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ORIGINAL ARTICLE

A novel mutation in *TFL1* homolog sustaining determinate growth in cucumber (*Cucumis sativus* L.)

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Abstract

Key message **BSA-seq combined with whole-genome resequencing map-based cloning delimited the cucumber *determinate-novel* locus into a 44.5 kb region in chromosome 6 harboring a putative candidate gene encoding a phosphatidylethanolamine-binding protein (*CsCEN*).**

Abstract Determinate and indeterminate growth habits of cucumber can affect plant architecture and crop yield. The *TERMINAL FLOWER 1* (*TFL1*) controls determinate/indeterminate growth in *Arabidopsis*. In this study, a novel mutation in cucumber *TFL1* homolog (*CsCEN*) has shown to regulate determinate growth and product of terminal flowers in cucumber (*Cucumis sativus* L.), which is similar to the function of *CsTFL1* as previously reported. Genetic analysis in two determinate genotypes (D226 and D082) and indeterminate genotype (CCMC) revealed that a single recessive gene is responsible for this determinate growth trait. With the combination of BSA-seq and whole-genome resequencing, the locus of *determinate-novel* (*det-novel*) trait was mapped to a 44.5 kb genomic region in chromosome 6. Sequence alignment identified one non-synonymous SNP mutation (A to T) in the third exon of *CsCEN*, resulting in an amino acid substitution (Thr to Pro), suggesting that determinate growth might be controlled by a novel gene *CsCEN* (*Csa6G152360*) which differed from the reported *CsTFL1* gene. The *CsCEN* expression level in shoot apexes and axillary buds was significantly lower in D226 compared to CCMC, suggesting its essential role in sustaining indeterminate growth habit. Identification and characterization of the *CsCEN* in the present study provide a new insight into plant architecture modification and development of cucumber cultivars suited to mechanized production system.

Introduction

Cucumber (*Cucumis sativus* L.) is an economically important vegetable crop widely cultivated in the world. Most cultivated cucumber have indeterminate growth habit which is characterized by tall plant which continuously produce inflorescences, nodes and branches, while stems have prominent nodes and internodes. Indeterminate growth habit brings about sustainable fruit production from different parts of the

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plant, as harvesting is usually performed at different times. Indeterminate plants are difficult to manage in cultivation under field conditions. Moreover, their growth habit tends to bring about asynchronous fruit maturity from different parts of the plant, and harvesting is usually performed at different times. On the other hand, in determinate cucumber, the stem growth is interrupted by a flower cluster on the stem apex, significantly reducing the plant height and number of nodes. Also, its causes cessation of growth of branches with terminal flowers. Therefore, determinate growth habit is suitable for high-density planting and mechanical harvesting, because of its synchronized flowering and homogeneous fruit maturation (Pierce and Wehner 1990; Çagirgan 2006). In addition, the determinate types usually flower much earlier than indeterminate cucumber and are thus harvested much earlier in some cultivation production systems (Zhang et al. 2018).

The growth habit, as a part of plant architecture, is controlled by genetic mechanisms associated with environmental factors and largely dependent on the activity of shoot apical meristem (SAM). The *TERMINAL FLOWER 1*/*CENTRORADIALIS* (*TFL1*/*CEN*) gene family shares high homology in regulating inflorescence indeterminacy and is alternatively named as phosphatidylethanolamine-binding protein (PEBP). *TFL1* is found in *Arabidopsis*, which is reported to highly conserve in maintaining SAM indeterminacy and has an additional effect on flowering time (Shannon and Meeks-Wagner 1991; Bradley et al. 1997). *tfl1* mutants flower early and the SAM is converted to terminal flowers, unlike in the indeterminate growth as in wild type (Hanzawa et al. 2005; Hanano and Goto 2011). The *CEN* in *Antirrhinum* can also control the determinate growth habit; *CEN* loss-of-function mutations results in determinate inflorescences without affecting the time of flowering (Bradley et al. 1996). Besides, the *TFL1*/*CEN* homologs in other plant species have also been found such as *SELF-PRUNING* (*SP*) in tomato (*Solanum lycopersicum*) (Pnueli et al. 1998; Soyk et al. 2017), *Dt1* in soybean (*Glycine max* L.) (Pañeda et al. 2008; Liu et al. 2010), *PvTFL1*y in common bean (*Phaseolus vulgalis*) (Koinange et al. 1996), *RCN1* and *RCN2* in rice (*Oryza sativa*) (Nakagawa et al. 2002), *PsTFL1c* in pea (*Pisum sativum*) (Foucher et al. 2003), whose functions are to regulate plant architecture by controlling flowering transition and determine the fate of SAM. Beyond that, several other genes can determine the plant architecture and regulate flower development to control SAM, including *LEAFY* (*LFY*), *APETALA1* (*AP1*), *APETALA3* (*AP3*), *AGAMOUS* (*AG*) and *SEPALLATA* (*SEP*) (Wang and Li 2008; Zhang and Yuan 2014).

Recently, limited advances have been made in the gene identification, mapping, cloning and molecular mechanism of determinate growth in cucumber. Genetic analysis indicated that a single recessive gene located on cucumber chromosome 6 controlled the determinate growth habit in cucumber (Fazio et al. 2003; Sato et al. 2009; Weng et al. 2010). Wen et al. (2019) have identified that the determinate growth habit was controlled by the *determinate* (*det*) locus. Further analyses indicated that there was a non-synonymous SNP in *CsTFL1* (*Csa6G452100*), which could interact with CsNOT2a-CsFDP to regulate growth habit in cucumber. Furthermore, the *Arabidopsis LFY* homolog gene *CsLFY* also affects the formation of plant architecture, and determines SAM maintenance through interaction with *CsWUS* in cucumber (Zhao et al. 2018).

In the current study, a cucumber determinate genotype D226 was found to present determinate growth habit and also showed sensitivity to the environment. No mutation was detected in *CsTFL1* gene of D226 when compared to indeterminate genotypes of cucumber. In this study, with the main objectives of identifying genomic regions and candidate genes responsible for the *det-novel* phenotype in D226, we performed whole-genome resequencing which was expedited with BSA-seq approach, we identified a novel gene *CsCEN* in cucumber that affects the formation of determinate growth in different ways. The loss-of-function of *CsCEN* gene resulted in determinate growth and hastened the process of 'blunt with blossom' in cucumber mutants (D226) which led to an extremely decreased plant height and number of nodes, in addition the D226 mutant was shown to be sensitive to low temperatures. Further, we found that the determinate growth habit in D226 mutant was caused by a 1 bp mutation in the coding region of the *CsCEN* gene, encoding a phosphatidylethanolamine-binding protein, which plays an important role in the formation of determinate growth in cucumber.

Materials and methods

Plant materials and mapping population

The inbred lines D226 and D082, spontaneous mutants with determinate growth habit, are Europe cucumber cultivars. CCMC is the North China-type inbred line that has indeterminate growth habit. D226, D082 and CCMC were crossed to establish 3 F_1 hybrids, which were self-pollinated to generate F_2 plants. For genetic mapping of *det-novel* trait, F_2 plants including 508 plants from the cross between D226 with CCMC, and 697 plants from the cross between D082 with CCMC were generated from two seasons in 2019. In addition, we developed about 498 F_2 plants from cross between two determinate genotypes (D082 × D226) for confirming the source of inheritance. All plant materials were grown in plastic greenhouses in the spring and autumn of 2019 at the Baima Cucumber Research Station of Nanjing Agricultural University, Nanjing, China. The phenotype of

plant growth habit was assessed by visual inspection, classified as either indeterminate or determinate growth habit. The segregation ratios of the F_2 populations were analyzed using the Chi-square test (χ^2).

Field phenotyping

Plant height measurement was taken every week up to 56 days after transplanting (transplant after one leaf) in plastic greenhouses. Ten different plants per genotype were measured in spring and autumn of 2019. Number of nodes was also counted on weekly basis. The representative photograph of the whole plant was taken when determinate genotypes had terminal flowers.

Histological examination

The morphology of CCMC and D226 shoot apexes was observed at 25 days after planting. Histological examination was performed using the method described by Wicart et al. (1984). The slides were observed and photographed under the microscope (Olympus BX51, Japan).

DNA isolation

Fresh leaves were collected from each plant including parents, F_1 and F_2 populations, and genomic DNA was extracted using the CTAB method as previously described by Tel-Zur et al. (1999). DNA concentration was determined on the NanoDrop. DNA was diluted to a concentration of 100 ng/µL and stored at − 20 °C for further use.

Molecular mapping of the *det-novel* locus

Initial mapping of the *det-novel* gene was performed in the D226 × CCMC F_2 population using bulked segregant analysis (BSA-seq). Two bulks consisted of the T_D pool with extreme determinate phenotype and T_ID pool with extreme indeterminate phenotype. For each pool, genomic DNA from 20 individual plants from F_2 population was generated for whole-genome resequencing with an Illumina HiSeq 2500. High-quality reads obtained from the two DNA pools were aligned against the 'Chinese Long (v2)' cucumber reference genome to obtain a consensus sequence (Huang et al. 2009). Single nucleotide polymorphism (SNP) calling, aligned data filtering, SNP index calculation and sliding window analysis were performed.

For fine mapping, based on the initial interval detected using BSA-seq approach, new polymorphic markers in this interval were developed and applied in D226 × CCMC F_2 population to delimit the *det-novel* gene in the final interval. Whole-genome resequencing was conducted for D226 and CCMC to develop InDel and SNP markers within the interval. Polymorphisms of the InDel markers were detected by polyacrylamide gel electrophoresis. General information regarding all primer sequences used for fine mapping in this study is listed in Table S1. The candidate genes in the fine mapping region harboring the *det-novel* locus were identified using the Cucurbit Genomics Database (http://cucurbitgenomics.org).

Phylogenetic analysis

The sequences of CsCEN protein and other known related proteins in different species were obtained in the GenBank accession numbers from the NCBI database, which were used in phylogenetic analysis. Multiple alignment of the protein sequences was conducted by Clustal W using the default settings, and the neighbor-joining tree was constructed using MEGA 6 based on 1000 bootstrap replications.

Cloning and expression analysis of *det-novel* candidate gene

The *CsCEN* gene was amplified from the genomic DNA of the CCMC, D226 and D082 using the primers described in Table S2. We used qPCR to examine the expression pattern in D226 and CCMC. Total RNA was extracted from young leaves, flowers, male buds, female buds, shoot apexes and axillary buds with the Trizol reagent (Invitrogen, USA), and cDNA was synthesized using the Prime Script™ RT Reagent Kit (TAKARA, Japan) following the manufacturer's instructions. *CsActin* (*Csa2G301530*) was used as the internal reference. The qPCR was performed with a TB Green® Premix Ex Taq™ Kit (TAKARA, Japan) in a Bio-Rad CFX96 PCR system. Each test was performed with three biological and three technical replications. All primers are listed in Table S2.

Scanning electron microscopy

To compare differences of shoot apexes between CCMC and D226, scanning electron microscopy (SEM) was employed to study in the plants using S-3000N scanning electron microscope (HITACHI, Japan). Shoot apexes were fixed in 2.5% glutaraldehyde for 2 h, and washed three times with PBS. After this, the samples were dehydrated through different concentrations of alcohol, dried, coated with gold particles. The method of meristem size determination was followed as previously explained by Landrein et al. (2015). The meristem diameter was estimated by drawing a circle roughly overlapping with the center of meristem surface using the ImageJ.

Results

Description of indeterminate and determinate growth phenotype

The performance of cucumber plants with different growth habits is shown in Fig. 1. In indeterminate genotype (CCMC), the shoot apex growth developed continually, with the elongation of stem, and its inflorescence growing definitely (Fig. 1a, e). Also, result indicated that the shoot apex of CCMC had regular differentiation (Fig. 1C). On the other hand, determinate genotype (D226) produced a cluster of flower buds at an early stage about 25 days after planting (Fig. 1b). We also observed significantly higher number of floral primordia at the shoot apex in the section of D226 compared to CCMC (Fig. 1d). More so, as the development of the stem terminates, a cluster of flowers was formed at the shoot apex of D226, significantly reducing the number of nodes and shortening the plant height (Fig. 1f). Moreover, a different cucumber genotype (D082) displayed determinate growth habit, with differences in plant morphology compared to D226 (Fig. S1).

To determine whether the determinate phenotype had a genetic stability, cucumber cultivars with different growth habits were planted in different growing seasons of spring and summer of 2019. From the growth cycle, CCMC displayed indeterminate growth habit, with the plant height and number of nodes increasing steadily in the two seasons (Fig. 2), whereas D226 had growth cessation with terminal flowers in both spring and autumn within 50 days after transplanting. Notably, D226 terminated more extremely with less number of nodes and shorter plant height in spring than in autumn season (9.5 ± 0.5 and 23.0 ± 4.0 cm in spring, compared to 19.8 ± 0.5 and 90.3 ± 4.0 cm in autumn), showing that D226 was sensitive to the environment (Fig. 2).

Inheritance of growth habit

The segregation pattern of growth habit using different F_2 populations was investigated and analyzed. All F_1 individuals between determinate and indeterminate genotype displayed a complete indeterminate phenotype, indicating the dominance of indeterminate over the determinate growth habit. However, D082 × D226 F_1 plants had determinate growth; it demonstrated that indeterminate growth is dominant over the determinate growth trait of D082 and

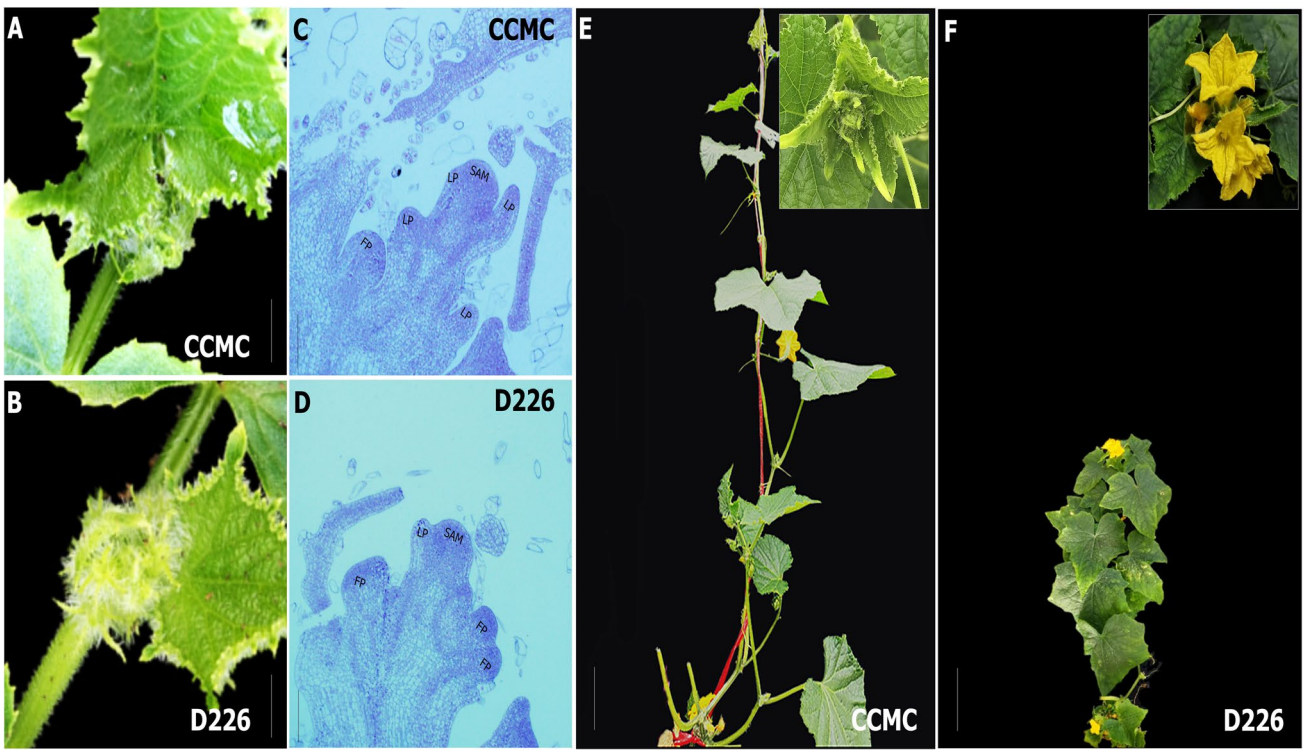

Fig. 1 Phenotypic differences in growth habit between cucumber genotypes with indeterminate (CCMC) and determinate (D226) growth habit. **a**–**d** Show the changes at shoot apexes of CCMC and D226, 25 days after planting, *SAM* shoot apical meristem, *LP* leaf primordium, *FP* floral primordium. CCMC (**e**) maintained growth steadily, while D226 (**f**) already had terminal flowers at the same time of assessments. **a**, **b** bar = 1 cm, **b**, **d** bar = 100 µm, **e**, **f** bar = 10 cm

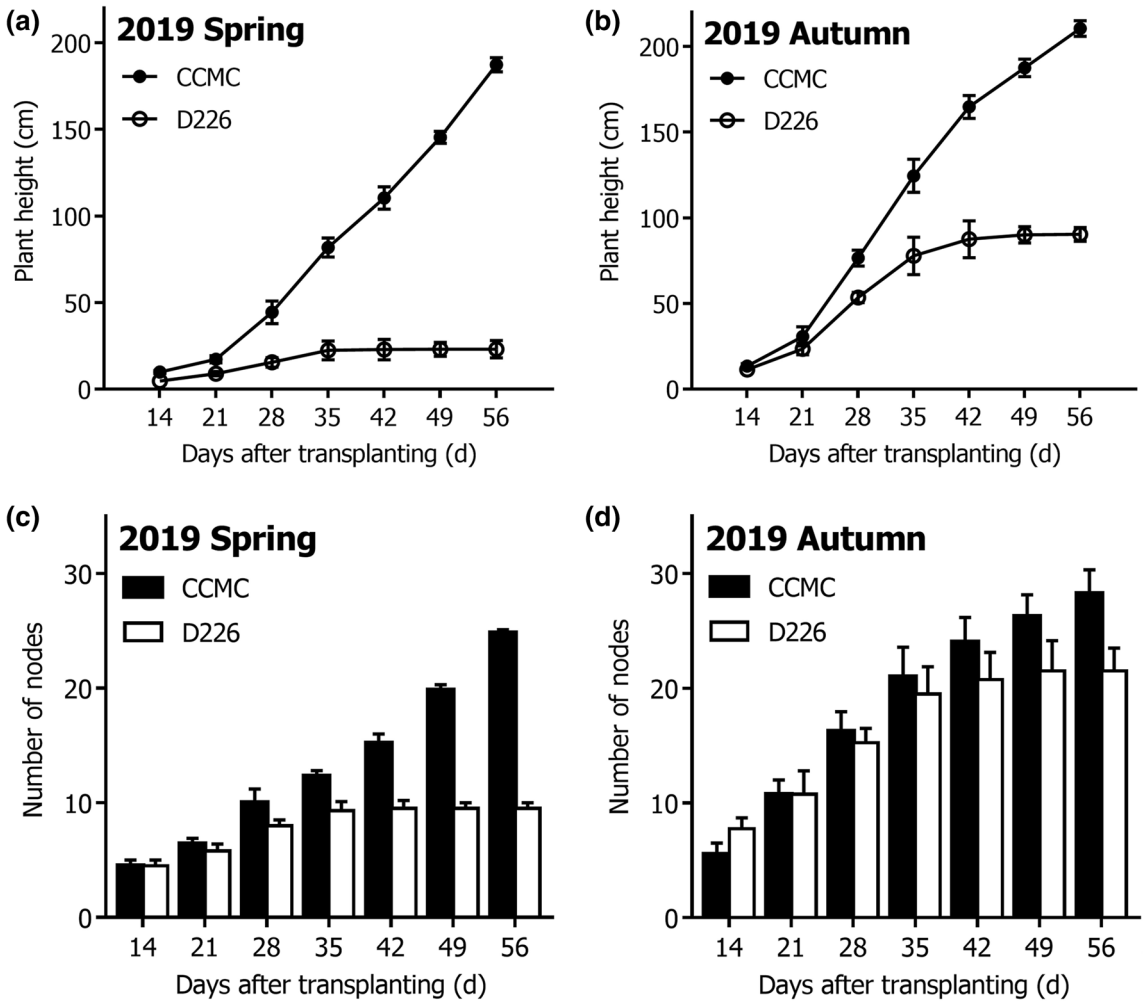

Fig. 2 Comparison of plant height (**a**, **b**) and the number of nodes (**c**, **d**) between CCMC (indeterminate) and D226 (determinate) genotypes after transplanting during spring and autumn seasons of 2019. Data are means of at least ten replicates (± SD)

D226 which is controlled by recessive alleles of *det-novel* genes. In addition, two segregating populations including D226 × CCMC and D082 × CCMC were investigated for growth habit. In D226 × CCMC F_2 plants, 114 and 48 plants had indeterminate and determinate growth, respectively, in spring of 2019, while 263 and 83 plants had indeterminate and determinate growth habit, respectively, in autumn of 2019, which were consistent with 3:1 segregation ratio. Also D082 × CCMC F_2 plants segregated in an approximate 3:1 ratio during spring and autumn in 2019 (Spring: indeterminate: determinate = 423:123; autumn: indeterminate: determinate = 107:44). However, 498 F_2 individuals from D082 × D226 plants were all determinate growth. This further confirmed that *det-novel* gene was inherited from determinate genotypes. Thus, the results were in agreements with previous studies that determinate growth habit was controlled by a single recessive gene.

Primary mapping of *det-novel* locus

Primary mapping of *det-novel* gene was first performed based on the acquirement of genotypes with two pools of extreme phenotypes in the F_2 population; BSA-seq analysis of determinate growth locus T_D bulk and T_ID bulk was pooled for whole-genome resequencing as well as two parental lines. For BSA-seq between D226 × CCMC F_2 population, a total of 17.8 GB data for determinate parent (D226), 5.4 GB data for indeterminate parent (CCMC), 12.3 GB data for T_D bulk, 18.3 GB data for T_ID bulk were obtained (Table S3). Mapping the reads of CCMC to the reference genome resulted in 98.65% coverage and 16.46 × reads depth, while D226 to the cucumber reference genome resulted in 98.37% coverage and 45.82 × reads depth. A similar mapping method was performed for the two bulks, and T_DT bulk achieved 99.05% coverage and

32.9 × reads depth, while T_ID bulk achieved 99.1% coverage and 50.41 × reads depth (Table S3).

Following the principle of BSA-seq analysis, a primary significant genomic region (6.2–14.3 Mb) on chromosome 6 was identified (Fig. 3a). The positive value of △(SNP index) in the region suggested that the determinate trait was derived from D226. The only one genomic region detected in the BSA-seq analysis further confirmed that the determinate trait in D226 was controlled by one single locus. This 8.1 Mb genomic contained numerous SNPs and polymorphic insertion/deletion (InDel) which were developed as useful markers for fine mapping of *det-novel* locus (Fig. 3a). Based on this information, seven InDel markers were developed in the region (Table S1) and used for genotyping 40 F_2 plants from D226 × CCMC population with extreme phenotype. The genotype of the recombinant individuals, which was detected by these markers, delimited the *det-novel* locus between indel markers TIPIN-009 and TIPIN-011, which were 10396856–11330614 kb apart (Fig. 3b).

Fine mapping of *det-novel* locus

For fine mapping of the cucumber *det-novel* locus, segregating F_2 population containing 508 individuals was constructed from a cross between D226 (determinate genotype) and CCMC (indeterminate genotype) and employed more SSR markers in the targeted region for genotyping to narrow down the candidate genomic region. A total of 47 pairs of SSR primers were specially developed in the primary mapped genomic region, and 16 of them were identified to be

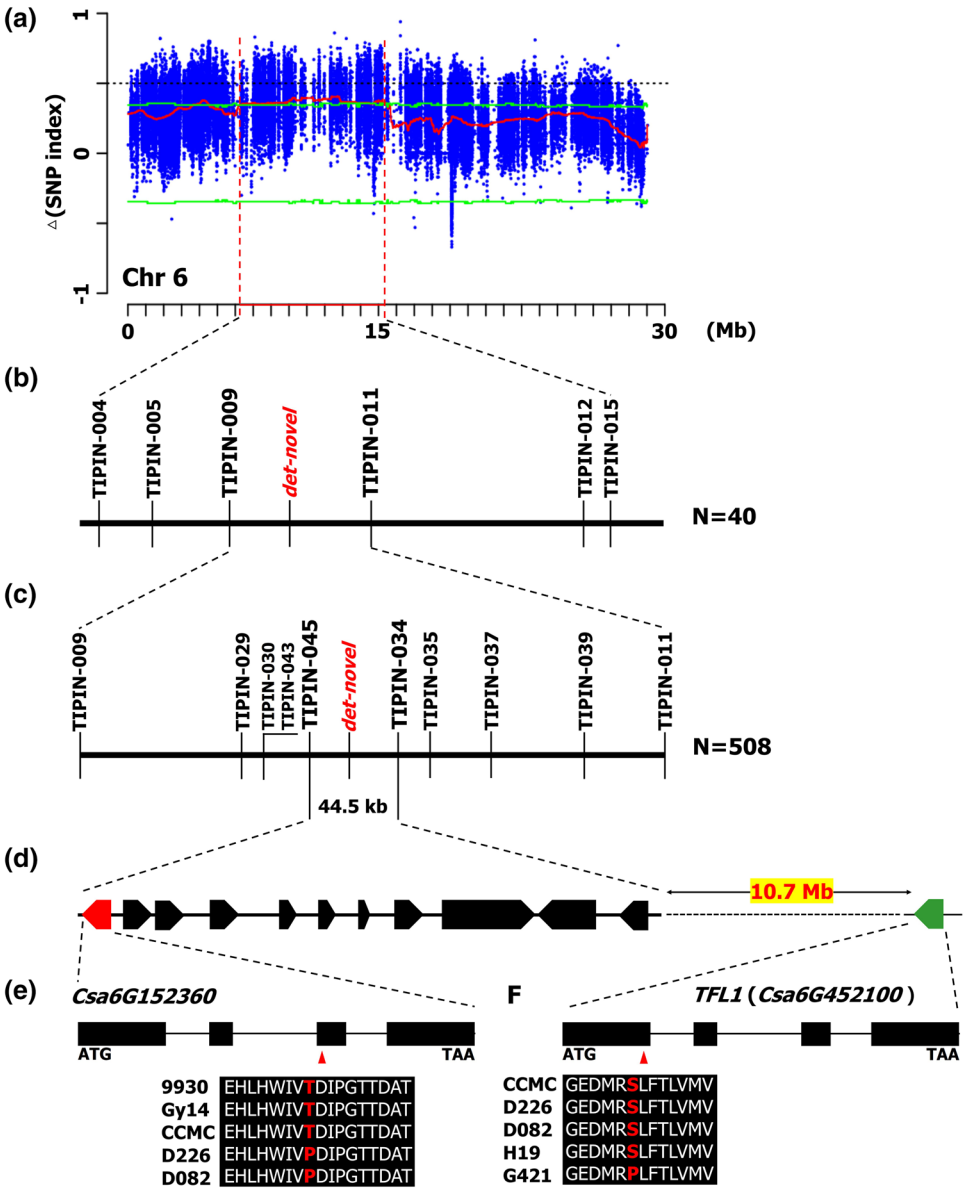

Fig. 3 Map-based cloning of *det* locus. **a** △(SNP index) graph was generated from BSA-Seq, where the *det* locus from 6.2 to 14.3 Mb on chromosome 6 is represented by the red curve. **b** A genetic map that delimited the *det* locus between TIPIN-009 and TIPIN-011 (933.8 kb) region using F_2 plants with extreme phenotype. **c** High-resolution map for the *det* locus into a 44.5 kb region between indel TIPIN-045 and TIPIND-034. **d** The annotated candidate gene *Csa6G152360* within the *det-novel* locus according to http://cucurbitgenomics.org has four exons and three introns. Black rectangles and black lines represent the exon–intron structure, respectively. **e** An SNP in the third exon (arrowed) of *Csa6G152360* resulted in an amino acid change from T in different indeterminate genotypes (9930, Gy14 and CCMC) to P in the determinate genotypes (D226 and D082). **f** *TFL1* (*Csa6G452100*) the reported *det* gene, indicating non-synonymous mutation substituted an amino acid from S (H19, indeterminate genotype) to P (G421, determinate genotype) confirming the *det* mutation (Wen et al. 2019). However, the mutation site was not detected in the current study when we compared both D082 and D226 with CCMC

polymorphic between D226 and CCMC parents. These polymorphic SSR markers were subsequently utilized for genotyping 508 F_2 individuals from the whole D226 × CCMC F_2 population. A total of 22 recombinant plants for determinate type were identified for further genotyping and based on the genotype and phenotype of these recombinant plants. Finally, the fine mapping placed the *det-novel* locus in a genomic region flanked by markers TIPIN-045 and TIPIN-034 which were 10803030–10847512 kb apart, a 44.5 kb on chromosome 6 (Fig. 3c).

Candidate genes analysis

A total of eleven candidate genes were identified in the 44.5 kb region between TIPIN-045 and TIPIN-034 by searching the cucumber reference genome database (Fig. 3d, Table S4). According to the whole-genome resequencing, the results of variation analysis indicated that there were no highly reliable SNP and InDel from both the intron and promoter regions (1.5 kb upstream of the ATG start codon) within this region. After comparing the candidate genes CDS sequences, a total of nine SNPs and one InDel were found in the CDS region including *Csa6G152360* (SNP position: 10805577 and 10805824), *Csa6G152380* (SNP position: 10812303, 10812566, 10812590, 10812656, 10812708 and 10812884) and *Csa6G152390* (SNP position: 10815547 and 10816258; InDel position: 10815552) between D226 and CCMC genomic sequences. However, the CDS comparison between D082 and CCMC genomic sequences indicated only one SNP (position: 10805824) in these candidate genes, which was in *Csa6G152360* CDS region and also overlapping with D226/CCMC data (Table S4). We also examined the expression levels of these eleven genes in D226 and CCMC by qPCR. In addition to the gene of *Csa6G152360*, the expression level of other candidate genes was no significant difference between CCMC and D226 shoot apexes at 15 days after transplanting (Fig. S4). Further, we cloned and compared the variations of *Csa6G152360* between indeterminate (9930, Gy14 and CCMC) and determinate genotypes (D226 and D082). The result showed that single SNP caused a non-synonymous mutation from A to C in the CDS of *Csa6G152360* resulting in an amino acid substitution from Threonine to Proline (Fig. 3e, Fig. S2). These data suggested that this single SNP may be the causal SNP for *det-novel* trait. Based on the functional annotation of these genes, *Csa6G152360* was predicted as *TFL1/CEN* gene family, encoding a phosphatidylethanolamine-binding protein, whose function was participated in the formation of plant architecture and regulating of inflorescence development. The previous study showed a single non-synonymous mutation resulted in an amino acid substitution from Serine in H19 (indeterminate genotype) to Proline in G421 (determinate genotype) of *Csa6G452100* (*CsTFL1*); however, in the current study there was no mutation found in the same position among CCMC, D226 and D082 (Fig. 3f). Furthermore, there was no difference after comparing the promoter and CDS regions of *CsTFL1* in CCMC, D226 and D082 (Fig. S3A-B). qPCR analysis of *CsTFL1* also found no significant difference in shoot apexes of CCMC and D226 (Fig. S3C). Therefore, all these lines of evidences left *Csa6G152360* (*CsCEN*) to be speculated as the most likely candidate gene.

Temporal and spatial expression patterns of *CsCEN*

The phylogenetic tree was constructed using the neighbor-joining (NJ) method to better understand the structural and functional relationship in CsCEN and its homologs among different species. The phylogenetic tree indicated that CsCEN was closely related to AtCEN (*Arabidopsis*), CEN (*Antirrhinum*) and SP (*S. lycopersicum*) and formed a subclade, but far away to CsTFL1 and AtTFL1 (*Arabidopsis*) (Fig. S5). We examined the relative expression of *CsCEN* in different plant tissues (young leaves, shoot apexes, flowers, male and female buds and axillary buds) in both CCMC and D226 plants by qPCR (Fig. 4a). *CsCEN* was highly expressed in male buds, shoot apexes and axillary buds in CCMC plants and there was no significant difference in the expression level of female buds between CCMC and D226 plants; however, in young leaves, flowers, male buds, shoot apexes and axillary buds, *CsCEN* exhibited lower expression levels in D226 than in CCMC (Fig. 4A). The significantly higher expression level of *CsCEN* in axillary buds and shoot apexes in CCMC may further suggest that *CsCEN* may be involved in sustaining the indeterminate growth habit in the shoot apexes.

To test if *CsCEN* had any effect on the shoot apical meristem (SAM) development, we visualized SAMs at 15 and 30 days after planting (DAP) by scanning electron microscopy (Fig. 4b–f). We found that the SAM size at 15 DAP plants was significantly larger in D226 plants than in CCMC, but also dramatically reduced SAM size at 30 DAP in D226 plants (Fig. 4b–f). Furthermore, no significant change of SAM size was found in CCMC plants at 15 and 30 DAP (Fig. 4b, d, f). In addition, qPCR was performed in shoot apexes at 15 and 30 DAP to analyze the expression levels of two SAM-related genes (*CsSTM* and *CsWUS*) in CCMC and D226 plants (Fig. 4g, h). The result indicated higher expression levels of *CsSTM* and *CsWUS* in D226 shoot apexes compared to CCMC, which was more significant in D226 at 15 DAP (Fig. 4g, h). In summary, loss-of-function of *CsCEN* resulted in altered SAM size and changed *CsSTM* and *CsWUS* expression levels in shoot apexes.

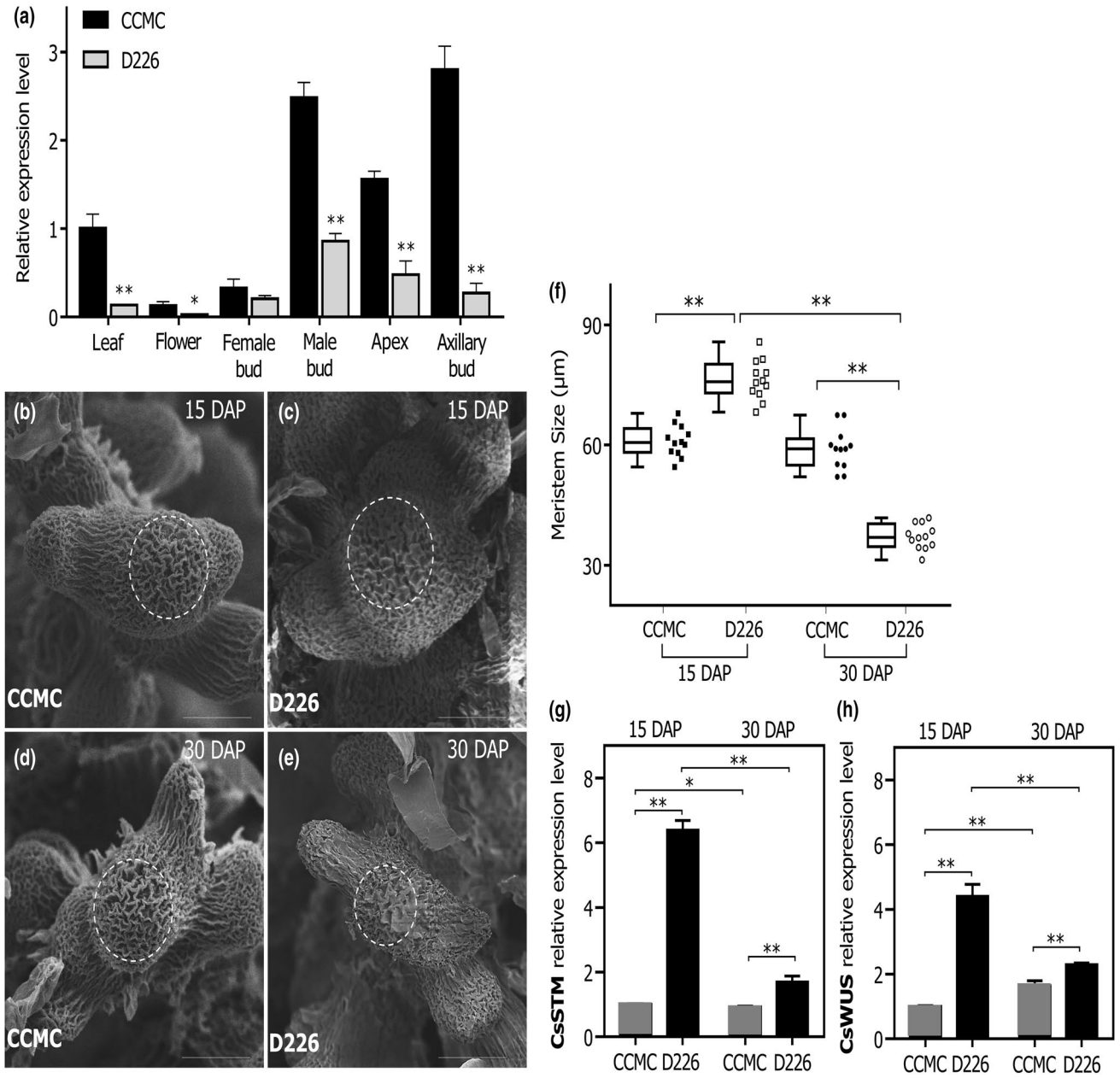

Fig. 4 Comparison of expression of *CsCEN* and SAM size between CCMC and D226 cucumber genotype. **a** Tissue-specific expression pattern of *CsCEN* in CCMC and D226. **b**–**e** The scanning electron microscopy image of SAM at 15 and 30 DAP between CCMC and D226. Dotted circles indicate the SAM size. Scale bar = 80 μm. **f** Boxplot indicating mean SAM size of CCMC and D226, data are average of 12 plants. **g**, **h** Expression level of *CsSTM* and *CsWUS* genes in shoot apexes of CCMC and D226 at 15 and 30 days after planting. Data are means of three replicates (± SD). Asterisks indicate a significant difference by *t* test, $*P < 0.05$; $**P < 0.01$

Discussion

The determinate/indeterminate growth habit is an important agronomical trait in the production of cucumber and many other vegetable crops. In cucumber production under protected environments, indeterminate growth habit is ideal as it can be harvested throughout the production period hence leads to fruits sustainability and extend the growing season to maximize yield, while in certain production systems, like the once-over machine harvest system, determinate cucumber may be advantageous (Fazio et al. 2003; Weng et al. 2010).

Isolation and characterization of genes associated with determinate growth habit are important for cucumber breeding, germplasm assessment and other fundamental studies. At present, two mains genes namely *CsTFL1* (Wen et al.

2019) and *CsLFY* (Zhao et al. 2018) involved in the formation of plant architecture have been explored in cucumber. *CsTFL1* had the function of *AtTFL1* homolog, which resulted in determinate growth and formation of terminal flowers in cucumber, on the other hand, *CsLFY* played a critical role in maintaining SAM and regulating floral meristem development. In this study, the mutant D226 is a recessive trait that exhibited a determinate growth habit, with extreme phenotype depending on the environments. The current study revealed that the phenotype of determinate growth habit in D226 mutant was caused by a mutation of *CsCEN*, which is a novel *TFL1/CEN* homolog gene (Fig. 3, Fig. S4) and the expression level of *CsCEN* in D226 (determinate genotype) was remarkably lower in shoot apexes compared to CCMC (indeterminate genotype) (Fig. 4a). However, no previous studies had reported about *CsCEN* gene roles in plant growth habit and its difference from the previously reported *CsTFL1* gene in cucumber.

The roles of *TFL1*/*CEN* gene family were known to be involved in regulation of floral transition, meristem indeterminacy and formation of plant architecture, demonstrating a conserved function in other crops (Shannon and Meeks-Wagner 1991; Bradley et al. 1997; Pnueli et al. 1998; Carmel-Goren et al. 2003; Zhang et al. 2018). For example, mutant in *TFL1* led to alteration of shoot apex growth, which influenced both the plant height and number of nodes (Bradley et al. 1997). In the present study, genotype with determinate growth habit (D226) showed significant differences in plant height and number of nodes in both spring and autumn of 2019 (Fig. 2). *CEN* expression in *Antirrhinum* was reported to be more expressed in the inflorescence meristem; this localization of *CEN* expression in the apex acted upon SAM to prolong the inflorescence phase (Bradley et al. 1996), which is in agreement with our study where the novel gene *CsCEN* was found to be expressed in the axillary buds and shoot apexes of indeterminate cucumber plants, suggesting its essential roles in sustaining indeterminate growth habits. Also the *SP* gene is the functional homolog of *CEN* in tomato and has high similarity with *CEN* and *TFL1* (Pnueli et al. 1998). Moreover, *SP* gene plays an essential role in maintaining plant growth at the shoot apexes and over-expression of *SP* or *CEN* in tomato can rescue *sp* mutant phenotype (Pnueli et al. 1998, 2001; Soyk et al. 2017).

The development of high plants is the maintenance of organogenesis in plant throughout their life cycle by the meristems (Birnbaum and Alvarado 2008). It is known that *TFL1*/*CEN* gene could maintain the meristem indeterminacy, loss-of-function mutations resulted in dramatic changes in plant architecture, causing the formation of terminal flowers that exhausts the SAM (Périlleux et al. 2019). The enlarged SAM in D226 at 15 DAP and the decreased SAM size in D226 at 30 DAP, as well as insignificant change in CCMC SAM within 15 and 30 DAP, demonstrated that *CsCEN* may function to maintain SAM size (Fig. 4b–f). Notably, *CsSTM* and *CsWUS* expression was upregulated in the SAM of D226 (Fig. 4g, h). Therefore, we hypothesized that *CsCEN* regulated SAM development by inhibiting the expression of *CsSTM* and *CsWUS* genes. However, more research is still needed to elucidate the mechanisms used by *CsCEN* gene in regulating the formation of growth habit which influences the development of SAM in cucumber, thus affecting the regulatory networks of stem cells by cell division and differentiation which is key question for understanding the formation of growth habit.

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Authors contribution statement JFC and JOO conceived and supervised the projects. MKN, FY and XYW collected phenotypic data from field. MKN and FY performed the experiments. MKN, FY and JL analyzed the data and wrote manuscript. JL, JFC and JOO contributed to revising the manuscript. All authors reviewed and contributed in drafting the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards The experiments were performed in accordance with all relevant Chinese laws.

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