Genetic analysis of a photoperiod response gene on the short arm of chromosome 2(2H) of *Hordeum vulgare* (barley)

DAVID A. LAURIE,^{†*} NICKY PRATCHETT,[†] JEREMY H. BEZANT[‡] & JOHN W. SNAPE[†]

[†]Cambridge Laboratory, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UJ and

[‡]School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

RFLP mapping of 94 doubled haploid lines from a winter × spring barley cross (Igri × Triumph) identified a previously undescribed major photoperiod response locus (*Ppd-H1*) on the short arm of barley chromosome 2(2H). Lines with the Igri allele (winter parent) flowered 10 days earlier under long days in a glasshouse experiment. The *Ppd-H1* locus also had a major effect in field experiments, giving differences of 12 days (spring sowing) and 7 days (autumn sowing). The *Ppd-H1* locus accounted for 60 per cent of the genetic variation in flowering time in the spring sowing and 46 per cent in the autumn sowing. The most likely location of *Ppd-H1* was in the 6 cM interval between the RFLP loci *XMWG858* and *XpsrB9*, 1 cM proximal to *XMWG858*. The map position of *Ppd-H1* suggests that it may be a homoeoallele of the wheat photoperiod response gene *Ppd1*. Field data also showed that the barley *Ppd-H1* locus was associated with highly significant effects on plant height, biomass and yield components which were probably the direct results of the variation in flowering time. Flowering time and other agronomic characters were also significantly affected by an additional developmental rate gene on the same chromosome.

Keywords: barley, *Hordeum vulgare*, photoperiod, QTL, RFLP mapping.

Introduction

Genes controlling flowering time (heading date) are important for adapting cereal varieties to particular environments and hence for maximizing yield potential. Their regulation of the change from vegetative growth (production of leaf primordia) to reproductive growth (production of spikelet primordia) also makes them of interest for fundamental studies of plant development.

Numerous studies of flowering time have been reported in barley (*Hordeum vulgare*) but the specific genes that have been identified are those giving constitutive early flowering or controlling vernalization response. Of the former, the best characterized are the recessive mutations *ea7*, *ea_k*, *ea_c* and *ea_{sp}* (Takahashi & Yasuda, 1971; Søgaard & von Wettstein-Knowles, 1987; Gallagher *et al.*, 1991; von Wettstein-Knowles,

1992), each of which results in early flowering when homozygous.

Comparative mapping of wheat (*Triticum aestivum*), rye (*Secale cereale*) and barley using RFLP markers shows that when translocations are taken into account the order of loci on equivalent (homoeologous) chromosome segments is conserved (Devos *et al.*, 1993a, b). This predicts that genes controlling morphological and physiological traits will also show conserved genetic map locations in different species. If this were true the short arm of barley chromosome 2(2H) should have a major gene (a homoeoallele) corresponding to the wheat photoperiod response genes *Ppd1*, *Ppd2* and *Ppd3*. However, no such gene has previously been identified in barley.

Allelic variation for photoperiod response in barley is most likely to be detected in crosses between genotypes adapted to different growth conditions. We therefore analysed a doubled haploid population derived from a cross between two European barleys, the winter variety Igri and the spring variety Triumph. Data from

*Correspondence.

field and glasshouse experiments were combined with RFLP mapping to show that a major photoperiod response gene is located on the short arm of chromosome 2(2H). This paper reports its map location, effects on agronomic performance and discusses its possible relationship to the wheat *Ppd* genes.

Materials and methods

Plants

A population of 94 doubled haploid (DH) lines was produced from F_1 plants of a cross between the winter barley variety Igri and the spring barley variety Triumph by the *Hordeum bulbosum* method (Simpson & Snape, 1981). Igri and Triumph are useful parents for mapping populations because they feature extensively in European barley breeding programmes. Thus, genes from these varieties are likely to be important in many current commercial barley varieties.

Field experiments

The DH population was characterized in two separate field microplot experiments: one from a spring sowing and one from an autumn sowing. The field experiments consisted of five randomized blocks each containing one row of 11 plants of each DH line and two rows of each parent. Flowering time was recorded when half the plants in the row had half the ear emerged from the flag leaf on the leading tiller. Final plant height, plant grain weight, plant biomass, tiller biomass, tiller grain weight and ear grain number were recorded at maturity. Tiller biomass, tiller grain weight and ear grain number were estimated from samples of five main tillers taken from each row. Tiller grain weight and tiller grain number were used to calculate 1000 grain weight. Harvest indices (per cent) were calculated as tiller grain weight/tiller biomass and were analysed after angular transformation.

Glasshouse experiment

Plants were given 0, 3 or 6 weeks vernalization at 5°C with 8 h light and were then grown under 10, 13 or 16 h days of natural daylight in a photoperiod glasshouse. Vernalization times were staggered to enable the whole experiment to be planted in the glasshouse on the same day. Four replicate plants of each genotype and eight plants of each parent were grown under each of the nine regimes. Flowering time was recorded for each plant as days to awn emergence. Because of space limitations, this experiment was restricted to 69 DH lines selected at random from the full population.

RFLP analysis

DNA was extracted from each DH line and from the parents using the method of Sharp *et al.* (1988) or a modified CTAB method (Murray & Thompson, 1980). Polymorphisms were selected by hybridizing DNA of the parental varieties digested with *EcoRI*, *EcoRV*, *DraI* or *HindIII* with a range of previously mapped cDNA and genomic clones using the methods of Devos *et al.* (1992). Linkage maps were made with MAPMAKER v2.0 (Lander *et al.*, 1987) and the recombination frequencies converted to centiMorgans using the Kosambi mapping function.

Statistical analysis

Analysis of variance (ANOVA) was used to detect differences between DH lines and, for the glasshouse experiment, to partition variation into effects of genotype, vernalization and photoperiod treatment and their interactions. Within each treatment or field experiment further ANOVAs were then used to detect associations between RFLP alleles and all scored traits by comparing variation between the two allele classes at each marker locus with variation between lines within classes. The mean value of each line for each trait was also used for MAPMAKER/QTL analysis.

Results

ANOVA comparisons revealed highly significant differences between lines for flowering time in both glasshouse and field experiments. Further analysis of the glasshouse experiment showed that there were highly significant interactions between lines and vernalization time and between lines and daylength, showing that the cross was segregating for vernalization and photoperiod response genes. ANOVA comparisons of allele class means showed that the vernalization effects were strongly associated with RFLP markers on chromosomes 4(4H) and 7(5H) due to segregation of the *Sh* and *Sh₂* genes (data not shown). Photoperiod effects were strongly associated with RFLP markers on the short arm of chromosome 2(2H).

The RFLP map of chromosome 2(2H)

To localize the photoperiod response locus, 11 RFLP markers previously mapped on chromosome 2(2H) in two other barley crosses (Laurie *et al.*, 1993) and one additional marker (*Xpsr87*) were mapped in the Igri × Triumph DH population (Fig. 1). The markers, which are listed in Table 1, were selected to give good coverage of the published maps of Heun *et al.* (1991), Graner *et al.* (1991) and Laurie *et al.* (1993).

Fig. 1. RFLP map of chromosome 2(2H) in the Igri $\times$ Triumph cross. The interval containing the centromere is indicated in black.

Analysis of photoperiod response in the glasshouse

For plants given 6 weeks vernalization and grown under long days (16 h), the greatest differential response in flowering time was at the 2(2H)S loci XMWG858 and *XpsrB9*, where the mean flowering time of lines with the Triumph allele was about 10 days later than lines with the Igri allele (Fig 2a). These differences were highly significant in ANOVA tests. Under short days (10 h) the difference in flowering time was reduced to about 4 days which was not signifi-

Table 1. RFLP probes mapped on chromosome 2(2H) in the Igri $\times$ Triumph cross

Clone	Locus	Type
PSR87	<i>Xpsr87</i>	Wheat cDNA
PSR108	<i>Xpsr108</i>	Wheat cDNA
PSR331	<i>Xpsr331</i>	Wheat genomic DNA
PSR571	<i>Xpsr571</i>	Wheat genomic DNA
PSR666	<i>Xpsr666</i>	Wheat genomic DNA
PSRB9	<i>XpsrB9</i>	Barley genomic DNA
PSRB24	<i>XpsrB24</i>	Barley genomic DNA
MWG858	<i>XMWG858</i>	Barley genomic DNA
MWG865	<i>XMWG865</i>	Barley genomic DNA
MWG878	<i>XMWG878</i>	Barley genomic DNA
BCD266	<i>XBCD266</i>	Barley cDNA
BCD410	<i>XBCD410</i>	Barley cDNA

PSR and PSRB probes were from Cambridge Laboratory IPSR libraries. MWG clones were previously mapped in barley by Graner *et al.* (1991) or A. Graner (unpublished data). BCD clones were previously mapped in barley by Heun *et al.* (1991).

cant ($P < 0.10$) in the ANOVA. These results showed that there was a major photoperiod response gene (designated *Ppd-H1*) in this chromosome region. The data from 13 h and 16 h days were similar, suggesting that 13 h days largely satisfied the photoperiod response.

Analysis of flowering time in the field

Data from the spring sowing of the full population of 94 DH lines also showed a highly significant effect on flowering time associated with RFLP markers on 2(2H)S (Fig. 2b). The largest effect was at XMWG858, where the difference between the allele class means was 12 days. In the autumn sowing this locus was again the most strongly associated with variation in flowering time although the effect was smaller with a maximum difference of seven days. As in the glasshouse test, later flowering was always associated with the Triumph alleles. This indicates that flowering time in the field was strongly affected by the *Ppd-H1* gene.

Map location of the photoperiod response gene

Analysis using MAPMAKER/QTL showed a major flowering time QTL in the 6 cM XMWG858 $\rightarrow$ *XpsrB9* interval (Fig. 2c). This was consistent with ANOVA results where the highest variance ratios from comparisons of allele class means were at XMWG858 (field experiments) and *XpsrB9* (glasshouse experiment). The location was confirmed using the RFLP classification of individual DH lines and data from the spring sowing

and the glasshouse test (the experiments where differences in flowering time were clearest) as flowering time could effectively be scored as a qualitative character (Fig. 3). Of the five recombinants in the *XMWG858*→*XpsrB9* interval four could be classified as late flowering (Triumph allele) and one as early flowering (Igri allele). On this interpretation, the position of the *Ppd-H1* gene was 1 cM proximal to *XMWG858* (Fig. 2).

Additional genes affecting flowering time

Flowering time also appeared to be significantly affected by a second gene on chromosome 2H. This was most easily seen in the glasshouse test where lines with the Triumph allele of *Xpsr571* flowered significantly later under all three daylengths (Fig. 2a). In the field experiments the effect of the second gene was not clear but its presence was indicated by the failure of the graphs to fall away smoothly in the chromosome region proximal to *Ppd-H1* (Fig. 2b). MAPMAKER/QTL analysis also suggested the presence of a second gene whose most likely location was in the *XpsrB24*→*Xpsr571* interval (Fig. 2c). *Xpsr571* has previously been shown to be the most proximal long arm marker in wheat (Devos *et al.*, 1993b) while *XpsrB24* has been mapped about 25 cM from the centromere in barley (Laurie *et al.*, 1993). This suggests that most of the *XpsrB24*→*Xpsr571* interval is likely to be on the short arm, suggesting that the second gene was also on 2(2H)S.

In the glasshouse test, the difference between the *Xpsr571* allele class means was 6.7 days under 16 h days and 5 days under 10 h days. It is estimated that 1.6 days of this 1.7 days difference is attributable to coupling linkage with the Triumph allele of *Ppd-H1* as about 30 per cent of the *Ppd-H1* effect should be detectable at the second locus if the map positions of the two genes are correct. This suggests that the gene associated with *Xpsr571* affected flowering time largely independently of photoperiod and vernalization (latter

Fig. 2. Mean allelic differences in flowering time for chromosome 2(2H). For each locus the graphs show the mean of all lines with the Triumph allele minus the mean of all lines with the Igri allele. (a) Results from the glasshouse experiment. (b) Results from spring sown (—) and autumn sown (---) field experiments. Significances from ANOVA tests are indicated by asterisks (a) or letters (s for spring and a for autumn). *, s or a = $P < 0.05$; **, ss or aa = $P < 0.025$; ***, sss or aaa = $P < 0.01$. (c) LOD scores from MAPMAKER/QTL for the spring sown (—) and autumn sown (---) experiments. Solid and open arrowheads show the map positions of peak LOD scores for the spring and autumn sowings, respectively.

Fig. 3. Mean flowering time distributions of lines from the spring sown field experiment. The scale is days difference from the earliest flowering line (day 1). Of the five recombinants in the *XMWG858*→*XpsrB9* interval, four (shaded) are interpreted as having the Triumph allele of *Ppd-H1* and one as having the Igri allele (unshaded) as they lie outside the flowering time ranges of the non-recombinant lines. Mean flowering times of Igri and Triumph are indicated by open and solid arrowheads, respectively.

data not shown). In wheat, such factors have been termed earliness *per se* or developmental rate genes. The latter term is used in this paper.

When the coupling linkage of late flowering alleles is taken into account, approximate estimates of the independent effects of the genes can be made. In the glasshouse, the difference in flowering time between Igri and Triumph alleles of *Ppd-H1* is probably about 8.5 days under 16 h days and 3.5 days under 10 h days. The 5-day difference between long and short days is not altered. The difference between the Igri and Triumph alleles of the developmental rate gene is probably about 4 days under all daylengths. Confirmation of these conclusions requires the development of near-isogenic lines for the alternative alleles.

Analysis of other agronomic traits

Triumph alleles of RFLP markers on the short arm of chromosome 2(2H) were significantly associated with

increases in plant height (Fig. 4a), plant grain weight (Fig. 4b), tiller grain weight (Fig. 4c), ear grain number (Fig. 4d) and, in the spring sowing, 1000 grain weight (Fig. 4e) when analysed by ANOVA or by MAPMAKER/QTL. Analysis of tiller biomass gave very similar results to tiller grain weight (data not shown).

The *XMWG858*→*XpsrB9* and *Xpsr571* regions had similar effects on tiller biomass, tiller grain weight and ear grain number (both sowings) and plant height (spring sowing only). In the autumn sowing, plant height was more strongly affected by the *Xpsr571* region. For plant grain weight the *XMWG858*→*XpsrB9* region had the greater effect in both sowings. As is often seen in studies of cereals, alleles giving increased height were significantly associated with lower harvest index (data not shown).

The positions of the graph peaks suggested that these differences were the result of pleiotropic effects of the *Ppd-H1* and developmental rate genes. A large part of the effects can be attributed to the longer growing season of lines with the later flowering (Triumph) alleles. However, comparison of Figs 4c and d with Fig. 2b shows that the tiller yield effects associated with *Xpsr571* were greater than would be predicted if there was direct proportionality with variation in flowering time. This must mean that *Ppd-H1* or the developmental rate gene had additional effects not directly related to flowering time or that there were other closely linked genes. The latter possibility is also suggested by the MAPMAKER/QTL results for plant grain weight, tiller biomass, tiller grain weight and 1000 grain weight. In these plots the peak LOD scores are in the *XpsrB9*→*XpsrB24* interval and are not coincident with the predicted *Ppd-H1* location. However, this shift in peak position may be an artefact of the coupling linkage of alleles giving higher mean values.

There was also a marked difference in the relationship between tiller yield components and plant grain weight. Tiller yield components were strongly affected by both the *XMWG858*→*XpsrB24* and *Xpsr571* regions (Fig. 4c, d, e) but the effect of the *Xpsr571* region on plant yield was weak in the spring sowing and not significant in the autumn sowing (Fig. 4b). As there was no significant variation in tiller number (data not shown), these results suggest that the effect of the *Xpsr571* region (the developmental rate gene) was to increase the size of the main tillers at the expense of the remainder, giving no overall increase in plant yield. In contrast, the increased tiller yield in the *XMWG858*→*XpsrB24* region was correlated with increased plant yield, probably because of a more general effect of the *Ppd-H1* gene on plant growth.

As only a single RFLP marker (*XpsrB24*) separated the two regions affecting plant performance, an

Fig. 4. Mean allelic differences in other agronomic characters. (a) Plant height; (b) plant grain weight; (c) tiller grain weight; (d) ear grain number; (e) 1000 grain weight. For each locus the line graphs (thick lines) show the mean of all lines with the Triumph allele minus the mean of all lines with the Igri allele. Solid and dashed lines indicate spring and autumn sowing, respectively. The significance of each mean difference in ANOVA tests in spring and autumn sowings is shown below each diagram (s or a = $P < 0.05$; ss or aa = $P < 0.025$; sss or aaa = $P < 0.01$). For each character there is also a graph of the LOD scores from MAPMAKER/QTL (thin lines). Solid and dashed lines again indicate the spring and autumn sowings, respectively. Solid and open arrowheads show the map positions of peak LOD scores for the spring and autumn sowings, respectively.

additional test was made to confirm that two genes were present. Lines with each *Ppd-H1* allele (Fig. 3) were divided into two groups on the basis of their RFLP scores for the *XpsrB24*→*Xpsr571* interval, the predicted location of the developmental rate gene. The resulting pairs of classes were compared by ANOVA to determine if significant differences remained when the effect of *Ppd-H1* was removed. Lines with Triumph alleles of *XpsrB24* and *Xpsr571* had significantly higher mean values in both spring and autumn sowings for height ($P < 0.01$), tiller biomass ($P < 0.01$), tiller grain weight ($P < 0.01$), ear grain number ($P < 0.01$) and harvest index ($P < 0.01$). Ear emergence time was significantly different only in the spring sowing ($P < 0.05$). These results support the existence of two genes and confirmed the lack of effect of the *XpsrB24*→*Xpsr571* region on overall plant yield.

Components of variance from ANOVAs were also used to estimate the percentage of genetic variation attributable to the *Ppd-H1* locus (Table 2). The table shows that the *Ppd-H1* locus was the major source of variation in flowering time in the spring sowing and was a major factor in all other traits. However, the percentage may be overestimated because of linkage with the developmental rate gene in the *XpsrB24*→*Xpsr571* region.

Analysis of RFLP markers on the long arm of chromosome 2(2H) showed that the Igri allele of *Xpsr331* was associated with increased tiller biomass, tiller grain weight and 1000 grain weight. For the latter character *XMGW865* also showed a significant increase associated with the Igri allele. These data

suggest the existence of a tiller size QTL in this chromosome region which was not associated with variation in flowering time.

Discussion

Agronomic effects

The data clearly show that there are at least two major genes affecting agronomic characters on chromosome 2(2H) in the Igri×Triumph cross, both of which are associated with variation in flowering time. One gene is clearly involved in photoperiod response because variation in daylength markedly affects flowering time. The other we have described as a developmental rate gene as its effect appeared to be much less dependent on daylength (Fig. 2).

The strong effect of the *Ppd-H1* region on height and yield can reasonably be ascribed to a pleiotropic effect of the variation in flowering time because this will affect the length of the growing season. This interpretation also accounts for the smaller effects of the *Ppd-H1* locus on plant height and 1000 grain weight in the autumn sowing as plants would be less influenced by long day responses during their early development. However, variation in flowering time does not satisfactorily account for all the observed effects of the developmental rate gene on yield components and it is possible that additional genes are involved. An alternative interpretation is that the effect on flowering time of the developmental rate gene is an indirect result of a role in increasing leading tiller size. To resolve these possibilities, and to characterize fully barley *Ppd* and developmental rate effects, additional mapping populations and isogenic lines for each allele are needed. This approach has been used successfully in wheat to define the pleiotropic effects of the *Ppd1* locus on height and yield components (Worland *et al.*, 1988).

Homoeologies of cereal *Ppd* genes

It is known that major photoperiod response genes in wheat are located on the group 2 chromosomes (Welsh *et al.*, 1973; Law *et al.*, 1978), which are homoeologous to chromosome 2(2H) of barley. *Ppd2* has been assigned to 2BS, *Ppd1* to 2DS and *Ppd3* to 2AS (Scarath & Law, 1983; Law *et al.*, 1993). *Ppd1* is weakly linked to the centromere region and is approximately 30 cM proximal to *Xpsr566*, a marker previously mapped 3 cM distal to *XMWG878* in barley (Laurie *et al.*, 1993). These data show that *Ppd1* in wheat (and by implication *Ppd2* and *Ppd3*) and *Ppd-H1* in barley are in broadly similar map locations and may therefore be homoeoalleles.

Table 2. Estimates of the percentage of the genetic variation attributable to the *Ppd-H1* locus for agronomic traits

Trait	Glasshouse experiment		
	10 h days	13 h days	16 h days
Ear emergence time	—	55.7	56.2
	Field experiments		
	Spring	Autumn	
Ear emergence time	60.3	46.6	
Plant height	37.4	15.7	
Plant grain weight	29.4	38.2	
Tiller grain weight	50.0	42.7	
Ear grain number	51.1	47.1	
1000 grain weight	15.1	—	

Estimates are only included for comparisons with significant ($P < 0.05$) differences between allele class means.

In the Igri × Triumph cross flowering time differences in vernalized material were greatest under long days. In contrast, wheat photoperiod response in vernalized material is greatest under short days (Scarth & Law, 1984). Thus, wheat lines with the photoperiod insensitive allele of *Ppd1* or *Ppd2* flower earlier than lines with the sensitive allele under short days but have more similar flowering times under long days. More detailed mapping, particularly of the wheat *Ppd* genes, will help to determine if this difference in behaviour results from the involvement of different genes or whether it can be accounted for by the presence of different alleles at homoeologous loci. MWG858 hybridizes well to wheat DNA and provides a useful potential marker for such a study. Also, the existence of recombinants between the flanking markers in the Igri × Triumph population should enable markers closer to *Ppd-H1* to be selected.

If the wheat and barley *Ppd* genes prove to be homoeoalleles, this will provide good evidence for the value of comparative mapping in the analysis of agronomically important traits in cereals (Ahn *et al.*, 1993; Bennetzen & Freeling, 1993; Moore *et al.*, 1993) and will provide a basis for analysing the relationship with photoperiod response genes in other species. It is of importance to determine the extent of such 'trait co-linearity', as well as RFLP marker co-linearity, because similar phenotypes could be derived by manipulating different genes. Thus, the relative importance of a particular gene in controlling a trait might differ substantially between related species or even between accessions within a species.

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