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Molecular mapping of the photoperiod response gene *ea*$_7$ in barley

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Abstract The gene *ea*$_7$ determining photoperiod insensitivity under short day length was mapped on the short arm of chromosome 6H near the centromere. The gene was linked to the two flanking markers *Xmwg2264* and *Xmwg916* by 6.7 and 13.0 cM, respectively. Compared to *Ppd-H1* (chromosome 2H) and *Ppd-H2* (chromosome 1H), *ea*$_7$ determines the strongest effect on flowering time with 55 and 18 days difference compared to photoperiod sensitive genotypes grown under short and long photoperiods, respectively. Allelic and homoeologous relationships to major genes and quantitative trait loci controlling flowering time in barley and wheat are discussed.

Key words Genetic mapping · RFLP · Flowering time · Photoperiod response · Barley

Introduction

Genes which regulate the time of flowering are divided into vernalization response genes, photoperiod response genes, and earliness *per se* genes acting independent of environmental effects. Flowering time genes are important for adapting cereal varieties to particular environments. Recent studies show a highly significant association of such genes with plant height, biomass and yield components (Laurie et al. 1995). The knowledge of the effects of these genes makes it possible to maximize plant performance.

A genetic analysis of flowering control by photoperiod sensitivity has been carried out by many researchers. Both in short day plants, such as rice and maize, and long day plants, such as barley and wheat, genes for photoperiod response determine sensitivity to the length of the photoperiod. In cereals photoperiod insensitive genotypes enable the initiation of floral primordia without the requirement for long day photoperiods, whereas sensitive genotypes need a long day period for floral primordia initiation.

In rice the major photoperiod response gene, *Se-1*, has been located on chromosome 6 (Kinoshita 1995). Probably the same locus on chromosome 6, *Hd-1*, designated however as, was identified by Yano et al. (1997) conducting a QTL analysis. The homoeologous region of rice chromosome 6 in maize (chromosome 9) also encompasses a QTL for flowering time (Lin et al. 1995; Paterson et al. 1995).

Another locus on chromosome 7 in rice, designated *Hd-2*, aligns with the *Ppd-H1* region of barley (Laurie 1997). *Ppd-H1*, located on the short arm of chromosome 2H (Laurie et al. 1994), is one of two mapped loci which determine the photoperiod response in barley (Laurie et al. 1995). This locus causes most significant differences in flowering time under long day conditions. The second locus, *Ppd-H2*, was mapped on the long arm of the chromosome 1H and shows explicit differences in flowering time only under short days (Laurie et al. 1995).

Comparative mapping indicated that *Ppd-H1* is located at a similar position as the homoeologous *Ppd* genes on the short arms of the group 2 chromosomes of wheat (Worland et al. 1998). However, there are differences in the impact of the flowering-time genes. The photoperiod insensitive allele of *Ppd-H1* induces early flowering under long day length. Genotypes with the homozygous photoperiod sensitive allele show a reduced or no induction. Photoperiod insensitive, dominant alleles of wheat *Ppd* genes confer early flowering both under short and long days (Worland 1996). Loci homoeologous to *Ppd-H2* have not been identified in wheat so far.

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An additional four mutants determining earliness under short day length are known in spring barley (Gallagher et al. 1991). The mutants, designated ea_k (Dormling and Gustafsson 1969, Takahashi and Yasuda 1971), ea_7 syn *ec* (Ramage and Suneson 1958), ea_c (Yasuda and Hayashi 1980) and ea_{sp} (Gallagher et al. 1991), are located on chromosomes 1H, 6H, 4H and 3H, respectively. Besides photoperiod sensitivity these loci also control maturity. Segregation analysis indicated that these loci interact with each other and that there are recessive epistatic interactions amongst the loci (Gallagher et al. 1991). In the present study, we report molecular mapping experiments aimed at intrachromosomal mapping of the ea_7 locus on chromosome 6H.

Materials and methods

Plant materials

A mapping population was produced from a cross between the spring barley genotypes 'Atsel' and 'Betzes'. The mutant 'Atsel', derived spontaneously from the variety 'Atlas' in the field, is homozygous recessive for the ea_7 gene on chromosome 6H and confers extreme earliness and relative photoperiod insensitivity (Arieas et al. 1983, Gallagher, personal communication). The seed of 'Atsel' was kindly provided by L. W. Gallagher, University of California, Davis, USA. 'Betzes' is photoperiod sensitive. The F_2 population consisting of 134 individuals was generated by the selfing of one individual F_1 plant.

Evaluation of photoperiod response

For segregation analysis, the 134 F_2 plants were grown together with their parents (12 plants each) in a growth chamber with 10 h light and 14 h darkness at 25°C and 18°C, respectively. The photoperiod response was measured in days to flowering. In addition, derived F_3 seedlings (10–15 per F_2 plant) were tested, allowing a re-classification of the F_2 plants into homozygous (sensitive or insensitive) or heterozygous genotypes. 'Betzes', the wild-type 'Atlas' and the mutant 'Atsel' were also grown under controlled long day conditions to verify the photoperiodic influence of ea_7.

Molecular marker analysis

DNA was extracted by the procedure of Saghai-Maroof et al. (1984) from fresh leaf material cut from 5–6 week old F_3 seedlings (10–15 plants per F_3 family). RFLP-analysis was performed according to Devos et al. (1992). Genomic DNA (10 µg per sample) was digested with the restriction enzymes *Eco*RI, *Eco*RV and *Hin*dIII, respectively, applying 2 units per µg of DNA for 8 h. The restriction fragments were size-separated on a 0.8% agarose gel and transferred to a nylon membrane (Hybond N^+, Amersham). RFLP clones known to be mapped on chromosome 6H were provided by A. Graner, IPK Gatersleben, and used to screen for polymorphisms. The probes were labelled with the Megaprime DNA labelling system (Amersham), according to the suppliers instructions. Individual plants were genotyped for each locus to construct a linkage map with the MAPMAKER 2.0 computer program (Lander et al. 1987) using the Kosambi map-unit function.

Results

Controlled-environment tests of parents 'Atsel' and 'Betzes' showed a clear difference under short days. On average 'Atsel' flowered 55 days earlier than 'Betzes'. Figure 1 shows the differences among the phenotypes of 'Atsel' and 'Betzes' 60 days after sowing. Under long days 'Atsel' flowered only 18 days earlier than 'Betzes' and the wild-type 'Atlas'.

The F_2 population of the cross 'Atsel' × 'Betzes' segregated into 38 early flowering : 96 late-flowering plants (Fig. 2) fitting the Mendelian segregation ratio of 1 : 3, as tested by χ^2 ($P > 0.30$). There was a clear classification with a break of 15 days between early and late-flowering plants. After scoring the 98 derived F_3 families (Fig. 3) a distorted segregation ratio of 33 : 35 : 30 was observed ($\chi^2 = 8.184$; $P > 0.025$). This distortion was characterized by an under-representation of the heterozygous class compared to the two homozygous classes.

Of the 14 RFLP probes used for hybridization, seven (50%) were polymorphic detecting six loci. The markers *Xmwg2100* and *Xmwg820* co-segregated. Five loci could be scored as co-dominant markers, whereas for *Xcmwg679* a dominant inheritance was observed. All segregation data along with the χ^2-test values are given in Table 1. The target gene locus ea_7 was mapped in the centromere region on the short arm of chromosome 6H (Fig. 4) linked to *Xmwg2264* and *Xmwg916* by 6.7 and 13.0 cM, respectively.

Fig. 1 Phenotypes of the two parents 'Atsel' (ea_7) and 'Betzes' (Ea_7) grown for 60 days under a short photoperiod

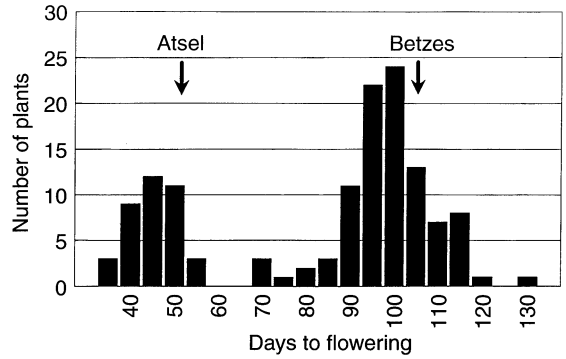

Fig. 2 F_2 segregation pattern (134 plants) for days to flowering of the cross 'Atsel' (ea_7) × 'Betzes' (Ea_7). The parental means are marked by *arrows*

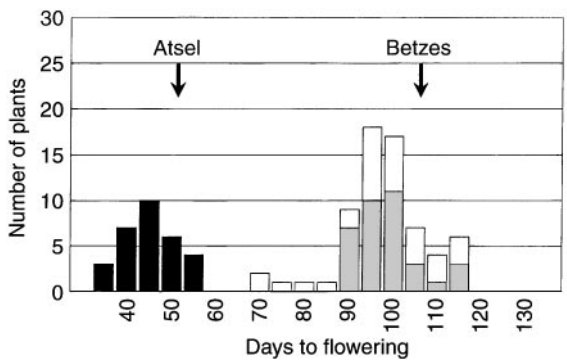

Fig. 3 F_2 segregation pattern for days to flowering of the cross 'Atsel' (ea_7) × 'Betzes' (Ea_7) based on F_3-derived scoring data (98 families). *Black*, *white and grey bars* show the number of homozygous early, homozygous late and heterozygous genotypes, respectively. The means of parents are marked by the *arrows*

Table 1 Segregations and χ^2 test values for the placed markers

Locus	Expected segregation	Observed segregation	χ^2 value	*P* value
Xmwg916	1:2:1	21:48:29	1.346	$P > 0.50$
ea_7	1:2:1	33:35:30	8.184	$P > 0.025$
Xmwg2264	1:2:1	18:46:34	5.592	$P > 0.05$
Xabg458	1:2:1	20:45:33	4.101	$P > 0.10$
Xmwg2313	1:2:1	20:44:34	5.012	$P > 0.05$
Xmwg2100	1:2:1	19:46:33	4.368	$P > 0.10$
Xmwg820	1:2:1	19:46:33	4.368	$P > 0.10$
Xcmwg679	3:1	79:19	1.647	$P > 0.10$

Discussion

The genetic control of photoperiod sensitivity is complex. Five of the seven barley chromosomes are reported to carry genes determining photoperiod response. Whereas the two loci *Ppd-H1* and *Ppd-H2* have been mapped already on chromosomes 2H and 1H, respectively (Laurie et al. 1995), no mapping data were available for the genes ea_k, ea_{sp}, ea_c and ea_7, although they are known to be located on chromosomes 1H, 3H, 4H and 6H, respectively (Gallagher et al. 1991). Whether *Ppd-H2* and ea_k, both located on chromosome 1H, are allelic can only be postulated.

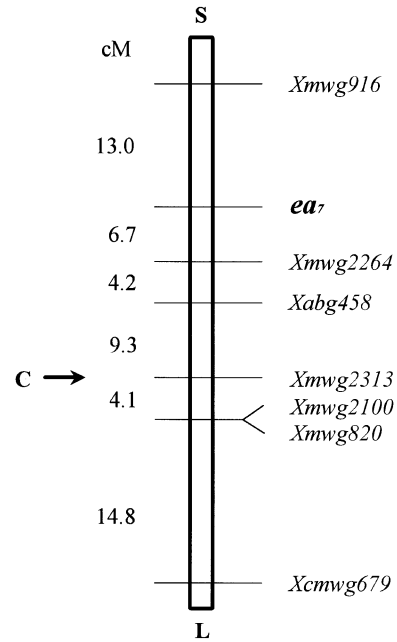

Fig. 4 Partial RFLP map of chromosome 6H derived from an F_3 population of the cross 'Atsel' × 'Betzes' including the gene ea_7 determining photoperiod insensitivity. Genetic distances (cM) are given on the left. The approximate position of the centromere is indicated by the *arrow*

With respect to ea_7 mapped in the present study it should be noted that Laurie et al. (1995) described two quantitative trait loci (QTLs) controlling flowering time on chromosome 6HL and designated as *eps6L.1* and *eps6L.2*. However, the effects of both loci were not attributable to photoperiod response.

Using a different mapping population Bezant et al. (1996) again detected two regions on chromosome 6H having significant effects on early flowering. Whereas one locus was identified by QTL marker-regression analysis on the long arm of chromosome 6H and closely linked to *αAmy1* another significant ear emergence time effect was detected by ANOVA and associated with the marker *Xmgw652*. Although *Xmgw652* is located on 6HS it maps distal to *Xmwg916* at a distance of > 20 cM (Graner 1996) and, therefore, the ear emergence time effect detected by Bezant et al. (1996) seems unrelated to ea_7 located 13 cM proximal to *Xmwg916* on our map (Fig. 4).

In wheat, photoperiod insensitivity is primarily determined by a homoeologous series of dominant genes located on the group 2 chromosomes. No recessive

mutants have so far been described. However, it should be mentioned, that genes on other wheat chromosomes including chromosome 6B have been implicated in determining photoperiod response (Worland 1996).

A comparison of common markers on the map presented here to the RFLP map for chromosome 6H of Graner (1996) reveals a very similar order. The differences observed for *Xabg458*, *Xcmwg679* and *Xmwg2264* may be due to the relatively low number of individuals used or, in the case of cMWG679, to the fact that it is not a single-copy probe and therefore might result in a different map position.

Surprisingly, the effect of ea_7 on flowering time under short (55 days) and long photoperiods (18 days) was very marked compared to the *Ppd* genes of barley. For *Ppd-H1*, which shows the greatest effect under long photoperiods, a difference of 10 days was observed under long days while there was no difference under short days. For *Ppd-H2*, which is comparable to ea_7 because both have the greatest effect on photoperiod response under short days, the difference was 17 days under a short photoperiod whereas under long day length no difference was observed (Laurie et al. 1995).

In barley, chromosome regions showing a distorted segregation of genes or markers are detected frequently whilst making genetic or mapping studies. Two genes, *Ga* located on chromosome 7H (Tabata 1961) and *Ga2* on chromosome 2H (Konishi et al. 1990), were identified as being responsible for local segregation distortion. Heun et al. (1991) describe five regions within the barley genome showing distorted segregation, one of which was located on chromosome 6HL. A further region on chromosome 6H seems to be near the ea_7 locus on the short arm where all markers in the centromere region exhibit rather higher χ^2 values.

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