Introduction

Maize grain yield (GY) is the most important breeding goal, and is one of the most complex traits [1], because it comprises several yield components including kernel number (KN) and kernel weight (KW). Furthermore, the KN per ear measurement can be further classified into kernel row number (KRN) and kernel number per row (KNPR). Previous studies suggested that yield components always show higher heritability than GY [1–2], and selection of certain yield components could be more effective than direct selection for GY itself [3–4]. Thus, geneticists and breeders have strived to understand the genetic basis underlying maize GY, and its components, by quantitative trait locus (QTL) mapping approaches. Numerous QTLs for GY-related traits have been identified on maize chromosomes [5]. Those identified chromosome regions also provide targets for QTL pyramiding and gene cloning.

Our current knowledge of the molecular regulation and genetic basis of GY mainly comes from cloned mutant genes that are involved in the regulation of inflorescence architecture and development [6]. Typically, three *rasmus* genes, *ra1* on 7.02bin, *ra2* on 3.03bin and *ra3* on 7.04bin, separately encode a C2H2 Zinc-finger protein [7], a LOB domain protein [8] and a Trehalose phosphatase [9]. Mutation of each of the three genes results in the spikelet-pair meristems (SPMs) at the base of inflorescence to transition into branch meristems (BMs), leading to branched ears and tassels with increased degrees of branching. The second type of cloned genes is associated with meristem initiation and maintenance. *Barren stalk1 (ba1)* encodes a non-canonical bHLH (basic helix-loop-helix) domain protein [10] that regulates the initiation of all axillary meristems. Mutations in *ba1* result in mutant plants lacking vegetative tillers, car tassel branches and spikelets [11]. *Barren inflorescence2 (bf2)* encodes a serine/threonine protein kinase that regulates polar transport of auxin [12]. *Bif2* mutants produce rudimentary ears and tassels that occasionally produce spikelets [13]. Moreover, *thick tassel dwarf1 (td1)* and *fasciated ear2 (fes2)* encode two homologs of Arabidopsis CALVATA proteins [14–15]. Mutation of either of the two genes influences inflorescence development by affecting the size or maintenance of the inflorescence meristem (IM). Maize GY is highly associated with female inflorescence development. The cloning of mutant genes involved in the regulation of ear architecture and development is undoubtedly helpful to understand the developmental regulation of GY and its components, but the genetic basis of the quantitative variation of GY and its components remains unknown.
Numerous QTLs and/or QTL clusters for GY-related traits have been identified in diverse populations; however, the cloning of QTLs remains difficult in the maize genome because of its large size and highly repetitive sequence [16]. Among identified QTLs, a clustered QTL for GY and its components on 6.01–6.03 bin of the maize genome was repeatedly detected in previous studies. Ajmone-Marsan et al. (1995) reported a clustered QTL for GY in umc396–ume21 of 6.02–6.03bin that explained 24.5% of the phenotypic variance [17]. Frascaroli et al. (2009) identified a QTL for GY and KN in phi075–bnlg1371 (6.01bin) in two testcross populations [18]. Peng et al. (2011) also found a pleiotropic QTL simultaneously controlling GY and KN on 6.02–6.03bin across multiple environments [19]. Moreover, many QTLs for GY-related traits were identified on 6S of the maize chromosome by multiple groups, such as a QTL for ear length (EL) in phi126–bnlg1371 [20], bnlg1338–bnlg291 [21] and phi126–np235 [1]. Liu et al. (2011) identified a QTL for KNPR around phi031 in 1r–ume1257 (6.01–6.02bin) [22], and Tan et al. (2011) identified a QTL for kernel weight in umc1656–ume1796 (6.02–6.04bin) [23]. Our group identified a chromosome segment around gce2 harboring a QTL for KNPR across four environments, designated as qKNPR6 [24].

These results highlight the importance of 6.01–6.03 bin for determining GY and its components, as well as providing a key target for QTL cloning. An optional strategy to finely map and clone a QTL is to create a large segregating population by crossing nearly isogenic lines (NILs) that differ only in the allelic constitution at the short chromosome segment harboring the target QTL (QTL-NILs). In such a population, because of the absence of other segregating QTLs, the target QTL becomes the major source of genetic variation, i.e., the QTL is considered Mendelian [25]. Thus, QTL detection power, resolution, and genetic effect can be significantly improved [16]. Previous studies in our laboratory confirmed a line, SL57, containing an introgressed segment flanked by gce2 and umc1857, which showed higher KNPR, longer ears and higher GY than the recurrent parent Ye478, an elite inbred in the Reid heterotic group. This implied that the introgressed segment encodes a pleiotropic gene, or several linked genes, which could affect the performance of multiple traits. In the present study, we used two inbred lines, SL57 and Ye478, to develop a new mapping population. We then combined linkage mapping and substitution mapping strategies to: 1) re-evaluate qKNPR6 and its genetic effect underlying the introgressed segment in SL57; 2) fine map qKNPR6 to a ≈1 cm interval; and 3) infer potential candidate genes responsible for qKNPR6.

Materials and Methods

Plant Materials

The SL57 is a chromosome segment substitution line that harbors a QTL for KNPR across four environments, designated as qKNPR6 [24]. Subsequently, Ye478 and SL57-6 were crossed to develop a QTL mapping population. Approximately 600 SL57-6×Ye478 F2 plants were grown during the summer of 2009, of which 193 individuals were open-pollinated for linkage map construction and QTL mapping, 201 individuals were self-pollinated to develop F2 as families for remapping of the target QTL, and the remaining individuals were genotyped to detect the recombinants that were then self-pollinated to develop homozygote recombinant lines. The phenotypes of the F2.3 families and homozygote recombinant lines were evaluated by a field experiment using a randomized complete block with three replications, with twenty-two individuals per block, in summer 2010 at Baoding (Northern China). 12,800 F2 plants were grown in summer 2011 to finely map the QTL. 1000 randomly selected F2 plants were genotyped and used for QTL mapping. Using PCR-based markers within gKNPR6, 363 recombinants were selected. Of these recombinants, 191 plants carrying recombinant chromosomes were open-pollinated to evaluate the phenotype. The other recombinants were selfed to develop homozygous lines with overlapping in the introgression segment (hereafter termed sub-NIL). Ten sub-NILs, Ye478 and SL57-6 were grown at Sanya (Southern China) in winter 2011 with three replications, with twelve individuals per block. The GY-related traits were measured, including KNPR, EL, EW and GW per plant.

Molecular Marker Development and Linkage Analysis

The DNA sequence flanked by umc1656 and umc1857 in the B73 genome [26] was retrieved to develop SSR markers using the Simple Sequence Repeat Identification Tool [27], with the maximum motif-length set at four base pairs, and the minimum number of repeats set at 10 base pairs. Primers were designed using Primer Premier 5.0 with a product size under 300 bp. Similarly, PCR-based markers were developed in the chromosomeal interval flanked by N6M19 and N6M66. Those developed SSRs from the Umc1656–Umc1857 interval were used to identify the genotypes of the F2 population in summer 2009, and to construct a linkage map. The PCR-based markers within N6M19–N6M66 interval were used to identify the genotypes of the large F2 population in summer 2011. All of the developed markers that were used to screen the F2 population for recombinant chromosomes are listed in Table 1.

QTL Analysis

The phenotypic variance among NILs was estimated by analysis of variance (ANOVA). Broad-sense heritability was estimated on a family mean basis. QTL analysis was first performed by ANOVA, testing the significance of the difference between phenotypic values of the genotypic classes (homozygous for the SL57-6 allele and homozygous for the Ye478 allele) at each marker position. A significance threshold of P = 0.01 was chosen for declaring linkage between a marker and qKNPR6. QTL mapping was performed using QTL IciMapping [28].

Quantitative RT-PCR

Immature ears representing two different car development stages (1 to 2 mm long) were collected from Ye478 and SL57-6, respectively. Total RNA was extracted using TRIzol® Reagents (Promega, Madison, WI, USA) according to the manufacturer’s instructions. Gene-specific primers (Table 1) were designed to assay the expression of six candidate genes by real-time RT-PCR. Total RNA was treated with RNase-free DNase (Promega) to remove contaminating DNA. DNA-free RNAs were mixed with
1.0 μg oligo dT on ice, and then heated to 70.0°C for 10 minutes, followed by three minutes on ice. First strand cDNA was synthesized using 1.0 μL M-MLV reverse transcriptase according to the manufacturer’s instructions (Takara, Otsu, Japan). cDNA (1.0 μL) was used as the template in PCR reactions, which contained 10.0 μL 2 × Smart SYBR QPCR Mix (DoGene, Shanghai, China), 0.8 μL 10 μM primer mixture and 8.2 μL water. Reactions were performed using the Chromo4 Real-Time PCR Detection System. The threshold value was empirically determined using the observed linear amplification phase of all primer sets. Sample cycle threshold (Ct) values were standardized for each template, based on an Actin (GRMZM2G030169) control reaction. The comparative Ct method (ΔΔCt) was used to determine the relative transcript abundance of each gene [29].

Figure 1. Distribution of the introgressed segments and phenotype of SL57. 1a: Distribution of the introgressed segments on maize chromosome 4 and 6 in SL57. The introgressed segments were identified by SSR screening. The white box represents the chromosome with the same genotype as Ye478, and the black box represents the introgressed segment. The genetic similarity between Ye478 and SL57 is 97.6%. 1b: The kernel number per row of SL57 and Ye478 under four environments. The white bar represents Ye478, and the black bar represents SL57. The horizontal axis shows four environments: Baoding in 2008 (2008BD), Baoding in 2009 (2009BD), Baoding in 2010 (2010BD) and Baoding in 2011 (2011BD). The vertical-axis shows the kernel number per row. N: The number of samples. 1c: The ear length, ear weight and grain weight of SL57 and Ye478 detected in 2010 at Baoding (northern China).

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Results

qKNPR6 Mapping

Using 193 F2 plants developed by crossing SL57×Ye478 in 2009, qKNPR6 was remapped to the umc1656-umc1857 interval (24.6 cM) on 6.02-6.04 bin, explaining 39.45% of the phenotypic variance. No QTL was detected in the introgression segment on 4.08 bin (Figure 2). The allele of SL57 showed a positive additive effect and a negative dominant effect. Individuals with only one introgression segment on 6.02-6.04 bin or on 4.08 bin were selected on the basis of marker genotype and were self-pollinated to produce two segmental isolines: SL57-6 harboring the introgressed segment flanked by umc1656-N6M30 interval, a QTL for EL was also detected that

Table 1. Primers for QTL mapping and gene specific primers for real-time PCR.

| Marker/gene | Primer_F | Primer_R |
|-------------|----------|----------|
| Primers for QTL mapping | | |
| N6M19 | TAGTTAGGCCTAGCTAGGATG | AAGAAGACATAAATGAGGACG |
| N6D139 | GGCTGAGGTGAGGAGGAGA | GGCTCTAGGAGGAATG |
| N6M30 | GCTATGTTGCTAGGCTAGG | AAGAAGAAGAGGAGGAG |
| N6D262 | CCGCAGCTAGCTAGGATG | CGTCTGGAAGAGAGGAG |
| N6D354 | CTGCGCCGAAGACATTT | AGGAGAAGAGGAGGAG |
| N6D389 | CGTCTGGAAGAGGAGGAG | CTGCTGGAAGAGGAGGAG |
| N6M46 | TGAACCTGGAAGAGGAGGAG | TGAAGCTGGAAGAGGAGG |
| N6D543 | GTCCAGCTAGCTAGGATG | ATGCCAGCTAGCTAGGATG |
| N6M66 | TACATTACCTGGAAGAGGAG | TACATTACCTGGAAGAGGAG |
| N6M123 | GAATTGCAAGCTGGAAGGAG | CAACAGGGAAGAGGAGG |
| N6M137 | CGAAGAAAGAGGAGGAGGAG | TACATTACCTGGAAGAGGAG |
| Primers for real-time PCR | | |
| GRMZM2G128485 | GGAAAGCTAGCTAGGATG | CAGGCAAGCTAGGATG |
| GRMZM2G428518 | CGACCTGCTAGCTAGGATG | GTTGTGGAGAGGAGGAG |
| GRMZM2G128574 | AGCTGCTAGCTAGGATG | GGAAGAGAGGAGGAGGAG |
| GRMZM2G119678 | ATGCTGCTAGCTAGGATG | CGTCTGGAAGAGGAGGAG |
| GRMZM2G119714 | CTTGCTGGAAGAGGAGGAG | CTTGCTGGAAGAGGAGGAG |
| GRMZM2G128560 | TCAGTTACGCTAGGATG | CTTGCTGGAAGAGGAGGAG |
| Actin (GRMZM2G030169) | GGCTCAAGCTAGGATG | GGCTCAAGCTAGGATG |

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Fine Mapping of qKNPR6

A larger segregating population with 12,800 F2 individuals was developed from the SL57-6×Ye478 cross and was used to finely map qKNPR6. Furthermore, newly developed markers were used to produce a high-resolution linkage map of the target QTL interval by genotyping 1,000 randomly selected plants. Using the genotype and phenotype of these random F2 samples, qKNPR6 was further narrowed down to a 0.91 cM interval flanked by N6M19 and umc1656-umc1857. In the same interval, QTLs for EL, EW and GW were also detected (Table 3). The logarithms of odds (LOD) scores of these QTLs increased rapidly, up to 69.95 for KNPR. However, the phenotypic variance explained by the QTLs decreased, to only 23.34% by the EL QTL (Table 4). The unexpected decline could be attributed to the high environmental sensitivity of these QTLs. To decrease the environmental effect on the phenotypic of these traits, 365 recombinant chromosomes were detected by genotyping all of F2 plants using
markers within and flanking qKNPR6. Of these recombinant chromosomes, 191, representing 13 meiotic events, were open-pollinated to evaluate their phenotypes in summer 2011. The phenotypic means of recombinants with the same genotype were used to detect QTLs by a t-test. The result demonstrated that Rec1 to Rec7 carried the SL57-6 allele in the N6M19-umc1257 interval, while Rec8 to Rec13 carried the Ye478 allele in the corresponding interval. The KNPR of Rec1 to Rec7 was distinctly higher than that of Ye478, as well as being higher than that of Rec8 to Rec13 (Table 5), indicating that N6M19-umc1257 interval might contain a QTL for KNPR. The remaining recombinants (172) were self-pollinated to develop homozygous recombinant lines (sub-NILs), and were then phenotypically assessed in winter 2011 at Sanya (Southern China). The result of QTL mapping using the sub-NILs was consistent with the results of linkage mapping in the F2 population and of the substitution mapping using the recombinants, supporting the view that qKNPR6 is harbored within the N6M19-umc1257 interval (Figure 3a,3b). Notably, those sub-NILs holding the favorable allele of qKNPR6 also showed greater performance in EL, EW and GW per ear than Ye478 (Figure 3c–3e), indicating that the N6M19-umc1257 region might affect the performance of multiple traits, i.e., it could be a pleiotropic locus.

Prediction of Candidates and Expression Assays

The N6M19-umc1257 interval on the B73 genome is about 198 Kb long, and contains six genes annotated in B73 RefGen_v2. GRMZM2G119714 encodes a serine/threonine protein kinase receptor (STKR) protein that is highly homologous with receptor protein kinase PERK-like in Oryza sativa, but markedly differs from Bif2 of maize (Figure 4a). GRMZM2G119678 encodes a SET domain-containing protein group 102 (SDG102), which is highly homologous with Arabidopsis ASHH2/SDG8 [30]. GRMZM2G428518 encodes a protein

Figure 2. QTL interval for kernel number per row identified in different populations. 2a: The QTL interval detected using different segregation populations; the high LOD score supported the QTL interval. 2b: The genotypes of sub-NILs derived from the recombinant chromosomes. The black boxes or bars represent marker genotypes of SL57-6, the white boxes represent marker genotypes of Ye478. 2c: The phenotypes of sub-NILs detected in summer 2010 at Baoding (northern China). The amount in parentheses showed the number of samples. *** Significant difference at P = 0.01. doi:10.1371/journal.pone.0049836.g002
homologous with alpha-glucosidase in Oryza sativa, and GRMZM2G128485 encodes a BTB superfamily protein that is 61% homologous with a Brachypodium distachyon BTB/POZ domain-containing protein that is involved in auxin signaling. The other two genes are of unknown function. A qPCR assay revealed that the six genes were expressed in the immature ears of both SL57-6 and Ye478. The expression of STKR in Ye478 at the spikelet meristems (SMs) stage was significantly higher than in SL57-6. The expression of SDG102 in Ye478 was markedly higher than in SL57-6 at SPM and SM developmental stages (Figure 5). The expressions of the other candidates showed no significant differences between Ye478 and SL57-6 at the p = 0.01 level.

Sequencing demonstrated that the coding regions of both STKR and SDG102 were highly conserved in Ye478 and SL57-6. Four SNPs were identified in the coding region of STKR, of which two C/G SNPs led to amino acid substitutions: Leu to Var and Gln to Glu (Figure 4b). The two amino acids are not conserved among plant species. The 5'-upstream sequence of SDG102 has a few SNPs between Ye478 and SL57-6, and only one C/T SNP was found in the coding region of SDG102 (Figure 4c). However, the promoter region of the STKR gene showed a large insertion/deletion variation (Figure 4b), which might affect the expression level of the gene in the two NILs.

Table 3. Pearson correlations among the studied traits and the heritability of these traits.

| Trait | Correlation Coefficient | Heritability (%) |
|-------|-------------------------|-----------------|
| KNPR  | 1.00                    | 91.1            |
| EL (mm)| 0.83***                 | 90.8            |
| EW (g) | 0.81***                 | 83.8            |
| GW (g) | 0.83***                 | 83.0            |

**Note:** ***indicates significance at p < 0.001.

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Table 4. QTLs detected in the F2,3 families in summer 2010.

| Population | Trait | Marker Interval | LOD | PVE (%) | Add | Dom |
|------------|-------|-----------------|-----|---------|-----|-----|
| 2010 F2,3 families | KNPR | N6M19-N6M30 | 33.28 | 56.14 | 3.01 | 0.19 |
| | EL (mm) | N6M19-N6M30 | 32.08 | 44.99 | 11.81 | 5.44 |
| | EW (g) | umc1556-N6M19 | 23.55 | 41.76 | 12.36 | 2.99 |
| | GW (g) | umc1556-N6M19 | 22.44 | 40.24 | 11.22 | 2.72 |
| 2011 F2 | KNPR | N6M19-umc1257 | 69.95 | 23.34 | 2.01 | 0.93 |
| | EL (mm) | N6M19-umc1257 | 20.43 | 7.48 | 5.18 | 0.67 |
| | EW (g) | N6M19-umc1257 | 30.41 | 11.04 | 7.28 | 1.78 |
| | GW (g) | N6M19-umc1257 | 26.60 | 9.72 | 6.42 | −0.53 |

**Note:** KNLPR, kernel number per row. EL, ear length. EW, ear weight. GW, grain weight. LOD, logarithms of odds. Add, Additive. Dom, Dominant. PVE (%), Percentage of phenotypic variance explained.

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**Table 5.** Genotype and phenotype of homozygous recombinants selected from the F2 in 2011.

| NIL     | bnlg1422 | umc1656 | N6M19 | umc1257 | N6D139 | N6M30 | N6D262 | N6D354 | umc1595 | N6D389 | N6M46 | N6D543 | N6M64 | No. | Mean ± SD | P Value |
|---------|----------|---------|-------|---------|--------|-------|--------|--------|---------|--------|-------|--------|-------|-----|-----------|---------|
| SL57-6  | A        | A       | A     | A       | A      | A     | A      | A      | A       | A      | A     | A      | A     | 30  | 32.9 ± 2.5 | 1.19E-06 |
| Rec1    | B        | B       | A     | A       | A      | A     | A      | A      | A       | A      | A     | A      | A     | 3   | 29.2 ± 3.3 | 2.76E-04 |
| Rec2    | A        | A       | A     | A       | A      | A     | A      | A      | A       | A      | A     | B      | 39    | 30.0 ± 2.4 | 9.42E-05 |
| Rec3    | B        | B       | A     | A       | A      | A     | A      | A      | A       | A      | A     | B      | B     | 4   | 30.7 ± 2.1 | 6.07E-05 |
| Rec4    | B        | A       | A     | A       | A      | A     | A      | A      | A       | A      | B     | B      | B     | 2   | 30.1 ± 2.7 | 3.78E-04 |
| Rec5    | A        | A       | A     | A       | A      | A     | A      | A      | A       | B      | B     | B      | B     | 14  | 27.9 ± 1.8 | 8.25E-03 |
| Rec6    | A        | A       | A     | A       | A      | A     | B      | B      | B       | B      | B     | B      | B     | 7   | 29.3 ± 2.5 | 1.19E-03 |
| Rec7    | A        | A       | A     | A       | B      | B     | B      | B      | B       | B      | B     | B      | B     | 4   | 29.3 ± 1.5 | 2.21E-04 |
| Rec8    | A        | A       | B     | B       | A      | A     | A      | A      | A       | A      | A     | A      | A     | 5   | 23.3 ± 3.8 | 0.07    |
| Rec9    | A        | A       | B     | B       | B      | B     | B      | A      | A       | A      | A     | A      | A     | 5   | 21.0 ± 3.08 | 0.97    |
| Rec10   | B        | B       | B     | B       | B      | B     | B      | A      | A       | A      | A     | A      | A     | 11  | 22.4 ± 2.39 | 0.06    |
| Rec11   | B        | B       | B     | B       | B      | B     | A      | A       | A      | A      | A     | 10   | 24.8 ± 1.64 | 0.69    |
| Rec12   | B        | B       | B     | B       | B      | B     | B      | B      | A       | A      | A     | 67   | 23.2 ± 3.24 | 0.09    |
| Rec13   | B        | B       | B     | B       | B      | B     | B      | B      | B       | A      | A     | 20   | 250 ± 2.16 | 0.08    |
| Ye478   | B        | B       | B     | B       | B      | B     | B      | B      | B       | B      | B     | 30   | 244 ± 1.40 |         |

A and B represent marker genotypes that are the same as SL57-6 and Ye478, respectively. No. shows the number of recombinants with the same genotype.

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Discussion

The qKNPR6 Might be a Pleiotropic Locus

Most agronomically important traits in crop plants, such as GY, quality, and resistance to biotic and abiotic stresses, are complex quantitative traits that are usually controlled by multiple QTLs which are frequently clustered on the genome [1–31], [32–33], [34–35]. The clustered QTLs can be genetically explained by QTL pleiotropy or tight linkage of QTLs. QTL analysis typically produces a large confidence interval spanning 10-30 cM, comprising several hundred genes; therefore, it is usually uncertain whether a QTL represents one or multiple genes, which makes it difficult to ascertain whether a QTL cluster results from a pleiotropic gene or from multiple linked genes. In one case, clustered QTLs were resolved in linked QTLs with minor effects. QTL mapping studies in maize clearly showed that clustered QTLs for complex traits, such as plant height and GY, could be separated into multiple QTLs when the resolution of the linkage
analysis improved from a 10–30 cM to a 1–5 cM interval [36–37], [38–39]. In another case, a QTL cluster was mapped as a major QTL affecting multiple traits that correlated with each other: the QTL acts as a regulator in multiple biological pathways [35–40], [41–42]. Several cloned QTLs have provided evidence of gene pleiotropy. For example, vgt1 controls flowering time and plant node number in maize [40], tb1 affects the plant and inflorescence architecture [43], and Ghd7 and Gha8 simultaneously affect GY, plant height and heading date in rice [41–42].

In the present study, KNPR in maize was significantly correlated with EL, as well as with EW and GW (Table 3). A developmental association of the two traits could explain the highly positive correlation between KNPR and EL. The correlation between KNPR and GW could be attributed to the indirect effect of alteration of KN on EW per plant. QTL mapping in F2:3 families indicated that multiple QTLs for the above-mentioned traits were clustered in a ~2.8 cM interval (Table 4). When the clustered QTLs were further narrowed down to an approximately 198Kb region by substitution mapping, the QTL's simultaneous effects on the performance of the four association traits were still observed (Figure 3). This suggested that qKNPR6 might be a pleiotropic locus that simultaneously affects KNPR and EL, and indirectly affects EW and GW per plant in maize.

Possible Candidate Gene Underlying the qKNPR6

Maize female inflorescence originates from an axillary meristem at the tip of a lateral shoot in the axil of a leaf. When the plant transitions from vegetative to reproductive development, the axial...
meristem becomes an IM, and the IM produces multiple rows of SPMs, a kind of short BM, which then form two SMs. Each SM uniquely initiates two floral meristems (FMs) (upper FM and lower FM), and each FM subsequently forms the floral organs. Subsequently, the lower floret and the stamens abort, resulting in the formation of single female florets. Obviously, a kernel in maize ear is developed from a pollinated floret. This implies that the number of fertile florets in a given row of SMs determine the possible KN in the row. In other words, maintaining a high differentiating activity in the SM is important for developing more FMs that produce fertile florets. Thus, those genes that are involved in regulation of the differentiating capacity of IMs are probable candidates for genetic control of KNPR in maize. Within the 198Kb region of \textit{qKNPR6}, the B73 genome encodes six genes with EST evidence and all six genes are expressed in maize immature ears [44].

The expression level, sequence variation and the annotated biological function in model species of the six genes in SL57-6 and Ye478 could provide clues to identity the candidate gene. Among the six predicted candidates for \textit{qKNPR6}, quantitative PCR revealed that the \textit{STKR} and \textit{SDG102} were differentially expressed in two NILs, Ye478 and SL57-6, while the other genes displayed similar expression levels in the two NILs and had similar expression patterns during the different developmental stages of the immature ear (Figure 5). Temporal and spatial expression patterns showed that the serine/threonine-protein kinase receptor gene is highly expressed in the immature ear, roots, 20-day

**Figure 5. Relative level of candidate gene expression in immature ears.** S2: Differentiation stage of the spikelet-pair meristem. S3: Differentiation stage of the spikelet meristem. **: Significant difference at $P = 0.01$, ***: Significant difference at $P = 0.001$.\r
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**Figure 6. Schematic diagram of inflorescence development along with genes that are likely to affect the various developmental stages.** SAM, shoot apical meristem; AM, axillary meristem; IM, inflorescence meristem; SPM, spikelet-pair meristem; SM, spikelet meristem; FM, floret meristem. The genes were: \textit{Fea2}, \textit{fasciated ear2}; \textit{Td1}, \textit{thick tassel dwarf1}; \textit{Bif2}, \textit{barren inflorescence2}; \textit{Bd1}, \textit{branched silkless1}; \textit{qKNPR6}, a QTL for kernel number per row on chromosome 6.\r
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endosperm, ovule, and tassel, but is not expressed in pollen or leaves [44]. Previous studies demonstrated that *Barren inflorescence2 (Bif2)* encodes a serine/threonine protein kinase [12] that phosphorlates ZmPIN1a to regulate the subcellular localization of ZmPIN1a [45]. *Bif2* mutants produce rudimentary ears that occasionally produce spikelets [13]. In the 196Kb segment, one candidate gene encodes a receptor protein serine/threonine kinase (STKR) that plays a role in the regulation of cell proliferation, cell differentiation, and embryonic development by phosphorylating receptor proteins. Sequencing revealed that the coding regions of the STKR gene was highly conserved, while the promoter region showed a large insertion/deletion variation, which might affect the expression level of the gene in Ye478 and SL57-6. Another differentially expressed gene, *SDG102*, is a homolog of Arabidopsis *ASHH2/SDG6* [30], which is a class of proteins that have been implicated in regulating gene expression through H3K36 trimethylation modification [46]. *ASHH2/SDG6* is a repressor of the transition from vegetative to reproductive growth [47–48], and is a regulator of shoot branching. Flower morphology and fertility in Arabidopsis [49]. *ASHH2/SDG6* mutants can downregulate expression of the floral organ identifying genes *APETALA1 (AP1)* and *AP2*, display homeotic changes of floral organs and have very low seed sets because of developmental defects of reproductive organs. By inference, we suggest that one of the two genes is a likely candidate for gKNPR6, owing to their function in inflorescence development and floral organ differentiation, but direct evidence from map-based cloning, genetic transformation and mutants are still required.

If one of the two genes is responsible for the gKNPR6, how does it act to control KNPR in maize? Upadhyayula et al. (2006) summarized the genetic steps of the functions of several cloned genes in the development of the tassel and ear (Figure 6) [50]. Both *Fasciated ear2* (*Fae2*) and *Thick tassel dwarf1* (*td1*) have similar functions in the transition from AM or SAM to IM. *Barren inflorescence2 (Bif2)* affects the transition from IM to SPM or BM, and both *R1* and *R2* have similar functions in the transition from SPM to BM. *Branched silkless1* (*Bds1*) is required for FM identity, and regulates the transition from SM to FM. Notably, *Bif2*, which encodes a serine/threonine protein kinase co-orthologous to PINOID [12], is required for maintenance of the BM, SM and FM in the inflorescence. During inflorescence development, BIF2 directly phosphorlates ZmPIN1a, and regulates auxin transport by regulation of the subcellular localization of ZmPIN1a [45]. In addition, the heterochronic expression of a gene is considered a regulator of phenotypic variation of a quantitative trait, such as *fcs2* [51] and *Ghd7* [41]. Thus, we postulate that the STKR protein might function together with BIF2 to positively regulate auxin efflux by phosphorylation of PIN1a. The heterochronic expression of qKNPR6 in an inflorescence might regulate auxin concentration and its temporal and spatial distribution, affecting the activity duration of the BM, SM and FM, which in turn would regulate the KN in a maize ear. If the *SDG102* is responsible for the qKNPR6, on the basis of function of its homolog of Arabidopsis *ASHH2/SDG6*, then qKNPR6 is postulated to be a repressor of transition from vegetative to reproductive growth. More FMs (or kernels) in female inflorescence of SL57 than that of Ye478 might be explained by lower expression of *SDG102* in SL57 inflorescence allowing earlier transition from vegetative to reproductive growth.

**Author Contributions**

Conceived and designed the experiments: ZZ YZ. Performed the experiments: RL HJ XC FL JH. Analyzed the data: RL ZZ YT. Wrote the paper: ZZ RL FQ. Critically read and approved the manuscript: ZZ RL HJ XC JH YT FQ YZ.

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