7	NS	NS									32.9	3.0	NS	NS
4	44.4	*Xibf29*	3	46.5	8.7	NS	NS	50.0	37.6	NS	NS									26.7	2.7	NS	NS
4	50.5	*Xrgc496a*	1	33.9				30.8								8.8				12.1			
4	52.4	*Xibf543a*	3	35.1	8.1	NS	NS	39.8	33.8	NS	NS					13.5	21.56	NS	NS	18.9	2.1	NS	NS
4	53.7	*Xrz531*	1	35.1				34.0								12.9				9.0			
4	54.3	*Xwg644*	1	39.2				38.3								9.8				17.6			
4	55.0	*Xcsu33*	1	42.1				38.9								11.3				15.9			
4	57.7	*Xwg114*	3	45.5	9.4	NS	NS	42.9	36.7	NS	NS									20.0	2.2	NS	NS
4	58.4	*Xibf514*	3	38.0	8.1	NS	NS	38.1	34.7	NS	NS									18.5	2.3	NS	NS
4	60.5	*p76m66_6*	1	23.9				24.8															
4	68.1	*Pgm*	2	33.0				25.1								16.2							
6	9.6	*Dhn6c*	1	22.8				23.2								11.2							
7	41.0	*Xcdo17*	3									16.0	NS	5.71	5.03								
7	44.2	*Xdjf20b*	3													13.1	NS	NS	NS				
7	50.7	*Xibf596d*	1									14.0											

[a]For a description of traits, see footnote in Table 1. The number of individuals analysed for each trait/marker combination were 112–134 for NPN, NPL and VR and 60–76 for NPNH and NPLH

[b]Degrees of freedom

[c]Kruskal-Wallis test statistic. All QTLs for NPN and NPH were significant at a 0.0001 level. QTLs for NPNH, NPLH and VR were significant at 0.005, 0.001 or 0.0001 levels. Marker-trait associations with a significance level below 0.005 were regarded as not significant

[d]Number of panicles (NPN, NPL, NPHN, NPLH) or classes (VR). *M* effect of maternal alleles, *P* effect of paternal alleles. Parental marker effects for NPN, NPL and VR were all significant at $P \leq 0.001$ or non-significant (NS) at $P > 0.05$. Parental marker effects on NPNH and NPLH were significant at $P \leq 0.05$ or non-significant

respectively. There was a maternal effect of QDth1F-1 and QDth5F-1 on DTH12 (6.1 and 5.8 days, respectively). In addition, there was a paternal effect and a maternal × paternal interaction effect of QDth1F-1 on DTH12 (5.8 and 3.7 days, respectively).

Interval mapping detected four QTLs affecting PHS12, one at 50.6 cM (*Xcdo580(Ivd)*) on 1F, one at 5.7 cM (*Dhn4/5 g*) on 4F and two closely linked at 30.3 cM (*Xibf529a*) and 39.7 cM (*Xwg364a*) on 5F (Table 4, Fig. 1). After two rounds of MQM, the peak on 4F and the peak at 30.3 cM on 5F were insignificant or had disappeared, the peak on 1F had shifted to 46.3 cM (*Gpi-2*) and one new QTL at 51.6 cM (*Xibf547*) on 6F was revealed (Table 4, Fig. 1). The three QTLs in the final MQM model, QPhs1F-1, QPhs5F-1 and QPhs6F-1 had LOD scores of 5.7, 11.5 and 4.7 and explained 12, 26 and 10% of the variation in PHS12, respectively. There was a maternal effect of QPhs1F-1 and QPhs6F-1 on PHS12 (both 10%). QPhs5F-1 had both maternal and paternal effects on PHS12 (18 and 7%, respectively).

One QTL affecting NP12 was detected on 4F by both IM and MQM. It was located at 38.5 cM (*Xcdo678a*), and had a LOD score of 4.3 and explained 14% of the variation in NP12 in the MQM model (Table 4, Fig. 1). No maternal or paternal effect of this locus could be detected, but there was a maternal × paternal interaction effect (3.9 panicles per pot).

Mapping of a *VRN1* orthologue

A single 319-bp fragment was PCR-amplified using the lpVRN1 primers. The 279 bp between the primers [designated *FpVRN1* (DQ108934)] cover 53 of the 60 amino acid residues of the MADS-box domain as well as a part of the 5′UTR. At the amino acid level, the MADS-box domain was identical to that of *TmAP1* (AY188331), *TaVRT1* (AY380870), *LpMADS1* (AY198326) and *LtMADS1* (AF035378), indicating that it was indeed a homologue of the Vrn1-series of genes that had been amplified. One single nucleotide polymorphism for which the female parent (BF14/16) was heterozygous and the male parent (HF2/7) homozygous was found in the 5′UTR. It resided in a recognition site of the restriction enzyme BsmAI, and this was utilized in the genotyping of the mapping population. In wheat, Vrn1 is closely linked to the marker *Xwg644* (Galiba et al. 1995). Both *Xwg644* and the polymorphism detected in *VRN1* were polymorphic in the female parent

Table 4 Quantitative trait loci detected for heading traits in the greenhouse experiment

Trait[a]	LG	QTL[b]	Interval mapping				MQM mapping				Marker effect[c]		
			Position (cM ±2LOD)	Marker peak	LOD	Variation explained (%)	Position (cM ±2LOD)	Marker peak	LOD	Variation explained (%)	*M*	*P*	*M*×*P*
DTH12	1	QDth1F-1	41.3 (32.5–50.6)	*Xibf580b – Gpi-2*	4.9	27	41.3 (32.5–46.3)	*Xibf580b – Gpi-2*	4.8	22	6.1**	NS	NS
	5	QDth5F-1	31.0 (20.8–44.3)	*Xcdo412*	4.4	19	31.0 (29.7–32.6)	*Xcdo412*	3.6	12	5.8**	5.8**	3.7*
NP12	4	QNp4F-1	38.5 (32.2–44.4)	*Xcdo678a*	4.3	14	38.5 (32.2–40.6)	*Xcdo678a*	4.3	14	NS	NS	3.9***
PHS12	1	QPhs1F-1	50.6 (36.3–52.4)	*Xcdo580*	3.3	11	46.3 (36.3–50.6)	*Gpi-2*	5.7	12	10**	NS	NS
	4	QPhs4F-1	5.7 (0.0–15.7)	*Dhn4/5g*	3.6	15							
	5	QPhs5F-1	30.3 (21.6–32.6)	*Xibf529a*	7.8	23					18***	NS	NS
	5	QPhs5F-2	39.7 (37.6–44.3)	*Xwg364a*	8.6	26	39.7 (37.6–40.3)	*Xwg364a*	11.5	26	18**	7*	NS
	6	QPhs6F-1					51.6 (50.4–53.2)	*Xibf547*	4.7	10	10**	NS	NS

[a]For a description of traits, see footnote in Table 1
[b]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 LOD scores above the chromosome-wide significance thresholds
[c]Number of days (DTH12), number of panicles (NP12) or percent heading shoots (PHS12). *M* effect of maternal alleles, *P* effect of paternal alleles, NS not significant
*, $0.05 \geq P \geq 0.01$; **, $0.01 \geq P \geq 0.005$; ***, $0.005 \geq P \geq 0.001$

only. The genotype of the *VRN1* locus was therefore compared to that of *Xwg644* and surrounding markers on the maternal map (Alm et al. 2003) for all individuals in the mapping population. Analysis of the recombinants indicated that *VRN1* is closely linked to *Xwg644* (one recombinant) (Fig. 1).

Discussion

Although meadow fescue has been reported to have a very strong vernalisation requirement for the induction of flowering (Cooper and Calder 1964; Bean 1970; Heide 1988), HF2/7 and many of the progenies flowered to a limited extent without vernalisation in the present study. Fjellheim (2004) also found ten Nordic cultivars to be able to flower marginally after being sown in the greenhouse in April and planted in the field 2–3 months later, and Aamlid (2005) obtained 15–35% flowering of both seedlings and clonal plants after only 6 weeks of vernalisation in a pot experiment. One possible explanation for the apparent discrepancy regarding a strong and absolute vernalisation requirement in meadow fescue may be that temperatures as high as 12–15°C have a slight vernalising effect, as shown by Heide (1988).

Vernalisation requirement and number of panicles

Most of chromosome 4F was strongly associated with variation in the number of panicles of non-vernalised plants at Norderås and Landvik (NPN, NPL) and vernalisation requirement (VR) in the greenhouse. This indicates that there is more than one locus on 4F controlling these traits. Most of the variation in VR was due to the presence/absence of an ability to flower without vernalisation (most of the individuals obtained either score 1 or 4), similarly to the variation in NPN and NPL. The fact that many of the markers were also associated with variation in the number of panicles among genotypes that headed (NPNH, NPLH) indicates that these loci not only affect the presence/absence of a vernalisation requirement, but also the number of panicles produced. Separate KW analyses of parental alleles and maps, as well as analyses of parental effects by orthogonal contrasts, showed that all effects of 4F on VR, NPN and NPL were due to maternal alleles. QNp4F-1, a QTL for the number of panicles in the 12-week vernalisation treament (NP12), was located at *Xcdo678a* on 4F. The fact that there was a maternal × paternal interaction effect and no maternal or paternal effects of *Xcdo678a* on NP12 indicates that QNp4F-1 represents a gene different from those controlling VR and NP in the field. The QTLs detected for NPNH or NPLH on 7F were neither detected for NPN, NPL nor VR, and may therefore represent genes controlling other characters than the presence/absence of a vernalisation requirement.

Comparative mapping has shown that chromosome 4F has regions that are syntenic to regions of Triticeae

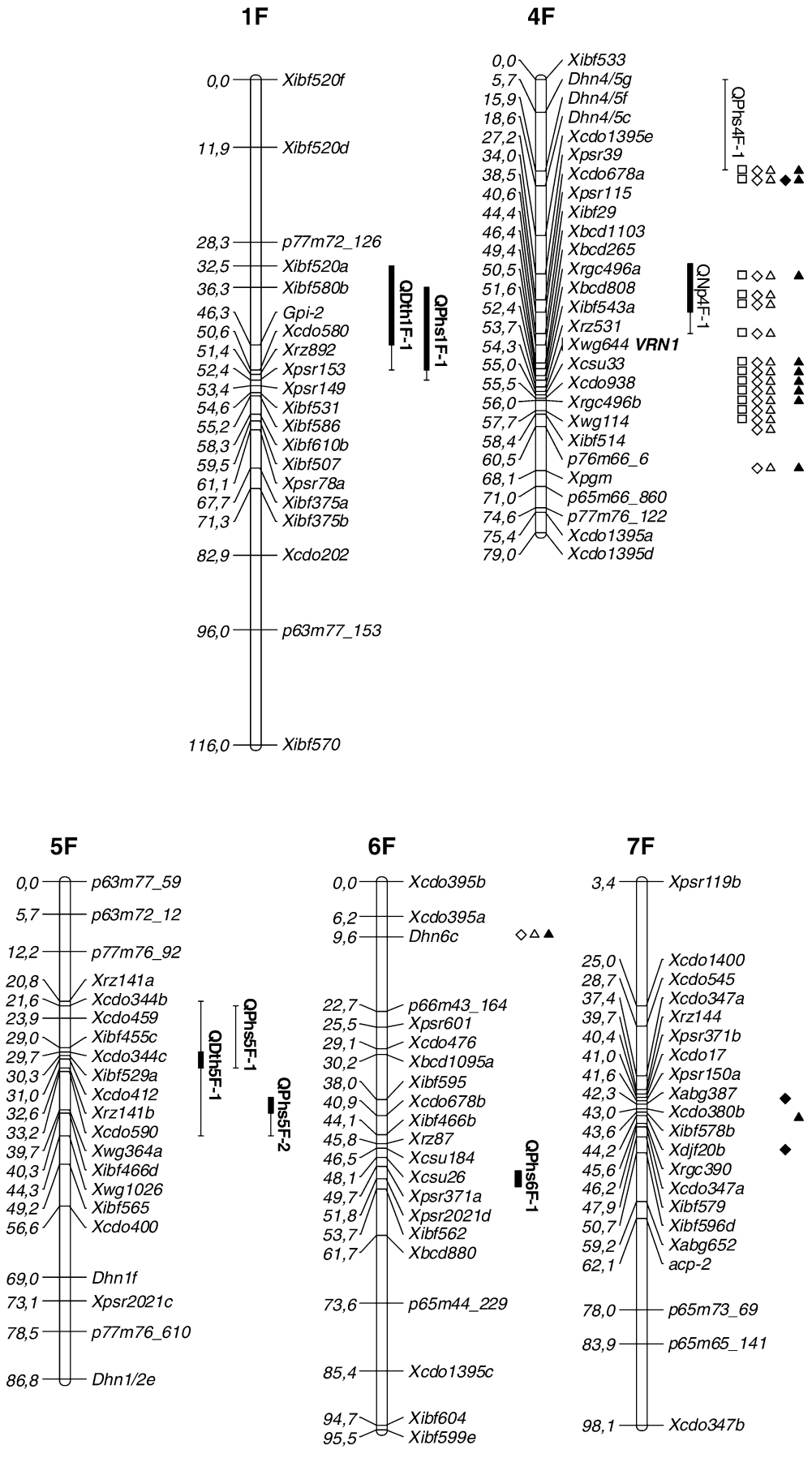

Fig. 1 Meadow fescue chromosomes with positions of *VRN1* and QTLs for number of panicles in non-vernalised plants (NPN, NPL, NPNH, NPLH), vernalisation requirement (VR), and days to heading (DTH), number of panicles (NP) and proportion of heading shoots (PHS) in vernalised plants. See Table. 1, 3 and 4 for further description of traits and QTLs. *Thin lines* indicate 2LOD support intervals for QTLs detected by interval mapping, *thick lines* indicate the same for multiple QTL mapping. *Symbols* indicate markers that were significantly associated with VR (*square*), NPN (*diamond*), NPL (*triangle*), NPNH (*filled diamond*) and NPLH (*filled triangle*) according to the Kruskal Wallis test

chromosomes 4, 5 and 6 (Alm et al. 2003). The segment between *Dhn4/5 g* and *Dhn4/5c* (5.7–18.6 cM) in the proximal end of 4F corresponds to Triticeae chromosome 6. QTLs around these markers could be orthologous to the barley earliness *per se* QTL eps6L.2 located between *Xpsr154* and *Xwg514* on 6H (Laurie et al. 1995), as *Dhn5* is also located between these markers in barley (Qi et al. 1996). The marker *Xwg644* is closely linked to the Vrn1-series on Triticeae chromosome 5L that control spring/winter habit type. We have shown that meadow fescue has an orthologue of *VRN1* close to *Xwg644* at 54.3 cM on 4F. This marker has a strong effect on vernalisation requirement in the present population. Armstead et al. (2004) identified a QTL for heading date in perennial ryegrass at *Xcdo795*, which is co-located with *Xrz531* at 53.7 cM on the "BF14/16 × HF2/7" map. This QTL is likely due to the action of the *VRN1* gene, which has also been mapped at the same position as a heading date QTL in perennial ryegrass by Jensen et al. (2005). Alternatively, it could correspond to wheat *TaCK2a*, an orthologue of the photoperiod-responsive heading date gene *HD6* in rice (Kato et al. 2002). Although *VRN1* appears to affect the presence/absence of a vernalisation requirement in our mapping population, markers proximal to *Xwg644* have stronger effects on this trait. Yamada et al. (2004) identified a QTL for heading time approximately 20 cM proximal to *Xcdo795* on perennial ryegrass chromosome 4. This QTL could correspond to one of the effects detected proximal to *Xwg644* of our study. Vrn-A^{m}2 map approximately 30 cM distal to the 4AL/5AL translocation breakpoint near *Xwg114* in *T. monococcum* (Dubcovsky et al. 1998). Our QTLs for NPN and NPL near *Pgm* (68.1 cM) could therefore correspond to Vrn-A^{m}2.

Markers *Xcdo17* (41.0 cM), *Xdjf20b* (44.2 cM) and *Xibf596d* (50.7 cM) on 7F were associated with either NPNH or NPLH. Chromosome 7F has strong syntenic relationships with chromosome 7 of perennial ryegrass and the Triticeae and a region distal to *Xcdo545* is also syntenic to rice chromosome 6 (Alm et al. 2003; Sim et al. 2005, Fig. 2). Armstead et al. (2004) mapped two flowering time QTLs on perennial ryegrass chromosome 7. A major QTL was located in a region with synteny to the region of rice chromosome 6 containing the heading date gene *Hd3a* and a minor QTL was located at marker *LtCOa*, which is detected by *LtCO* used as a RFLP probe. Armstead et al. (2005) sequenced *LpHD1*, a likely orthologue of rice *HD1*, and mapped it between the two QTLs, closely linked to *Xrz144*. A highly similar gene was also sequenced from meadow fescue. Griffiths et al. (2003) isolated and mapped 16 *CO*-like genes in barley. A likely orthologue of *HD1*, *HvCO1*, maps between

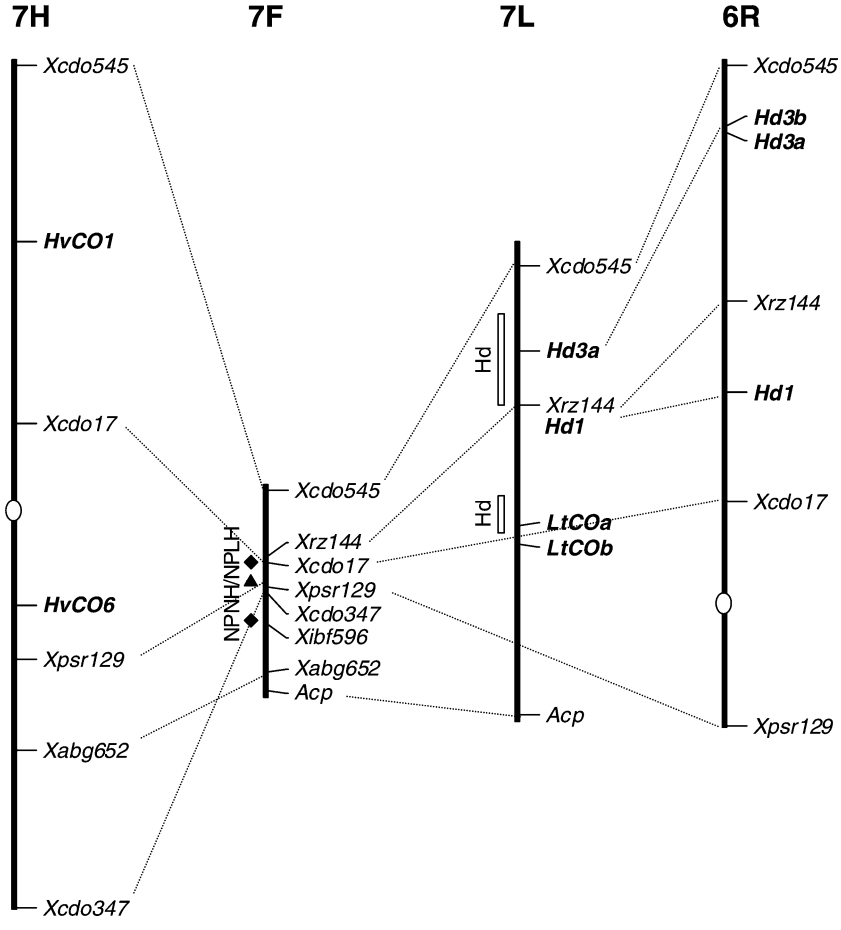

Fig. 2 A comparative map of the region of meadow fescue chromosome 7 containing QTLs for number of panicles among genotypes that were able to head without vernalisation (*filled diamond*, *filled triangle*), and syntenic regions of barley (*H*), wheat (*A*, *B*) and rice (*R*) chromosomes. *Circles* indicate centromere positions. The maps have been compiled from the following sources: Alm et al. (2003), Armstead et al. (2004, 2005), Griffiths et al. (2003), Qi et al. (1996), Yano et al. (2000) and rice maps at the NCBI Plant Genomes Query (http://www.ncbi.nlm.nih.gov/mapview/)

Xrz144 and *Xcdo17* on 7HS while another *CO*-like gene, *HvCO6*, is located between *Xcdo17* and *Xpsr129* on 7HL. QTLs detected in the present study could correspond to one or several of the CO-like genes and/or heading time QTLs found in syntenic regions of perennial ryegrass, barley and rice. The CO-like genes in barley do not map to loci known to influence photoperiodic regulation of flowering in barley. However, perennial ryegrass *CO* is able to complement the *A. thaliana* co-2 mutant (Martin et al. 2004) and *T. aestivum HD1-1* is able to complement *HD1* function in rice (Nemoto et al. 2003), indicating that one or several *CO*-like genes function in photoperiod-regulated flowering in these species. Sourdille et al. (2000) mapped QTLs for eps and/or heading time approximately 10 cM proximal to the centromere on 7BS, 20 cM distal to *Xpsr129* on 7BL and 10 cM distal to the centromere on 7DL. These QTLs could correspond to the three QTLs detected in the present study.

Days to heading and proportion of heading shoots

The QTLs for days to heading and proportion of heading shoots on 1F (QDth1F-1 and QPhs1F-1) are located in the same region and could be due to the same gene(s). QDth5F-1 at *Xcdo412* (31.0 cM) and QPhs5F-2 at *Xwg364a* (39.7 cM) are close to each other, but in the MQM model the 2LOD confidence intervals do not overlap, indicating that they represent segregation at different loci. The region around QDth5F-1 and QPhs5F-2 contains several markers that map in a collinear manner on homoeologous chromosomes 5F, 5H, 5A and 5B (Fig. 3). In the Triticeae, the region appears to carry at least two eps-loci. The QTLs Eps5L and Eps-5BL1 are located close to the centromere on 5H (Laurie et al. 1995) and 5B (Tóth et al. 2003), respectively. Sourdille et al. (2000) also detected an eps-QTL close to the centromere on 5AL. These QTLs may correspond to the grain yield QTL QYld.ocs-5A.2 detected on 5A by Kato et al. (2000) and possibly also to QDth5F-1. QTLs for eps are also located between *Xcdo412* and *Xbcd9* on 5A (QEet.ocs-5A.2, Kato et al. 1999) and approximately 20 cM distal to the centromere on 5B (Eps-5BL2, Tóth et al. 2003). These QTLs are likely to correspond to each other and to QPhs5F-2. An eps-QTL 40 cM distal to the centromere on 5D (Sourdille et al. 2000) may belong to the same series of genes. Two flowering time QTLs in rice (FLTQ2 and FLTQ3) have also been located to a region of rice chromosome 9 that has common markers with this region of Triticeae chromosome 5 (Sarma et al. 1998).

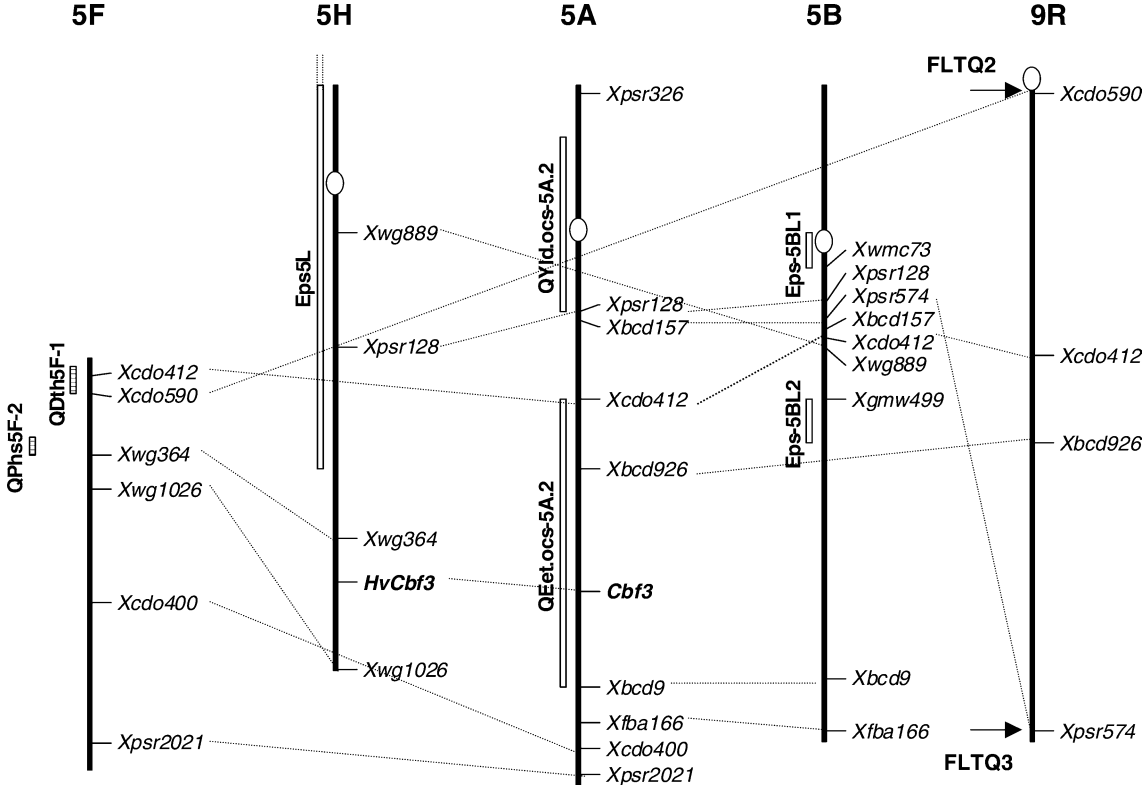

Fig. 3 A comparative map of the region of meadow fescue chromosome 5 containing QTLs for days to heading and proportion of heading shoots, and syntenic regions of barley (*H*), wheat (*A*, *B*) and rice (*R*) chromosomes. *Circles* indicate centromere positions. The maps have been compiled from the following sources: Alm et al. (2003), Choi et al. (2002), Kato et al. (1999, 2000), Laurie et al. (1995), Nelson et al. (1995), Qi et al. (1996), Sarma et al. (1998), Tóth et al. (2003), Vágújfalvi et al. (2003) and Wilson et al. (1999)

Relation to QTLs for other traits in the same population

A number of QTLs for seed production characteristics expressed in the field (Fang 2003) as well as QTLs for winter survival, frost- and drought tolerance (Alm et al. 2005) in the same mapping population were co-located with QTLs identified in the present study. On 4F, QTLs for thousand seed weight and drought tolerance are located in the proximal end, QTLs for heading date and drought tolerance are located near *Xpsr39*, QTLs for heading date, number of fertile tillers, a number of other traits related to seed production, frost tolerance and drought tolerance are located around *Xwg644*, and QTLs for several seed production related traits are found near *Pgm*. QTLs for heading date, other seed production traits and winter survival are located near QDth5F-1 and QPhs5F-2 on chromosome 5. Finally, a QTL for heading date is located in the region of 7F that affects NPNH and NPLH. The co-location of QTLs controlling the induction of flowering and QTLs controlling winter survival, frost- and drought tolerance could be due to cold-inducible genes affecting all these traits, or it could be due to clustering of genes of different function that are under control of similar environmental or developmental signals. Barley plants are less able to cold acclimate and/or maintain a high level of freezing tolerance and expression of cold-inducible genes after the transition to reproduction has occurred (Mahfoozi et al. 2000, 2001; Fowler et al. 2001). This led Fowler et al. (2001) to suggest that developmental genes (vernalisation and photoperiod) regulate the expression of low-temperature-induced genes related to freezing tolerance.

In conclusion, we have identified QTLs involved in the induction of flowering in meadow fescue on chromosomes 1F, 4F, 5F, 6F and 7F. Several QTLs were found in similar positions as QTLs for seed yield components, indicating that they are important for seed yield. Based on comparative mapping we have related some of the QTLs to genes and QTLs that are involved in the induction of flowering in other Poaceae species.

Acknowledgements The Norwegian Research Council has funded this research (project no. 140986/110). Experiments were performed in the Centre of plant research in controlled climate (SKP), Ås, Norway. Dr Heidi Rudi and Dr Vibeke Alm are acknowledged for valuable comments.

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