Detection of photoperiod responsive and non-responsive flowering time QTL in barley

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A QTL analysis was performed to determine the inheritance of flowering time in barley, using a set of recombinant inbred lines developed from a winter-type × spring-type cross. Two photoperiod responsive loci, *Ppd-H1* and *Ppd-H2*, were detected on chromosome arms 2HS and 1HL respectively. Segregation for *eam8* (mapping to the terminus of chromosome arm 1HL) and *Eam5* (close to *Sgh2* on chromosome arm 5HL) was also observed. These latter two genes functioned under 12 h to 24 h photoperiods. In addition, *eps2S* and *eps7S*, known to lie on chromosome arms 2HS and 7HS respectively, were detected. A new QTL for flowering time, *qDHE.ak-1HS*, was mapped 23 cM from the terminus of chromosome 1HS, and appears to be expressed under extremely short day lengths.

Key Words: Earliness *per se*, photoperiod response, spring growth habit, flowering time, flowering date.

Introduction

The control of flowering time is central for the reproductive success of plants and has a major impact on grain yield (Cockram *et al.* 2007, Sameri *et al.* 2006, Sameri and Komatsuda 2007). Flowering is often restricted to a particular time of year. In barley, three classes of genes control flowering time: those responsive to photoperiod (day length), those responsive to vernalization, and earliness *per se*. All three classes influence the transition from vegetative to reproductive growth (Laurie *et al.* 1995, Takahashi and Yasuda 1970).

Particular photoperiod responsiveness has been selected for in many crops to adapt the plants to specific environments and farming practices (Turner *et al.* 2005). Barley is a long-day photoperiod plant, so that its transition to reproductive growth is significantly delayed when grown under short day conditions (Laurie 1997, Stockinger *et al.* 2007). Extremely early flowering winter-type cultivars can be damaged by late frost, whereas in the Far East region, late flowering ones typically yield poorly and produce poor quality grain due to seasonal rainfall patterns. Photoperiod also plays an important role in determining winter hardiness (Hayes *et al.* 1997, Szűcs *et al.* 2006, Takahashi and Yasuda 1970). The photoperiod response gene *Ppd-H1*, located on chromosome arm 2HS, regulates flowering time under long

days, while *Ppd-H2* on chromosome arm 1HL functions only under short days (Laurie *et al.* 1995). *Ppd-H1* encodes a pseudo-response regulator (PRR) (Turner *et al.* 2005), which is different from the major photoperiod response genes *Hd1* and *Hd3a* in rice (Kojima *et al.* 2002, Yano *et al.* 2000). *Ppd-H2* encodes *HvFT3*, an *FT*-like gene (Faure *et al.* 2007, Kikuchi *et al.* 2009).

Earliness *per se* affects flowering time independently of day length and vernalization. Several recessive mutants generate an early flowering phenotype, including *ea7* (*eam7*), *eac* (*eam9*), *ea_k* (*eam8*) and *ea_{sp}* (*eam10*) (Börner *et al.* 2002, Franckowiak *et al.* 1997, Lundqvist *et al.* 1997, Takahashi and Yasuda 1970, Yasuda *et al.* 1965). None of the genes responsible for this phenotype has yet been isolated, although some candidates have been suggested (Kikuchi and Handa 2009). Barley germplasm can be broadly divided into winter, facultative, and spring types (Mahfoozi *et al.* 2001, Szűcs *et al.* 2006). Three epistatic genes *Vrn-H1* (*Sgh2*), *Vrn-H2* (*sgh1*), and *Vrn-H3* (*Sgh3*), located on chromosomes 5H, 4H and 7H, respectively, are responsible for the spring growth habit (Sameri and Komatsuda 2004, Takahashi and Yasuda 1956). These genes are homologues of *API*, *ZCCT* and *FT*, respectively (Yan *et al.* 2003, 2004, 2006).

Although many genetic studies of flowering time have been performed—leading to the recognition of the major genes responsible for photoperiod sensitivity, earliness *per se* and the vernalization response (Cockram *et al.* 2007, Kikuchi and Handa 2009)—the genetic resources analysed in these studies have mostly excluded oriental barley

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germplasm. Thus the objective of the current study was genetically to characterize the determination of flowering time in barley, with a special emphasis on photoperiod responsiveness and earliness *per se*.

Materials and Methods

Mapping population

A set of 99 recombinant inbred lines (RILs) at the F₁₂ generation were bred from the cross Azumamugi (winter-type) × Kanto Nakate Gold (spring-type) by single seed descent. Azumamugi is a Japanese cultivar with characteristics of typical oriental-type cultivars (Sameri *et al.* 2006).

Flowering under controlled photoperiods

The RILs (two plants per RIL) and their parental cultivars were grown in a randomized complete block design as replicated sets in two identical growth chambers (Koito Kogyo, Tokyo). The temperature for the first two weeks post germination was 20°C and the daylength set to 8 h. Vernalization was achieved by maintaining the seedlings for six weeks at 4°C under an 8 h photoperiod. After vernalization, the temperature was raised to 20°C, and five different photoperiod treatments (10 h, 12 h, 14 h, 16 h and 24 h: respectively, Ppd-10 h, Ppd-12 h, Ppd-14 h, Ppd-16 h and Ppd-24 h) were examined. The main effect of the photoperiod treatments (Ppd-MEP) was defined for each line by subtracting the number of days from sowing to flowering in the Ppd-24 h treatment from the number of days from sowing to flowering in the Ppd-12 h treatment. Flowering was defined by the awn emergence from the flag leaves.

Flowering in the field

Three field experiments were conducted. In the first, vernalized RIL seedlings were transplanted in early April (Ppd-SV); in the second, the RILs were sown without vernalization in early April (Ppd-SNV); and in the third, the RILs were sown in mid October (Ppd-autumn), as for a conventional winter-type crop. The photoperiod was 11 h 12 min in mid October, 9 h 32 min in late December (the winter solstice), 12 h 47 min in early April, 14 h 39 min in late June (the summer solstice) and 14 h 18 min in mid July, and the mean daily temperature ranged from 4.3°C to 34.2°C, with an overall mean of 18.0°C. Rows of each RIL contained 10 plants, and days to flowering indicated the number of days from sowing to the time when in 50% of the ears the base had emerged from the flag leaf sheath.

QTL detection

The linkage map of Sameri *et al.* (2009) was used for the QTL analysis. This map is based on that of Mano and Komatsuda (2002), with the addition of 37 microsatellite loci. Composite interval mapping (CIM) was performed using Windows QTL Cartographer v2.0 (Wang *et al.* 2004). The LOD threshold for the presence of a QTL was estimated from a 1,000 permutation test.

Results

Photoperiod sensitive flowering time QTL

Days to flowering varied widely among the RILs grown in the growth chamber (Fig. 1). Two major genes governing flowering time were located—one on chromosome arm 1HL and the other on 2HS, corresponding to *Ppd-H2* and *Ppd-H1*, respectively (Table 1). Both *Ppd-H1* and *Ppd-H2* were expressed exclusively in the Ppd-12 h treatment. The Azumamugi *Ppd-H2* allele delayed flowering by 17.6 days compared to the Kanto Nakate Gold allele, while the Azumamugi *Ppd-H1* allele accelerated it by 19.1 days (Table 1). The MEP parameter detected only *Ppd-H2* and *Ppd-H1*, with the map location of *Ppd-H2* placed ~10 cM distal to that detected under the Ppd-12 h treatment. A flowering time QTL was detected under the Ppd-10 h treatment, mapping 23 cM from the terminus of chromosome arm 1HS (hereafter *qDHE.ak-1HS*), and was flanked by *Bmac0213* and *e12m22.8* (Table 1). The Azumamugi *qDHE.ak-1HS* allele accelerated flowering by 34.4 days. This QTL was not detected under any of the other photoperiod treatments.

Photoperiod insensitive flowering time QTL

Two QTL were detected on chromosome arms 1HL and 5HL under the Ppd-12 h, -14 h, -16 h and -24 h treatments, suggesting that their expression was relatively insensitive to photoperiod (Table 1). The location of the QTL corresponded to, respectively, *eam8* (formerly *ea_k*, Franckowiak *et al.* 1997, Yasuda *et al.* 1965) and *Eam5* (Franckowiak 2002). The two loci were mapped in classical linkage maps in these studies but they were mapped in molecular linkage maps in this study. The two genes were detectable in varying photoperiod (Table 1) indicating their expression is photoperiod insensitive. The additive effect of the Azumamugi *eam8* allele delayed flowering by 5.9–23.1 days, whereas that of the Azumamugi *Eam5* allele accelerated it by 2.8–22 days. These effects were also noticeable in the Ppd-SV and Ppd-autumn field experiments, although the additive effects were smaller than those detected in the growth chamber experiments. Neither *eam8* nor *Eam5* was detectable in the Ppd-10 h treatment, suggesting that there is a minimum photoperiod necessary for their expression—in other words, the genes are sensitive to photoperiod to some degree.

eps2S and *eps7S* have also been described as earliness *per se* genes (Laurie *et al.* 1995), but here they were only expressed in the Ppd-24 h treatment. Thus, they only function under photoperiods which occur only at the poles in mid summer. This implies that the two genes are in fact sensitive to photoperiod. The additive effect of both *eps2S* and *eps7S* was small (2.1 and 1.7 days, respectively).

Flowering time genes detected under field conditions

The Ppd-SV experiment allowed the detection of four loci: *eam8*, *eps2S*, *Eam5* and *qDHE.ak-7H*, located on chromosome arms 1HL, 2HS, 5HL and 7H, respectively

Fig. 1. Frequency distribution of the number of days to flowering in response to variation in the photoperiod in the Azumamugi/Kanto Nakate Gold RILs. The mean performance over two plants and two growth chambers is given for each RIL. The performance of Azumamugi and Kanto Nakate Gold is indicated by a black and a white arrow, respectively. After vernalization, the temperature was raised to 20°C, and five different photoperiod treatments (10 h, 12 h, 14 h, 16 h and 24 h: respectively, Ppd-10 h, Ppd-12 h, Ppd-14 h, Ppd-16 h and Ppd-24 h) were examined. In the field experiments, vernalized RIL seedlings were transplanted in early April (Ppd-SV); non-vernalized RILs were sown in early April (Ppd-SNV); and the other RILs were sown in mid October (Ppd-autumn),

(Table 1). Arm location of *qDHE.ak-7H* is unknown. *qDHE.ak-7H* was expressed in the Ppd-SV treatment, and thus is responsive to the lengthening of the photoperiod as spring advanced. The same QTL was also detected in a spring sowing of non-vernalized barley (Sameri and Komatsuda 2004), which indicates that its action is independent of vernalization. In the Ppd-SNV experiment, the flow-

ering time of the 20 winter habit RILs was greatly delayed, and these were therefore eliminated from the QTL analysis. The analysis detected the presence of two flowering time genes, one corresponding to *sgl1* on chromosome arm 4HL, and the other to *Eam5* on chromosome arm 5HL. RILs carrying the Azumamugi allele at each locus accelerated flowering (Table 1). In the Ppd-autumn experiment, one QTL,

Table 1. Flowering time QTL detected in the Azumamugi/Kanto Nakate Gold RILs

Trait	Chr.	Flanking markers	Pos. cM	LOD	AE ^a	PVE ^b	Locus
Ppd-10 h	1HS	<i>Bmac0213–e12m22.8</i>	23.31	4.42	–34.46	0.198	<i>qDHE.ak-1HS</i>
Ppd-12 h	1HL	<i>e02m18.4–e06m18.10</i>	101.31	4.45	17.68	0.109	<i>Ppd-H2</i>
	1HL	<i>ABC261–ABG055</i>	151.91	6.18	22.27	0.165	<i>eam8 (eak)</i>
	2HS	<i>e08m18.7–ABG602</i>	39.51	3.40	–19.11	0.121	<i>Ppd-H1</i>
	5HL	<i>e07m25.3–e12m19.9.1</i>	125.81	6.03	–22.03	0.168	<i>Eam5</i>
Ppd-14 h	1HL	<i>ABC261–ABG055</i>	151.91	15.60	23.16	0.392	<i>eam8 (eak)</i>
	5HL	<i>e07m25.3–e12m19.9.1</i>	129.81	8.13	–15.23	0.169	<i>Eam5</i>
Ppd-16 h	1HL	<i>ABC261–ABG055</i>	151.91	18.88	12.59	0.458	<i>eam8 (eak)</i>
	5HL	<i>e07m25.3–e12m19.9.1</i>	131.81	7.76	–6.73	0.136	<i>Eam5</i>
Ppd-24 h	1HL	<i>ABC261–ABG055</i>	151.91	20.00	5.94	0.473	<i>eam8 (eak)</i>
	2HS	<i>EBmac0607–EBmac0623</i>	48.41	4.16	–2.18	0.064	<i>eps2S</i>
	5HL	<i>e07m25.3–e12m19.9.1</i>	129.81	6.17	–2.88	0.115	<i>Eam5</i>
	7HS	<i>ABG616–e15m31.6</i>	28.41	2.93	–1.77	0.044	<i>eps7S</i>
Ppd-MEP ^c	1HL	<i>e02m18.4–e06m18.10</i>	111.31	3.79	25.63	0.337	<i>Ppd-H2</i>
	2HS	<i>e08m18.7–ABG602</i>	35.51	3.45	–18.52	0.176	<i>Ppd-H1</i>
Ppd-SV	1HL	<i>ABC261–ABG055</i>	151.91	9.10	2.52	0.167	<i>eam8 (eak)</i>
	2HS	<i>e13m23.6–e13m31.7.1</i>	52.61	11.97	–2.89	0.226	<i>eps2S</i>
	5HL	<i>e07m25.3–e12m19.9.1</i>	127.81	14.00	–3.60	0.347	<i>Eam5</i>
	7H	<i>e11m17.10.2–e12m19.10.3</i>	100.61	3.55	1.56	0.063	<i>qDHE.ak-7H</i>
Ppd-SNV	4HL	<i>e12m19.10.2–e04m32.8</i>	96.21	3.77	–7.05	0.096	<i>sgh1</i>
	5HL	<i>Hvlox–e07m25.3</i>	123.51	10.60	–12.02	0.281	<i>Eam5</i>
Ppd-autumn	1HL	<i>MWG2077–e02m18.4</i>	97.81	5.33	3.30	0.126	<i>Ppd-H2</i>
	1HL	<i>ABC261–ABG055</i>	149.91	4.92	3.00	0.117	<i>eam8 (eak)</i>
	2HS	<i>e13m23.6–e13m31.7.1</i>	52.61	8.39	–3.68	0.176	<i>eps2S</i>
	5HL	<i>e07m25.3–e12m19.9.1</i>	127.81	13.33	–4.93	0.317	<i>Eam5</i>

^a The additive effect of the Azumamugi allele.

^b The proportion of phenotypic variance explained by the detected QTL.

^c Ppd-MEP (main effect of photoperiod) = (Ppd-12 h) – (Ppd-24 h).

corresponding to *Ppd-H2*, was detected on chromosome arm 1HL, close to *e02m18.4* (Table 1).

Discussion

Photoperiod sensitive genes

Two QTL affecting photoperiod response in barley were detected in the growth chamber experiments. One mapped in the same region as *Ppd-H2*, according to the barley consensus map (<http://www.graingenes.org>), and was only expressed under a 12 h photoperiod. The spring-type parent Kanto Nakate Gold carries a photoperiod-responsive *Ppd-H2* allele, which accelerated flowering under short photoperiods. The segregation of a flowering time QTL in the Apex × Prisma mapping population (Yin *et al.* 2005), which also maps to this region, probably also corresponds to *Ppd-H2*. We also detected a photoperiod sensitive QTL on chromosome arm 2HS, since it was only detectable under the Ppd-12 h and MEP treatments (Table 1). At this locus the Azumamugi allele accelerated flowering by 18 days. The location and action of this QTL corresponds to *Ppd-H1*. Indeed, a study by Turner *et al.* (2005) demonstrated that cultivars carrying the dominant *Ppd-H1* allele flower 20 days

earlier than cultivars carrying the recessive *ppd-H1* allele, further supporting our conclusion. This study detected both *Ppd-H1* and *Ppd-H2* in the 12 h treatment (Table 1). In general, *Ppd-H1* is a long-day regulator and *Ppd-H2* is a short-day regulator for flowering. Kanto Nakate Gold allele for *Ppd-H1* delayed flowering at only the 12 h condition, and it did not function in the longer day-length condition. The results suggest that the Kanto Nakate Gold allele was different from the alleles of northern European spring cultivars. The *qDHE.ak-1HS* locus was detected in an extremely short day-length condition, probably not occurring in nature, and was not detected under any of the other photoperiod treatments. Effects of *qDHE.ak-1HS* were as large as 30 days difference of flowering. However the biological function of the *qDHE.ak-1HS* locus can not be discussed until the gene products encoded by the gene are clarified. This QTL requires further study to understand its biological roles. Near-isogenic lines for this gene may allow further characterization of the genes as for the case of other agronomic traits (Sameri *et al.* 2009).

Earliness per se genes

We have shown earlier (Sameri *et al.* 2006, 2009) that the

QTL lying close to *ABC261* on chromosome arm 1HL is likely to be *eam8*, as identified by Takahashi and Yasuda (1970) (Table 1). This locus here was one of the major determinants of flowering time under a wide range of photoperiod treatments. Yin *et al.* (2005) have similarly mapped a flowering time QTL to the terminus of chromosome arm 1HL in their Apex $\times$ Prisma mapping population. A further major QTL insensitive to photoperiod, believed to be *Eam5*, mapped close to *Sgh2* (*Vrn-H1*) locus in all of the treatments except Ppd-10 h. As this QTL was also detected under natural day length conditions from a spring sowing (Sameri and Komatsuda 2004), the detection of this locus may have been thanks to the pleiotropic effect of *Sgh2*. Yasuda and Okinaga (1977) have shown that the effect of *Sgh2* on flowering time depends on the genetic background of the cultivar. In *Triticum monococcum*, the vernalization gene *Vrn1* encodes an APETALA1 transcription factor, with a number of other genes implicated in the control of flowering physically located close to it (Yan *et al.* 2003). Thus it is possible that *Eam5*, rather than being a homologue of *Sgh2*, is related to one of these linked genes. Some support for this notion comes from the observation that the Azumamugi *Eam5* allele accelerates early flowering, while the Azumamugi *Sgh2* allele delays it in the absence of vernalization. At the QTL described by Cuesta-Marcos *et al.* (2008), the winter-type allele delayed flowering, indicating that their QTL corresponded to *Sgh2* rather than to *Eam5*. This study clearly indicated the major effects of the two earliness *per se* genes over different day-length conditions, and near-isogenic lines for these genes (Sameri *et al.* 2009) may allow a fine mapping and further characterization of the genes.

The *eps2S* gene on chromosome arm 2HS was detected in the Ppd-24 h, the Ppd-SV and the Ppd-autumn treatments (Table 1). As Laurie *et al.* (1995) detected this locus under 10 h, 13 h and 16 h photoperiod treatments after vernalization and autumn sowing, it has been considered as being photoperiod neutral and classified among the earliness *per se* genes. Here, however, *eps2S* was detected in the 24 h photoperiod treatment in addition to the field-grown vernalized material, suggesting that *eps2S* is not necessarily photoperiod insensitive. An alternative explanation could be that *eps2S* is photoperiod insensitive, but that its effect is considerably weaker than that of either *eam8* or *Eam5* and was therefore not detected in our QTL analysis. The chromosome arm 7HS locus, likely to be *eps7S*, behaves similarly to *eps2S* in terms of its photoperiod insensitivity. The map position defined by Laurie *et al.* (1995) for *eps7S* is consistent with the present one. The outcome of the Ppd-24 h experiment confirmed that this locus determines earliness *per se*. However, as for *eps2S*, *eps7S* is not necessarily photoperiod insensitive—either it is photoperiod sensitive to some extent, or its effect is much weaker than that of either *eam8* or *Eam5*.

Vernalization response genes

A recessive spring growth habit gene, *sgh1* (*vrn-H2*), was

identified by Takahashi and Yasuda (1956) and located on chromosome 4H (Laurie *et al.* 1995, Takahashi *et al.* 1958, Yan *et al.* 2004). Sameri and Komatsuda (2004) found a close linkage between *sgh1* and *e04m32.8*. Here, the Ppd-SNV treatment detected a QTL lying close to *vrn-H2*. The transcription of the likely wheat homologue of *vrn-H2* is down-regulated by vernalization (Yan *et al.* 2004), so it is understandable that the QTL was not recognized in experiments where the plants had been vernalized.

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