Physical map of the *Eps-A^m1* gene region in *Triticum monococcum* L.

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INTRODUCTION

Wheat varieties (*Triticum aestivum* L.) can be grown in very different environments with contrasting temperatures and photoperiods. This broad adaptability is favoured by wheat's extensive genetic diversity in flowering time. However, optimum grain yields in different environments can only be obtained when plants flower within a narrow window of time that maximizes resource utilization.

Earliness *per se* (*Eps*) genes are responsible for the fine tuning of flowering time independently of vernalization and photoperiod, the main environmental signals regulating the initiation of reproductive development in wheat. A quantitative trait locus for earliness *per se* was mapped in the distal region of chromosome arm 1A^mL in a cross between cultivated (DV92) and wild (G3116) *T. monococcum* L. accessions, which was designated *Eps-A^m1* [1]. Allelic variation at this locus resulted in differences in heading time of several weeks when plants where fully vernalized and grown under long days, and those differences were significantly greater at 16 °C than at 23 °C, indicating that the effect of this gene was modulated by temperature [1]. This locus was then mapped within a 0.8-cM interval between flanking genes *Adk1* and *VatpC* using a mapping population of 1,026 gametes and markers generated from the colinear region on rice chromosome 5 [2]. This initial genetic map was the starting point of the efforts aiming at identifying the *Eps-A^m1* gene using a positional cloning approach.

Both the complete high-density genetic and physical *T. monococcum* maps of the *Eps-A^m1* region are presented in this paper. The effects of the *Eps-A^m1* region on both duration of developmental phases and spikelet number in diploid wheat are also reported. Number of spikelets per spike is an important component of potential grain yield in wheat. The presence of a gene with effects similar to *Eps-A^m1* in common hexaploid wheat is also shown here.

MATERIALS AND METHODS

Mapping population. The high-density genetic map was completed using 3,298 BC_6F_2 near isogenic lines (NILs) developed 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-l*) and wild *T. monococcum* ssp. *aegilopoides* accession G3116 (winter) carrying the *Eps-A^m1* allele for early heading (hereafter *Eps-A^m1-e*). 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 lines are >99% identical to DV92 and have a spring growth habit. The final high-density genetic map was based on 3,811 plants (adding 417 $F_{2:3}$ and 96 F_5 single-seed descent lines derived from the same cross [2]).

The BC_6F_2 lines were screened with PCR markers flanking the *Eps-A^m1* gene [2], and those with recombination events close to this locus were self-pollinated to immortalize the recombinant chromosomes. The *Eps-A^m1* alleles present in the recombinant lines were determined by progeny tests.

BAC selection and sequencing. *T. monococcum* accession DV92 BAC library was screened with markers closely linked to *Eps-A^m1* (Fig. 1). Positive BAC clones were *Hind*III-fingerprinted and BAC-end sequenced, and contigs were assembled. Selected BAC clones covering the *EpsA^m1* region were sequenced and assembled using Phred/Phrap/Consed [3, 4]. Sequences were annotated using a combination of comparative genomic analyses, BLAST searches, and gene-finding programs. Gaps in the sequences were filled by primer walking. Genomic DNA extraction, PCR, hybridization, and Southern blot procedures were performed as described before [2, 5].

Phenotypic experiments. All plants were grown in a growth chamber at 16 ºC and long day photoperiod (16 h, 90 μEm^-2s^-1).

Diploid wheat. Plants from 12 NILs carrying either the *Eps-A^m1-e* or the *Eps-A^m1-l* allele were used in this experiment. The phenological stage of the apex of the main tiller from several plants of each NIL was determined every five days, and times 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) were determined [6]. Number of spikelets of the main spike was recorded for the remaining plants.

Hexaploid wheat. Chinese Spring (CS) nullisomic-tetrasomic N1AT1B, ditelosomic Dt1AS [7], and deletion 1AL1 [8] lines were grown as described above, using CS as a control line. Average heading time and number of spikelets per spike were determined from three spikes. Phenotypic differences 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.
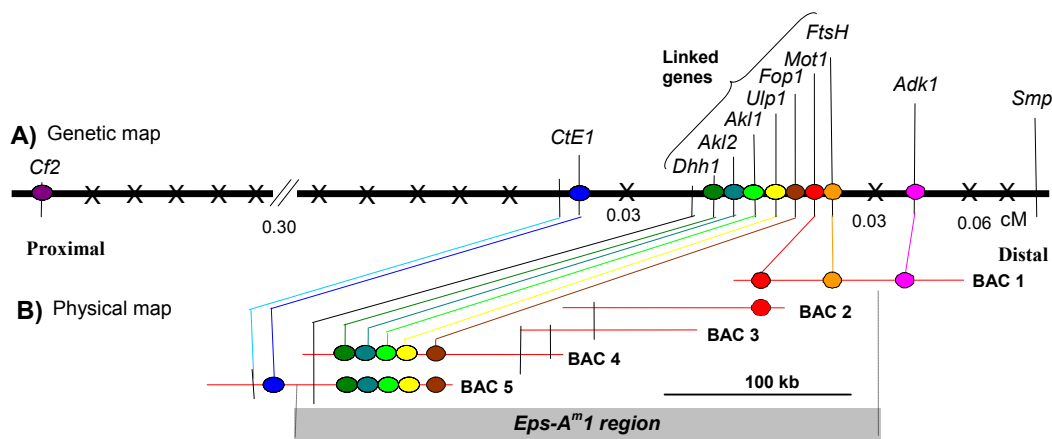
Figure 1. *T. monococcum* high-density genetic (**A**) and physical (**B**) maps. In the genetic map, X indicates a recombination event. BAC clones are indicated as red bars, genes as coloured circles, and other markers as black bars.

RESULTS

High-density genetic and physical maps of the *Eps-A^m1* region. In a previous map, the *Eps-A^m1* locus was mapped between genes *Adk1* and *VatpC* [2]. *Adk1* was used as the starting point for the chromosome walk. Sequencing of BAC 1 selected with *Adk1* yielded genes *FtsH* (membrane-bound protease) and *Mot1* (transcriptional regulator). These genes were completely linked to each other and mapped 0.03 cM proximal to *Adk1*, closing the distal gap of the physical map (Fig. 1).

Mot1 was then used to select BAC 2, which did not include additional genes. Markers from regions flanking the insertion of repetitive elements were used to select BACs 3 and 4. BAC 4 included genes *Fop1* (flavonoid 3'-monooxygenase), *Ulp1* (protease), *Akl1* and *Akl2* (ankyrin-like proteins), and *Dhh1* (DNA helicase). These genes were completely linked to each other and to *Eps-A^m1*. Sequencing of BAC 5 selected with *Dhh1* included the same genes plus the additional gene *CtE1* (cysteine-type endopeptidase), which was mapped 0.03 cM proximal to *Eps-A^m1*, closing the proximal gap of the physical map (Fig. 1). Based on these results we concluded that *FtsH*, *Mot1*, *Fop1*, *Ulp1*, *Akl1*, *Akl2*, and *Dhh1* are candidate genes for *Eps-A^m1*.

Effects of the *Eps-A^m1* region on both duration of developmental phases and spikelet number in diploid wheat. NILs carrying the *Eps-A^m1-l* allele initiated the transition between the vegetative and reproductive stages 35 days later than those carrying the *Eps-A^m1-e* allele ($P<0.0001$, Fig. 2A). In addition, the duration of the transition from double ridge stage to terminal spikelet stages was significantly longer in the isogenic lines carrying the *Eps-A^m1-l* allele than in those carrying the *Eps-A^m1-e* allele ($P<0.0001$, Fig. 2B). Non-significant differences ($P=0.79$) were detected between *Eps-A^m1* alleles in the stem elongation period (TS-H).

NILs carrying the *Eps-A^m1-l* allele had 6.5 more spikelets per spike (32% increase) than the isogenic lines carrying the *Eps-A^m1-e* allele ($P<0.0001$, Fig. 2C).
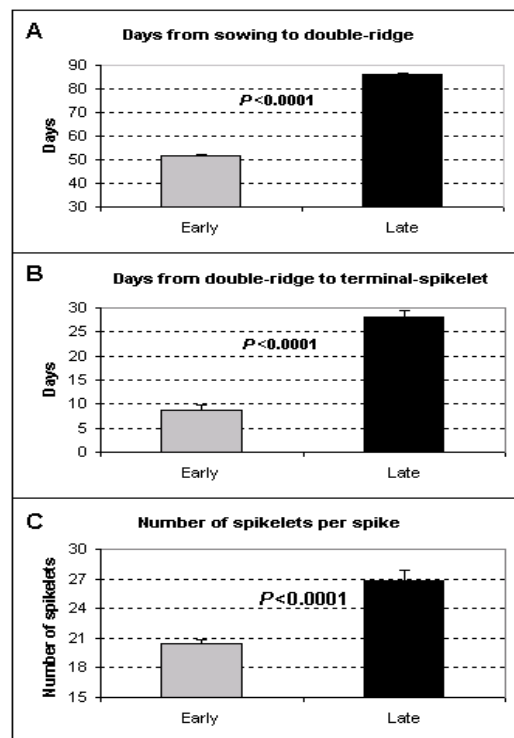

Figure 2. Effects of *Eps-A^m1* early and late alleles on both duration of developmental phases and spikelet number in diploid wheat.

Effects of chromosome arm 1AL on both heading time and spikelet number in hexaploid wheat. Lines N1AT1B, Dt1AS, and 1AL1 lacking the complete chromosome 1A, its long arm (1AL), or the 83% distal region of 1AL, respectively, flowered significantly earlier (11, 7, and 6 days earlier respectively, $P<0.01$) and had a significant reduction in number of spikelets per spike (27%, 39%, and 32% reduction respectively, $P<0.01$) than the control line CS. These results suggest that a gene similar to *Eps-A^m1* is present in the distal region of chromosome arm 1AL of common wheat.

DISCUSSION

Genetic and physical maps of the *Eps-A^m1* region. The large mapping population used in this study (7,622 chromosomes) facilitated the mapping of *Eps-A^m1* within a 0.06-cM region flanked by genes *Adk1* and *CtE1*. This small genetic distance corresponds to a physical distance of ~302-kb, within the 404-kb complete physical map (Fig. 1). Sequencing of the five overlapping BAC clones covering the *Eps-A^m1* region revealed the presence of nine genes, resulting in a gene density of 1 gene every 45 kb. Seven of these genes (*FtsH, Mot1, Fop1, Ulp1, Akl1, Akl2,* and *Dhh1*) were completely linked to *Eps-A^m1* (Fig. 1) and are candidates for this gene. TILLING mutants of these genes are being generated to determine which of them *Eps-A^m1* is.

In spite of the high gene density and distal location, the minimum ratio between physical and genetic distances for this region, 11.5 Mb cM^{-1}, is much larger than the estimated genome-wide average ratio (3 Mb cM^{-1}) [9] or the ratio estimated for the distal region of chromosome arm 1AS (1.4 Mb cM^{-1}) [10]. A possible explanation for the reduced recombination observed in this region may be the presence of structural differences (e.g. deletions or inversions) between the two parental lines.

The *Eps-A^m1* region affects both duration of developmental phases and spikelet number in diploid wheat. Results from the experiment using diploid NILs showed that the *Eps-A^m1* region affects both the timing of the transition from the vegetative to reproductive stages and the duration of spike development, but not the later stages of development.

In this experiment, the differences in heading time were highly correlated with the differences in spikelet number ($R=0.96, P<0.0001$). The effect of the flanking markers on spikelet number was less significant than the effect of the *Eps-A^m1* locus indicating that the gene controlling spikelet number is within this interval. This suggests that the differences in spikelet number are either a pleiotropic effect of the *Eps-A^m1* gene or the effect of a separate but tightly linked gene within the *Eps-A^m1* region. The first hypothesis is favored because a gene affecting the duration of spike development is likely to have pleiotropic effects on the number of spikelets per spike. An extended period of spike development provides time for the development of additional spikelets. Number of spikelets per spike is an important component of potential grain yield since it is associated with higher number of grains per spike. Therefore, a better understanding of the genetic factors controlling spike development can have an impact on the ability to improve grain yield.

A gene with effects similar to *Eps-A^m1* is present in chromosome arm 1AL in common wheat. Results from the experiment using hexaploid CS lines showed that a gene with effects similar to *Eps-A^m1* is present in the corresponding region in common wheat. Line N1AT1B lacking the complete chromosome 1A flowered significantly earlier and had a reduction in spikelet number when compared with CS. This locus was mapped on the distal 83% region of chromosome arm 1AL, since both Dt1AS and deletion 1AL1 lines also flowered earlier and had a smaller number of spikelets per spike than the control line CS.

Because the A genome of both common and pasta wheat species was contributed by the diploid species *T. urartu* Thum.[11], the effect of domestication of *T. monococcum* on the A^m genome has been independent from the effect of domestication of polyploid wheat species on the A genome. It is possible that the allele for lateness and increased spikelet number present in *T. monococcum* chromosome arm 1A^mL has a larger effect than the homoeologous alleles in the A genome of both bread and pasta wheat species. The *Eps-A^m1* allele is being transferred to common wheat to test this hypothesis.

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