Theor Appl Genet (2006) 112: 634–647
DOI 10.1007/s00122-005-0166-1

ORIGINAL PAPER

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Development and QTL assessment of *Triticum aestivum–Aegilops tauschii* introgression lines

Received: 13 September 2005 / Accepted: 17 November 2005 / Published online: 9 December 2005

Abstract A set of 84 bread wheat lines, each containing a single homozygous introgression of the *Aegilops tauschii* genome was produced in the 'Chinese Spring' background via backcrossing of the D-genome chromosome substitution lines 'Chinese Spring'/Sears's 'Synthetic 6*x*' with the recurrent parent and subsequent selfing. The development of the lines was accompanied by microsatellite marker assisted selection. With the exception of three telomeric regions at chromosomes 1DL, 4DL and 7DS, and a region of less than 24 cM on the chromosome arm 3DL, the genome of *Ae. tauschii* is fully represented in these lines. The newly developed lines were used for the discovery of morphological and agronomical quantitative trait loci (QTLs) from the wild species. Fifty-two introgression lines were grown in the field and evaluated for six traits including flowering time, plant height, ear length, spikelet number, fertility and grain weight per ear. Seventeen significant QTLs were detected, *Ae. tauschii* contributed favourable alleles at nine loci influencing five traits. The whole set of 84 homozygous lines provides a tool for further testing the effects and stability of the detected QTLs and for the evaluation of new traits.

Communicated by B. Friebe

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Introduction

Bread wheat, *Triticum aestivum* L. ($2n=6x=42$, AABBDD) has been cultivated for over 8,000 years (Helbaek 1959). A bottleneck effect in the origin of hexaploid wheat and a long breeding process have led to a narrow genetic base of bread wheat (Talbert et al. 1998; Bryan et al. 1999). Therefore, the wild relatives of wheat form a source with high potential for the improvement of wheat. A large number of wild accessions related to wheat has been collected in gene banks throughout the world. However, the existing collections are often not actively utilized particularly with respect to complex traits such as yield and nutritional quality (Tanksley and McCouch 1997).

The diploid goatgrass, *Aegilops tauschii* (Coss.) Schmal. ($2n=2x=14$, DD), the donor of the bread wheat D-genome (Kihara 1944; McFaden and Sears 1946), grows across large areas of southwest Asia and reveals extensive polymorphism in morphology, seed storage protein composition, isozymes, and molecular markers (Hammer 1980; Lagudah et al. 1987; Kam-Morgan et al. 1989; Lubbers et al. 1991; Dvorak et al. 1998; Pestsova et al. 2000a; Yan et al. 2004). Disease and pest resistance of this species is well documented (Lutz et al. 1995; Yang et al. 2003; Smith and Starkey 2003). Recent data obtained by using analysis of recombinant inbred line (RILs) of the International Triticeae Mapping Initiative (ITMI) population (Börner et al. 2002) and by advanced backcross (AB-QTL) analysis in wheat (Huang et al. 2003, 2004) showed that despite its inferior phenotype, *Ae. tauschii* contains genes that can improve quantitative traits.

Conventional populations used for the dissection of quantitative traits in self-pollinated plants are usually F2 or backcross (BC) generations, as well as RILs and double-haploids (DHs). The search for QTLs using such populations can result in an underestimation of the number of involved loci or the effect of loci, due to the overshadowing of minor loci by a major QTL or inter-

action between unlinked loci (Eshed and Zamir 1995). Therefore, the availability of precise genetic stocks, such as monosomic and substitution lines which have been developed during the last 40 years using cytogenetic methods (Law and Worland 1996), presents a particular advantage for QTL analysis in wheat. Single chromosome recombinant lines derived from these stocks enabled accurate QTL location and the estimation of the effects of QTLs in a uniform genetic background (Kato et al. 2000; Toth et al. 2003).

The construction of a population consisting of *Lycopersicon esculentum* lines with introgressions of *L. pennellii* genetic material was reported by Eshed and Zamir (1995). These ILs proved to be very efficient in identification and fine mapping of quantitative traits (Eshed and Zamir 1995; Monforte et al. 2001; Fridman et al. 2000, 2002, 2004). Recently, several sets of introgression lines in barley were developed and characterized (Matus et al. 2003; von Korff et al. 2004).

Based on a set of *T. aestivum* cv. 'Chinese Spring'/'Synthetic 6*x*' chromosome substitution lines, we created single chromosome recombinant lines for seven D-genome wheat chromosomes by backcrossing with 'Chinese Spring' (CS). Microsatellite marker selection resulted in the development of a set of homozygous introgression lines (ILs) containing different segments of individual chromosomes of *Ae. tauschii* in the CS background. The ILs represent near-isogenic lines (NILs) with a relatively large average introgression length of 55.5 cM. With the exception of three telomeric regions and a region of not more than 24 cM on the chromosome arm 3DL, the genome of *Ae. tauschii* is fully represented in the ILs. In the present paper, we describe the architecture of the lines and show their benefit in the identification of agronomically important QTLs.

Materials and methods

Introgression lines (ILs)

ILs were developed based on a set of substitution lines CS/'Synthetic 6*x*' in which single chromosomes of CS were replaced by homologous chromosomes of 'Synthetic 6*x*'. The substitution lines were produced by C.N. Law and A.J. Worland at the John Innes Centre, UK (Arraiano et al. 2001). The parental 'Synthetic 6*x*' had been obtained earlier by McFadden and Sears (1947) from a cross of tetraploid emmer and wild grass *Aegilops tauschii* (*T. dicoccoides* var. *spontaneovillosum* × *Ae. squarrosa* ssp. *eusquarrosa*). Thus, in the set of substitutions for the D-genome, individual chromosomes of *Ae. tauschii* replaced the homologous chromosomes of CS.

The scheme of IL development through repeated backcrossing to the recurrent parent CS and microsatellite markers selection was described previously by Pestsova et al. (2001). Forty-two ILs were obtained by selfing and ten lines by double-selfing of the BC1 progeny (Table 1). In order to select a set of homozygous ILs representing the whole *Ae. tauschii* genome, 450 BC2 plants were genotyped with microsatellite markers and 60 were selected and selfed for development of homozygous lines. Genotyping of 600 plants from the BC2F2 progeny resulted in the detection of 47 homozygous ILs. Of these, 15 were later discarded since they had the same marker compositions as previously obtained lines. In total, 84 different ILs were developed from the BC1 and BC2 progenies. Seeds of the ILs were transferred into the gene bank of IPK (Gatersleben, Germany) for multiplication and maintenance. They are openly available and can be requested from A. Börner.

Genotyping

DNA was isolated from the leaf material at the seedling stage according to a modified procedure of Plaschke et al. (1995). Genotyping was carried out using microsatellite markers previously mapped on the chromosomes of the D-genome of wheat (Röder et al. 1998; Pestsova et al. 2000b). Unpublished *Xgwm* primer sequences are available upon request. *Xbarc* markers were described by Song et al. (2002) and are available at the web site http://www.scabusa.org. Polymerase chain reactions (PCR) and gel electrophoresis were performed as described by Röder et al. (1998). The chromosomal location of the markers polymorphic between the 'Synthetic 6*x*' and CS is shown in Fig. 1. Linkage map positions of the markers were inferred from the data obtained for the International Triticeae Mapping Initiative (ITMI) mapping population (Röder et al. 1998). The same order of the markers in the CS/'Synthetic 6*x*' population was confirmed by manual examination of the BC1 and BC2 genotypic data for the minimum number of double recombinants.

Phenotyping

The BC1, BC1F2, BC2 and BC2F2 generations were grown under greenhouse conditions and individual plants were analysed for different quantitative traits. The results of QTL analysis of the BC1, BC1F2 and BC2 were published by Pestsova et al. (2001, 2003). Field trials for 52 ILs developed from the BC1 progeny were conducted during the summer of 2002 at Gatersleben (Germany). Forty-three ILs derived from the BC1F2 were represented by 10–40 plants, while the number of plants for another nine ILs selected from the BC1F3 was limited to 2–7 individuals (Table 3). The recurrent parent and seven chromosome substitution lines were grown as a control, each represented by ten plants. Six phenotypic traits were measured. Flowering time (*Flt*) and plant height (*Ht*) were evaluated for each single plant. Ear length (*El*), spikelet number (*Spn*), fertility (*Fr*) and grain weight per ear (*Gwe*) were measured on the selected ten spikes belonging to one line (usually one

Table 1 Number of microsatellite markers polymorphic between 'Chinese Spring' and 'Synthetic 6*x*', which were successfully used for genotyping during development of introgression lines (ILs), and the number of ILs produced per chromosome

Chromosome	Number of microsatellite markers	Number of ILs developed from BC1	Number of ILs developed from BC2	Total number of developed Ils
1D	9	6	3	9
2D	16	12	8	20
3D	9	9	2	11
4D	7	3	4	7
5D	17	8	3	11
6D	9	8	5	13
7D	13	6	7	13
Total	80	52	32	84

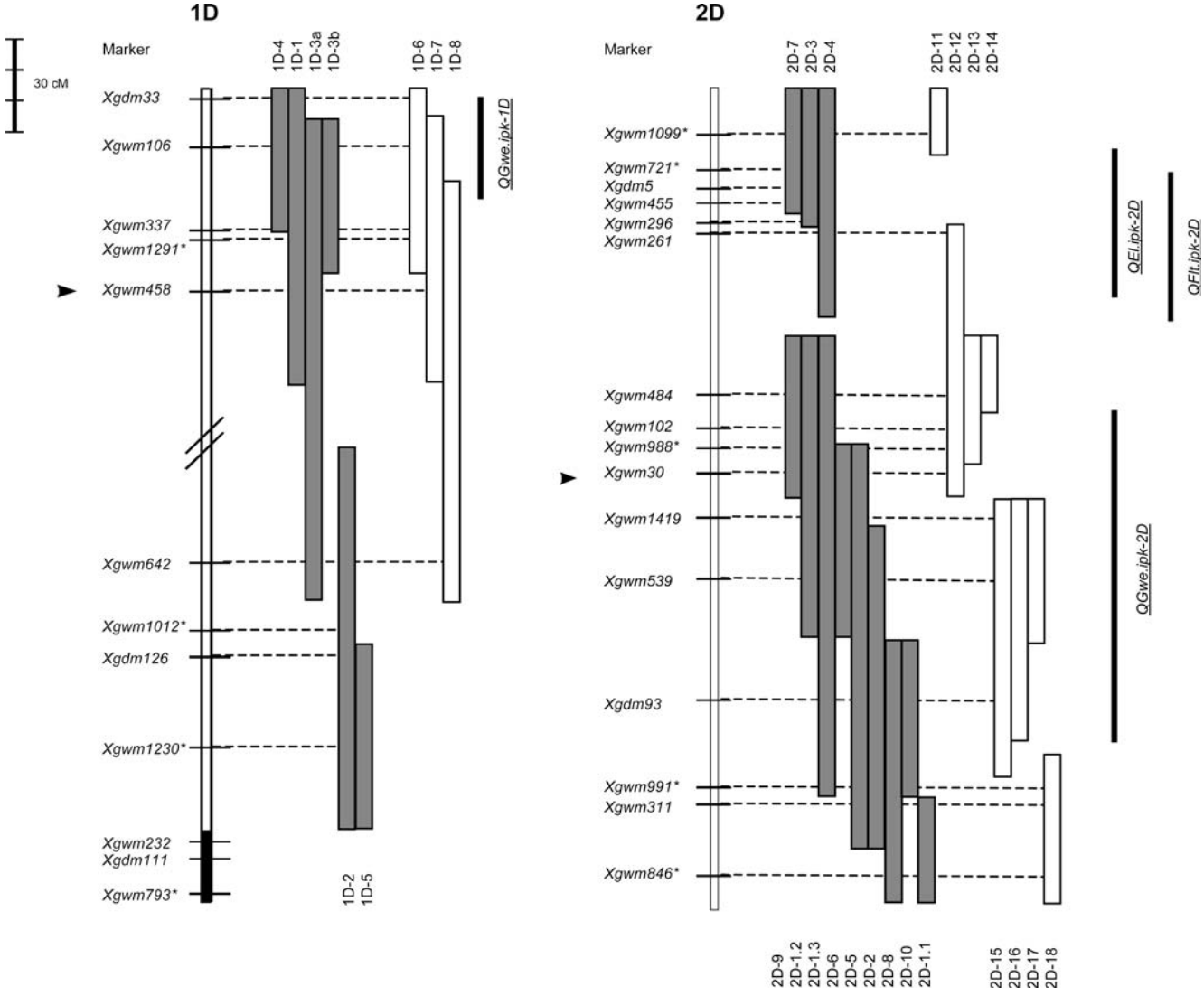


Fig. 1 Graphical genotypes of 84 introgression lines (ILs) developed for seven chromosomes of the D-genome of bread wheat. Each IL contains a single introgression of the *Ae. tauschii* genome, with introgressed segments shown in *wide bars*. *Grey bars* indicate the 52 ILs used in the field trials. The chromosome linkage maps are adapted from Röder et al. (1998). Only polymorphic microsatellite markers between the parental 'Synthetic 6*x*' and 'Chinese Spring' are shown. Unpublished microsatellite markers are marked with an *asterisk* and are available upon request. Short arms of the chromosomes are at *top*, with *arrows* showing the position of the centromeres. Chromosomal regions with detected incorrect substitutions are *coloured in black*. Detected QTLs (LOD threshold of >3.0) are shown to right of the chromosomes in *narrow black bars*. Underlined QTLs mark loci for which *Ae. tauschii* contributes the favourable alleles

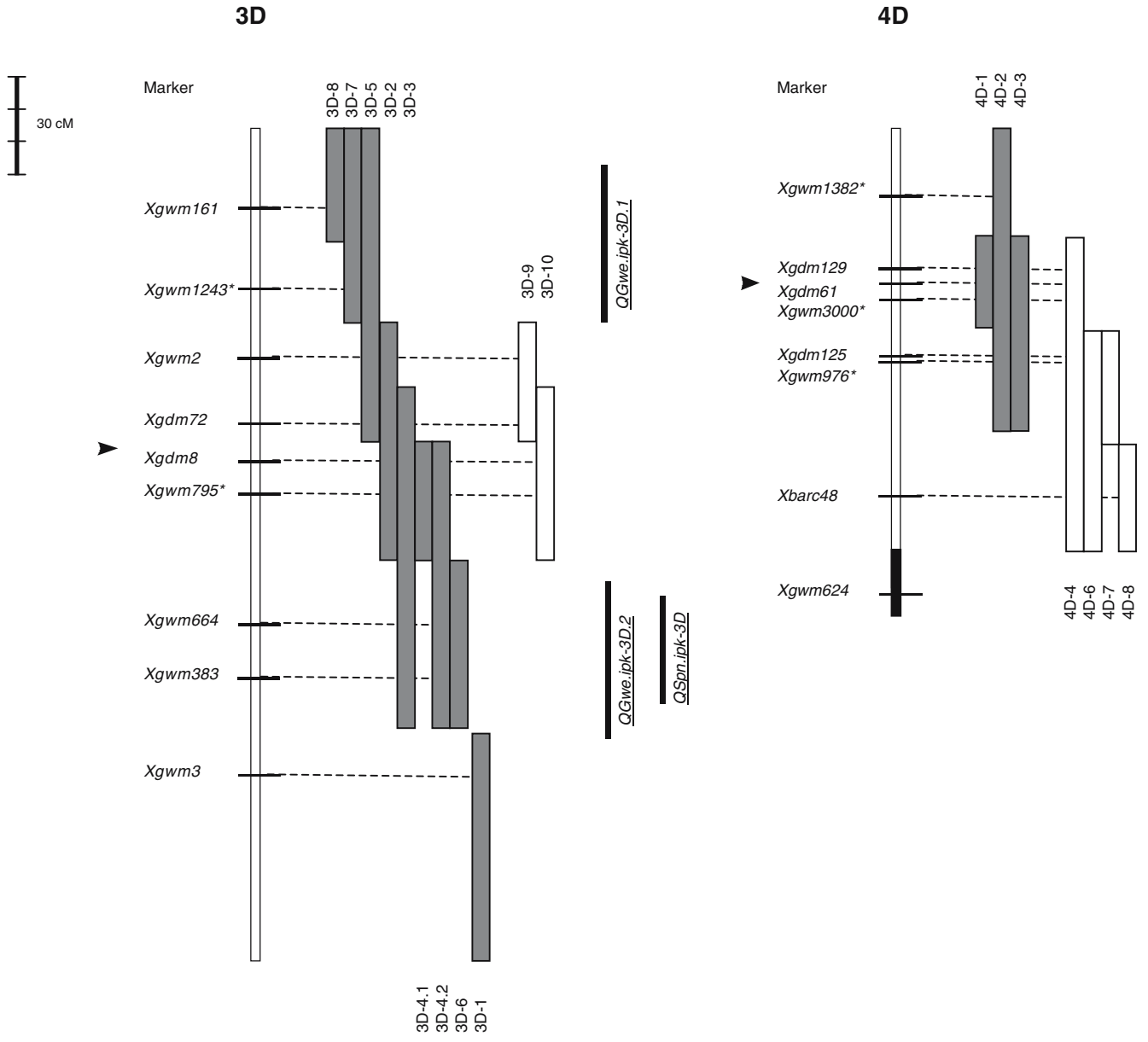


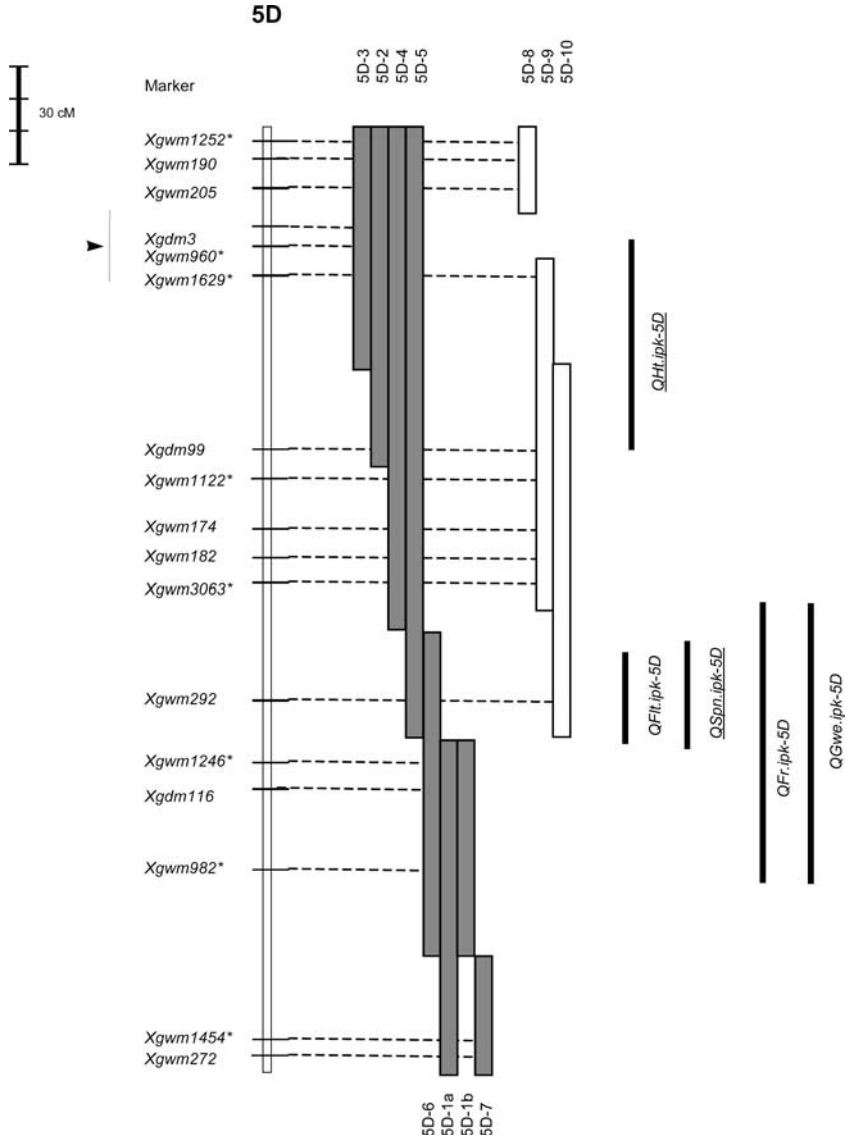
Fig. 1 (Contd.)

main spike per plant was chosen). *Fr* was calculated as the number of grains divided by the total number of spikelets per ear.

Data analysis

Statistical comparison of the measured parameters between the ILs and CS was performed by unpaired *t* test using computer software StatView 4.0 (Abacus Concept, Inc., Berkeley, CA, 1992). For each trait the coefficients of variation (CVs) within ILs were calculated by dividing the standard deviations over all plants belonging to one line by the line means. The mean of the CVs are presented in Table 2. The association between phenotype and marker genotype data was investigated using single-marker regression analysis of the QGENE software application (Nelson 1997). For any detected QTL, the additive effect (A%) was measured as the percentage of phenotypic change associated with the presence of the donor allele: A% = 100(BB-AA)/AA, where AA is the phenotypic mean of individuals homozygous for the CS alleles at the marker locus and BB is the phenotypic mean of individuals homozygous for the *Ae. tauschii* alleles at the same locus.

Fig. 1 (Contd.)



Results

Choice of informative microsatellites and verification of the substitution lines

A set of molecular markers evenly distributed over the genome is the necessary prerequisite for successful development of ILs covering the whole genome. A total of 226 microsatellite markers previously mapped on the chromosomes of the D-genome of wheat were tested for polymorphism between the parents 'Synthetic 6*x*' and CS, and 136 microsatellites (60.2%) were found to be polymorphic. These markers were used for checking the authenticity of the seven D-genome substitution lines. In all, 125 markers confirmed the presence of 'Synthetic 6*x*' alleles in the respective substitution lines. The remaining 11 microsatellites located in the distal regions of chromosome arms 1DL, 4DL and 7DS (Fig. 1) amplified the fragments specific to the recipient parent CS in the respective 1D, 4D and 7D substitution lines. Those markers were discarded from further analysis since they could not be polymorphic in the ILs.

The remaining 125 polymorphic markers were critically analysed to avoid markers amplifying many fragments, dominant (presence–absence) markers and markers difficult to score as a result of small allele size differences between the parents. Finally, a set of 80 informative and well amplifying microsatellite markers was identified (Fig. 1). The number of markers per chromosome varied from 7 to 17, with an average of 11.4 (Table 1). The mean distance between the markers was 18.7 cM. Although we tried to obtain optimal marker coverage of the D-genome, gaps of more than 50 cM were still present on the maps of five chromosomes (1D, 4D, 5D, 6D and 7D).

For substitution line development, five to eight and usually seven backcrosses to the recurrent parent are

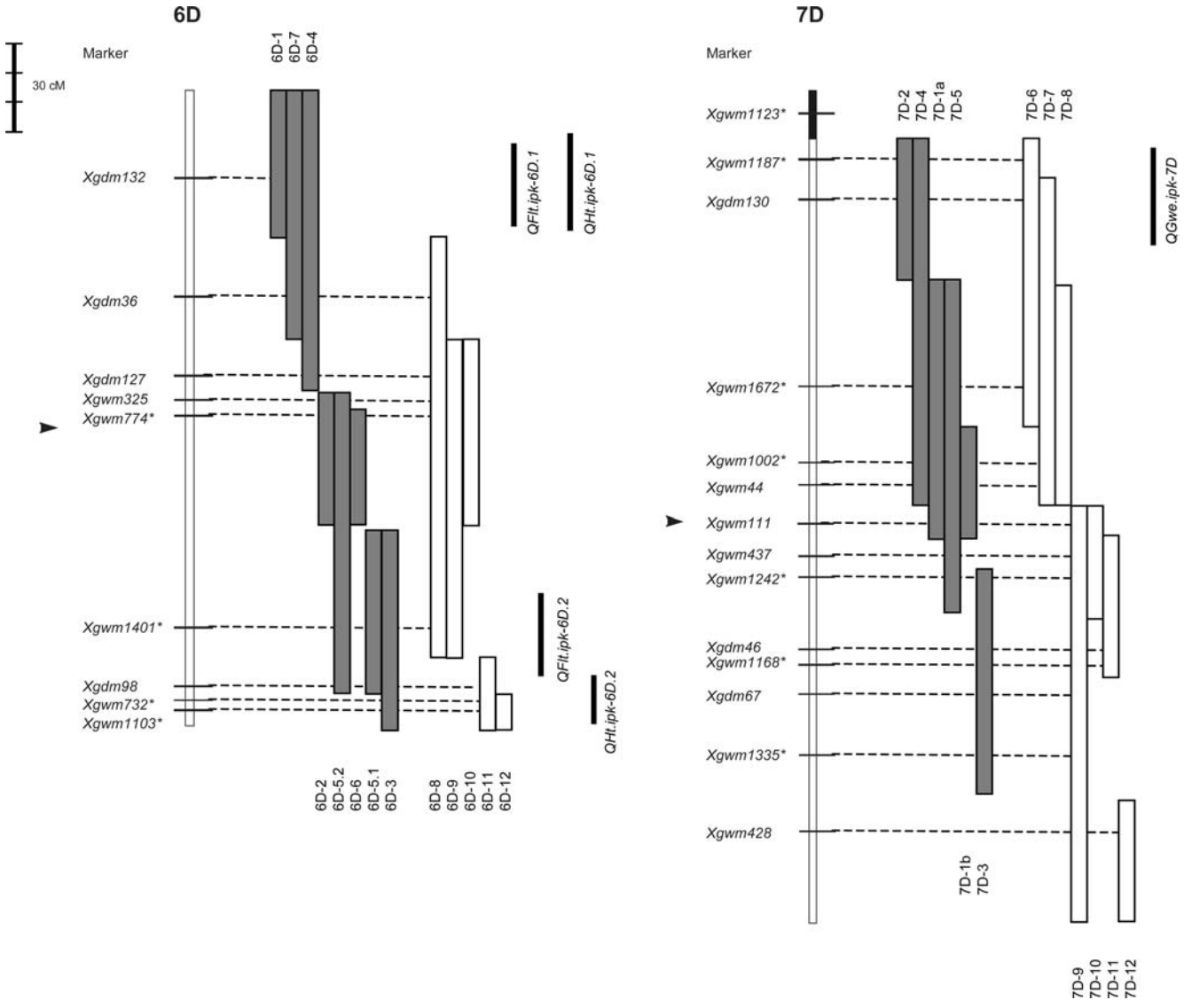


Fig. 1 (Contd.)

performed (S. Reader, personal communication). However, the developed substitution lines can still contain some residual background contamination by genetic material of a donor parent (Salina et al. 2003). In the present study, it could be alleles of 'Synthetic 6*x*' wheat (*Ae. tauschii* and tetraploid emmer alleles). In order to test the D-genome substitution lines for background contamination by *Ae. tauschii* alleles, the DNA of each line was amplified with ca. 6–9 microsatellites mapped to the unsubstituted D-genome chromosomes. No amplification of *Ae. tauschii* allele was detected. Although we did not systematically test the A and B genomes of substitution lines for background contamination by the wild tetraploid wheat alleles, our use of chromosomal substitution lines as base for IL development implies that the genetic background of the substitution and the corresponding introgression lines is much more uniform than the genetic background of any conventional population or advanced backcross population used for QTL analysis.

Characterization of introgression lines

Marker-assisted selection applied during five generations (BC1, BC2, BC1F2, BC1F3 and BC2F2) resulted in the development of a set of 84 lines containing single homozygous introgressions of *Ae. tauschii* genome in the CS background. Genetic composition of the lines, the regions of introgressions and their approximate length are shown in Fig. 1. The estimated average donor segment length was 55.5 cM or 4.3% of the total map length of the D-genome based on the D-genome size of 1289.7 cM published by Röder et al. (1998). The introgressed segments are overlapping with the exception of the region between the loci *Xgwm383* and *Xgwm3* (24 cM) on chromosome 3D. Except for this region and the telomeric segments of chromosomal arms 1DL, 4DL and 7DS, the genome of *Ae. tauschii* is fully represented in the ILs.

Phenotypic analysis

A field trial using the set of 52 homozygous ILs developed by selfing and double selfing of the BC1 was carried out during the 2002 season at Gatersleben. Six phenotypic traits directly or indirectly related to yield were evaluated (see Material and methods). The mean phenotypic values for the set of ILs did not differ sharply from the parental cultivar CS (Table 2). In general, the ILs were slightly higher than CS, had lower fertility, and had a higher spikelet number per spike probably because of the longer spike length. *Flt*, *Ht*, *El* and *Spn* showed relatively low variation over all the lines and within the individual ILs (CV within the lines up to 9.8%) while *Fr* and *Gwe* varied wider (up to 25.3% within the lines).

Phenotypic values for each individual IL were evaluated and statistically compared with the control CS. ILs differing significantly from CS were detected for all traits under consideration (Table 3). As an alternative method of analysis, the set of ILs was considered as one population and a search for putative QTLs was performed. Twenty-five QTLs with LOD scores more than

Table 2 Mean trait phenotypic values and standard deviations for parental cultivar 'Chinese Spring' and for a set of 52 introgression lines (ILs) observed in field trials 2002 at Gatersleben (Germany)

	Plant No.	Days for flowering	Plant height (cm)	Ear length (cm)	Spikelet number	Fertility	Grain weight per ear (g)
CS	10	83.1 ± 3.7	82.9 ± 10.9	7.2 ± 0.5	17.7 ± 1.8	2.5 ± 0.5	1.4 ± 0.3
52 ILs	763	84.1 ± 4.2	86.6 ± 9.0	7.5 ± 0.8	19.6 ± 2.2	2.1 ± 0.7	1.3 ± 0.4
Mean CV (%)		3.1	8.5	9.8	9.2	25.3	23.8

Mean coefficient of variation (CV) represents mean of the CVs within ILs

Table 3 Trait means for introgression lines (ILs) observed in field trials 2002 at Gatersleben (Germany) expressed as the difference in percentage from the control 'Chinese Spring'

IL	Plant No.	Flowering time (*Flt*)	Height (*Ht*)	Ear length (*El*)	Spikelet number (*Spn*)	Fertility (*Fr*)	Grain weight per ear (*Gwe*)
1D	**10**	**-0.5**	**7.1**	**15.3*** **	**11.3** **	**-1.6**	**0.7**
1D-4	30	ns	ns	ns	15.3***	ns	ns
1D-1	10	ns	ns	ns	ns	ns	ns
1D-3a	2	ns	ns	18.1**	24.3**	ns	31.7***
1D-3b	2	ns	ns	ns	21.5*	ns	13.7**
1D-2	30	ns	ns	ns	10.7***	-18.4*	ns
1D-5	25	ns	ns	16.7***	14.7***	ns	17.3*
2D	**10**	**-4.3**	**2.9**	**11.1** **	**10.7**	**-20.0***	**-4.3**
2D-7	20	ns	ns	8.3*	ns	ns	ns
2D-3	20	ns	ns	9.7***	10.2*	ns	ns
2D-4	30	-2.5*	ns	12.5***	ns	ns	ns
2D-9	10	ns	ns	ns	ns	ns	ns
2D-1.2	10	ns	ns	ns	ns	ns	17.3*
2D-1.3	2	ns	ns	ns	ns	ns	18.0***
2D-6	20	ns	ns	ns	10.2*	-12.4*	ns
2D-5	10	ns	10.4*	22.2***	22.0***	ns	18.7**

Table 3 (Contd.)

2D-2	10	ns	ns	ns	ns	ns	ns
2D-8	2	ns	ns	ns	ns	ns	ns
2D-1.1	10	ns	ns	ns	11.3*	ns	15.1*
2D-10	20	ns	ns	ns	ns	ns	ns
3D	**10**	**-1.8**	**6.0**	**11.1****	**13.6****	**10.0**	**2.2**
3D-8	10	ns	13.4*	ns	15.2**	ns	ns
3D-7	10	ns	14.2**	ns	21.5***	ns	43.2***
3D-5	10	ns	ns	ns	15.8**	ns	ns
3D-2	30	ns	ns	ns	9.6*	ns	ns
3D-3	10	ns	ns	ns	9.04*	ns	ns
3D-4.1	10	ns	ns	11.1***	13.6*	ns	ns
3D-4.2	10	ns	ns	ns	14.1*	ns	18.0*
3D-6	30	ns	ns	ns	20.9***	ns	14.4*
3D-1	10	ns	ns	8.3**	9.6*	ns	21.6**
4D	**10**	**-1.1**	**1.5**	**6.0**	**-0.6**	**-10.4**	**-6.5**
4D-1	30	ns	ns	8.3**	ns	ns	ns
4D-2	10	ns	ns	ns	11.9**	ns	14.4*
4D-3	10	ns	ns	13.9***	18.6***	ns	20.9**
5D	**10**	**22.1*****	**-**	**4.6**	**22.0***	**-90.0*****	**-86.3*****
5D-3	20	ns	ns	ns	12.4**	-29.2*	-29.5*
5D-2	40	ns	ns	ns	ns	ns	ns
5D-4	10	4.1*	-17.3*	ns	ns	-54.8***	-58.3**
5D-5	10	19.1***	-	ns	28.8***	-38.8**	-38.1**
5D-6	20	21.7***	-	ns	33.3***	-90.0***	-89.9***
5D-1a	7	ns	ns	ns	ns	-40.0***	-30.9***
5D-1b	3	-6.1*	ns	ns	ns	-32.0*	-18.7*
5D-7	10	6.8**	10.3*	ns	12.4**	-44.0***	ns
6D	**10**	**1.2**	**10.0**	**8.1**	**6.8**	**-17.6**	**-10.8**
6D-1	30	5.7***	16.2***	ns	15.8***	-24.4***	ns
6D-7	10	7.3***	11.7*	ns	14.1**	-28.4**	-22.3*

Table 3 (Contd.)

6D-4	30	ns	10.3**	ns	14.1***	-14.8*	ns
6D-2	30	ns	12.4**	ns	ns	ns	ns
6D-5.2	22	4.8**	ns	ns	67.8***	-30.8**	-25.2*
6D-6	20	ns	ns	ns	10.2*	-21.2*	-24.5*
6D-5.1	10	ns	ns	8.3*	11.9**	ns	ns
6D-3	20	5.4**	13.8**	ns	ns	-22.8**	ns
7D	**10**	**-2.8**	**1.5**	**-8.3***	**9.6***	**-16.4**	**-23.7***
7D-2	10	ns	10.3*	ns	ns	-18.4*	-28.1*
7D-4	10	6.6**	ns	ns	ns	-44.8***	-52.5**
7D-5	10	ns	ns	ns	ns	ns	ns
7D-1a	3	ns	19.1*	ns	ns	ns	ns
7D-1b	3	7.9*	ns	12.5*	ns	ns	ns
7D-3	2	ns	ns	ns	ns	ns	-28.8***

For graphical genotype of the corresponding ILs, please see Fig. 1. *Boxed areas* correlate with detected QTLs (see Table 4)
Asterisks indicate a significant difference from the control (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), *ns* nonsignificant difference from control

3.0 were found. Notably the results obtained by the direct comparison of the lines with the recurrent parent and by the QTL analysis were not completely identical mainly because different reference values served for a calculation of significance in the two methods. While the first method provides a direct comparison of each IL with CS, the QTL analysis compares the phenotypic mean of the lines carrying the *Ae. tauschii* allele at a given locus with that of the lines carrying the CS allele. Accordingly, most of the discrepancies between the methods were found for the traits *Spn* and *Fr* characterized by large differences in the mean phenotypic values between the ILs and CS (Table. 2, 3). To avoid the generation of false positive loci, the detected QTLs were assumed to be significant only if at least one IL containing an introgression in the region of the detected QTL differed significantly from the control. According to these criteria, 17 significant QTLs are described in Table 4 and their positions are presented in Fig. 1

Flowering time (*Flt*)

The parental varieties CS and 'Synthetic 6*x*' differed in their response to vernalization. CS is insensitive to vernalization and carries the *Vrn-D1a* (*Vrn3*) allele on the long arm of chromosome 5D while the synthetic wheat is sensitive to vernalization (*Vrn-D1b*). The overall range of flowering time observed across the material was 25 days. Microsatellite locus *Xgwm292* was the closest marker to *Vrn-D1* based on the map of the chromosome 5D published by Nelson et al. (1995). A major QTL for *Flt* was detected near *Xgwm292* which accounted for 69.5% of the phenotypic variation. The lines 5D-5 and 5D-6 carrying an *Ae. tauschii* allele at this locus had 16 and 18 days delay in *Flt*, respectively (19.1 and 21.7% difference from CS, $P < 0.001$; Table 3). These values are comparable with the phenotypic effect of the whole 5D chromosome substitution causing an 18-day delay in *Flt*. Three other lines with introgressions on chromosome 5D showed significant but weaker effects on *Flt*.

Besides chromosome 5D, three chromosomes, 2D, 6D and 7D, were found to be involved in the regulation of flowering time. Two QTLs accounting for 3.3 and 2.5% of phenotypic variation were detected on chromosome 6D in the distal regions of both chromosomal arms. Four lines with introgressions in chromosome 6D (6D-1, 3, 5.2 and 7) flowered 4–6 days later than CS and the regions of introgressions coincided with the position of the detected QTLs.

A significant QTL was found on chromosome 2D near the locus *Xgwm296* explaining 4.9% of phenotypic variance of *Flt*. The IL 2D-4 with an introgression of *Ae. tauschii* genetic material in the terminal region of 2DS flowered 2 days earlier than CS. The position of the detected QTL coincided with the location of the photoperiodic insensitivity gene *Ppd-D1* (Worland et al. 1998; Pestsova and Röder 2002). No significant QTL

was detected on chromosome 7D but two overlapping ILs, 7D-1b and 7D-4, showed significant delay in *Flt* (5–6 days). The detection of QTL could be hampered by the length of *Ae. tauschii* substituted segment, occupying almost the whole 7DS arm. Moreover, only three plants were available for analysis of line 7D-1b.

Plant height (*Ht*)

Three loci important for the determination of plant height were revealed on the chromosomes 5D and 6D. Two significant QTLs for *Ht* detected on chromosome 6D explained together 19.3% of phenotypic variation. Five ILs (6D-1, 2, 3, 4 and 7) were significantly higher than CS; in four of them the regions of introgressions coincided with the position of the detected QTLs. A height effect was also found for the whole chromosome substitution, but the difference of 10% between substitution line 6D and CS did not reach the significance level. Possibly the region between loci *Xgdm127* and *Xgwm325* not covered by the ILs carries *Ae. tauschii* alleles reducing height.

Although phenotypic data for several lines with introgressions on the chromosome 5D were missing, an effect of this chromosome on *Ht* was detected. Locus *QHt.ipk-5D* in the proximal region of 5DL explained 14.5% of phenotypic variation in plant height. The corresponding IL 5D-4 was on average 17.3% shorter than the control CS.

Two ILs, 3D-7 and 3D-8, carrying overlapping introgressions in the distal region of 3DS, were 13–14% taller than CS. At the same time, no effect was detected for another overlapping line 3D-5. We speculate that the line 3D-5 carries an undetectable recombination site distal to marker *Xgwm161* and does not include the locus influencing *Ht*. Only a minor QTL (LOD 2.0) was detected in the corresponding region of 3DS. Four ILs (2D-2, 5D-7, 7D-2 and 7D-1a) with significant differences from CS ($P < 0.05$) did not correlate with any of the detected QTLs.

Ear length (*El*)

Ae. tauschii contributed favourable alleles for *El*. Three substitution lines (1D, 2D and 3D) and 12 ILs showed significantly longer *El* than CS. Only substitution line 7D revealed an opposite effect, with slightly shorter ears ($P < 0.05$). QTL analysis revealed just one significant QTL for *El*, lying on the short arm of chromosome 2D (*QEl.ipk-2D*) and explaining 7.6% of the trait phenotypic variation. The QTL was confirmed by three ILs (2D-3, 2D-4 and 2D-7).

Two non-overlapping ILs for chromosome 1D (1D-3a and 1D-5) had significantly longer ears than CS though the line 1D-3a represented by two plants should be considered with caution. An overlap of the significant line 1D-5 with the non-significant line 1D-2 at the bottom of the chromosome could be explained either by deleterious alleles of *Ae. tauschii* in the proximal part of the 1D-2 introgression or by an undetectable recombination distal to *Xgwm1230* in line 1D-5 resulting in an absence of the locus influencing *El*. Putative QTL at the bottom of 1D would explain also a high *El* detected for substitution line 1D. Two lines carrying introgressions on 3D (3D-1 and 3D-4.1) had significantly longer ears than CS. Further experiments are needed for precise mapping of the QTL influencing *El* on the chromosome. A negative effect of substitution of chromosome 7D on *El* was not confirmed by any IL, suggesting that the locus influencing the traits might occur in one of the terminal parts of the chromosome not represented in the lines.

Spikelet number (*Spn*)

Four substitution lines (1D, 3D, 5D and 7D) and 30 ILs had more spikelets per spike than CS, meaning that *Ae. tauschii* contributed favourable alleles for the trait. As expected from the plant architecture, *Spn* and *El* were positively correlated ($r = 0.44$, Table 5). Despite this correlation the major QTLs for *Spn* differed from those for *El*.

A major QTL for *Spn* with an estimated phenotypic effect of 24.7% was detected on chromosome 5D and supported by two ILs, 5D-5 and 5D-6. A second significant QTL explaining 3.9% of the phenotypic variation was found on 3DL and confirmed by three ILs, 3D-3, 3D-4.2 and 3D-6. Another minor QTL at the top of chromosome 3D (see phenotypic values of the lines 3D-5, 3D-6 and 3D-7, Table 3) was detected with LOD score 1.9 and not included in Table 4. Interestingly, the combined magnitude of the two 3D QTL effects was larger than the magnitude of the whole chromosome effect, which could be explained by possible epistatic interaction between the QTLs or presence of undetectable deleterious alleles in the IL regions of the chromosome badly covered by markers (the region *Xgwm383–Xgwm3* and the distal part of 3DL). Saturation of the ILs with new molecular markers, development of new lines for the *Xgwm383–Xgwm3* gap and further phenotypic characterization of the ILs should help us distinguish between the two hypotheses.

Five ILs carrying introgressions on chromosome 1D showed significantly higher *Spn* than CS. These overlapping ILs covered the whole chromosome and so no distinct QTL could be assigned to the chromosome. A similar situation was observed for chromosome 6D where six ILs differed significantly from CS but no QTL with a LOD score above 3.0 could be found. In contrast to substitution line 7D, neither of the 7D-ILs showed significant effects on *Spn*. Additional ILs with introgressions on the distal part of 7D need to be analysed to explain this phenomenon.

Table 4 List of QTLs detected for six phenotypic traits, microsatellite markers most closely associated with the QTLs, QTL significance thresholds (LOD scores), phenotypic variances (%PV), and additive effects (%A)

Trait	QTL	Marker	LOD	%PV	A% 100 (BB-AA)/AA	Genes mapped in the region	QTLs mapped in the same or homoeologous region	QTL reference
Flowering time	*QFlt.ipk-5D*[a]	*Xgwm292*	61.0	69.5	20.8	*Vrn-D1*	5DL	Börner et al. (2002)
	QFlt.ipk-2D	*Xgwm296*	7.5	4.9	−4.1	*Ppd-D1*	2DS	Börner et al. (2002)
	QFlt.ipk-6D.1[c]	*Xgdm132*	6.8	3.3	3.5	–	–	–
	QFlt.ipk-6D.2	*Xgwm1401*	5.0	2.5	3.3	–	6AL	Huang et al. (2003)
Plant height	*QHt.ipk-6D.1*[d]	*Xgdm132*	11.7	14.5	8.8	–	6AS	Börner et al. (2002)
	QHt.ipk-6D.2	*Xgwm1103*	3.8	4.8	7.4	–	–	–
	QHt.ipk-5D[c]	*Xgdm99*	11.6	14.5	−10.9	–	–	–
Ear length	*QEl.ipk-2D*[c]	*Xgwm1099*	4.6	7.6	6.0	*Ppd-D1*	2DS, 2BS	Börner et al. (2002); Sourdille et al. (2003)
Spikelet number	*QSpn.ipk-5D*	*Xgwm292*	20.9	24.7	17.6	*Vrn-D1*	5AL	Sourdille et al. (2003)
	QSpn.ipk-3D[c]	*Xgwm383*	3.2	3.9	5.7	–	–	Peng et al. (2003)
Fertility	*QFr.ipk-5D*[a,b,c]	*Xgwm982*	60.9	53.1	−74.0	*Vrn-D1*	–	–
Grain weight per ear	*QGwe.ipk-5D*[a,c]	*Xgwm982*	61.0	41.7	−72.8	*Vrn-D1*	–	–
	QGwe.ipk-7D	*Xgwm1187*	7.5	4.5	−30.3	–	7DS	Huang et al. (2004)
		Xgwm106	6.2	3.6	20.2	*Gli-D1,*	–	–
	QGwe.ipk-1D	*Xgwm30*	5.7	3.3	18.0	*Glu-D1*	2DL	Börner et al. (2002)
	QGwe.ipk-2D[c]	*Xgwm383*	5.9	3.5	20.2	–	–	–
	QGwe.ipk-3D.1	*Xgwm161*	4.8	2.8	21.7	–	3BL	Huang et al. (2004)
	QGwe.ipk-3D.2					–		

Known genes and QTLs mapped to the same or homoeologous chromosome regions are shown
QTLs also observed in [a]BC1, [b]BC2, [c]BC1F2 and [d]BC2F2

Fertility (*Fr*)

Neither a substitution nor an introgression line was detected with increased fertility compared to CS, though many lines had the same *Fr* as the control. A strong decrease in *Fr* up to 90% was caused by *Ae. tauschii* introgressions on chromosome 5D; sterile plants were found among ILs 5D-3, 5D-4, 5D-5 and 5D-6. Interestingly, low *Fr* in lines 5D-5 and 5D-6 could be explained by a negative effect of the *Vrn-D1b* gene, while that in lines 5D-1a, 5D-1b and 5D-7 was a result of the influence of an unknown gene. One very significant QTL on chromosome 5D accounted for 53.1% of phenotypic variation. Six lines carrying partly overlapping introgressions on chromosome 6D and two lines having overlapping introgressions on 7D showed significantly decreased *Fr*, but those effects were not detectable by QTL analysis.

As mentioned above, *Fr* and *Spn* were among the traits showing a large difference between the mean trait values of the whole IL set and the CS control, so that comparison of individual IL means with CS means frequently gave different results than regression of phenotype on marker genotype. In general, the first method was more sensitive and allowed the detection of more genetic loci involved in the trait regulation. Further experiments will be done in order to verify the significance of the loci.

Grain weight per ear (*Gwe*)

Five chromosomes were found to be involved in the regulation of the yield component *Gwe*. Table 5 shows that *Gwe* was strongly correlated with *Fr* ($r = 0.70$). Consistent with this correlation, the major QTL for *Gwe* on the chromosome 5D coincided with the major QTL for *Fr* and covered the position of the vernalization response gene. *Ae. tauschii* alleles at this locus caused strong negative effects on the trait and explained as much as 41.7% of the phenotypic variation.

Introgressions of *Ae. tauschii* alleles on chromosome 7D also had negative effects on *Gwe*, *QGwe.ipk-7D* explained 4.5% of the trait phenotypic variation. Positive effects of *Ae. tauschii* alleles were detected for chromosomes 1D, 2D and 3D. The location of *QGwe.ipk-1D* with an estimated QTL effect 3.6% was confirmed by two ILs, 1D-3a and 1D-3b, and coincided with the position of glutenin and gliadin loci. The QTL on 2D supported by 2D-1.2, 2D-1.3 and 2D-2 and explaining 3.3% of phenotypic variation was detected earlier in the BC2 population grown under greenhouse conditions. Two QTLs with the combined effect of 6.3% were found on 3D. Several single ILs significantly differing from CS did not correspond to any QTL while overlapping ILs (4D-2 and 4D-3; 6D-5.2 and 6D-6) coincided with two minor QTLs (LOD < 3.0).

Table 5 Correlation coefficients between traits (only correlation coefficients detected with $P < 0.001$ are shown)

	Flt	*Ht*	*El*	*Spn*	*Fr*
Ht	0.20				
El	−0.13	0.16			
Spn	0.18	0.26	0.44		
Fr	−0.48	–	0.27	−0.2	
Gwe	−0.49	–	0.21	–	0.70

Discussion

A total of 84 bread wheat lines containing single introgressions of *Ae. tauschii* genome was produced. The data presented in the paper will be incorporated into the public database (http://www.wheat.pw.usda.gov) in the nearest future. The number of lines developed per chromosome varied from 7 for chromosome 4D to 20 for chromosome 2D and in general corresponded to the number of markers used for chromosome genotyping (Table 1). The average introgression length was 55.5 cM, greater than the 46.0 and 48.1 cM of barley ILs (von Korff et al. 2004), the 38.6 cM of barley recombinant chromosome substitution lines (RCSLs, Matus et al. 2003) and the 33 cM of tomato ILs (Eshed and Zamir 1995). The larger insert size compared to barley is explained by the line developmental scheme. Around two-thirds of our ILs were produced via selfing of the BC1 progeny and the rest via selfing of the BC2, while barley ILs and RCSLs were represented by BC2DH and BC2F7 lines, respectively.

Previously, it was reported for tomato that the population comprising of 50 overlapping ILs was suitable for mapping of yield-associated QTLs (Eshed and Zamir 1995). In the present study, 52 homozygous ILs developed from BC1 were tested in the field. In several cases, the introgressions of the lines were not overlapping, gaps with maximal sizes of 46.1, 24.1 and 8.7 cM being present on chromosomes 2D, 3D and 6D, respectively. Also, the distal parts of chromosome arms 4DL and 7DL of *Ae. tauschii* represented by markers *Xbarc48* and *Xgwm428* were absent in the ILs (Fig. 1). The best coverage was reached for chromosomes 1D and 5D by six and eight overlapping lines, respectively, and the worst was for chromosome 4D represented by three lines.

We were able to detect 17 significant QTLs with LOD scores above 3.0 whose positions and effects were confirmed by the corresponding ILs. Six of the detected loci explained more than 10% of phenotypic variation of the traits and could be considered as major QTLs. Four major QTLs clustered together and coincided with the position of the vernalization response gene *Vrn-D1b*. The *Ae. tauschii* allele of the gene (*Vrn-D1b*) determined late flowering of the substitution line 5D and the corresponding ILs having introgressions on 5D. The same lines were found to have higher *Spn*, suggesting a pleiotropic effect of *Vrn-D1b* on *Spn*. However, a strong negative effect of wild alleles on yield parameters *Fr* and *Gwe* could not be entirely explained by late flowering time and pleiotropic effects of the *Vrn-D1b* gene since low *Fr* and *Gwe* were observed also in the control greenhouse conditions where late flowering should not limit seed formation. Moreover, two ILs, 5D-1a and 5D-4, carrying the *Vrn-D1a* allele of the genes showed very low yield parameters, suggesting that chromosome 5D of *Ae. tauschii* contains other yield deleterious alleles located in the vicinity of *Vrn-D1b*. Multiple QTLs on 5DL permit us to attribute this locus to domestication syndrome factor (DSF) which is a genomic region influencing multiple domestication-related traits (Peng et al. 2003). Homoeologous DSF was detected on the chromosome 5A of wild tetraploid wheat *Triticum dicoccoides* (Peng et al. 2003).

Clustering of QTLs was observed also on chromosomes 2D, 3D and 6D. The phenomenon has been reported for many cereals (Doebley and Stec 1993; Paterson et al. 1995; Poncet et al. 2000; Peng et al. 2003; Sourdille et al. 2003; Huang et al. 2004) and can be explained either by linkage of several genes or by pleiotropy of one gene. The generation of ILs with shorter introgressions may help distinguish between these two possibilities. The power of this approach was demonstrated by fine mapping of a 9 cM introgression, originating from the wild tomato species *L. pennellii*, which contained a major QTL for fruit Brix. As a result, the QTL position was delimited first to 484 bp within an invertase gene (Fridman et al. 2000) and then to a single nucleotide polymorphism within the gene (Fridman et al., 2004). Additively a second QTL for an altered growth habit influencing Brix was detected 0.3 cM apart from the first one (Fridman et al. 2002).

Recently, the potential of *Ae. tauschii* for improvement of quantitative traits in bread wheat was studied by Börner et al. (2002) and Huang et al. (2003, 2004). The first two studies used the same source of wild alleles, synthetic wheat W-7984, the parent of the ITMI (International Triticeae Mapping Initiative) population. Huang et al. (2004) reported the analysis of synthetic wheat XX86 originating from another *Ae. tauschii* accession. In the present study a third different source of wild alleles was investigated. Five identified QTLs were probably orthologous to the published ones thus reflecting common features of the *Ae. tauschii* species. In total, nine loci could be assigned to known wheat QTLs detected at the same or homoeologous chromosome positions (Table 4). Eight QTLs were new and probably specific for the *Ae. tauschii* accession analysed.

An increasing number of publications report the finding of beneficial alleles in wild relatives of cultivated species where they are hidden in a sea of deleterious alleles (Fulton et al. 2000; Poncet et al. 2000; Börner et al. 2002; Huang et al. 2003; Peng et al. 2003: Matus et al. 2003). In our study, 9 out of 17 QTLs revealed beneficial effect from *Ae. tauschii* alleles if earliness and short stature were considered as positive effects. Four of those loci represented novel QTLs. Two genomic regions were of special interest since they combined favourable QTLs originating from the wild goatgrass. *Ae. tauschii* alleles on chromosome arm 2DS contributed earliness and higher *El*, while chromosome arm 3DL carried favourable QTLs for *Gwe* and *Spn*. Unfortunately, all detected beneficial loci represented minor QTLs that make them unattractive for fine mapping. One of the possible reasons for the finding of minor favourable loci alone is the long size of introgressions: beneficial alleles of *Ae. tauschii* could be still masked by many deleterious

alleles located on the same chromosomal segment. If this is the case, the development of sub-NILs could reveal beneficial QTLs with stronger effects on the traits. Additive experiments for validation of QTL effects and stability should be performed before any fine mapping experiments are started.

It would be interesting to analyze the baking quality properties of the lines with introgressions on 1DS where the QTL for *Gwe* was detected. Some investigations have shown that the HMW glutenin genes from *Ae. tauschii* have a significant influence on bread-making properties in synthetic hexaploid wheats (Lagudah et al. 1987; Hsam et al. 2001; Wieser et al. 2003), and some of the synthetic wheats exhibited a shorter mixing time and improved milling and baking characteristics when compared to parental lines (Tilley et al. 2000; Yan et al. 2004).

It should be noted that the number of QTLs detected using the set of 52 ILs is possibly underestimated because of insufficient genome coverage. Further experiments with the whole set of 84 lines will improve QTL detection. Besides the assessment of QTLs for morphological and yield-associated traits, the set of developed ILs can be used for analysis of the influence of *Ae. tauschii* alleles on grain protein content and bread-making properties, biotic (pest and disease) and abiotic (drought and salt) stresses. The introgression of new genetic material will contribute in the long-term to the broadening of the genetic base of elite germplasm.

Acknowledgements This work was supported by a grant of the Deutsche Forschungsgemeinschaft (grant no. Ro1055/1-5). We thank Renate Voß, Ellen Weiß, Bärbel Apel, Angelika Flieger and Sonja Allner for their excellent technical assistance.

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