

GENETIC CONTROL OF HEADING DATE IN SPRING BARLEY

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Abstract:

A basic vegetative period (BVP) of short duration and sensitivity to increasing photoperiod are the two critical traits contributing to the adaptation of spring barley (*Hordeum vulgare*) cultivars grown under winter growing season conditions in Australia. Cultivars adapted to summer growing season conditions are characterised by BVP's of long duration and insensitivity to increasing photoperiods, making these traits important in the evaluation and exploitation of improved germplasm from Europe and North-America.

Conventional genetic analysis indicates that short BVP can be inherited as a Mendelian recessive, but molecular analysis has so far failed to identify an appropriate molecular marker. Cultivar variation in the duration of BVP is correlated with variation in minimum main stem leaf number suggesting that this variable, combined with variation in the rates of leaf initiation, leaf appearance and stem extension could explain the nature of BVP and the influence of growing season conditions on its duration.

Molecular genetic analysis of the response to increasing photoperiod suggested the involvement of two independent genes, but this interpretation was confounded by segregation distortion and evidence of epistasis with the Ppd-H1 gene on 2HS only expressed when in combination with *Eam8* on 1HL.

Keywords:

BVP, Photoperiod, Adaptation, Spring Barley.

Introduction and Background:

Developmental variation among flowering plants is a major factor determining the geographic distribution of plant species. It is also a major factor determining the adaptation of crop genotypes to diverse growing season conditions and their associated cultural practices. For that reason the timing of flowering is a major selection criterion in all plant breeding programs and an appreciation of its genetic control would enhance the efficiency of that selection.

The critical event in a plant's development is floral induction which initiates the transition from vegetative to reproductive phases in the life-cycle (Garcia del Moral *et al.*, 2002). The timing of floral induction, which is correlated with the timing of flowering, is mediated through light induced activity of photo-reversible phytochrome pigments (Lang, 1965), and varies in its timing with the duration of exposure to light (ie. with photoperiod). This phenomenon, defined as "photoperiodism" by Garner and Allard (1920) established that plant species, and genotypes within species, differ in their flowering response to seasonal changes in photoperiod. Barley is classified as a quantitative "long-day" species, implying only that increase in photoperiod advances flowering. Measures of this advance, based on heading dates under short and long days, indicate that almost all Australian spring barley's released for commercial production are highly responsive to increase in photoperiod. In contrast, most spring cultivars released in Europe / North-America are unresponsive.

Notwithstanding the critical role of light, emerging barley seedlings are not immediately competent to respond to its inductive effects (Roberts *et al.*, 1988). This is illustrated by the fact that few barley genotypes come to ear with less than 7 main stem leaves. The widely recognised cause for this delay is that of a requirement for a period of exposure to low temperatures before reproductive competence is attained. This phenomenon, known as vernalisation, was first reported in 1929 (Salisbury, 1963), and is a characteristic of wild barley. Genotypes differ in the influence of (or requirement for) vernalisation depending on the duration of exposure to low temperature, and differences among genotypes in the effective range of those temperatures (Ritchie, 2002). Among barley genotypes response to vernalisation ranges in a quantitative manner between the extremes from "zero" (in true spring types) to an obligate requirement (in true winter types). All spring cultivars released for

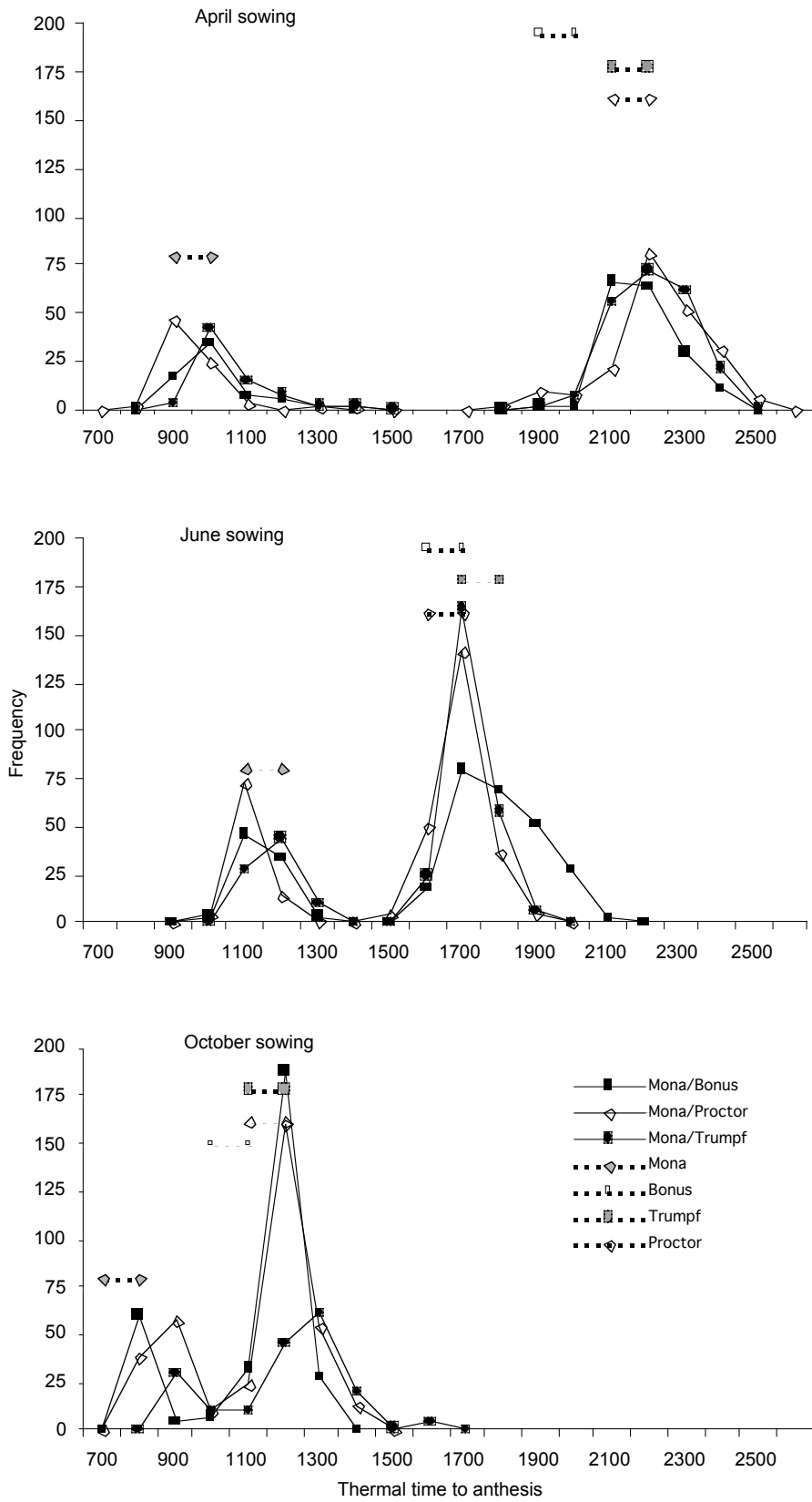


Figure 1. Frequency distributions of thermal time (degree-days) to anthesis of parents and F₂ populations in each of 3 planting dates for crosses between Mona (M) and Bonus (B), Trumpf (TR), and Proctor (P). Mean thermal times and errors shown. Due to the bimodal distribution among progeny, early (E) and late (L) refer to the early and late subpopulations.

commercial production in Australia are insensitive to the influence of vernalisation. This characteristic also applies to many cultivars released for Europe / North-America.

Although photoperiod and vernalisation have long been regarded as the major factors contributing to developmental variation, other variously defined factors are also reported to vary the timing of floral induction (hence flowering). This can best be illustrated with reference to the Western Australian data presented in Fig. 1. These data represent F₂ segregation patterns for heading date from each of three planting dates for each of three barley populations involving parents of European origin that are insensitive to the developmental influences of both photoperiod and vernalisation. At all planting dates cvs. Bonus, Trumpf and Proctor are late flowering, relative to Mona. Each was crossed to cv. Mona. Conventional analyses of these F₂ data confirm that a single major gene discriminates between the distinctly early and late parents and sub-populations, with early heading the recessive trait. There is, however, a major influence of seeding date on the differential between early and late heading parents and sub-populations and, within both categories, considerable transgressive segregation at all three seeding dates.

We have interpreted this major distinction between parents (and their early and late sub-populations) as the manifestation of variation in the duration of a “basic vegetative period” (or BVP). Given that the April (autumn) and October (spring) plantings approximate commercial practice in Australia and Europe/N.America respectively, the influence of this single gene has major implications for the adaptation of cultivars to winter and summer growing season conditions, and for the exchange of improved germplasm between S. and N. hemisphere breeding programs. Most cultivars grown over the mild winter growing season conditions in Australia are characterised by BVP’s of short duration. In contrast, BVP’s of long duration are a characteristic of cultivars adapted to the summer growing season conditions of Europe and N.America.

The influence of seeding date on the duration to heading, and on the expression of the proposed major gene for BVP, could be interpreted to reflect differential genotype responses to the seasonal and correlated trends of photoperiod, temperature and incoming radiation at the time of planting and over the period up to floral induction. With reference to Fig. 1 these environmental variables decline toward their winter perigee from the April (autumn) planting, are at their seasonal lows before increasing rapidly after planting in June (winter), and are approaching their summer apogee’s from the October (spring) planting. This effect of seeding date on the timing of heading cannot be explained on the influences of vernalisation or photoperiod (as all parents are insensitive to their inductive effects) or attributed to the modifying influence of variation in seasonal temperature (as that impact has been negated by presenting the data in thermal time: °Cd). A possible explanation for the effect of seeding date, and of the transgressive segregation within the early (short BVP) and late (long BVP) parents and sub-populations, is that it reflects inherent variation in growth related progress to flowering. Hay and Ellis (1998) proposed that seasonal events, including environmental factors contributing to photosynthate production and respiration, have an influence on heading date..

The results presented in this paper derive from a study conducted to identify molecular markers for the two traits important to the adaptation of spring cultivars grown under the mild winter growing season conditions in Australia – namely a short BVP combined with a strong response to increasing photoperiod. The exploitation of such markers would be a valuable aid to selection in populations derived from crosses between locally adapted cultivars and unadapted sources of improved germplasm. Variation in heading date among two doubled haploid populations is reported: one in which the parents (Mona and Bonus) do not respond to vernalisation or photoperiod but differ markedly in their durations of BVP and, a second population in which the parents (Mona and Turk) differ in response to extended photoperiod and duration of BVP.

Methods:

Parents and DH progeny were grown from the same winter planting date (13th June) in 2005 and 2006 when natural photoperiod would be about 10.2h at the time of emergence, and in an adjacent plot over which photoperiod was extended to 18h. Days to awn appearance were recorded. The data presented in Figs. 2 and Table 1 represents means over replicates and years. The reason for this averaging was that patchy transient waterlogging in both years induced high experimental errors in the extended photoperiod treatment. Under natural photoperiod awn appearance occurred over the range of 94 to 140 days in 2005 and 97 to 139 days in 2006, with standard errors of 2.35 and 2.11 days, respectively. Under extended photoperiods, where heading among replicates, within years, varied by more than 4 days the earlier heading date was considered a better estimate of phenotype than the replicate mean. On that basis the standard errors were similar to those under the natural photoperiod treatment.

The molecular markers used include a gene – specific component of the Ppd-H1 gene, identified by Turner et al., (2005) as a pseudo-response regulator, and sequenced from the Mona X Turk in the present study, and a gene-specific marker (WMC1E8) for the eam8 gene.

Results:

Mona X Bonus

Mona headed 37 and 36 days earlier than of Bonus under both natural (short) and extended photoperiod treatments, respectively (Fig. 2). As response to the extension of photoperiod was negligible (+4 and +3 days, respectively), the differential of 36 days under a long (18h) photoperiod is interpreted as a measure of BVP. It is hypothesised that the DH progeny would segregate for that trait.

The 27 DH lines available segregated in a ratio of 12 (early):15 (late) in both photoperiod treatments, in both years, with negligible responses to extended photoperiod in both early and late sub-populations (Fig. 2). Although the DH population size is low the repeatability of a near 1:1 segregation ratio over years, replicates and photoperiod treatments suggests segregation for a single gene governing the duration of BVP – with long BVP being the dominant trait.

Marker analysis of the parents and progeny is currently incomplete due to negligible polymorphism between parents among any of the markers tested to date. This includes the gene-specific marker (WMC1E8) for “eam8” (1HL) and reported by Lundqvist (1995) to govern the early flowering mutant (cv. Mari) from which Mona was derived. Possible explanations will be considered in the discussion.

Mona X Turk

Turk, like Mona, does not respond to vernalisation but responds strongly to extended photoperiod. Under natural (short) photoperiod Turk headed 55 days later than Mona. When the photoperiod was extended to 18h, heading in Turk was advanced by 39 days and the differential between parents was reduced to 12 days - reflecting a differential among the parents in the duration of their BVP's (Turk > Mona). It was hypothesised that the DH progeny would segregate for the two traits by which the parents differ: BVP and response to extended photoperiod.

Under natural (short) photoperiod 20 of the 80 DH progeny grown headed over the range from 87 to 106 days (mean 95) with the remaining 60 over the distinctly greater range from 131 to 155 days (mean 144). This 1 early : 3 late segregation ratio implies the involvement of 2 major independent genes accounting for 94% of the phenotypic variation recorded in this short day treatment. That interpretation would suggest single major genes for each of BVP and response to extended photoperiod, and the expectation parental and recombinant phenotypes would be identified, in equal proportions among the DH progeny. Neither expectation is confirmed (Table 1), due to epistasis and segregation distortion.

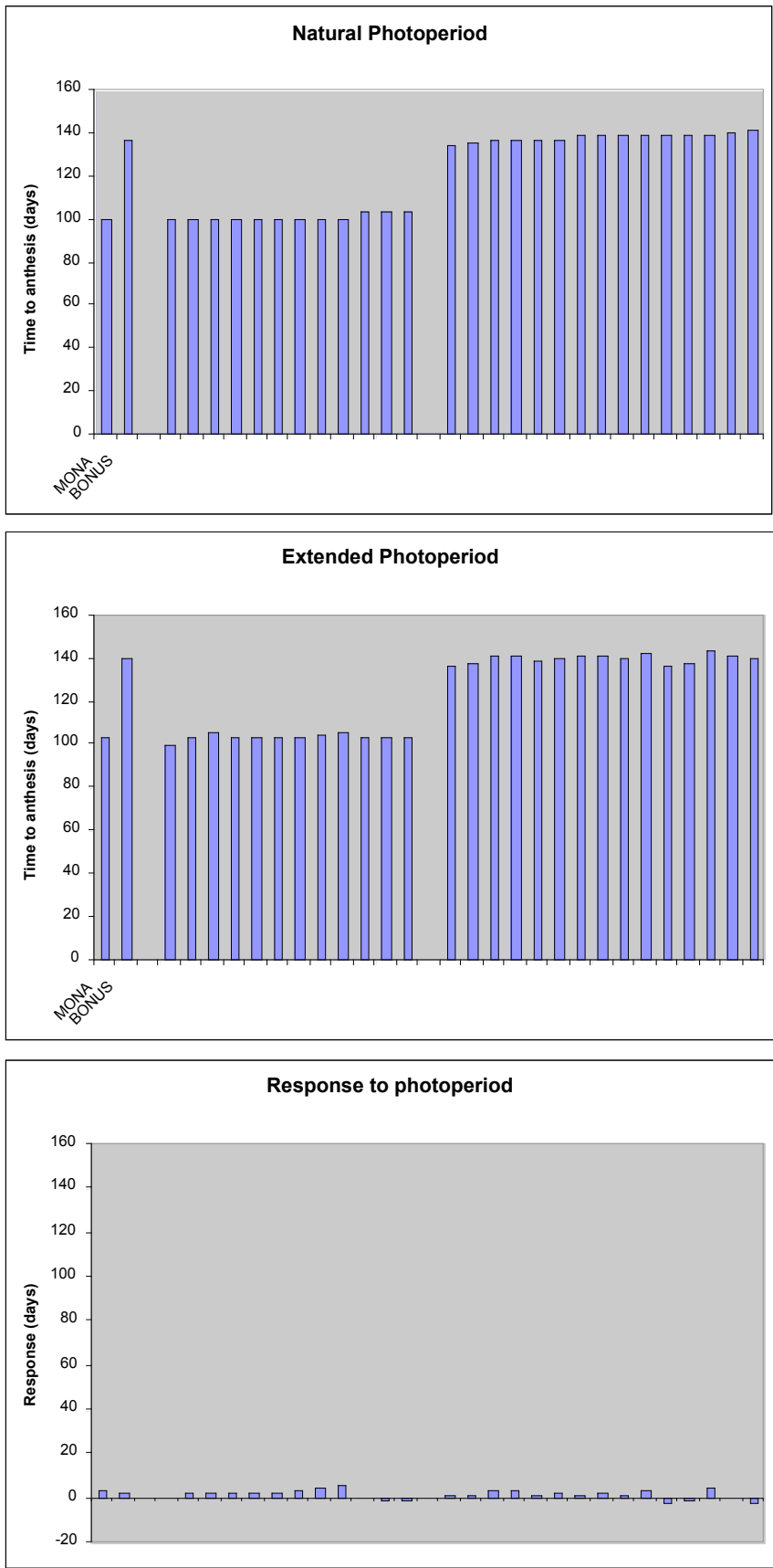


Figure 2. Days to awn appearance (anthesis) among parents and doubled haploid progeny of the cross between Mona and Bonus under natural and extended (18h) photoperiods from a mid-June planting

Table 1. Segregation among 80 DH Mona X Turk progeny for marker based assessment of their genotypes together with details of mean durations (days) to heading under natural and extended photoperiods, and calculated responses to photoperiod.

Genotype	Natural Ppd (short)	Extended Ppd (long)	Response to Ppd	Number of DH lines	Phenotype
M1M2	96	104	-8	14	Mona
T1M2	93	102	-9	6	Recombinant
M1T2	142	135	+7	13	Recombinant
T1T2	146	114	+32	47	Turk

M1 and T1 refer to the Mona and Turk alleles, respectively located on 2HS with the gene specific marker Ppd-H1
M2 and T2 refer to the Mona and Turk alleles, respectively located on 1HL with the gene specific marker WMC1E8 for *eam8*

- The M2 allele for *eam 8* is associated with early flowering under both short and long photoperiods irrespective of the presence of either the Mona or Turk alleles for Ppd-H1. The T2 allele for *Eam-8* is associated with delayed flowering under both and long photoperiod, and permits a strong response to change in photoperiod when combined with the T1 allele for Ppd-H1. This evidence implies that the expression of Ppd-H1, reportedly a major gene for sensitivity to extended photoperiod (Laurie et al., 1994), is governed / permitted by the *Eam8/eam8* locus.
- Since the phenotype of the male parent is in excess, misidentification of the grossed F1 plants can not be responsible for the unexpected ratios. However, segregation distortion is commonly reported in doubled haploid populations derived from anther culture, and specifically for chromosomes 1H and 2H (Castillo et al., 2005).

Discussion:

The genetic control of variation in heading date in spring barley is more complex than would be assumed from historical studies characterised by segregation for 1 to 3 genes (Smith, 1951). Since then major genes governing response (sensitivity) to extended photoperiod have been identified: Ppd-H1 on 2HS by Laurie *et al.* (1994) with another, “*Eam 6*” in the centromeric region of 2H (Frankowiak pers comm.) A gene on 1 HL, designated Ppd-H2, is reported by Laurie *et al.* (1995) to govern variation in flowering under short days in winter barley. Five other genes -“*Eam-5*”- (Frankowiak, pers comm.), and *eam 7*, *eam 8*, *eam 9*, and *eam 10* (Gallagher *et al.* (1987), and located on chromosomes 5HL, 6HS, 1HL, 4HL and 3HL respectively, have been associated with insensitivity to photoperiod, and with confounding interactions. *Eam 5* is the most widely used gene in international breeding programs for early flowering under short-day conditions, despite its response in different genetic backgrounds varying from very early to late under short days, including extreme earliness when combined with Ppd-H2 (Frankowiak, pers. comm.). Gallagher *et al.* (1987) report that epistasis is a recurring theme when certain of the other 4 genes for photoperiodic insensitivity are combined. Additional genes influencing heading date include 8 QTL's for *earliness per se (eps)* genes located by Laurie *et al.* (1994) on 2H, 3H, 4H, 5H, 6H (x2) and 7H (x2). The influence of these *eps* genes is mainly characterised by their relatively minor individual contributions considered by Laurie *et al.*, (1995) and Hay and Ellis (1998) to impact on variation in growth related progress to flowering.

The trait, defined in this paper as BVP is not recognised in literature from the N. hemisphere. This is understandable given the volatility in the expression of the trait, as was illustrated in Fig.1, from a major variable in an April planting *viz a viz* a much reduced influence in an October planting. In the N. hemisphere (>45°N), where most the global production of barley occurs, and the bulk of research information derives, spring barley is planted under photoperiods already longer than those prevailing during the grain filling stage in S. hemisphere areas of production. Under such summer growing season conditions genotypes with BVPs of short duration would “bolt” to heading – as may Australian introductions do in (Rosnagel, pers comm.). Canada. In those areas, therefore, a BVP of long duration mimics vernalisation by delaying competence to respond to the inductive influence of photoperiod

where as, in the S. hemisphere, such a delay would have the effect of extending the date of grain filling to conditions of declining rainfall, increasing temperatures and high evaporative demand. A BVP of short duration removes this constraint leaving photoperiod the primary determinant of heading date in the S. hemisphere. Thus, segregation for a gene controlling BVP among DH progeny derived from the cross between Mona and Bonus (Fig. 2) reflects segregation for a trait of major adaptive importance for cultivars grown under a mild winter growing season..

Here, in Australia, we have adopted the term BVP for physiological reasons. The essential element of which is that vegetative growth proceeds during the period of delay until floral induction occurs. Support of the concept of has been provided by evidence of a period, following seedling emergence, during which spring barley plants are insensitive to the influence of an otherwise inductive photoperiod (Roberts *et al.*, 1988), and the report by Inagaki and Musuda (1984) of a “minimum growth requirement” before floral induction can proceed. Genotypes do differ, and consistently over environments, in the minimum number of main stem leaves initiated (Mona = 7-8 < Bonus = 10-12), and in the rates of leaf and spikelet initiation, leaf appearance and stem internode elongation (Mona > Bonus). These inherent differences would be expected to impact on the timing of floral induction, hence flowering, and contribute to differences among genotypes in the rate of developmental progress to flowering. As this speculation conforms to the proposed influence of *eps* genes proposed by Hay and Ellis (1998), it is likely that the gene(s) for BVP, and for which we are seeking a marker(s), involves interactions among the *eps* genes identified by Laurie *et al.* (1995) – with that (those) determine main stem leaf number responsible for the major difference in heading illustrated in Fig.1 for the autumn planting.

Regrettably no BVP specific markers have been detected to date although, in the study of seven doubled haploid populations developed from locally adapted x adapted parents in Australia by participants of the National Barley Molecular Marker Program (Boyd *et al.*, 2003). 2 QTL's were of dominant influence: one on 2HS (presumably Ppd-H1) was associated with response to extended photoperiod and the other, located near the centromere of 2H (marker Bmy2) with variation for reasons other than photoperiod. This raises the possibility that the Eam6/eam 6 locus for photoperiod response, and located in the centromeric region of 2H, could be involved in some interaction governing the duration of BVP.

Although phenotypic segregation for 2 independent genes in the Mona x Turk population was confirmed by molecular marker analysis, the combination of segregation distortion and evidence of epistasis confound the search for appropriate molecular markers

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