Genetic analysis and mapping of a short-internode gene (cladw) in watermelon (Citrullus lanatus L.)

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Abstract Internode length (IL) is an important characteristic of plant architecture of watermelon (Citrullus lanatus L.). A dwarf type plant phenotype can support the greater planting density and land utilization for well growth of crop plants. In this study, two watermelon lines "W1-1 (standard vine) and ZXG01061 (dwarf vine)" were used as parental lines and F₁, F₂, BC₁P₁ and BC₁P₂ generations were developed for dwarf trait inheritance analysis and candidate gene identification. Genetic analysis of two year’s collected phenotypic data indicated that watermelon dwarfism was regulated by a single recessive gene (cladw). Bulked segregant analysis sequencing (BSA-seq) total of 1.24-Mb genomic region harbouring the candidate dwarfism gene on chromosome 9. Fine genetic with 1,097 F₂ plants signified that the cladw locus was finally delimited to a 203-kb region containing 10 candidate genes (including five genes annotated as GID1L2 gibberellin (GA) receptors). Endogenous hormone quantification analysis also showed that the internode GA content of ZXG01061 was higher than that of W1-1. When ZXG01061 plants were treated with exogenous application of GA3, then original plant height was not recovered, indicating that ZXG01061 is GA insensitive. Further, Cla010254 and Cla010256 (annotated as gibberellin receptor GID1L2) exhibited base deletions in ZXG01061 compared with W1-1. The expression of Cla010254 in W1-1 was significantly higher than that of ZXG01061. In conclusion, our results indicated that Cla010254 is a candidate gene for regulating the watermelon dwarfism trait.

Keywords Watermelon · Dwarfism trait · Bulked segregant analysis · Fine mapping

Introduction

The core achievements of the “Green Revolution” beginning in the 1950s were the reduction of plant height and large increases in global food production
by improving the characteristics of the plants themselves (Zhang 2010; Peng 1999; Sasaki 2002). Substantial progress in increasing lodging resistance by shortening the stem has been achieved in corn, wheat, rice and other crops. Some of these modified crops have been applied in production. In the middle of the last century, scientists believed that it was very important to mine genetic resources and cultivate ideal plant types in breeding practices. In the main cultivated crops (such as corn, rice, and wheat), breeding work has been carried out on dwarf varieties and improved plant types and has yielded important progress. Plant height is a key agronomic trait in crops. If plants are too tall, lodging readily occurs and reduces yield. In contrast, dwarf varieties have the advantages of relatively high lodging resistance, amenability to close planting, and a high light utilization rate, and their yield is significantly improved. Therefore, dwarf breeding is very important for cultivating ideal plant types and has yielded important progress. Dwarf breeding is very important for the cultivation of ideal plant types, and the exploration and application of dwarfism genes.

Lines with dwarfism traits, including short internodes, short main stems and bushy growth habits, have been reported in watermelons (Citrullus lanatus L.), such as dw-1, dw-1 s, dw-2, dw-3, and dw-4 (Liu and Loy 1972; Huang 1995; Yang 2010). The first dwarf watermelon was a mutant of the standard vining watermelon inbred line WB-2 that contained a recessive dw-1 gene and exhibited short internodes resulting from abnormal internode cells (Mohr 1956). Bush Desert King is a short vining watermelon variety with the recessive gene dw-2, which results in substandard internodes. Another simply inherited recessive gene, dw-1 s, was identified in the short vining watermelon variety Somali Local (Dyutin and Afanaseva 1987). A short-vine mutant with stable heredity was found by Yang and is controlled by a single recessive gene named dw-4 (Yang 2010). Furthermore, Dong and colleagues found that Cla010726 (annotated as gibberellin 20-oxidase-like protein) on chromosome 7 is a potential dwarf candidate gene (Dong et al. 2018). Zhu reported Cldw-1, encoding an ATP-binding cassette (ABC) transporter-related protein, as a candidate gene on chromosome 9 in watermelon (Zhu et al. 2019a, b).

In cucumber, researchers have found that cp, cp2, and dw are the three genes responsible for compact and dwarf phenotypes (Robinson and Mishanec 1965; Kauffman and Lower 1976). The cp locus was fine mapped and predicted as a cytokinin oxidase on cucumber chromosome 4 (Li et al. 2011). A dwarf gene was mapped to an interval of 2.29 cM using random amplified polymorphic DNA (RAPD) markers in near-isogenic lines (NILs) derived from the pumpkin Ai10 variety (Li et al. 2007). Three recessive loci, si-1, si-2, and si-3, in the varieties UC Top Mark Bush, Persia202, and Maindwarf have been shown to be related to compaction or dwarfism in melon (Knavel 1988, 1990; Paris 1984). Furthermore, the dwarf gene mdw1 on chromosome 7 of the melon PNU-WT1 dwarf mutant was identified by Hwang et al. (2014) and is highly homologous to CKX.

Plant height is a key agronomic trait of crops. If a plant grows too tall, it is likely to droop or fall, causing yield reductions. In contrast, dwarf varieties have the advantages of relatively high lodging resistance, amenability to close planting, and a high light utilization rate, with significantly improved yields. Therefore, dwarf breeding is very important for the cultivation of ideal plant types, and the exploration and application of dwarfism genes are receiving increasing attention from breeders.

Plant stem elongation is the result of continuous division and elongation of apical meristem and intermediate meristem cells (Beavis 1991). Thus, a change in plant height must be caused by a change in the size or number of internode cells. At present, there are two main ways to study the function of dwarfism genes in plants. One is to examine the alteration of hormone synthesis pathways, and the other is to investigate the alteration of hormone signalling pathways. The main changes in hormone synthesis pathways are increases or decreases in gibberellin (GA), auxin, brassinolide or cytokinin synthesis. Such changes usually occur in the plant itself, but these variations may be accompanied by negative traits such as a low yield or flower deformity. On the other hand, concerning hormone signalling pathways, recent research has shown that problems generally originate in hormone receptors; thus, the plant hormone content may be normal, but the plant still shows a dwarf phenotype because of a mutation in the receptor. Recent studies have shown that GA plays a major role in most crops (Liu 1994), with the ability to regulate different growth and development processes, such as seed germination, stem node contact, leaf extension, and flower and fruit development. Most of the genes involved in...
GA biosynthesis and metabolism have been isolated and cloned, which provides favourable conditions for the study of the GA metabolic pathway in greater detail. As a major negative regulator of the GA signalling pathway, the DELLA protein has attracted much attention in recent years (Peng et al. 1997; Silverstone et al. 1998; Cheng et al. 2004; Wild et al. 2012; Davière J M. 2014). At present, GA is widely used in agricultural production and is an efficient and broad-spectrum plant growth regulator that plays an important role in the cultivation of new varieties with improved stress resistance and yield.

Materials and methods

Plant materials and phenotypic data collection

The male parent “ZXG01061 (P1)” is a dwarf type watermelon variety and female parent “W1-1 (P2)” is a standard type watermelon variety. The F1 generation was obtained from a cross between ZXG01061 and W1-1, and the F2 generation was derived from F1 self-crosses (Fig. 1). In the summer of 2017, P1 (n = 30), P2 (n = 30), F1 (n = 30), and F2 (n = 213) individuals were grown in a greenhouse at the Xiangyang Experimental Agricultural University, Harbin, China, and genetic inheritance analysis were performed based on bulked segregant analysis sequencing (BSA-seq). In the summer of 2018, another 1,097 F2 individuals were grown in the greenhouse and genetic inheritance verification and initial mapping of the cladw locus was done. Each ZXG01061, W1-1, F1, and F2 plant was photographed and preserved, and watermelon internode length was measured with a ruler at 50 days after sprouting (DAS). Segregation analysis was performed on the basis of chi-square analysis of the expected segregation ratios of a single gene using IBM SPSS Statistics 22.0 software (International Business Machines Corp., New York, United States).

The eighth internode tissues of ZXG01061 and W1-1 were collected in the reproductive growth period. These samples were immediately fixed in a formaldehyde-acetic acid–ethanol (FAA) mixture and stored. From the fixed samples, paraffin sections of the stem tips and flower buds were generated according to standard procedures. First, the samples were dehydrated in an alcohol series, cleared, and subjected to paraffin embedding. Then, the samples were deparaffinized with xylene, cut to a thickness of 6 to 8 microns, dewaxed, fixed with red and green dyes, and

Fig. 1 Different physiological characteristics of watermelon stem growth. (A) ZXG0106 (P1, short-vine type) showing the plant growth at 35 day; (B) W1-1 (P2, standard type) showing the plant growth at 35 day; (C) measurement of stem parameters at 6-10 internode length with different plant materials; (D) Microscopic observation of the slitted tissue at the 8th node of the stem of ZXG0106; (E) Microscopic observation of the slitted tissue at the 8th node of the stem of W1-1
sealed. Representative specimens with a complete structure according to the staining of the cleared paraffin sections were selected, sealed with neutral gum, and sliced to obtain the final specimens (Li 2009). The slices were observed and photographed with a Nikon TE2000-U microscope.

**DNA extraction and BSA-seq**

DNA extraction was performed on undamaged young leaf tissue of ZXG01061, W1-1, F1, and F2 plants, and samples were stored at −80 °C. Total genomic DNA was extracted using the modified hexadecyl trimethyl ammonium bromide (CTAB) method. The concentration and quality of DNA were verified by using 1% agarose gels and a DNA ultraviolet spectrophotometer. Two bulked DNA samples were prepared by mixing DNA equally from 30 standard-internode-length and 30 short-internode F2 plants. DNA from two bulked samples and the parental lines was selected for sequencing and then subjected to ultrasonic fragmentation, purification, end repair, sequencing, and adapter ligation. Next, the samples were filtered by 1% agarose gel electrophoresis to obtain a target insert size of 500 bp for further analysis and purification. PCR amplification was used to construct a paired-read sequencing library. The DNA of parental lines and gene pools was sequenced (20 × sequencing depth) on the Illumina X10 platform at the Beijing Genomics Institute (BGI) (Shenzhen, China).

**BSA and primary mapping**

The total resequenced reads were analysed by removing low-quality reads, reads containing adaptors, and reads with >10% unknown bases. The cleaned reads were aligned across the watermelon reference genome (97,103) (Guo et al. 2013) by using Burrows–Wheeler Aligner (BWA) software (Li and Durbin 2009). The raw reads showing single nucleotide polymorphisms (SNPs) and insertions and deletions (InDels) were sorted, and low-quality reads (<20 read depth) were removed with the SAMtools rmdup command (Li et al. 2009). The Unified Genotyper module of GATK was used to detect SNPs in multiple samples (McKenna et al., 2010). The chromosome regions related to dwarfism were determined by the Δ(SNP index) value derived from locally estimated scatterplot smoothing (LOESS) regression ($P$ value ≤ 0.01) curves at each SNP position of both bulks according to previously reported equations (Li et al. 2017). $\Delta$(SNP index) analysis of each chromosome was conducted for both types of bulks on the basis of read depth to test the significance of the SNPs according to a $P$ value ≤ 0.01 and LOESS regression, and the detected region showing a value above the threshold was designated as the main region responsible for controlling dwarfism.

**Cleaved amplified polymorphism sequence (CAPS) marker development and genetic mapping**

CAPS markers were developed based on resequencing data of the two parents (ZXG01061 and W1-1). The reads were filtered to remove all unusable regions by applying an in-house Perl program and were mapped to the watermelon reference genome (http://www.icugi.org/, 97,103 v1) using BWA with the default parameters (Li and Durbin 2009). We used SAMtools software to sort and index map reads with mapping scores > 20 (Li et al. 2009) and obtained 500 bp of flanking sequences on both sides of each candidate SNP locus identified between ZXG01061 and W1-1 with SAMtools software. The candidate SNP loci were transformed into CAPS markers using SNP2CAPS (Thiel et al. 2004). Ten restriction endonucleases (EcoRI, MboI, HindIII, PstI, MspI, BclI, TaqI, MboII, Scal, and XhoI) were used to detect the restriction enzyme cutting site and design PCR primers based on the target chromosome using the results of BSA-seq. Primers were designed with Primer Premier 6.0 (http://www.premierbiosoft.com/) and synthesized by Sangon Biotech (Table S1). The PCR amplification procedure and system were as described by Amanullah (Amanullah et al. 2018). The enzyme digestion experiment was performed according to the instructions for each restriction enzyme. Other SNP sites that could not be converted into CAPS markers were designated as Kompetitive Allele-Specific PCR (KASP) markers and genotyped at the Vegetable Research Center of the Beijing Academy of Agricultural and Forestry Sciences. The codominant polymorphic markers were selected for genotyping, with the selected individuals exhibiting recessive traits.

**Exogenous gibberellin (GA$_3$) treatment**

The two parental lines, ZXG01061 and W1-1, were exogenously treated with GA$_3$ in the greenhouse.
The GA$_3$ used for exogenous treatment was obtained from Solarbio Science and Technology Ltd. (Beijing, China). The GA$_3$ powder was dissolved in a small quantity of ethanol and diluted in distilled water (ddH$_2$O) to obtain the final solution. All plants were exogenously treated with various GA$_3$ concentrations (0.3 mmol/L, 0.9 mmol/L, and 1.5 mmol/L), while control plants were treated by simple spray application of an ethanol and ddH$_2$O mixture. Plant height was subsequently measured once per week after seed germination.

GA extraction and endogenous content determination

When the parental lines reached their reproductive growth stage (50 days after sprouting), the eighth internodes were collected from three individuals per line, and all plants showed the same growth rate according to visual observation. Fresh internode tissues were ground to a powder in liquid nitrogen. A 50 mg ground sample was weighed, and an appropriate amount of internal standard was added, followed by extraction with methanol:water:formic acid at a ratio of 15:4:1. After concentration, the extract was redissolved in 100 μL of an 80% methanol and water solution, passed through a 0.22 μm PTFE filter membrane, and placed in an injection bottle for LC–MS/MS analysis. Then, the same samples were analysed by qRT–PCR.

Gene expression analysis and candidate gene cloning

The eighth internodes of W1-1 and ZXG01061 were collected, and total RNA was extracted from 500 mg frozen tissue samples by using an EasyPure Plant RNA Kit (TransGen Biotech, China) according to the manufacturer’s instructions. The total RNA was evaluated by running a 2 μL sample in a 1% formamide denaturing gel. The total RNA sample obtained from a pool composed of material from 6 representative plants was used for cDNA synthesis. A 1 μg total RNA sample was initially used for first-strand synthesis, and double-stranded cDNA was synthesized by using the SMART™ cDNA Library Construction Kit for the determination of total RNA and cDNA.

Gene expression levels were identified by quantitative real-time polymerase chain reaction (qRT–PCR) using a real-time PCR system (Analytik Jena AG, Germany) with SYBR Green Master Mix reagent (Novogene, Beijing, China) according to the manufacturer’s instructions. Each sample was analysed with three biological replicates. Cla020175 was used as the internal control (Wang et al. 2016). Specific transcript amplification was verified by the observation of a single peak in the melting curve analysis after completion of the amplification reaction. Negative controls without any cDNA template were included in each run to test for potential impurities. The relative gene expression levels were determined by the $2^{ΔΔCT}$ method (Livak and Schmittgen 2001). The primers used for qRT–PCR are listed in Table S2. The cloning of the candidate gene sequence was performed in W1-1 and ZXG01061 with the primers listed in Table S3. The amplified targeted fragments were then inserted into the pMD18-T vector and sent to Sangon Biotech (Shanghai, China) for sequencing. The protein structure of the candidates was predicted with the online software PredictProtein through https://predictprotein.org/.

Results

Phenotypic and genetic analysis of short vine traits

ZXG01061 plants showed slower growth, shorter internodes and a shorter plant height than W1-1 plants. Another obvious feature of ZXG01061 was that it had fewer branches. The length of the main vine and average internode length of ZXG01061 (4.31 ± 0.98 cm, Fig. 1A) were shorter than those of W1-1 (8.77 ± 0.64 cm, Fig. 1B), showing significant differences compared with those of normal vines (Fig. 1 C). The middle eighth internodes of ZXG01061 and W1-1 were selected to analyse the number and size of internode cells after sampling. The cytological results showed that the number of ZXG01061 (Fig. 1D) cells per unit area was significantly greater than that of W1-1 cells (Fig. 1E), and the cell size of ZXG01061 was obviously smaller than that of W1-1. The internode cells of ZXG01061 were arranged in a disorderly manner. Therefore, the main reason for the short vine phenotype of ZXG01061 was smaller cell size.

A total of 162 normal-vine plants and 51 short-vine plants were obtained in 2018 year, while in 2019 year, the numbers of normal vine and short-vine individuals were 850 and 247, respectively, which
were consistent with the segregation ratio of 3:1. For BC\textsubscript{1}P\textsubscript{1} and BC\textsubscript{1}P\textsubscript{2}, the normal vine and the short-vine plants showed segregation ratios of 1:0 and 1:1, respectively (Table 1). These data indicated that the short vine trait of ZXG01061 was controlled by a single recessive gene, which was tentatively named as \textit{cladw}.

### BSA-seq and genetic mapping of the \textit{cladw} locus

According to the BSA-seq results, one obvious signal (approximately 1.24 Mb, Fig. 2A) related to \textit{cladw} was detected on chromosome 9 ranging from 29.27 Mb to 30.51 Mb (Fig. 2B). Nine polymorphic pairs of CAPS primers were designed based on the parental line resequencing data in the BSA-seq region. Individuals with the short vine phenotype from 2019 were selected for genotyping and initial \textit{cladw} locus mapping. The \textit{cladw} locus was preliminarily mapped to a 312.527-Kb region (from 29,275,926 bp to 29,588,453 bp) between markers 1061C920 and 1061C926 (Fig. 2C). To narrow this region, 23 recombinant plants were selected from 800 F\textsubscript{2} plants with CAPS markers 1061C920 and 1061C926. Five KASP markers were then developed in the initial mapping region to genotype the 23 recombinants for recombinant event detection. Finally, the \textit{cladw} locus was delimited to a 203.087-Kb physical distance (from 29,306,232 bp to 29,509,319 bp) between markers mc908 and mc912 (Fig. 2D).

### Candidate gene analysis in the mapping region

According to the gene annotation results of the watermelon reference genome, a total of 10 candidate genes were detected in the shortened 203-Kb mapping region (Table 2). \textit{Cla010253}, \textit{Cla010254}, \textit{Cla010255}, \textit{Cla010256} and \textit{Cla010258} encode GA receptors. The coding sequences of five GA receptor genes were compared between ZXG01061 and W1-1 with the resequencing data. The results significantly revealed a G base deletion in the coding region of \textit{Cla010254} in ZXG01061. Additionally, in \textit{Cla010256}, a C base deletion in the coding region was also detected. This base deletion caused amino acid sequence alterations. Other GA receptor genes (\textit{Cla010253}, \textit{Cla010255}, \textit{Cla010256}, and \textit{Cla010258}) did not contain any sequences or structural variations corresponding to the trait. To further confirm the best candidate gene regulating the \textit{cladw} locus, we detected the relative gene expression patterns of the five GA receptor genes. The results showed that the expression levels of \textit{Cla010254} in W1-1 were significantly higher than those in ZXG01061 (Fig. 3). This indicated that W1-1 synthesizes more GA receptors, which is consistent with its phenotype. \textit{Cla010255} and \textit{Cla010256} also exhibited an expression difference, but we did not detect any variations in the coding region between the parental lines, and the expression pattern did not correspond to our endogenous GA verification. Based on the above results, we predicted that \textit{Cla010254} is the best candidate gene for the \textit{cladw} locus. There was only one exon for the \textit{Cla010254} coding sequence, with 921 base pairs encoding 306 amino acids. We also cloned the coding sequences of \textit{Cla010254} from ZXG01061 and W1-1 and found the G base deletion (Fig. 4A). Moreover, the protein structures of ZXG01061 and W1-1 exhibited an obvious discrepancy based on the G base deletion (Fig. 4B and C).

### Verification of the dCAPS3 marker

In a previous study reported by Zhu (Zhu et al., 2019a, b), \textit{cldw-1} was also inferred to be a dwarf trait gene located near our mapping region, but the candidate

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**Table 1** Segregation ratios of the plant types in developed six generations

| Year | Group | Standard type | Dwarf type | Expected ratio | Actual ratio | \(\chi^2\) |
|------|-------|---------------|------------|----------------|--------------|---------|
| 2018 | P\textsubscript{1} | 30 | 0 | – | – | – |
| 2018 | P\textsubscript{2} | 0 | 30 | – | – | – |
| 2018 | F\textsubscript{1} | 30 | 0 | – | 0 | – |
| 2018 | BC\textsubscript{1}P\textsubscript{1} | 100 | 0 | 1.0 | 1.1:1 | 0.693 |
| 2018 | BC\textsubscript{1}P\textsubscript{2} | 55 | 50 | 3.0 | 3.17:1 | 0.637 |
| 2018 | F\textsubscript{2} | 162 | 51 | 3.0 | 3.44:1 | 0.06 |
| 2019 | F\textsubscript{2} | 850 | 247 | 3.0 | 3.44:1 | 0.06 |
A gene was different from ours. *dcap3* is a gene marker reported by Zhu (Zhu et al., 2019a, b) that co-segregated with the dwarf trait in their F₂ mapping population and the natural population. Ninety-two short-vine watermelon plants with a short vine phenotype in the F₂ group were randomly selected and genotyped with *dcap3*. However, in these F₂ individuals, the marker did not co-segregate with the short-vine gene, indicating that the marker was suitable for locating the

cldw-1 gene but not the cladw gene (Fig. 4D). *Cladw* is a new dwarfism gene in watermelon.

Determination of endogenous GA levels

To determine the relationship between dwarfism and GA in the ZXG01061 parent, various exogenous GA₃ treatments were carried out (Fig. 5A to D, E to H). The results of endogenous GA₃ quantification
(ng/g) showed that GA3 insensitivity was the only factor leading to dwarfism in ZXG01061 parental plants (Fig. 5A to D) compared with W1-1 plants (Fig. 5E–H). The plant height for W1-1 increased as the GA3 concentration increased (18.5 ± 1.3 cm, 21.6 ± 1.4 cm, 25.8 ± 3.6 cm, and 30.3 ± 2.1 cm for the control, 0.3, 0.9, and 1.5 mmol/L treatments, respectively). For ZXG01061, the plant height was 9.0 ± 1.5 cm, 10.2 ± 2.6 cm, 10.3 ± 1.9 cm, and 10.7 ± 0.9 cm at the same GA3 concentration levels. GA15, GA19 and GA3 were also analysed in the middle internodes of ZXG01061 and W1-1 plants. The results showed that the endogenous GA3 content in ZXG01061 was significantly higher than that in W1-1 (Fig. 6). Therefore, the selected candidate genes were appropriate in terms of physiology.

### Discussion

Short-vine plants with short internodes are compact and suitable for high-density cultivation. To a certain extent, this phenotype can reduce the requirement for land resources, contribute to the full use of light resources and improve the yield per unit area. From the perspective of farmers, dwarf watermelon does not require pruning and shows precocious development; these characteristics help reduce labour requirements and costs and improve economic benefits. Therefore, an important step in watermelon production is the selection of excellent varieties, and the breeding of dwarf plants is an important goal. In the production and cultivation of watermelon, mostly long-vine watermelon varieties are used, while there are fewer available short-vine watermelon varieties.

The normal short-vine watermelon variety ZXG01061 shows reduced agronomic size characteristics relative to those of the cultivated variety W1-1. It has the advantages of early maturity, a high yield per plant and amenability to high planting density and can be used to develop new high-quality germplasm resources. The plants of this variety have only one main vine and few lateral branches, which greatly reduces the workload in production.

Our genetic mapping results showed that the dwarfism gene cladw was located between markers mc908 and mc912 on chromosome 9, a region including ten candidate genes. In determining which of the ten genes was the gene regulating the dwarfing of ZXG01061, the following four lines of evidence proved that Clad010254 was the candidate gene: A. The length of the main vine did not differ among

### Table 2  Functional annotation of candidate genes regulating short-vine trait in watermelon

| Gene ID    | nsSNP | Gene annotation                  | Physical location                      |
|------------|-------|----------------------------------|----------------------------------------|
| Clad010253 | 0     | Gibberellin receptor GID1L2       | Chr9: 29,306,920 to 29,307,876 (−)     |
| Clad010254 | 1     | Gibberellin receptor GID1L2       | Chr9: 29,339,351 to 29,340,271 (−)     |
| Clad010255 | 0     | Gibberellin receptor GID1L2       | Chr9: 29,353,298 to 29,356,377 (−)     |
| Clad010256 | 2     | Gibberellin receptor GID1L2       | Chr9: 29,372,950 to 29,373,876 (−)     |
| Clad010257 | 0     | Unknown Protein (AHRD V1)         | Chr9: 29,397,681 to 29,397,989 (+)     |
| Clad010258 | 0     | Gibberellin receptor GID1L2       | Chr9: 29,400,906 to 29,401,829 (−)     |
| Clad010259 | 1     | Nucleic acid binding protein      | Chr9: 29,440,544 to 29,441,505 (+)     |
| Clad010260 | 1     | AR781 similar to yeast pheromone receptor | Chr9: 29,455,218 to 29,456,893 (+)     |
| Clad010261 | 0     | Peptidyl-prolyl cis–trans isomerase D | Chr9: 29,500,015 to 29,504,845 (−)     |
| Clad010262 | 1     | Peroxidase 72                     | Chr9: 29,519,125 to 29,521,220 (−)     |
the tested GA₃ concentrations, which indicated that ZXG01061 was a GA-insensitive mutant and that its dwarf phenotype was due to the dysfunction of key genes in the GA pathway. B. The endogenous GA₃ content of ZXG01061 was significantly higher than that of W1-1, which indicated that ZXG01061 could synthesize GA₃ normally and that a large amount of GA accumulated in the plants due to defects in the transmission pathway. C. Real-time PCR results showed that the expression level of the Cla010254 gene in W1-1 was significantly higher than that in ZXG01061, indicating that ZXG01061 synthesized
fewer bioactive GA receptors than W1-1. D. According to the \textit{Cla010254} gene sequence information in the published watermelon reference genome, the coding sequence of \textit{Cla010254} was amplified in the W1-1 and ZXG01061 parents, and a mutation was found in the coding region of \textit{Cla010254} in ZXG01061. Our results indicate that ZXG01061 is a GA transmission-deficient mutant and that the reason for its dwarf phenotype is that endogenous GA3 cannot be transmitted normally. Because of the mutation in the coding region of the \textit{Cla010254} gene, the translation of the \textit{Cla010254} gene is disturbed at the transcription step, resulting in amino acid substitutions that change the structure of the original protein. Thus, normal \textit{Cla010254} gene expression fails, and the original protein function is lost.

Both \textit{cladw} and \textit{cldw-1} (Zhu et al. 2019a) are located on chromosome 9, and the distance between the two genes is short. The dCAPS3 marker which was reported in Yang et al., 2019, which cosegregated with the dwarfism trait in the F2 mapping population and natural population, indicating that the functional \textit{dcap3} marker of the \textit{cldw-1} gene can be used for marker-assisted selection (MAS) in plant height
breeding or as a basis for watermelon genomic selection breeding. However, this marker could not distinguish the plants in our F2 population. Thus, the short-vine gene of ZXG01061 may not be the *cldw-1* gene and was thus named *cldw*. The plant height of the ZXG01061 inbred line is 60–80 cm, and its field characteristics are stable. Our results showed that the phenotype was controlled by a pair of recessive gene alleles. Following a cross with a conventional inbred line, the internodes of F2 dwarf vine plants were shortened, while other plant characteristics remained unchanged. Furthermore, the plant height of the inbred line did not change after treatment with different concentrations of GA3 in different periods, indicating that it may be a GA-insensitive inbred line, which needs further study. Therefore, the short-vine gene should have good application prospects in watermelon breeding. This material can be used as a new dwarf resource to breed new watermelon varieties by crossing with other excellent inbred lines, and this gene can potentially be transferred into excellent corn varieties or other crops to play a unique role in crop breeding and production.

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**Author contributions** FL and HL supervised the project and participated in revision of the manuscript. JL designed the experiments, performed the studies and wrote the draft of the manuscript. PG and XW participated in sample preparation and phenotypic data collection. SM and JW provided the seeds of ZXG01061. All authors have read and approved the final manuscript.

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**Declarations**

**Conflict of interest** The authors have no competing interests to declare that are relevant to the content of this article.

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