Journal of Experimental Botany, Vol. 59, No. 13, pp. 3595–3607, 2008
doi:10.1093/jxb/ern209
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Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

The chromosome region including the earliness *per se* locus *Eps-A^{m}1* affects the duration of early developmental phases and spikelet number in diploid wheat

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Received 11 April 2008; Revised 10 July 2008; Accepted 21 July 2008

Abstract

Earliness *per se* genes are those that regulate flowering time independently of vernalization and photoperiod, and are important for the fine tuning of flowering time and for the wide adaptation of wheat to different environments. The earliness *per se* locus *Eps-A^{m}1* was recently mapped within a 0.8 cM interval on chromosome 1A^{m}L of diploid wheat *Triticum monococcum* L., and it was shown that its effect was modulated by temperature. In this study, this precise mapping information was used to characterize the effect of the *Eps-A^{m}1* region on both duration of different developmental phases and spikelet number. Near isogenic lines (NILs) carrying the *Eps-A^{m}1-l* allele from the cultivated accession DV92 had significantly longer vegetative and spike development phases (P <0.0001) than NILs carrying the *Eps-A^{m}1-e* allele from the wild accession G3116. These differences were paralleled by a significant increase in the number of spikelets per spike, in both greenhouse and field experiments (P <0.0001). Significant interactions between temperature and *Eps-A^{m}1* alleles were detected for heading time (P <0.0001) but not for spikelet number (P=0.67). Experiments using NILs homozygous for chromosomes with recombination events within the 0.8 cM *Eps-A^{m}1* region showed that the differences in number of spikelets per spike were linked to the differences in heading time controlled by the *Eps-A^{m}1* locus. These results indicate that the differences in these two traits are either pleiotropic effects of a single gene or the effect of closely linked genes. A similar effect on spikelet number was detected in the distal region of chromosome 1AL in common wheat (*T. aestivum* L.).

Key words: Development, earliness *per se*, heading time, spikelet number, wheat.

Introduction

Wheat varieties can be grown in very different environments with contrasting temperatures and photoperiods. This broad adaptability is probably favoured by the extensive genetic diversity in flowering time in wheat. Wheat varieties are divided into winter and spring growth habit classes based on the presence or absence of a requirement for a long exposure to cold temperatures to induce flowering (vernalization). In both spring and vernalized winter wheat varieties, photoperiod and growing temperature are the main factors affecting development rate and time to heading. The physiological and genetic basis of the regulation of flowering time by vernalization and photoperiod have been well documented in wheat and barley, and the main genetic factors controlling these responses have been cloned (Yan *et al*., 2003, 2004, 2006; Turner *et al*., 2005; Beales *et al*., 2007). However, the genetic factors responsible for the differences in flowering time once vernalization and photoperiod requirements are fulfilled are not well understood. These residual differences in flowering time are

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usually referred to as earliness *per se*, also named ear-emergence *per se,* earliness in narrow sense, intrinsic earliness, or basic development rate (Flood and Halloran, 1984; Hoogendoorn, 1985*a*; Worland and Law, 1986; Kato and Yamagata, 1988; Slafer, 1996; Law and Worland, 1997; Snape *et al*., 2001). This variability is important to fine-tune flowering time and can be exploited to maximize yield potential in different environments.

Most studies about earliness *per se* have been focused on the final differences in heading time. However, these differences are the result of the integration of differences in the duration of several developmental phases, including the transition from the vegetative to the reproductive apices, early and late spike development, and stem elongation. It is not well known how the different genetic factors regulating earliness *per se* affect these different phases of wheat development, although in some cases earliness *per se* effects have been restricted to the duration of the vegetative phase (Aitken, 1974; Major, 1980; Wallace, 1985; Evans and Blundell, 1994). Slafer (1996) concluded that earliness *per se* affects both the vegetative and early reproductive phases in independent and contrasting ways. As important grain yield components are determined during these phases (Slafer and Whitechurch, 2001), it is relevant to determine the effect of individual earliness *per se* genes (*Eps*) on each of these developmental phases. Since *Eps* genes include all those genes affecting flowering time that are not involved in either vernalization or photoperiod requirements, they are likely to be a heterogeneous group with variable effects on different developmental phases.

The analysis of the effect of individual *Eps* genes on different wheat developmental phases requires the precise mapping of the genes responsible for those effects. *Eps* genes have been identified on different wheat chromosomes, and a few of them have been mapped as quantitative trait loci (QTL) for heading time (Scarth and Law, 1984; Hoogendoorn, 1985*a*; Worland and Law, 1986; Miura and Worland, 1994; Kato *et al*., 1999, 2002; Sarma *et al.,* 2000; Sourdille *et al*., 2000; Toth *et al*., 2003; Kuchel *et al*., 2006). Bullrich *et al*. (2002) mapped a QTL for earliness *per se* in the distal region of chromosome $1A^mL$ in a cross between cultivated (DV92) and wild (G3116) *Triticum monococcum* L. accessions, which was designated *Eps-A^m1*. Even though allelic variation at this locus resulted in differences in heading time of only a few days under natural conditions, those differences extended to several weeks when plants were fully vernalized and grown under a long-day photoperiod. Differences in heading time between *Eps-A^m1* allelic classes were significantly larger at 16 °C than at 23 °C, indicating that the effect of this gene was modulated by temperature (Bullrich *et al*., 2002). This locus was then mapped within a 0.8 cM interval between flanking genes *Adk1* and *VatpC* using a high-density mapping population and markers generated from the colinear region on rice chromosome 5 (Valárik *et al*., 2006).

The precise mapping of the *Eps-A^m1* locus together with the development of isogenic sister lines for this chromosome region provide a unique opportunity to analyse the effect of an individual *Eps* gene on wheat developmental phases. The effect of the *Eps-A^m1* chromosome region on both duration of developmental phases and number of spikelets per spike, an important component of grain yield, is reported here. A better understanding of the contributions of individual *Eps* genes to the duration of individual developmental phases and the interactions with different environmental factors is essential to manipulate wheat heading time and maximize yield potential in different environments.

Materials and methods

Lines and cytogenetic stocks used in the different experiments

Lines used in this study are all derived from the cross between cultivated *T. monococcum* ssp. *monococcum* accession DV92 (spring) carrying the *Eps-A^m1* allele for late heading (hereafter *Eps-A^m1-1*) and wild *T. monococcum* ssp. *aegilopoides* accession G3116 (winter) carrying the *Eps-A^m1* allele for early heading (hereafter *Eps-A^m1-e*) (Bullrich *et al*., 2002).

Two different sets of genetic materials were developed from the same DV92×G3116 cross. The first one was a population of F_6 single-seed descent (SSD) lines from which 35 lines including all possible combinations of spring and winter types and the two different *Eps-A^m1* allelic classes were selected (Bullrich *et al*., 2002). These lines were only used in experiment 1.

Lines used in experiments 2, 3, and 4 were BC_6F_2 near isogenic lines (NILs; Table 1). These lines were developed by backcrossing the *Eps-A^m1-e* allele from G3116 into DV92 for six generations, and then self-pollinating the resulting heterozygous BC_6 lines. These NILs are expected to be more than 99% identical to DV92, and they all have a spring growth habit. The BC_6F_2 lines were screened with markers flanking the *Eps-A^m1* locus, and those with recombination events close to this locus were self-pollinated to immortalize the recombinant chromosomes. Sister BC_6F_3 NILs homozygous for the non-recombinant chromosome were selected as controls. The recurrent parental line DV92 and two pairs of sister NILs homozygous for either the DV92 or the G3116 alleles at all markers encompassing the *Eps-A^m1* region were also included as additional controls (Table 1).

In experiment 5, hexaploid wheat Chinese Spring (CS) and several cytogenetic stocks for homoeologous groups in the CS genetic background were used. These stocks included nullisomic-tetrasomic lines of homoeologous group 1 N1AT1B, N1AT1D, N1BT1A, N1BT1D, and N1DT1B (the N indicates the missing chromosome and the T the chromosome in tetrasomic state), and ditelosomic line Dt1AS (missing the complete 1AL arm) developed by Sears (1954), and also deletion line 1AL1 missing the distal 83% of the 1AL arm (Qi *et al*., 2004).

The *Eps-A^m1* alleles present in the recombinant lines used in experiments 2, 3, and 4 were determined by progeny tests for heading time (growth-chamber, 16 °C, 16 h light; Table 2). Time from sowing to heading was above 140 d for all control and recombinant NILs carrying the DV92 allele and less than 110 d for the lines carrying the G3116 allele, except for NIL 502-19, which headed 126 d after sowing. Heading time for this NIL was still

Table 1. *NILs used in experiments 2, 3, and 4*

Eps-A^{m}1 alleles for each line were determined by the progeny tests described in Table 2. Lines indicated in bold letters are the ones carrying chromosomes with recombination events within the *Eps-A^{m}1* region. A, DV92 allele (*Eps-A^{m}1-l*); B, G3116 allele (*Eps-A^{m}1-e*).

Recombinant line	Objective[a]	PCR markers										Experiment
		wg241/VatpC		*Eps-A^{m}1*		*Adk1*		*Smp*		*Barc287*		
DV92	C	A		A		A		A		A		**3, 4A, 4B**
853-1	C	A		A		A		A		A		**2**
408-15	C	A		A		A		A		A		**2**
408-1	C	B		B		B		B		B		**2**
853-74	C	B		B		B		B		B		**2**
529-1	PR	B	**X**	A		A		A		A		**2, 3, 4A, 4B**
529-3	PR-C	A		A		A		A		A		**2, 3, 4A, 4B**
500-14	PR	B	**X**	A		A		A		A		**2**
500-1	PR-C	B		B		B		B		B		**2, 3**
508-7	PR	A	**X**	B		B		B		B		**2, 3**
518-3	PR	A	**X**	B		B		B		B		**2, 3, 4A, 4B**
518-2	PR-C	B		B		B		B		B		**4A, 4B**
502-19	DR	B		B	**X**	A		A		A		**3, 4B**
502-5	DR	B		B	**X**	A		A		A		**4A**
502-3	DR-C	B		B		B		B		B		**3, 4A**
505-9	DR	B		B		B	**X**	A		A		**3, 4B**
268-5	DR	A		A		A		A	**X**	B		**2, 4A, 4B**
268-3	DR-C	B		B		B		B		B		**2, 4A, 4B**

[a] C, control; PR, proximal recombinant; DR, distal recombinant (-C after either PR or DR indicates sister control line). Lines with the same initial number are sister lines.

significantly earlier than the control lines carrying the DV92 allele. In addition, sister line 502-5 carrying the same recombinant chromosome headed 107 d after sowing, confirming that the recombinant chromosome present in the 502 family carries an *Eps-A^{m}1-e* allele. Differences between NILs and non-recombinant control lines were analysed using ANOVA, and means were compared using Tukey's test (Table 2).

Since these genetic materials were developed in parallel to the experiments presented here, different sets of lines were included in the different experiments, as described in the last column of Table 1. The genotype of all the lines used in this study was confirmed using PCR markers developed by Valárik *et al*. (2006) (Table 1). Genomic DNA extraction and PCR procedures were performed as described by Valárik *et al*. (2006).

Experiment 1: interactions between Eps-A^{m}1 *alleles, heading time, spikelet number, and temperature*

The objective of this experiment was to study the effect of the *Eps-A^{m}1* alleles on spikelet number at different temperatures, using the same SSD lines analysed for heading time by Bullrich *et al*. (2002). Thirty-five homozygous F_6 SSD lines derived from the DV92×G3116 cross were grown under two different temperatures (air temperature based on chamber sensors 16 °C and 23 °C). These lines included all possible combinations of spring and winter types with the two different *Eps-A^{m}1* allelic classes. Among the spring SSD lines, eight were *vrn-A^{m}2-Eps-A^{m}1-l* and ten were *vrn-A^{m}2-Eps-A^{m}1-e*. Among the winter SSD lines, five were *Vrn-A^{m}2-Eps-A^{m}1-l* and 12 were *Vrn-A^{m}2-Eps-A^{m}1-e*.

For each treatment, 15 seedlings from each line were vernalized at 5 °C for 8 weeks under a short-day photoperiod and then transferred to different growth-chambers (16 °C and 23 °C) under a long-day photoperiod (16 h, fluorescent light, 45 μE m^{-2} s^{-1}, extended to 24 h with incandescent light) to saturate both photoperiod and vernalization requirements. Lines were grown as a single plant per pot. Pots (experimental units) were organized in a Completely Randomized Design. Heading time (measured in calendar days) and number of spikelets per spike (from the main spike) were recorded, and differences among alleles were analysed using ANOVA. Thermal time to heading was also calculated by multiplying calendar days to heading by growth temperature, and it was expressed in degree days (°Cd). A base temperature of 0 °C

Table 2. *Progeny tests for heading time to determine the* Eps-A^{m}1 *alleles present in the recombinant lines*

Lines in bold letters are the ones with recombination events within the *Eps-A^{m}1* region. A, DV92 allele (*Eps-A^{m}1-l*); B, G3116 allele (*Eps-A^{m}1-e*).

Recombinant line	PCR markers			Heading time[a]
	wg241/VatpC	*Eps-A^{m}1*	*Barc287*	
853-1	A	A	A	149.3±2.9 a
853-74	B	B	B	106.1±3.1 b
408-15	A	A	A	169.0±0.0 a
408-1	B	B	B	100.3±3.5 b
529-3	A	A	A	146.5±1.5 a
529-1	B	A	A	150.5±0.5 a
500-1	B	B	B	99.4±3.5 a
500-14	B	A	A	166.0±0.0 b
268-5	A	A	B	143.0±0.0 a
268-3	B	B	B	97.2±6.6 b
518-3	A	B	B	104.4±3.1 b
268-5	A	A	A	143.0±0.0 a
268-3	B	B	B	97.2±6.6 b
508-7	A	B	B	102.0±2.4 b
529-3	A	A	A	150.6±1.4 a
502-19	B	B	A	125.9±3.4 b
502-5	B	B	A	106.8±2.7 c
DV92	A	A	A	144.3±1.9 a
505-9	B	B	A	93.1±1.3 b

[a] Means ±standard error of the means followed by different letters are significantly different (P <0.05).

was used since temperatures in this experiment were constant and always well above developmental base temperatures for wheat (Slafer and Savin, 1991).

Experiment 2: effect of Eps-A^{m}1 *alleles on developmental phases*

The objective of experiment 2 was to determine the effect of the *Eps-A^{m}1* alleles on the relative duration of wheat developmental phases. In this study, six NILs with the *Eps-A^{m}1-e* allele and six with the *Eps-A^{m}1-l* allele were used. Four of the NILs (500-14, 508-7, 518-3, and 529-1) had recombination between *Eps-A^{m}1* and the proximal marker *VatpC* and one (268-5) had recombination between *Eps-A^{m}1* and the distal marker *barc287* (Tables 1, 2). Plants were grown in a growth-chamber at constant temperature (16 °C) and a long-day photoperiod (16 h, fluorescent light, 90 μE m^{-2} s^{-1}). Apices were dissected and microscopically checked from several plants of each genotype every 5–7 d, and the time from sowing to double ridge stage (S-DR), from double ridge stage to terminal spikelet (DR-TS), and from terminal spikelet to heading (TS-H) was determined following the scales proposed by Kirby and Appleyard (1987). The number of days between sowing and heading was recorded for the remaining plants, and the main spike of each plant was harvested to count spikelet number.

Experiment 3: mapping the locus affecting spikelet number

The objective of experiment 3 was to test if the locus controlling the differences in spikelet number was linked to the *Eps-A^{m}1* locus, responsible for the differences in heading time. This experiment included three NILs with close recombination events proximal to *Eps-A^{m}1* (508-7, 518-3, and 529-1) and two with close recombination events distal to *Eps-A^{m}1* (502-19, and 505-9; Tables 1, 2), together with four control lines without recombination within this region: DV92 and 529-3 carrying the DV92 allele, and 500-1 and 502-3 carrying the G3116 allele, for all the markers in the *Eps-A^{m}1* region. All lines were homozygous and were represented by 8–10 plants each. However, fewer plants were available for lines 502-5 (5 plants), 529-1 (3 plants), and 529-3 (5 plants). Plants were grown under the same conditions as experiment 2. The number of days between sowing and heading was recorded for the individual plants, and the main spike per plant was harvested to count the number of spikelets. Values from all plants within a line were averaged and lines were used as replications in the statistical analysis.

Experiment 4: effect of Eps-A^{m}1 *alleles on spikelet number in the field*

The objective of experiment 4 was to test if the differences in spikelet number were also detectable in field-grown plants. Two different experiments were conducted, one at the Intermountain Research and Extension Center at Tulelake, California, USA (41°57′ N, 121°28′ W, experiment 4A), and the other one at Davis, California, USA (38°32′ N, 121°46′ W, experiment 4B). Solar radiation was obtained from http://www.ipm.ucdavis.edu/WEATHER/wxretrieve.html.

Experiment 4A included parental accession DV92, two lines with recombination proximal to *Eps-A^{m}1* (518-3, and 529-1) and two with recombination distal to *Eps-A^{m}1* (502-5, and 268-5), with their respective non-recombinant sister control NIL (Tables 1, 2). Lines were sown on 1 May 2007 (spring-planted), and harvested on 11 September 2007. The average solar radiation at Tulelake for this period was approximately 1400 μE m^{-2} s^{-1}. Genotypes were organized in a Completely Randomized Design.

Experiment 4B included parental accession DV92 and the same two NILs with recombination proximal to *Eps-A^{m}1* (518-3 and 529-1), and three NILs with recombination distal to *Eps-A^{m}1* (268-5, 505-9, and 502-19). Non-recombinant sister lines were included for 518-3, 529-1, and 268-5 but were not available for 505-9 and 502-19 (Tables 1, 2). These lines were sown on 16 November 2007 (autumn planted), and harvested on 11 June 2008. For this period, the average solar radiation at Davis was approximately 800 μE m^{-2} s^{-1}. Genotypes were organized in a Randomized Complete Block Design with three replications.

For both experiments, three separate ANOVAs were performed for the proximal, distal, and *Eps-A^{m}1* loci, using alleles as levels and genotypes as replications. Genotypes were represented by a single row with 20 plants each, and for each line the average number of spikelets per spike was calculated from eight random spikes (subsamples).

Experiment 5: effect of homoeologous group 1 chromosomes on spikelet number in hexaploid wheat

To test the hypothesis that a gene with an effect similar to *Eps-A^{m}1* is present in common wheat *T. aestivum* L., an experiment using CS nullisomic-tetrasomic lines (Sears, 1954) was first conducted at Buenos Aires, Argentina (experiment 5A). Nullisomic-tetrasomic lines N1AT1B, N1AT1D, N1BT1A, N1BT1D, and N1DT1B were grown in a growth-chamber at constant temperature (16 °C) and with a long-day photoperiod (16 h, fluorescent light, 90 μE m^{-2} s^{-1}), using CS as a control line. Individual plants were used as replications (nine to 16 plants per genotype), and the number of spikelets per spike for each plant was estimated from the average of two spikes.

A second experiment was conducted at Davis, CA, USA to determine the intra-chromosomal location of the locus affecting the number of spikelets per spike on chromosome 1A (experiment 5B). This second experiment included nullisomic-tetrasomic line N1AT1D, ditelosomic line Dt1AS (Sears, 1954), and deletion line 1AL1 (Qi *et al*., 2004). Three to four plants of each line were grown in a greenhouse at 20–25 °C temperature and a long-day photoperiod (16 h light, 602 μE m^{-2} s^{-1}). The number of spikelets per spike for each plant was estimated from the average of three spikes.

Differences in number of spikelets per spike were analysed using ANOVA with plants used as replications. Dunnett's test was used to compare the control line CS with the different cytogenetic stocks.

Statistical analyses

All the statistical analyses were performed using SAS statistical package version 9.0. PROC GLM was used for the ANOVAs. Homogeneity of variances was tested using Levene's test and normality of residuals using Shapiro–Wilk test.

Results

Experiment 1: interactions between Eps-A^{m}1 *alleles, heading time, spikelet number, and temperature*

A three-way factorial model including growth habit, temperature, *Eps-A^{m}1* allele, and all their possible interactions explained a large proportion of the variation in both heading time (R^2=0.96) and number of spikelets per spike (R^2=0.85). Non-significant differences were observed for these two traits between lines with winter and spring growth habits. This was the expected result because

the plants were vernalized at 5 °C for 8 weeks, eliminating the effect of growth habit on heading time.

Highly significant differences in both heading time and number of spikelets per spike were found between *Eps-A^{m}1* alleles (P <0.0001; Table 3; Fig. 1). The SSD lines with the *Eps-A^{m}1-l* allele from DV92 headed 61 d later (76% increase across temperatures) and had on average 8.7 more spikelets per spike (56% increase across temperatures) than the lines with the *Eps-A^{m}1-e* allele from G3116 (Fig. 1). Significant differences (P <0.0001) in both parameters were also detected between the two different temperatures used in this experiment. Combining both *Eps-A^{m}1-l* and *Eps-A^{m}1-e* alleles, lines grown at 16 °C headed 31 d later (32% increase) and showed on average 4.6 more spikelets per spike (26% increase) than those grown at 23 °C (Fig. 1).

The differences in heading time between *Eps-A^{m}1* alleles were also highly significant (P <0.0001) when heading time was measured as thermal time (Table 3; Fig. 1B). The thermal time to heading for the lines carrying the *Eps-A^{m}1-l* allele (2675 °Cd) was 1118 °Cd longer (72% increase across temperatures) than that for the lines carrying the *Eps-A^{m}1-e* allele (1557 °Cd).

Significant interactions (P <0.0001; Table 3) were detected between temperature and *Eps-A^{m}1* alleles for both calendar and thermal times to heading. These interactions can be visualized as non-parallel lines in the interaction graphs presented in Fig. 1A and B. The differences between the *Eps-A^{m}1* alleles in both calendar and thermal times to heading were larger at 16 °C than at 23 °C. It is interesting to point out that when calendar time to heading was converted into thermal time, lines carrying the *Eps-A^{m}1-l* allele from cultivated accession DV92 showed almost no difference between temperatures (21 °Cd difference). On the contrary, lines carrying the *Eps-A^{m}1-e* allele from the wild accession G3116 showed a shorter thermal time to heading at 16 °C than at 23 °C (336 °Cd difference). The accelerated development (thermal

Table 3. *ANOVAs (three-way factorial with all interactions) for heading time, thermal time to heading, and spikelet number*

Allele: *Eps-A^{m}1-e* and *Eps-A^{m}1-l*, Temperatures 16 °C and 23 °C, Habit: dominant and recessive *Vrn-A^{m}2* alleles. Significant factors and interactions are indicated by bold letters.

Source	Heading time				
	DF	Type III SS	MS	*F*	*P*
Allele	1	58105.31	58105.31	897.01	**<0.0001**
Temp	1	14527.21	14527.21	224.27	**<0.0001**
Allele*Temp	1	5771.84	5771.84	89.10	**<0.0001**
Habit	1	77.71	77.71	1.20	0.2778
Allele*Habit	1	90.49	90.49	1.40	0.2419
Temp*Habit	1	12.69	12.69	0.20	0.6596
Allele*Temp*Habit	1	2.02	2.02	0.03	0.8605
Error	60	3886.58	64.78		
Corrected Total	67	87264.08			
Source	Thermal time to heading				
	DF	Type III SS	MS	*F*	*P*
Allele	1	19664185.07	19664185.07	873.02	**<0.0001**
Temp	1	506929.84	506929.84	22.51	**<0.0001**
Allele*Temp	1	407128.26	407128.26	18.08	**<0.0001**
Habit	1	25263.02	25263.02	1.12	0.2938
Allele*Habit	1	32617.06	32617.06	1.45	0.2336
Temp*Habit	1	1516.33	1516.33	0.07	0.7962
Allele*Temp*Habit	1	32.48	32.48	0.00	0.9698
Error	60	1351457.87	22524.30		
Corrected Total	67	23008975.47			
Source	Spikelet number				
	DF	Type III SS	MS	*F*	*P*
Allele	1	975.45	975.45	170.27	**<0.0001**
Temp	1	272.41	272.41	47.55	**<0.0001**
Allele*Temp	1	1.04	1.04	0.18	0.6711
Habit	1	2.97	2.97	0.52	0.4749
Allele*Habit	1	9.95	9.95	1.74	0.1932
Temp*Habit	1	0.89	0.89	0.15	0.6959
Allele*Temp*Habit	1	0.09	0.09	0.02	0.9002
Error	52	297.90	5.73		
Corrected Total	59	1930.06			

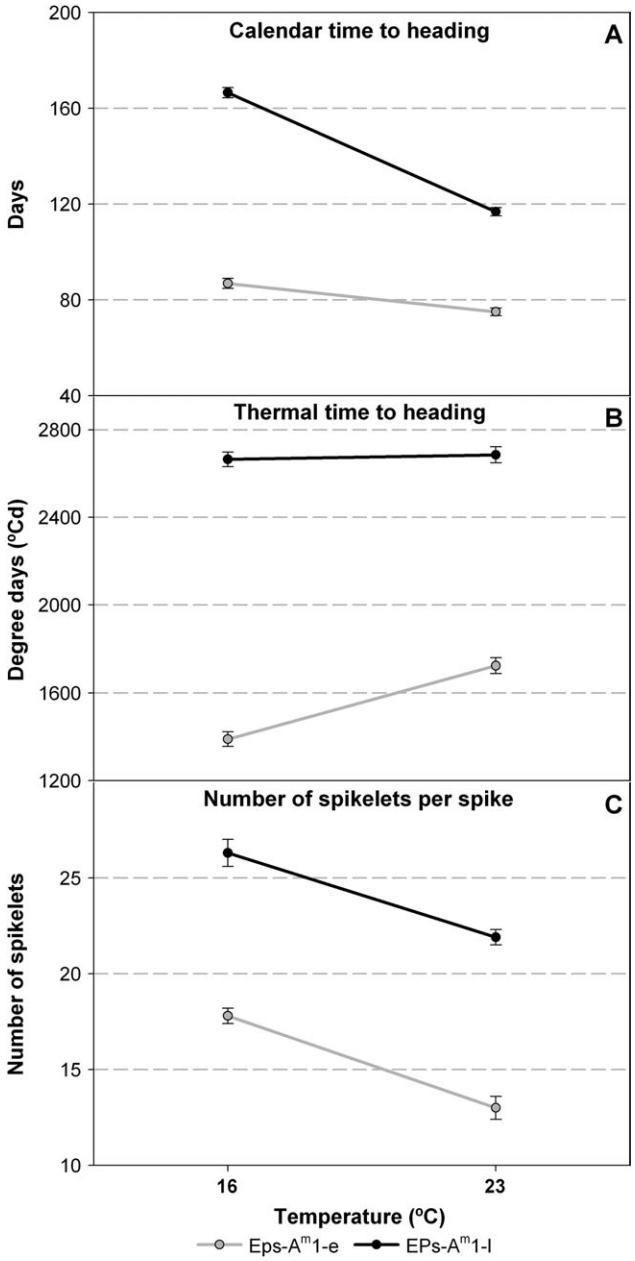

Fig. 1. Interaction graphs between temperature and *Eps-A^{m}1* alleles for time to heading measured as (A) calendar time and (B) thermal time, and for (C) spikelet number. Data points are averages of SSD lines carrying the different *Eps-A^{m}1* alleles ±1 standard error of the mean (including both spring and winter *Vrn-A^{m}2* alleles). Differences in the slopes of the lines within one graph indicate interaction (significant for A and B and non-significant for C; Table 3).

time to heading) of lines carrying the *Eps-A^{m}1-e* allele from G3116 (Fig. 1B) was associated with smaller differences between temperatures in calendar time to heading compared with the ones observed for the lines carrying the *Eps-A^{m}1-l* allele from DV92 (Fig. 1A).

In contrast to the significant interactions for heading time detected between temperature and *Eps-A^{m}1* alleles, non-significant interaction was detected between the same factors for spikelet number (P=0.67; Table 3; Fig. 1C). This indicates that the effect of the *Eps-A^{m}1* alleles on this parameter was similar at both temperatures (differences between alleles of 8.3 and 8.9 spikelets at 16 °C and 23 °C, respectively).

Experiment 2: effect of Eps-A^{m}1 *alleles on developmental phases*

To determine which developmental phases were affected by the *Eps-A^{m}1* alleles, the time from sowing to heading was divided into three phases: (i) from planting to the initiation of the double ridge (DR) stage, (ii) from DR to terminal spikelet (TS), and (iii) from TS to heading (H). The experiment included six NILs with the DV92 allele and six with the G3116 allele for *Eps-A^{m}1*. Lines carrying the *Eps-A^{m}1-l* allele initiated the transition between the vegetative and reproductive apices 35 d later (67% increase) than the isogenic lines carrying the *Eps-A^{m}1-e* allele (P <0.0001; Fig. 2A). In addition, the transition between DR to TS was significantly longer (P <0.0001) in the lines carrying the *Eps-A^{m}1-l* allele relative to those carrying the *Eps-A^{m}1-e* allele (Fig. 2B). Non-significant differences (P=0.79) were detected between alleles in the stem elongation period from TS to H (Fig. 2C). These results indicate that the *Eps-A^{m}1* region affects both the timing of the transition between the vegetative to reproductive stages and the duration of the transition between DR to TS.

As in experiment 1, significant differences in number of spikelets per spike were detected between *Eps-A^{m}1* alleles (P <0.0001; Fig. 2D). Lines carrying the *Eps-A^{m}1-l* allele showed, on average, 26.8 spikelets per spike, which is 6.5 spikelets per spike more (32% increase) than the isogenic lines carrying the *Eps-A^{m}1-e* allele (P <0.0001; Fig. 2D).

Since this analysis included four lines with recombination between *VatpC* and *Eps-A^{m}1* (Table 1), an additional analysis of variance for the *VatpC* instead of the *Eps-A^{m}1* alleles was performed. Non-significant differences in heading time (P=0.36), duration of individual developmental phases (P >0.25), or spikelet number (P=0.73) were detected between *VatpC* alleles, indicating that the gene(s) regulating these traits was (were) distal to *VatpC*.

Experiment 3: mapping the locus affecting spikelet number

To map the locus controlling the differences in spikelet number more precisely, and to determine its position relative to *Eps-A^{m}1*, an additional experiment was performed including NILs with close recombination events to the *Eps-A^{m}1* locus, three proximal and two distal to this locus (Table 1). Results from the different ANOVAs using loci as classification variables showed that the F values for number of spikelets per spike peaked at the *Eps-A^{m}1* locus, which also showed the most

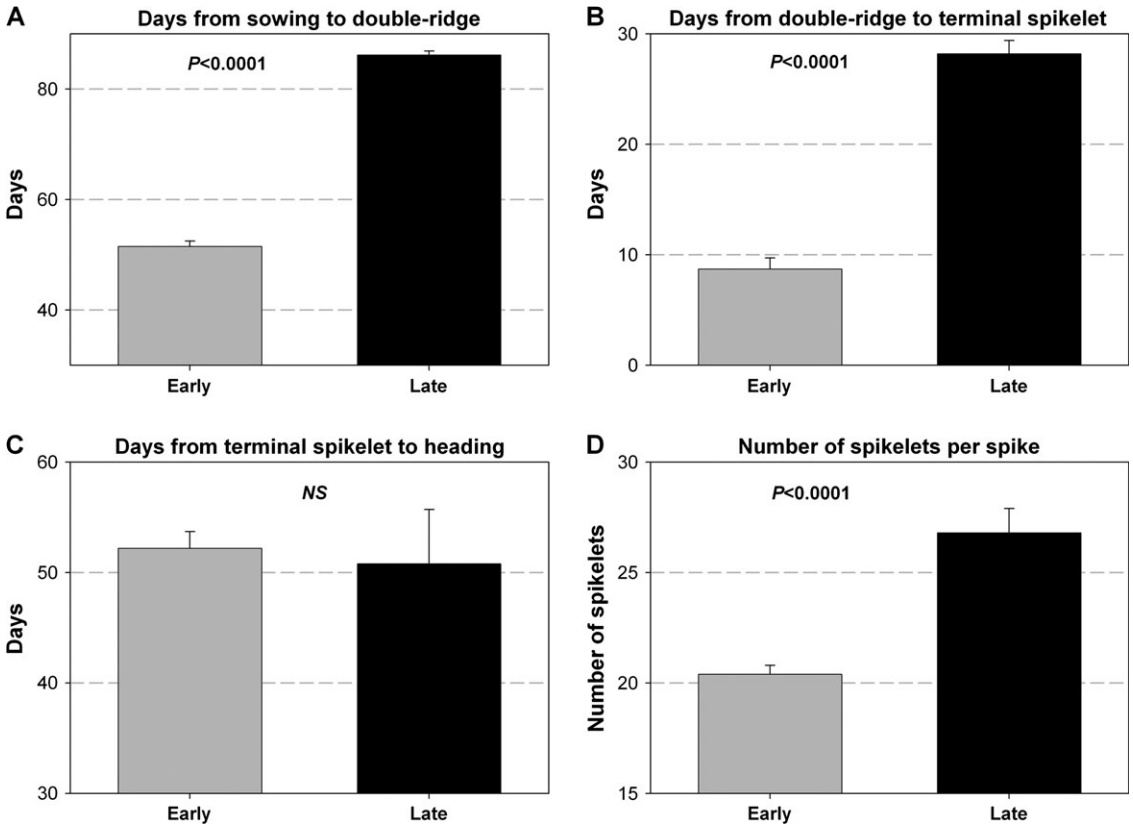

Fig. 2. Comparison between NILs used in experiment 2 (Table 1), carrying *Eps-A^{m}1-e* or *Eps-A^{m}1-l* alleles. (A) Days from sowing to double ridge stage. (B) Days from double ridge stage to terminal-spikelet. (C) Days from terminal spikelet to heading. (D) Number of spikelets per spike. Values for each allele are averages of six lines ±standard errors of the means. NS, not significant.

significant P values (P=0.003), the largest proportion of explained variance (R^2=0.73), and the largest difference in spikelet number (5.1 spikelets) between the *Eps-A^{m}1-l* (24.7 spikelets per spike) and *Eps-A^{m}1-e* (19.6 spikelets per spike) alleles (Fig. 3A). All these parameters decreased when the lines were classified using either proximal or distal markers (Table 4). As expected, differences in heading time also peaked at the *Eps-A^{m}1* locus (Table 4), confirming the previous determination of the *Eps-A^{m}1* alleles for these lines based on progeny tests (Table 2). In this experiment, lines carrying the *Eps-A^{m}1-l* allele headed 36.4 d later (33% increase) than lines carrying the *Eps-A^{m}1-e* allele. These results indicate that the locus controlling the differences in number of spikelets per spike is closely linked to the *Eps-A^{m}1* locus regulating heading time.

Experiment 4: effect of Eps-A^{m}1 *alleles on spikelet number in the field*

Highly significant differences in the number of spikelets per spike were detected between NILs carrying early and late alleles at the *Eps-A^{m}1* locus in experiments 4A (P=0.003, Table 5, Fig. 3B) and 4B (P <0.0001; Table 5; Fig. 3C). This locus alone explained 74% of the variation in the number of spikelets per spike in experiment 4A and 90% in experiment 4B. Although the differences in spikelet number between *Eps-A^{m}1* alleles in the field experiment 4A (2.7 spikelets) were smaller than those observed in the growth-chambers (more than 5.0 spikelets), the differences in the field experiment 4B (5.6 spikelets) were slightly larger than the differences observed in the growth-chamber experiment 3 (Table 4).

For both field experiments, separate ANOVAs using loci proximal and distal to *Eps-A^{m}1* as classification variables (Table 5) showed smaller F values, significance levels, per cent of explained variation, and differences between *Eps-A^{m}1* alleles than the ANOVA using *Eps-A^{m}1* as the classification variable. These results confirm that the locus controlling the differences in number of spikelets per spike is closely linked to the *Eps-A^{m}1* locus.

Experiment 5: effect of homoeologous group 1 chromosomes on spikelet number in hexaploid wheat

Highly significant differences in the number of spikelets per spike were found among lines in both experiment 5A, including only the nullisomic-tetrasomic lines (P <0.0001, R^2=0.39) and in experiment 5B including ditelosomic and deletion lines for chromosome 1A (P=0.0007, R^2=0.78).

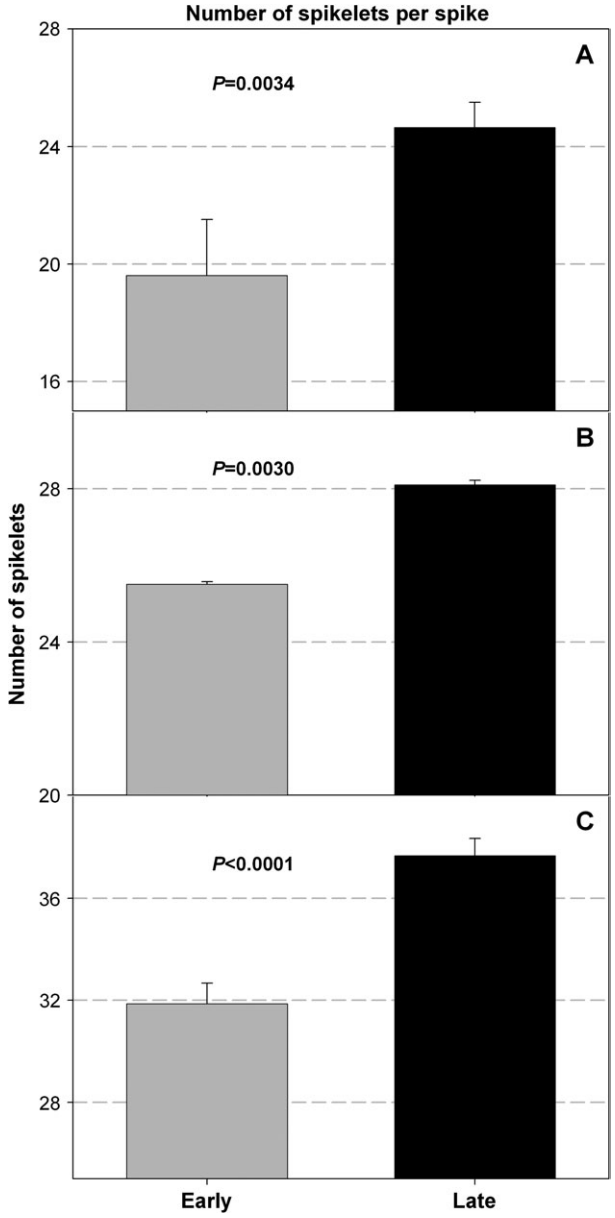

Fig. 3. Differences in the number of spikelets per spike between NILs carrying the *Eps-A^{m}1-l* or *Eps-A^{m}1-e* alleles. Results from (A) experiment 3, (B) experiment 4A, (C) experiment 4B. Values for each allele are averages ±standard errors of the means.

Lines N1AT1B and N1AT1D lacking chromosome 1A and line N1DT1B lacking chromosome 1D had a significantly smaller number of spikelets per spike than CS (P <0.01; Table 6). The reduction was larger in the lines lacking chromosome 1A (22% on average) than in the one lacking chromosome 1D (18%). Interestingly, none of the nullisomic-tetrasomic lines lacking chromosome 1B (N1BT1A and N1BT1D) showed significant differences in the number of spikelets per spike with CS (Table 6).

To determine the intra-chromosomal location of the locus affecting number of spikelets per spike on chromosome 1A, a second experiment was performed (experiment 5B) including ditelosomic and deletion lines of chromosome 1A. As in the previous experiment (5A), line N1AT1B showed a significant reduction (26.7%) in number of spikelets per spike relative to CS (P <0.01). Both Dt1AS and deletion line 1AL1 also showed a significant reduction in this trait when compared with CS (P <0.01). The result observed for the ditelosomic line indicates that the locus controlling number of spikelets per spike is located on the long arm of chromosome 1A. This result was confirmed by the deletion line 1AL1, which is missing the distal 83% of the 1AL arm, and which was used to map this locus to the distal 83% region of this chromosome arm (Table 6).

Discussion

Effect of the Eps-A^{m}1 *region on spikelet number in* T. monococcum

The same light conditions identified in a previous study that maximized the differences in heading time between the *Eps-A^{m}1* alleles (fluorescent light at low intensity; Bullrich *et al*., 2002) were used in the growth-chamber experiments presented here to study the effect of these alleles on both the duration of the developmental phases and the number of spikelets per spike. Since the light intensity in the growth-chambers (45 and 90 μE m^{-2} s^{-1}) was significantly lower than the one observed in the field (800 and 1400 μE m^{-2} s^{-1}), two different field experiments were performed to validate the differences observed

Table 4. *Summary of statistical analyses for experiment 3 (growth-chamber, 16 °C, long-day photoperiod)*

Different loci were analysed in separate ANOVAs, with the two *Eps-A^{m}1* alleles as levels and nine lines as replications. Genotypes are described in both the Materials and methods and in Table 1.

Locus used as classification var.	Spikelets per spike				Heading time			
	F	*P*	R^2	Dif. alleles	*F*	*P*	R^2	Dif. alleles
wg241/VatpC	6.64	0.037	0.49	3.9	1.61	0.245	0.19	16.8
Eps-A^{m}1	**18.79**	**0.003**	**0.73**	**5.1**	**26.51**	**0.001**	**0.79**	**36.4**
Adk1	2.84	0.136	0.29	3.0	25.6	0.002	0.78	34.4
Smp	0.82	0.397	0.10	1.8	3.01	0.126	0.30	21.3

Table 5. *Summary of statistical analyses for field experiments 4A and 4B*

Different loci were analysed in separate ANOVAs. Genotypes are described in both the Materials and methods and in Table 1.

Locus used as classification var.	Experiment 4A (Tulelake, CA, USA)				Experiment 4B (Davis, CA, USA)			
	F	*P*	R^2	Dif. alleles	*F*	*P*	R^2	Dif. alleles
wg241/VatpC	2.44	0.1625	0.26	1.6	9.95	0.0044	0.31	3.3
Eps-A^{m}1	**19.65**	**0.0030**	**0.74**	**2.7**	**194.45**	**<0.0001**	**0.90**	**5.6**
Adk1	8.56	0.0222	0.55	2.3	21.28	0.0001	0.49	4.1
Smp	2.29	0.1739	0.25	1.5	9.08	0.0062	0.29	3.3

Table 6. *Number of spikelets per spike and heading time of nullisomic-tetrasomic, ditelosomic, and deletion CS lines evaluated at Buenos Aires, Argentina, and at Davis, CA, USA*

Experiment 5A[a] (Buenos Aires, Argentina)			Experiment 5B[a] (Davis, CA, USA)		
Line	Spikelets per spike	No. plants	Line	Spikelets per spike	No. plants
CS	17.8±0.7	9	CS	24.3±1.3	3
N1BT1A	17.6±0.8 NS	9	N1AT1B	17.8±0.9**	4
N1BT1D	16.6±0.8 NS	10	Dt1AS	14.8±1.7**	3
N1DT1B	14.5±0.6**	16	Del. 1AL1	16.6±1.3**	3
N1AT1B	13.8±0.5**	9			
N1AT1D	14.0±0.4**	10			

[a] P <0.01. Significantly different from control line CS by Dunnett's test. Average values are followed by standard errors of the means.

in the growth-chambers. Although the differences in spikelet number between the *Eps-A^{m}1* alleles were generally larger in the growth-chamber (5.0–8.7 spikelets) than in the field experiments (2.7–5.6 spikelets), the differences were significant under both conditions, confirming the relevance of the allelic differences in this gene under natural light conditions. An ANOVA combining the spikelet number results from experiments 1–4 (considering the two temperatures of experiment 1 as two separate experiments, and using experiment as a block), showed highly significant differences between the *Eps-A^{m}1* alleles (P=0.0013).

It is interesting to point out that the total number of spikelets per spike (average combining both *Eps-A^{m}1* alleles) observed in the growth-chamber experiment 2 at low light intensity (23.6 spikelets) was not very different from the results observed in the field experiment 4A at high light intensity (26.9 spikelets). Larger differences in average spikelet number were observed between the previous two experiments and the field experiment 4B (34.9 spikelets), suggesting that environmental factors other than light intensity play a more significant role in the determination of the final number of spikelets per spike.

The differences in the number of spikelets per spike between *Eps-A^{m}1* alleles were correlated with the differences in heading time across and within experiments. Experiment 1 showed the largest difference in both heading time (61 d) and number of spikelets per spike (8.7 spikelets). Experiment 2 showed a smaller difference in both heading time (52.8 d) and number of spikelets per spike (6.5 spikelets). The same trend was observed in experiment 3, which showed differences between *Eps-A^{m}1* alleles of 36.4 d in heading time and 5.1 spikelets per spike. However, the field experiment carried out at Davis (experiment 4B) showed a small significant difference in heading time between *Eps-A^{m}1* alleles (3.5 d, P=0.0004) but a relatively large difference in number of spikelets per spike (5.6 spikelets). The field experiment conducted at Tulelake (experiment 4A) showed the smallest difference in number of spikelets per spike (2.7 spikelets), but unfortunately heading time was not recorded for this experiment.

The correlation between average heading time and number of spikelets per spike across the different *Eps-A^{m}1* alleles for the experiments for which both parameters were available is very high (R=0.91, P=0.002, n=8). Correlations between these two parameters are also highly significant within each experiment (R=0.87, P <0.0001 in experiment 1; R=0.96, P <0.0001 in experiment 2; R=0.77, P=0.006 in experiment 3; R=0.55, P=0.003 in experiment 4B). These results indicate that these two parameters are highly correlated, and that the variation in the differences in spikelet number between *Eps-A^{m}1* alleles across experiments are probably associated with the differences in heading time in each particular experiment (e.g. different times of the year, pre-planting conditions, differences in light intensity, etc).

Different levels of correlation between heading time and spikelet number have been found in other earliness *per se* studies (performed with fully vernalized plants grown under long-day photoperiod). A significant positive

correlation between these two parameters (R=0.62, P=0.0001) was found for the 33 wheat varieties analysed by Hoogendoorn (1985*b*). However, when Hoogendoorn (1985*b*) divided these varieties into groups with similar vernalization and photoperiod requirements, the correlations were smaller and non-significant.

Two studies using reciprocal F_1 monosomic lines derived from crosses between early and late hexaploid wheat varieties found an overall positive correlation between earliness *per se* and spikelet number (Hoogendoorn, 1985*a*; Millet, 1987). It is interesting to point out that even though most of the reciprocal F_1 monosomic lines followed the overall positive correlation between spikelet number and earliness *per se* (e.g. 4A, 4B, and 6B), two of them (3A and 4D) combined late ear emergence with reduction in number of spikelets per spike (Hoogendoorn, 1985*a*). These results confirm that different *Eps* genes may affect these parameters in different ways.

Interaction between temperature and Eps-A^{m}1 *alleles for growth rate and spikelet number*

Results from experiment 1 show how environmental differences, such as temperature, can modulate the effects of different *Eps-A^{m}1* alleles on both heading time and number of spikelets per spike. Plants grown at 16 °C showed, on average, 4.6 more spikelets per spike than plants grown at 23 °C. The differences in spikelet number between *Eps-A^{m}1* alleles at both temperatures were very similar, resulting in a non-significant interaction between temperature and *Eps-A^{m}1* alleles for this trait. Based on these results it is concluded that the effects of both genotype and temperature on spikelet number were mainly additive.

By contrast, a highly significant interaction between *Eps-A^{m}1* alleles and temperature was observed for heading time. Plants grown at 16 °C showed larger differences between alleles than those grown at 23 °C. A similar interaction has been reported before (Bullrich *et al*., 2002; Appendino and Slafer, 2003) indicating that the effect of the *Eps-A^{m}1* alleles on heading time is modulated by temperature. These results are in agreement with previous studies from Pirasteh and Welsh (1980) and Slafer and Rawson (1995), who found significant interactions between genotypes and temperatures for heading time in vernalized common wheat plants grown under a long-day photoperiod. Slafer and Rawson (1995) concluded that earliness *per se* is not an intrinsic value of a genotype, but the result of the interaction between genotype and temperature, with genotypes flowering either earlier or later at different temperatures.

Both Pirasteh and Welsh (1980) and Slafer and Rawson (1995) found that the interactions between genotype and temperature were still significant when heading time was expressed as thermal time. These observations are somehow unexpected, since thermal time is expected to correct for the effect of different temperatures on growth. A possible interpretation of these results is that genotypes differ in their ability to adjust developmental thermal time to temperature. It is interesting to point out that in both studies the differences between high and low temperatures in thermal time to heading were inversely correlated with the same differences expressed in calendar time across genotypes. Using published data from Slafer and Rawson (1995) and Pirasteh and Welsh (1980), the negative correlations were R= –0.74 and R= –0.73, respectively.

Similarly, in the present study thermal time to heading was 336 °Cd shorter at 16 °C than at 23 °C for the lines carrying the *Eps-A^{m}1-e* allele, but only 21 °Cd shorter for the lines carrying the *Eps-A^{m}1-l* allele. This was reflected in a significant interaction between *Eps-A^{m}1* alleles and temperature for thermal time to heading (Fig. 1B). As in the two studies on common wheat described above, the larger differences between high and low temperatures in thermal time to heading for the lines carrying the *Eps-A^{m}1-e* allele were associated with smaller differences between the same temperatures when time to heading was expressed in calendar time (*Eps-A^{m}1-e* allele 12 d difference versus *Eps-A^{m}1-l* allele 50 d difference; Fig. 1A, B). These results suggest that the *Eps-A^{m}1-e* allele from the wild accession has a better ability to 'adjust' thermal time to heading at different temperatures than the *Eps-A^{m}1-l* allele from the cultivated accession. This negative correlation between the differences in thermal and calendar times to heading at different temperatures suggests that the ability to adjust thermal time to heading can buffer the variation in calendar time to heading. It is tempting to speculate that this ability to adjust their developmental rates may help plants to target narrow windows for optimum flowering time, limiting the negative impact that annual fluctuations in temperature may have on flowering time in calendar days. Relaxation of selection pressures under cultivation may have facilitated the loss of this ability in *T. monococcum* accession DV92.

Effect of the Eps-A^{m}1 *region on the duration of developmental phases in* T. monococcum

To understand better the effect of the *Eps-A^{m}1* alleles on heading time and spike development, their effect on different developmental phases was characterized. These analyses showed that the differences in time to heading between *Eps-A^{m}1* alleles were determined mainly by differences in the vegetative and early reproductive phases. Non-significant differences were observed in the later stem elongation phase (experiment 2). The significant effect of the *Eps-A^{m}1* locus on the timing of the transition between the vegetative and reproductive apices and the duration of the transition from the double ridge stage to terminal spikelet was confirmed in an independent replication of experiment 2 (data not shown).

Since this experiment was performed at a single temperature, it is not possible to determine which of the

different phases is responsible for the significant interactions between temperature and *Eps-A^{m}1* alleles for heading time observed in experiment 1. However, since experiment 1 showed no interaction between temperature and *Eps-A^{m}1* alleles for number of spikelets per spike, it is more likely that this interaction is the result of differences in the duration of the vegetative phase rather than differences in the duration of the spike development phase. This speculation is based on the assumption that number of spikelets per spike is more dependent on the differences in the duration of spike development than on the differences in the duration of the vegetative phase. An experiment studying the effect of the duration of both the vegetative and early reproductive phases at different temperatures will be necessary to test this hypothesis.

Halloran and Pennell (1982) studied the duration of different developmental phases in six wheat cultivars and two environments, and concluded that their duration within each cultivar appeared to be independent of each other. In addition, Slafer and Rawson (1995) showed that individual developmental phases between sowing and anthesis are differentially sensitive to temperature among genotypes. Their studies, using four wheat varieties and six different temperatures (10–25 °C) showed that the relative contribution of each phase to earliness and their individual responses to temperature varied among genotypes. This variability can be interpreted as the result of allelic differences in different subsets of *Eps* genes present in different varieties. Consequently, a better understanding of wheat diversity in earliness *per se* will require a more precise characterization of the effects of individual *Eps* genes on individual developmental phases. Particularly important will be the characterization of those *Eps* genes that affect the duration of spike development because of its importance in the determination of the number of spikelets per spike.

Mapping of the locus controlling spikelet number in T. monococcum

The cloning of the gene controlling the differences in number of spikelets per spike can provide an important entry point to study the gene network involved in the regulation of spike development. The first step to complete this long-term objective is to construct very precise high-density maps of the target trait. This was achieved in this study using BC_6F_2 NILs, which reduced the genetic variance across lines. DV92 is a cultivated accession of *T. monococcum* ssp. *monococcum*, whereas G3116 is a wild accession of *T. monococcum* ssp. *aegilopoides*. Therefore, the SSD population showed segregation in a wide range of traits, complicating the mapping of the target traits. The introgression of the *Eps-A^{m}1-e* allele into the cultivated parent by six backcross generations eliminated most of the variation in genes outside the region flanking the *Eps-A^{m}1* locus, resulting in morphologically homogeneous NILs.

Using selected NILs with recombination between *Eps-A^{m}1* and the closest proximal (*wg241* and *VatpC*) and distal markers (*Adk1*, *Smp*, and *barc287*), the locus controlling number of spikelets per spike was mapped completely linked to the *Eps-A^{m}1* locus controlling heading time. These results indicate that the differences in both heading time and number of spikelets per spike are either different pleiotropic effects of a single gene or the effect of multiple genes tightly linked within the 0.8 cM region delimited by *VatpC* and *Adk1*.

The following argument favours the 'single gene' hypothesis. Results from experiment 2 have shown that part of the differences in heading time between *Eps-A^{m}1* alleles is due to differences in the duration of the spike development phase. Since the duration of this phase is critical for the determination of the number of spikelets per spike (Rahman and Wilson, 1977), it is logical to assume that a gene affecting the duration of this phase may have pleiotropic effects on the number of spikelets per spike. However, a final validation of either the 'single' or the 'multiple' gene(s) hypotheses will require the cloning of the *Eps-A^{m}1* gene.

Differences in spikelet number in hexaploid wheat

Several wheat chromosomes have been shown to affect number of spikelets per spike. Millet (1987) identified a recessive major gene on chromosome 2D affecting both heading time and spikelet number. F_1 monosomic lines carrying the allele from the late parental line and their derived F_2 progeny headed significantly later and had more spikelets than the euploid line. The same author showed that chromosome 6B also has a minor effect on heading time and spikelet number (Millet, 1987). In a different cross, Hoogendoorn (1985*a*) confirmed that chromosomes 3A, 4B, 4D, and 6B have significant effects on earliness *per se* and spikelet number.

This study showed that, in common wheat, chromosomes of homoeologous group 1 also affect the number of spikelets per spike. Nullisomic-tetrasomic CS lines lacking chromosomes 1A or 1D showed a significantly smaller number of spikelets per spike than the euploid CS, whereas no differences were detected for the nullisomic 1B lines (N1B). To interpret these results correctly, it is important to take into consideration that the nullisomic lines have a compensatory tetrasomic dose of one of the other two homoeologues. The non-significant reduction in spikelet number in the N1B lines shown in this study was interpreted as evidence of a null or reduced effect of the 1B homoeo-alelle. The N1DT1B showed a significant reduction of 3.3 spikelets per spike with respect to CS, but this reduction was smaller than the one observed in the N1AT1D (3.8 spikelets) and N1AT1B (4 spikelets). The

lower reduction in the N1AT1D relative to the N1AT1B may be caused by the compensatory effect of the tetrasomic 1D chromosome present in the N1AT1D line.

Based on the previous results, it is possible to rank the locus present on chromosome 1A as the one with the strongest effect on spikelet number, and the one present on chromosome 1B as the weakest one. These results parallel the effect of these chromosomes on heading time. Law *et al*. (1998) reported the presence of earliness *per se* genes causing a delay in ear emergence in each of the homoeologous group 1 chromosomes. According to these authors, the genes on chromosome 1A had the major effect on delaying days to ear emergence (1.94 d, $P < 0.001$), whereas the genes on chromosome 1B had the smallest one (0.09 d, $P < 0.001$).

The A genome of both common and pasta wheat species was contributed by the diploid species *T. urartu* Thum. (Nishikawa, 1983; Dvorak *et al*., 1988, 1993; Tsunewaki *et al*., 1991). Although this wild species is closely related to *T. monococcum,* their hybrids are sterile (Johnson and Dhaliwal, 1976). As a consequence, the effect of *T. monococcum* domestication on the A^m genome has been independent from the effect of domestication of polyploid wheat species on the A genome, creating multiple opportunities for selection of different favourable alleles in these species.

For example, it is possible that the allele for increased spikelet number present in the distal region of *T. monococcum* chromosome arm $1A^mL$ has a larger effect than the homoeologous alleles currently present in the A genome of both bread and pasta wheat species. To test this hypothesis the *T. monococcum Eps-A^m1* region is being introgressed into hexaploid wheat by backcrossing and marker-assisted selection. Since cultivated *T. monococcum* has been selected for increased productivity over thousands of years, it is not surprising that the cultivated parent DV92 carries the allele for higher number of spikelets per spike.

Conclusion and relevance of the study

In most environments, there is a narrow window for optimum flowering time that will maximize wheat grain yield potential. The *Eps* genes have an indirect effect on yield potential by fine-tuning wheat flowering time to this narrow window. In addition to this indirect effect, the results presented here show that some *Eps* genes may have a more direct impact on yield by affecting basic developmental processes and individual grain yield components.

The longer duration of the spike development phase and the increase in spikelet number associated with the presence of the *Eps-A^m1-l* allele from cultivated diploid wheat relative to the allele from the wild accession may have contributed to the high productivity of the cultivated *T. monococcum* accession. It is possible that the higher number of spikelets per spike associated with the *Eps-A^m1-l* allele found in the cultivated diploid wheat accession was originated as a result of selection pressures during thousands of years of domestication and cultivation.

Number of spikelets per spike is an important component of grain yield potential since it is associated with a higher number of grains per spike. However, the relationship between the increase in spikelet number and grain yield is not simple because of the ability of the wheat plant to adjust different yield components to available resources (for a recent discussion see Sinclair and Jamieson, 2008 and Fischer, 2008). In spite of these limitations, a better understanding of the genetic factors controlling the developmental phases affecting grain yield will be useful for a more intelligent manipulation of wheat yield components in wheat improvement.

Acknowledgements

This project was supported by the National Research Initiative of the USDA Co-operative State Research, Education and Extension Service, grant number 2003-00703 and proposal number 2008-02508, and by the Argentinean grant INTA BID 1728/OC-AR PICT 08-13442.

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