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Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.)

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Abstract Advanced backcross QTL (AB-QTL) analysis was used to identify quantitative trait loci (QTLs) for yield and yield components in a BC_2F_2 population derived from a cross between the German winter wheat variety 'Prinz' and the synthetic wheat line W-7984 developed by CIMMYT. Two hundred and ten microsatellite markers were employed to genotype 72 pre-selected BC_2F_2 plants and phenotypic data were collected for five agronomic traits from corresponding BC_2F_3 families that were grown at four locations in Germany. Using single-marker regression and interval mapping, a total of 40 putative QTLs derived from W-7984 were detected, of which 11 were for yield, 16 for yield components, eight for ear emergence time and five for plant height. For 24 (60.0%) of them, alleles from the synthetic wheat W-7984 were associated with a positive effect on agronomic traits, despite the fact that synthetic wheat was overall inferior with respect to agronomic appearance and performance. The present study indicated that favorable QTL alleles could be transferred from wild relatives of wheat into an elite wheat variety for improvement of quantitative trait loci like yield by the advanced backcross QTL strategy and molecular breeding. To our knowledge, the results presented here were the first report on AB-QTL analysis in wheat.

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Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important crops in the world. It is an allohexaploid carrying the genomes AABBDD (2n = 6x = 42). Wild emmer wheat, *Triticum dicoccoides* Korn (2n = 28, AABB) and diploid *Aegilops tauschii* (2n = 14, DD) are the tetraploid progenitor and the D-genome donor of cultivated hexaploid wheat, respectively (Kihara 1944). Many genes for resistance to leaf rust, strip rust, stem rust and powdery mildew have been transferred from relatives of wheat such as durum wheat and *Aegilops tauschii* into bread wheat (Knot 1989; Lutz et al. 1995). For increasing yield, however, wild relatives were not employed as a potential source for favorable alleles to-date, since crosses were usually performed within the gene pool of high yielding varieties. This kind of modern breeding has led to a narrowing of genetic diversity by concentrating favorable alleles. So far, no attempt has been made to utilize wild and unadapted germplasm for improvement of yield in wheat, because agriculturally desirable alleles for yield are often masked by the effects of deleterious alleles and hence cannot be identified phenotypically in the wild germplasm (Xiao et al. 1998).

Grain yield and its component traits, such as ear number per plant, ear grain weight, spikelet number per ear, thousand-grain weight and plant height, are generally controlled by a number of quantitative trait loci (QTLs). Molecular markers and maps allow one to detect QTLs controlling traits for yield and the relationship between grain yield and its components. Many scientists have reported on mapping QTLs for grain yield and its components by using various segregating populations such as F_2, recombinant inbreeding lines (RILs) and a double-haploid (DH) population in diploid crop plants like rice (Lin et al. 1996; Xiao et al. 1996), barley (Bezant

et al. 1997) and maize (Veldboom and Lee 1994; Austin and Lee 1996). In hexaploid wheat, however, QTL analyses of grain yield and its components using molecular marker systems are limited (Hyne et al. 1994) because RFLP (Chao et al. 1989) and RAPD markers (Devos and Gale 1992) show a low level of polymorphism in wheat, especially in a cross between two cultivars and, hence, detected polymorphic markers cannot cover the entire genome. Several studies concerning mapping QTLs for grain yield and its components on chromosomes 3A (Shah et al. 1999), 4A (Araki et al. 1999) and 5A (Kato et al. 2000) of wheat have been recently reported by using recombinant substitution lines.

Microsatellite markers, also termed simple sequence repeats (SSRs) in wheat, were chromosome-specific and evenly distributed along chromosomes (Röder et al. 1998a, b). Such markers reveal a higher level of polymorphism than RFLP markers. Microsatellite markers have been widely used for tagging resistance genes (Peng et al. 1999; Huang et al. 2003), marker-assisted selection in wheat (Huang et al. 2000a), and assessment of genetic diversity in closely related bread wheat (Plaschke et al. 1995) and a large number of wheat accessions (Huang et al. 2002). A large number of wheat microsatellite markers recently developed by Röder et al. (1998b, unpublished data; Pestsova et al. 2000; Huang et al. 2001) enables the identification of QTLs for yield and its components in wheat.

Advanced backcross (AB) QTL analysis has been proposed as an approach for combining marker-based QTL discovery with elite variety improvement (Tanksley and Nelson 1996). This strategy has been successfully applied in detecting and transferring valuable QTLs from unadapted germplasm into elite breeding lines for several crop plants like tomato (Tanksley et al. 1996; Bernacchi et al. 1998) and rice (Xiao et al. 1998; Moncada et al. 2001). Here, we report for the first time an AB-QTL analysis in winter wheat. The objectives of the present study were to detect and map QTLs controlling grain yield and its components using a BC_2F_2 population derived from a cross between the German winter wheat variety 'Prinz' as the recurrent parent and a synthetic wheat W-7984 as donor.

Materials and methods

Plant materials

The German winter wheat cultivar 'Prinz' and a synthetic hexaploid wheat, W-7984, were used as the recurrent and donor parent in this study, respectively. W-7984 was produced from the cross between durum wheat (*Triticum turgidum* L.) cultivar 'Altar 84' and *Triticum tauschii* CIMMYT accession WPI 219, as described by Nelson et al. (1995a). This line was used as a parent to develop the International Triticeae Mapping Initiative (ITMI) population 'Opata' × W-7984 for mapping of RFLP markers (Nelsen et al. 1995a, b; Marino et al. 1996) and microsatellite markers (Röder et al. 1998b).

Population development

W-7984 was crossed as the female parent to 'Prinz'. F_1 plants were grown in the greenhouse in Monsanto GmbH, Silstedt, and the six most vigorous F_1 plants were backcrossed to 'Prinz' (as the male). Ninety five BC_1F_1 plants were obtained, which were grown in the field. The best 42 individuals based on phenotypic selection were backcrossed a second time to 'Prinz' to produce approximately 164 BC_2F_1 seeds. They were grown in the field to produce BC_2F_2 and subsequently BC_2F_3 families. The best 72 BC_2F_3 families were selected for measurement of agronomic traits.

Field trials and trait evaluation

The field trials were conducted in the year 2001 at four different locations spread over the north and south of Germany: Wetze (WE), Böhnshausen (BÖ), Moosburg (MO) and Herzogenaurach (HA). A complete randomized block design was used for the field trials. The 72 BC_2F_3 families were sown in two replications at WE, BÖ and MO, and with one replication in HA. Each family was grown in 5.9 m × 1.25 m plots. In total, 190 kg of urea (nitrogen)/ha were applied at five different growth points. All trials were kept free of weeds and diseases, with two applications of broad-range herbicides and fungicides, respectively.

Grain yield per plot (YLD) was evaluated based on the grain harvest from all plants in each plot. Ear emergence time (EET) was evaluated based on morphological characters in each plot. Plant height (HT) was calculated as the average height of ten plants in cm from the soil surface to the tip of the spike (awns excluded). Tiller number per m^2 (TN) was calculated as the spike number of 1 square meter from each plot. Thousand-grain weight was measured in grams as the average weight of two different samples of 1,000 grains from each plot.

Trait correlations and analysis of variance (ANOVA)

Correlations between traits were calculated for each trait/location combination based on the field data using the QGene software (Nelson 1997).

Using the package Minitab for Windows (MINITAB Inc., State College, Pa.), one-way ANOVA was performed to determine the significances of differences between the genotypes of the population lines and between the locations (environments).

Microsatellite marker analyses

DNA from the two parents 'Prinz' and W-7984 was investigated for polymorphism using microsatellite markers. The order and distribution of the microsatellite markers were based on the ITMI population of wheat described by Röder et al. (1998b; unpublished data). Segregation ratios of individual markers were statistically determined for each marker locus and deviations from the expected ratios were determined using the chi-square (χ^2) test. Six plants from each of 72 BC_2F_3 families were bulked for DNA extraction. Total genomic DNA was extracted from young leaf tissue, frozen in liquid nitrogen, as previously described by Huang et al. (2000b). Polymorphic microsatellite markers were used for genotyping. PCR reactions of microsatellite markers were performed according to Röder et al. (1998b). Microsatellite fragments were detected on an automated laser fluorescence (A.L.F.) sequencer and analysed using the computer program Fragment Analyser Version 1.02 (Pharmacia) by comparison with the internal size standard (Röder et al. 1998b).

QTL analysis

The software Qgene developed by Nelson (1997) was used for QTL analysis. The association between phenotype and marker genotype

was investigated using single-marker regression. The positions of detected QTLs were determined using interval mapping. Each trait/location combination was treated separately. According to Fulton et al. (1997a, 2000) and Tanksley et al. (1996), regions of the genomes were identified as putatively containing a QTL if the results met one or more of the following criteria: a significant effect was observed for a single marker/trait combination at a single location with $P < 0.001$; significant effects were observed in the same direction (i.e. either all positive effects or all negative effects) for a single marker/trait combination at two or more locations with $P < 0.01$; significant effects were observed in the same direction for a single marker/trait combination at three or more locations with $P < 0.1$.

The percent phenotypic variation (%PV) associated with each significant QTL was calculated from the regressions of each marker/phenotype combination. The percent phenotypic change (A%) of each significant QTL, associated with the presence of the donor allele at a given marker locus, was estimated as A% = 100(AB-AA)/AA (Fulton et al. 1997a, 2000), where AA is the phenotypic mean for the individual homozygous for Prinz alleles at specified markers and AB is the phenotypic mean for the heterozygotes Prinz/W-7984.

Results

Microsatellite polymorphism and marker segregation

Out of 298 microsatellite markers, 210 (70.5%) that detected polymorphism between the recurrent parent 'Prinz' and the donor parent W-7984 were used to genotype the BC_2F_2 population. These SSR markers covered the whole genome of wheat. The distribution of the markers is shown in Fig. 1. The highest polymorphism with 84 markers was found in the D genome, followed by the B genome (69) and the A genome (57). On average, there were ten markers on each chromosome, varying from six markers on chromosomes 4B, 4D and 5A to 15 markers on chromosome 7D. Large gaps (more than 45 cM) remained on chromosomes 2B, 3B, 3D, 4D, 5A, 5D and 6A (Fig. 1). The average number of heterozygotes per locus was 18.9%, close to the expected 25% heterozygotes in a BC_2F_2 population. The segregation ratio at 32 loci (15.2%) deviated from the expected ratio ($\chi^2 < 3.84$, $P < 0.05$). Twenty four loci were skewed towards W-7984, while eight loci deviated towards 'Prinz' of which six loci were on chromosome 4B. This may be the result of the low recombination frequency on chromosome 4B as suggested by Huang et al. (2002).

Correlations between traits and ANOVA for genotypes and environments

Correlation coefficients between traits were calculated separately for each location (Fig. 2). No clear correlations were found between total yield and its components for four locations. It appears that ear emergence time was not correlated to total yield. A significant positive correlation between plant height and total yield was found for one location (BÖ). Total yield showed a negative correlation with thousand-grain weight in MO, but a significant positive correlation in HA. There was a significant negative correlation between total yield and thousand-grain weight averaged for four locations ($r = –0.41$, $P < 0.001$). Thousand-grain weight was significantly correlated with plant height at four locations.

The *F*-value of ANOVA for the genotypes and environments is presented in Table 1. When ANOVA for total yield was performed in four locations, there was no significant difference between total yield of the population lines but significant differences between the four locations ($F = 76.65$, $P < 0.0001$). It appeared that the environment had a great affect on total yield. The reason was that the field trial in HA was performed under non-uniform soil conditions. When ANOVA for total yield was carried out for the other three locations without HA, significant differences between the total yield of the population lines ($F = 7.35$, $P < 0.0001$), and no significant differences between the three locations, were observed. Significant differences were found for ear emergence time as well as plant height. Differences between thousand-grain weight (TGW) of the population lines were not significant, but differences for TGW between the four locations were highly significant ($F = 249.19$, $P < 0.0001$). There were significant differences between tiller number/m^2 of the population lines ($F = 1.66$, $P < 0.05$) and no significant differences between the two locations, WE and MO.

QTLs detection

Putative QTLs for each trait are listed in Table 2 and their map positions are shown in Fig. 1. As stated in the methods section, criteria for the definition of a QTL were set in accordance with Tanksley et al. (1996) and Fulton et al. (1997a, 2000). Based on these criteria, a total of 40 putative QTLs were identified, ranging from 5 to 11 QTLs for each trait.

Total yield

Eleven QTLs were detected for total yield, explaining from 9.6% to 21.6% of the phenotypic variance with a LOD of 1.6 to 3.8. For seven QTLs, the Prinz allele increased total yield. For four QTLs, *QYld.ipk-1B*,

Table 1 The *F*-value of ANOVA for genotype and environment in the BC_2F_2 population of the cross Prinz × M6

Item	Genotype		Environment	
	df	*F*-value	*df*	*F*-value
Total yield	71	0.93 ns	3	76.65****
Total yield without HA	71	7.35****	2	1.30 ns
Ear emergence time	71	3.67***	3	38.45****
Plant height	71	2.01***	3	74.06****
Thousand-grain weight	71	0.53 ns	3	249.19****
Tiller number/m^2	71	1.66*	1	0.89 ns

Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

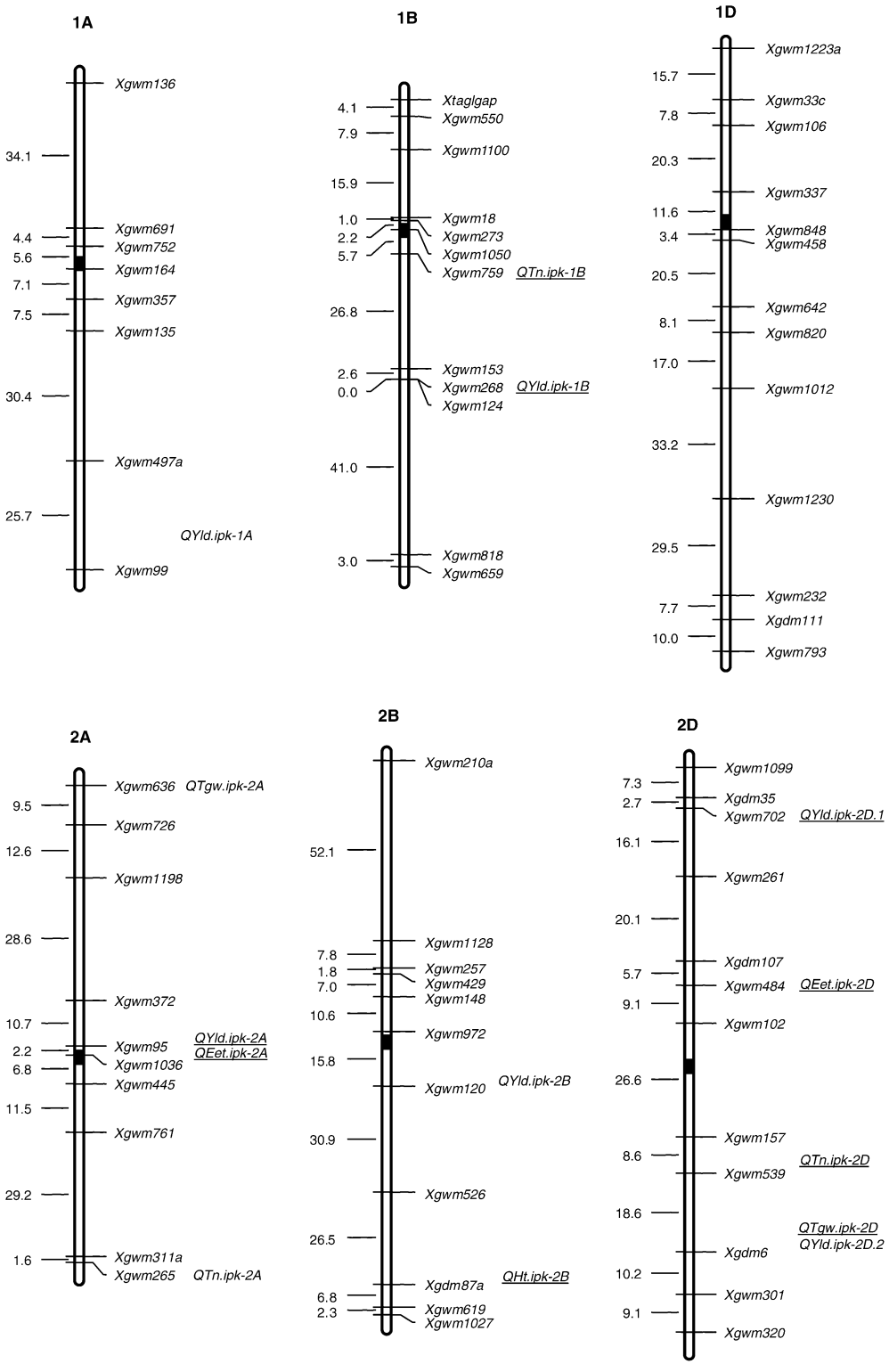


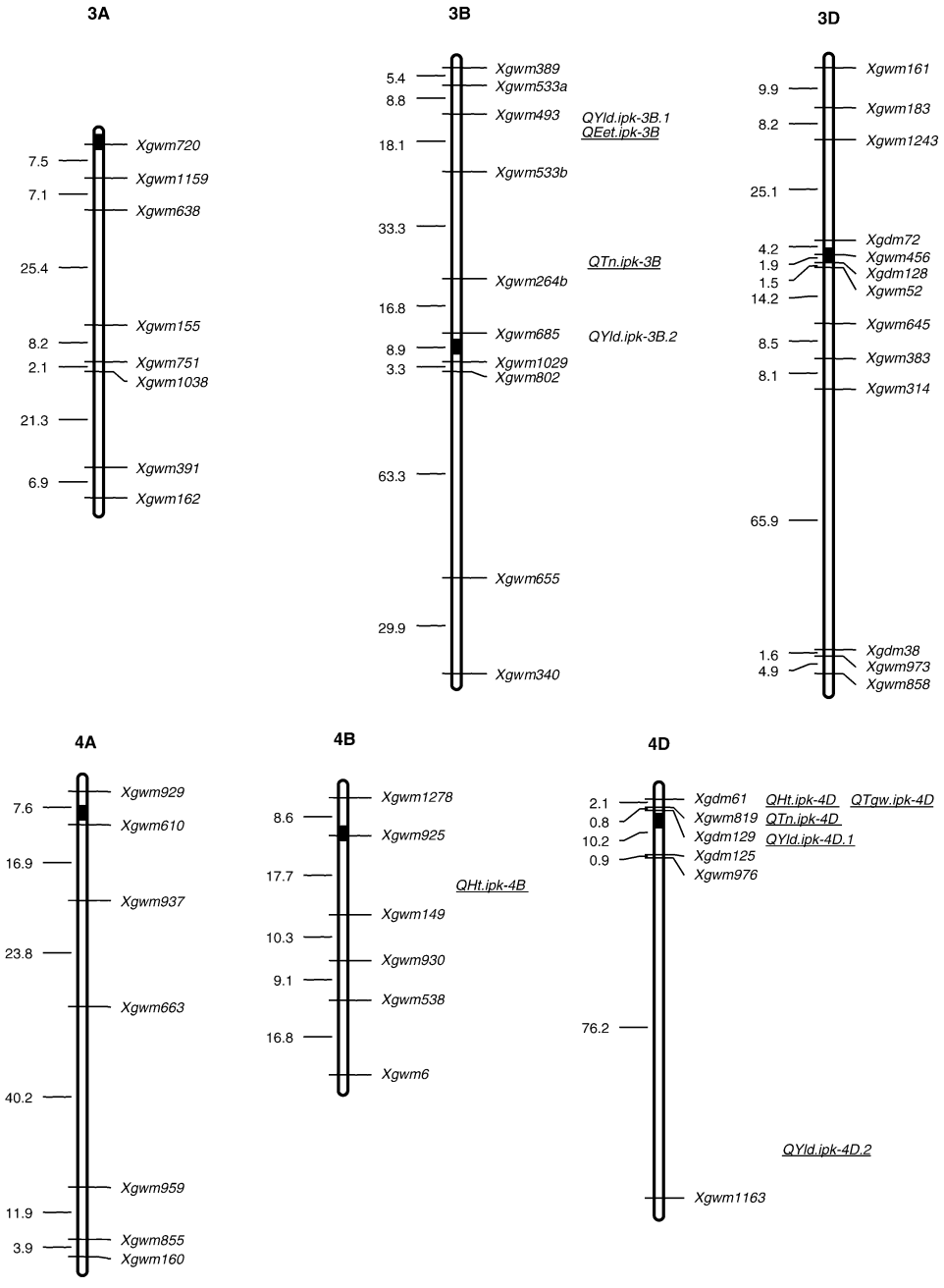
Fig. 1 Linkage map of microsatellite markers used for BC_2F_2 QTL analysis. The marker order and relative distances (in Kosambi mapping units) are based on the ITMI population. The centromeres are indicated in *black*. Putative QTLs are shown on the right side. *Underlined QTL*, the allele from synthetic wheat W-7984 is favourable for the traits. Abbreviations for traits: *Eet* = ear emergence time, *ht* = plant height, *tgw* = thousand-grain weight, *tn* = tiller number per m^2, *yld* = yield

QYld.ipk-2A, *QYld.ipk-2D.1* and *QYld.ipk-5B*, the wild allele had an effect that increased total yield by 5.0%, 15.0%, 6.0% and 14.5%, respectively (Table 2). However, the QTLs *QYld.ipk-2A* and *QYld.ipk-5B* were only detected in one location (HA).

Ear emergence time

Eight QTLs were significantly associated with ear emergence time. For all the QTLs that were located on chromosomes 2A, 2D, 3B, 5A, 5B, 6A and 7B, respectively, the W-7984 allele reduced the number of days to

Fig. 1 (continued)



ear emergence. The variation explained by these individual QTLs ranged from 9.0% to 16.9%.

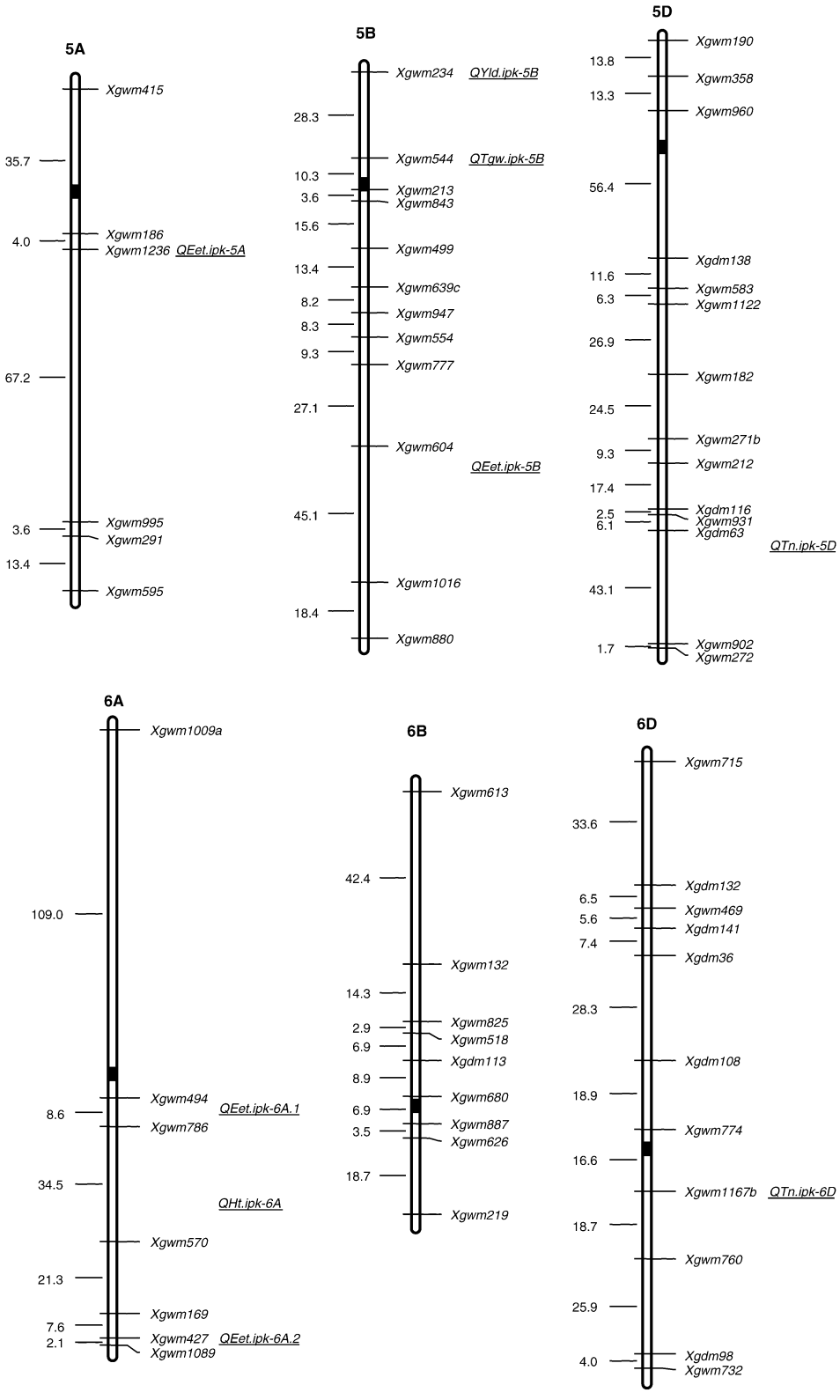
Plant height

Five putative QTLs significantly influenced plant height. These single QTLs explained 9.4%–29.5% of the phenotypic variance with a LOD of 1.6 to 5.5. For four QTLs, the wild alleles increased plant height, while for the other QTLs, *QHt.ipk-2B*, the alleles from W-7984 decreased plant height by 3.9%.

Tiller number/m^2

Tiller number/m^2 was evaluated only in two locations, WE and MO. Eight QTLs were detected for tiller number/m^2, on chromosomes 1B, 2A, 2D, 3B, 4D, 5D, 6D and 7A. All QTLs explained more than 9.0% of the phenotypic variance. For four QTLs, *QTn.ipk-1B*, *QTn.ipk-2D*, *QTn.ipk-3B* and *QTn.ipk-5D*, the W-7984 alleles increased tiller number by 5.7%–6.0%.

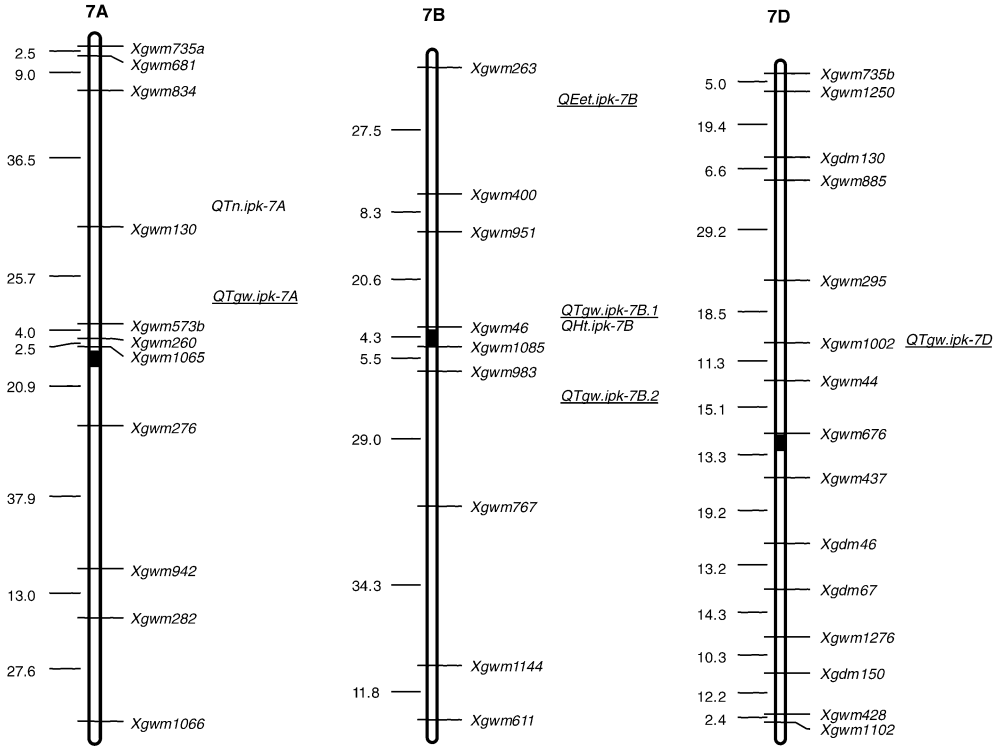
Fig. 1 (continued)



1,000-grain weight

Eight QTLs were significantly associated with 1,000-grain weight. Seven of them had an effect from the wild alleles on increasing 1,000-grain weight. The QTL on chromosome 7B explained 25.9% of the variance with a LOD = 4.7, whereas the QTL on chromosome 4D increased 1,000-grain weight by 11.7%. It is very interesting to note that four QTLs were detected in homoelogous group 7. The QTLs *QTgw.ipk-7A*,

Fig. 1 (continued)



	Yld		Ht		Eet		Tn	
Ht	0.097	-0.327**						
	-0.086	0.111						
Eet	0.015	0.123	0.028	0.143				
	-0.054	0.180	-0.081	-				
Tn	0.234*	-	0.099	-	0.016	-		
	-0.014	-	-0.135	-	0.201	-		
Tgw	-0.055	-0.174	0.337**	0.423***	0.446***	0.136	-0.036	-
	-0.303**	0.602***	0.401***	0.448***	0.137	0.390**	-0.137	-

WE	BÖ
MO	HA

Fig. 2 Correlations between traits in the BC_2F_2 Population from the cross Prinz × M6. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. *WE* = Wetze, *BÖ* = Böhnshausen, *MO* = Moosburg, *HA* = Herzogenaurach, – = no data, *eet* = ear emergence time, *ht* = plant height, *tgw* = thousand-grain weight, *tn* = tiller number per m^2, *yld* = yield

QTgw.ipk-7B.1 and *QTgw.ipk-7D* were mapped in the homoelogous positions of group 7.

Discussion

Conventional wheat breeding for increase of yield is usually performed by selecting for combinations of genotypes from a cross between two high yielding varieties. This approach has increasingly narrowed the genetic diversity of wheat. Advanced backcross QTL (AB-QTL) analysis provides a possibility to use wild, unadapted relatives of crops for the improvement of cultivated varieties. This strategy has been successfully applied in diploid crop plants such as rice and tomato for genetic improvement of quantitatively inherited traits like yield, fruit size and others (Fulton et al. 1997a; Xiao et al. 1998; Moncada et al. 2001).

Co-dominant and locus-specific molecular markers are required to identify donor alleles containing specific QTLs from wild relatives of crops. Bread wheat is a hexaploid wheat possessing A, B and D genomes. Among four major marker systems, RAPD markers and AFLP markers were not suitable for AB-QTL analysis because they are dominant markers and are not easily transferable between different populations. RFLP probes from cereals that were digested with different restriction enzymes can be mapped to three different homoeologous chromosomes (e.g. 1A, 1B or 1D) of hexaploid wheat; therefore, RFLP markers are very useful for comparative mapping, but extremely difficult for AB-QTL analysis. Microsatellite markers are PCR-based markers, co-dominant and locus-specific (Röder et al. 1998b), hence they are an ideal molecular marker for the identification of donor segments. In cases where the amplified fragment is present in one parent, but absent in the other parent, only the microsatellite marker that generates the products in the donor parent can be used for genotyping.

In the present study, 210 polymorphic microsatellite markers were used to detect the QTLs for yield and its components in the BC_2F_2 population from the cross 'Prinz' × W-7984. These markers spanned 3,006 cM in the ITMI population. The average spacing of the used microsatellite markers was 14 cM. The highest polymorphism was found in the D genome. This could be explained by the fact that the parents of the synthetic hexaploid wheat W-7984 were cultivated tetraploid wheat for the genomes AABB, but a wild species (*T. tauschii*) for the D genome. It has been found that W-7984 possesses QTLs associated with resistance to tan spot and

Table 2 Putative QTLs detected in a BC_2F_2 Population from the cross Prinz × M6. (WE = Wetze, BÖ = Böhnshausen, MO = Moosburg, HA = Herzogenaurach, ns = not significant, nd = no data)

Trait	QTL	Marker	WE	BÖ	MO	HA	LOD[a]	%A[b]	%PV[c]
Total yield	*QYld.ipk-1A*	*Xgwm99*	**–	*–	*–	ns	1.9	–7.3	11.7
	QYld.ipk-1B	*Xgwm268*	ns	**+	*+	*+	1.6	5.0	9.7
	QYld.ipk-2A	*Xgwm95*	ns	ns	ns	***+	2.6	15.0	15.2
	QYld.ipk-2B	*Xgwm120*	ns	ns	ns	***–	3.0	–25.9	17.6
	QYld.ipk-2D.1	*Xgwm702*	**+	*+	*+	ns	1.9	6.0	11.5
	QYld.ipk-2D.2	*Xgdm6*	**–	ns	*–	*–	1.6	–4.8	9.6
	QYld.ipk-3B.1	*Xgwm493*	**–	*–	*–	ns	1.6	–5.3	9.6
	QYld.ipk-3B.2	*Xgwm685*	****–	**–	***–	ns	3.8	–8.1	21.6
	QYld.ipk-4D.1	*Xgdm129*	**–	**–	ns	ns	1.7	–6.6	10.1
	QYld.ipk-4D.2	*Xgdm1163*	**–	**–	**–	ns	2.1	–5.0	12.3
	QYld.ipk-5B	*Xgwm234*	ns	ns	ns	***+	2.6	14.5	15.0
Ear emergence time	*QEet.ipk-2A*	*Xgwm95*	*+	**+	ns	*+	2.2	2.7	11.5
	QEet.ipk-2D	*Xgwm484*	*+	ns	**+	***+	2.6	2.6	15.0
	QEet.ipk-3B	*Xgwm493*	*+	+*	**+	ns	1.6	2.9	9.8
	QEet.ipk-5A	*Xgwm1236*	**+	*+	*+	ns	1.5	2.2	9.0
	QEet.ipk-5B	*Xgwm604*	*+	*+	**+	*+	1.6	2.1	9.8
	QEet.ipk-6A.1	*Xgwm494*	*+	**+	ns	**+	2.0	2.2	11.9
	QEet.ipk-6A.2	*Xgwm427*	**+	***+	**+	*+	2.9	3.7	16.9
	QEet.ipk-7B	*Xgwm263*	**+	*+	**+	**+	2.3	3.7	13.7
Plant height	*QHt.ipk-2B*	*Xgdm87a*	***–	*–	ns	ns	3.0	–3.9	17.4
	QHt.ipk-4B	*Xgwm149*	ns	**+	ns	**+	2.0	5.8	11.8
	QHt.ipk-4D	*Xgdm61*	*+	****+	**+	****+	5.5	7.3	29.5
	QHt.ipk-6A	*Xgwm570*	*+	*+	**+	***+	2.8	8.5	16.5
	QHt.ipk-7B	*Xgwm46*	**+	ns	*+	ns	1.6	3.1	9.4
Tiller number/m^2	*QTn.ipk-1B*	*Xgwm759*	ns	nd	**+	nd	1.8	5.8	10.5
	QTn.ipk-2A	*Xgwm265*	**–	nd	ns	nd	2.1	–7.0	12.6
	QTn.ipk-2D	*Xgwm539*	*+	nd	**+	nd	2.0	5.9	11.7
	QTn.ipk-3B	*Xgwm264b*	ns	nd	**+	nd	1.9	6.0	11.0
	QTn.ipk-4D	*Xgwm819*	**–	nd	ns	nd	1.6	–6.3	9.2
	QTn.ipk-5D	*Xgdm63*	ns	nd	**+	nd	2.1	5.7	12.3
	QTn.ipk-6D	*Xgwm1167b*	**–	nd	ns	nd	1.8	–8.4	10.8
	QTn.ipk-7A	*Xgwm130*	**–	nd	ns	nd	1.8	–6.5	10.8
1000-grain weight	*QTgw.ipk-2A*	*Xgwm636*	ns	***–	*–	ns	3.0	–5.6	17.2
	QTgw.ipk-2D	*Xgdm6*	*+	*+	*+	***+	2.6	10.3	15.4
	QTgw.ipk-4D	*Xgdm61*	ns	**+	ns	***+	2.5	11.7	14.3
	QTgw.ipk-5B	*Xgwm544*	**+	*+	***+	***+	2.8	4.8	16.0
	QTgw.ipk-7A	*Xgwm573b*	***+	*+	**+	*+	2.5	3.8	14.5
	QTgw.ipk-7B.1	*Xgwm46*	***+	**+	****+	***+	3.6	4.9	20.6
	QTgw.ipk-7B.2	*Xgwm983*	***+	**+	****+	**+	4.7	5.8	25.9
	QTgw.ipk-7D	*Xgwm1002*	**+	***+	**+	**+	3.0	6.6	17.3

Significance levels: * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

[a] LOD score from the location with the underlined *P*-value

[b] A (%) = 100(AB–AA)/AA where AA is the phenotypic mean for the individual homozygous for Prinz alleles at specified markers and AB is the phenotypic mean for the heterozygotes Prinz/W-7984

[c] % PV = phenotypic variance estimated from marker regression against phenotype

[d] +/– indicate a positive or negative effect from the W-7984 allele

[e] Underlined *P*-value indicates location for which A (%) and % PV were calculated

leaf rust in the ITMI population (Faris et al. 1999). Recently, Börner et al. (2002) mapped some quantitative trait loci for agronomically important characters which originated from W-7984 in the ITMI population. Hence, W-7984 was selected for AB-QTL analysis to identify beneficial alleles in the BC_2F_2 population.

In many QTL analyses by the Advanced Backcross method it is accepted that inferior types are selected against, either morphologically or genotypically. Al-

though 164 lines were available for this investigation, the selection to investigate only 72 was made due to the fact that in such a population there was an extreme variation of phenotypes, of which many are not interesting for the plant breeders. A population of 72 plants is certainly at the lower end of the plant numbers that have been analyzed in such studies. The increase in significance that might be gained by the larger population would at least be partially offset by such negatively acting genes. However, a larger population is also a much higher burden for population generation (due to sterility in the material) and population analysis.

There were no significant correlation coefficients between yield and its components, indicating the complexity of the trait yield. The results of ANOVA indicated that environmental factors influenced genotypes for yield and yield components in the AB-QTL analysis (Table 1). Alleles from the synthetic wheat W-7984 were associated with a positive effect on yield for four of the 11 QTLs detected for this trait. The four QTLs for yield-increase were mapped on chromosomes 1B, 2A, 2D and 5B, respectively (Table 2 and Fig. 1). The yield-increasing QTL *QYld-ipk-2D.1* may be a pleiotropic effect of the gene *Ppd1* for day length insensitivity that is important for the adaption to short days and which increases yield in southern Europe (Börner et al. 1993; Worland et al. 1998).

For the eight QTLs identified for ear emergence time, alleles from W-7984 were associated with earlier ear emergence. These QTLs were mapped to chromosomes 2AS, 2DS, 3BS, 5AL, 5BL, 6AL and 7BS, respectively. Law and Wolfe (1966) located a genetic factor for ear emergence time on chromosome 7BS. The QTL on chromosome 2DS, *QEet.ipk-2D*, might be the same as that found in the ITMI population (Börner et al. 2002). In hexaploid wheat, the vernalization response gene *Vrn-A1* on chromosome 5A appears to contribute a major effect on controlling ear emergence time (Law et al. 1976). The QTL on chromosome 5A, *QEet.ipk-5A*, is located in a similar position as *QEet.ocs-5A.1* reported by Kato et al. (1999). The QTLs controlling ear emergence time identified on chromosomes 7A (Hyne et al. 1994) and 4A (Araki et al. 1999) were not detected in the present study.

A reduction in plant height can improve lodging resistance and indirectly increase yield. However, Law et al. (1978) found that there was a positive correlation between reduced height and reduced yield, and the genetic control of plant height is known to be complex involving many genes. In the present study, five QTLs were associated with plant height, of which one QTL from W-7984 decreasing plant height was identified on chromosome 2BL. The alleles from W-7984 at loci *Xgwm149* on chromosome 4B and *Xgdm61* on chromosome 4D increased height (Table 2). A possible explanation is the presence of the *Rht-B1* and *Rht-D1* alleles in 'Prinz' (Börner et al. 1997). The QTL on chromosome 6A, *QHt.ipk-6A*, observed in the present study is possibly the same as that previously detected by Börner et al. (2002). The other QTLs on chromosomes 1AS, 1BL and 7AL found by Cadalen et al. (1998) and Börner et al. (2002) were not identified here.

Eight QTLs for tiller number/m^2 were detected in this study; in four cases *QTn.ipk-5D*, *QTn.ipk-2D*, *QTn.ipk-3B* and *QTn.ipk-1B*, the W-7984 alleles were associated with an increase in the tiller number (Table 2). Few studies on the identification of QTLs for tiller number were reported. By using single-chromosome recombinant lines, QTLs for tiller number were discovered on chromosomes 3A, 5A and 7B, respectively (Law 1967; Shah et al. 1999; Kato et al. 2000).

The alleles derived from W-7984 were associated with an increase of 1,000-grain weight for seven of eight QTLs detected for this trait. Three of these loci, *QTgw.ipk-7A* and *QTgw.ipk-7B.1* and *QTgw.ipk-7D*, were located in the homoeologous position of group 7. The QTLs on chromosomes 7B and 7D are in similar positions to the 1,000-grain weight QTLs reported by Börner et al. (2002) based on the same donor W-7984 in the ITMI population. The QTL on chromosome 4D, *QTgw.ipk-4D*, may be homoeologous to the 50-grain-weight QTL on chromosome 4A identified by Araki et al. (1999).

A total of 40 QTLs were identified for the five traits in the present study. For 24 (60.0%) of them, alleles from the synthetic wheat W-7984 were associated with a positive effect on plant performance. It is worth noting that for all five QTLs mapped in homoeologous group 5, the synthetic wheat W-7984 alleles showed the positive phenotypic effect (Table 2 and Fig. 1). Yield QTLs and QTLs for yield components were mapped independently in most cases. This was consistent with the result that there was no significant correlation between yield and its components. Kato et al. (2000) found that yield was highly correlated with yield components. This could be explained by the fact that they used single-chromosome recombinant lines. Yield can be increased by a few heavy grains or many light grains, and a few large ears or many smaller ears. Tiller number can increase yield directly by increasing the number of ears. But late-developing tillers often fail to produce ears and compete with ear-bearing tillers for resources and thus reduce yield indirectly (Bezant et al. 1997). For two QTL loci, yield seemed to be negatively correlated with thousand-grain weight. The allele from W-7984 flanked by the markers *Xgwm539* and *Xgdm6* on chromosome 2D, as well as between the flanking markers *Xgdm61* and *Xgwm129* on chromosome 4D, decreased yield but increased thousand-grain weight. The W-7984 allele at the locus *Xgwm95-2A* reduced the number of days to ear emergence, but increased yield. In another case, a yield QTL coincided and was positively correlated with a QTL for plant height, for example on the short arm of chromosome 4D.

QTLs for yield and yield components detected in the present study were mainly distributed on chromosomes 2D, 3B, 4D, 5B, 6A and 7B. Several chromosomal regions were associated with more than one trait, indicating either pleiotropic or linkage effects. For instance, in the 2.9-cM interval between *Xgdm61* and

Xgdm129 on chromosome 4DS, there were four QTLs for plant height, thousand-grain weight, tiller number/m^2 and yield. Four QTLs for yield, ear emergence time and tiller number/m^2 were identified on the short arm of chromosome 3B (Fig. 1). It is important to mention that relatively low LOD scores were used to identify the putative QTLs in the present study, because it is difficult to detect QTLs using a high threshold in smaller populations (Lin et al. 1998). These putative QTLs need to be confirmed in a large BC_3 population. The development of a large BC_3 population is in progress, because more favorable QTLs could be detected in a BC_3 population rather than in a BC_2 population (Fulton et al. 1997b). Moreover, it is necessary to develop near-isogenic lines containing introgressions associated with QTLs for further genetic characterization of yield and its components.

The present study suggests that favorable QTL alleles could be transferred from wild relatives of wheat into an elite wheat variety for improvement of yield by advanced backcross QTL strategy and microsatellite markers.

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References

Araki E, Miura H, Sawada S (1999) Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat. Theor Appl Genet 98:977–984

Austin DF, Lee M (1996) Comparative mapping in F2:3 and F6:7 generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet 92:817–826

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey M (1997) Mapping QTLs controlling yield and yield components in a spring barley (*Hordeum vulgare* L.) cross using marker regression. Mol Breed 3:29–38

Börner A, Worland AJ, Plaschke J, Schumann E, Law CN (1993) Pleiotropic effects of genes for reduced height (*Rht*) and day length insensitivity (*Ppd*) on yield and its components for wheat grown in middle Europe. Plant Breed 111:204–216

Börner A, Röder M, Korzun V (1997) Comparative molecular mapping of GA insensitive *Rht* loci on chromosomes 4B and 4D of common wheat (*Triticum aestivum* L.). Theor Appl Genet 95:1133–1137

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci for agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105:921–936

Cadalen T, Sourdille P, Charmet G, Tixier MH, Gay G, Boeuf C, Bernard S, Leroy P, Bernard M (1998) Molecular markers linked to genes affecting plant height in wheat using a doubled-haploid population. Theor Appl Genet 96:933–940

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495–504

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS (1999) Candidate gene analysis of quantitative disease resistance in wheat. Theor Appl Genet 98:219–225

Fulton TM, Beck-Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (1997a) QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. Theor Appl Genet 95:881–894

Fulton TM, Nelson JC, Tanksley SD (1997b) Introgression and DNA marker analysis of *Lycopersicon peruvianum*, a wild relative of the cultivated tomato, into *Lycopersicon esculentum*, followed through three successive backcross generations. Theor Appl Genet 95:895–902

Fulton TM, Grandillo S, Beck-Bunn T, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *Lycopersicon parviflorum cross*. Theor Appl Genet 100:1025–1042

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000a) Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding. Theor Appl Genet 101:407–414

Huang XQ, Zeller FJ, Hsam SLK, Wenzel G, Mohler V (2000b) Chromosomal location of AFLP markers in common wheat (*Triticum aestivum* L.) utilizing nulli-tetrasomic stocks. Genome 43:298–305

Huang XQ, Röder MS, Pestsova E, Börner A, Ganal MW (2001) Development and use of wheat microsatellite markers for the characterization of germplasm of hexaploid wheat (*Triticum aestivum* L.). In: the Plant and Animal Genome IX Conference, January 13–17, 2001, San Diego, California, USA, p 260

Huang XQ, Börner A, Röder MS, Ganal MW (2002) Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers.Theor Appl Genet 105:699–707

Huang XQ, Wang LX, Xu MX, Röder MS (2003) Microsatellite mapping of the wheat powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.). Theor Appl Genet (in press)

Hyne V, Kearsey MJ, Martinez O, Gang W, Snape JW (1994) A partial genome assay for quantitative trait loci in wheat (*Triticum aestivum*) using different analytical techniques. Theor Appl Genet 89:735–741

Kato K, Miura H, Sawada S (1999) QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. Theor Appl Genet 98:472–477

Kato K, Miura H, Sawada S (2000) Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor Appl Genet 101:1114–1121

Kihara H (1944) Die Entdeckung des DD Analysators beim Weizen. Agric Hort 19:889–890

Knot DR (1989) The wheat rusts: breeding for resistance. Monographs on Theoretical Applied Genetics 12, Springer-Verlag, Berlin

Law CN (1967) The locations of genetic factors controlling a number of quantitative characters in wheat. Genetics 56:445–461

Law CN, Wolfe MS (1966) Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. Can J Genet Cytol 8:462–470

Law CN, Worland AJ, Giorgi B (1976) The genetic control of ear-emergence time by chromosomes 5A and 5D of wheat. Heredity 36:49–58

Law CN, Snape JW, Worland AJ (1978) The genetic relationship between height and yield in wheat. Heredity 40:133–151

Lin HX, Qian HR, Zhuang JY, Lu J, Min SK, Xiong ZM, Huang N, Zheng KL (1996) RFLP mapping of QTLs for yield and related

characters in rice (*Oryza sativa* L.). Theor Appl Genet 92:920–927

Lin SY, Sasaki T, Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. Theor Appl Genet 96:997–1003

Lutz J, Hsam SLK, Limpert E, Zeller FJ (1995) Chromosomal location of powdery mildew resistance genes in *Triticum aestivum* L. (common wheat). 2. Genes *Pm2* and *Pm19* from *Aegilops squarrosa* L. Heredity 74:152–156

Marino C, Nelson J, Lu Y, Sorrels M, Leroy P, Tuleen N, Lopes C, Hart G (1996) Molecular genetic maps of the group-6 chromosomes of hexaploid wheat (*Triticum aestivum*). Genome 39:359–366

Moncada P, Martínez CP, Borrero J, Chatel M, Gauch Jr H, Guimaraes E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theor Appl Genet 102:41–52

Nelson J (1997) QGene: software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Nelson J, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995a) Molecular mapping of wheat. Homoeologous group 3. Genome 38:525–533

Nelson JC, Van Deynze A, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995b) Molecular mapping of wheat. Homoeologous group 2. Genome 38:516–524

Peng JH, Fahima T, Röder MS, Li YC, Dahan A, Grama A, Ronin YI, Korol AB, Nevo E (1999) Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. Theor Appl Genet 98:862–872

Pestsova E, Ganal WM, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Röder MS, Korzun V, Gill BS Ganal MW (1998a) The physical mapping of microsatellite markers in wheat. Genome 41:278–283

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998b) A microsatellite map of wheat. Genetics 149:2007–2023

Shah MM, Gill KS, Baenziger PS, Yen Y, Kaeppler SM, Ariyarathne HM (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:1728–1732

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines.Theor Appl Genet 92:191–203

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed T, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92:213–224

Veldboom LR, Lee M (1994) Molecular-marker facilitated studies of morphological traits in maize. II. Determination of QTLs for grain yield and yield components. Theor Appl Genet 89:451–458

Worland AJ, Korzun V, Röder MS, Ganal MW, Law CN (1998) Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor Appl Genet 96:1110–1120

Xiao J, Li J, Yuan L, Tanksley SD (1996) Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor Appl Genet 92:230–244

Xiao JH, Li JM, Grandillo S, Ahn SN, Yuan LP, TanksleySD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909