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Effect of added barley chromosomes on the flowering time of new wheat/winter barley addition lines in various environments

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Abstract The heading characters and morphological traits of two partial sets of wheat–barley disomic addition lines, namely Mv9kr1/Igri and Asakaze/Manas, were evaluated under controlled environmental conditions in a phytotron under long-day, short-day and non-vernalised conditions and in field-sown experiments. The winter barley chromosome additions significantly influenced the flowering time of wheat both in the controlled environment test and under field-sown conditions. Of all the barley addition lines, the effect of the 4H and 7H additions was the most characteristic. The 7H addition lines were the earliest in both cultivar combinations in each treatment. In the Mv9kr1/Igri combination the 4H addition was the latest under all the environmental conditions. In the Asakaze/Manas combination 4H addition was the latest under short-day and long-day illumination in the phytotron but the 6H addition was the latest without vernalisation and in the field in 2012. There was 12 and 11 days difference between the flowering times of the 7H and 4H Mv9kr1/Igri and Asakaze/Manas addition lines in the field in 2012, which increased to 52 and 44 days under short-day illumination in the phytotron. In the winter wheat background, the addition of 2H carrying the photoperiod sensitivity gene *Ppd-H1* decreased the flowering time under the short photoperiod regime, but had a very strong delaying effect under field-grown conditions. Considering the yield components under field conditions, 4H was the most fertile of the addition lines, while 7H showed the highest tillering capacity, and Igri 3H had good tillering capacity and the highest number of seeds per plant.

Keywords Wheat–barley addition lines · Earliness · Flowering time · Heading characters · Morphological parameters

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Introduction

The control of flowering plays a central role in the reproductive success of plants, and has a major impact on grain yield in crop species. To maximize yield under different agro-ecological conditions, cereals have to adjust their life cycle and optimize the flowering time. The transition from vegetative to reproductive growth is a critical developmental switch and a key adaptive trait that ensures that crop plants set their flowers at the optimum time for pollination and seed development. In major cereals such as bread wheat and barley the initiation of flowering is determined both by external signals such as cold temperatures (vernalisation requirement) and long days (photoperiod sensitivity), and internal signals such as

intrinsic earliness (earliness per se) (Snape et al. 2001). The major genes for both vernalisation response and photoperiod sensitivity have been identified, and their effects on the determination of heading have been characterised in detail both in wheat and barley (Distelfeld et al. 2009). In spite of the strong similarities between the two species for these features, there are also definite differences. Due to the hexaploid versus diploid natures of wheat and barley, respectively, the gene copy number is probably one of the major factors leading to differences in the genetic control of plant development (Distelfeld et al. 2009; Greenup et al. 2009). In addition however, differences in the gene sequence bases of functional polymorphisms, and differences in the presence/absence of genes with significant effects on flowering are also important factors (Cockram et al. 2007). The flowering and ripening of barley usually occurs several days or almost a week earlier than that of wheat under field conditions, but the genetic mechanism of this phenomenon has not yet been exactly dissected. Almost all the barley chromosomes carry major genes for plant development. Of the vernalisation response (*Vrn*) genes, *Vrn-H1* is located on chromosome 5H, *Vrn-H2* on 4H, and *Vrn-H3* on 7H, while the two major photoperiod response genes, *Ppd-H1* and *Ppd-H2*, are located on chromosomes 2H and 1H, respectively (Cockram et al. 2007).

Parallel to the theoretical aspects of this phenomenon, earliness is also a significant trait in wheat breeding, as a hot, dry period is experienced in early summer in several wheat growing areas. If the grain-filling period is not completed before the start of the extremely dry season, there is not enough time for normal seed development. The production of wheat–barley hybrids thus represents a specific tool both for studying genetic differences in the determination of heading time between the two species and for efforts to transfer the earliness of barley into wheat.

Wheat–barley disomic addition lines were first produced by Islam et al. (1978) from the hybrids of spring wheat (*Triticum aestivum* L.) cultivar Chinese spring and spring barley (*Hordeum vulgare* L.) cultivar Betzes. The effect of the added barley chromosomes on heading characters was studied by Murai et al. (1997) using these addition lines together with the 5H and 6H Shinchunaga/New Golden additions produced by Koba et al. (1997). These addition lines, however, were produced partly with model genotypes of both species and partly in a spring growth habit background, which narrows both the resolving power of the genetic studies and the practical application of the lines in breeding. To overcome these shortcomings new wheat–barley hybrids were produced in Martonvásár by crossing a facultative and a winter wheat cultivar with agronomically adaptable winter barley cultivars (Molnár-Láng et al. 2000). Two series of wheat/barley disomic addition lines were developed from the hybrids, one with Igri (German two-rowed) and one with Manas (Ukrainian six-rowed) winter barley cultivars (Szakács and Molnár-Láng 2007, 2010; Molnár-Láng et al. 2012). The new wheat/barley addition lines make it possible to study the effects of chromosomes from winter barley cultivars in the wheat genetic background under various environmental conditions.

The aim of the present work was to evaluate the effects of added barley chromosomes on heading characters and morphological parameters, comparing these characteristics under controlled environmental conditions and in field-sown experiments. The flowering times of the various wheat/barley addition lines were evaluated using long (16 h) and short (12 h) day length, and non-vernalised conditions. A further goal was to differentiate the wheat and barley genotypes in terms of their sensitivity to vernalisation and day length, and to evaluate their intrinsic earliness-related traits.

Materials and methods

Plant material

Two partial sets of wheat (*Triticum aestivum* L.) -barley (*H. vulgare* L.) disomic addition lines along with their parental lines were used to determine heading characters. The parental wheat genotypes were the line Martonvásári 9 kr1 (Mv9kr1), which carries the recessive crossability allele kr1 and has winter growth habit (Molnár-Láng et al. 1996), and Asakaze komugi (Asakaze), a Japanese facultative wheat cultivar. Both wheat parental genotypes carry the recessive *vrn-A1*, *vrn-B1* and *vrn-D1* alleles and the photoperiod-insensitive (dominant) allele at the *Ppd-D1* locus. Heading characters were studied for the 2H, 3H, 4H, 6HS and 7H disomic addition lines of Mv9kr1/Igri and the 2H, 4H, 6H and 7H lines of

Asakaze/Manas. The allele types of the major genes determining the flowering time (*Vrn1* series, *Ppd-D1*) were checked in all the experimental plants using gene allele-specific molecular markers (Yan et al. 2004a; Fu et al. 2005; Beales et al. 2007). The barley parental genotypes Igri (German 2-row winter barley cultivar) and Manas (Ukrainian 6-row barley cultivar) have recessive *vrn-H1* (Igri: length of intron 1 insertion is 5200 bp, Manas: intron 1 insertion is 5300 bp), dominant *VRN-H2*, dominant *Ppd-H1* and recessive *ppd-H2* alleles (von Zitzewitz et al. 2005; Karsai et al. 2005; Turner et al. 2005; Faure et al. 2007).

Genomic in situ hybridization (GISH)

The presence of the added barley chromosomes was detected by GISH in the disomic wheat/barley addition lines (Fig. 1). The seeds were germinated and root tips were fixed for chromosome preparations as described earlier (Molnár-Láng et al. 2000). Total barley genomic DNA was used as a probe and unlabelled wheat genomic DNA was used as blocking DNA. Labelling, in situ hybridization and detection were carried out as reported by Molnár-Láng et al. (2012). The slides were screened using a Zeiss Axio Imager M2 (Zeiss) fluorescence microscope with the appropiate filter sets. Images were captured with a Zeiss AxioCam MRm CCD camera and processed with Zeiss Axiovision 4.8.2. software.

Growing conditions

Phytotron

The plants were grown in phytotron chambers (Conviron PGV96) in Martonvásár. Germinated seeds were planted in Jiffy peat pellets. In the short-day (12 h) and long-day (16 h) treatments young seedlings were subjected to vernalisation for six weeks at 4 °C, a light intensity of 12 µmol m^{-2} s^{-1} and a day/night period of 10/14 h. Vernalised and unvernalised plants were grown in 2 L pots filled with a 2:1:1 mixture of garden soil, humus and sand. The plants were grown until tillering under an initial day/night temperature of 15/10 °C, a light intensity of 200 µmol m^{-2} s^{-1} (at pot level) and 75 % relative humidity (Tischner et al. 1997). After tillering, both the day and night temperatures were increased by 2 °C.

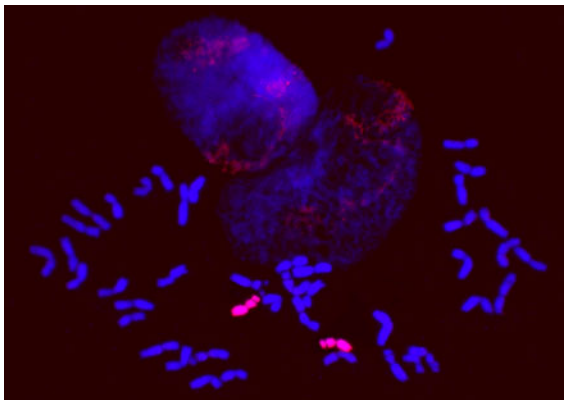

Fig. 1 Detection of the added barley 6H chromosomes on mitotic chromosomes of an Asakaze/Manas disomic addition line. Barley chromosomes were detected with GISH using total barley genomic DNA as a probe (labelled with digoxigenin-11-dUTP and detected with anti-digoxigenin-Rhodamin; red). Wheat chromosomes are blue as a result of counterstaining with DAPI

The plants were grown under three climatic programmes: short day (SD, 12 h of light/12 h of dark) and long day (LD, 16 h of light/8 h of dark) illumination, and a programme without vernalisation (NV) and a long photoperiod (16 h of light/8 h of dark). Five plants of each genotype were grown in each treatment. The heading date (DEV59 on the scale of Tottman 1987) and the flowering time (DEV61) were recorded and defined as the number of days elapsing from planting to ear emergence and to the beginning of anthesis (when the first stamen emerged from the flowers), respectively. The photoperiod sensitivity of the genotypes was determined as the difference between the flowering times (DEV61) measured in the 12 and 16 h photoperiod regimes, while the vernalisation requirement was characterised by the difference in flowering time measured with and without vernalisation in the 16 h photoperiod regime.

Field trials

The addition lines and parents were grown in chernozem soil in the field in Martonvásár in the 2011–2012 season. Each genotype was sown in 10 × 1 m rows with a row distance of 15 cm and 10 seeds per row on 15th October 2011. Ten plants were randomly selected from the ten rows. The developmental phase when the ear is in the last leaf sheath and the awns just visible (DEV49, Tottman 1987), the heading date (DEV59) and the flowering time (DEV61) were recorded in

2012. Plant height and tillering were determined immediately before harvest, and the length of the main spike, spikelets/main spike, seeds/main spike and seeds/plant were determined after harvest.

Statistical analysis

The statistical analyses were carried out using the Excel for Windows programs. Student's t test for paired data was applied at the $P = 0.05$ and $P = 0.01$ significance levels.

The contribution of the wheat genetic background and the added barley chromosomes to the heading characters was analysed using two-factorial ANOVA without replications, with the wheat genetic background and the added barley chromosome as the two factors. The morphological traits were analysed with one-way ANOVA.

Results

Genetic stability of the addition lines

When the genetic stability of each line was studied using GISH, 100 % of the seeds analysed were found to contain the added barley chromosome pairs in the Mv9kr1/Igri 2H, 4H and 7H, and in the Asakaze/Manas 4H, 6H and 7H additions. A low level of instability was observed in the Mv9kr1/Igri 3H and 6HS and the Asakaze/Manas 2H additions, as GISH showed that one of the 16 seeds analysed (6.75 %) did not contain the two added barley chromosomes. Only plants which carried the two barley chromosomes were included in the experiment (Fig. 1).

Effect of added barley chromosomes on heading characters

The effects of the added barley chromosomes on the heading characters were examined in two partial sets of wheat–barley chromosome addition lines under different day length and vernalisation conditions in the phytotron. Generally, the time required for flowering was the shortest under long-day conditions when the vernalisation requirement was fulfilled, while unfavourable growing conditions, i.e. short-day length and the lack of vernalisation, increased the developmental periods of all the genotypes examined (Table 1). The same responses were observed for the heading date (DEV59) and flowering time (DEV61).

Under long-day conditions the 7H Mv9 kr1/Igri addition line flowered significantly earlier than the Mv9kr1 wheat. In both combinations the 7H addition lines flowered significantly earlier than the average of the addition lines. Significantly later heading was observed for the 4H additions compared to the wheat parents. The flowering of Mv9kr1/Igri addition line 2H and the ditelosomic line 6HS was earlier than the average of the addition lines. The 2H, 4H and 6H Asakaze/Manas additions flowered significantly later than the wheat parent.

Under a short photoperiod most of the added barley chromosomes had the same effect on the flowering time relative to the average as under a long photoperiod, as chromosome 4H increased the number of days to flowering while 7H decreased it, irrespective of the crossing combination. The 2H addition showed a specific effect depending on the wheat genetic background, being earlier in the Mv9kr1/Igri combination and later in the Asakaze/Manas combination. In comparison with the wheat parental genotype, the Mv9kr1/Igri 2H and 7H lines were significantly earlier than Mv9kr1. Under short-day conditions barley cv. Manas flowered more than twenty days later than Asakaze komugi, so all the Asakaze/Manas addition lines flowered significantly later than the parental wheat Asakaze komugi. 7H was the earliest of all the Asakaze/Manas addition lines.

The NV treatment (long photoperiod, no vernalisation) showed a combination-specific effect on the flowering and heading times. Mv9kr1 is a winter wheat, but Asakaze komugi is a facultative wheat, which resulted in a 50-day difference between the flowering times of the two parental genotypes. A significantly higher number of days to flowering was detected in all the genotypes of the Mv9kr1/Igri population, compared with the results obtained under long or short photoperiod. On the other hand, the flowering time of the Asakaze/Manas addition lines decreased significantly under non-vernalised conditions relative to that recorded under short photoperiod, but still remained higher than that observed under long photoperiod. At the same time, the effect of the added barley chromosomes on the flowering time, in comparison with the average, was the same as that observed for the short photoperiod treatment. The 7H Asakaze/Manas addition line flowered significantly

Table 1 Effect of short day length (12 h) and the lack of vernalisation (NV) on the heading time (determined at DEV59) and flowering time (determined at DEV61) of Mv9kr1 (Mv) × Igri (Ig) and Asakaze komugi (Ak) × Manas (Ma) wheat/barley addition lines grown in a phytotron compared with the wheat (Mv, Ak) and barley (Ig, Ma) parental lines and with the average of the addition lines (AVG) for each combination

Genotype	Vern. resp[a] (no. of days)	Photoper. sens[b] (no. of days)	Heading date (no. of days)			Flowering time (no. of days)		
			16 h	12 h	NV	16 h	12 h	NV
Mv	62.0	22.0	73	94	138	74.2	96.2	136.2
Ig	36.6	12.4	89.4**	–[c]	126**	89**	101.4	125.6**
AVG	71.1	22.5	76.9	99.2	149.7	78.6	101.1	149.7
Mv-Ig2H	77.6	18.2	$71.6^{\#}$	$88.6^{*,\#\#}$	152*	$73.2^{\#}$	$91.4^{*,\#}$	150.8*
Mv-Ig3H	75.8	24.6	74.2	98	151.8**	75.4	100	151.2**
Mv-Ig4H	58.4	33.8	$99^{**,\#\#}$	$133^{**,\#\#}$	$159.7^{**,\#\#}$	$102.4^{**,\#\#}$	$136.2^{**,\#\#}$	$160.8^{**,\#\#}$
Mv-Ig6HS	74.2	22.2	$71.4^{\#}$	92.8	145.2*	$72.2^{\#}$	94.4	146.4**
Mv-Ig7H	69.4	13.8	$68.4^{\#}$	$83.4^{**,\#\#}$	$139.4^{\#\#}$	$69.8^{\#}$	$83.6^{**,\#\#}$	$139.2^{\#}$
Ak	30.0	15.8	51.4	69.8	84.4	56.2	72	86.2
Ma	59.0	9.8	83.7**	–	141**	83.2**	93	142.2**
AVG	18.4	39.3	66.3	103.9	85.7	67.7	107	86.1
Ak-Ma2H	18.0	41.8	69**	105**	86.6	68.8**	110.6**	86.8
Ak-Ma4H	15.8	49.2	$73.2^{**,\#}$	$121.8^{**,\#\#}$	$89.2^{*,\#}$	$72.8^{**,\#}$	$122^{**,\#\#}$	88.6
Ak-Ma6H	19.2	45.2	70**	$114^{**,\#}$	$90.2^{**,\#\#}$	71.8*	$117^{**,\#}$	$91^{*,\#\#}$
Ak-Ma7H	20.8	21.2	$53^{\#\#}$	$74.6^{*,\#\#}$	$76.6^{**,\#\#}$	$57.2^{\#\#}$	$78.4^{*,\#\#}$	$78^{**,\#\#}$

The results are means of data for five plants per treatment

*,** Significantly different from the corresponding wheat parental line at the $P < 0.05$ or $P < 0.01$ significance levels; #, ## Significantly different from the average of the addition lines within the corresponding combination at the $P < 0.05$ or $P < 0.01$ significance levels

[a] Vernalisation response was determined as the difference in flowering time (DEV61) measured without and with vernalisation treatment under a 16 h photoperiod regime

[b] Photoperiod sensitivity was determined as the difference in flowering time (DEV61) measured under the 12 and 16 h photoperiod regimes in the vernalised treatment

[c] Under the 12 h photoperiod Manas and Igri did not head completely, so DEV59 could not be observed

earlier than the wheat parent and the 7H Mv9kr1/Igri addition line significantly earlier than the average of the addition lines. The Mv9kr1/Igri 2H, 3H, 4H and 6HS additions and the Asakaze komugi/Manas 6H addition flowered significantly later than the wheat parents. These results were in agreement with the analysis of variance (Table 2), indicating that phenotypic variance in the heading characters originated almost exclusively from the wheat genetic background under unvernalised conditions.

The relative role of the wheat genetic background, the added barley chromosomes and the interaction between them in the development of phenotypic variance was investigated by two-way ANOVA under three different conditions (Table 2). Under long day length conditions (16 h, LD) the added barley chromosomes played a dominant role (45.6 %) in the development of the phenotypic variance for flowering time (DEV61), although the role of the wheat genetic background and the interaction between them was also considerable (20.5 and 18.6 %, respectively) (Table 2). Under short day length (12 h, SD), the role of the added barley chromosomes became even more pronounced (70.3 %), while the effect of the wheat background was almost negligible (1.88 %) and the interaction remained unchanged (14.8 %). The lack of vernalisation (NV) caused the opposite effect, as the wheat genetic background was the main source of flowering time variation (94.2 %), with no interaction (0.8 %) and a negligible role for the barley chromosomes (3.1 %). The same tendency could be observed for the variance in the heading time (DEV59).

Both the two wheat maternal partners and the two winter barley donors differed with respect to their

Table 2 Two-way ANOVA on Mv9kr1 (Mv) × Igri (Ig) and Asakaze komugi (Ak) × Manas (Ma) wheat/barley addition lines grown under long-day (16 h, LD), short-day (12 h, SD) and no vernalisation (NV) programmes

Source of variation	d.f.	MS					
		Flowering 12 h	Flowering 16 h	Flowering NV	Heading 12 h	Heading 16 h	Heading NV
Genetic background	1	313.6*	1380.6***	39879.2***	193.6	1276.9***	40322.5***
Additional barley chr.	3	3902.5***	1024.4***	442.1***	4008.9***	1103.6***	479.7***
Interaction	3	818.8***	418.5***	119.1**	703.2***	334.0***	108.0**

df degrees of freedom, *MS* mean square

*,**,*** Significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$ respectively

vernalisation requirements (Table 1). Of the two barley genotypes, Manas had almost twice the vernalisation requirement of Igri, although both barleys have winter growth habit based on the allele composition of the *Vrn* genes. In the case of the wheat genotypes, the winter growth habit Mv9kr1 had a significantly higher vernalisation requirement than the facultative Asakaze komugi. There was less difference in the photoperiod sensitivity of the four parents. In the first combination both Mv9kr1 and Igri proved to be more sensitive to photoperiod than the parents in the second combination (Asakaze and Manas). In the case of the Mv9kr1/Igri combination, the average values of the barley addition lines were close those of the winter wheat parent with respect to both vernalisation requirement and photoperiod sensitivity. In the case of Asakaze/Manas, however, the barley addition lines proved to be significantly more photoperiod-sensitive and had a much lower vernalisation requirement than either of the parental lines. In spite of the differences in magnitude, the photoperiod sensitivity of the individual addition lines showed similar tendencies in both wheat genetic backgrounds. Photoperiod sensitivity was increased by the addition of chromosome 4H and decreased by the addition of 7H. In the case of vernalisation response only the effect of the 4H addition was similar in the two wheat genetic backgrounds, as it led to the lowest vernalisation response amongst the addition lines.

The heading characters of the Mv9kr1/Igri and Asakaze/Manas genotypes were also determined under field conditions in 2011/2012, where the plants were sown in autumn and were consequently vernalised naturally (Table 3). The earliest flowering was observed in the 7H addition lines in both wheat cultivar combinations. The 7H Asakaze/Manas addition flowered significantly earlier ($t = -6.171$; $P = 4e^{-6}$) than the wheat parental line Asakaze komugi. In comparison with the average of the addition lines, a longer flowering time was detected for the 4H additions in both combinations, and for the Mv9kr1/Igri 2H and Asakaze/Manas 3H and 6H additions. The 6H Asakaze/Manas addition flowered later than 4H in the field in 2012.

The major difference between the flowering times measured in the controlled growth chamber and the field tests lay in the fact that the barley cultivars had the earliest heading and flowering times in the field, but not under artificial conditions. Both barley cultivars flowered later than the wheat genotypes in the phytotron, irrespective of the photoperiod regime. The barley addition lines, however, showed more similar behaviour over the different environments. In both wheat genetic backgrounds, the strongest significant correlations were found for the flowering times measured under 16 h NV and field-grown conditions ($r = 0.94$* for the Mv9kr1 addition lines, and $r = 0.97$* for the Asakaze addition lines). In both combinations the photoperiod sensitivity of the addition lines exhibited stronger, though not significant correlations with the flowering time measured under field conditions ($r = 0.79$ and 0.88 for the Mv9kr1 and Asakaze addition lines, respectively) than the vernalisation response ($r = -0.19$ and -0.50). The controlled environment test magnified the differences between the genotypes. Thus, the differences between the flowering times of the 7H and 4H Mv9kr1/Igri and Asakaze/Manas addition lines were 12 and 11 days in the field in 2012, respectively, but this increased to 52 and 44 days under short-day illumination (12 h) in the phytotron.

Effect of added barley chromosomes on morphological parameters

Traits related to plant and spike architecture and yield were also investigated under field-sown conditions (Table 4). The two-rowed Igri and Mv9kr1 differed

Table 3 Days elapsing from sowing to the late booting (DEV49), heading (DEV59) and flowering (DEV61) stages of Mv9kr1 (Mv) × Igri (Ig) and Asakaze komugi (Ak) × Manas (Ma) wheat/barley addition lines, the wheat (Mv, Ak) and barley (Ig, Ma) parental lines and the average of the addition lines (AVG) for each combination in the field experiment in 2012, Martonvásár

Genotype	2012, Martonvásár		
	Late booting stage (no. of days)	Heading date (no. of days)	Flowering time (no. of days)
Mv	201.4	206.9	207.8
Ig	197.8**	208*	202.7**
AVG	207.1	214.3	214.3
Mv-Ig2H	208.8**,#	216.8**,##	217.1**,##
Mv-Ig3H	207.5**	215.6**	215.2**
Mv-Ig4H	210.4**,##	219.2**,##	218.7 **,##,a
Mv-Ig6HS	207.1**	214.3**	213.2**
Mv-Ig7H	201.5##	205.6**,##	207.2##
Ak	199.2	204.2	205.2
Ma	196.9**	203**	201.1**,b
AVG	205.2	209.5	211.8
Ak-Ma2H	203.6**,#	208.2**	210.4**
Ak-Ma3H	206.2**	210.8**	213.3**,#
Ak-Ma4H	206.1**	210.6**	213.8**,#
A k-Ma6H	211.8**,##	216.3**,##	218.6**,##
Ak-Ma7H	198.2*,##	201.8**,##	202.9**,##

The results are means of data for ten plants per genotype

*,** Significantly different from the corresponding wheat parental line at the $P < 0.05$ or $P < 0.01$ significance levels

#, ## Significantly different from the average of the addition lines within the corresponding combination at the $P < 0.05$ or $P < 0.01$ significance levels

[a] Date of the beginning of flowering in the latest genotype: 22nd May, 2012

[b] Date of the beginning of flowering in the earliest genotype: 4th May, 2012

significantly from each other for all the traits studied. The barley parent was shorter and produced more tillers than the wheat parent. Its shorter spike was paralleled by a higher number of spikelets per spike. In spite of this, the wheat parent produced more seeds per plant, as the two-rowed barley had a lower number of seeds per spike. Most of the yield-related traits showed a significant decrease in the addition lines compared to the better parent. In the winter wheat Mv9kr1 genetic background the 4H addition caused the greatest decrease in the seed number per plant, which was primarily due to the strong decline in the tiller number, as it had the highest number of seeds per spike. On the other hand, the least decrease in seed number per plant was recorded for the chromosome 3H addition, which was due to its better tillering capacity and the largest spikelet number per spike among the addition lines. The earliest heading 7H addition was characterised by the smallest number of spikelets and seeds per spike. In spite of this, its yielding ability was the second best among the addition lines, as it had the highest tillering capacity.

The facultative Asakaze and the six-rowed winter barley Manas showed similar tillering capacities, but in this combination the barley parent produced the higher number of seeds per plant due to the significantly higher number of spikelets and seeds per spike. In this set of addition lines, the 4H addition resulted in the highest spikelet and seed number per spike, and thus the highest seed number per plant. The earliest addition 7H again resulted in the highest tillering capacity, but in this case this feature was not able to compensate for the low seed number per spike.

Discussion

The effect of added barley chromosomes on heading and flowering time was studied in two partial sets of wheat-barley addition lines, namely the Mv9kr1/Igri and Asakaze/Manas disomic additions. Both Igri and Manas are winter barley cultivars which need vernalisation. Unfortunately the 5H addition was missing from both combinations, because 5H was the first chromosome to be eliminated from the backcross derivatives (Szakács and Molnár-Láng 2010; Molnár-Láng et al. 2012), as also observed by Koba et al. (1991). The 1H addition line is also missing from both combinations, as this chromosome causes sterility in a wheat background (Islam and Shepherd 1990; Taketa et al. 2002; Molnár-Láng et al. 2005, 2012). Thus, the added effects of *Vrn-H1* and *Ppd-H2* on plant development in a wheat genetic background could not be evaluated.

Of all the barley addition lines, the effects of the 4H and 7H additions were the most pronounced. The 4H additions headed and flowered latest in all the treatments except in the field in 2012, where the 6H Asakaze/Manas addition line was the latest. The 7H addition lines were the earliest in both cultivar combinations in all the treatments. Both chromosomes

Table 4 Morphological traits of Mv9kr1 (Mv) × Igri (Ig) and Asakaze komugi (Ak) × Manas (Ma) wheat/barley addition lines grown in the field (2012)

Genotype	Plant height (cm)	Tillering (spikes/plant)	Length of main spike (cm)	Spikelets/ main spike	Seeds/main spike	Seeds/ plant
Mv	69.7	5.7	10.4	20.3	55.8	244.4
Ig	62.2	8.9	8.3	25.3	26.7	187.9
Mv-Ig2H	65.5	4.3	10.1	18.6	41.7	126.1
Mv-Ig3H	51.2	5.4	8.0	18.8	41.3	160.0
Mv-Ig4H	46.9	3.3	8.9	18.2	42.5	95.6
Mv-Ig6HS	58.5	4.4	8.8	18.4	42.4	136.7
Mv-Ig7H	61.8	6.0	9.8	17.1	35.3	146.5
$LSD_{1\ \%}$	6.1	1.2	0.8	1.9	5.4	37.6
Ak	81.9	7.6	9.5	17.2	50.6	252.2
Ma	65.9	8.0	7.1	29.0	55.1	322.7
Ak-Ma2H	85.6	5.6	8.3	18.2	46.8	190.0
Ak-Ma3H	78.7	4.1	9.2	17.6	38.4	100.8
Ak-Ma4H	98.7	5.9	11.1	21.0	47.9	196.5
Ak-Ma6H	84.6	5.7	9.5	20.1	39.2	155.4
Ak-Ma7H	87.9	7.8	9.5	19.9	39.3	150.8
$LSD_{1\ \%}$	5.7	1.2	1.2	1.5	5.8	41.8

The results are means of data for ten plants per genotype. The least significant difference (LSD) values of the one-way ANOVA were calculated for each morphological trait at the $P = 0.01$ level

carry several plant developmental genes. In addition to *Vrn-H2*, the *Phytochrome A* and *B* genes are also located on chromosome 4H (Szűcs et al. 2006), while in the case of 7H, *HvVRT2*, and various members of the *CONSTANS* gene family (*HvCO1*, *HvCO6*, and *HvCO8*) are also present, in addition to *Vrn-H3* (Griffiths et al. 2003; Kane et al. 2005). However, as *Vrn-H2* and *Vrn-H3* have the greatest role in determining the heading date in barley, it was probably the effect of these two genes that could be detected in the wheat-barley addition lines.

In the case of the 7H chromosome, it was probably the effect of *Vrn-H3,* located on the short arm, that was detected. The *FT1* candidate gene for this locus promotes the transcription of *Vrn1* under long-day conditions after the saturation of the vernalisation requirement, thus accelerating flowering (Chao et al. 1989; Yan et al. 2006; Faure et al. 2007). The ability of *Vrn3* to promote flowering is directly associated with its activity levels in both wheat and barley (Yan et al. 2006; Faure et al. 2007). Thus, the addition of an extra copy of *Vrn-H3* to the *Vrn3* series of wheat would be expected to result in hastened plant development, as was indeed the case in the 7H addition lines. It is interesting to note that the effect of the 7H addition was also dependent on the wheat genetic background. In the winter wheat background it resulted in earlier flowering as compared to the wheat parent in the controlled environment tests with vernalization, but not under field conditions. In the case of the facultative wheat genetic background the hastening effect of *Vrn-H3* was significant under field-grown conditions, but not in the controlled environment tests.

In the case of the *Vrn-H2* locus, both barley genotypes have dominant alleles, as they carry the *ZCCT-H* genes (Dubcovsky et al. 1998; Yan et al. 2004b; Karsai et al. 2005). These genes encode for a flowering repressor, the major role of which is to repress the activation of *Vrn-H3* and *Vrn-H1* under long photoperiods until the vernalisation requirements of the genotypes are saturated (Trevaskis 2010). In wheat, *VRN2* was shown to play a similar role at the diploid level, but less is known about its effect in hexaploid wheat, probably due to the three copies of the *Vrn1* and *Vrn3* genes, and due to a chromosome rearrangement involving 4AL and 5AL (Yan et al. 2004b; Zhu et al. 2011). The role of *Vrn2* is, however, more complex (Diallo et al. 2010). In barley it also exhibits an association with photoperiod sensitivity, as genotypes with the dominant allele are less sensitive to a long photoperiod than those with the recessive allele (Karsai et al. 2006). The addition of *Vrn-H2,* located on chromosome 4H, to the wheat genetic background, had the opposite effect to what was expected, as the 4H addition lines showed the highest photoperiod sensitivity and the lowest vernalisation response among all

the addition lines in both wheat genetic backgrounds. This combination of the component traits of flowering resulted in the latest flowering under almost all the environmental conditions. The difference in flowering between 7H and 4H was 12 and 11 days in the field (2012) in the Asakaze/Manas and Mv9kr1/Igri combinations, respectively, and this increased to 44 and 52 days under short-day conditions in the phytotron. Only two days difference was observed between the CS/Betzes 7H and 4H addition lines by Murai et al. (1997) under short-day illumination in the phytotron, which could be primarily due to the fact that Betzes, being a spring barley, did not carry the *ZCCT-H* genes at the *VRN-H2* locus, giving further indirect proof that the effect of *Vrn-H2* was detected in these addition lines.

The major photoperiod sensitivity locus *Ppd-H1* is located on chromosome 2H, and both barley genotypes carry the photoperiod sensitivity allele, which results in hastened plant development under long photoperiods in barley (Turner et al. 2005). Thus the addition of chromosome 2H was expected to have a significant effect on plant development in the wheat genetic background, too. In the winter wheat background, the addition of *Ppd-H1* decreased the time to flowering under the short photoperiod regime, but had a very strong delaying effect under field-grown conditions. In the facultative wheat background, however, the delaying effect of *Ppd-H1* was evident not only in the field test but also under controlled environment conditions, especially with a short photoperiod regime. The effect and molecular basis of the *Ppd1* locus were distinctly different in barley and wheat, including differences in the regulation of the circadian rhythms of several downstream genes such as *Vrn3* (*FT1*) (Turner et al. 2005; Beales et al. 2007). Thus, the combination of *Ppd-H1* with *Ppd-D1* could lead to various discrepancies in downstream regulation, leading to altered plant development. The higher positions of both *Ppd1* and *Vrn2* in the hierarchical regulation systems of plant development compared to *Vrn3* could also lead to more substantial changes in downstream regulation when the genes of different species are combined in one genome, a phenomenon which will require further studies.

The agronomic performance of all the wheat-barley addition lines was poorer than that of both the wheat and barley parents in the one-year field test, which could be due to the adverse gene interactions originating from the extra chromosome pair and the effect of aneuploidy. In studying the yielding ability of the Chinese Spring/Betzes wheat-barley addition lines, Farshadfar et al. (2012) established that these addition lines had various levels of yield stability and genotype x environment interactions, and identified the CS-Be7H addition line as the genotype with the highest mean yield and low stability (larger variance among environments). In the present study the yielding ability of the earliest (7H) addition in both combinations was close to the average of the addition lines, while the highest fertility was observed for the 4H additions in agreement with the previous results of Hoffman et al. (2010).

In conclusion, the addition of winter barley chromosomes significantly influenced the flowering time of wheat both in the controlled environment tests and under field conditions. In the Mv9 kr1/Igri combination the 7H additions were the earliest flowering and the 4H additions the latest in both the phytotron and the field in 2012. In the Asakaze komugi/Manas combination 7H was the earliest flowering in all the environments, but 6H was the latest in the field and without vernalization in the phytotron.

It is planned to use the additions to develop wheat/barley ditelosomic and translocation lines where the effect of various genes located on smaller chromosome segments could be studied on flowering time and morphological traits.

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