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

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Abstract

Internode length is an important plant type characteristic of watermelon (*Citrullus lanatus* L.). A dwarf phenotype can increase plant density and land utilization. In this study, W1-1 (standard vine) and ZXG01061 (dwarf) were used as the parental lines to create F_1, F_2, BC_1P_1 and BC_1P_2 progenies for dwarf trait inheritance analysis and candidate gene identification. Genetic analysis indicated that the watermelon dwarf trait was regulated by a single recessive gene (*cladw*) with phenotypic data collected over two years. The bulked segregant analysis (BSA-seq) revealed a 1.24 Mb genomic region harbouring the candidate dwarfing gene on chromosome 9. Through initial and fine mapping with 1,097 F_2 plants, the *cladw* locus was finally delimited into a 203 kb region containing 10 candidate genes (including five genes annotated as GID1L2 gibberellin GA receptors). Hormone analysis showed that the GA content of the ZXG01061 internode was higher than that of W1-1. When ZXG01061 plants were treated with exogenous GA, the original plant height could not be recovered, indicating that ZXG01061 is GA insensitive. *Cla010254* and *Cla010256* (annotated as gibberellin receptor GID1L2) in ZXG01061 had base deletions compared with W1-1. The expression pattern of *Cla010254* in W1-1 was significantly higher than that in ZXG01061. In conclusion, our results indicate that *Cla010254* is a candidate gene for watermelon dwarfing.

Introduction

The core of the “green revolution” started in the 1950s was the reduction of plant height and greatly increasing world food production by improving the characteristics of the plants themselves (Zhang 2010; Peng 1999; Sasaki 2002). Great success in increasing lodging resistance by reducing the length of the stem has been achieved in corn, wheat, rice and other crops. Some of these crops have been applied in actual production. In the middle of the last century, scientists believed that it was very important to excavate genetic resources and cultivate ideal plant types in breeding practice. 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 achieved important research progress. Plant height is a key agronomic trait of crops. If plant height is too high, lodging readily occurs and causes yield reduction. In contrast, dwarf varieties have the advantages of relatively high lodging resistance, amenability to close planting, and a high light energy utilization rate, and their yield is significantly improved. Therefore, dwarf breeding is very important to cultivate ideal plant types, and increasing attention has been given to the discovery and application of dwarf genes.

Crops with dwarf traits, including short internodes, short main stem length and bush-type 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 mutation from the standard vining watermelon inbred line WB-2, which 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 due to the recessive gene *dw-2*, which has substandard internodes. Another simply inherited recessive gene *dw-1 s* for the short vining watermelon variety was identified from “Somali Local”

(Dyutin and Afanaseva 1987). A short vine mutant with stable heredity has been 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 may be a potential dwarf candidate gene (Dong et al. 2018). Zhu reported that (an ATP-binding cassette transporter ABC transporter-related protein) was the candidate gene for *Cldw-1* in watermelon on chromosome 9 (Zhu et al., 2019).

In cucumber, researchers have found that *cp*, *cp2*, and *dw* are the three genes responsible for compact and dwarf phenotypes, respectively (Robinson and Mishanec 1965; Kauffman and Lower1976). The *cp* locus was fine mapped and predicted as 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, respectively, in melon (Knavel 1988; Paris 1984; Knavel 1990). Furthermore, the dwarf gene *mdw1* of the melon PNU-WT1 dwarf mutant on chromosome 7 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 high, 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 dwarfing 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). 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 dwarfing genes. One is to examine the alteration of hormone synthesis pathways in plants, and the other is to investigate the alteration of hormone signalling pathways in plants. 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, current research results show 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). GA is a widely existing plant hormone that can 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 plant stress resistance and crop yields.

Materials And Methods

Plant materials and phenotypic data collection

The male parent, ZXG01061 (P_1), is a dwarf watermelon variety. The female parent, W1-1 (P_2), is a standard watermelon variety. The F_1 generation was obtained from a cross between ZXG01061 and W1-1, and the F_2 generation was derived from a self-cross of F_1 (Fig. 1). In the summer of 2017, individuals of P_1 (n = 30), P_2 (n = 30), F_1 (n = 30), and F_2 (n = 213) were all grown in a greenhouse at the Xiang Yang Experimental Agricultural Station of Northeast Agricultural University, Harbin, China, for genetic inheritance analysis and BSA-seq analysis. In the summer of 2018, 1,097 F_2 individuals were grown in the greenhouse for genetic inheritance verification and initial mapping of the *cladw* locus. Each plant of ZXG01061, W1-1, F_1, and F_2 individuals was photographed and preserved, and watermelon internode length was measured with a ruler at 50 days after sprouting. Segregation analysis was based on 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 dye, and sealed. Representative specimens with a complete structure according to the staining of the cleared paraffin sections were selected, sealed with neutral gum sealed, 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, F_1, and F_2 plants, and samples were stored at -80°C. Total genomic DNA was extracted using the modified CTAB (hexadecyl trimethyl ammonium bromide) 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- and 30 short-internode-length F_2 plants. DNA from two bulked samples and the parental lines were 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 analysis 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 (97103) (Guo et al., 2013) by using the Burrows–Wheeler Aligner (BWA) software package (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 region related to dwarfism was 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). Δ(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 of ≤ 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/, 97103 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 (*Eco*RI, *Mbo*I, *Hin*dIII, *Pst*I, *Msp*I, *Bcl*I, *Taq*I, *Mbo*II, *Sca*I, and *Xho*I) 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 1). The PCR amplification procedure and system were described by 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 individuals exhibiting recessive traits.

Exogenous gibberellin (GA3) treatment

The two parental lines, ZXG01061 and W1-1, were exogenously treated with GA3 in the greenhouse. The GA3 used for exogenous treatment was obtained from Solarbio Science and Technology Ltd. (Beijing,

China). The GA3 powder was dissolved in a small quantity of ethanol and diluted in distilled water (ddH_2O) to obtain the final solution. All plants were exogenously treated with various GA3 concentrations (0.3 mmol/L, 0.9 mmol/L, and 1.5 mmol/L), while control plants were treated by the simple spray application of an ethanol and ddH_2O mixture. Plant height was subsequently measured once a week after seed germination.

GA extraction and content determination

When the parental lines reached their reproductive growth stage (50 days after sprouting), the eighth internodes were collected from three individuals for each 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 water solution, passed through a 0.22 μm PTFE filter membrane, and place in an injection bottle for LC–MS/MS analysis. Then, the same sample was 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^{TM} 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 $2^{\Delta\Delta CT}$ method (Livak and Schmittgen. 2001). The cloning of the candidate gene sequence was performed in W1-1 and ZXG01061 with the primers in Table 1. The amplified targeted fragments were then inserted into the pMD18-T vector and sent to Sangon Biotech (Shanghai, China) for sequencing.

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 (Fig. 1). 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) were shorter than those of W1-1 (8.77±0.64 cm), showing significant differences from normal vines. The middle eighth internodes of ZXG01061 and W1-1 were selected for the study of the number and size of internode cells after sampling. The cytological results showed that the number of ZXG01061 cells per unit area was significantly greater than that of W1-1 cells, 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 the smaller cell size (Fig. 1).

A total of 162 normal vine plants and 51 short vine plants were obtained in 2018, while in 2019, the numbers of normal vine and short vine individuals were 850 and 247, respectively, which fit for the segregation ratio of 3:1. For BC_1P_1 and BC_1P_2, the normal vine and the short vine plants showed ratios of 1:0 and 1:1, respectively (Table 4). These data indicated that the short vine trait of ZXG01061 was controlled by a single recessive gene tentatively named *cladw*.

BSA-seq and genetic mapping of the *cladw* locus

According to the BSA-seq results, one obvious signal (approximately 1.24 Mb) related to *cladw* was detected on chromosome 9 ranging from 29.27 Mb to 30.51 Mb (Fig. 4). Nine polymorphic pairs of CAPS primers were designed based on the parental lines resequencing data in the BSA-seq region. Individuals with the short vine phenotype from 2019 were selected for genotyping and initial *cladw* locus mapping. The *cladw* locus was preliminarily mapped into a 312.527-Kb region (from 29,275,926 bp to 29,588,453 bp) between markers *1061C920* and *1061C926* (Fig. 4). To narrow this region, 23 recombinant plants were selected from 800 F_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 *cladw* locus was delimited into a 203.087-Kb physical distance (from 29,306,232 bp to 29,509,319 bp) between markers *mc908* and *mc912* (Fig. 5).

Determination of endogenous GA levels

To determine the relationship between dwarfing and GA in the ZXG01061 parent, various exogenous GA3 treatments were carried out (Fig. 2). The results of endogenous GA3 quantification (ng/g) showed that GA3 insensitivity was the only factor leading to dwarfing in ZXG01061 parental plants. 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. 3). Therefore, the appropriateness of the selected candidate genes was supported from a physiological point of view.

Candidate gene analysis in the mapping region

According to the gene annotation of the watermelon reference genome, 10 candidate genes were detected in the 203-Kb mapping region (Table 5). *Cla010253*, *Cla010254*, *Cla010255*, *Cla010256* and *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 showed that there was a G base deletion in coding region ^{847} of *Cla010254* in ZXG01061. Additionally, in *Cla010256*, a C base deletion in coding region 104 was also detected. These variations caused amino acid sequence alterations. Other candidate genes did not contain any sequences or structural variation. To confirm which gene was the best candidate gene for the *cladw* locus, we identified the expression pattern of the five GA receptor genes. The results showed that the expression levels of *Cla010254* in W1-1 were significantly higher than those in ZXG01061. This indicated that W1-1 synthesizes more GA receptors, which is consistent with its phenotype. *Cla010255* 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 *Cla010254* may be the best candidate gene for the *cladw* locus (Fig. 3). We also cloned the coding sequences of *Cla010254* from ZXG01061 and W1-1 and found that a G base deletion existed.

Verification of the *dcaps3* marker

In a previous study reported by Zhu (Zhu et al., 2019), *cldw-1* was also a dwarf trait gene which located near our mapping region but the candidate gene was different from our results. The *dcap3* was a gene marker reported by Zhu (Zhu et al., 2019) which cosegregated with the dwarf trait in their F_2 mapping population and the natural population. Ninety-two short vine watermelon plants with a short vine phenotype in the F_2 group were randomly selected and genotyped with *dcap3*. However, in these F_2 individuals, the marker did not cosegregate with the short vine gene, indicating that the marker was only suitable for locating the *cldw-1* gene and not the *cladw* gene (Fig. 6). *Cladw* is a new dwarf trait gene in watermelon.

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 contribute to reducing labour requirements and costs and improving 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, it is mostly long vine watermelon varieties that 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 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.

The results of our genetic mapping showed that the dwarfing gene *cladw* was located between markers *mc908* and *mc912* on chromosome 9, 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 *Cla010254* was the candidate gene: A. The length of the main vine did not differ among the tested GA 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 *Cla010254* gene sequence information in the published watermelon reference genome, the coding sequence of *Cla010254 was* amplified from the W1-1 and ZXG01061 parents, and a mutation was found in the coding region of *Cla010254* in ZXG01061. Our results indicate that ZXG01061 is a GA transmission-deficient mutant and that the reason for its dwarfing phenotype is that endogenous GA3 cannot be transmitted normally. Because of the mutation in the coding region of the *Cla010254* gene, the translation of the *Cla010254* gene is disturbed at the transcription step, resulting in amino acid substitutions that change the structure of the original protein. Thus, normal *Cla010254* gene expression fails, and the original protein function is lost.

Both *cladw* and *cldw-1* (Yang. 2019) are located on chromosome 9, the distance between the two genes is short. The dcap3 marker was reported in Yang et al, 2019, which cosegregated with the dwarf trait in the F_2 mapping population and the natural population, indicating that the functional *dcap3* marker of the *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 F_2 population. Thus, the short vine gene of ZXG01061 may not be the *cldw-1* gene and was named *cladw*.

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 F_2 dwarf vine plants were shortened, while other plant characteristics were unchanged. Furthermore, the plant height of the inbred line did not change after treatment with different concentrations of GA in different periods, indicating that it may be a gibberellin-insensitive inbred line, which needs further study. Therefore, the short vine gene should have good application prospects for use 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.

Declarations

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Author contributions Conceptualization

F. S. and H.L. supervised the project and participated in revision of the manuscript. J. L. designed the experiments, performed the studies and writing the draft manuscript. P. G. and X. W. participated in sample preparation and phenotypic data collection. S. M. and J. W. provided the seed of ZXG01061. All authors have read and approved the final manuscript.

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

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Tables

Table 1 PCR primer sequences used in this study

CAPS markers	Primer sequences (5'→3')	Enzyme
1061C916	F: GTCCAAACACCCACCAAAGT	TaqI
	R: TGATCTAGTTCCAAGTTGCCTT	
1061C920	F: TGGAGATATAGAGGGAGGAGTC	MboI
	R: GGTTGTGTTCTTCAGCTTCAAT	
1061C924	F: CACTAGAATCAAACACTCAACC	ScaI
	R: GATTTGTCGAGGGCTTTGC	
1061C926	F: ACCTTCCTTCTTCACCTTCATT	XhoI
	R: CAGCAGCAACCACAACCA	
1061C935	F: TTCATATTCACGACAACCCTCT	TaqI
	R: TCATCATCCCTTTGTTCACACT	
1061C940	F: TCCTCAATCAATGACTCCTTCC	EcoRI
	R: TTGCGTATAATGTTGCTGATGC	
1061C948	F: AGAAGCACATGAATGAGGGAAA	TaqI
	R: CAACACACAAAGGGTTCAATGA	

Table 2 PCR primer sequences and candidate genes clone primer sequences used in this study

Marker name	Primer sequences (5'→3')
10253	AGGTGAGAGGACGGCATTAC
	TCGCAGGTCGGATTGAACA
10254	TACCACCGCCATCTCAACTC
	GCAATTCCATTCAACCACTCCT
10255	GCCTGCTACGACGACTGTT
	AGTTGCTCTGCTGAAGAACCT
10256	CCTGCCTCTCCAATGTTCCA
	CCGTCCAAGCGTCCTCATAT
10258	GTCTGGTTCATCCGTTCTTCAT
	AGATCCGCCGCCAATATCA

Table 3 Candidate genes clone primer sequences used in this study

Marker name	Primer sequences (5'→3')
Cla010253	ATGGCTTCCGATGAAATTGCC
	TTAACCACTGCGGCCGCTGCTGGTGAA
Cla010254	ATGCTCATCGTTTACAAAGACGG
	TCACTCAGGGCGCCCGCCATTGATGAA
Cla010255	ATGGCTTCTTCTATAAAC
	CTAATCTAAGTTGATGAAAG
Cla010256	ATGGATTCCCAGGAAGACGAAAT
	TTAGGATAACGGTGATTG
Cla010258	ATGGATTCTGAGGCCGGCGAT
	TTACATTGATTGGATAAAGGAAGCG

Table 4. Segregation ratios of the plant types in six generations

Years	Group	Standard type	Dwarf type	Expected ratio	Actual ratio	c2
2018	P_1	30	0	-	-	-
2018	P_2	0	30	-	-	-
2018	F_1	30	0	-	0	-
2018	BC_1P_1	100	0	-	-	-
2018	BC_1P_2	55	50	1:01	1.1:1	0.693
2018	F_2	162	51	3:01	3.17:1	0.637
2019	F_2	850	247	3:01	3.44:1	0.06

Table 5 Functional annotation of candidate short vine genes

Gene ID	nsSNP	Gene annotation	Physical location
Cla010253	0	Gibberellin receptor GID1L2	Chr9: 29,306,920 to 29,307,876 (-)
Cla010254	1	Gibberellin receptor GID1L2	Chr9: 29,339,351 to 29,340,271 (-)
Cla010255	0	Gibberellin receptor GID1L2	Chr9: 29,353,298 to 29,356,377 (-)
Cla010256	2	Gibberellin receptor GID1L2	Chr9: 29,372,950 to 29,373,876 (-)
Cla010257	0	Unknown Protein (AHRD V1)	Chr9: 29,397,681 to 29,397,989 (+)
Cla010258	0	Gibberellin receptor GID1L2	Chr9: 29,400,906 to 29,401,829 (-)
Cla010259	1	Nucleic acid binding protein	Chr9: 29,440,544 to 29,441,505 (+)
Cla010260	1	AR781 similar to yeast pheromone receptor	Chr9: 29,455,218 to 29,456,893 (+)
Cla010261	0	Peptidyl-prolyl cis-trans isomerase D	Chr9: 29,500,015 to 29,504,845 (-)
Cla010262	1	Peroxidase 72	Chr9: 29,519,125 to 29,521,220 (-)

Figures

Figure 1

Differences in plant characteristics between the dwarf watermelon line ZXG01061(A) , the standard vining line W1-1(C), F_1 (E), and F_2 (F)cultivated in a plastic greenhouse. Differences in internode cells (eighth internodes) between cultivated plants of the dwarf watermelon line ZXG01061 (B) and the standard vining line W1-1 (D) determined by cytological observation.

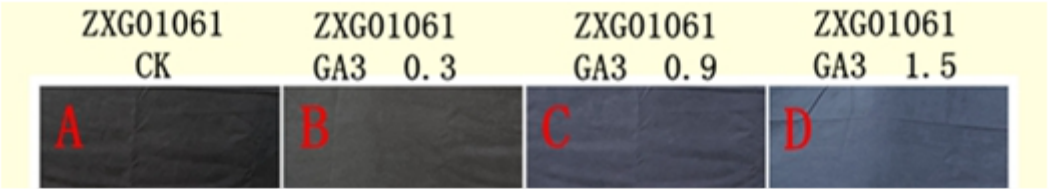

Figure 2

Differences in plant characteristics (height and internodal length) between parental lines W1-1 (P_1, standard type) and ZXG010161 (P_2, dwarf type). (A to D) Various exogenous GA3 treatments applied to ZXG01061; (E to H) Various exogenous GA3 treatments applied to W1-1.

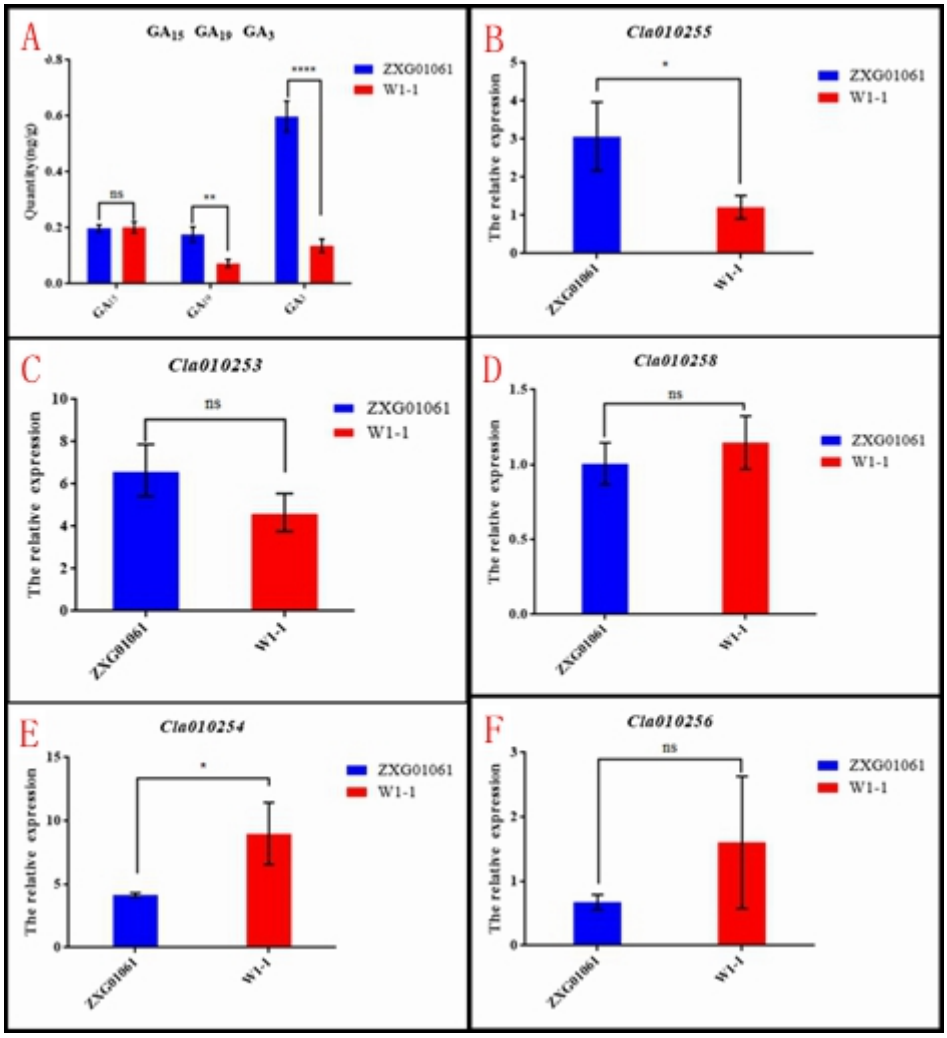

Figure 3

(A) Analysis of gibberellin receptor expression in 45-day-old watermelon seedlings of the two parental lines, ZXG01061 and W1-1. (B, C, D, E, F) Expression levels of the *Cla010255*, *Cla010253*, *Cla010258*, *Cla010254*, and *Cla010256* genes in the reproductive growth period (eighth internodes) in the two parental lines.

Figure 4

Plots of the calculated SNP index across the whole genome. The x-axis indicates the positions of 11 chromosomes of watermelon, and the y-axis represents the SNP index. The green line represents the threshold value calculated by LOESS regression (0.5). The red circle on chromosome 9 represents a significant fluctuation peak. Plots of the single nucleotide polymorphism index across chromosome 9. A candidate gene region is framed with the blue line.

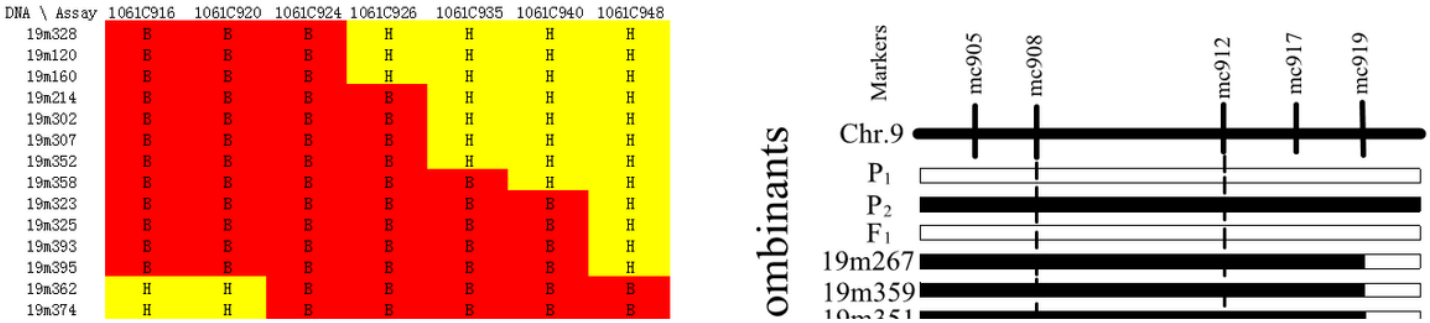

Figure 5

Genetic mapping of c*ldw* gene. The first blue column represents F_2 individuals, the first blue row represents CAPS markers; B is the genotypic code for ZXG01061 (P_2), A is the genotypic code for W1-1 (P_1), H is the genotypic code for F_1: ZXG01061 × W1-1.

Fine mapping of candidate genes controlling dwarfism in watermelon in recombinant recessive individuals. The white bar represents W1-1 (P_1), the black bar represents ZXG01061 (P_2), and the grey bar represents F_1.

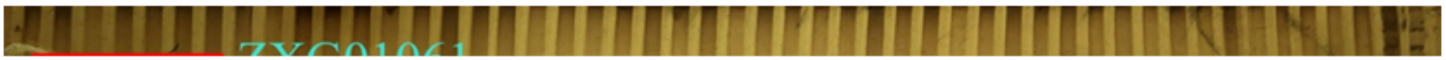

Figure 6

The 48 short internode genotypes in the F_2 population based on the analysis of dCAPS marker *dcaps3*.

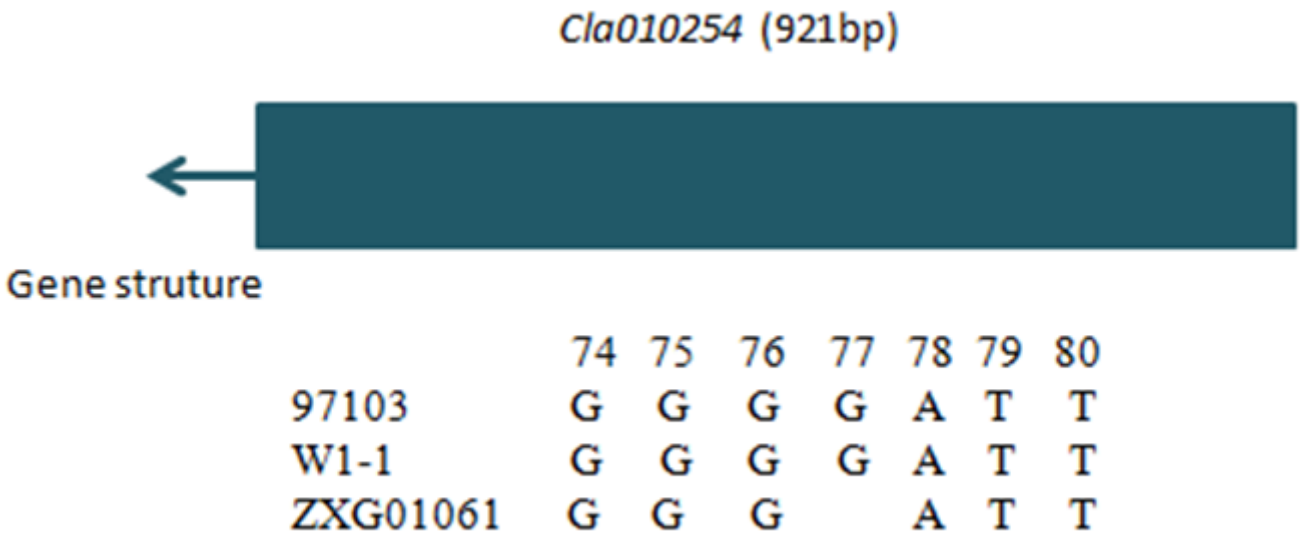

Figure 7

Sequence analysis of the watermelon candidate dwarf gene *Cla010254*.