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Article

Loci underlying leaf agronomic traits identified by re-sequencing celery accessions based on an assembled genome

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SUMMARY

Celery is one of the most popular vegetables in the world. The main edible parts of celery are the leaf blade and petiole. The celery petiole is usually green, red, or white, with a hollow or solid pith. However, the loci/genes controlling these petiole-related traits have not been reported. In this study, we present a chromosome-level celery genome assembly with a total size of 3.339 Gb. Simultaneous bursts of long-terminal repeats (78.43%) contributed greatly to the large genome size. Re-sequencing and population structure analysis of 79 celery accessions revealed that they could be divided into Chinese celery and Western celery. By combining genome-wide association studies (GWAS) and mapping data, we located the hollow petiole (hp) loci in an 807.6-kb region on chromosome 11. This study provides valuable resources for genetic research on celery and is also helpful for the identification and cloning of genes controlling leaf agronomic traits in celery.

INTRODUCTION

Celery (Apium graveolens L., 2n = 2x = 22) is an annual or biennial herbage species that belongs to the Apiaceae family and originates from the Mediterranean and Middle East. Celery was initially domesticated as a medicinal plant, and later became a popular vegetable worldwide (Sturtevant, 1886; Li et al., 2018). It was introduced to China during the Han Dynasty (second century B.C.), and the current area under celery cultivation is ~650,000 hectares, which is almost 3% of the total Chinese vegetable planting area and produces approximately 20 million tons of celery each year. Celery is rich in nutrients, such as vitamins (especially vitamins K and C), apigenin, carotenoids, and cellulose (Fazal and Singla, 2012; Li et al., 2014; Dianat et al., 2015), and is also a good source of flavonoids, volatile oils, and antioxidants (Sowbhagya et al., 2010; Sowbhagya, 2014). In addition, celery can be utilized in the chemical and medical industries (Nagella et al., 2012; Kooti et al., 2014).

Based on their edible parts, celery can be classified as celeriac (Apium graveolens var. rapaceum) and leaf celery (Apium graveolens var. dulce). Celeriac is also called root celery or turnip-rooted celery, and its expanded spherical root is the major edible part. Leaf celery is often abbreviated as celery, of which the edible parts are the leaf blades and petioles. Celeriac is mainly grown in Europe and the USA but is barely cultivated in China. Leaf celery is grown in Western Europe, the USA, Japan, and China (Rozek, 2013; Salehi et al., 2019). In China, based on its origin and morphological features, leaf celery is generally classified as Chinese celery (also known as local celery) or Western celery. Chinese celery has been grown and selected in China for a long time and has the following features: a long, slender, and hollow petiole (hp) with a white or light green color, easy to cook, and strong fragrance. Western celery, introduced in China in modern times, is generally tall and dark green, difficult to bolt, develops short, thick, solid petioles, and emits light flavors.

In most plants, the petiole connects the leaf and the stem. In some leafy vegetables, the petiole is not only the major edible part but also plays a stem-like function. In addition, the petiole is also an important channel for transporting water, nutrients, and photosynthetic products and plays an important role in maintaining leaf angle and plant architecture (Tsukaya et al., 2002; Kozuka et al., 2005, 2010). In celery, petioles can
have a hollow or solid pith. Petiole structure is an important horticultural trait that influences customer decisions. However, the loci/genes that control this trait remain unclear.

Among the Apiaceae species, the carrot and coriander genome has been reported with N50 scaffold lengths of 12.7 Mb and 2.15 Mb, respectively (Iorizzo et al., 2016; Song et al., 2020). Two versions of the celery genome have been reported in recent years, and the sequencing variety was Q2-JN11 and Ventura, respectively (Li et al., 2020; Song et al., 2021). Q2-JN11 belongs under Chinese celery derived from “Jinnan Shiqin,” while the Ventura belongs under Western celery. With improvements in sequencing and assembly technology, the genome size and N50 scaffold length of Ventura were found to be much higher than those of Q2-JN11. All previously published genomes of the Apiaceae species provide valuable information for researchers.

In this study, we generated a high-quality chromosome-scale genome assembly of the celery inbred line, Baili. Based on this genome, re-sequencing of 79 celery accessions, including Chinese and Western celeries, was performed; and later genome-wide association studies (GWAS) for several leaf agronomic traits were conducted. The data obtained in this study not only provide a better understanding of the genetic evolution and divergence of the Chinese and Western celeries but also clarify the loci/genes underlying the hp. The celery genome assembly in this study will provide valuable resources for facilitating celery genetic research and improvement as well as for studying the evolution and speciation of Apiaceae species. Moreover, the identification of hp loci is not only useful for celery genetic improvement but also deepens our understanding of the molecular mechanisms underlying the hp.

RESULT
Genome sequencing, assembly, and annotation

Baili (Apium graveolens L.), a highly inbred celery line that belongs to the group of Western celery and derived from California celery via single plant selection, was used for genome sequencing. The characteristics of Baili were similar to those of most Western celery: tall and light green, with developed thick and solid petioles (Figure 1A). The genome was sequenced using Illumina HiSeq, PacBio Sequel, and chromosome conformation capture interaction mapping (Hi-C) platforms. A total of 173.81 Gb (53.24 \( \times \)) Illumina paired-end short reads (270 bp) were obtained and used to estimate the heterozygosity ratio of the sampled individuals (0.02%). Based on the 21-mer depth distribution of the Illumina reads (Table S1; Figure S1), the celery genome size was estimated at 3.26 Gb, which was close to the estimates by flow cytometry (3.10 Gb) (Figure S2). A total of 181.61 Gb (54.40 \( \times \)) of clean subreads with a mean read length of 14.34 kb were obtained using the PacBio Sequel system and then used to assemble a 3.338 Gb genome with a contig N50 of approximately 1.03 Mb (Tables S2 and S3). With the aid of Hi-C interaction data, the genome was assembled to 3.339 Gb; 96.59% (3.22 Gb) of the assembled genome was anchored onto 11 pseudo-chromosomes, and the scaffold N50 was approximately 258.97 Mb (Tables 1 and S4). The genome size, contig, and scaffold N50 values of the Baili assembly genome and Ventura assembly genome were much higher than the Q2-JN11 assembly genome (Li et al., 2020; Song et al., 2021, Table S5); and the genome size, contig, and scaffolds N50 values, as well as the comparison of genomic structure, showed that our assembly genome had a similar quality to the Ventura assembly genome (Table S5), indicating that both the Baili and Ventura assembly genomes were of high quality.

To evaluate the quality of the celery genome assembly, Illumina and RNA-seq reads were first aligned against the genome, and the proper mapping rates were 95.36% and 78.49%, respectively (Tables S6 and S7). Next, the core eukaryotic genes (CEGs) and BUSCO database were searched, and the majority of the CEGs (95.2%) and genes in the BUSCO dataset (97.03%) were identified (Table S8). Furthermore, based on the long high terminal repeat (LTR) assembly index (LAI), our celery genome LAI reached 11.31, indicating high quality of our assembly. Finally, a heatmap was drawn with the Hi-C data, and all bins could be clearly classified into 11 pseudochromosomes (Figure 1B). Taken together, these evaluations...
indicate that our genome assembly was of high quality, and the overall completeness was acceptable at the chromosome scale.

A total of 32,599 genes were predicted in our assembly genome, which is similar to the two previous celery assemblies (Figure 1C; Table S5). Among these predicted genes, 32,156 (98.64%) shared homology with the annotated genes (Table S9) and 24,082 (73.87%) could be supported by RNA-seq data (Figure S3; Table S10). The predicted genes showed average exons and average CDS lengths similar to the Ventura and coriander genomes (Song et al., 2020, 2021) (Table S11; Figure S4). Among the noncoding RNAs, 70 miRNAs, 780 rRNA, and 647 tRNA were predicted (Table S12). The predicted motifs and pseudogenes are listed in Table S12.

Genomic variation between the Baili and Ventura genomes

We conducted genomic collinearity analysis between the Baili (this study) and Ventura genomes (Song et al., 2021) (Figure 2A). Large structural variations (SVs) between the Baili and Ventura genomes were found on Chr03, Chr06, and Chr11 (Figure 2C; Table S13). A total of 952 inversions, 2,789 translocations, and 18,069 duplications were identified in the syntenic blocks between the two genomes (Table S13). The distribution lengths of these SVs are shown in Figure 2B.

Repetitive sequence contributed to the large genome size of celery

Repetitive sequences accounted for the majority (2.90 Gb, 81.49%) of the celery genome and were 1.2 times that of coriander (Coriandrum sativum) (70.59%) and 1.8 times that of carrot (Daucus carota) (46%) (Figure 1C; Table S14). Most transposable elements (TEs) belong to the long terminal repeat (LTR) category, with a total length of over 2.79 Gb, accounting for 78.43% of the whole-genome size. Most of the LTRs were Copia and Gypsy elements, accounting for 36.94% and 23.16% of the whole genome, respectively (Table S14). Therefore, the substantial accumulation of TEs, especially LTRs, contributed greatly to the large genome size of celery.

To trace the history of the greatly expanded repetitive sequences in celery, the insertion times of all LTRs were estimated. A peak of increased insertion activity was found at ~0.35 Ma (Figure 3A), suggesting that the expansion of the celery genome occurred quite recently. In addition, compared to the other two Apiaceae species, the accumulation of LTRs was much higher and faster in celery and coriander (Figure 3A).

The insertion time analyses of Copia and Gypsy showed that both LTR retrotransposons had the highest insertion activity after Apiaceae species diverged, which made them the most abundant retrotransposons in the celery genome (Figures 3B and 3C). Analysis of the phylogenetic topology of Copia and Gypsy clades showed that there were many species-specific LTRs, especially in the celery and coriander genomes (Figures S5 and S6).

### Table 1. Statistics for the celery genome

| Assembly Feature                      | Number | Length (bp) | Percentage (%) |
|---------------------------------------|--------|-------------|----------------|
| Assembled scaffold sequences (>1 kb) | 5,358  | 3,339,076,396| –              |
| N50 scaffold                          | –      | 258,965,703 | –              |
| N90 scaffold                          | –      | 412,247     | –              |
| Max scaffold                          | –      | 315,280,121 | –              |
| Assembled contig sequences (>1 kb)   | 11,246 | 3,338,485,196| 99.98         |
| N50 contig                            | –      | 600,000     | –              |
| N90 contig                            | –      | 179,658     | –              |
| Max contig                            | –      | 3,697,775   | –              |
| GC content                            | –      | –           | 35.86          |
| Chromosome                            | 11     | 3,224,725,386| 96.59         |
| Anchored and oriented scaffolds       | 5,923  | 2,948,500,488| 91.43         |
Figure 2. Genomic variation between the Baili and Ventura genomes
(A) Colinearity between the Baili and Ventura genomes. Each dot indicates an aligned region with a length of at least 20 kb.
(B) The distribution length of SVs.
(C) Whole-genome alignment between the Baili and Ventura genomes. See also Tables S5 and S13.
Gene family analysis of celery

To identify the unique and common gene families in celery, celery genes were clustered with the genes of coriander, carrot, sunflower (Helianthus annuus), and grape (Vitis vinifera). The result showed that there were 14,829 genes in celery that had more than one orthologous gene, and 6,795 gene families were shared between the five species (Figure 4A). Additionally, 1,216 gene families (2,882 genes) were unique to celery. Gene Ontology (GO) analysis showed that 757; 1,418; and 984 of these unique genes were enriched in the cellular component, molecular function, and biological process, respectively; and they were mainly enriched in cell (GO:0044464), catalytic activity (GO:0003824), and metabolic process (GO:0008152) (Figure S7; Table S15). KEGG analysis showed that these unique genes were mainly involved in RNA polymerase, glycosaminoglycan degradation, sphingolipid metabolism, and diterpenoid biosynthesis (Figure S8; Table S16).

Figure 3. Comparison of transposable elements

(A) distribution of insertion times for LTR retrotransposons in coriander (C. sativum), carrot (D. carota), fennel (F. vulgare), Arabidopsis (A. thaliana), rice (O. sativa), and celery (A. graveolens).
(B) Distribution of insertion times for Gypsy in coriander (C. sativum), carrot (D. carota), fennel (F. vulgare) and celery (A. graveolens).
(C) Distribution of insertion times for Copia in coriander (C. sativum), carrot (D. carota), fennel (F. vulgare), and celery (A. graveolens). Mya: million years ago.

See also Table S14.
The annotated genes were clustered with those of carrot, coriander, sunflower, pepper (Capsicum annum), tomato (Solanum lycopersicum), soybean (Glycine max), cucumber (Cucumis sativus), grape, Medicago truncatula, poplar (Populus trichocarpa), rice (Oryza sativa), and Arabidopsis thaliana using OrthoMCL. A total of 16,045 gene families were identified in the celery genome, of which 419 gene families appeared nearly 91.2 Mya. Furthermore, there were 3,238 and 6,968 gene families that expanded and contracted in our celery genome, respectively (Figure 4B). Among these expanded and contracted gene families, there were 31 and 47 gene families that expanded and contracted statistically significantly (p < 0.05) in the celery genome (Figure 4B).

Whole-genome duplication in celery
In evolutionary history, most plants have undergone whole-genome duplication (WGD) or polyploidization. Similar to carrot and coriander, there were two peaks in the 4DTv value in the celery genome, indicating that two WGD events occurred in the celery genome lineage (Figure 4C). This result was further confirmed by Ks analysis, in which the Ks values between celery paralogous gene pairs displayed two peaks at 0.618 and 1.1 (Figure 4D).

The Ks of the orthologous genes in celery and coriander peaked at 0.2 and the diverged time between celery and coriander was 25.08 Mya (Figure 4B). We used the substitution rate that was obtained from Song et al. (2021) and calculated that the two WGD events in celery might have occurred at 58-67 and 103-119 Mya, respectively. These results suggest that celery shares WGD with Apiaceae.

Genomic variations and population structure of celery
To explore genetic variations in the celery germplasm, we re-sequenced 79 celery accessions, including 34 Chinese celery accessions, 34 Western celery accessions, 10 hybrid selections from Chinese celery cross Western celery, and 1 wild type (Table S19). A total of 1,376.51 Gb of clean data with an average of ~4× and 97.36% coverage rate of the celery genome was generated with a Q30 of 93.51% (Table S20). After alignment against the celery genome, we identified a total of 17,157,833 high-quality SNPs and 10,662,508 InDels. Among these SNPs, there were 1,660,755 missense variant SNPs and 90,167 stop-gained SNPs (variants causing a STOP codon). In addition, 16,117 and 13,339 SNPs were located at the splice site acceptors and donors, respectively (Table S22). We also detected SVs among the above 79 celery accessions, and a final set of 496,924 SVs, ranging from 30 bp to 5 Mb, were identified, which included 15,496 insertions, 182,127 deletions, 12,337 inversions, 36,128 duplications, and 250,836 translocations (Tables S23 and S24). These data provide valuable resources for celery biology and genetic breeding.

To further infer the population structure of celery, we performed phylogenetic, model-based ADMIXTURE, and principal component analyses (PCA) for the 79 celery accessions. The results showed that the celery accessions could be classified into two groups: the Chinese celery (CC) group and the Western celery
Forty-one celery accessions were classified into the WC group and thirty-seven celery accessions were classified into the CC group. Wild-type celery formed an outgroup (Figures 5A–5C). Most of the American (13/14) varieties, except for 18Q210; most of the Holland (8/10) varieties, except for 18Q257 and 18Q265; and all of the French varieties (8/8) were classified into the WC group. Structural analysis showed that 18Q210 carried a genetic background closer to Chinese celery than Western celery. The two Holland celery varieties 18Q257 and 18Q265, which were the only two red petiole accessions, were classified into the CC group. All Thailand (2/2) and most of the Chinese (26/33) varieties were classified into the CC group and the 7 remaining Chinese varieties were assigned to the WC group. Among the seven Chinese varieties, 18Q146 was Chinese celery with the unclear origin, and the other six Chinese varieties (18Q148, 18Q214, 18Q224, 18Q232, 18Q246, and 18Q253) were hybrids that came from crosses between the Chinese celery and Western celery (Figures 5A–5C; Table S19). These results indicated that these varieties carried more Western celery genetic backgrounds than Chinese celery. In addition, most Chinese varieties (21/23) carried a Western celery genetic background to a certain degree (Figure 5B).

Figure 5. Population structure of the 79 celery accessions
(A) Phylogenetic tree generated from whole-genome SNPs. The 79 accessions could be assigned to the Chinese celery group (CC), Western celery group (WC), and wild type (WT).
(B) Model-based clustering analysis with the cluster numbers was 2. The y-axis quantifies cluster membership, and the x-axis lists the different accessions. The orders of the 79 accessions on the x axis are consistent with those in the phylogenetic tree.
(C) Principal component analysis of the 79 celery accessions.
(D) Genome-wide average LD decay estimated from the Chinese celery group (CC) and Western celery group (WC). See also Tables S19, S20, S21, S22, S23, S24, and S25.
Divergence between the Chinese celery and Western celery

To obtain genetic insights into Chinese celery and Western celery, we analyzed nucleotide diversity (π). The π values of Chinese celery and Western celery were 6.96 \times 10^{-4} and 5.61 \times 10^{-4}, respectively (Table S25), which is consistent with the result of Watterson estimator analysis (θW (Chinese celery) = 4.79 \times 10^{-6}, θW (Western celery) = 4.55 \times 10^{-6}). Notably, the π values of Western celery were lower than those of Chinese celery, and the LD decay of Western celery was higher than that of Chinese celery, suggesting that Western celery may have experienced a more severe bottleneck during domestication. In addition, both Chinese and Western celery had quite low π values, suggesting that celery had low genetic diversity and a narrow genetic background, and the genetic relationship between the Chinese and Western celery was close.

It is well known that both Chinese and Western celery are cultivated species. Geographically, Western celery is cultivated worldwide, whereas Chinese celery is concentrated in China. The major morphological difference between Chinese and Western celery is the plant architecture. Generally, Chinese celery plants are small and light (0.25-0.5 kg each), and their petioles are slender, leading to loose plant architecture. In contrast, plants of Western celery are large and heavy (1.5-2.5 kg each), and their petioles are thick, leading to compact plant architecture (Figures S11A and S11B). To identify the loci underlying these plant architecture-related traits, we measured the population fixation statistics (FST) of SNPs in Chinese and Western celery. The average FST value between Chinese and Western celery was estimated to be 0.122, and the top 5% had FST ≥ 0.315 (Table S26). These results indicate moderate population differentiation between the two subspecies (Wright, 1978). Based on FST, 366 divergent genomic regions were identified, which included 1,770 predicted genes and covered 5.03% (168.1 Mb) of the genome (Figure S11C; Table S27).

To better understand the function of the genes in divergent genomic regions, we performed GO analysis for these genes. Interestingly, several GO terms related to cell development and cell wall construction-related GO terms were enriched. For example, in the "Biological Process" term, cell growth (GO:0016049), developmental cell growth (GO:0048588), plant-type secondary cell wall biogenesis (GO:0009834), cell differentiation (GO:0030154), and plant-type cell wall organization (GO:0009664) were enriched; in the "Cellular Component" term, plant-type cell wall (GO:0009505), cell wall (GO:0005618) and plant-type vacuole membrane (GO:009705) were enriched; and in the "Molecular Function" term, cellulose synthase (UDP-forming) activity (GO:0016760) and pectate lyase activity (GO:0030570) were enriched (Table S27).

Identification of genes or loci underlying the hollow petiole locus in celery

Petiole structure is an important quality trait for both Chinese and Western celery, which affects yield and mouth feel. Celery petioles are either hollow or solid (Figure 6A). In this study, experiments were conducted to identify genes or loci underlying the hp locus. From the GWAS results obtained from the 79 celery accessions (Table S19), strong association signals were identified on chromosome 11 (Figure 6A; Table S28).

To further validate the above signal, an F2 segregating population was generated from a cross between line 308 and solid petiole line 314. A 3:1 segregation ratio for the hollow and solid petioles was discovered in the population (Table S29), indicating that the hp is controlled by a single gene and the hp is dominant over the solid petiole. Subsequently, SSR markers were developed and screened with a 5 Mb interval. Using these markers, hp was narrowed to an 807.6-kb region on chromosome 11 (Figures 6B and 6C). Twenty-five genes were identified in this region (Table S30), and we conducted the expression analysis for these genes, the result showed that Ag11G009560, Ag11G009610, Ag11G009700, Ag11G009780, and Ag11G009790 were highly expressed in the solid petiole line 314, while only Ag11G009710 was highly expressed in the hp line 308. The other genes were barely detected in the petiole 308 and 314 (Figure 6D). Gene annotation showed that Ag11G009780 encodes a homolog of Arabidopsis FUCOSYLTRANSFERASE 1 (FUT1)
(Table S30). FUT1 catalyzes the transfer of fucose from GDP fucose to terminal galactose residues on the side chain of xylan (Rocha et al., 2016). Mutations in FUT1, also called the mur2 mutant, showed a 98% reduction in L-fucose levels (Vanzin et al., 2002), and may have a load-bearing effect on the xyloglucan cellulose network (Ryden et al., 2003). Ag11G009710 encodes a homolog of Arabidopsis MALATE DEHYDROGENASE (MDH, AT3G47520) (Table S30), and mutations in PLASTIDAL NAD-DEPENDENT MALATE DEHYDROGENASE can rescue ROS accumulation and PCD phenotypes in mod1 (Zhao et al., 2018). As we know, the hp line 308 undergoes a process from solid to hollow during development. At development, the degradation of cytoplasm was observed, and then the intercellular space between pre-cavity cells appeared; the parenchyma cells that formed the pith collapsed and broke down, resulting in the petiole becoming hollow (Figure S12). It has been reported that the formation of cavities in many plant stems or leaves is caused by PCD, such as the cavities in the stems of sorghum and wheat (Fujimoto et al., 2018; Nilsen et al., 2020), the pith cavity of bamboo (Guo et al., 2019), and the fistular leaves of Allium fistulosum (Ni et al., 2015). Therefore, combined with the annotation and expression analysis of these candidate genes, we speculated that Ag11G009780 and Ag11G009710 may be strong candidate genes for celery hp.

In addition, signals for petiole color (green, white, and red) and leaflet margin (mucronate or obtuse) were also identified to be located on chromosome 4 and chromosome 7 in the GWAS analysis (Figures S13, and S14, S31, and S32).

**DISCUSSION**

In this study, a high-quality celery genome assembly was obtained, of which the genome size was larger than that of carrot (~6.8 times) and coriander (~1.5 times) (Iorizzo et al., 2016; Song et al., 2020), and was close to that of the newly reported celery Ventura genome (Song et al., 2021).

In our celery genome, repetitive sequences, most of which were LTRs, accounted for the vast majority of the genome (2.90 Gb, ~81.49%) (Table S14), which is similar to the coriander genome (1.50 Gb, 70.59%; Song et al., 2020). In addition, we found that the celery and coriander genomes shared an expansion of the LTR, whereas the carrot and fennel genomes did not (Figure 3), which may be the reason why the genomes of celery and coriander were much larger than those of carrot and fennel.

In addition to LTR expansion, WGD and polyploidization also significantly affect the genome size of angiosperms (Piegou et al., 2006; El Baidouri and Panaud, 2013). In this study, two WGD events were identified in our celery genome (Figures 4C and 4D), which were also observed in coriander and carrot (Iorizzo et al., 2016; Song et al., 2020), and the WGD events may have occurred 58-67 and 103-119 Mya, respectively. However, this differs from the Ventura genome, where WGD events occurred 34-38 and 66-77 Mya, respectively (Song et al., 2021). The earliest WGD (103-119 Mya) was confirmed to be shared by all eudicots and is similar to the γ event (115-130 Mya) in the Ventura genome. The latter WGD (58-67 Mya) is similar to the α event (66-77 Mya) in the Ventura genome (Song et al., 2021); however, the α event in the Ventura genome was not found in our celery genome, this maybe owing to the expansion of repetitive sequences were occurred at recently (the peak of LTR increased insertion was 0.35 Mya) in Baili celery genome, so the latest WGD (α event) has not yet occurred.

In this study, based on phylogenetic analysis, the 79 investigated celery accessions could be classified into three groups, which was in good agreement with the plant taxonomy. However, two Chinese celery lines, 18Q146 and 18Q148, were assigned to the Western celery group, while three Western celery lines, 18Q210, 18Q257, and 18Q265, were assigned to the Chinese celery group (Figures 5A–5C). As Chinese celery was domesticated from Western celery, it is not surprising that some of the Chinese celery varieties harbored a certain degree of genetic background originating from Western celery. Based on our records, the three Western celery varieties that were assigned to the Chinese celery group originated in the USA and Holland. However, it was unclear whether they were crossed with Chinese celery before or after they were introduced into China. A similar phenomenon was observed by Wang et al. (2011) and Fu et al. (2013, 2014). Traditionally, celery is classified into China and Western celery based on its origin and morphological features. However, based on our results, morphological data and original records were not fully consistent with the genomic analysis, indicating that the classical division method based only on the origin and morphological characteristics is not accurate. However, by analyzing the nucleotide diversity (π) of the 79 celery accessions, we found that the genetic diversity of celery was low, indicating that the genetic background of celery was narrow.
Most researchers accept that Chinese celery is highly likely to be selected and domesticated from Western celery. In this study, phylogenetic analysis supported the above hypothesis. Considering the differences between Chinese and Western celery at the morphological, structural, and physiological levels, it can be presumed that after celery was introduced into China, based on their own preferences, Chinese breeders selected and bred a special type of celery, which has a long, slender, and hp with white or light green color, strong fragrance, and is easy to cook. Meanwhile, the long, slender, and hp of Chinese celery also affected the plant architecture, which made the petiole of the Chinese celery less erect and stronger than that of the Western celery. In this study, the $F_{ST}$ of SNPs between Chinese and Western celery was calculated using the re-sequenced data of 79 accessions, and a number of divergent genomic regions that may determine the plant architecture were identified. Genes within these regions were enriched in cell development and cell wall construction in GO analysis, suggesting that these genes may be selected during Chinese celery domestication. GWAS was also conducted to map the hp trait. An 807.6-kb region on chromosome 11 was identified to harbor hp(Figure 6). Twenty-five genes were identified in the hp region, among which Ag11G009780 and Ag11G009710 may be responsible for the hp in celery.

In summary, in this study, we assembled a high-quality celery genome and provided a genomic variation map of celery, which not only deepens our understanding of the genome evolution of Apiaceae species but also provides a genomic framework for germplasm research and celery quality improvement in the future. Moreover, our data are also valuable for the fine-mapping and cloning of genes controlling leaf agronomic traits in celery.

Limitations of the study

We reported a high-quality assembly of celery (Baili) genome, performed GWAS on several important leaf agronomic traits, and identified several genes that are potentially related to hp. However, further experiments are needed to determine the final candidate gene that controls the hp. Furthermore, the samples used for population structure analysis were inadequate.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Plant materials
- METHOD DETAILS
  - Genome size estimation
  - De novo genome assembly using PacBio reads
  - Chromosome assembly using Hi-C
  - Transcriptome sequencing
  - Gene annotation
  - Comparative genomic analysis
  - Analysis of full-length LTR retrotransposons
  - Sequence alignment and variation calling
  - Phylogenetic and population analysis
  - Selective sweep regions for celery
  - Identification candidate regions related to leaf agronomic traits in celery
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104565.

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AUTHOR CONTRIBUTIONS

HS and QC conceived and designed the research. HS and QC conducted sample preparation and sequencing. QC, LS, and ZL performed the assembly and annotation. HQ and ZL worked on genome comparative and population genomic analyses. ML, XC, and WL performed fine mapping. SL and HW prepared material for re-sequencing and phenotype investigation. QC and SL wrote the article. WY and HS revised the article. All authors have read and approved the final article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Deposited data      |        |            |
| Re-sequencing data  | This study | Genome Sequence Archive : CRA003635 |
| RNA sequence data   | This study | Genome Sequence Archive : CRA003635 |
| Genome assembly data| This study | Genome Sequence Archive : GWHBGBL00000000 |
| Software and algorithms |        |            |
| BUSCO (v2.0)        | Simão et al. (2015) | http://busco.ezlab.org |
| Canu (v1.5)         | Koren et al. (2017) | https://github.com/marbl/canu |
| WTDDBG v1.2.8       | NA | https://github.com/ruanjue/wtdbg |
| FaCon (v0.732)      | Chakraborty et al. (2016) | |
| Pilon (v1.22)       | Walker et al. (2014) | http://broadinstitute.org/software/pilon/ |
| CEGMA (v2.5)        | Parra et al. (2007) | http://korflab.ucdavis.edu/Datasets |
| LAI method          | Ou et al. (2018) | https://github.com/oushujun/LTR_retriever |
| LACHESIS software   | Burton et al. (2013) | https://github.com/shendurelab/LACHESIS |
| LTR_FINDER (v1.05)  | Xu and Wang (2007) | https://github.com/xzhub/LTR_Finder |
| RepeatScout (v1.0.5)| Price et al. (2005) | https://github.com/mmcco/RepeatScout |
| PASTECclassifier    | Hoede et al. (2014) | https://urgi.versailles.inrae.fr/download/repet/PASTEC_linux-x64-2.0.tar.gz |
| RepeatMasker v4.0.6 | Tarailo-Graovac and Chen (2009) | http://www.repeatmasker.org/RMDownload.html |
| Augustus (v2.4)     | Stanke and Waack (2003) | http://bionfi.uni-greifswald.de/augustus/ |
| SNAP (v2006-07-28)  | Korf (2004) | http://korflab.ucdavis.edu/Software |
| GeMoMa (v1.3.1)     | Keilwagen et al. (2016), 2018 | http://www.jstacs.de/download.php?which=GeMoMa |
| HISAT2              | Kim et al. (2015) | http://daehwankimlab.github.io/hisat2/download/index |
| Stringtie (v1.2.3)  | Pertea et al. (2015) | https://ccb.jhu.edu/software/stringtie/index.shtml |
| GeneMarkS-T (v5.1)  | Tang et al. (2015) | http://exon.gatech.edu/license_download.cgi |
| Trinity (v2.1.1)    | Grabherr et al. (2011) | https://codeload.github.com/trinitymaseq/trinitymaseq/zip/master |
| PASA (v2.0.2)       | Campbell et al. (2006) | https://github.com/PASAppipeline/PASAppipeline/wiki |
| EVM (v1.1.1)        | Haas et al. (2008) | https://github.com/EVidenceModeler/EVidenceModeler/archive/v1.1.1.tar.gz |
| tRNAscan-SE (v 1.3.1)| Lowe and Eddy (1997) | https://github.com/UCSC-LoweLab/tRNAscanSE |
| GenBlastA (v1.0.4)  | She et al. (2009) | http://genome.sfu.ca/genblast/download.html |
| Gene-Wise (v2.4.1)  | Birney et al. (2004) | https://www.ebi.ac.uk/~birney/wise2/ |
| OrthoMCL package (v 2.0.9) | Li et al. (2003) | http://orthomcl.org/orthomcl |
| CAFÉ software (v 4.2) | Han et al. (2013) | http://sourceforge.net/projects/cafehahlab/ |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests should be directed to the lead contact, Huolin Shen (shl1606@cau.edu.cn).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The raw sequence data reported in this study have been deposited in the Genome Sequence Archive in National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession number CRA003635 that are publicly accessible at https://bigd.big.ac.cn/gsa. The whole genome sequence data reported in this paper have been deposited in the Genome Warehouse in National Genomics Data Center (Members from National Genomics Data Center, 2020), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession number GWHBGBL00000000 that is publicly accessible at https://bigd.big.ac.cn/gwh.

All original code has been deposited at Genome Sequence Archive and is publicly available as of the date of publication. DOIs are listed in the key resources table.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant materials
The celery inbred cultivar Baili, which was belong to the Western celery and was derived from California celery via single plant selection, and it had self-crossed eight generations when used for genome sequencing. A total of 79 celery samples were re-sequenced in this study, these included 34 Chinese celery accessions, 34 Western celery accessions, 10 hybrid selections from Chinese celery cross Western celery and one wild type (Table S19). All celery accessions were maintained and cultivated grown in the plastic-covered tunnels at China Agricultural University in Beijing, China.

REAGENT or RESOURCE SOURCE IDENTIFIER

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| PAML (v 3.15)      | Yang (2007) | http://abacus.gene.ucl.ac.uk/software/paml.html |
| McscanX            | Wang et al. (2012) | https://github.com/wyp1125/MCScanX |
| MUSCLE (v3.8.31)   | Edgar (2004) | http://www.drive5.com/muscle/ |
| BWA (v 0.7.17-r1188) | Li and Durbin (2009) | https://github.com/lh3/bwa.git |
| SAMtools (v1.6.3-g200708f) | Li et al., 2009 | http://www.htslib.org/ |
| GATK (v v3.2-2-gec30cee) | McKenna et al. (2010) | https://gatk.broadinstitute.org/hc/en-us |
| Manta tool         | Chen et al., 2016 | https://github.com/Illumina/manta/releases/download/v1.6.0/manta-1.6.0.centos6_x86_64.tar.bz2 |
| MEGA X             | Kumar et al. (2018) | https://www.megasoftware.net/ |
| admixture          | Alexander et al. (2009) | http://software.genetics.ucla.edu/admixture/download.html |
| EIGENSOFT63 (v.6.0.1) | Price et al. (2006) | https://github.com/DRichLab/EIG |
| Software plink     | Purcell et al. (2007) | https://www.cog-genomics.org/plink/ |
| PopGenome Software | Pfeifer et al. (2014) | https://cran.r-project.org/web/packages/PopGenome/index.html |
METHOD DETAILS

Genome size estimation
Genomic DNA was extracted from celery using a standard CTAB protocol. We constructed five two paired-end (PE) libraries with insert sizes of 270 bp following the standard Illumina protocols, and then sequenced on the Illumina HiSeq 2500 platform. We used these data to calculate and plot the k-mer frequency distribution. Genome size can be estimated by the formula \( G = \frac{K_{\text{num}}}{\text{peak depth}} \) (K_num: the total number of k-mers; peak depth: the depth of the major peak) (Li et al., 2010). The K value was 21. After filtering abnormal depth k-mers, we finally obtained a total of 27,144,018,011 k-mers, and the major peak depth was 44.38. The celery genome size was thus estimated to be 3.26 Gb.

About 1 g leaf from 3-months-old celery was used to obtain the cell nucleus, then the cell nucleus DNA was stained with propidium iodide in dark for 20 min (Dolezel, 2010). DNA was quantified by flow cytometry on a FACSCalibur (BD company, America) and the data was acquired and analyzed by CellQuest (BD company, America) and ModFit (Yerity Software House), respectively. Zea mays was used as an internal reference. The estimated celery genome size was 3.10 Gb.

De novo genome assembly using PacBio reads
For PacBio library construction, celery genomic DNA was sheared to 20 kb using G-tubes, then purified, damage repair, hairpin adaptor ligation, and digestion with exonuclease to remove the damaged DNA and fragments that without adaptors. The target fragments were size-selected by Blue Pippin electrophoresis. Then the PacBio library was sequenced on a PacBio Sequel sequencer (Pacific Biosciences, CA, USA).

For genome assembly, we first used Canu (v1.5) (Koren et al., 2017) to correct the PacBio data, then we used WTDDBG v1.2.8 (https://github.com/ruanjue/wtdbg), FaCCon (v0.732) (Chakraborty et al., 2016) and Canu (v1.5) to independently assemble the high-quality PacBio subreads. These packages yielded 2,087 Gb, 3,184 Gb and 3,335 Gb assemblies, with contig NS0 values of 79.57 kb, 1,041 Mb and 1,032 Mb, respectively. The assembly by the Canu (v1.5) resulted in the optimal assembly and was used as a reference. Then the most well assembly genome was corrected with the Illumina data using Pilon (v1.22) (Walker et al., 2014) for three times. To evaluate the assembled genome’s quality, we first mapped the Illumina data and PacBio data to it using BWA (Li and Durbin, 2009), then mapped core eukaryotic genes (CEGs) using CEGMA (v2.5) with 458 conserved core eukaryotic genes (Parra et al., 2007), and finally applied a BUSCO (v2.0) test (Simão et al., 2013), with 1440 genes in Embryophyta-odb9 to examine its gene content. We conducted the LAI method followed Ou et al. (2018).

Chromosome assembly using Hi-C
We constructed Hi-C fragment libraries from 300 to 700 bp insert size following Rao et al. (2014) and sequencing through Illumina NovaSeq 6000 with the PE150 model. The adapter sequences of the raw reads were trimmed, and low-quality PE reads were filtered to obtain clean data. Totally we obtained 157.79 Gb (47.27×) clean Hi-C data, which was used for chromosome-level genome assembly. We then evaluated the alignment efficiency and insert length distribution for valid pair fragments and valid interaction pairs using HiC-Pro (Servant et al., 2015). We first correct errors in scaffolds by splitting the scaffolds into 50 kb segments on average. Then we used LACHESIS software (Burton et al., 2013) to assemble the genome, with the parameters set as follow: CLUSTER_MIN_RE_SITES = 103; CLUSTER_MAX_LINK_DENSITY = 1; CLUSTER_NONINFORMATIVE_RATIO = 5; ORDER_MIN_N_RES_IN_TRUN = 60; ORDER_MIN_N_RES_IN_SHREDS = 60. Finally we performed artificial correction of the LACHESIS-assembled results and gap filling or sequence de-duplication to increase the accuracy and completeness of the assembled genome (Servant et al., 2015).

We then visualized the interaction matrix of all chromosomes and heatmaps with a resolution set at 500kb to assess the accuracy of the Hi-C assembly. Additionally, the final genome assembly was also validated using the next-generation sequencing (NGS) short reads, PacBio long reads and RNA sequencing (RNA-seq) reads.

Transcriptome sequencing
RNA was isolated from the different tissues of celery (fresh leaf, petiole, root) following the manufacturer’s protocol provided in the TaKaRa MiniBEST Universal RNA Extraction Kit. The integrity, purity, and concentration of the RNA were assessed using an Agilent 2100 Bioanalyzer, a NanoDrop, and a Qubit 2.0. RNA-seq
library was constructed by mixing an equal amount of RNA from the above different tissues following the NEBNext Ultra RNA Library Prep Kit (NEB) following the manufacturer’s instructions and then sequenced on an Illumina HiSeq 2500 platform. Eventually, 11.86 Gb of RNA-seq data with Q30 higher than 93.51% were obtained.

Gene annotation
For repeat annotation, we used the structural prediction and the ab initio prediction methods by using LTR_FINDER (v1.05) (Xu and Wang, 2007) and RepeatScout (v1.0.5) (Price et al., 2005) to construct a primary repeat sequence database. And used the PASTEClassifier (Hoede et al., 2014) to classify the primary database, then formed a final repeat sequence database by combing with the Repbase database (Jurka et al., 2005). Finally, we predicted the repetitive sequence by using RepeatMasker v4.0.6 (Tarailo-Graovac and Chen, 2009) based on the final repeat sequence database.

For protein-coding gene prediction, we first masked and excluded the repeat elements from the genome assembly, then we used ab initio predictions, homology-based gene models and unigene prediction to predict the high quality protein-coding genes. For ab initio prediction, we used the Augustus (v2.4) (Stanke and Waack, 2003) and SNAP (v2006-07-28) (Korf, 2004). Homologous species prediction was using GeMoMa (v1.3.1) (Keilwagen et al., 2016, 2018) based on Arabidopsis thaliana, Apium graveolens (Ventura) (Song et al., 2021), Coriandrum sativum, Daucus carota and Lactuca sativa. All RNA-Seq reads were initially aligned against the celery genome using HISAT2 (Kim et al., 2015) and assembled into transcripts using Stringtie (v1.2.3) (Pertea et al., 2015), then open reading frames (ORFs) were predicted using GeneMarkS-T (v5.1) (Tang et al., 2015). RNA-seq reads were de novo assembled using Trinity (v2.1.1) (Grabherr et al., 2011) and then analyzed using PASA (Campbell et al., 2006). We then used EVM (v1.1.1) (Haas et al., 2008) to integrate these three prediction methods and performed final modifications using PASA (v2.0.2) (Campbell et al., 2006).

For noncoding RNAs, we predicted microRNAs and rRNAs to search the RFam database (Griffiths-Jones et al., 2005), and used tRNAscan-SE (v 1.3.1) (Lowe and Eddy, 1997) to predict the tRNA. And we used GenBlastA (v1.0.4) (She et al., 2009) alignment and GeneWise (v2.4.1) (Birney et al., 2004) to predict the pseudogene.

For gene functional annotation, we blasted the predicted genes against the non-redundant protein (NR) (Marchler-Bauer et al., 2011), KOG (Koonin et al., 2004), GO (Dimmer et al., 2012), KEGG (Kanehisa et al., 2005), TrEMBL (Boeckmann et al., 2003), EggNOG (http://eggnoG5.embl.de/download/eggnoG_5.0/) (Jaime Huerta-Cepas et al., 2018), SWISS-PROT (http://ftp.ebi.ac.uk/pub/databases/swissprot) (Boeckmann et al., 2003) and Pfam (http://pfam.xfam.org) (Finn RD, 2006) databases using BLAST (v2.2.31) (-evalue 1e-5) (Altschul et al., 1990). For motifs annotation, we used InterProScan (Zdobnov and Apweiler, 2001) by aligning with the PROSITE (Bairoch, 1991), HAMAP (Lima et al., 2009), Pfam (Finn et al., 2006), PRINTS (Attwood and Beck, 1994), ProDom (Bbru et al., 2005), SMART (Letunic et al., 2004), TIGRFAMs (Haft et al., 2003), PIRSF (Wu et al., 2004), SUPERFAMILY (Gough and Chothia, 2002), CATHGene3D (Lees et al., 2012), and PANTHER (Thomas et al., 2003) databases.

Comparative genomic analysis
We used the OrthoMCL package (v 2.0.9) (Li et al., 2003) to identify orthologous genes between celery and 11 other plant species, including Daucus carota, Coriandrum sativum, Helianthus annuus, Capsicum annum, Solanum lycopersicum, Vitis vinifera, Medicago truncatula, Populus trichocarpa, Oryza sativa, and Arabidopsis thaliana. Gene family expansion and contraction was analyzed using CAFE software (v 4.2) (Han et al., 2013) with a probabilistic graphical model. Phylogenetic tree between these 13 plant species was constructed using PHYML (Guindon et al., 2010) based on the 320 single-copy orthologous genes with the parameters (-gapRatio 0.5 -badRatio 0.25 -model HKY85 -bootstrap 1000). Divergence times were estimated using MCMCtree in PAML (v 3.15) (Yang, 2007), based on the predicted divergence time from A. thaliana and P. trichocarpa (105.97~107.96 Mya) and V. vinifera and O. sativa (151.98~159.97 Mya).

The all-versus-all tblastp method (E-value< 1e-5) was used to detect paralogous genes in Apium graveolens, Daucus carota, Coriandrum sativum as well as orthologous genes in Apium graveolens-Daucus carota, Apium graveolens-Coriandrum sativum and Apium graveolens-Vitis vinifera. Then gene pairs were
detected using McscanX (Wang et al., 2012) and the 4DTv-value of these gene pairs was calculated using the HKY model.

Paralogs within Apium graveolens and orthologs between Apium graveolens and Coriandrum sativum and Daucus carota were identified using BLASTP (e-value was 1e-10). Then we used MCscanX to analyze chromosome collinearity (Wang et al., 2012) with the following parameters: match_score (k) = 50; genes required to call a collinear block (s) = 5; gap penalty (g) = 1; maximum gaps allowed (m) = 25; and alignment significance = 1e-10.

Analysis of full-length LTR retrotransposons
We used LTR_Finder (v1.0.5) (Xu and Wang, 2007) to de novo detect full-length LTR retrotransposons in genome of Apium graveolens, Daucus carota, Coriandrum sativum and Foeniculum vulgare. Next, we screened the LTR sequence of scores higher than 6 by using the PS SCAN (Prestridge, 1991), and filtered those overlapped LTR sequence. The LTR retrotransposons protein sequences were aligned using MUSCLE (v3.8.31) (Edgar, 2004), and we built the neighbor-joining (NJ) trees of Copia and Gypsy superfamilies using MEGA X (Kumar et al., 2018) with the default parameters. The nucleotide distance was estimated using the Kimura two-parameter (K2p) (transition–transversion ratio) criterion by using DistMat software, and the rate of nucleotide substitution was using the dicotyledon mutation rate, which was used $7.3 \times 10^{-9}$.

Sequence alignment and variation calling
The 79 celery accessions genomic DNA was extracted from leaves using the CTAB method. Illumina genomic libraries with insert sizes of 300–500 bp were constructed following the manufacturer’s instructions, the libraries then sequenced on an Illumina NovaSeq 6000 platform (Illumina Inc., USA) with 150 bp paired-end reads.

To call SNPs, reads of each accession were mapped to the celery reference genome using BWA (v 0.7.17-r1188) (Li and Durbin, 2009) with the default parameters. Then we count the map results by using SAMtools (v1.6–3-g200708f) (Li et al., 2009). Before calling SNPs and small InDels, we used Picard (http://sourceforge.net/projects/picard/) to filter the MarkDuplicates reads, then used GATK (v v3.2-2-gec30cee) (McKenna et al., 2010) to detect SNPs and small InDels. SNPs were further filtered using the following criteria: (1) we filtered out SNPs that located nearby InDels within 5 bp and adjoined InDels within 10bp; (2) the variant SNPs in 5 bp window should not more than two; (3) an overall quality (QUAL) score of <30; (4) a variant quality by depth (QD) score of <40; (5) a phred-scaled p value (FS) > 60; (7) the other variable filter parameters were used as default parameters. For SV detection, we used Manta tool (Chen et al., 2016) followed the default parameters.

Phylogenetic and population analysis
A subset of 7,629,138 SNPs with a minor allele frequency (MAF) ≥ 0.05 and missing rate ≤ 0.2 from 79 celery accessions were used for phylogenetic and population structure analyses. We used MEGA X (Kumar et al., 2018) to build the phylogenetic treewith 1000 bootstrap replicates. And we used admixture (Alexander et al., 2009) to construct the population structure. Cross-validation error was tested for obtaining the most likely K value varying from 1 to 10. In addition, principal component analysis (PCA) was performed with EIGENSOFT63 (v.6.0.1) (Price et al., 2006) using the above SNP data set. Two dimensional coordinates were plotted for the 79 celery accessions. For linkage disequilibrium (LD) analysis, We used the above SNPs to perform LD using Software plink (Purcell et al., 2007), and LD decay was calculated on the basis of the $r^2$ value and corresponding distance between any two SNPs within a 1000 kb window.

Selective sweep regions for celery
A 100-kb sliding window along with a step size of 10 kb was used to estimate population polymorphisms through the population fixation index ($F_{ST}$), nucleotide diversity ($\pi$) and watterson estimator ($\theta W$)between the Chinese celery and Western celery. $F_{ST}$, $\pi$ values and $\theta W$ were calculated at each window using the PopGenome Software (Pfeifer et al., 2014). Sliding windows with the top 5% highest $F_{ST}$ values were selected initially. Then we merged the neighboring windows into one fragment, and if the distance between two fragments was <100 kb, we also merged them into one region. Finally, these merged regions were considered as highly diverged regions between local and Western celery.
Identification candidate regions related to leaf agronomic traits in celery

The agronomic traits in celery were evaluated three times during the winter of 2017, 2018, and 2019 at China Agricultural University in Beijing, China (Table S18). A total of 7,629,138 SNPs with MAF $\geq 0.05$ and missing rate $\leq 0.2$ were used to carry out GWAS, and GWAS were performed using Emmax program (Kang et al., 2010). Finally, the signals with $p < 10^{-8}$ were considered as the significantly and $p < 10^{-9}$ were considered as extremely significant. The methods used for mapping of $ph$ loci were followed Cheng et al. (2020), and the primers used in this study were shown in Table S33.

Total RNA was exacted from the young petiole of line 308 and 314 using the Quick RNA isolation Kit (Huayueyang, China), following manufacturer protocol. First-strand cDNA was synthesized using the SuperScript III First-Strand Synthesis System Kit (Huayueyang, China). The primers used for real-time PCR were designed using the Primer 5.0 (http://www.premierbiosoft.com/primerdesign/) and are listed in Table S34. The real-time PCR was performed with a TB Green® Premix Ex Taq™ (Takara, China), following manufacturer’s instructions, on an ABI 7500 real-time PCR system. The thermocycling conditions were set as follows: 95°C for 30s, 40 cycles of 95 °C for 5s and 60 °C for 30s, then melt curve. Relative expression values were calculated using the $2^{-\Delta\Delta CT}$ method.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification and statistical analysis used in the genome sequencing and assembly, genome quality assessment, evolutionary analysis and comparative genome analysis can be found in the relevant sections of the method details.