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An intervarietal genetic linkage map of Indian bread wheat (*Triticum aestivum* L.) and QTL maps for some metric traits

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Summary

Bread wheat (*Triticum aestivum* L.) exhibits very narrow genetic diversity and hence there is high relatedness among cultivated varieties. However, a population generated from an intervarietal cross, with the parents differing in a large number of traits, could lead to the generation of QTL maps which will be useful in practice. In this report a genetic linkage map of wheat is constructed using a cross between two Indian bread wheat varieties: Sonalika and Kalyansona. The linkage map consisted of 236 markers and spanned a distance of 3639 cM, with 1211·2 cM for the A genome, 1669·2 cM for the B genome, 192·4 cM for the D genome and 566·2 cM for unassigned groups. Linkage analysis defined 37 linkage groups of which 24 were assigned to 17 chromosomes. The genetic map was used to identify QTLs by composite internal mapping (CIM) for three metric traits, viz. culm length (CL), flag leaf length (FLL) and flag leaf breadth (FLB). Of 25 QTLs identified in this study, 15 have not been reported previously. Multitrait CIM (MCIM) analysis was carried out for traits that were significantly correlated such as FLB–FLL and CL–FLB–FLL. Detection of a large number of QTLs for the three traits analysed suggests that in parent cultivars that are not too diverse, the differences at genetic level detected as polymorphisms may be mostly associated with QTLs for the observed differences.

1. Introduction

Wheat (*Triticum aestivum* L.) is a major food crop of the world and the second most important crop in India. It is a segmental allopolyploid containing three distinct but genetically related (homoeologous) genomes: A, B and D. It is a hexaploid containing 42 chromosomes. The haploid DNA content of bread wheat genome is approximately $1{\cdot}7 \times 10^{10}$ bp.

In bread wheat several genetic linkage maps have been published either in the form of separate homoeologous groups, such as groups 1 to 7 (Phillips & Vasil, 2001), or as complete maps (Liu & Tsunewaki, 1991; Gale *et al.*, 1995; Messmer *et al.*, 1999). Owing to the poor levels of polymorphism often encountered in wheat, mapping strategies most often used wide crosses involving either a synthetic wheat and a variety such as Chinese Spring (Gale *et al.*, 1995) or Opata (Nelson *et al.*, 1995*a–c*) as parents, or crosses between Chinese Spring and *Triticum spelta* (Liu & Tsunewaki, 1991; Messmer *et al.*, 1999).

The development of genetic maps is a prerequisite for the understanding of QTLs governing complex agronomic traits and their use in plant breeding via marker-assisted selection. The first intervarietal map of bread wheat, based on restriction fragment length polymorphism (RFLP) markers, was published by Cadalen *et al.* (1997). An updated version of this Chinese Spring–Courtot genetic map was published by Sourdille *et al.* (2003). More recently three intervarietal maps based on Australian bread wheat varieties were reported by Chalmers *et al.* (2001) and other intervarietal maps by Paillard *et al.* (2003), Liu *et al.* (2005), Quarrie *et al.* (2005), Suenaga *et al.* (2005) and Torada *et al.* (2006). Some of these maps have also been used for QTL analysis (Sourdille *et al.*, 2003; Liu *et al.*, 2005; Quarrie *et al.*, 2005; Suenaga *et al.*, 2005; Semagn *et al.*, 2006).

The major trait governing plant stature is plant height (culm length). Several major genes reducing

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plant stature have been identified in wheat. Introduction of genes *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) for reduced height (Peng *et al*., 1999) into commercial wheat cultivars resulted in the Green Revolution. The other *Rht* genes in hexaploid wheat include *Rht4*, *Rht5*, *Rht7* and *Rht12*.

Leaf size has a positive effect on biomass and yield of plants. In wheat, the flag leaf makes important contribution of photosynthates, particularly during grain filling. Several flag leaf morphogenetic parameters have been identified which contribute to the moisture stress tolerance of wheat. Flag leaf length and flag leaf breadth are components which contribute to photosynthesis. Searching for loci controlling these quantitative traits will be useful as they are of agronomic importance (Lupton, 1987).

The aim of this study was to obtain a genetic linkage map of wheat (*Triticum aestivum* L.) using a cross between the Indian bread wheat varieties Kalyansona and Sonalika and to obtain a QTL map for three metric traits: length and breadth of flag leaf blade, and culm length. For this purpose an F_2 population was used. Most often a genetic linkage map is prepared using populations obtained from two highly diverse genotypes. However, the markers from such a map may not be useful in the breeding programme as they may not be polymorphic among the varieties used in breeding. Kalyansona and Sonalika have served as popular cultivars and have been used as parents in a breeding programme, and hence the markers thus obtained would be useful for a future Indian wheat-breeding programme involving parents related to the two varieties.

2. Materials and methods

The mapping population consisting of 150 F_2 plants was derived from a cross between the varieties Sonalika and Kalyansona (bread wheat: *Triticum aestivum* L.). A set of nullitetrasomic lines derived from Chinese Spring were used. All plants were grown at Trombay under field conditions.

(i) *DNA extraction and estimation*

DNA was isolated and quantitated from leaf tissue by a new method of DNA isolation suitable for long-term storage (Nalini *et al*., 2004).

(ii) *Phenotypic data collection*

The data on three agronomic traits, viz. culm length (CL), flag leaf length (FLL) and flag leaf breadth (FLB), among 150 F_2 individuals were recorded at different stages of growth.

(iii) *PCR analysis*

All PCR amplifications were carried out on an Eppendorf Mastercycler-Gradient Thermal Cycler.

(a) *AP-PCR analysis*

PCR amplification was carried out in a volume of 25 μl containing 100 ng of template DNA, 2 mM $MgCl_2$, 25 pmol of primers, 200 μM each of dNTPs and 1 unit of *Taq* DNA polymerase. The cycling condition was as follows: 1 cycle of 5 min at 94 °C, 5 min at 45 °C and 5 min at 72 °C, and 35 cycles of 1 min at 94 °C, 1 min at 45 °C and 1 min at 72 °C, followed by a final 10 min extension at 72 °C.

(b) *RAPD analysis*

PCR amplification was carried out in a volume of 25 μl containing 100 ng of template DNA, 2 mM $MgCl_2$, 10 pmol of 10mer primers, 200 μM each of dNTPs and 1 unit of *Taq* DNA polymerase. The cycling condition was as follows: 1 cycle of 5 min at 94 °C, 5 min at 42 °C and 5 min at 72 °C, and 45 cycles of 1 min at 94 °C, 1 min at 42 °C and 1 min at 72 °C, followed by a final 10 min extension at 72 °C.

(c) *ISSR analysis*

PCR amplification using a 3′ anchored I SSR primer was carried out in a volume of 25 μl. The reaction mixture contained 100 ng of template DNA, 2 mM $MgCl_2$, 25 pmol of ISSR primer, 200 μM each of dNTPs and 1 unit of *Taq* DNA polymerase. The cycling condition was as follows: 1 cycle of 5 min at 94 °C, 5 min at 50 °C and 5 min at 72 °C, 45 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C, followed by a final 10 min incubation at 72 °C.

The PCR products of AP-PCR, RAPD and ISSR were separated by electrophoresis using 1× TBE buffer on a 2% agarose gel. The DNA fragments were stained with ethidium bromide and viewed under ultraviolet light and photographed.

(d) *AFLP analysis*

The AFLP analysis was carried out essentially by the method described by Vos *et al*. (1995). Genomic DNA (100 ng) was digested with *Eco*RI and *Mse*I. Ligations of the *Eco*RI and *Mse*I adapter sequences, the preselective amplifications and the selective amplifications were carried out using the primer pairs E_{A+3}/M_{C+2} as described previously. Equal amounts of the selective amplification products and formamide loading dye were mixed. The samples were denatured for 3 min at 90 °C, chilled on ice and fragments were separated by electrophoresis on a denaturing 5%

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polyacrylamide gel in a vertical cassette. The DNA fragments were stained by the silver staining method.

(e) *STMS analysis*

PCR amplifications were carried out using 84 STMS primers for the A, B and D genomes of bread wheat, viz. two each on either arm of the seven A, B, D chromosomes (Roder *et al*., 1998). The PCR reaction mixture (25 μl) contained 10 mM Tris-HCl pH 9·0, 2 mM $MgCl_2$, 10 pmol of each left and right primer, 200 μM of each dNTP, 1·0 unit of *Taq* DNA polymerase (Bangalore Genei, India) and 100 ng of template genomic DNA. The cycling condition was as follows: 1 cycle of 3 min at 94 °C and 45 cycles of 1 min at 94 °C, 1 min at 62 °C and 20 s at 72 °C followed by a final 10 min incubation at 72 °C. The PCR products were separated on 2·5% agarose gel and some STMS were analysed on denaturing 5% polyacrylamide gels.

(iv) *Extraction and analysis of seed proteins*

Total protein was extracted from five seeds each of Sonalika, Kalyansona and F_3 seeds from F_2 plants. High molecular weight (HMW) glutenin subunit and other seed proteins were analysed by SDS-PAGE (Payne & Lawrence, 1983).

(v) *Analysis of gene-specific loci*

(a) *PCR-RFLP of the ITS region*

ITS region from 18S-5.8S-26S rRNA was amplified using the primers G_1: 5′-TCCGTAGGTGAACCTGCGG-3′ and C_2: 5′-TCCTCCGCTTTATTGATATGC-3′ as detailed by Saini *et al*. (2000). An aliquot of the PCR product was digested with a restriction endonuclease (4 units) in a 10 μl reaction mixture according to the manufacturer's instructions. Digested fragments were separated on a 3% high-resolution agarose gel in TBE at 8 V/cm for 1·5–2 h and then stained with ethidium bromide.

(b) *PCR of puroindoline genes*

The wild-type allele of pinA (*Pina-D1a*) was PCR-amplified using the allele-specific primers Pina-D1F-5′-CCCTGTAGAGACAAAGCTAA-3′, Pina-D1R-5′-CACCAGTAATAGCCAATAGTG-3′ as described by Gautier *et al*. (1994).

(c) Rht-B1 *and* Rht-D1 *amplification*

The wild-type and mutant alleles of *Rht-B1* and *Rht-D1* were PCR-amplified using allele-specific primers according to Ellis *et al*. (2002).

(vi) *Nullisomic-tetrasomic analysis*

A series of nullisomic-tetrasomic lines of Chinese Spring (CS) (Sears, 1966) was used to physically map 22 AFLP primer combinations.

(vii) *Data analysis and linkage mapping*

In the case of a dominant marker the polymorphic band positions were scored as '0' or '1' for absence or presence of band, respectively. In the case of a co-dominant marker, the allele from the female parent was scored as '1', the allele from the male parent was scored as '2' and presence of the two alleles together (heterozygotes) was scored as '3'. The observed segregation ratios were tested by chi-square analyses (3 : 1). The linkage analysis was performed using MapMaker v.3.0b (Lander *et al*., 1987) for the F_2 population. Recombination frequencies were converted to centimorgans (cM) using Kosambi's mapping function (Kosambi, 1944). The linkage groups were constructed using the 'two-point/group' command with a LOD threshold of 3·0 and a maximum distance of 50 cM.

(viii) *QTL mapping*

QTL Cartographer v.2.5 (Wang *et al*., 2005) was used for QTL analysis. Zmap QTL, Model 6 with a window size of 10 cM, was used for composite internal mapping (CIM) analyses. The number of markers for the background control was set to five. For each trait, a minimum LOD value of 2·5 was used for the identification of putative QTLs. Association of a marker with a QTL was analysed by a two-population *t*-test. The F_2 population was divided into two groups based on the alleles of a marker closest to a QTL. The trait means of the two groups were subjected to a *t*-test for significance. QTL effects (R^2 values), also referred to as phenotypic variation, were obtained from the output file of CIM. Two combinations of the quantitative traits, viz. (1) FLB–FLL, (2) CL–FLB–FLL, were used for joint multitrait CIM (MCIM) using the module JZmapqtl available in QTL Cartographer.

3. Results

(i) *Genetic linkage map*

A genetic linkage map referred to as an 'SK' map consisting of 236 loci with a marker density of 15·4 cM was obtained. The map consisted of 37 linkage groups and spanned 3639 cM, with 1211·2 cM for the A genome, 1669·2 cM for the B genome, 192·4 cM for the D genome and 566·2 cM for unassigned groups. Twenty-four linkage groups were assigned to

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Table 1. *Number of markers and length of each linkage group*

Linkage group	Chromosome	No. of markers	Length in cM (Kosambi)
1	1A	04	57·9
2	1A	02	5·9
3	2A	03	20·2
4	3A	13	271·0
5	3A	02	17·1
6	4A	03	35·1
7	5A	08	72·1
8	5A	12	263·1
9	5A	09	165·1
10	5A	07	145·8
11	6A	05	96·0
12	7A	04	61·9
13	3D	07	97·6
14	3D	04	48·1
15	5D	04	24·4
16	6D	02	22·3
17	1B	26	453·5
18	2B	07	114·3
19	2B	07	89·0
20	3B	12	137·3
21	4B	06	40·4
22	5B	11	285·5
23	6B	17	343·9
24	7B	11	205·6
25	–	04	21·2
26	–	03	15·7
27	–	02	18·2
28	–	04	29·2
29	–	03	31·0
30	–	02	13·2
31	–	02	15·2
32	–	02	28·5
33	–	02	3·8
34	–	03	40·4
35	–	12	206·0
36	–	07	112·2
37	–	04	31·6
Total	**–**	**236**	**3639**

17 chromosomes; however, none were assigned to chromosomes 1D, 2D, 4D and 7D (Table 1). The number of markers mapped was highest in the B genome (97) followed by the A genome (72) and D genome (17) (Fig. 1).

(ii) *Segregation distortion*

Of the 280 markers analysed, 89 (31%) deviated significantly ($P<0{\cdot}05$) from a 3:1 ratio and this was not specific to any marker type. Of the 89 distorted markers, 74 were mapped and 15 remained unlinked. Thirty-nine mapped markers (53%) showed a segregation distortion in favour of Kalyansona and 35 (47%) in favour of Sonalika, indicating no bias towards a particular parent.

(iii) *Frequency distribution, ANOVA and correlation among the traits*

The frequency distribution of each of the three traits in the segregating population was found to be different. While FLB showed a normal distribution, FLL showed a distribution skewed towards shorter leaf length. A skewed distribution indicates higher frequency of a phenotype. The skewed distribution could arise due to (a) dominance of the alleles responsible for shorter leaf length, (b) epistatic action of leaf length inhibitor as well as (c) genotype × environment interactions. CL showed a double bell-shaped curve distribution. This is due to segregation for the two major semi-dwarfing genes present in the parents, viz. Kalyansona harbours *Rht-B1b* while Sonalika harbours the *Rht-D1b* gene. In the absence of major dwarfing genes the population would have shown a normal distribution. Further analysis showed that three combinations, viz. CL–FLL, CL–FLB and FLL–FLB, among the three quantitative traits were positively correlated.

(iv) *Composite interval mapping (CIM) of QTLs*

Twenty-five QTLs with LOD scores above 2·5 spread over seven chromosomes were detected for the three traits (Table 2). Eight QTLs for CL with LOD scores ranging from 2·5 to 4·3 and phenotypic variation (QTL effect, R^2) ranging from 21·6% to 66·5% were found on chromosomes 2B, 3A, 5A, 6A, 6B, 7B and linkage group 12. Twelve QTLs for FLL with LOD scores ranging from 2·5 to 7·2 and phenotypic variation ranging from 8·2% to 39·0% were found on chromosomes 1B, 2B, 5A, 6A, 6B and 7B. Five QTLs for FLB with LOD scores ranging from 2·5 to 3·3 and phenotypic variation ranging from 10·9% to 34·9% were found on chromosomes 2B, 5A, 6A, and linkage groups 1 and 11.

(v) *Multitrait composite interval mapping (MCIM)*

Of the various combinations of the quantitative traits that were used for correlation analysis, two combinations, viz. FLB–FLL, CL–FLB–FLL, which showed positive correlation, were chosen for MCIM analysis. The results are described below.

(a) *Flag leaf breadth and flag leaf length*

The results of MCIM are given in Table 3. Twenty-two QTLs were detected in joint MCIM, of which nine were also detected by CIM.

(b) *Culm length, flag leaf breadth and flag leaf length*

The results of MCIM are given in Table 4. Forty-three QTLs were detected in joint MCIM, of which 17

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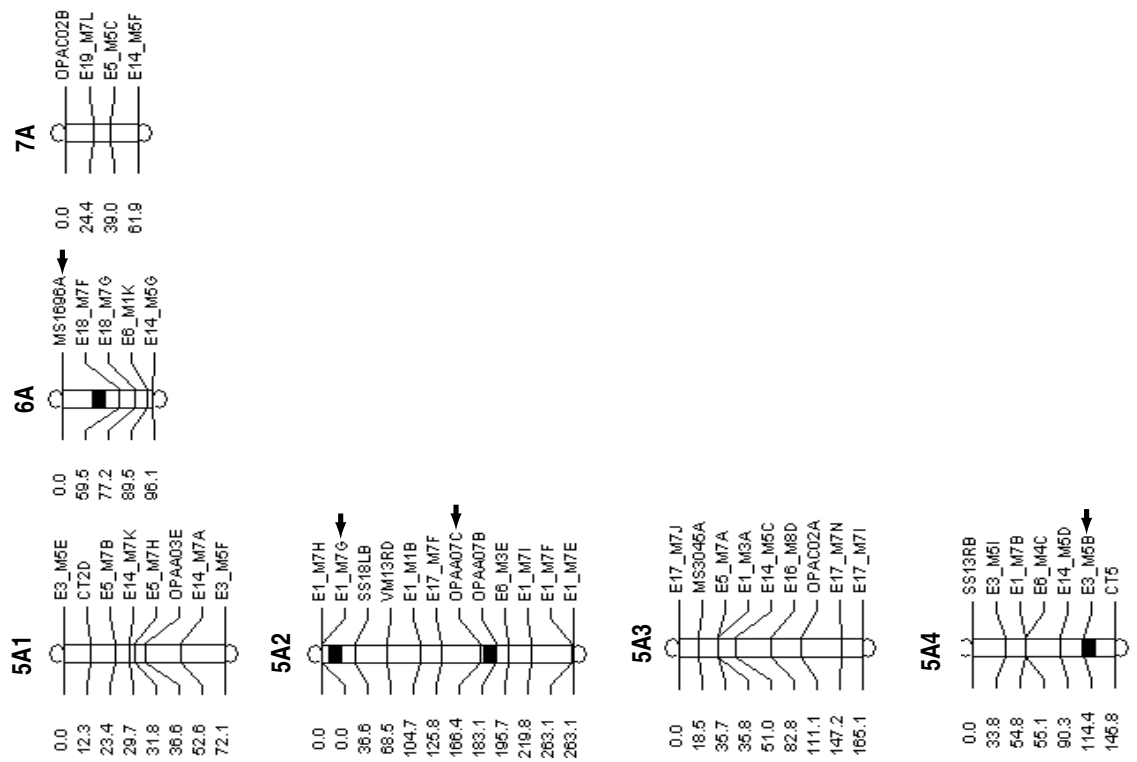

Fig. 1.

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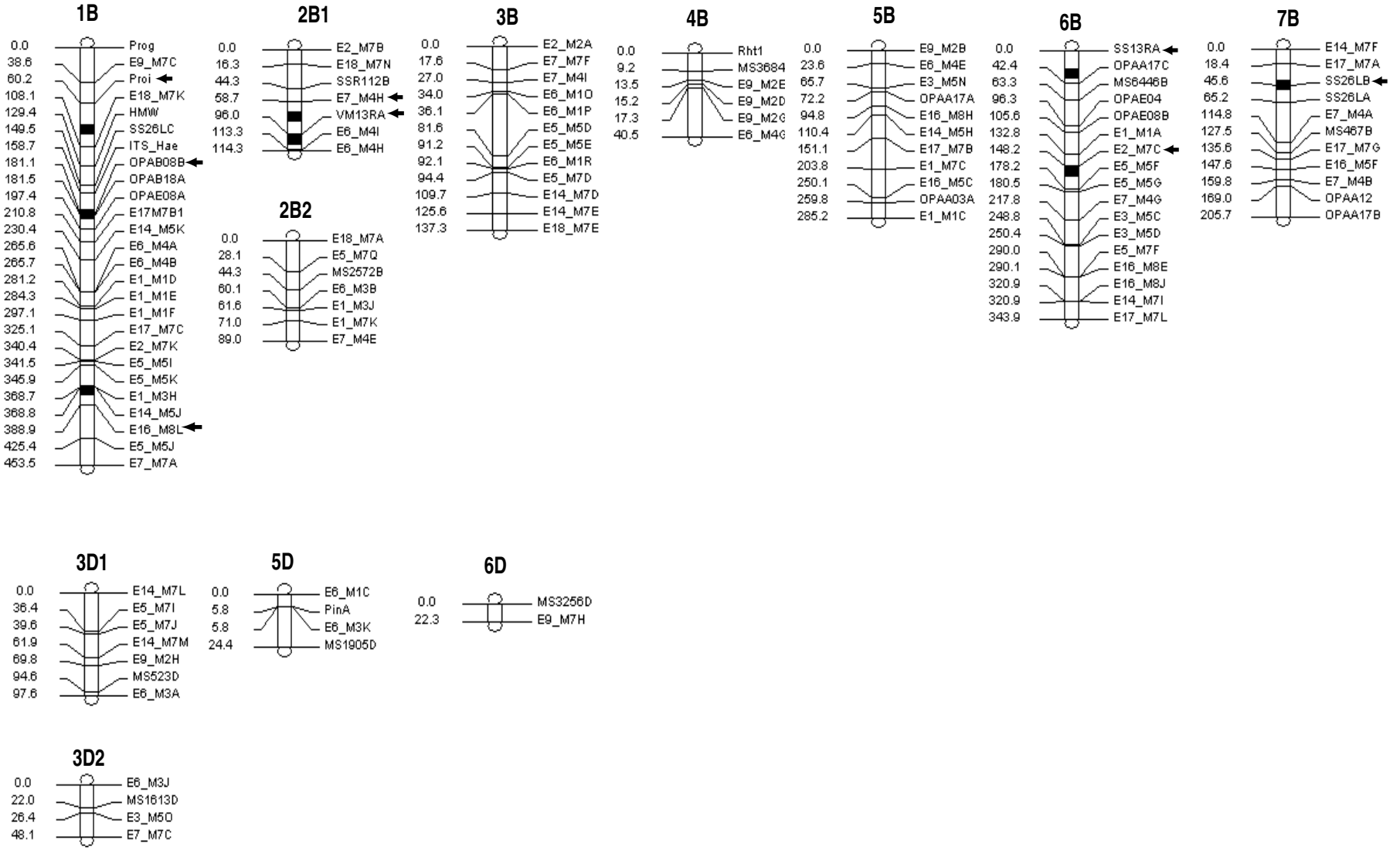

Fig. 1. (*cont.*)

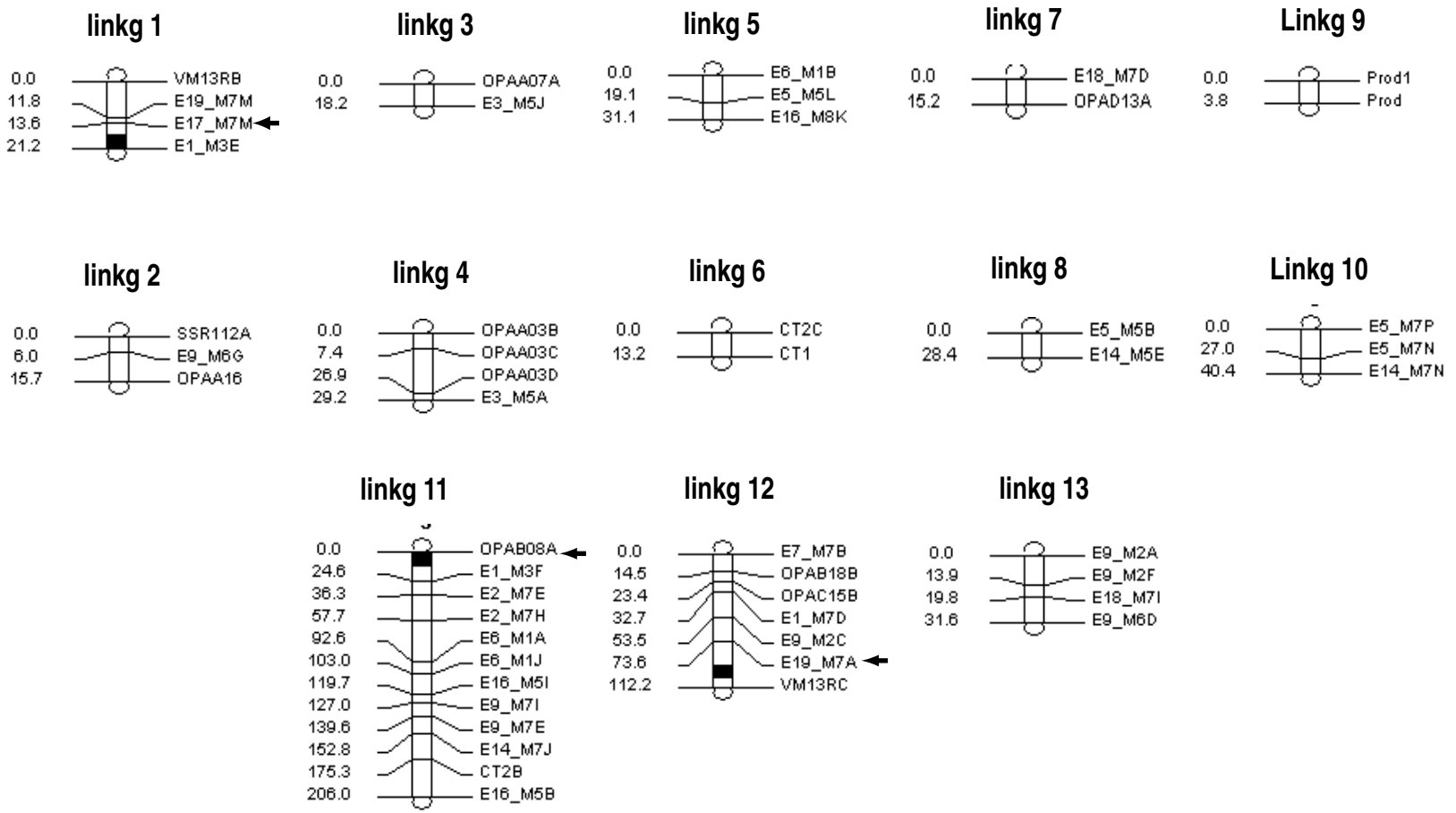

Fig. 1. Genetic linkage map of bread wheat using an F_2 population derived from a cross between Sonalika and Kalyansona. The name of the chromosome or group is given on the top of individual linkage group. The names of markers are given on the right-hand side and the distance (in centimorgans) between markers on the left-hand side of the linkage groups. The arrows on chromosomes 3A1, 5A4 and 6A indicate the markers E16_M5D, E3_M5B and Xgwm1696A, respectively, which are lying closest to the QTLs for the trait culm length (CL). The arrows on chromosome 5A2 indicate the markers E1_M7G and OPAA07C, which are lying closest to the QTLs for the trait flag leaf length (FLL). The black coloration within the linkage group indicates the QTL at these locations. The STMS markers indicated as 'MS' are the 'Xgwm' series of STMS described by Roder *et al*. (1998). The arrows on chromosomes 2B1 and 6B indicate the markers E7_M4H, SS13RA and E2_M7C, respectively, closest to the QTLs for CL. The arrows on chromosomes 1B, 2B1 and 7B indicate the markers E14_M5J, Proi, OPAB18A, VM13RA, and SS26LB, respectively, closest to the QTLs for FLL. The arrow on linkage group 1 indicates the marker E17_M7M closest to the QTL for trait flag leaf breadth (FLB). The arrows on linkage groups 11 and 12 indicate the markers OPAB08A, and E19_M7A closest to the QTLs for traits FLB and CL, respectively.

Table 2. *Composite interval mapping (CIM) for three quantitative traits*

Sr. no.	Trait and linkage group	LOD score	QTL position and interval (cM)[a]	Closest marker[b]	QTL effect (R^2 %)
	Culm length (CL)				
1	2B1	3·3	76·7 (18)	E7_M4H (18)	40·5
2	3A1	4·3	205·7 (24)	E16_M5D (20)	65·3
3	5A4	3·1	120·0 (24)	E3_M5B (5·6)	46·8
4	6A	3·7	40·0 (30)	Xgwm1696A (40)	66·5
5	6B	4·3	30·0 (56)	SS13RA (30)	21·6
6	6B	3·2	156·2 (40)	E2_M7C (8)	30·2
7	7B	2·5	22·4 (36)	**E17_M7A* (4)**	21·6
8	Linkg12	3·3	99·6 (28)	E19_M7A (26)	59·2
	Flag leaf breadth (FLB)				
1	2B1	2·7	56·3 (14)	SSR112B (12)	11·7
2	5A3	2·6	24·4 (28)	**Xgwm3045A* (6)**	10·9
3	6A	2·5	77·1 (8)	**E18_M7G*** (0·1)	20·9
4	Linkg1	3·1	19·6 (6)	E17_M7M (6)	34·9
5	Linkg11	3·3	8·0 (24)	OPAB08A (8)	14·4
	Flag leaf length (FLL)				
1	1B	3·9	372·7 (12)	**E14_M5J** (4)**	20·7
2	1B	3·9	90·1 (40)	Proi (30)	32·9
3	1B	3·3	181·5 (19)	OPAB18A (0)	8·2
4	2B1	7·2	104·0 (17)	VM13RA (8)	26·5
5	5A1	2·9	12·3 (6)	**CT2D* (0)**	8·5
6	5A2	5·1	16·0 (16)	E1_M7G (16)	34·8
7	5A2	5·5	178·3 (19)	OPAA07C (12)	29·4
8	5A3	2·5	6·0 (18)	E17_M7J (6)	11·3
9	6A	2·7	59·5 (16)	E18_M7F (0)	39·0
10	6B	2·6	81·3 (37)	Xgwm6446B (18)	22·6
11	6B	2·9	247·8 (41)	E7_M4G (30)	8·9
12	7B	3·4	45·6 (13)	SS26LB (0)	11·6

*, ** Means for marker allele classes, which differed significantly at $P<0·05$ and 0·01 respectively. The markers associated with the traits are shown in bold.
[a] Intervals in cM were obtained by marking positions ± 1 LOD from the peak.
[b] Values in parentheses are the distances (cM) of the marker from the peak.

were also detected by CIM. As an example a representative QTL Cartographer plot involving chromosome 6B obtained using MCIM involving three correlated metric traits – trait 1, culm length; trait 2, leaf breadth; trait 3, leaf length – is shown in Fig. 2.

(vi) *Association of molecular markers with quantitative traits by* t-*test*

Thirty markers that were closest to the QTLs were analysed further. The means for the trait concerned were estimated for each of the two-allele classes and subjected to a *t*-test. Among five of the markers, the differences between the allele classes were found to be significant, indicating that these markers show association with the respective traits (Table 2), viz. one marker associated with CL, two for FLB and two for FLL.

4. Discussion

The aim of the study was to develop a linkage map based on varieties which were extensively used in cultivation and in wheat breeding, with the purpose of identifying associations between markers and QTLs. In this study an intervarietal map has been constructed based on the Indian bread wheat cultivars Kalyansona and Sonalika. In general, cultivated wheat varieties exhibit narrow genetic diversity. However, the two varieties used in this study showed differences in 10 agronomically important traits and also exhibited considerable DNA polymorphisms. The differences at DNA level detected as polymorphisms were observed to be mostly associated with QTLs for the observed differences. An F_2 population was used as it is available earlier and expected to be unbiased.

(i) *Genetic linkage map*

The length of the SK map (3639 cM) is comparable with reported wheat maps lengths such as the Courtot × Chinese Spring intervarietal map (3685 cM; Sourdille *et al.*, 2003) and the Cranbrook × Halberd intervarietal map (4110 cM; Chalmers *et al.*, 2001), and is less than the Synthetic W7984 × Opata 85 (ITMI) map (> 5000 cM; for review see Langridge

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Table 3. *QTLs detected by joint MCIM involving two correlated quantitative traits: flag leaf breadth (FLB) and flag leaf length (FLL)*

	QTL detected by joint MCIM		
Linkage group	LOD score	Position (cM)	Closest marker[a]
1A1	3·4	17·8	E16_M5B
1B	3·8	368·7	E14_M5J
1B	3·0	181·5	OPAB18A
2B1	7·4	104·0	VM13RA
2B1	3·8	66·7	E7_M4H
3A1	3·1	61·1	E16_M8B
3A1	2·6	163·5	E14_M5I
4A	2·8	34·9	E1_M3D
5A2	5·3	14·0	E1_M7G
5A2	5·8	182·3	OPAA07C
5A3	4·1	10·0	E17_M7J
5A3	3·4	163·2	E17_M7N
6A	3·3	59·5	E18_M7F
6B	2·7	290·1	E16_M8E
6B	3·5	96·3	OPAE04
7B	5·8	36·3	E17_M7A
Linkg1	3·8	19·6	E17_M7M
Linkg4	2·7	6·0	OPAA03B
Linkg5	3·2	19·1	E5_M5L
Linkg11	4·2	100·0	OPAB08A
Linkg11	4·2	119·6	E16_M5I
Linkg13	3·8	25·7	E18_M7I

[a] The closest marker is the one lying next to the QTL locus influencing the QTL at the respective LOD value.

Table 4. *QTLs detected by joint MCIM involving three correlated quantitative traits: culm length (CL), flag leaf breadth (FLB) and flag leaf length (FLL)*

	QTL detected by joint MCIM		
Linkage group	LOD score	Position (cM)	Closest marker[a]
1A1	4·4	19·8	E16_M5B
1B	2·6	90·1	Proi
1B	3·9	368·7	E14_M5J
1B	3·4	181·5	OPAB18A
2A	4·3	19·8	E2_M7D
2B1	8·1	108·0	VM13RA
2B1	3·4	62·7	E7_M4H
2B1	2·5	16·0	E2_M7B
2B2	2·6	52·3	MS2572B
3A1	5·7	205·7	E16_M5D
3A1	4·0	30·6	E14_M5A
3A2	2·8	0·0	E7_M7E
3B	3·4	58·1	E6_M1P
3D2	2·9	26·3	E3_M5O
4A	3·0	34·9	E1_M3D
4B	3·4	37·3	E9_M2G
5A2	6·4	182·3	OPAA07C
5A2	4·7	18·0	E1_M7G
5A2	3·7	110·7	E1_M1B
5A3	4·2	10·0	E17_M7J
5A3	4·0	155·2	E17_M7N
5A4	4·4	120·3	E3_M5B
5B	3·1	259·0	OPAA03A
5B	2·8	175·0	E17_M7B
5B	2·7	76·1	OPAA17A
6A	5·2	59·5	E18_M7F
6B	3·2	83·3	MS6446B
6B	5·3	32·0	SS13RA
6B	3·1	292·1	E16_M8E
7A	3·6	12·0	OPAC02B
7B	3·6	182·9	OPAA12
7B	7·0	36·3	E17_M7A
Linkg1	4·1	19·6	E17_M7M
Linkg4	3·5	6·0	OPAA03B
Linkg5	3·6	6·0	E6_M1B
Linkg6	2·8	8·0	CT2C
Linkg7	3·5	14·0	E18_M7D
Linkg8	3·2	26·0	E5_M5B
Linkg11	4·5	10·0	OPAB08A
Linkg11	4·9	143·6	E9_M7E
Linkg11	4·1	92·6	E6_M1A
Linkg12	4·2	103·6	E19_M7A
Linkg13	4·3	27·7	E18_M7I

[a] The closest marker is the one lying next to the QTL locus influencing the QTL at the respective LOD value.

et al., 2001). The number of markers in the ITMI map is 1065, while it is 659 in the Courtot × Chinese Spring map and 902 in the Cranbrook × Halberd map. The mean interval between two markers on the ITMI map is ~5·8 cM, while on the Courtot × Chinese Spring intervarietal map it is 5·6 cM and on the Cranbrook × Halberd map it is 4·5 cM and on the SK map it is 15·4 cM. The large difference in the marker frequency could be attributed to the number of markers on the map.

In the SK map, maximum number and proportion of markers were mapped onto the B genome (97, 41%), followed by the A genome (72, 31%) and then the D genome (17, 7%). Chromosomes 1D, 2D, 4D and 7D were not represented at all. A low level of polymorphism in the D genome observed is in agreement with the reports available in the literature and with the hypothesis suggesting a recent and monophyletic introduction of the D genome in bread wheat (Lagudah *et al*., 1991). The higher proportions of markers placed on the chromosomes 1B, 6B and 5A indicated that the two parents could be carrying more variations in these chromosomes than the rest.

The lengths of chromosomes 3A, 2B, 5B, 6B, 7B and 3D in the SK map are comparable to the sizes reported by others. The lengths of chromosomes 1A, 2A, 4A, 6A, 7A, 5D, 6D, 3B and 4B in the SK map are shorter than the lengths reported in the ITMI genetic linkage map. This could be due to the lower number of markers in the map. In contrast, the lengths of chromosomes 5A and 1B in the SK map are longer than the lengths reported in previous maps. Apparently addition of more markers was not the only

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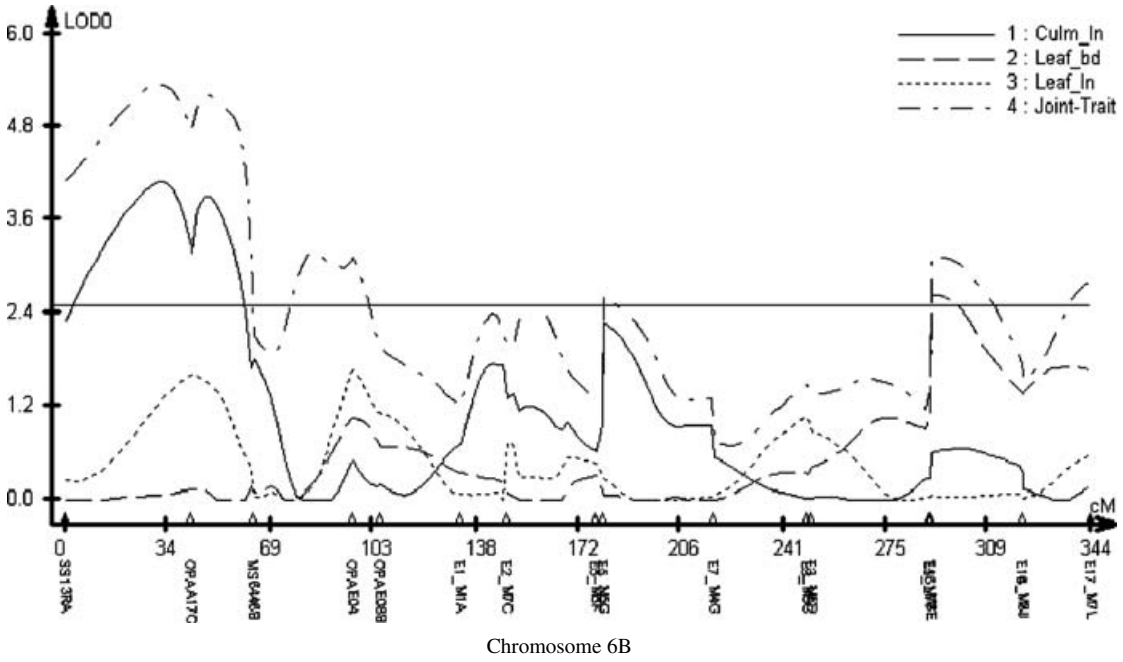

Fig. 2. A representative QTL Cartographer plot involving chromosome 6B obtained using multitrait composite interval mapping (MCIM) involving three correlated metric traits: trait 1, culm length (CL); trait 2, flag leaf breadth (FLB); trait 3, flag leaf length (FLL). The LOD value is given on the *y*-axis and the name of the markers and the distance between them in centimorgans is given on the *x*-axis.

reason for the increase in the length of chromosomes 5A and 1B, because for chromosome 6B, which had a similar number of markers to 5A and 1B, the chromosome length was comparable to that reported in the literature. The majority of the markers on chromosomes 5A and 1B were AFLP markers, and this may have led to map stretching. To test the effect of the type of marker on chromosome length, the lengths of chromosomes 1B and 5A were estimated using two modifications: (1) selectively withdrawing AFLP markers located on these chromosomes and (2) withdrawing non-AFLP markers. The results showed that withdrawal of AFLP markers resulted in a larger change in chromosome length (Table 5, chromosome 1B, rows 2–6) while withdrawal of non-AFLP markers resulted in a smaller change in length (Table 5, chromosome 1B, rows 7–9). It was also observed that withdrawal of AFLP markers resulted not only in compression at the interval *per se* but also all over the chromosome (Table 5). For example, when AFLP marker E6_M4A from chromosome 1B was removed the compression at the interval *per se* was only 7 cM but the overall length of the chromosome reduced by 20 cM. Similar results were found for other chromosomes including chromosome 6B. Stretching of linkage maps with the incorporation of AFLP markers in the map along with other markers such as RAPD, ISSR and SSR has been shown previously in several studies on cereals (Becker *et al*., 1995; Maheswaran *et al*., 1997; Castiglioni *et al*., 1999; Lotti *et al*., 2000; Saal & Wricke, 2002). The stretching of the map in the case of durum wheat (Lotti *et al*., 2000), barley (Becker *et al*., 1995) and rice (Maheswaran *et al*., 1997) was 52·5%, 70·9% and 68·9%, respectively. Map stretching could occur due to the addition of map distances as new markers are discovered and also due to differences in methods used in the construction of an existing map and the new data being superimposed. In the present case the methods used for the earlier mapping and the superimposed markers were same; therefore the increase in map distances could be due to addition. Genetic distances are subject to modification as new loci are discovered between the existing ones.

The distortion in segregation ratios for the markers observed (31%) in the SK map is comparable to the segregation distortion reported by others in wheat (27%, Cadalen *et al*., 1997; 35%, Messmer *et al*., 1999). Segregation distortion is reported among F_2 progenies of wheat (Liu & Tsunewaki, 1991). The segregation distortion in the SK map was not biased towards a particular marker type; also, when all markers were considered together, the segregation distortion was not found to be biased towards any parental allele. The segregation distortion observed could be (a) due to the polymorphic band being amplified from more than one loci or (b) the phenotypes associated with the marker may influence selection towards a particular allele. Segregation distortion is also reported among F_2 progenies in other plants such as rice (McCouch *et al*., 1988), lettuce (Landry *et al*., 1987) and tomato (Helentjaris *et al*., 1986).

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Table 5. *Effect of removal of a marker on the length of chromosome 1B*

Sr. no.	Markers removed	Chromosome length (cM)	Interval around the marker (cM)		Change in length (cM)
			With marker	Without marker	
1	None	453·5	–	–	–
2	E6_M4A	433·5	35·2	28·0	7·2
3	E6_M4B	433·5	15·6	0·2	15·4
4	E17_M7C	444·9	43·4	35·1	8·3
5	E14_M5K	406·7	54·8	12·0	42·8
6	E16_M8L	436·9	56·7	40·7	16·0
7	OPAB18A	447·7	16·0	14·5	1·5
8	OPAB08B	457·7	22·8	19·5	3·3
9	SS26LC ITS_Hae	451·4	51·4	47·4	4·0

Rows 2–6: AFLP markers, Rows 7–9: Non-AFLP markers.

Marker order and distances of some regions on the SK map and the reported maps were found to be comparable. Specific gene/loci markers such as *Rht-B1b*, *Glu-B1* loci and the *Nor-B1* locus along with the STMS loci allowed the comparison of two different intervals on the SK map with microsatellite (Roder *et al*., 1998) and consensus genetic (Somers *et al*., 2004) maps. The distance between *Rht-B1* and the microsatellite marker Xgwm368-4B on chromosome 4B in the SK map was 9·2 cM, which is similar to the reported distance of 9·0 cM (Roder *et al*., 1998). The reported distance between *Glu-B1* and *Nor-B1* loci on chromosome 1B (22 cM) (Payne *et al*., 1984; Ellis *et al*., 2002; Ram *et al*., 2002) is shorter than the distance estimated in this study (29 cM). This could be due to addition of a marker between the two loci and/or the computational method used.

(ii) *QTL mapping*

In recent years the availability of DNA markers and powerful biometric analytical tools has led to considerable progress in QTL mapping in plants. There are several types of experimental designs for QTL analysis and the choice of method depends on the mating system of the crop species. Most QTL analyses in plants involve populations derived from pure lines and use several approaches to associate QTLs with molecular markers. In this study an F_2 population was used to detect the loci significantly contributing to the traits of interest.

The SK map was used for QTL analysis of three metric traits that differed between the parents. We used CIM and MCIM, which are often recommended for power and precision of QTL analysis. CIM and MCIM have been used recently in bread wheat (Kulwal *et al*., 2003; Campbell *et al*., 2003; Marza *et al*., 2006). CIM is an extension of simple interval mapping (SIM) that considers both the markers flanking the QTL and background markers, which could be or need not be linked to the QTL. CIM is said to give more power and precision in the detection of QTLs than SIM. CIM has been used in QTL mapping in wheat (Shah *et al*., 1999; Campbell *et al*., 2003; Kulwal *et al*., 2003). One of the most important advantages of CIM is that the markers can be used as boundary conditions to narrow down the most likely QTL position. The resolution of QTL locations can be greatly increased.

(a) *Culm length* (*CL*)

Culm length (or plant height) is an important trait that contributes to the plant's stature. Classical genetic studies have shown that genetic control of CL in bread wheat is complex, and most chromosomes harbour factors (loci) that can affect it (Law *et al*., 1973). To date 21 loci with major effect on plant height have been identified (Worland *et al*., 1998). The two most common semi-dwarfing genes, *Rht-B1b* and *Rht-D1b*, are present on the short arms of chromosomes 4B and 4D (Ellis *et al*., 2002), respectively, and are gibberellic acid (GA)-insensitive. Both Sonalika and Kalyansona are semi-dwarf genotypes, and harbour *Rht-D1b* and *Rht-B1b*, respectively. Of the eight QTLs that were detected for CL in this study, the QTL on chromosome 6A showed the highest phenotypic variation (66·5%). The QTL with the highest LOD score of 4·3 and a phenotypic variation of 65·3% was on chromosome 3A1 with E16_M5D as the closest marker. The STMS marker Xgwm169-6A is closest to the QTL for plant height on chromosome 6A. Since genes for plant height are known to be present on chromosome 6A, this STMS marker could be linked to one of these genes.

In addition to *Rht-B1b* and *Rht-D1b*, a large number of QTLs for CL have been reported by many workers (Table 6). Two major QTLs on chromosome 6B, with LOD scores of 3·2 and 4·3, have been observed in this study. A QTL for CL on chromosome 6B has not

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Table 6. *List of QTLs detected in the present study and reported in literature for the four quantitative traits*

Name of trait	QTLs detected in the present study[a]	QTLs reported in literature	References
Culm length (CL)	2B1, 3A1, 5A4, 6A, **6B**, 7B, Linkg12	4BS, 4DS, 6A 7AL, 7BL	Cadalen *et al.* (1998)
		1AS, 2DS, 4AL, 6AS	Borner *et al.* (2002)
		1BL, 2AS	Kulwal *et al.* (2003)
		3A	Shah *et al.* (1999)
		2BL, 2BS, 2DL, 3BL, 4B, 6A	Marza *et al.* (2006)
Flag leaf breadth (FLB)	**2B1**, 5A3, 6A, Linkg1, Linkg11	1A, 1B, 2A, 3B, 5A, 5B, 6A	Keller *et al.* (1999)
		1A, 2A, 3A, 3B, 4B, 6D, 7D	Iqbal & Vahidy (1992)
Flag leaf length (FLL)	**1B, 2B1, 5A1, 5A2, 5A3, 6A, 6B, 7B**	1A, 2A, 5A, 6A, 2B, 3B, 4B, 5B, 6D	Iqbal & Vahidy (1992)

QTLs in bold are detected in this study.

been reported, although previous cytogenetic studies have indicated that a gene for plant height is also present on chromosome 6B (Goud & Sridevi, 1988). The two parents Sonalika (average CL = 57·75 ± 1·6 cm) and Kalyansona (average CL = 49·3 ± 2·34 cm) are semi-dwarf genotypes. The phenotype exhibited is contributed to by the eight QTLs in addition to the two dwarfing genes.

(b) *Flag leaf length* (*FLL*) *and flag leaf breadth* (*FLB*)

FLL and FLB determine area, which is an important trait. QTLs for leaf breadth reported in the literature are listed in Table 6. Monosomic analysis has shown that chromosomes 1A, 2A, 3A, 3B, 4B, 6D and 7D affect FLB (Iqbal & Vahidy, 1992). Among the five QTLs identified for FLB in this study, the QTL with the highest phenotypic variation (34·9 %) is present on unassigned linkage group 1. STMS marker Xgwm3045A was lying closest to the QTL on 5A showing a phenotypic variation of 10·9 %. A QTL on chromosome 5A with a phenotypic variation of 14·9 % has been reported by Keller *et al.* (1999). QTLs for FLB on chromosome 2B have not been reported previously.

Monosomic analysis of FLL in bread wheat had indicated that chromosomes 1A, 2A, 5A, 6A, 2B, 3B, 4B, 5B and 6D affected FLL (Iqbal & Vahidy, 1992). Of the 12 QTLs for FLL identified in this study, those on chromosomes 5A, 6A and 2B were also identified by monosomic analysis and the one exhibiting maximum phenotypic variation (39 %) was on chromosome 6A.

(c) *General observation*

For CL and FLL, often, more than one QTL for the same trait was identified on a chromosome. However, more than one QTL within the same interval, if present, cannot be identified since CIM does not have the power to resolve such linked QTLs. Among QTLs for a trait located on the same chromosome, two QTLs for CL were located on 6B and three QTLs for FLL were located on 1B, two on chromosome 5A2 and two on chromosome 6B. Such QTLs on the same chromosome were not linked. In all cases QTLs on the same chromosome were separated by long genetic distances ranging from 13 to 280 cM, thus suggesting absence of close linkage or no linkage between the QTLs.

(iii) *Multitrait composite interval mapping* (*MCIM*)

MCIM has been used recently as a means of improving the power and precision of QTL detection for correlated traits, as information on the traits acts like repeated measurements. Using MCIM, loci showing pleiotropy on the traits CL, FLB and FLL were analysed.

(a) *Flag leaf breadth – flag leaf length*

The traits FLB and FLL showed significant positive correlation ($r = 0{\cdot}53$; $P < 0{\cdot}01$), indicating that there could be some loci affecting both the traits. Of the 22 QTLs detected by joint MCIM, the nine QTLs which were also detected by CIM in addition to joint MCIM are the QTLs which influences both the traits in the combination. Of these nine, the QTL on chromosome 7B was also detected for CL. Two of these nine QTLs located on chromosomes 1B and 5A1 were the same as those for which the corresponding molecular markers showed significant association with the traits in question (see later).

(b) *Culm length – flag leaf breadth – flag leaf length*

Positive correlation was observed between CL and FLB ($r = 0{\cdot}22$; $P < 0{\cdot}05$) and between CL and FLL

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($r = 0{\cdot}22$; $P < 0{\cdot}05$), indicating that there could be some loci influencing all three traits. MCIM analysis on FLB, FLL and CL detected a few more QTLs in addition to the ones detected by MCIM analysis on FLB and FLL. In addition to MCIM analysis on FLB and FLL, additional QTLs were detected when all three traits were analysed together for multitrait analyses. Seven QTLs located on chromosomes 3A1, 3B, 5A4, 6B, 7B and linkage group 12 were detected exclusively for CL by CIM but were also detected by joint MCIM for all three traits. Joint MCIM detected more QTLs jointly affecting CL, FLL and FLB than the QTLs jointly affecting only FLL and FLB. Of the 43 QTLs detected by joint MCIM, 17 which were also detected by CIM in addition to joint MCIM were the QTLs which influence both the traits in the combination. Three of the 17 QTLs located on chromosomes 1B, 3A2 and 7B are the same as those for which the corresponding molecular markers showed significant association with the traits in question (see later).

In both the above MCIM analyses the LOD scores for the common QTLs were higher in MCIM than in CIM. This shows that the level of confidence in MCIM for QTL detection is higher even than for CIM. The repeated finding of a QTL by two methods such as CIM and MCIM confirms the QTL only in the available data. However, a separate set of data would be needed to confirm the presence of a QTL. A similar finding has been reported by Kulwal *et al.* (2003), although with a different combination of traits. The QTLs detected by joint MCIM could suggest pleiotropy as the possible cause of correlation among the correlated traits. This inference may be taken into account while designing experiments involving molecular MAS aimed at improving more than one trait simultaneously.

(iv) *Markers associated with quantitative traits*

The significance of marker–trait association was analysed by *t*-test to check their usefulness in MAS and in detecting probable false QTLs. For instance OPAB18A (FLL) and SS26LB (FLL) were coincident with the QTL positions but did not show a significant association with the trait. It was noticed (Table 2) that the marker–trait association was not always correlated either with the distance of the marker from the QTL position or with the size of confidence interval. Also, no significant correlation between marker–trait associations and the magnitude of the LOD score was detected, in contrast to that reported by Kulwal *et al.* (2003). Markers having lower LOD values but showing association could be due to a lower confidence interval and the marker being very close to the QTL position. A few markers with LOD values higher than 3·9 did not show association with the trait in question. This could be due to the lower contribution to phenotypic variation or the larger distance between the marker and the QTL position or larger confidence interval. Of the five marker–trait associations found, four were also detected by MCIM for the trait(s) in question and these markers could prove to be useful in MAS.

5. Conclusions

An intervarietal genetic linkage map based on a cross between the two wheat Indian varieties Sonalika and Kalyansona was developed. These two varieties have served as the starting material for many of the later-developed cultivars and hence the markers thus obtained would be useful for future breeding programmes involving parents related to the two varieties. Several QTLs were detected for three quantitative traits, of which 15 have not been reported previously. Cultivated Indian bread wheat varieties have narrow diversity; however, we found many agronomically important traits which differed among Kalyansona and Sonalika and also sufficient polymorphisms at the DNA level. The four markers that showed association with quantitative traits could be useful in MAS.

Although genetic maps of wheat have been developed previously, many of which using the ITMI populations, there is a need to develop maps for specific populations for actual use. The Indian cultivars, for example, are spring wheats many of which carry cytogenetic variations. Also varieties adapted to certain agroclimatic conditions could carry variations. Hence an independent map based on Indian wheat varieties will thus be more useful.

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