Phenotypic Characterization and QTL/Gene Identification for Internode Number and Length Related Traits in Maize

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Abstract

Internode number and length are the foundation to constitute plant height, ear height and the above-ground spatial structure of maize plant. In this study, segregating populations were constructed between EHel with extremely low ear height and B73. Through the SNP-based genotyping and phenotypic characterization, 13 QTL distributed on the chromosomes (Chrs) of Chr1, Chr2, Chr5-Chr8 were detected for four traits of internode no. above ear (INa), average internode length above ear (ILaa), internode no. below ear (INb), and average internode length below ear (ILab). Phenotypic variation explained (PVE) by a single QTL ranged from 6.82% (qILab2-2) to 12.99% (qILaa5). Zm00001d016823 within the physical region of qILaa5, the major QTL for ILaa with the largest PVE was determined as the candidate through the genomic annotation and sequence alignment between EHel and B73. Product of Zm00001d016823 was annotated as a WEB family protein homologous to At1g75720. qRT-PCR assay showed that Zm00001d016823 highly expressed within the tissue of internode, exhibiting statistically higher expression levels among internodes of IN4 to IN7 in EHel than those in B73 (P < 0.01), implying a negative regulating trend to internode elongation in maize. Functional dissection of Zm00001d016823 might provide novel insight into molecular mechanism beyond phytohormones controlling internode development in maize.

*These authors contributed equally to this work.
Keywords

Maize (Zea mays L.), Internode No., Average Internode Length, Phenotypic Characterization, Candidate Gene Discovery

1. Introduction

Plant architecture plays a crucial role in determining the yield performance of maize varieties. Key factors influencing plant architecture are plant height (PH) and ear height (EH). Both PH and EH are closely related to the final yield performance of maize varieties [1] [2]. Therefore, optimizing these factors is crucial for improving the overall productivity of maize. Extensive research has been conducted on PH and EH, resulting in the identification and collection of over 250 quantitative trait loci (QTL) distributed throughout the maize genome (MaizeGDB, http://www.maizegdb.org). Additionally, stalk lodging performance is closely linked to both PH and EH, particularly the ratio of EH to PH (EH/PH). Stalk lodging can have a significant impact on plant architecture and can result in substantial yield losses during maize production [3] [4].

The performances of both PH and EH are determined by the internode no. and length of maize stalk, and significant progress has been made in identifying candidate genes associated with PH, EH, and even internode no. and length related traits in maize [5] [6]. Phytohormones play important roles in structuring many tissues in plants [7]-[9]. And genes involved in biosynthesis, signaling, and regulating of phytohormones, including auxin, CK, GA, and BR, are intensively documented in maize for their significant potential roles in stress responding and organ growth and development [10]-[13]. Dwarf plant related genes, such as dwarf plant1 (d1)/3/8/9 that are involved in GA biosynthesis or signal transduction, are reported to control both PH and EH in maize [14]-[17]. A recent report documented by Paciorek and colleagues of Bayer Crop Science demonstrated that the suppression of two GA biosynthesis related genes, i.e., ZmGA20ox3 and ZmGA20ox5, resulted in the reduced GA levels in internodes, which finally led to significantly reduced PH and EH in maize [6]. Similar performances of shortened internode lengths and PH caused by the repression of RIN1 (REDUCED INTERNODE) to two gibberelin-oxidase genes of GA2ox7a/7b in soybean were also reported and functionally dissected [18]. Another study based on mutant of m30 with decreased internode no. and length, documented ZmCYP90D1 as a candidate gene that was involved in regulating internode development through modulation of BR-mediated cell division and growth [19]. Furthermore, a most recent report documented a novel module of ZmBZR1-ZmIBH1-ZmXTH1 that joint both BR and JA in regulating the internode elongation in maize [20].

Continuous and further gene discovery and functional dissection of height related traits, especially those focused on internodes, could provide informative
references for the understanding of growth and development of internodes, then the regulating of height related traits, and even stalk improvement in maize. Intensively genotyping and omics strategies provide high-throughput and global tools to dissect genes or pathways for candidate traits, such as PH, EH, and internode related traits, through whole development stages. Based on B73, Le et al. correspondingly collected 12 and 17 mass samples of internodes at elongation (V14) and maturity (R6) stages, and dissected the dynamic transcriptome features and spatiotemporal expression patterns of genes involved in maize internodes via RNA-seq [21]. They discovered vast genes and several regulatory networks for cell elongation and cell division responding to internode development in maize [21]. Another study by Wu and colleagues identified 85 significant SNPs and five candidate genes that were associated with internode length, diameter, and other stalk lodging resistance-related traits. These candidate genes are involved in various biological processes, such as cell division, growth, and hormone signaling pathways [22]. An integrated study of metabolites detection and RAN-seq focused on stalk strength identified >2000 up-regulated genes potentially associated with stalk-lodging resistance in maize, among which 28 genes that encoding cellulose synthase, chitinase, and COBRA-like protein 4 were found to be associated with stalk strength, providing insights into the molecular mechanisms underlying this trait [23].

In the previous work of germplasm characterization and improvement, a distinct inbred line of EHel with extremely low EH was identified [24]. The ratio of EH/PH of EHel is less than 0.25, extremely lower than that of the reference line B73. The corresponding F2 and F2:3 populations were constructed through the crossing of EHel and B73. In the present study, both parental lines of EHel and B73, and F2 were genotyped with chipset-based SNPs. Combining with phenotyping of PH, EH, EH/PH, and internode related traits, we performed candidate gene discovery controlling internode no. and length related traits in maize. The results will deepen and extend our understanding to the growth and development of maize internodes, as well as provide informative references for the construction of idea plant architecture in maize.

2. Materials and Methods

2.1. Plant Materials

Female line of EHel was screened out from the self-pollinating descendants between the cross of Dan 299 and Pioneer hybrids ten years ago [24]. The typical character of EHel is the extremely low ratio of EH/PH, less than 30% to that of B73. In the spring of 2016, we collected pollens from B73, and crossed with EHel. The F1 seeds were planted and all F1 plants were self-pollinated to construct F2 in 2017. In the next spring, one F2 ear was randomly selected and planted, and F2:3 family lines were constructed with the self-pollinating of F2 individuals. In the present study, both parental lines of EHel and B73, and populations of F2 and F2:3 were used for the phenotyping, genotyping, QTL map-
ping, and further candidate gene discovering.

2.2. Phenotyping and Data Analyzing

In the spring of 2019, EHel, B73, and 127 family lines of F2:3 were all planted in the field of college farm in Xiema (29°45’39” N, 106˚23’32” E, Beibei, Chongqing, China) with the same experimental design described by Gul et al. [24]. One week after anthesis, 10 continuous plants of each F2:3 family line were labelled, and the phenotypic datasets of PH and EH were collected referred to Shi et al.

and Norman et al. [25] [26]. At harvesting time, all leaves and leaf sheaths of these same 10 plants of each family line were removed, and datasets of four internode related traits, including internode number above ear (INa), internode length above ear (ILa), internode number below ear (INb), and internode length below ear (ILb) were collected. Then the average internode length above ear (ILaa) and average internode length below ear (ILab) were calculated accordingly. When collecting the datasets of these four traits, the node that bearing the topmost ear was labelled as the initiation internode, and the internodes above the topmost ear were correspondingly marked as IN1, IN2, IN3, etc. in ascending order based on the distance between internodes and the labelled node. On the contrary, all internodes below the topmost ear were marked as IN-1, IN-2, IN-3, etc. in the same way.

All collected datasets were input into Microsoft-Excel 365 to calculate the average and standard deviation (S.D.) and carried out the T-test. SAS (Version 9.0) was used to carry out summary statistics analysis and normal distribution test for the frequency of all traits, as well as the correlation analysis.

2.3. DNA Extraction, Genotyping, and Linkage Map Construction

In the spring of 2018, tips of the 3rd developed leaves of EHel, B73 and 127 individuals of F2 were cut for DNA extraction with CTAB procedure. The extracted DNA solutions with RNase were sent to China Golden Marker Biotechnology Co., Ltd. (Beijing, China) for chipset-based genotyping, as described by Gul et al. [24]. Among ~10000 SNPs integrated within the chipset, a total of 2108 polymorphism SNPs between EHel and B73 were screened out for the construction of linkage map via QTL IciMapping V4.5.3 [27].

2.4. QTL Mapping and Genomic Annotation of Target QTL

To detect the QTL for INa, INb, ILaa, and ILab, the ICIM-ADD model implemented in the BIP procedure of QTL IciMapping was used with the procedure of fixed LOD of 2.5 [27]. All detected QTL were named by the way described by McCouch et al. [28], while gene action and phenotypic variation explained (PVE, %) of each detected QTL were determined according to Stuber et al. [29]. Physical chromosomal region of target QTL was determined according to the integrated information of maize genome database (MaizeGDB, https://www.maizegdb.org/). Candidate genes within the physical region of tar-
2.5. Candidate Gene Screening

Referred to Walley et al., RNA-seq based FPKMs among 23 tissues, i.e. tissue 1 to 23, of all annotated genes were collected from MaizeGDB (https://www.maizegdb.org) [30]. The FPKMs of all annotated genes in the tissue of 7-8 internode (Tissue 2) were standardized to 1, then all the FPKMs of other tissues were divided by that of Tissue 2 for each annotated gene, to get the relative FPKMs of all genes among 23 tissues. According to the annotated description and the relative FPKMs of all genes, Zm00001d016823 was screened out as the candidate of qILaa5 with the largest PVE. Another gene, Zm00001d016831 also exhibited relatively higher FPKMs within the internode tissues (Tissue 1 and 2), and was also screened for sequence comparison.

2.6. Gene Cloning and qRT-PCR Based Validation

Primers of both Zm00001d016823 (Forward: 5’-ACGATGTCTACTTCACCAGCC-3’, Reverse: 5’-ATACGGGACGACTCTCAGC-3’) and Zm00001d016831 (Forward: 5’-ATATGAAGGTCGCTTGGACCC-3’, Reverse: 5’-CCTGTCCTGGTTAGTGAATCCT-3’) were designed by the on-line tool of NCBI—Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to clone the corresponding sequences from EHel and B73. The PCR products of Zm00001d016823 and Zm00001d016831 from EHel and B73, i.e., Zm00001d016823EHel, Zm00001d016823B73, Zm00001d016831EHel, Zm00001d016831B73, were sequenced and aligned through DNAMAN (v8.0). Vector NTI advance® v11.5 was used to predict the coding amino acids (aa), and compare the aa sequences between EHel and B73.

During the spring season of 2019, tissues of leaf samples at the middle part of the 8th leaf, immature female inflorescences (about 2 to 3 cm), internodes and leaf sheaths above ground, and roots samples of both EHel and B73 were collected at V12 stage. RNA of all samples were extracted with the kit of DP432 (TianGen Biotech (Beijing) Co., Ltd., Beijing, China). Cause no polymorphisms were detected between Zm00001d016831EHel and Zm00001d016831B73, qRT-PCR assays were only focused on Zm00001d016823 between tissues of EHel and B73 referred to Xiao et al. [31]. actin1 was used as the internal control, and the relative transcription levels were calculated through the method of 2-∆∆CT [32].

3. Results

3.1. Phenotypic Features of Parental Lines and Segregating Populations

The phenotyping results showed that the plant height (PH), ear height (EH), and
ratio of EH/PH of EHel were extremely lower than those of B73 (Figure 1(A)-(E)). The phenotypic performances of ILaa, INb, and ILab of EHel were also extremely lower than those of B73 (Figure 1(F); Figure 1(G) & Figure 1(I)), while the INa exhibited contrary trends in both 2018 and 2019 (Figure 1(H)). In addition, except IN5 in 2018, the lengths of all internodes of EHel were statistically lower than those of B73 (Figure 1(J) & Figure 1(K)).
Correlation analysis among F2:3 family lines showed that INa presented negative correlations to the other six traits, especially to EH, EH/PH, and ILaa (P < 0.01, Table 1). Positive correlations were observed among the trait pairs of PH, EH, EH/PH, INb, ILaa, and ILab, and the P values of all correlation coefficients were less than 0.01 except those of ILab vs EH/PH and ILaa vs INb (Table 1). Specially, we constructed the correlation network among four internode related traits of INa, INb, ILaa, and ILab (Figure 2). Significant correlations were observed among three trait-pairs of INa vs ILaa, ILaa vs ILab, and ILab vs INb, among which INa vs ILaa exhibited significant negative correlation, while positive correlations were detected for the other two pairs (Figure 2).

![Correlation network among four internode related traits](image)

Note: Blue (negative) and red (positive) solid lines refer to significant correlations between the connected traits, while those of blue (negative) and red (positive) dashed lines for non-significant correlations between the connected traits.

**Figure 2.** Correlation network among four internode related traits.
Table 1. Correlation of seven target traits among F2:3 family lines.

<table>
<thead>
<tr>
<th>Trait</th>
<th>EH</th>
<th>EH/PH</th>
<th>INa</th>
<th>INb</th>
<th>ILaa</th>
<th>ILab</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>0.79**</td>
<td>0.58**</td>
<td>−0.05</td>
<td>0.59**</td>
<td>0.57**</td>
<td>0.80**</td>
</tr>
<tr>
<td>EH</td>
<td>0.95**</td>
<td>−0.23**</td>
<td>0.82**</td>
<td>0.25**</td>
<td>0.78**</td>
<td></td>
</tr>
<tr>
<td>EH/PH</td>
<td>−0.27**</td>
<td>0.81**</td>
<td>0.08</td>
<td>0.67**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INa</td>
<td>−0.16</td>
<td>−0.36**</td>
<td>−0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INb</td>
<td>0.08</td>
<td>0.49**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILaa</td>
<td>0.51**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: ** refers to the corresponding significance level of $P < 0.01$.

3.2 Construction of Linkage Map and QTL Mapping

A total of 2108 polymorphic SNPs were screened out between EHeI and B73, and the linkage map was constructed through the genotyping of these 2108 SNPs, with a total length of 3299.18 cM and average SNP interval of 1.57 cM (Figure 3). Integrated the constructed linkage map and phenotypic performances of INa, INb, ILaa, and ILab of F2:3, 13 QTL were detected for these four traits, including four (qINa2, qINa7-1, qINa7-2, and qINa8) for INa, three (qILaa1, qILaa2, and qILaa5) for ILaa, one (qINb8) for INb, and five (qILab2-1/2/3/4 and qILab6) for ILab (Table 2). Among these 13 QTL, qILaa5 presented the largest PVE (phenotypic variation explained) of 12.99%, followed by qINa7-1 (12.19%) and qINb8 (12.22%), while the rest 10 possessed PVE less than 10% (Table 2).

Table 2. QTL mapping results of four internode related traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>Position (cM)</th>
<th>LOD</th>
<th>PVE (%)</th>
<th>A</th>
<th>D</th>
<th>Gene action</th>
<th>Confidence interval (cM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INa</td>
<td>qINa2</td>
<td>297</td>
<td>2.51</td>
<td>9.89</td>
<td>−0.02</td>
<td>−0.30</td>
<td>OD</td>
<td>292.5 - 299.0</td>
</tr>
<tr>
<td></td>
<td>qINa7-1</td>
<td>5</td>
<td>3.06</td>
<td>12.19</td>
<td>−0.26</td>
<td>−0.02</td>
<td>A</td>
<td>3.5 - 7.5</td>
</tr>
<tr>
<td></td>
<td>qINa7-2</td>
<td>64</td>
<td>2.69</td>
<td>9.82</td>
<td>0.17</td>
<td>0.28</td>
<td>OD</td>
<td>55.5 - 65.5</td>
</tr>
<tr>
<td></td>
<td>qINa8</td>
<td>317</td>
<td>2.72</td>
<td>9.52</td>
<td>−0.10</td>
<td>−0.26</td>
<td>OD</td>
<td>312.5 - 320.5</td>
</tr>
<tr>
<td></td>
<td>qILaa1</td>
<td>300</td>
<td>2.99</td>
<td>8.71</td>
<td>0.61</td>
<td>−0.15</td>
<td>PD</td>
<td>299.5 - 300.5</td>
</tr>
<tr>
<td>ILaa</td>
<td>qILaa2</td>
<td>1</td>
<td>2.94</td>
<td>8.96</td>
<td>−0.45</td>
<td>−0.86</td>
<td>OD</td>
<td>0 - 7.5</td>
</tr>
<tr>
<td></td>
<td>qILaa5</td>
<td>303</td>
<td>4.50</td>
<td>12.99</td>
<td>−0.59</td>
<td>0.48</td>
<td>PD</td>
<td>302.5 - 304.5</td>
</tr>
<tr>
<td>INb</td>
<td>qINb8</td>
<td>338</td>
<td>3.20</td>
<td>12.22</td>
<td>−0.23</td>
<td>0.52</td>
<td>OD</td>
<td>335.5 - 343.5</td>
</tr>
<tr>
<td></td>
<td>qILab2-1</td>
<td>3</td>
<td>3.05</td>
<td>7.32</td>
<td>−0.98</td>
<td>0.24</td>
<td>PD</td>
<td>0 - 7.5</td>
</tr>
<tr>
<td></td>
<td>qILab2-2</td>
<td>176</td>
<td>4.02</td>
<td>6.82</td>
<td>−0.49</td>
<td>0.85</td>
<td>OD</td>
<td>172.5 - 177.5</td>
</tr>
<tr>
<td>ILab</td>
<td>qILab2-3</td>
<td>200</td>
<td>4.23</td>
<td>6.98</td>
<td>−0.67</td>
<td>0.73</td>
<td>D</td>
<td>197.5 - 203.5</td>
</tr>
<tr>
<td></td>
<td>qILab2-4</td>
<td>214</td>
<td>4.16</td>
<td>6.85</td>
<td>−0.72</td>
<td>0.72</td>
<td>D</td>
<td>211.5 - 214.5</td>
</tr>
<tr>
<td></td>
<td>qILab6</td>
<td>45</td>
<td>3.77</td>
<td>6.87</td>
<td>−0.61</td>
<td>0.42</td>
<td>PD</td>
<td>44.5 - 47.5</td>
</tr>
</tbody>
</table>

Note: a refers to the phenotypic variation explained, b to additive effect, and c to dominant effect. A, D, PD, and OD of Gene action refer to additive effect, dominant effect, partial dominant effect, and over dominant effect, respectively.
3.3. Candidate Gene Discovering for qILaa5

Among all identified QTL, qILaa5 presented as the major QTL with the largest PVE for average internode length above ear (ILaa), and candidate genes were discovered for this QTL. Within the physical interval of 1 Mb fixed by the flanking SNPs of qILaa5 (177.26 Mb - 178.26 Mb), a total of 28 genes were annotated according to the reference genome of B73 (Table 3). Among the annotated genes, 17 genes exhibited expression signals in the tissues of internodes based on the RNA-seq datasets, while only Zm00001d016823 exhibited the highest relative FPKMs in the tissues of 6 - 7 Internode and 7 - 8 Internode (Figure 4). Considering the significant tissue-specific expression patterns, Zm00001d016823 was screened out as the candidate of qILaa5 for further validation.
### Table 3. Annotated genes within the physical interval of qILLa5.

<table>
<thead>
<tr>
<th>ID</th>
<th>Gene</th>
<th>Beginning site (bp)</th>
<th>Ending site (bp)</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Zm00001d016821</td>
<td>177246416</td>
<td>177261273</td>
<td>Function unknown</td>
</tr>
<tr>
<td></td>
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<td>177260095</td>
<td>177285934</td>
<td>Signal transduction</td>
</tr>
<tr>
<td></td>
<td>Zm00001d016823</td>
<td>177286413</td>
<td>177287057</td>
<td>Function unknown</td>
</tr>
<tr>
<td>2</td>
<td>Zm00001d016824</td>
<td>177354271</td>
<td>177357801</td>
<td>Recombination and repair</td>
</tr>
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<td></td>
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<td>177530082</td>
<td>177539300</td>
<td>Function unknown</td>
</tr>
<tr>
<td></td>
<td>Zm00001d016826</td>
<td>177539021</td>
<td>177540697</td>
<td>Function unknown</td>
</tr>
<tr>
<td></td>
<td>Zm00001d016827</td>
<td>177540939</td>
<td>177547554</td>
<td>Chromatin structure and dynamics, Transcription DDT domain-containing protein [Zea mays]</td>
</tr>
<tr>
<td>3</td>
<td>Zm00001d016828</td>
<td>177557935</td>
<td>177560415</td>
<td>Pentatricopeptide repeat-containing protein At2g27610 [Zea mays]</td>
</tr>
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<td></td>
<td>Zm00001d016831</td>
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<td>177774278</td>
<td>Posttranslational modification, protein turnover, chaperones Tubulin-folding cofactor C [Zea mays]</td>
</tr>
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<td>4</td>
<td>Zm00001d016832</td>
<td>177779774</td>
<td>177784519</td>
<td>Function unknown</td>
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<tr>
<td></td>
<td>Zm00001d016833</td>
<td>177789995</td>
<td>177803672</td>
<td>Protein AIG1 [Zea mays]</td>
</tr>
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<td></td>
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<td>177815819</td>
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<td></td>
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<td>177822681</td>
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<td>Zm00001d016840</td>
<td>178096482</td>
<td>178097375</td>
<td>Replication, recombination and repair DNA repair protein XRCC3 homolog [Zea mays]</td>
</tr>
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<td></td>
<td>Zm00001d016841</td>
<td>178195938</td>
<td>178198322</td>
<td>E3 ubiquitin-protein ligase EL5 [Zea mays]</td>
</tr>
<tr>
<td>6</td>
<td>Zm00001d016842</td>
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![Graphs](image-url)
Note: Vertical axis of each section refers to the relative FPKM value of each gene. FPKM values of all genes were referred to Walley et al. (2016), and downloaded from MaizeDGB (http://www.maizegdb.org). For each annotated gene, the relative FPKM in the tissue of 7-8 Internode (with tissue ID of 2) was standardized to 1, and then the relative expression values of the rest 22 tissues were calculated according to the downloaded FPKMs of the corresponding tissue divided by that of 7-8 Internode.

Figure 4. Relative expression patterns of 17 annotated genes among different tissues via RNA-seq.
3.4. Sequence Comparison and qRT-PCR Based Validation

The results of sequence analysis showed that Zm00001d016823 has only one exon with the coding sequence of 645 bp for 214 amino acids (aa). The sequence of Zm00001d016823\(^{EHel}\) exhibited 11 polymorphisms to that of Zm00001d016823\(^{B73}\), including InDel I (deletion of 12 bp), InDel II (deletion of 6 bp), and 9 SNPs (Figure 5(A)). We also cloned another annotated gene of Zm00001d016831 that exhibited relatively higher expression levels within the tissues of internodes, while no polymorphisms were detected between the sequences of Zm00001d016831\(^{EHel}\) and Zm00001d016831\(^{B73}\) (Figure 5(B)). We compared the coding products by both Zm00001d016823\(^{EHel}\) and Zm00001d016823\(^{B73}\). The results showed that InDel I and II of Zm00001d016823\(^{EHel}\) led the missing of 6 aa, comparing to the coding product of Zm00001d016823\(^{B73}\) (Figure 5(C)). Additionally, only the 1\(^{st}\) (G in B73 while A in EHel) and 9\(^{th}\) (A in B73 while G in EHel) SNPs presented as synonymous mutations, the other 7 SNPs observed within the sequence of Zm00001d016823\(^{EHel}\) served as nonsynonymous mutations, and led to the changes of aa (Figure 5(C)).

**Figure 5.** Chromosomal distribution of detected QTL and candidate validation of qILaa5.

The resultsof qRT-PCR assay showed that Zm00001d016823 highly expressed in the tissues of internode and ear of EHel, while nearly no expression signals were observed in both tissues of root and leaf of EHel (Figure 5(D)). Considering the relative expression of Zm00001d016823 among different internodes from IN3 to IN7 between EHel and B73, statistical higher expression levels were observed among the IN4 to IN7 of EHel than those of B73 (Figure 5(E)). The rela-
tive expression levels of Zm00001d016823 among IN3 to IN7 exhibited negative correlation to the relative length of IN3 to IN7 $(R = -0.4165, P = 0.4854)$, suggesting that Zm00001d016823 might negatively regulate the internode length above the ear in maize.

4. Discussion

Moderate height is essential for the construction of idea plant type. In 1960s, numerous varieties with dwarf or semi-dwarf plant height, improved resistance, higher-yielding and better-quality performance were released in both rice and wheat, leading a revolution in the increasing of grains around the world, trigging a global Green Revolution [33]-[35]. Differed from both rice and wheat, maize possesses tall plant with higher PH, and corresponding higher EH. This special plant structure results in an inherent weakness of lodging, i.e., stalk lodging and root lodging, over strong winds [36]. It was documented that a record-breaking windstorm in 2020 swap across Iowa, destroyed about 16% maize production there, causing total losses of more than $10 billion [36]. Similar losses were also documented in other maize producing areas around the world [37]-[39]. Breeding novel maize varieties with shorter PH and EH might be the potential way out for lodging related resistances [40]. While few genes were discovered beyond phytohormone related pathways involving in regulating the development of internode no. and length, and then PH and EH, in maize.

In the present study, we identified 13 QTL for internode no. and length related traits in maize, including three major QTL for both internode no. and average internode length above ear (ILaa) (Table 2). Among all the annotated genes, Zm00001d016823 was determined as the candidate of qILaa5, the major QTL for average internode length above ear in maize (Table 2, Figure 5(A), Figure 5(D)-(E)). Zm00001d016823 is annotated to encode a WEB family protein with unknown function (Table 3). WEB (Weak Chloroplast Movement under Blue Light) is a plant-specific protein with coiled-coil domain, and is reported to respond the chloroplast photo-relocation under different light intensities [41] [42]. The chloroplasts in the corresponding mutants of web1 and web2/pmi2 (plastid movement impaired2) in Arabidopsis are defective in moving toward or away from weak or strong light, respectively [41] [43]. Meanwhile, WEB1 can physically interact with WEB2/PMI2, forming a WEB1-PMI2 complex to suppress JAC1, another key protein with J-domain that essential for chloroplast moving toward weak light intensity [41] [42]. Except Arabidopsis, there are few documents focused on WEB gene family in other plants, especially WEB family members involved in controlling the phenotypic performance of internode length in maize.

We compared the relative expressions ratio of this gene between EHel to B73 and the corresponding relative internode length among INa4 to INa7 between EHel to B73. The results indicated a negative trend between the relative expression levels and the relative internode length, suggesting a negative regulating of Zm00001d016823 to the average internode length above ears in maize. Considering
the annotated information, further functional dissection of Zm00001d016823 might discovery novel molecular mechanism beyond phytohormone of WEB family member in controlling internode length and PH in maize, and will shed new light into the construction of ideotype with short stature for maize varieties.

5. Conclusion

In the present study, 13 QTL controlling four internode no. and length related traits in maize were identified, distributing on six chromosomes of Chr1, Chr2, and Chr5-8. Genomic annotation of qILa5, the major QTL for average internode length above ear with the largest PVE suggests Zm00001d016823 as the candidate. Zm00001d016823 is annotated to encode the product that belongs to WEB protein family. Combined comparisons between the qRT-PCR assay and average internode lengths of IN3 to IN7 showed negative correlation between the gene expression levels and the lengths of internodes above ear, implying a negative regulating of Zm00001d016823 to the average internode length above ear in maize.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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