Quantitative trait loci analysis for kernel length and width in wheat (*Triticum aestivum* L.)

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Abstract:【**Objective**】Kernel length and width in wheat are very important because of their relationship with yield and milling quality.【Method】Quantitative trait loci(QTL) analysis for kernel length(LEN) and kernel width(WID) in wheat was conducted using a set of 115 recombinant inbred lines(RILs) derived from a cross between the synthetic hexaploid wheat W7984 and the spring wheat cultivar Opata 85. Synthetic W7984 has larger kernels, especially in length, compared to Opata 85. The stability of these QTLs was evaluated in two environments.【Result】Corresponding to genome-wide single marker regression analysis($P<0.01$), five markers were linked to LEN QTL, three markers were linked to WID QTL in two different environments. Based on composite interval mapping(LOD>2.5), two intervals with contributions of 20.20% to 20.81%, 13.54% to 13.91% were found to be linked to long LEN on chromosomes 5BL and 7DS in two different environments. The LOD score was 4.50 to 4.55, 2.94 to 3.20. One interval with contributions of 13.71% to 19.30% were found to be linked to wide WID on chromosomes 2BS. The LOD score was 2.98 to 4.18.【Conclusion】The marker *tam*72*c* on 5B, *bcd*707 on 7D and closest *cdo*405*b* to *ksuf*11*a* on 2B identified in this study may prove useful for marker-assisted selection in LEN, WID and facilitate molecular cloning of this gene for wheat kernel length and width.

Key words: kernel length; kernel width; recombinant inbred line(RIL); quantitative trait loci (QTL); *Triticum aestivum* L.

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小麦粒长和粒宽的 QTL 定位分析

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[**摘 要**] 【目的】粒长、粒宽是小麦种子重要的形态性状, 该性状对籽粒的外观商品品质、产量及磨粉品质均至关重要, 研究不同环境条件下小麦粒长、粒宽的单个标记和复合区间作图的 QTL 定位, 对小麦粒长、粒宽的分子标记辅助选择具有重要参考作用。【方法】应用一个由 115 个系组成的 W7984/Opata 85 重组自交系(RIL)群体, 建立了由 394 个 DNA 分子标记组成的遗传连锁图, 在 2 种不同环境条件下对小麦粒长、粒宽进行了单个标记的回归分析和复合区间作图的 QTL 定位。【结果】在单个标记的回归分析中检测到 5 个粒长的 QTLs、3 个粒宽的 QTLs; 复合区间作图分析结果表明, 控制粒长的 QTLs 分别位于 5BL 和 7DS 上, 在 5BL 上的贡献率为 20.20%～20.81%, LOD 值为 4.50～4.55; 在 7DS 上的贡献率为 13.54%～

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13.91%,LOD值为2.94～3.20。控制粒宽的QTL位于2B上,贡献率为13.71%～19.30%,LOD值为2.98～4.18。【结论】位于5B和7D上的控制粒长的QTL和位于2B上的控制粒宽的QTL在2种条件下均能检测到。

[关键词] 粒长;粒宽;重组自交系(RIL);数量性状基因座位(QTL);小麦

Kernel length and width determine wheat seed size and appearance, and affect yield and milling quality, and are therefore important agronomic traits in wheat breeding. The market value of wheat grain is determined by kernel morphology, texture, test weight and others. In bread wheat, which is used to manufacture different products requiring specific grain characteristics, kernel shape and uniformity influence its milling quality[1]. Similarly, kernel weight, a function of kernel size and density, has a favourable effect on the agronomic and flour yield of wheat[2].

Understanding the genetic basis of seed shape and size could improve knowledge about the domestication of cereals[3], humans tended to select large seeds during the early domestication, as evidenced by the fact that most cultivated species have larger seeds than their wild relatives. However, small seed is usually favored by natural selection because it is frequently associated with more seeds per plant, early maturity, and wider geographic distribution. Therefore, from the standpoints of both biological development and breeding, it is necessary to understand the genetic basis of wheat kernel shape. In addition, larger kernels could have a good effect on seedling vigor and consequently promote yield increase[4]. From the crop improvement perspective, Kernel size and shape are important for their relationship with yield potential.

Kernel size is an important component of grain yield and also has high phenotypic stability/heritability[5], and the improvement of the trait as a component of grain yield has been recommended[6]. However, phenotypic selection for grain weight is laborious and time consuming, and there is usually a compensation effect between seed size and seed number[7]. Experimental attempts to obtain yield progress by simply selecting larger kernels in wheat have been unsuccessful[8-9].

Wheat kernel size, like most of the traits of biological interest and agricultural importance, is a complex character and is suggested to be quantitative in nature[1,10]. In another set of studies, QTL analyses for grain weight and other yield component traits were conducted using molecular maps either for individual chromosomes[6,11-12] or for the whole genome[13-15]. Attempts were also made to utilize advanced-backcross QTL(AB-QTL) analysis for the study of kernel size[16-17], and association mapping for the study of genetics of kernel size[18].

Previous studies detected quantitative trait loci (QTL) for kernel morphology on several wheat chromosomes. In the population NY18 × Clark's Cream, homoeologous group 3 was the most influential for kernel size[1]; in the bread wheat population Chinese Spring × RS111, a QTL was found on 1A and other significant loci were detected on 1D, 2D and 6B[10]. In another bread wheat population, the most important QTL was detected on chromosome 2D[19].

In the present study, the kernel traits were analysed in a different recombinant inbred line (RIL) population, using polymerase chain reaction (PCR)-based DNA markers, to find additional new controlling kernel length and width.

1 Material and methods

1.1 Plant material

Analyses were performed on a population of 115 recombinant inbred lines (RILs) developed at Cornell University. From a cross made at CIMMYT between the synthetic hexaploid wheat W7984 (*T. turgidum* cultivar Altar 84 × *Aegilops tauschii* Coss. LineWPI 219, also known as M6) and the spring wheat cultivar Opata 85. W7984 has larger kernels, especially in length, compared with Opata 85.

The population was grown at Ithaca, New York (42.5°N, 76.5°W, 335 m above sea level) in the spring of 2002 and 2006. The experiment was

conducted in the field with two replicates in three row plots, 2 M long, and a uniform spacing of 30 plants/row. Cropping conditions in the location were suitable for full plant development and grain filling.

1.2 Phenotypic data

The phenotypic measurement for each trait was made in two sets of 30 grains, and the mean values of two replicates obtained were used in this study. Phenotyping for kernel length and width was achieved by measuring the length and width of grains with vernier calipers.

1.3 Genetic map

The W7984×Opata map used in the analysis included 394 loci, consisting of 292 RFLP (BCD, CDO, KSU, MWG and others), 94 SSR(*GW*3-1 and *IND*109 come from rice), and 8 gene-specific hybridzation probes (Glu1A, Glu1B, Glu1D, pBS128a, pBS128b, Waxy, ATPasea, hor1, 2c). RFLP genotyping of the RILs was described elsewhere[20]. The linkage map was constructed with MapManager QTXb20 [21] using the Kosambi mapping function and p-value 0.01. Markers were selected to minimize missing data ($n>60$), avoid segregation distortion and give even coverage of the chromosomes as much as possible. The total map length was 4 627 cM. The 21 wheat chromosomes were mapped with 10 to 26 markers per chromosome.

1.4 QTL analysis

QTL analysis involving detection of main effect QTL was conducted following composite interval mapping (CIM) using Cartographer v. 2.5[22] Using model 6, stepwise selection of cofactors with $SLE=SLS=0.01$, window size 10 cM and testing step 2 cM. A threshold of $LOD=2.5$ was applied for declaring QTL by single-trait composite interval mapping (ST-CIM).

2 Results

2.1 Phenotypic assessment of LEN and WID

The LEN (8.159 0 mm in 2002 environment, 8.217 0 mm in 2006 environment) and WID (3.482 0 mm in 2002 environment, 3.511 7 mm in 2006 environment) of the W9784 parent was significantly different ($P<0.01$) from Opata 85 (LEN 6.910 0 mm in 2002 environment, 7.041 8 mm in 2006 environment; WID 3.065 0 mm in 2002 environment, 3.121 7 mm in 2006 environment) and the LEN RILs ranged from 6.728 6 mm to 8.544 0 mm with a mean of 7.606 3 mm in 2002 environment, from 6.678 1 to 8.698 1 mm with a mean of 7.759 3 mm in 2006 environment; WID RILs ranged from 2.968 1 mm to 3.703 2 mm with a mean of 3.364 7 mm in 2002 environment, from 2.971 8 mm to 3.710 8 mm with a mean of 3.425 2 mm in 2006 environment. The values for LEN and WID in the RILs population showed a continuous distribution (Fig. 1), indicating the polygenic nature of those traits.

2.2 Single marker analysis

The single marker analysis (SMA) of LEN (2002) showed significant associations with 10 markers at 4 locations (2D, 5B, 6A and 7D) in particular, one marker in 2D, 3 markers in 5B, 5 markers in 6A and one marker in 7D. In 2006 it showed significant associations with 14 markers at 6 locations (1B, 5A, 5B, 6A, 7A and 7D) in particular, one marker in 1B, one marker in 5A, 6 markers in 5B, 4 markers in 6A, one marker in 7A and one in 7D. 6 markers were detected on chromosomes 5B, 6A and 7D for two environments. The markers tam72c ($P<0.001$) on chromosomes 5B respectively (Table 1).

The SMA of WID (2002) showed significant associations with 7 markers at 5 locations (2B, 2D, 4A, 5D and 7A) in particular, 3 markers in 2B, one marker in 2D, one in 4A, one in 5D, one in 7A. In 2006 it showed significant associations with 9 markers at 5 locations (2B, 2D, 5B, 5D and 7B) in particular, 2 markers in 2B, 2D, 5B and 5D, one marker in 7B. 3 markers were detected on chromosomes 2B, 2D and 5D for two environments, respectively (Table 2).

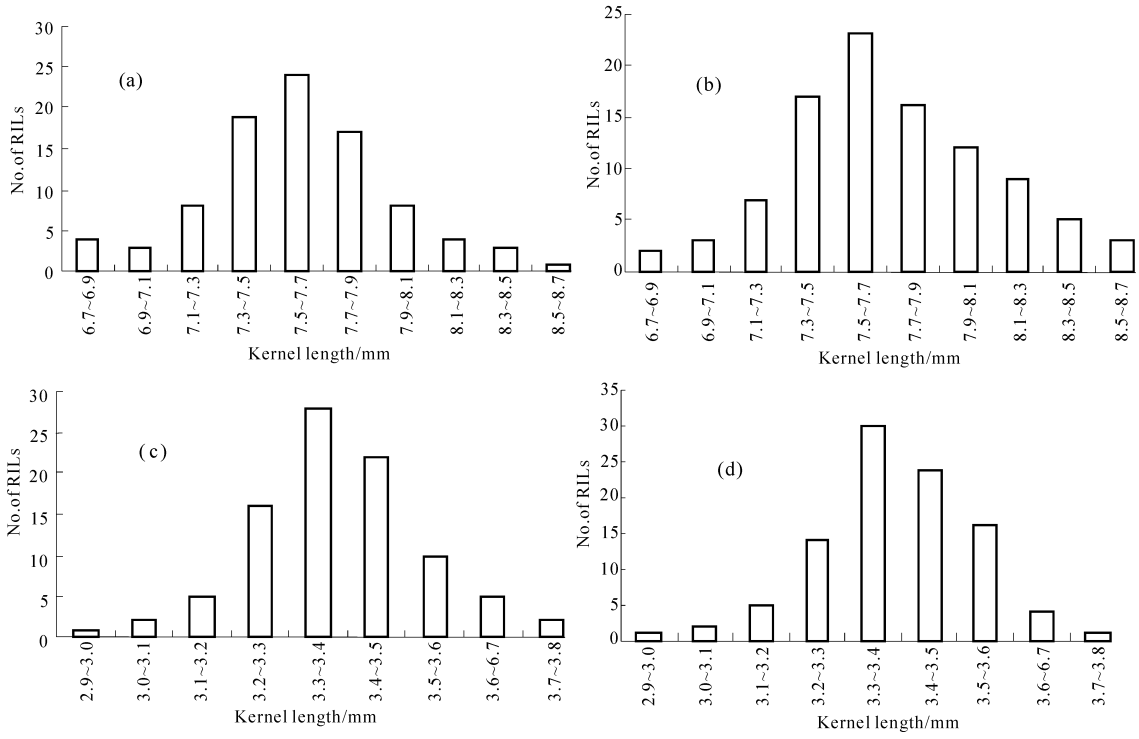

Fig. 1 Histograms showing frequency distribution of LEN in 2002(a), LEN in 2006(b), WID in 2002 (c) and WID in 2006(d) in an RIL population of W7984 × Opata 85

Table 1 F-value of markers association with QTL for LEN in the population of the cross M6×Opata

Environment	Chromosome	Marker	Likelihood ratio	*F*-value
2002	2D	Cdo1379	7.666	7.790**
	5B	Ksua1	7.997	8.138**
	5B	Spr574	8.968	9.164**
	5B	Tam72c	11.996	12.425***
	6A	Cdo388a	9.311	9.530**
	6A	Gwm570	7.053	7.147**
	6A	Fbb070c	8.322	8.481**
	6A	Ksud276	7.960	8.099**
	6A	Gwm617	7.960	8.099**
	7D	Bcd707	7.678	7.802**
2006	1B	Gwm140	9.025	9.225**
	5A	Abg391	8,150	8.298**
	5B	Gwm371	9.120	9.326**
	5B	Gwm540	6.811	6.895**
	5B	Ksua1	9.003	9.202**
	5B	Psr574	10.951	11.289**
	5B	Tam72c	13.757	14.360***
	5B	Cdo584	8.581	8.754**
	6A	Gwm494	8.972	9.169**
	6A	Cdo29	11.303	11.670***
	6A	Cdo388a	7.282	7.386**
	6A	Gwm570	8.787	8.972**
	7A	Cdo475b	7.386	7.495**
	7D	Bcd707	8.386	8.548**

Note: Significance levels: ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Table 2　F-value of markers association with QTL for WID in the population of the cross M6 × Opata

Environment	Chromosome	Marker	Likelihood Ratio	*F*-value
2002	2B	Mwg850	11.296	11.663***
	2B	Cdo405b	16.135	17.020*****
	2B	Ksuf11a	10.104	10.377**
	2D	Gwm261	7.089	7.185**
	4A	Bcd1975	7.499	7.614**
	5D	Gwm190	11.034	11.380**
	7A	Fbb218	9.897	10.155**
2006	2B	Ksuf11a	9.156	9.364**
	2B	Ksuf11c	8.474	8.641**
	2D	Gwm261	8.514	8.684**
	2D	Bcd611	9.813	10.065**
	5B	Gwm443	14.284	14.945***
	5B	Bcd873a	11.677	12.076***
	5D	Gwm190	7.431	7.543**
	5D	Bcd1874	7.688	7.813**
	7B	Gwm344	7.249	7.352**

Note:Significance levels: ** $P<0.01$, *** $P<0.001$, ***** $P<0.0001$.

2.3 **Composite interval mapping**

The experimentwise threshold for composite interval mapping was established by carrying out 1 000 permutations at $P<0.01$, corresponding to an average likelihood ratio of 11.5 and a $LOD \geqslant 2.5$. Framework linkage maps were prepared for chromosomes 2B, 5B and 7D, 5B and 7D carried genes for kernel length, 2B carried genes for kernel width as inferred from monosomic analysis. As many as 21 loci were mapped on 2B with a total length of 239.5 cM, 22 loci were mapped on 5B with a total length of 308.1 cM, 17 loci were mapped on 7D with a total length of 235.4 cM.

The composite interval mapping analysis produced two QTLs significantly linked to long LEN on chromosomes 5B and 7D with contributions of 20.20%, 13.91% in 2002 environment, the two markers are *tam*72*c* in 5B and *bcd*707 in 7D. The marker *tam*72*c* also significantly linked to long LEN with contribution of 20.81% in 2006 environment. One QTL significantly linked to wide WID on chromosomes 2B in two environments with contributions of 13.71% to 19.30%, the closest marker linked between *cdo*405*b* to *ksuf*11*a* on chromosome 2B.

3　Discussion

Giura and Saulescu[23] reported the positive influence of chromosome 1B, 4A, and 4B on kernel length. Campbell[1] reported that QTLs for kernel length were found on chromosome 1B, 2B, 2D, 3B and 7B in a cross of soft × hard winter wheat. Breseghello and Sorrells[24] found the RFLP locus *Xpsr*574, on 5B, which is a QTL for kernel length in W7985 × Opata 85, and the SSR locus *Xwmc*150*b*, on 5A, which were significantly associated with kernel length in their panel of cultivars. An interesting observation in our study was the identification of new loci associated with kernel length which have not been reported earlier-2D, 5B, 6A and 7D. We identified one significant marker *tam*72*c* on chromosome 5B, which explained 20.20% to 20.81% of the variance with a $LOD=4.50$ to 4.55 in two different environments; one significant marker *bcd*707 on chromosome 7D, it can explain 13.54% to 13.91% of variance with a $LOD=2.94$ to 3.20 in two different environments.

Giura and Saulescu[23] indicated that kernel width in wheat was increased by chromosome 1A and 1B; Dholakia[19] reported that only two markers, *Xgwm*261 and UBC881600, were found to be associated with kernel width on chromosome 2DL. Campbell[1] reported QTLs for kernel width were located on chromosomes 1A, 2A, 2B, 2D and 3D. This study, markers at 3 locations (2B, 2D and 5D) were found to be associated with kernel width in two different environment, one QTL significantly linked to wide WID on chromosomes 2BS, with

contributions of 13.71% to 19.30% variance with a $LOD = 2.98$ to 4.18 in two different environments.

QTLs for kernel length and width were detected. Kernel morphology is likely to be influenced by genes affecting plant growth and development. Further analysis of this population in other environments and use of a larger population of W7985×Opata 85 RILs would allow us to localize kernel QTLs with more accuracy. And then, these markers can be used for further application in wheat breeding.

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