Construction of SSR linkage map and QTL mapping for spike characters in common wheat RIL population

Manickavelu A, Kawaura K, Imamura H, Mori M, Ogihara Y

Kihara Institute for Biological Research, Yokohama City University, Maioka-cho, Yokohama, Japan

ABSTRACT

Linkage map with SSR markers are highly useful for further map based approach in wheat. In the present study, Recombinant Inbred Lines (RILs) developed from Chinese Spring/Spelta (*Duhamelianum*) was used for constructing the linkage map with SSR markers selected from so far published data and developed some functional markers. As a cost effective platform, for PCR reactions, home made *Taq* enzymes were used and the PCR products were genotyped mostly in Agarose gel. Out of 363 polymorphic primers between parents, 292 resulted as linked markers in linkage analysis. But after elimination of > 50 cM distanced markers, 253 loci were lingered uniformly in all chromosomes except chromosome 1D. Only one linkage break was occurred in chromosome 5A and no linked markers were found for chromosome 1D. The total map length was 2473.5 cM with average mapping distance of 9.77 cM between the markers. *A* genome accounts for 949.4 cM with 105 loci, followed by *B* genome of 837.9 cM with 102 markers, whereas the *D* genome had 686.2 cM with 46 markers. Because the parents phenotypically varied for prominent spike characters (*Q/q*), phenotypic data were recorded for spike length (SL), spikelet number (SN) and spike compactness (SC) for QTL analysis. Totally eleven QTLs were detected for three traits. For SL, out of four, two QTLs were located on chromosome 2A contributed by CS (*Q*). Another two QTLs were found on chromosome 5A contributed by Spelta parent (*q*). For SN, three out of six QTLs were found on chromosome 2A, two QTL located on 2B and the remaining one located on chromosome 4D. One major QTL for SC was found at chromosome 5A.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major food crop of the world. 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 1.7 x 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). Owing to the poor levels of polymorphism often encountered in wheat, mapping strategies most often used wide crosses involving either synthetic wheat with variety such as Chinese Spring or Opata as parents, or crosses between Chinese Spring and *Triticum spelta* (Liu & Tsunewaki, 1991). 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). Among the hexaploid wheat species, spelt has proved to be a rich-source of useful genes for tolerance to biotic and abiotic stress, and quality (Campbell 1997). The morphological characteristics of spelt, such as spike shape, hulled grain and brittle rachis, are believed to be principally controlled by the recessive *q* allele at the *Q* locus on chromosome 5A (Muramatsu 1963). Heterozygous plants with *Q/q* have a spike morphology that is like to speltoid, and plants that are nullisomic or monosomic for chromosome 5A also have a speltoid phenotype, thereby these phenotypic changes seems to be attributed to the *Q* gene dosage. However, these traits are also subjected to modification by other genes on the genetic background (Muramatsu 1986).

With the objectives of constructing linkage map and locating QTLs related to spike characters, the present investigation was carried out using the Recombinant Inbred Lines (RIL) population developed from Chinese spring/Spelta RIL population.

MATERIALS AND METHODS

Materials

The RILs used in this study were established at F8 generation by the single-seed descent method from an F2 family between *Triticum aestivum* cv. Chinese Spring (CS) and *Triticum spelta* var. *duhamelianum* KT19-1 (Sp) (Ahmed et al. 2000). These two parental lines were selected from the genetic resource bank of KIBR because of the relatively large genetic diversity and lack of any genetic distortion of segregation among them (Liu and Tsunewaki, 1991). CS and Sp are moderate and strong spring types, respectively. One hundred and forty four RILs in addition to the parents (CS and Sp) were grown in the experimental field of Kihara Institute, Yokohama, Japan. Seeds were sown first in nursery boxes in November, and were transplanted to the field in December in the season of 2006. The spike characters *viz*., Spikelet length (SL), Spiketlet number (SN) were observed in three spikes in three plants in each RIL. The Spike compactness (SC) was calculated by dividing SL with SN.

Microsatellite assay

DNA from parents and the RIL population was extracted following the CTAB method as modified by Saghai-Maroof et al. (1984) by pooling leaf tissue from three plants of each RIL. Nine hundred and thirty five SSR markers were used for screening the parental lines for polymorphism. The SSRs included the following markers: 232 GWM (Röder et al. 1998); 65 GDM (Pestsova et al. 2000); 251 WMC (Gupta et al. 2002); 94 CFD and CFA (Guyomarch et al. 2002; Sourdille et al. 2003), 29 BARC (Song et al. 2005), 207 HBG, HBE and HBD (Torada et al. 2006) and 58 GPW (Sourdille et al. 2004). Each PCR reaction was performed in a total volume of 10 µl containing 0.5 µM of each forward and reverse primer, 0.2 mM of each deoxynuleotide, 0.03 U/µl of Taq DNA Polymerase and 50 ng of template DNA. The PCR amplification consisted of an initial denaturation step of 5 min at 94^0C, followed by 45 cycles of three steps of 30 s each: denaturation at 94^0C, annealing at 50, 55 or 60^0C (depending on the individual marker), and extension at 72^0C. Products were electrophoresed in 3% (w/v) agarose gels and visualized after staining with ethidium bromide. Molecular markers that did not have easily discernible polymorphisms on agarose gels were visualized in non-denaturing 10% PAGE gel.

Data analysis and linkage mapping

Goodness of fit for all the loci to an expected 1:1 segregation ratio was tested using chi-square analysis. Linkage analysis of polymorphic marker loci was performed with MAPMAKER (Lander et al. 1987) for selfed RILs. Recombination frequencies were converted to centi-Morgans (cM) using Kosambi mapping function because of the independent cross-over events in different meiotic phases during the development of the population. Multipoint analysis was used on individual linkage groups, using an initial LOD threshold of 3.0 and maximum recombination fraction of 0.45. Non-distorted markers that could be mapped with a LOD threshold >3.0 were integrated first, followed by markers that showed distorted segregation. Markers that did not map at LOD 3.0 were then placed at preferred locations using LOD score 2.5. The polymorphic markers were ordered using the "compare" command. Additional markers were added afterwards on this frame using the "try" command. The final order was verified with the "ripple" command with a window-size of five and LOD threshold of 2.5.

RESULTS AND DISCUSSION

Genetic linkage mapping

Totally we selected 935 SSR primers from all published linkage maps and 625 primers showed good level of amplification in the parents of RIL population. Out of 625 primers, 363 showed clear polymorphism between the parents further used for genotyping in all 147 lines of RIL population. For genotyping, the cost effective ways were tried and succeeded. We tried less expensive Taq enzyme (Paq 5000) and own made Taq enzymes for PCR reaction and succeeded. Importantly the PCR products were mostly (~80%) visualized in Agarose gel (Nalam et al. 2006) further the gel was reused for more than 20 times. The approach not only reduced the cost and also reduced the time of developing a skeleton map of a crop. In linkage analysis, 292 from 363 markers were linked, but we eliminated the markers linked more than 50 cM for further linkage map construction in order to get the informative linkage map for QTL analysis. Finally 253 marker loci were used for map construction and they distributed uniformly in all the chromosomes except 1D. Microsatellites in hexaploid wheat are fairly evenly distributed along the linkage groups (Roder et al. 1998). We have not observed a significant clustering of such markers, with the exception of several centromeric regions on chromo somes 2A, 3A, 4A, 5A, 3B, 4B, 5B, and 6B. Thus, microsatellites are useful for complete coverage of the wheat genome in the same way as RFLP markers. Only one linkage break occurred in chromosome 5A and may be due to homozygous regions between mapping parents or lack of SSR marker sources on the regions (Torada et al. 2006). The total map length was 2473.5 cM and covered 61% of wheat genome with an average mapping distance of 9.77 cM between the markers (Sourdille et al. 2003). Among three genomes of hexapliod wheat, *A* genome mapped with 105 loci have total length of 949.4 cM followed by *B* genome of 102 loci with 837.9 cM mapping length, whereas, the *D* genome accounts only 46 marker loci with 686.2 cM. This agrees with other maps, where fewer markers were found on *D* genome (Paillard et al. 2003; Sourdille et al. 2003).

QTL analysis

The spike related traits *viz*., spike length, spikelet number and spike compactness were recorded because phenotypic differences were observed between the parents of the RIL population. In addition, the *Q* allele, which confers the square-headed phenotype and free-threshing character, is possessed by most of the cultivated wheats, but most wild wheats have the *q* allele and, therefore, speltoid spikes that are not free threshing (Muramatsu 1986). Hence the traits were measured to correlate the traits with *Q* gene. All three traits showed normal distribution of phenotypic data and were further used for QTL analysis (data not shown). In total, 11 QTLs were detected for three traits (Table 1.). Four QTLs were identified for SL, where two QTLs each located on chromosome 2A and chromosome 5A respectively. Among these, the QTLs in 2A were contributed by CS parent that has the *Q* gene. But the QTLs in 5A were contributed by *q* gene containing Sp parent. This QTL analysis further confirmed the location of *Q/q* allele's location. A maximum six QTLs were earmarked for SN, where three were located on chromosome 2A, two on chromosome 2B and the remaining one was in chromosome 5A. Only one QTL was derived for SC was bracketed at chromosome 5A. Although this trait is well correlated with other two traits, only one major QTL was observed.

CONCLUSION

Constructing linkage map is very important step to isolate a gene by map based approach. In this study the SSR linkage map was constructed in the intervarietal RIL population. The linkage map is useful to find the closest markers for any traits for marker assisted selection and further genes can be isolated by map based cloning approach. As a confirmation, we identified 11 QTLs for important spike traits genetically differed between the parents. In future we are planning to locate new kind of QTLs in this linkage map.

ACKNOWLEDGEMENT

This work was supported by Grants-in-Aid for Scientific Research on Priority Areas `Comparactive Genomics` from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

Ahmed TA, Tsujimoto H, Sasakuma T (2000). Identification of RFLP markers linked with heading date and its heterosis in hexaploid wheat. Euphytica 116: 111–119.

Cadalen, T., Boeuf, C., Bernard, S. & Bernard, M. (1997). An intervarietal molecular marker map in Triticum aestivum L. Em. Thell. and comparison with a map from a wide cross. Theor Appl Genetics 94: 367−377.

Campbell K.G. 1997. Spelt: agronomy, genetics, and breeding. Plant Breeding Rev. 15: 187−213.

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier MF, Joudrier P, Schlatter AR, Dubcovsky J, De la Pena RC, Khairallah M, Penner G, Hayden MJ, Sharp P, Keller B, Wang RCC, Hardouin JP, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernard M (2002a) Characterization of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D genome of bread wheat. Theor Appl Genet 104:1164–1172

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daley, M., Lincoln, S. & Newburg, L. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174−181.

Liu, Y. & Tsunewaki, K. (1991). Restriction fragment length polymorphism (RFLP) in wheat. II. Linkage maps of the RFLP sites in common wheat. Japanese Journal of Genetics 66: 617−633.

Muramatsu M. 1963. Dosage effect of the spelta gene q of hexaploid wheat. Genetics 48: 469−482.

Muramatsu M. 1986. The vulgare super gene Q; its universality in durum wheat and its phenotypic effects in tetraploid and hexaploid wheats. Can. J. Genet. Cytol. 28: 30−41.

Pestsova, E., Ganal, M. W. & Roder, M. S. (2000). Isolation and mapping of microsatellite markers specific for the D genome of bread wheat Genome 43: 689−697.

Phillips, R. L. & Vasil, I. K. (2001). DNA-Based Markers in Plants. Dordrecht: Kluwer Academic.

Roder, M. S., Korzun, V., Wandehake, K., Planschke, J., Tixier, M. H., Leroy, P. & Ganal, M. W. (1998). A microsatellite map of wheat. Genetics 149: 2007−2023.

Saghai-Maroof MA, Soliman KM, Jorgensen AR, Allard RW (1984) Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellitebased deletion bin system for the establishment of genetic–physical map relationships in wheat (*Triticum aestivum* L). Funct Integr Genomics 4:12–25

Sourdille, P., Cadalen, T., Guyomarc' h, H., Snape, J. W., Perretant, M. R., Charmet, G., Boeuf, C., Bernard, S. & Bernard, M. (2003). An update of the CourtotrChinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genetics 106: 530−538.

Torada, A., Koike, M., Mochida, K. & Ogihara, Y. (2006). SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor Appl Genetics 112: 1042−1051.

Table 1. QTL analysis of spike characters in CS/Sp RIL population

Trait	Ch #	Marker interval	QTL (cM)	LOD	R^2 (%)	Additive Effect
SL	2A	*Xhbg264a-Xhbg449*	0.0	2.53	7.7	-1.1267
	2A	*Xwmc198-Xwmc455*	20.0	2.72	9.5	-1.2463
	5AL	*Xcfa2163-Xcfa2155*	6.0	6.97	25.8	1.9989
	5AL	*Xcfa2155-Xhbg448*	18.0	9.95	49.4	2.7628
SN	2A	*Xhbg264a-Xhbg449*	0.0	3.02	9.1	-1.2276
	2A	*Xhbg494-Xhbg340*	8.0	2.68	9.6	-1.2600
	2A	*Xwmc455-Xgpw9kl*	0.0	2.67	8.1	-1.1588
	2B	*Xgwm429-Xgwm148*	0.0	4.49	13.3	1.4489
	2B	*Xwmc41-Xhbg405*	0.0	2.04	6.2	-1.0013
	4D	*Xgpw9k2-Xhbe341*	12.0	2.54	12.8	-1.4075
SC	5AL	*Xcfa2155-Xhbg448*	16.0	10.74	46.5	-0.4600