Theor Appl Genet (2013) 126:1545–1553
DOI 10.1007/s00122-013-2072-2

ORIGINAL PAPER

# Fine mapping a major QTL for kernel number per row under different phosphorus regimes in maize (*Zea mays* L.)

**Guodong Zhang · Xiaopeng Wang · Bin Wang · Yanchen Tian · Meng Li · Yongxin Nie · Qingcai Peng · Zeli Wang**

Received: 26 October 2011 / Accepted: 23 February 2013 / Published online: 15 March 2013


**Abstract** Phosphorus (P) is one of the essential macronutrients for plant growth and development. Grain yield is the primary trait of interest in maize breeding programs. Maize grain yield and yield-related traits are seriously affected by P deficiency. Kernel number per row (KN), as one of the major components of grain yield, has attracted the attention of more and more breeders. In our previous study, one major QTL (named *qKN*), controlling KN under different P regimes was mapped to the interval between molecular markers bnlg1360 and umc1645 on chromosome 10 using a $F_{2:3}$ population derived from the cross between maize inbreds 178 and 5,003 (107). In order to understand its genetic basis, we developed a population of near isogenic lines (NILs) and two P regimes were used to fine map and characterize *qKN*. The QTL *qKN* was finally localized in a region of ~480 kb. A single *qKN* allele of inbred 178 increased KN by 6.08–10.76 % in the 5,003 (107) background; *qKN* acted in a partially dominant manner. Our results will be instrumental for the future identification and isolation of the candidate gene underlying *qKN*. The tightly linked molecular markers that we developed for *qKN* will be useful in maize breeding programs for improving KN applying the marker-assisted selection.

Communicated by T. Würschum.

G. Zhang and X. Wang contributed equally to this work.

G. Zhang · X. Wang · Y. Tian · M. Li · Y. Nie · Q. Peng · Z. Wang (✉)
The State Key Lab of Crop Biology, College of Life Sciences, Shandong Agricultural University, Daizong Street 61, Taian, Shandong 271018, People's Republic of China
e-mail: wangzeli@sdau.edu.cn

B. Wang
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100190, People's Republic of China

**Abbreviations**

| | |
|---|---|
| cM | Centimorgan |
| KN | Kernel number per row |
| NIL | Near isogenic line |
| P | Phosphorus |
| QTL | Quantitative trait loci |
| RIL | Recombinant inbred line |
| SSR | Single sequence repeat |

## Introduction

Maize (*Zea mays* L.) is one of the most important crops worldwide, serving as a source of food, feed, and fuel. Grain yield is the primary trait of interest in maize breeding programs. By 2020, we will have to produce 31 % more maize to meet the demands of a constantly increasing population in China (Zhang et al. 2008). So, it is very important to study the mechanism of improving total maize grain yield. Studies about total grain yield are concentrating on grain yield per plant, 100 kernel weight, ear length, row number per ear, kernel number per row, ear diameter, and so on (Austin and Lee 1996; Thomson et al. 2003; Guo et al. 2008). All of these traits are major components of total grain yield. As an important yield determinant, kernel number per row has attracted the attentions of breeders (Sabadin et al. 2008; Liu et al. 2010; Tang et al. 2010). Sabadin et al. (2008) indicated that KN has a high heritability coefficient and a high correlation with total grain yield. Li et al. (2010) suggested that KN has a positive

coefficient with grain yield per plant, 100 kernel weight, ear length, ear diameter but has a negative coefficient with row number per ear.

With the development of molecular genetic markers, QTL mapping has become a routine strategy for the discovery of genes involved in complex quantitative traits. During the past decade, many attempts have been made to characterize QTL for grain yield and yield components in maize, and many QTLs have been mapped on the linkage map (Tang et al. 2007). However, most of the materials used in mapping were primary segregating populations such as $F_{2:3}$ lines, recombinant inbred lines (RILs), and doubled haploid lines (Lima et al. 2006; Ma et al. 2007; Li et al. 2008; Messmer et al. 2009). Primary segregating populations capture a single moment in time and thus cannot be used for multi-year phenotyping, nor is it likely that the genetic maps constructed will ever be improved. Lima et al. (2006) mapped a total of 16 QTLs for grain yield using 256 $F_{2:3}$ families. Ma et al. (2007) detected 31 QTLs for grain yield and yield components using 294 RILs. Although these populations are easy to develop, they are not suitable for fine mapping or cloning of QTL because of excessive genetic background noise.

The crucial step for identifying the candidate gene(s) is to delimit a QTL/gene into an interval as short as possible (Salvi and Tuberosa 2005). Advanced populations such as near isogenic lines (NILs) and chromosome segment substituted lines have the same genetic background as the recurrent parent except for the substituted segments, which makes it possible to narrow down the QTL to a small genetic interval and segregate the QTL as single Mendelian genes. Applying this approach, genes with large genetic effects on a quantitative trait have been accurately mapped and isolated by recurrent backcrossing combined with selection for the traits (Darvasi 1998; Salvi et al. 2007). Fan et al. (2006) detected a major QTL for grain length and weight using a similar NIL-based strategy in rice. The QTL locus explained 80–90 % of the variation for grain weight and length in the $BC_3F_2$ population. Quarrie et al. (2006) found a major QTL for wheat yield and identified the QTL as a candidate gene and identified a candidate responsible for the QTL. However, few loci segregating as single Mendelian genes have been reported for grain yield in maize and none have yet been cloned.

Phosphorus (P) is one of the essential macronutrients for plant growth and development (Raghothama 1999). Maize grain yield and yield-related traits are seriously affected by P deficiency (Plenet et al. 2000a, b). In order to increase maize production, maize breeders have exerted enormous efforts to breed hybrids with tolerance to low P (Reiter et al. 1991; Zhu et al. 2006; Chen et al. 2009). Therefore, detecting QTL for yield components and kernel-related traits, analyzing the stability of QTLs and exploiting constitutive QTLs under different P regimes are of great importance in marker-assisted breeding for P tolerance in maize (Chen et al. 2008).

At present, there are a lot of reports in the literature about mapping QTL for KN (Xiao et al. 2005; Yan et al. 2006), as well as there are plentiful papers about fine mapping of grain yield QTLs. Yan et al. (2006) used a $F_{2:3}$ population to detect three QTLs for KN, where these QTLs can explain 30 % of the phenotypic variation; however, very few reports of fine mapping KN QTLs exist (Liu et al. 2010). KN has been suggested as a very important contributor to total grain yield (Li et al. 2010; Liu et al. 2010) but we did not find any report about fine mapping of KN under different P regimes. It is, therefore, important to study the response of KN to high and low phosphorus treatments and the consequences for grain yield and grain yield components in maize.

In a previous study, we generated a $F_{2:3}$ family derived from the cross between inbred lines 178 and 5,003 (107) and found three major QTL clusters controlling maize yield component traits in high and low phosphorus treatments (Li et al. 2010). We identified five QTLs conferring KN including a major QTL, which was fine mapped by developing NILs. We named this major QTL as *qKN*, following the QTL naming principle indicated by McCouch et al. (1997). The objectives of this research were to fine map the major QTL conferring kernel number per row in maize, to develop new markers to saturate the major QTL region, and to fine map the major gene. Moreover, a high-resolution map of the *qKN* region with many cosegregating or closely linked markers will enable a marker-assisted selection program to improve KN and thus grain yield.

## Materials and methods

### Plant materials

In our previous study, one major QTL (named *qKN*), controlling KN, was mapped to the interval between molecular markers bnlg1360 and umc1645 on chromosome 10 using a $F_{2:3}$ population derived from the cross between inbred lines 178 and 5,003 (107) (Li et al. 2010). The genetic distance of the two markers is 12.6 cM. We selected the subsequent experimental materials by continuous backcrossing. The sequence developed to produce the experimental material is shown in Fig. 1. The $F_1$ plants, derived from a cross between 178 and 5,003 (107), were backcrossed with 5,003 (107) as the male parent. Inbred line 178 is a P efficient genotype, and 5,003 (107) is a P inefficient genotype. The genotypes of each individual were surveyed by simple-sequence repeat (SSR) analysis.

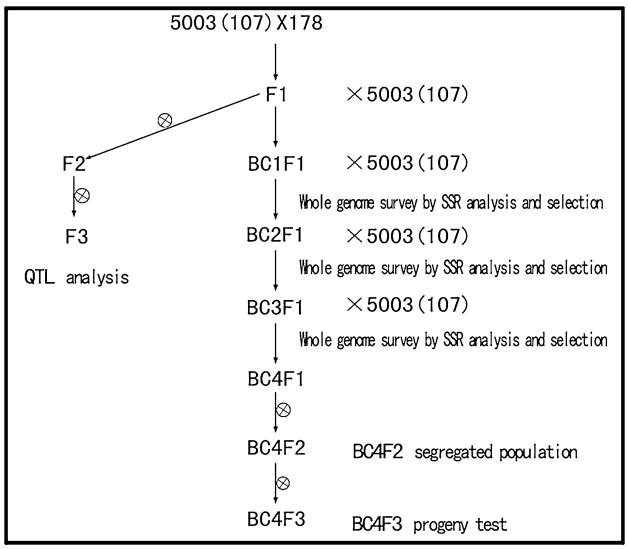


**Fig. 1** Plant material used for fine mapping of QTLs controlling kernel number per row in maize under high and low P treatments. Populations used in this study were derived from a cross between 178 and 5,003 (107). Primary QTL analysis for grain yield in maize under low P was carried out using an $F_{2:3}$ populations (Li et al. 2010)

From the $BC_2F_1$ generation, selections and backcrosses were carried out to obtain suitable $BC_4F_1$ plants for analysis. In order to screen the genetic background of $BC_2F_1$, 110 SSR marker evenly distributed on 10 chromosomes were selected from all polymorphic markers between 178 and 5,003 (107). Five chromosome fragments covering about 90 cM on chromosomes 2, 3, 4, 7, 8 were heterozygous except bin 10.07 in $BC_2F_1$. The markers situated in these five fragments were further used to screen several $BC_3F_1$ and $BC_4F_1$ plants. Finally, seven $BC_4F_1$ plants with the fewest background markers were selected for $BC_4F_2$ production. These $BC_4F_1$ plants were regarded as $F_1$ plants for fine mapping of target QTLs because the putative QTL regions were heterozygous while almost all other regions were homozygous for 5,003 (107) alleles. From these seven plants, a $BC_4F_2$ consisting of 1,036 plants was developed and grown in the field. As phenotyping in field conditions resulted in a large phenotypic variance, 287 $BC_4F_3$ lines homozygous at the SSR marker interval umc2351 and umc1344 were selected and grown in the field. Each BC4F3 line was represented by 10 individual plants.

## Experiment flow

The experimental design, trait evaluation, phenotypic data analysis, and SSR data analysis have been described in detail in a previous publication (Li et al. 2010). In brief, the experiment was designed using a split plot design with three replications at two locations, Taian (TA), Shandong province, China (36 16′ N latitude, 117 15′ longitude), where the average daytime temperature is 14 C and the average rainfall per year is 697 mm; and Yantai (YT), Shandong province, China (37 57′ N latitude, 121 16′ E longitude), where the daily temperature and average yearly rainfall are 12 C and 652 mm, respectively. We used two phosphorus treatments in each location, named as high phosphorus (HP) and low phosphorus (LP), respectively. The HP treatment was designed as follows: 50 kg $ha^{-1}$ of P, 63 kg $ha^{-1}$ of K in the form of potassium dihydrogen phosphate before sowing, 180 kg $ha^{-1}$ of N in the form of urea with 120 kg $ha^{-1}$ before sowing and 60 kg $ha^{-1}$ at stem elongation stage. The LP treatment was designed as same as the HP treatment except changing potassium dihydrogen phosphate into 63 kg $ha^{-1}$ of K in the form of potassium chloride.

Molecular linkage maps were constructed using MAPMAKER 3.0 (Lander et al. 1987). Analyses of QTL location, origin of positive alleles, and effects of QTLs were performed using the software Windows QTL Cartographer version 2.5 (Wang et al. 2005) based on composite interval mapping with a LOD threshold of 3.0.

## Development of specific markers in the target region

Sequences available in the major QTL *qKN* region, the QTL confidence interval extend six BAC contigs according to the B73 BAC-based whole-genome physical map (http://www.maizesequence.org/), including the anchored EST, IDP, BAC, and BAC-end sequences, were used to develop new polymorphic high-density markers. These sequences were compared to NCBI databases via tBLASTn to obtain possible longer sequences. Primers were designed using the PRIMER5.0 software along with the following parameter settings: primer length is 20 nucleotides with a 40–60 % GC content, no secondary structure, and no consecutive tracts of a single nucleotide. Primer pairs were used to amplify the corresponding segments from both parents. The PCR cycling parameters were set up followed the protocol described by Li et al. (2010), except for the annealing temperature that was adjusted for each primer pair. The microsatellite search tool SSRHunter1.3 (Li and Wan 2005) was employed to mine BAC sequences for SSR sequences and flanking BAC regions were used for primer design.

## Fine mapping *qKN*

Fine mapping of the major QTL controlling KN was performed as follows. First, high-density molecular markers within the QTL region were developed to resolve recombination events. Secondly, screening of new recombinants was conducted by genotyping all mapping populations using new markers within the QTL region. Then, a progeny test was performed to determine KN for the recombinant.

## Results

### Mapping and confirmation the major QTL

We divided BC4F1 population into two groups, high KN group and low KN group. A total of 110 SSR markers were used to test the frequency of 1/2 (heterozygote) and 1/1 (homozygote) in both groups, respectively. Significant biases in allele frequency were observed for those markers located in the bin 10.07 region, suggesting the presence of the putative QTL (Table 1). The markers in bin 10.07 showed no distortion from 1:1 ratios of heterozygote to homozygote in the whole $BC_4F_1$ population. However, percentages of heterozygote at these markers significantly ($P < 0.0001$) differ between the high and low KN groups (Table 1). In the high KN group, percentages of heterozygote (1/2) in bin 10.07 region was significant higher than those in the low KN group, suggesting the allele that increasing KN was derived from the donor parent 178.

Furthermore, using the Windows QTL Cartographer version 2.5 (Wang et al. 2005), a major QTL with the LOD value of 9.8 was detected on bin 10.07, interval with the SSR markers umc2351 and umc1344, the QTL could explain ~28 % of phenotypic variation.

### Phenotypic and genetic analysis

We evaluated BC4F2 individuals under both HP ($n = 617$) and LP ($n = 419$) conditions, respectively. The KN of donor parent 178 was 26.69, while for the recurrent parent 5,003 (107) it was 22.14 in high P treatment condition. For the low P treatment condition, the KN of 178 and 5,003 (107) were 24.54 and 20.33, respectively. We used SSR markers bnlg1360 and umc1645 to determine the plant genotypes in the *qKN* regions and recorded the KN of each plant in the field. After PCR reactions and silver staining, the genotype which was identical to the 5,003 (107) parent was named as 5,003(107)/5,003(107), the genotype which was identical to 178 parent was named as 178/178, while the genotype which was carried both 5,003(107) and 178 parental alleles was named as 5,003(107)/178. In high P treatment condition, the average KN of 178/178 plants was 25.88, 5,003(107)/5,003(107) plants was 23.16. The KN of 178/178 plants was 11.74 % more than 5,003 (107)/5,003 (107) plants. While in low P treatment condition, the average KN of 178/178 plants was 24.07, and that of 5,003 (107)/5,003 (107) plants was 20.88, i.e., the KN of 178/178 plants was 15.27 % larger than the KN of plants homozygous for the 5,003 (107) marker allele (Table 2). All of these results suggested a major QTL in this region with the allele from 178 increasing the number of KN by 11.74–15.27 %. The effect of this QTL on KN was more pronounced under low P treatment condition than under high P treatment condition (Figs. 2, 3).

**Table 1** Correlation of genotype and KN at makers in the *qKN* region

| Bins | Marker | Percentage of heterozygote (%)[a] | | $X^2$ | *P* value |
|---|---|---|---|---|---|
| | | In high KN group | In low KN group | | |
| 10.07 | umc1196 | 89.32 | 22.61 | 25.35 | <0.0001 |
| 10.07 | bnlg1360 | 83.66 | 25.93 | 21.79 | <0.0001 |
| 10.07 | umc2351 | 90.43 | 22.78 | 26.17 | <0.0001 |
| 10.07 | umc2203 | 92.38 | 27.64 | 28.39 | <0.0001 |
| 10.07 | bnlg1450 | 86.57 | 26.34 | 24.11 | <0.0001 |
| 10.07 | umc1344 | 94.72 | 30.18 | 31.69 | <0.0001 |
| 10.07 | umc2216 | 93.92 | 29.55 | 30.25 | <0.0001 |
| 10.07 | umc1645 | 91.36 | 31.62 | 27.74 | <0.0001 |
| 10.07 | bnlg1185 | 90.57 | 30.45 | 25.95 | <0.0001 |

*P* value Probability of H0 hypothesis that is independent between genotype and trait

[a] The percentage of plants heterozygous for molecular markers within the *qKN* region in each group

### Development of PCR-based markers in the *qKN* region

At first, a total of 21 SSR markers obtained from bin 10.07 (http://www.maizesequence.org/) were applied to test the polymorphism in parents and finally nine SSR markers (including bnlg1360 and umc1645) (Table 1) were used to construct genetic maps for fine scale QTL mapping. Using QTL Cartographer version 2.5, *qKN* was mapped in the region of umc2351 and umc1344 in BC4F1 population. After searching the position of the SSR markers umc2351 and umc1344 on B73 physical map, we found 24 BACs between umc2351 and umc1344, which represents too large a physical interval to easily work with further. However, by evaluating $BC_4F_2$ families (Fig. 4), we could narrow the QTL containing interval to umc2203 and umc1450, a physical interval contained within six BACs (AC209426, AC155500,

**Table 2** Genotypes and KN of $BC_4F_2$ segregation population in different P regimes treatments

| Genotypes | No. plants | | Means (*n*) | | Ranges (*n*) | |
|---|---|---|---|---|---|---|
| | HP | LP | HP | LP | HP | LP |
| 5,003 (107)/5,003 (107) | 135 | 103 | 23.16 | 20.88 | 15–33 | 15–30 |
| 178/178 | 161 | 126 | 25.88 | 24.07 | 18–36 | 15–33 |
| 5,003 (107)/178 | 321 | 240 | 24.19 | 22.85 | 15–36 | 15–33 |
| 5.003 (107) parent | | | 22.14 | 20.33 | 15–33 | 15–30 |
| 178 parent | | | 26.69 | 24.54 | 18–36 | 15–33 |

n, HP, LP represent number, high phosphorus treatment and low phosphorus treatment, respectively

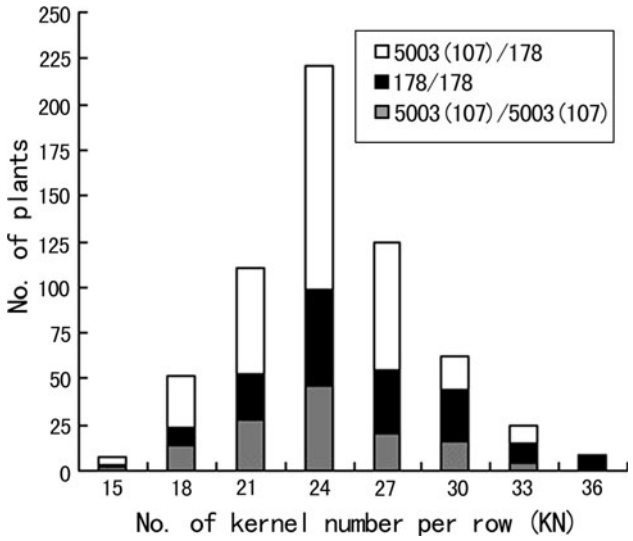


**Fig. 2** Frequency distribution for KN of $BC_4F_2$ Population in high P treatment

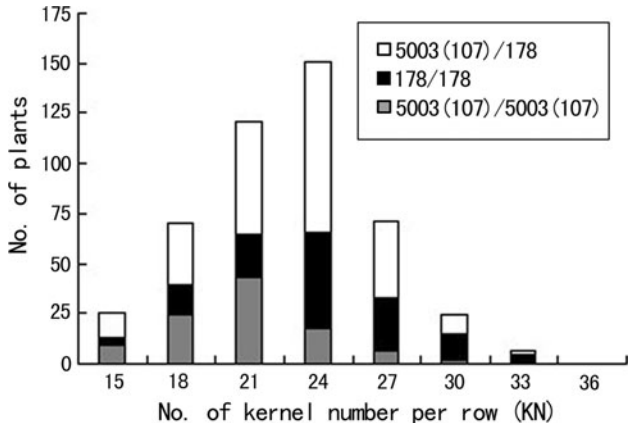


**Fig. 3** Frequency distribution for KN of $BC_4F_2$ Population in low P treatment

AC191572, AC197199, AC195335, AC190651). This represents a substantial improvement to our characterization of the *qKN* containing interval. These BACs were compared to NCBI and MAGI databases via tBLASTn to obtain possible longer sequences. Finally, SSR Hunter 1.3 was used to search SSR primers from obtained sequences and PRIMER5.0 was used to develop new markers, 133 SSR markers were designed and finally 30 new markers (Table 3) that represented good polymorphism in parents were used to narrow down the major QTL.

## Fine mapping of *qKN*

In 2009 in Taian and Yantai, 1,036 $BC_4F_2$ individuals were created for mapping. The QTL was located between umc2203 and umc1450, after using SSR marker umc2203 and umc1450 analyzing the whole BC4F2 populations, we identified six and three recombinant events, respectively.

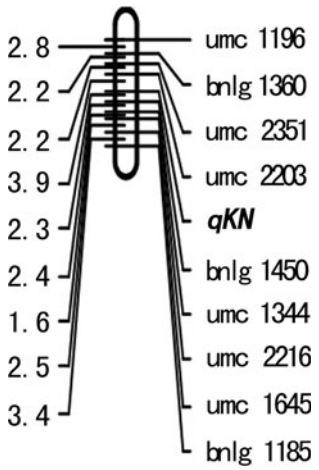


**Fig. 4** Molecular linkage of *qKN* in $BC_4F_2$ population

Using 30 new markers, we found different recombinants and noted the results under the genetic maps of BC4F2 (Fig. 5). Using new markers screening the recombinant events, *qKN* was limited between SSR11 and SSR27 with three and two recombinant events, respectively. SSR markers SSR14 and SSR23 showed co-segregation with these two markers. In order to fine map *qKN*, a much larger BC4F3 population derived from homozygous plants of BC4F2 was evaluated in the summer of 2010. Out of 2,870 plants, 609 individuals exhibited the low KN phenotype. 2,261 individuals exhibited normal and high KN phenotype. Two microsatellite markers (SSR11 and SSR27) flanking the QTL were used to detect recombinant events between the markers and the target gene. Analysis of SSR11 detected 12 recombinant events between the marker and *qKN* on one side, and analysis of SSR27 detected 7 recombinant events on the other side. Recombinant screening with three other markers (SSR15, SSR19, SSR23), which were more internal to *qKN*, detected five, two, three recombinants, respectively. SSR15 and SSR19 were developed from AC191572 and AC197199, respectively. The physical distance between these molecular markers is ~480 kb. By using the B73 reference genome sequence, RefGen_v1, the *qKN* was precisely defined in a ~480 kb region by SSR15 and SSR19 (Fig. 5). As expected, plants carrying the superior *qKN* region from cv 178 showed higher KN than those with the *qKN* from cv 5,003 (107) (Fig. 6).

## Estimation of the genetic effect of the major QTL

The genetic effect of *qKN* was estimated using $BC_4F_1$, $BC_4F_2$, $BC_4F_3$ populations during the year 2008–2010. As expected, plants carrying the *qKN* region showed higher KN than those without *qKN* (Fig. 6).

In 2008, the mean value of KN that the plants carrying *qKN* was 25.1 in $BC_4F_1$, while the value was 23.5 of without *qKN* plants. In 2009, 1,036 $BC_4F_2$ plants were

**Table 3** Newly developed molecular markers for fine-mapping of *qKN*

| Markers | BAC | Forward primers (5′–3′) | Reverse primers (5′–3′) | Annealing temperature (°C) | Predicted size (bp) |
|---|---|---|---|---|---|
| SSR1 | AC209426 | gttgtttgatgcaccatgcc | gttaggggataagggaaaga | 60 | 262 |
| SSR2 | AC209426 | gcgtgtgtggacgatggata | gaggagatggatcgaacgga | 62 | 265 |
| SSR3 | AC209426 | actcggcagacgaagagaca | cctcgacttatatggtggga | 61 | 247 |
| SSR4 | AC209426 | tgcacacattagaacggcga | gccacttgaaacgttgtctg | 60 | 291 |
| SSR5 | AC209426 | acatgtatatgggccaggcc | ctggagttctgtgcagctaa | 60 | 283 |
| SSR6 | AC155500 | gcactgctgtctaatctagg | ggcccgcctgtttttctaga | 61 | 213 |
| SSR7 | AC155500 | tgcggtgaagaggagaggac | tgcacacacacgcactccga | 64 | 267 |
| SSR8 | AC155500 | tcgatgggaggagatggatc | ctccaactccaaacgcgaca | 62 | 310 |
| SSR9 | AC155500 | cggacgaacggacgaacgaa | gctaacctcccactaatcct | 60 | 300 |
| SSR10 | AC155500 | cacccactcctgtaaagttc | ccacagacaacgcagcaaat | 60 | 217 |
| SSR11 | AC191572 | gacgagccgagaacgaacga | tctctgtgctgtgcatggga | 62 | 141 |
| SSR12 | AC191572 | ccgctgactccgatagatag | gcgtgtgtggacgatggata | 62 | 244 |
| SSR13 | AC191572 | tctctcctcgaagtcagcgc | gaagtcgcagggtgcttcca | 64 | 285 |
| SSR14 | AC191572 | actttacaggagtgggtggc | ctagcatctatccatcccgt | 61 | 304 |
| SSR15 | AC191572 | gaggcaccatccttcgatca | ccacaactgcggcaagcttg | 63 | 235 |
| SSR16 | AC197199 | gatgtcgattgagcagatgt | atcttcagcatccatggggt | 59 | 214 |
| SSR17 | AC197199 | ggagagaaaatgagagagag | gttgtttgatgcaccatgcc | 59 | 292 |
| SSR18 | AC197199 | acgcatcattctcccttctg | ccacctatgacctatccgtc | 60 | 312 |
| SSR19 | AC197199 | gttgattcagccagtcactc | gaacacggtgccagagagat | 61 | 305 |
| SSR20 | AC197199 | aactttacaggagtgggtgg | cggcacctgaagcgtctctt | 62 | 239 |
| SSR21 | AC195335 | ctgtaggccgtagctatgca | atccacgcctggtcagagag | 63 | 285 |
| SSR22 | AC195335 | cacagtgcgcagccaatctt | tgatgcagactcaggcagtt | 61 | 206 |
| SSR23 | AC195335 | accatgcatcgtcctgctgc | gttggctccttccataggcc | 64 | 296 |
| SSR24 | AC195335 | tcttttcttgtctccggcat | ctaagttagccacataatcc | 57 | 295 |
| SSR25 | AC195335 | acatcacttcgagcggttag | agagacccggtctttgtgac | 61 | 273 |
| SSR26 | AC190651 | cgctagaccggagaacccaa | cttccagagcttgatgaggt | 62 | 327 |
| SSR27 | AC190651 | gtgcaaccgaatcgaatccg | agagcccaagcctgagacga | 63 | 286 |
| SSR28 | AC190651 | gtggacgatgaagctagcac | gcgcgagtgcactctcaatt | 62 | 330 |
| SSR29 | AC190651 | agtgtgatccggcaacattc | ctcaccagccactaatatca | 60 | 204 |
| SSR30 | AC190651 | tgacgtacgcggttagtgtg | ctaaggtgtgtaatggtgga | 60 | 270 |

evaluated for KN, and the mean value of KN that with or without *qKN* was 24.8 and 22.9, respectively. A total of 2,870 $BC_4F_3$ plants were evaluated for KN, the mean value of KN that with or without *qKN* is 25.7 and 23.2, respectively. These data suggested that a single *qKN* allele derived from 178 could steadily increase the KN by 6.08–10.76 % in the 5,003 (107) background and the *qKN* acted in a partially dominant manner.

## Discussion

It has been confirmed that QTL mapping using early generation, biparental populations is imprecise, with the support or confidence interval for a QTL position spanning 10–30 cM or comprising 1–3 % of a genome (Dekkers and Hospital 2002; Salvi and Tuberosa 2005). Reasons for this imprecision include insufficient marker density and limited opportunities for recombination between closely linked loci due to the relatively small size of many mapping populations. Thus, using advanced populations has been proposed as a way to increase the accuracy of mapping experiments. It is reported that the confidence interval around a QTL was several times smaller in the advanced population compared to the initial population (Lee et al. 2002; Huang et al. 2010). Here, we developed a NIL population using $BC_4F_1$ derived families and located the known *qKN* to a physical ~480 kb interval 20 times smaller than we initially reported based on mapping in an $F_{2:3}$ population.

A common difficulty in mapping QTL is that QTL effects may show environment specificity and differ across environments due to the following reasons. First of all,

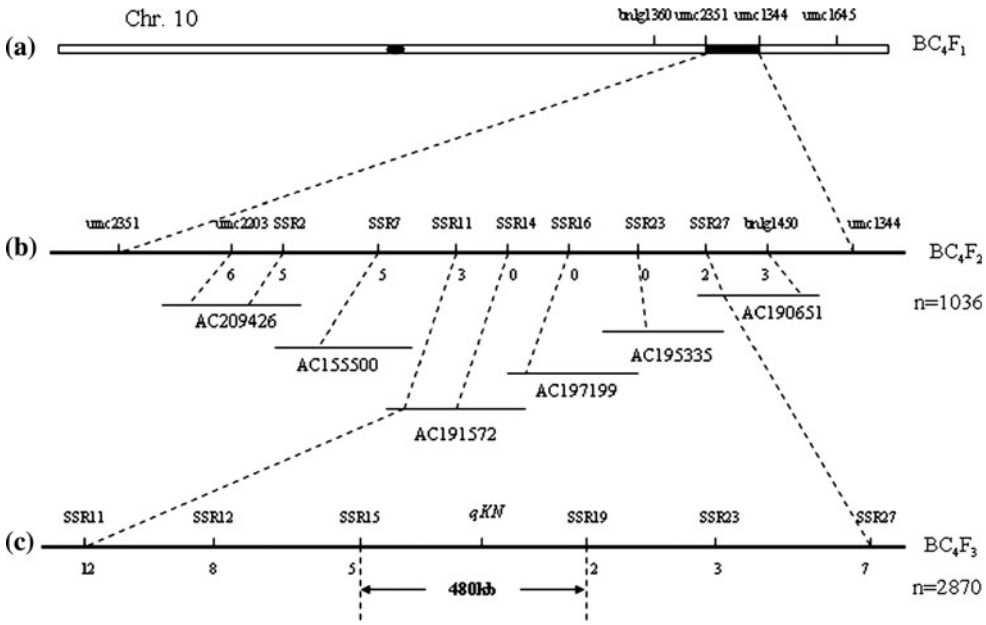


**Fig. 5** Genetic and physical maps of the *qKN*. **a** The introgression segment of *qKN* on chromosome 10 between SSR markers umc2351 and umc1344 on $BC_4F_1$, with the adjacent markers bnlg1360 and umc1645, respectively; **b** Mapping of the *qKN* on $BC_4F_2$ populations. The QTL was mapped to the region between markers SSR11 and SSR27, and the recombinants number between markers is indicated under the linkage map; **c** Mapping of the *qKN* on $BC_4F_3$ populations. The *qKN* was finally localized to a region of ~480 kb

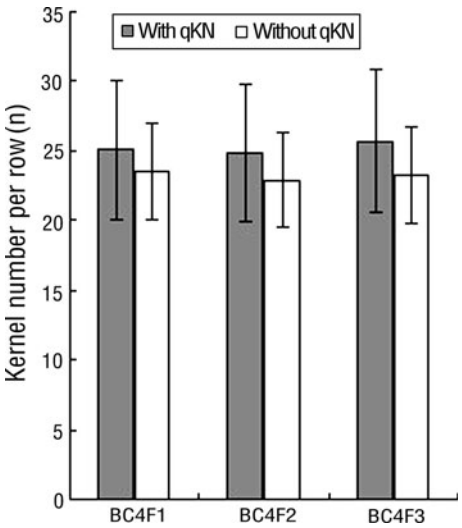


**Fig. 6** Kernel number per row in $BC_4F_1$, $BC_4F_2$ and $BC_4F_3$ populations

different studies used diverse mapping populations; these diverse populations have distinct genetic backgrounds, which induced dissimilar QTL restructure and exchange (Yan et al. 2006). Secondly, the parents of the mapping populations have different diversity in specific quantitative traits, causing dissimilar QTL polymorphism in different groups. Guo et al. (2008) and Liu et al. (2010) used different mapping populations gained dissimilar results about QTLs for grain yield. Thirdly, different mapping population sizes, marker types, and mapping saturation influence QTL detection and sensitivity. Finally, there are many different non-genetic factors, such as environmental conditions. Lukens and Doebley (1999) thought QTL effects were environmentally sensitive and this sensitivity resulted in phenotypic plasticity or the ability of organisms to take on alternative developmental fates depending on environmental cues. In this study, we detected a major QTL for kernel number per row in the bin 10.07 region using a NIL population; a new regions for a maize yield component QTL.

Several reports had shown that the marker-assisted backcross selection method is efficient for transferring QTL from elite or non-elite germplasm to elite inbred lines (Moreira et al. 2009). Kaeppler (1997) demonstrated that NIL-based QTL tests had less statistical power for QTL detection than RILs, but this comparison assumed that each NIL was paired with the recurrent parent in the experimental design. More efficient NIL experimental designs, where the recurrent parent is compared to multiple NILs simultaneously, can improve the relative efficiency of NIL experiments. Furthermore, although the power of detecting a single QTL may be greater in RILs than NILs, NILs may still offer more accurate QTL effect estimates than RILs when multiple QTLs are as segregating in the population. Typical population sizes that used in RIL mapping studies result in the confounding of effects of multiple segregating QTLs. This can lead to a reduced power of QTL detection and overestimation of the effects of the detected QTLs (Fazio et al. 2003). In contrast, phenotypic differences between the recurrent parent and a NIL should due primarily to the allelic differences at the chromosomal region surrounding the introgressed target locus. Essentially, this should reduce much of the "noise" caused by the effects of genetic background.

Expenses for marker assays are the major costs in marker-assisted backcrossing programs for transferring target genes from a donor into the genetic background of a

recipient genotype (Prigge et al. 2009). The efficiency of a marker-assisted backcrossing program depends on the selection strategy, population size, distance of the markers flanking the target gene, and length of the chromosome segment attached to the target gene. Sabadin et al. (2008) constructed a genetic map which had 117 microsatellite loci with average distance of 14 cM. Messmer et al. (2009) designed a RIL population and the genetic linkage map was constructed with 160 publicly available markers (81 SSRs and 79 RFLPs), it was 2,105.6 cM long and the average marker distance was 12.2 cM. Visscher (1996) suggested a marker distance of 10–20 cM should be appropriate. In our study, we used 110 SSR markers to control the genetic background of the recurrent parent 5,003 (107) from $BC_2F_1$ populations.

Development of high yield varieties is one of the most important goals in maize hybrids. KN, which is associated with ear length, row number per ear, ear diameter and other features, was shown to be highly correlated with yield and acted as a crucial component in determining maize yield (Li et al. 2010; Liu et al. 2010). Therefore, detection of its genetic basis would be of large value in breeding high yielding varieties. In the present study, we detected the major QTL on chromosome 10 for KN under different P regimes at two locations. This QTL was found to have an effect across different backcross populations (Fig. 6). Wang et al. (2009) combined a wealth of QTL mapping data from different publications and found 46 QTLs for KN, most of them were located on chromosome 1 and 5. This result was in agreement with our initial QTL mapping (Li et al. 2010). Only one of the reported QTL for KN was located on chromosome 10, the region of this QTL was in bin 10.01 and it is different with our result. Sabadin et al. (2008) obtained a segregating population by crossing inbreeding lines from different heterotic groups of maize and found five QTLs for KN, two on chromosome one, two on chromosome five, and one on chromosome 10, the region of the last QTL was in bin 10.04 between SSR markers bnlg1526-umc1930, which is also different with our result. Taken together, this indicates that the QTL identified here is a new QTL for KN.

Although P is one of the most important nutrient elements affecting many agronomic traits including KN, the previous ecological condition, designed for QTL identification controlling KN, were focused on different water content in soil according to literature (Xiao et al. 2005; Lu et al. 2006), and different nitrogen regimes (Liu et al. 2010), whereas, different P regimes have hardly been used in QTL analysis for KN. In our study, the major QTL could steadily enhance the KN under different P regimes (Figs. 2, 3). Under low P treatment condition, *qKN* increases the number of KN more than under the high P treatment condition. Chen et al. (2009) reported a QTL controlling whole phosphorus utilization efficiency located near the marker bnlg1839 in bin 10.07, which is adjacent to our *qKN*. We will validate whether our result is related with that report in the future. We also found five QTL for KN on the web (http://www.maizeGDB.org/). As none of them is located in the region of bin 10.07 suggests that the QTL identified here is a new QTL.

Over the past 10 years, researchers have gained an initial understanding of the genetic basis of yield traits in maize based on QTL mapping studies (Yan et al. 2006; Wang et al. 2009). Although very few QTL have been cloned, our results suggest that the QTL described here is a single gene. We can expect that with the improvement of mapping populations and statistical methods, more and more QTLs will be detected and used for fine mapping. With the help of bioinformatics, genome sequencing of model crops, and the principle of comparative genomics, these QTLs will be cloned, which in turn, will greatly promote the research of basic theory of complex traits and the development of molecular design breeding.

**Acknowledgments** This research was supported by the China National Major Basic research Program (973:2009CB118405). The authors greatly acknowledge Professor Dai JingRui from National Maize Improvement Center of China, China Agriculture University, for critical review of this manuscript.

## References

Austin DF, Lee M (1996) Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in mazie. Theor Appl Genet 92:817–826

Chen JY, Xu L, Cai YL, Xu J (2008) QTL mapping of phosphorus efficiency and relative biologic characteristics in maize (*Zea mays* L.) at two sites. Plant Soil 313:251–266

Chen JY, Xu L, Cai YL, Xu J (2009) Identification of QTLs for phosphorus utilization efficiency in maize (*Zea mays* L.) across P levels. Euphytica 167:245–252

Darvasi A (1998) Experimental strategies for the genetic dissection of complex traits in animal models. Nat Genet 18:19–24

Dekkers JCM, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Genetics 3:22–32

Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Fazio G, Staub JE, Stevens MR (2003) Genetic mapping and QTL analysis of horticultural traits in cucumber (*Cucumis sativus* L.) using recombinant inbred lines. Theor Appl Genet 107:864–874

Guo JF, Su GQ, Zhang GP, Wang GY (2008) Genetic analysis and QTL mapping of maize yield and associate agronomic traits under semi-arid land condition. Afr J Biotechnol 7:1829–1838

Huang YF, Madur D, Combes V, Ky CL, Coubriche D, Jamin P, Jouanne S, Dumas F, Bouty E, Bertin P, Charcosset A, Moreau L (2010) Comparing Intermitted and Conventional Populations Reveals the Genetic Architecture of Grain Yield and Related Traits in *Zea maize* L. Genetics. Published on line

Kaeppler SM (1997) Quantitative trait locus mapping using sets of near-isogenic lines: relative power comparisons and technical considerations. Theor Appl Genet 95:384–392

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Etoh T (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lee M, Sharopova N, Beavis WD, Grant D, Katt M, Blair D, Hallauer A (2002) Expanding the genetic map of maize with the intermitted B73 X Mo17 (IBM) population. Plant Mol Biol 48:453–461

Li Q, Wan JM (2005) SSRHunter: development of a local searching Software for SSR Sites. HEREDITAS (Beijing) 27:808–810

Li YL, Dong YB, Cui DQ, Wang YZ, Liu YY, Wei MG, Li XH (2008) The genetic relationship between popping expansion volume and two yield components in popcorn using unconditional and conditional QTL analysis. Euphytica 162:345–351

Li M, Guo XH, Zhang M, Wang XP, Zhang GD, Tian YC, Wang ZL (2010) Mapping QTLs for grain yield and yield components under high and low phosphorus treatments in maize (*Zea mays* L.). Plant Sci 178:454–462

Lima MLA, Jr CLS, Bento DAV, Souza AP, Garcia LAC (2006) Mapping QTL for grain yield and plant traits in a tropical maize population. Mol. Breed. 17:227–239

Liu XH, He SL, Zheng ZP, Huang YB, Tan ZB, Wu X (2010) QTL identification for row number per ear and grain number per row in maize. Maydica 55:127–133

Lu GH, Tang JH, Yan JB, Ma XQ, Li JS, Chen SJ, Ma JC, Liu ZX, E LZ, Zhang YR, Dai JR (2006) Quantitative trait loci mapping of maize yield and its components under different water treatments at flowering time. Integr Plant Biol 48:1233–1243

Lukens LN, Doebley J (1999) Epistatic and environmental interactions for quantitative trait loci involved in maize evolution. Genet Res Camb 74:291–302

Ma XQ, Tang JH, Teng WT, Yan JB, Meng YJ, Li JS (2007) Epistatic interaction is an important genetic basis of grain yield and its components in maize. Mol Breed 20:41–51

McCouch SR, Cho YG, Yano M, Paul E, Blinstrub M, Morishima H, Kinoshita T (1997) Report on QTL nomenclature. Rice Genet Newlett 14:11–13

Messmer R, Fracheboud Y, Banziger M, Vargas M, Stamp P, Ribaut JM (2009) Drought stress and tropical maize: qTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theor Appl Genet 119:913–930

Moreira JUV, Bento DAV, Souza AP, Souza CLS Jr (2009) QTL mapping for reaction to Phaeosphaeria leaf spot in a tropical maize population. Theor Appl Genet 119:1361–1369

Plenet D, Etchebest S, Mollier A, Pellerin S (2000a) Growth analysis of maize field crops under phosphorus deficiency. Plant Soil 223:117–130

Plenet D, Mollier A, Pellerin S (2000b) Growth analysis of maize field crops under phosphorus deficiency. II. Radiation-use efficiency, biomass accumulation and yield components. Plant Soil 224:259–272

Prigge V, Melchinger AE, Dhillon BS, Frisch M (2009) Efficiency gain of marker-assisted backcrossing by sequentially increasing marker densities over generations. Theor Appl Genet 119:23–32

Quarrie SA, Quarrie SP, Radosevic R, Rancic D, Kaminska A, Barnes JD, Leverington M, Ceoloni C, Dodig D (2006) Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. J Exp Bot 57:2627–2637

Raghothama KG (1999) Phosphate acquisition. Annu Rev Plant Physiol Plant Mol Biol 50:665–693

Reiter RS, Coors JG, Sussman MR, Gabelman WH (1991) Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphisms. Theor Appl Genet 82:561–568

Sabadin PK, Junior CLDS, Souza APD, Garcia AAF (2008) QTL mapping for yield components in a tropical maize population using microsatellite markers. Hereditas 145:194–203

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10:297–304

Salvi S, Sponza G, Morgante M, Tomes D, Miu XM, Fengler KA, Meeley R, Ananiev EV, Svitashev S, Bruggemann E, Li BL, Hainey CF, Radovic S, Zaina G, Rafalski JA, Tingey SV, Miao GH, Phillips RL, Tuberose R (2007) Conserved nocoding genomic sequences associated with a flowering-time quantitative trait locus in maize. Proc Natl Acad Sci USA 104:11376–11381

Tang JH, Yan JB, Ma XQ, Teng WT, Meng YJ, Dai JR, Li JS (2007) Genetic dissection for grain yield and its components using an "immortalized $F_2$ population" in maize. Acta Agron Sin 33:1299–1303

Tang JH, Yan JB, Ma XQ, Teng WT, Wu WR, Dai JR, Dhillon BS, Melchinger AE, Li JS (2010) Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized $F_2$ population. Theor Appl Genet 120:333–340

Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinez CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

Visscher PM (1996) Proportion of the variation in genetic composition in backcrossing programs explained by genetic markers. Heredity 87:136–138

Wang SC, Basten CJ, Zeng ZB (2005) Windows QTL Cartographer 2.5, Department of Statistics, North Carolina State University, Raleigh (http://www.statgen.ncsu.edu/qtl/crt/WQTL.html)

Wang BT, Wu JY, Ding JQ, Xi ZY (2009) Map Integration of QTLs for Grain Yield and Its Related Traits in Maize. Acta Agron Sin 35:1836–1843

Xiao YN, Li XH, George ML, Li MS, Zhang SH, Zheng YL (2005) Quantitative trait locus analysis of drought tolerance and yield in maize in China. Plant Mol Biol Rep 23:155–165

Yan JB, Tang H, Huang YQ, Zheng YL, Li JS (2006) Quantitative trait loci mapping and peristaltic analysis for grain yield and yield components using molecular markers with an elite maize hybrid. Euphytica 149:121–131

Zhang SH, Xu WP, Li MS, Li XH, Xu JS (2008) Challenge and opportunity in maize breeding program. J Maize Sci 16:1–5

Zhu JM, Mickelson SM, Kaeppler SM, Lynch JP (2006) Detection of quantitative trait loci for seminal root traits in maize (*Zea mays* L.) seedlings grown under differential phosphorus levels. Theor Appl Genet 113:1–10