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An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat

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Abstract We made an update of the intervarietal molecular marker linkage map of the wheat genome developed using a doubled-haploid (DH) population derived from the cross between the cultivars 'Courtot' and 'Chinese Spring'. This map was constructed using 187 DH lines and 659 markers. The genome was well covered (more than 95%) except for chromosomes from homoeologous group 4 and chromosomes 5D and 7D, which had gaps slightly larger than 50 cM. A core-map based on a set of 200 anchor loci (one marker each 18.4 cM) was developed. The total length of this map was 3,685 cM which is similar to the size of the international reference map of the ITMI population (3,551 cM). Map coverage was identical for the three genomes (A, B and D) and for the number of anchor loci, as well as for the size of the map. Using this map, QTLs for several agronomic traits were detected on phenotypic data from the population grown in Clermont-Ferrand (France) under natural field conditions over 6 years, and in Norwich (UK) in controlled conditions and under natural field conditions in 1 year. Almost all of the 21 chromosomes were involved in at least one trait. However, several regions seemed to contain gene clusters either for grain traits (and thus bread-making quality) or plant development traits.

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Introduction

In hexaploid bread wheat (*Triticum aestivum* L. em. Thell, 2n = 6*x* = 42), molecular marker linkage maps have been published either in the form of separate homoeologous groups such as groups 1 (Van Deynze et al. 1995), 2 (Devos et al. 1993; Nelson et al. 1995a), 3 (Devos et al. 1992; Nelson et al. 1995b), 4, 5 (Xie et al. 1993; Devos et al. 1995; Nelson et al. 1995c), 6 (Jia et al. 1996; Marino et al. 1996) and 7 (Chao et al. 1989; Nelson et al. 1995c), or as complete maps (Liu and Tsunewaki 1991; Gale et al. 1995; Cadalen et al. 1997; Messmer et al. 1999). However, owing to the poor levels of polymorphism often encountered in wheat (Chao et al. 1989; Cadalen et al. 1997), mapping strategies were most often directed toward the use of wide crosses, involving either a synthetic wheat and a variety such as 'Chinese Spring' (Gale et al. 1995) or 'Opata' (Nelson et al. 1995a, b, c) as parents, or crosses between 'Chinese Spring' and *Triticum spelta* (Liu and Tsunewaki 1991; Messmer et al. 1999).

One of the aims of developing such maps is the elaboration of strategies to access genes of interest underlying quantitative trait loci (QTLs). An elegant model for establishing genetic/physical map relationships was proposed by Gill and Gill (1993) from a set of deleted wheat lines (cv Chinese Spring). More than 400 deletion lines were isolated for the 21 chromosomes after introducing a gametocidal chromosome from *Aegilops cylindrica* that produced chromosomal breakages on the wheat chromosomes (Endo 1988; Endo and Gill 1996). This unique material was used to physically map RFLP probes onto sub-arm chromosomal regions for homoeologous groups 1 (Kota et al. 1993; Gill et al. 1996a; Tsujimoto et al. 1999; Ma et al. 2001), 2 (Delaney et al. 1995a), 3 (Delaney et al. 1995b; Ma et al. 2001), 4 (Mickelson-Young et al. 1995), 5 (Gill et al. 1996b), 6 (Gill et al.

Table 1 Different traits observed on the Courtot × Chinese Spring population which were submitted to QTL analysis

Trait	Abbreviations	No. of Plants	Years	Reference
Plant height	H	106	93, 94, 95	Sourdille et al. 1998
Pentosan viscosity	Visc.	91	94,95	Martinant et al. 1998
Crossability	K	187	97	Tixier et al. 1998
Powdery mildew	R	138	98	Sourdille et al. 1999
Bread making[a]	W, Hard., Prot.	169	94, 95, 96	Perretant et al. 2000
Heading time[a,b]	HT, LD, DLS	172, 189, 140	94, 95, 97, 98	Sourdille et al. 2000a
Ear compactness[a]	Cp, Ns, Sl	172, 189, 144	94, 95, 98	Sourdille et al. 2000b
Awning	Awn	144	98	Sourdille et al. 2002

[a] Several related traits were studied in these cases: Bread-making quality: W = strength of the dough; Hard. = grain hardness; Prot. = % protein content. Heading time: HT = heading time; LD = long day conditions; DLS = difference between long- and short-day conditions. Ear compactness: Cp = ear compactness; Ns = number of spikelets; Sl = spike length

[b] Photoperiod response and heading time were evaluated in Norwich in 1997 and 1998 respectively, on 140 entries. Heading time only was evaluated in Clermont-Ferrand in 1994 and 1995 on 172 and 189 entries respectively

1993a; Weng et al. 2000) and 7 (Werner et al. 1992; Hohmann et al. 1995a, 1995b). Röder et al. (1998b) also physically mapped a restricted set of microsatellites on chromosomes of homoeologous group 2. Zhang et al. (2000) saturated the1BS satellite region with AFLP markers. Deletion mapping strategy has allowed the delineation of chromosomal regions for some important genes like *Ph1* on 5BL (Gill et al. 1993b), *Vrn1* on 5AL (Sarma et al. 1998), *Ha* on 5DS (Sarma et al. 2000) and *Q* on 5AL with the isolation of 'candidate clones', by combining a fingerprinting cDNA approach, Northern analysis and deletion mapping (Kojima et al. 2000).

However, the genetic dissection of complex agronomic traits through QTL analyses requires the development of molecular-marker linkage maps in an intervarietal context. Such a population of doubled-haploid lines was developed using the French cultivar 'Courtot' and the well known line 'Chinese Spring' as parents (Cadalen et al. 1997). The original 'Courtot' × 'Chinese Spring' (CtCS) map, constructed using 293 RFLP markers, covered only 60% of the whole genome, mainly because of the scarcity of markers on the D-genome chromosomes.

Recently, however, we developed a set of D genome-specific microsatellites using *Triticum tauschii* as a genomic DNA donor for the production of a microsatellite-enriched library (Guyomarc'h et al. 2002). A large proportion of these microsatellites mapped on the D genome of the CtCS map. Thus, the objective of the present study was to establish a new version of the 'Courtot' × 'Chinese Spring' linkage map, and to use this to reanalyse all previous data for the QTLs that were detected using this cross for bread-making quality traits as well as for development traits (plant height, wheat/rye crossability, ear compactness, photoperiod response).

Materials and methods

Plant material and trait evaluation

The population consisted of 217 doubled-haploid (DH) lines and was produced through anther culture from Courtot (Ct) × Chinese Spring (CS) F_1-hybrids (Félix et al. 1996; Cadalen et al. 1997). One hundred and six lines were genotyped for all the markers and an additional set of 81 DH lines were genotyped only for anchor loci. Depending on the trait to be studied, various sets among these 187 entries were sown together with the parents (autumn sowing) at Clermont-Ferrand over several years (1993–1998; see Table 1). Three-row plots (1.5 m) were grown in a nursery with two replications under normal field conditions with fungicide application to control rusts and powdery mildew.

The traits were evaluated according to the different procedures described in the publications (for references see Table 1).

Chromosomal assignment of anchor loci was realised using a set of 19 nulli-tetrasomic (NT) and 35 di-telosomic (DT) lines, kindly provided by Dr. Steve Reader (John Innes Centre, UK). This was completed by a set of 85 characterized wheat deletion lines kindly provided by Dr. Bikram Gill (Kansas State University, USA).

Molecular analysis

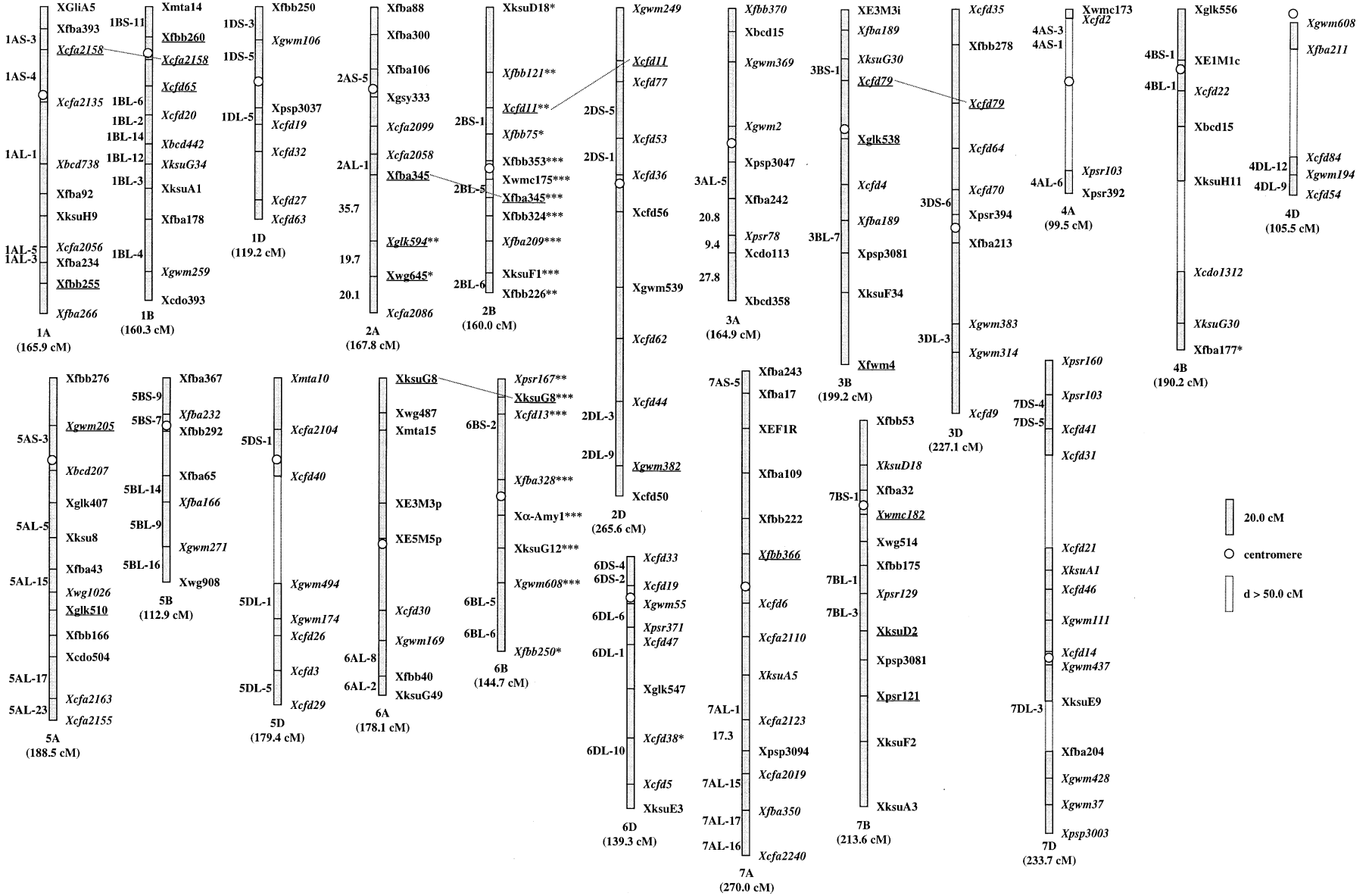
The probes used in this study, as well as the techniques for DNA extraction, digestion, electrophoresis, blotting and hybridization, were described by Cadalen et al. (1997). The protocol using non-radioactive probes was detailed in Lu et al. (1994) and Sourdille et al. (1996). Several microsatellites (Devos et al. 1995; Plaschke et al. 1995, 1996; Röder et al. 1995, 1998b; Bryan et al. 1997; Guyomarc'h et al. 2002) were also mapped on this population. Protocols for PCR reactions were as described in Tixier et al. (1998), and the detection of microsatellites using a non-radioactive silver-nitrate staining method was described by Tixier et al. (1997). Some AFLP markers obtained following the procedure described in Bert et al. (1999) were also added to the map.

Construction of the map

Segregation distortion for all the loci was tested using a chi-square test. Mapmaker/exp version 3.06 (Lander et al. 1987) was used to construct the maps for biased or unbiased markers separately. Linkage groups were established using LOD and θ thresholds of 5 and 0.25 respectively. The genetic distances were calculated using the Kosambi (1944) mapping function. Anchor loci were chosen according to the following criteria: absence of segregation distortion (except for chromosomes 2B and 6B), minimum of missing data and optimal spacing along the chromosomes.

QTL analysis

The associations between markers and the various traits were evaluated by a one-way ANOVA with $\alpha = 0.001$ to keep the overall type-I error risk below 5%, in order to determine the markers hav-


1A
(165.9 cM)
1B
(160.3 cM)
1D
(119.2 cM)
2A
(167.8 cM)
2B
(160.0 cM)
2D
(265.6 cM)
3A
(164.9 cM)
3B
(199.2 cM)
3D
(227.1 cM)
4A
(99.5 cM)
4B
(190.2 cM)
4D
(105.5 cM)
5A
(188.5 cM)
5B
(112.9 cM)
5D
(179.4 cM)
6A
(178.1 cM)
6B
(144.7 cM)
6D
(139.3 cM)
7A
(270.0 cM)
7B
(213.6 cM)
7D
(233.7 cM)
20.0 cM
centromere
d > 50.0 cM

ing the main effects. Then, estimates of the locations of the QTLs, origins of the positive alleles and additive values of the QTLs were assessed for each linkage group using the marker regression method according to Kearsey and Hyne (1994), the most significant markers from the other groups being used as covariates as proposed by Jansen and Stam (1994). The 95% confidence intervals of the QTL locations and effects were established by bootstrapping (Visscher et al. 1996) using 500 replicates.

Results

The first version of the CtCS map obtained from the DH population was constructed using 293 markers and was detailed by Cadalen et al. (1997). The map covered nearly 1,800 cM (60% of the genome) with an average of one marker each 7.6 cM. However, the D genome was poorly represented and no markers were assigned to chromosomes 2D, 4D and 5D. The development of microsatellites (Röder et al. 1998b; Guyomarc'h et al. 2002) has allowed a new map to be obtained. This second version of the map (Fig. 1) was constructed using a set of 200 anchor loci delimiting 178 linkage blocks ('bins'). This map now covers 3,685 cM which is slightly larger than the observed size for the ITMI map (3,551 cM) derived from the cross between a synthetic wheat [reconstructed by hybridization between *Triticum dicoccum* (AABB) and *T. tauschii* (DD)] and the variety Opata (Nelson et al. 1995a, b, c; Van Deynze et al. 1995; Marino et al. 1996). The whole genome was covered except for chromosomes from homoeologous group 4 and chromosomes 5D and 7D which showed residual gaps slighty larger than 50 cM. A similar percentage of anchor markers covered a similar length of map for the three genomes: 35% of the loci representing 34% of the size were assigned to the A genome, 34% of the loci representing 32.5% of the size were assigned to the B genome, 31% of the loci representing 33.5% of the size were assigned to the D genome. However, among the 659 mapped loci, the D genome was under-represented with only 171 loci (26%), while 37% and 34% of the loci were assigned to the A and B genomes, respectively. A small number of loci (only 3%) remained unlinked to any of the 21 linkage groups. The largest chromosome maps were those from chromosomes 2D (265.6 cM) and 7A (270.0 cM) while the shortests were observed for chromosomes 4A (99.5 cM) and 4D (105.5 cM).

Such a well-satured linkage map constitutes a powerful tool for QTL mapping. We previously used the CtCS population for the study of several agronomic traits (Table 1): plant height (H), wheat/rye crossability (K), powdery mildew resistance (R), bread-making quality through the study of the strength of the dough (W), grain hardness (Hard.), total protein content (Prot.), earliness through heading time (HT) and photoperiod response under long-day conditions (LD) or the difference between long- and short-day conditions (DLS), ear compactness (Cp) together with two related traits, spike length (Sl) and the number of spikelets (Ns) and awning (Awn). Using the new version of our map, we made an update of the QTLs detected for each trait (Table 2). Only three new QTLs were detected using this new version of the map. All of them were located on chromosomes from the D genome: one on chromosome 2D for awning, and two on chromosomes 5D and 6D for ear compactness. All these three QTLs were linked to microsatellite loci newly added to the map. We were also able to precisely locate these QTLs within confidence intervals. The shortest was observed for grain hardness for which a 5-cM confidence interval was detected on the short arm of chromosome 5D carrying the hardness locus (*ha*). The largest was computed for awning for which a confidence interval of 163.3-cM was located on chromosome 2D.

The position on the CtCS map of the 46 QTLs which were detected are summarized in Fig. 2. Nearly all the chromosomes contained at least one QTL. Only chromosomes 1D, 3A, and 3D exhibited none. On the other hand, some chromosomes were involved in the expression of several traits. For example, chromosome 1A was involved in bread-making quality [strength (W) and kernel hardness] with a QTL close to the storage-protein locus (Glu-1), but also in resistance to powdery mildew at the top of the short arm, in plant height with a strong interaction between chromosome arms 1AS and 1BL, and in spike morphology at the bottom of the long arm (compactness and spike length). In addition, QTLs frequently co-segregated not only when traits were related to each other (for example, a QTL for compactness co-segregated with those for spike length and the number of spikelets on chromosome arm 2BS), but also when the traits were unrelated, like a QTL for awning which co-segregated with those for compactness and spike length on chromosome arm 4AS.

Moreover, numerous genes associated with simply inherited traits have already been mapped, or at least assigned, using aneuploid stocks (Sears 1954) or substitution lines (Kuspira and Unrau 1957). In most of the cases, genes known to affect a particular trait were located in the close vicinity of QTLs associated to genetic variation for the same trait. This was clearly the case for plant height where two QTLs were located on chromosome arms 4BS and 4DS close to *Rht-B1* and *Rht-D1* respectively, two genes reducing plant height in wheat.

◀ **Fig. 1** Molecular marker linkage map developed for the 'Courtot' × 'Chinese Spring' population. Recombination fractions are in Kosambi cM (1944). Positions of the deletions are indicated on the left of the chromosomes. *Dotted chromosomal segments* represent recombination values > 50 cM. *Markers with dashed lines and/or underlined* are in homoeologous positions or present homoeologous loci. Markers in *italic* characters were assigned to chromosomes using aneuploid stocks or deletion lines. Markers that significantly deviated from the 1:1 ratio at the 5%, 1% and 0.1% levels are indicated with *, **, *** respectively. The approximate position of the centromere is indicated with a *circle*

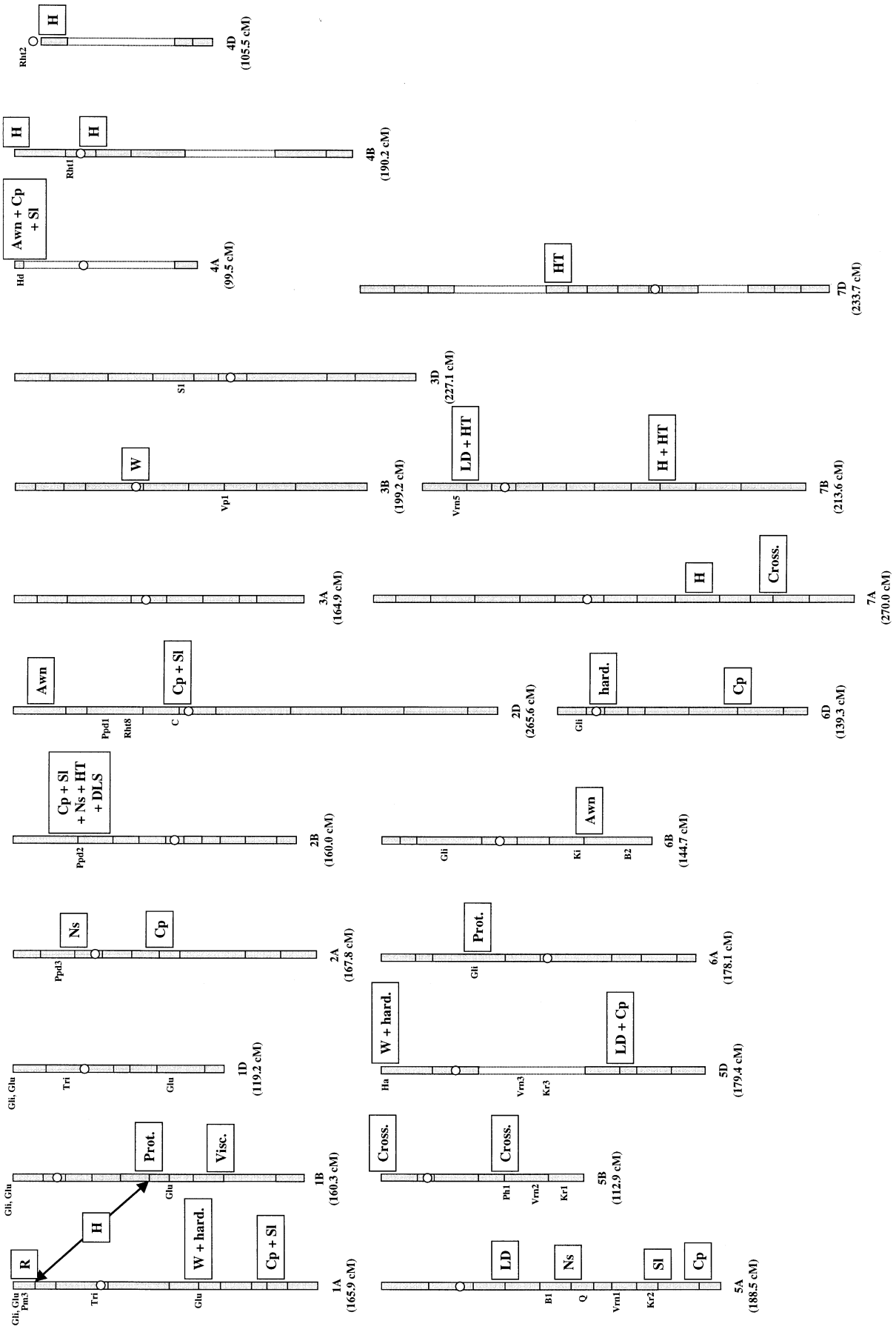

Gli, Glu
Pm3
R
Tri
H
Glu
Prot.
Visc.
W + hard.
Cp + Sl
1A
(165.9 cM)
Gli, Glu
Glu
1B
(160.3 cM)
Gli, Glu
Tri
Glu
1D
(119.2 cM)
Ppd3
Ns
Cp
2A
(167.8 cM)
Ppd2
Cp + Sl
+ Ns + HT
+ DLS
2B
(160.0 cM)
Ppd1
Rht8
Awn
C
Cp + Sl
2D
(265.6 cM)
3A
(164.9 cM)
Vp1
W
3B
(199.2 cM)
S1
3D
(227.1 cM)
Hd
Awn + Cp
+ Sl
4A
(99.5 cM)
Rht1
H
H
4B
(190.2 cM)
Rht2
H
4D
(105.5 cM)
B1
Q
Vrn1
Kr2
LD
Ns
Sl
Cp
5A
(188.5 cM)
Ph1
Vrn2
Kr1
Cross.
Cross.
5B
(112.9 cM)
Ha
Vrn3
Kr3
W + hard.
LD + Cp
5D
(179.4 cM)
Gli
Prot.
6A
(178.1 cM)
Gli
Ki
B2
Awn
6B
(144.7 cM)
Gli
hard.
Cp
6D
(139.3 cM)
H
Cross.
7A
(270.0 cM)
Vrn5
LD + HT
H + HT
7B
(213.6 cM)
HT
7D
(233.7 cM)

Table 2 QTLs detected using the new version of the map. New QTLs compared to previous analyses (Table 1) are indicated with *. Pos.: location of the QTL from the top of the chromosome (cM). CI: confidence interval. Power: % of significant models using bootstrap re-sampling. Add.: additive value of the QTL. The sign indicates the favourable allele (+ for 'Courtot' and – for 'Chinese Spring')

Trait	Loci	Chr.	Loc. (CI)	r2	Power	Pr(F)	Add.
H	Xglk556	4BS	6.1 (0–47.2)	19.1	0.978	1.14×10^{-4}	9.61
	Xfba211	4DL	36.8 (2.9–64.8)	16.7	0.668	8.50×10^{-5}	8.72
	XksuA5	7AL	168.5 (100.9–196.1)	11.9	0.926	3.89×10^{-5}	8.67
	XksuD2	7BL	98.0 (40.3–148.7)	12.5	0.722	7.63×10^{-4}	7.37
K	Xfba367	5BS	11.2 (0–32.6)	16.8	0.738	1.00×10^{-5}	13.6
	Xwg583	5BL	76.7 (49.7–96.7)	3.3	0.501	3.46×10^{-4}	6.1
	Xtam51	7AL	219.5 (145.3–262.1)	5.9	0.817	1.00×10^{-3}	8.1
Prot.	Xcdo1188	1BL	65.0 (39.0–103.0)	6.5	0.738	8.70×10^{-5}	–0.31
	$E38M60_{200}$	6AS	59.0 (36.0–86.0)	17.1	0.915	6.64×10^{-5}	–0.50
Hard.	Xfba92	1AL	83.0 (56.0–103.0)	3.1	0.502	7.46×10^{-4}	4.0
	Xmta10	5DS	1.0 (0–5.0)	66.9	1	0.10×10^{-7}	17.0
	Xgwm55	6AL	53.0 (34.0–70.0)	5.5	0.666	3.53×10^{-4}	–4.8
W	Xfba92	1AL	84.0 (64.0–97.0)	10.9	0.883	4.73×10^{-5}	17.0
	XksuE3	3BL	54.0 (12.0–120.0)	9.4	0.915	$2.47\ 10^{-4}$	15.0
	Xmta10	5DS	4.0 (0–13.0)	19.5	0.966	9.50×10^{-5}	21.0
LD	Xglk407	5AL	84.0 (58.9–158.7)	6.9	0.970	1.00×10^{-6}	2.12
	Xgwm174	5DL	150.6 (112.5–178.1)	9.4	0.500	4.19×10^{-4}	2.50
	XksuD18	7BS	25.3 (3.8–44.6)	7.3	0.890	2.11×10^{-3}	–2.20
DLS	Xfbb121	2BS	51.1 (30.6–87.5)	25.9	0.954	7.60×10^{-5}	–5.98
	Xglk556	4BS	37.5 (0–74.2)	6.3	0.506	1.73×10^{-4}	–2.89
HT	Xfbb121	2BS	43.5 (24.0–75.7)	44.4	0.999	2.20×10^{-5}	–3.15
	Xtam75	5AL	134.8 (69.0–185.8)	8.2	0.768	3.41×10^{-4}	1.13
	Xfbb53	7BS	52.1 (22.9–121.9)	9.4	0.679	3.23×10^{-6}	1.44
	XksuD2	7BL	237.8 (190.9–282.3)	5.3	0.664	1.43×10^{-3}	–1.64
	Xfbb366	7DL	123.8 (49.6–166.4)	5.8	0.966	8.10×10^{-4}	–0.92
Sl	XksuG34	1AL	126.9 (59.3–154.5)	11.6	0.926	3.89×10^{-5}	0.45
	Xfbb121	2BS	39.3 (11.9–73.9)	9.8	0.774	5.29×10^{-4}	0.49
	Xgwm261	2DS	15.3 (12.1–41.8)	7.7	0.810	6.16×10^{-4}	–0.36
	Xfba78	4AS	10.8 (0–41.3)	6.9	0.658	9.09×10^{-4}	0.40
	XksuE2	5AL	183.5 (124.9–212.5)	9.3	0.900	4.42×10^{-4}	–0.44
Ns	Xfba106	2AS	43.2 (6.7–73.3)	9.5	0.907	2.47×10^{-4}	–0.76
	Xfbb121	2BS	25.6 (11.2–43.8)	15.6	0.851	1.87×10^{-5}	0.97
	Xfba43	5AL	87.2 (46.9–118.9)	8.8	0.896	5.66×10^{-4}	–0.82
Cp	XksuG34	1AL	122.5 (51.5–140.2)	9.3	0.923	2.47×10^{-4}	–0.10
	Xwmc170	2AL	87.8 (42.3–108.9)	10.6	0.840	5.57×10^{-5}	–0.10
	Xfbb121	2BS	19.4 (5.7–36.4)	9.3	0.648	2.32×10^{-4}	0.10
	Xgwm261*	2DS	12.9 (0–48.1)	9.9	0.966	1.27×10^{-4}	0.10
	Xwmc173	4AS	8.7 (2.4–50.1)	10.1	0.874	1.13×10^{-4}	–0.10
	Xfba177	5AL	220.8 (136.5–220.8)	7.9	0.734	6.99×10^{-4}	0.09
	Xcfd26*	5DL	149.2 (88.0–174.8)	13.6	0.624	6.95×10^{-4}	0.12
	Xcfd38	6DL	68.0 (25.7–134.2)	12.2	0.700	6.75×10^{-4}	0.13
Awn	Xgwm249*	2DS	32.6 (21.9–185.2)	8.5	0.760	4.80×10^{-5}	0.262
	Xwmc173	4AS	29.1 (0–48.4)	8.4	0.756	7.40×10^{-5}	–0.379
	XksuG12	6BL	97.0 (50.1–141.5)	5.7	0.668	3.73×10^{-4}	–0.188

◀ **Fig. 2** Position of QTLs on the genetic wheat maps for plant height (*H*), wheat/rye crossability (*Cross*), powdery mildew resistance (*R*), strength of the dough (*W*), grain hardness (*Hard.*), total protein content (*Prot.*), heading time (*HT*), photoperiod response under long-day conditions (*LD*) or difference between long- and short-day conditions (*DLS*), ear compactness (*Cp*), spike length (*Sl*), number of spikelets (*Ns*) and awning (*Awn*). Approximate positions of the centromeres are indicated with a *circle*. *Dotted chromosomes* indicate that the linkage between the different groups was >50 cM. Approximate locations of known genes are indicated on the left of the chromosomes according to McIntosh et al. (1998)

Discussion

Molecular mapping in an intervarietal context in wheat is not a simple task, but is a need in molecular based breeding strategies. Breeders will frequently be confronted by the lack of polymorphism often mentioned in the literature for bread wheat (Chao et al. 1989; Cadalen et al. 1997). For example, using a population of 194 recombinant inbred lines from the cross between the two cultivars Renan and Récital, Groos et al. (2002) built a map comprising 436 loci but with a total length of 2,260 cM representing only 62% genome coverage. In order to im-

prove the saturation of such maps, microsatellites probably constitute the best tool because of their locus-specificity, their high level of polymorphism and their possibility for automation. Several groups have already derived microsatellites from Triticeae species (Bryan et al. 1997; Röder et al. 1998a; Guyomarc'h et al. 2002) but the effort is being continued in order to produce saturated microsatellite linkage maps as is the case for maize (1,735 public SSRs in the Maize Data Base). In our case, the previous map (Cadalen et al. 1997) was greatly improved by the addition of microsatellites, especially for the D genome where three different chromosomes had almost no RFLP loci mapped (chromosomes 2D, 4D and 5D).

In our map, the size of the individual chromosome maps and especially those from the D genome, were longer than those observed using the ITMI population (Nelson et al. 1995a, b, c; Van Deynze et al. 1995; Marino et al. 1996). This may be explained by the fact that recombination occurred more easily in an intervarietal F1 rather than in an interspecific one, which was the case for the D genome of the ITMI population, from a cross between a synthetic wheat and a cultivar. This is of great interest since breeders often look at wild related species to recover resistance genes and thus have a lot of difficulty in getting rid of deleterious traits. Furthermore, the size of our map was slightly higher than that obtained with the ITMI population (3,685 vs 3,551 cM). In addition several markers remained unlinked, suggesting that the final size of the genetic map of the wheat genome will be around 4,000 cM.

A significant number of markers (103) gave multiple loci. Even if the duplicated loci were frequently mapped at homoeologous positions (42%), they were also mapped at orthologous positions, either on the same genome (20%) or even on the same chromosome (24%). This suggests that the duplication of these loci probably occurred before polyploidisation rather than after it. Since the number of linked duplicated orthologous loci was not very high, it was not possible to define linkage blocks for duplication as this was done for the syntenic regions between rice and wheat (Moore et al. 1995). However, such blocks could probably be defined using a larger number of markers.

Concerning the QTL analyses, the present update revealed three new QTLs compared to previous analyses. They were all located on D-genome chromosomes that were not covered in the previous map (chromosomes 2D and 5D). This shows the importance of a good genome coverage for a powerful QTL detection. All the QTLs that were previously detected were confirmed and confidence intervals were computed. The mean size of these confidence intervals was 68.9 cM indicating that the precision of the location of the QTLs was not very good and that the linkage information between markers and traits of interest could not easily be used in breeding programs.

It is noteworthy that several QTLs co-segregated, and not only those involved in related traits. For example, three QTLs for the number of spikelets, spike length, compactness and heading time were located in the same region on the short arm of chromosome 2B. This type of result always gives rise to the problem of whether there is only one gene having a pleiotropic effect or there are several genes clustered in the same region and acting for different (even related) traits. More and more studies tend to indicate that genes are often grouped in clusters. This seems particularily true for resistance genes (Chantret et al. 2000; C. Feuillet, personal communication). However, this is also probably the same for other types of genes. For example, Tranquilli et al. (1999), showed that at least three different genes coding for puroindolines (a and b) or puroindoline-like proteins (grain-softness protein: GSP) were located on the unique *Triticum monococcum* BAC clone of 105 kb. This suggests that genes are not evenly dispersed thoughout the genome but are rather clustered in numerous regions separated by repeated or non-coding sequences. This suggests also that it would be possible to identify BAC clones containing genes among an entire library simply by total cDNA probing.

While some QTLs frequently co-segregated with genes known to influence a trait, in some cases, they also appeared located at different regions. For example, on chromosome arm 5BS, a QTL was detected for wheat/rye crossability (K) while the major genes (*Kr*) are known to be located on the long arm of this homoeologous group. This suggests that other genes are involved in the expression of this trait than those observed and mapped using aneuploid stocks or mutant phenotypes and having the strongest effect. It was also oberved that QTLs were detected close to genes known to be involved in the trait, although alleles at these loci were supposed to be identical between the parents of our segregating population. This indicates that a range of allelic variation exists at these loci.

This work showed that the 'Courtot' × 'Chinese Spring' segregating population was well adapted for QTL detection. We are currently developing a larger population in order to fine map the regions bearing QTLs of interest, which will be the first step toward the molecular cloning of the genes underlying these QTLs.

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