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Rapid Mining of Candidate Genes for the Branchless Phenotype in Watermelon by Bulked Segregant Analysis Using Whole-genome Resequencing

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Abstract. Lateral branching is an important agronomic trait in horticulture plants. The aim of this project was to reveal the genetic mechanism, map the gene localization, and predict candidate genes of watermelon lateral branching. An F_2 segregating population was derived from a cross between the multibranched maternal inbred line M6 and the branchless paternal inbred line N7. Two DNA pools were constructed using 20 multibranched plants and 20 branchless plants from the F_2 population. Whole-genome resequencing was performed for the DNA pools (25×) and the parents (30×) to identify the genomic region associated with lateral branching. Candidate genes were predicted based on the gene annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses; then, quantitative validation of these genes was performed. The results showed that the clean reads of four samples yielded 64,295,076 to 81,658,958 bp, with sufficient genome coverage and high quality. Based on single-nucleotide polymorphism and insertions/deletions association analyses, the candidate genes were mapped to a 2.01-Mb region on chromosome 4 (22,958,925–24,971,894 bp) containing 182 annotated genes. During the KEGG and GO enrichment analyses, these genes were annotated to 10 cellular components, 10 molecular functions, and 12 biological processes. Eight candidate genes responsible for the branchless phenotype in watermelon were identified: *Cla97C04G076340*, *Cla97C04G075820*, *Cla97C04G076060*, *Cla97C04G076250*, *Cla97C04G076280*, *Cla97C04G076380*, *Cla97C04G076830*, and *Cla97C04G075950*. These genes were involved in amino acid biosynthesis and catabolism, TCP transcription factor activity, and regulation of flower development. This study offers valuable insights into the molecular mechanisms governing the branchless phenotype in watermelon. These candidate genes serve as potential targets for gene cloning and marker-assisted selection of watermelon cultivars without lateral branches.

Watermelon (*Citrullus lanatus*) is a climbing vine species in the Cucurbitaceae family. According to the Food and Agriculture Organization, watermelon is an important melon crop that covers approximately 7% of the total land surface dedicated to the global production of vegetables (http://faostat.fao.org). The release of the reference genomes for watermelon cultivars 97103 and Charleston Gray (Guo et al. 2013, 2019; Wu et al. 2019) coupled with the discovery of an array of insertions and deletions (InDels), SSRs, single-nucleotide polymorphisms (SNPs), and SNP bin markers within these reference genomes (Ren et al. 2012, 2014, 2015; Zhu et al. 2016) established the foundation for the genetic analysis of various traits in watermelon. Building on this foundation, numerous genes governing important agronomic traits in watermelon have been mapped and studied, including the leaf delayed green gene *dg* (Gebremeskel et al. 2023), fruit shape gene *ClFS1* (Dou et al. 2018), rind hardness gene *ClERF4* (Liao et al. 2020), rind color gene *ClCGMenG* (Li et al. 2019), canary-yellow flesh gene *Cyf* (Liu et al. 2023), black seedcoat gene *ClCS1* (Li et al. 2020), lobed leaf gene *ClLL1* (Wei et al. 2017), and dwarfism gene (Dong et al. 2018; Wei et al. 2019; Zhu et al. 2019).

The branchless phenotype is an important agronomic trait in watermelon cultivation. Branchless cultivars have the advantages of reduced pruning needs, simplified management practices, and substantial savings in both labor and costs (Bao et al. 2022). However, the predominance of multibranched cultivars highlights a shortage of branchless watermelon germplasm resources. In this study, we constructed segregant pools using the branchless parent N7 and multibranched parent M6. We identified the genomic region associated with the branchless phenotype using a bulked segregant analysis with a whole-genome resequencing (BSA-seq) analysis. We predicted the candidate genes for the branchless phenotype in watermelon through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses and gene annotation against the Cucurbit Genomics Database. A quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed to quantitatively validate these candidate genes. Our results provide target genes and breeding materials that can be used directly for breeding watermelon cultivars with desired plant architectures. Furthermore, these findings lay a foundation for resource innovation, cultivar improvement, and watermelon cultivar breeding without lateral branches.

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Materials and Methods

Population construction and the χ^2 test. The multibranched inbred line M6 and branchless inbred line N7 were acquired using self-pollination and purification over a long period by the Watermelon and Melon Laboratory at the Vegetable Research Institute of Gansu Academy of Agricultural Sciences. The branchless plants in this experiment only exhibited minimal lateral branching at the nodes before extension. Multibranched plants in this experiment exhibited not only lateral branching at the nodes before extension but also lateral branching at the nodes after extension.

The watermelon plants were cultivated in a greenhouse located in Langou Village, Anning District, Lanzhou City, China. Conventional management practices for climbing plants were used. The parental lines were planted and crossed to obtain F_1 during Spring 2021. F_1 plants were planted and self-pollinated to obtain F_2 plants in 2022. The parents, F_1 and F_2, were planted in 2023, and their branching traits were determined. The segregation ratio of F_2 was calculated and evaluated using the χ^2 test.

The following formulas were used to calculate branching traits:

$$\text{Average number of lateral branches} = \frac{\text{total number of lateral branches}}{\text{number of plants}} \quad [1]$$

$$\text{Average total length of lateral branches} = \frac{\text{total length of lateral branches}}{\text{number of plants}} \quad [2]$$

$$\text{Average length of lateral branches} = \frac{\text{total length of lateral branches}}{\text{total number of lateral branches}} \quad [3]$$

The following formula was used for the χ^2 test:

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} \quad [4]$$

where O is the observed value and E is the expected value.

Construction and sequencing of mixed pools. Genomic DNA from young leaves was extracted using a Plant Genomic DNA Extraction Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. Watermelon DNA was extracted from 20 multibranched plants and 20 branchless plants in the F_2 population and from a single plant from each of the two parents. Equal amounts of DNA from 20 multibranched or 20 branchless plants were mixed to construct a mixed pool of multibranched plants (Hun-DUO) and a mixed pool of branchless plants (Hun-WU), respectively. Whole-genome resequencing was performed on an Illumina platform for the mixed pools (25×) and parents (30×). Libraries (350-bp fragments) were constructed for sequencing according to the manufacturer's instructions (Compass Biotechnology, Beijing, China) to generate 150-bp paired-end sequences.

Data processing. The raw reads were processed using FASTP software to remove low-quality sequences and those containing adaptors or poly-N. The resulting clean reads were used to calculate the Q20, Q30, GC content, and sequence duplication level. BWA-MEM2 software (Li and Durbin 2021) was used to align the clean reads to the reference genome Watermelon (97103) v2 Genome (http://cucurbitgenomics.org/organism/21). The mapping results were sorted and de-duplicated using SAMTOOLS (Danecek et al. 2021). The variants were called using the HaplotypeCaller algorithm of GATK (v3.8) (McKenna et al. 2010). Based on the physical positions of the variants, SNPs and InDels were annotated using SnpEff software (Cingolani et al. 2012). Association analyses were performed using a Euclidean distance (ED) algorithm (Hill et al. 2013) and SNP index/InDel index algorithms (Fekih et al. 2013; Takagi et al. 2013).

Functional annotation and prediction of genes in the candidate region. The candidate region was identified by the intersection of the SNP and InDel association results. The candidate region was aligned and annotated using BLAST software (Altschul et al. 1997) based on the following databases: NR (NCBI nonredundant protein sequences) (Deng et al. 2006), SwissProt (a manually annotated and reviewed protein sequence database), GO (Ashburner et al. 2000), KEGG Ortholog (Kanehisa et al. 2004), and COG (Clusters of Orthologous Groups of Proteins) (Tatusov et al. 2000). Candidate genes were identified based on GO and KEGG enrichment analyses of the candidate region and annotated against the Cucurbit Genomics Database (http://cucurbitgenomics.org/organism/21).

Validation of candidate genes. The qRT-PCR was used to determine the relative expression of candidate genes in the axils and stems of watermelon plants. Primers were designed using PrimerQuest Tool. The primer sequences are shown in Table 1. Actin was used as an internal control. RNA reverse-transcription was performed using a TRUEscript first Strand cDNA Synthesis kit (Aidlab, Beijing, China). Fluorescence quantification was performed using the abm® EvaGreen qPCR MasterMix-ROX kit. Three technical replicates were performed for each

Table 1. Primer sequence of candidate genes for the branchless phenotype in watermelon. Actin is the internal reference gene.

	Primer sequence (5′-3′)	
Gene	Forward primer	Reverse primer
Cla97C04G076340	GTAAGCATTCCTCATCATTG	AGTTCCTTCGTATCTTCATAA
Cla97C04G075820	GCACACTTCATCCTTCAA	TCGTCCTCTCCATTATTCA
Cla97C04G076060	GAGATAACAATCAAGGCTAATG	CATCAAGACCGACATCAATA
Cla97C04G076250	ATGTTATTATTGGTGTGCGATAC	CTGCGATATGCTGCTATACT
Cla97C04G076280	TTGATGAAGAAGTGAATG	GGAGTATTACCATAAGAAG
Cla97C04G076380	ATAAGCATTGGAGATGTATTAG	ATCAGCAACAGCACTATA
Cla97C04G076830	AAGAGTGATTGGAGATGTT	TTGAAGGTAAGCGAAGAA
Cla97C04G075950	TATCGGAGATCATTCTACAC	CGGAGATGAGTTACTACC
Actin	CCATGTATGTTGCCATCCAG	GGATAGCATGGGGTAGAGCA

Table 2. Statistics of lateral branches of different watermelon populations.

Traits	M6	N7	F1	Multibranched of F2	Branchless of F2
Average number of lateral branches	11.15	0.70	10.85	11.51	0.56
Average total length of lateral branches/cm	826.55	32.25	756.30	857.29	28.96
Average length of lateral branches/cm	74.13	46.07	69.71	74.48	51.48

treatment. The relative expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method.

Results

Lateral branching traits in the parental and F2 populations. A field investigation showed that the average number of lateral branches of M6 was 11.15, and the average total length of the lateral branches was 826.55 cm. The average number of lateral branches of N7 was 0.7, and the average total length of the lateral branches was 32.25 cm. Plants in the F_1 population from the cross of M6 and N7 showed multiple lateral branches. Its average number of lateral branches was 10.85, and the average total length of the lateral branches was 756.3 cm. These results indicated that the branchless phenotype was controlled by recessive genes (Table 2). F_1 was self-pollinated to generate 394 F_2 plants, including 298 multibranched plants and 96 branchless plants, which followed the segregation ratio conforming to the Mendelian segregation ratio of 3:1 ($\chi^2 = 0.04 < \chi^2_{0.05} = 3.84$) (Table 3). Therefore, the branchless phenotype in watermelon was controlled by a single recessive gene. The lateral branching traits of M6 and N7 are shown in Fig. 1. M6 plants showed lateral branches, tendrils, and flower buds at the axils, whereas N7 plants only showed flower buds at the axils.

Sequence quality analysis. The sequence quality was analyzed for the two mixed pools and two parental lines. The sequencing data from these four samples were filtered to yield 64,295,076 bp to 81,658,958 bp clean reads. The Q20 scores were >98%, the Q30 scores were >90%, the GC content ranged from 33.80% to 34.22%, and the ratios of alignment to the reference genome were >99%, indicating sufficient genome coverage. The genome coverages of the four samples were >97% at 5× and >89% at 10×. These results indicated that high-quality resequencing data with sufficient coverage were obtained and could be used for subsequent BSA-seq and association analyses (Table 4).

BSA-seq analysis and candidate region identification. In this study, the Δ(SNP index)/Δ(InDel index) was fitted using the SNPNUM method. Trait-associated regions were those with association values that exceeded the threshold. The SNP index and Δ(SNP index) of the two mixed pools are shown in Fig. 2, and a 2.01-Mb candidate region was identified using a confidence interval of 0.99. The InDel index and Δ(InDel index) of the two mixed pools are shown in Fig. 3, and a 3.29-Mb candidate region was identified with a confidence interval of 0.95 (Table 5).

The SNPNUM method was used to fit the ED values, and the association values are shown in Fig. 4. The median + 3 *SD*s of the fitted values for all loci was used as the association threshold. Based on the SNPs, the association threshold for the ED was estimated to be 0.04, and three regions with a total length of 2.63 Mb were identified according to this threshold. Based on the InDels, the association threshold for the ED was 0.02, and one region with a length of 4.31 Mb was identified according to this threshold (Table 5).

The regions identified through InDel and SNP association analyses were intersected. Consequently, the genes controlling the branchless phenotype in watermelon were mapped to a 2.01-Mb region (22,958,925 bp–24,971,894 bp) on chromosome 4.

Annotation and prediction of genes in the candidate region. The candidate region contained 198 genes. These genes were annotated using the BLAST algorithm against NR, SwissProt, GO, KEGG, and COG databases. A total of 182 genes were annotated, including 18 genes with nonsynonymous mutations and two genes with frame shift mutations between the parents (Table 6).

Using the GO database, 161 genes were annotated to 32 GO items (Fig. 5), including 10 cellular components, 10 molecular functions, and 12 biological processes. The biological processes genes were mainly involved in metabolic processes, cellular processes, reproductive processes, developmental processes, single-organism processes, multicellular organismal processes, signaling, localization, response to stimulus, cellular component organization, or biogenesis. The proteins encoded by these genes were located in the membrane, cell, and organelles. Their functions mainly included binding, catalytic activity, transporter activity, transcription factor activity,

Table 3. Plant numbers of different branch types in M6, N7, and progeny populations.

Group	Total plants	Multi-branched	Branchless	Multibranched: branchless	χ^2	$\chi^2_{0.05}$
M6	20	20	0			
N7	20	0	20			
F1	20	20	0			
F2	394	298	96	3.10:1	0.04	3.84

Fig. 1. Multibranched (M6) and branchless (N7) watermelon plants.

Table 4. Quality analysis of BSA-seq of two mixed pools and their parents.

Sample	Clean reads/bp	Clean base	Q20 (%)	Q30 (%)	GC (%)	Mapped(%)	Coverage rate (%)		
							1× (%)	5× (%)	10× (%)
M6	76121418	11352134204	99.12	95.84	34.02	99.53	99.54	98.05	93.91
N7	64295076	9590907804	98.91	95.06	34.02	99.47	99.53	97.44	89.94
Hun-DUO	81449206	12146812752	99.07	95.63	33.80	99.52	99.7	98.41	94.83
Hun-WU	81658958	12180027314	99.02	95.38	34.22	99.47	99.68	98.31	94.5

BSA-seq = bulked segregant analysis with a whole whole-genome resequencing; GC = guanine-cytosine.

structural molecule activity, antioxidant activity, and nutrient reservoir activity.

A KEGG analysis was performed to elucidate the metabolic pathways and biological functions associated with the genes in the candidate region. The top 20 KEGG pathways were related to amino acid biosynthesis, amino acid metabolism, carbon metabolism, nitrogen metabolism, phenylpropanoid biosynthesis, and phenylalanine metabolism (Fig. 6).

Through gene annotation and GO and KEGG enrichment analyses, eight candidate genes associated with the branchless

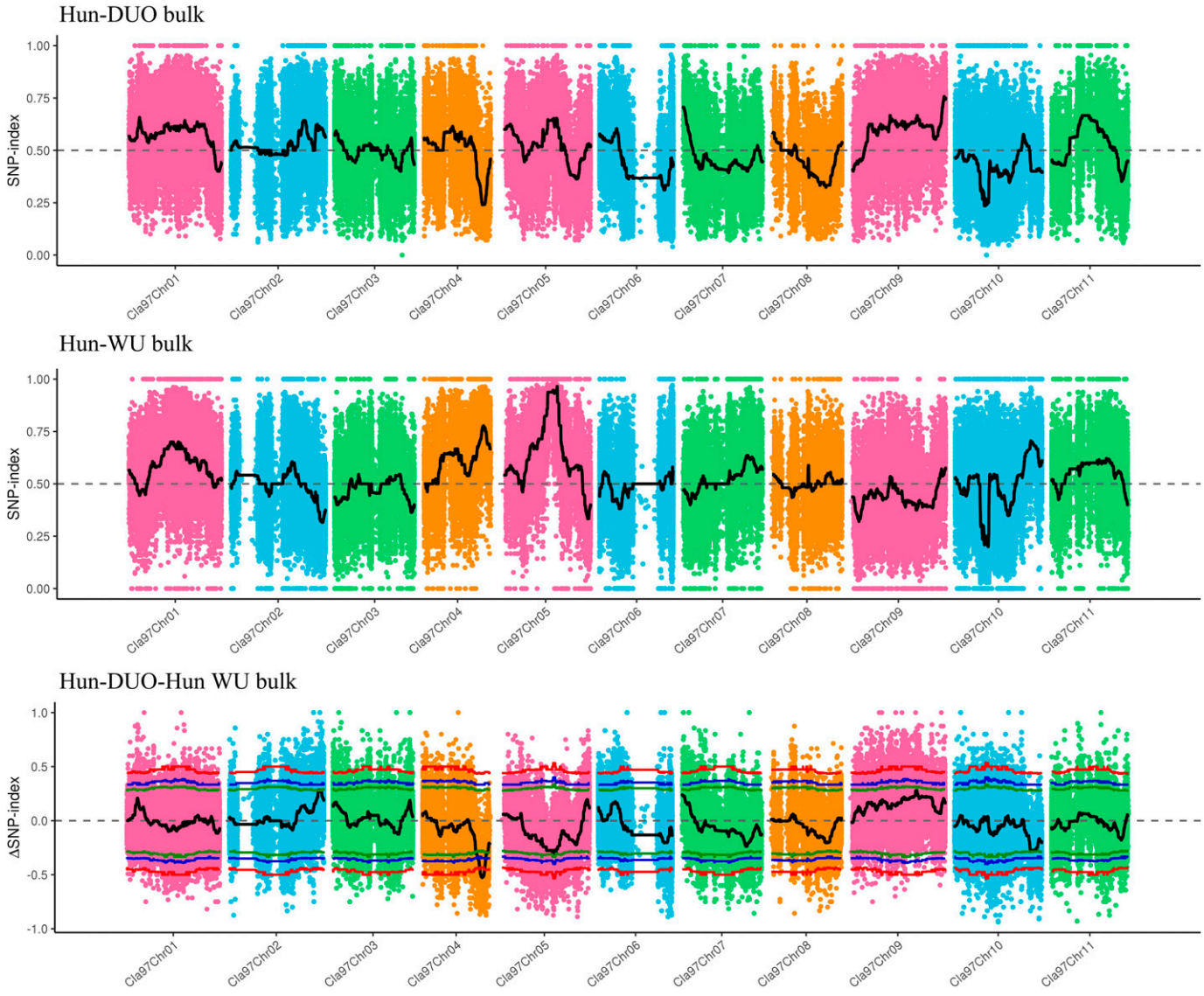

Fig. 2. Distribution of single-nucleotide polymorphism (SNP) index association values on chromosomes. The horizontal coordinate is the chromosome name. The scatter plot is the original SNP index value (or ΔSNP index value). The black curve is the fitting SNP index value (or ΔSNP index value). The top figure shows the distribution of SNP index values of the multibranched mix pool. The middle figure shows the distribution of SNP index values of the branchless mix pool. The bottom graph shows the distribution of the ΔSNP index values. The red line (99% confidence level) was selected as the threshold of the screen. The window beyond the red line was selected as the candidate interval.

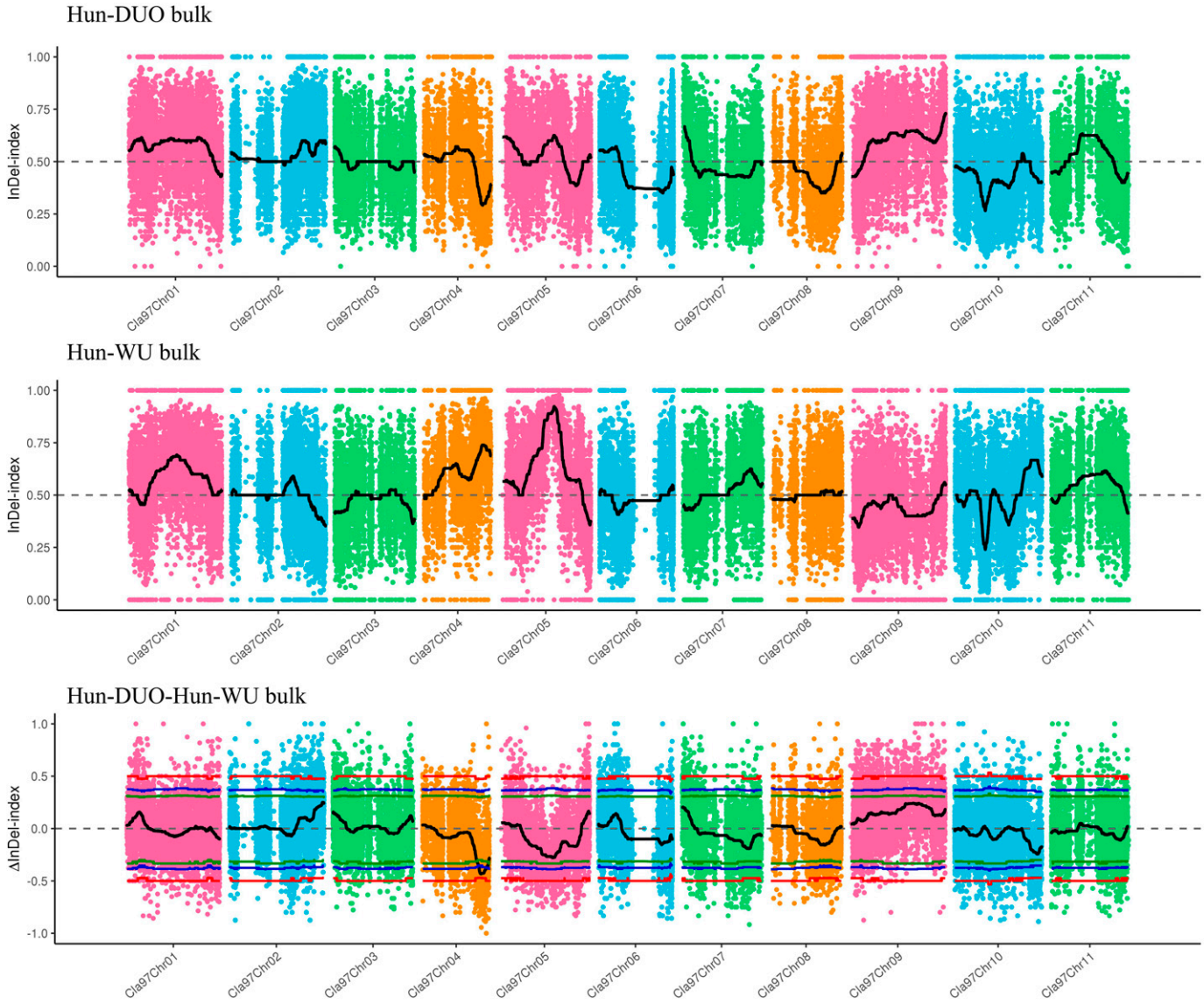

Fig. 3. Distribution of the insertions/deletions (InDel) index association values on chromosomes. The horizontal coordinate is the chromosome name. The scatter plot is the original InDel index value (or ΔInDel index value). The black curve is the fitting InDel index value (or ΔInDel index value). The top figure shows the distribution of the InDel index value of the multibranched mix pool. The middle figure shows the distribution of the InDel index value of the branchless mix pool. The bottom graph shows the distribution of the ΔInDel index value. The blue line (95% confidence level) was selected as the threshold of the screen. The window beyond the blue line was selected as the candidate interval.

phenotype in watermelon were identified. These genes were involved in amino acid biosynthesis and catabolism, Teosinte branched1/Cincinnata/proliferating cell factor (TCP) transcription factor activity, and regulation of flower development. There were 12 exonic SNPs with nonsynonymous mutations and two InDels with frame shift mutations (Table 7).

Table 5. Mapping results of watermelon branchless phenotype based on BSA-seq.

Correlation analysis		Chromosome ID	Start	End	Size (Mb)
SNP	ED	Cla97Chr04	22587652	22587652	0
		Cla97Chr04	22614284	22614305	0
		Cla97Chr04	22619385	25245837	2.63
	SNP index	Cla97Chr04	22958925	24971894	2.01
InDel	ED	Cla97Chr04	22111251	26418818	4.31
	InDel index	Cla97Chr04	22374188	25663596	3.29

BSA-seq = bulked segregant analysis with a whole whole-genome resequencing; ED = Euclidean distance; ID = identification; InDel = insertions/deletions; SNP = single-nucleotide polymorphism.

qRT-PCR validation of candidate genes. The expression levels of eight candidate genes in the axils and stems of M6 and N7 are shown in Fig. 7. The expression levels of *Cla97C04G075820*, *Cla97C04G075950*, *Cla97C04G076060*, *Cla97C04G076250*, *Cla97C04G076280*, and *Cla97C04G076340* were significantly higher in the M6 axils and stems than in the N7 axils and stems. The expression levels of *Cla97C04G076830* and *Cla97C04G076380* were significantly lower in the M6 axils than in the N7 axils. There was no significant difference in the expression of *Cla97C04G076830* in the stems of the parents, and *Cla97C04G076380* was not expressed in the stems of the parents. The qRT-PCR revealed significant variations in the expression of eight genes in the axils of the multibranched M6 and branchless N7. Our findings implied that these genes are

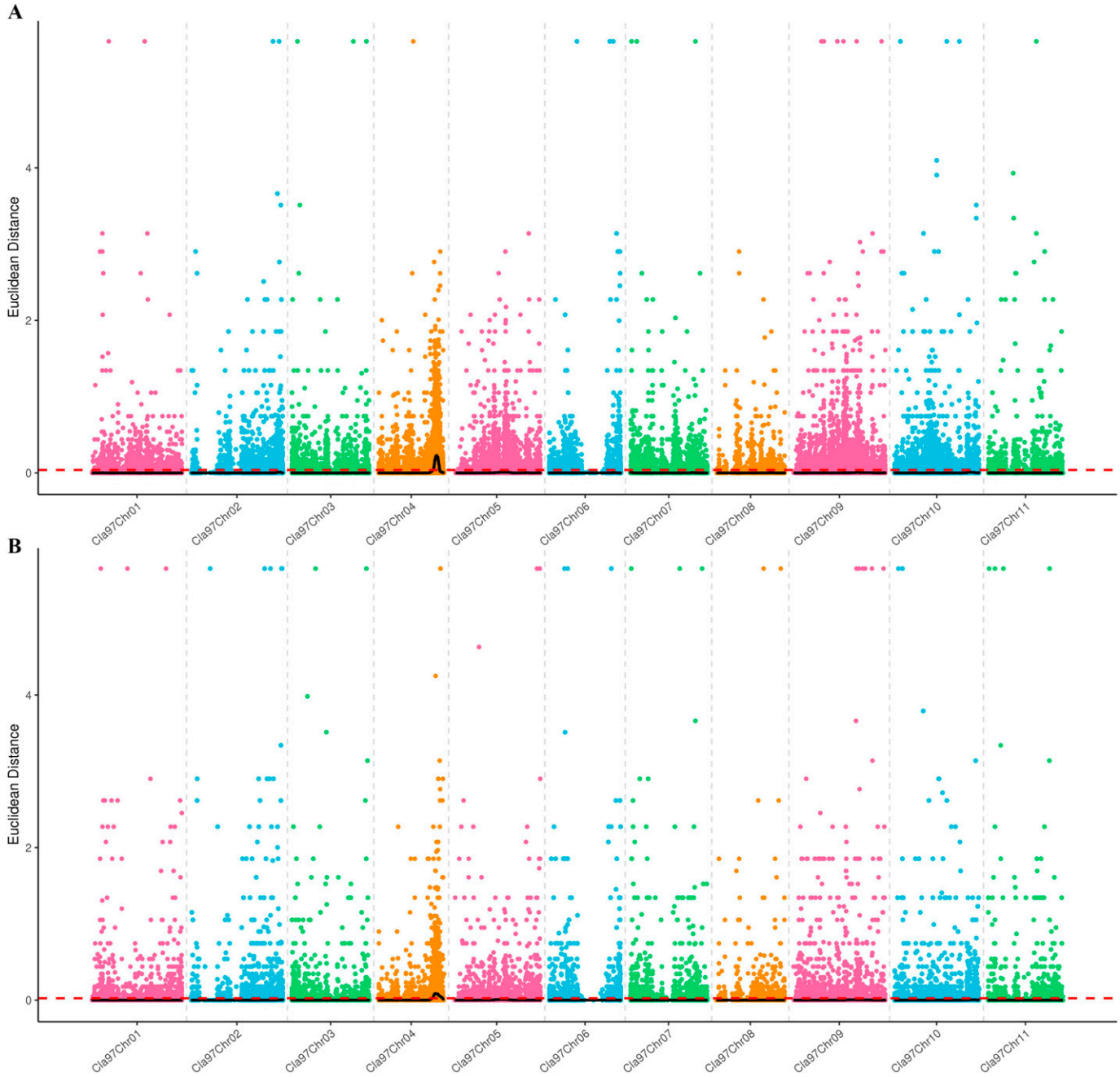

Fig. 4. Distribution of Euclidean distance (ED) association values on chromosomes. (**A**) The ED association values were calculated from single-nucleotide polymorphisms (SNPs). (**B**) The ED association values were calculated from insertions/deletions (InDels). The horizontal coordinate is the chromosome name. The scatter plot is the original ED value. The black curve is the fitting ED value. The higher the ED value, the better the association effect of this point. The red line (median + 3 *SD*s) was selected as the threshold of the screen. The window above the red line was selected as the candidate interval.

candidates for governing the branchless phenotype in watermelon; however, further validation through transgenic studies is necessary.

Discussion

Lateral branching represents an important agronomic trait in melon crops. Cultivating melon cultivars without lateral branches contributes to the reduction of labor required for pruning while simultaneously enhancing planting density by improving ventilation and light exposure. In this study, branchless N7 and multibranched M6 were used as parents to investigate the inheritance of the lateral branching trait in watermelon. Our results showed that the F_1 plants were multibranched, whereas the multibranched and branchless plants in F_2 conformed to the Mendelian segregation ratio of 3:1. Consequently, the branchless phenotype in watermelon was controlled by a single recessive gene. Genome-wide resequencing was performed for mixed pools of F_2 plants with highly multibranched and branchless phenotypes and the two parents. The four samples yielded 64,295,076 bp to 81,658,958 bp clean reads with

Table 6. Numbers of genes annotated through different databases.

Databases	Gene no.	Nonsynonymous gene no.	Frame shift gene no.
NR	181	18	2
NT	182	18	2
TrEMBL	181	18	2
SwissProt	144	14	1
GO	161	17	2
KEGG	145	16	2
COG	78	9	2
Total	182	18	2

COG = Clusters of Orthologous Groups of Proteins; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; NR = NCBI nonredundant protein sequences; NT = NCBI Nucleotide Sequences.

sufficient genome coverage and high quality. Through SNP/InDel association analyses, the candidate genes were confined to a region of 2.01 Mb (22,958,925 bp–24,971,894 bp) on chromosome 4. Dou (2018) studied the inheritance of a branching phenotype in watermelon using the cultivar 790010 with lateral branches and the cultivar Wuchazao without lateral branches as parental lines and found that the branchless phenotype in watermelon was controlled by a single gene in the 21.58 to 21.85 Mb region on chromosome 4. This finding aligns with our BSA-seq analysis findings that indicated that the branchless phenotype in watermelon was controlled by a single gene on chromosome 4, albeit with different candidate gene locations. This disparity may be attributed to the

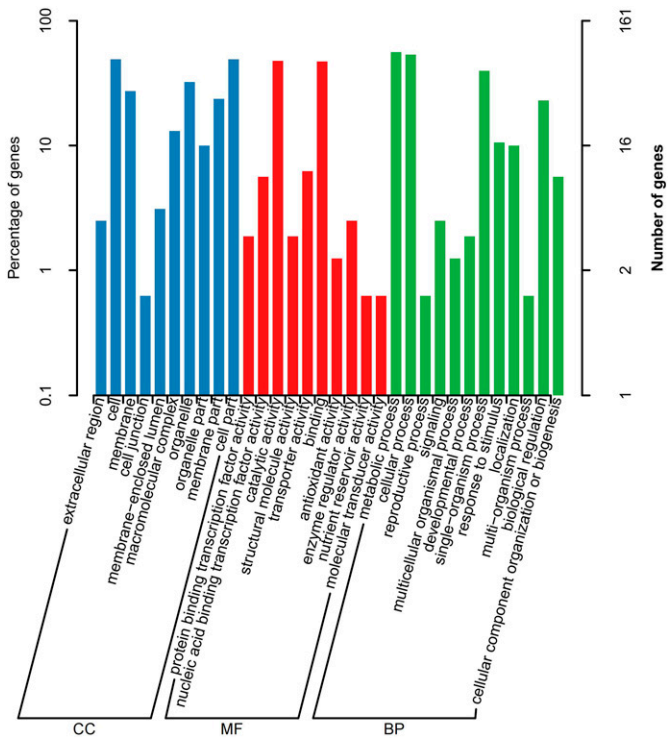

Fig. 5. Gene Ontology (GO) annotation cluster map of genes in candidate regions. The horizontal coordinate is the content of each GO classification. The left side of the vertical coordinate is the percentage of the number of genes. The right side is the number of genes. This figure shows the gene classification of each secondary function of GO in all gene backgrounds in the associated region. BP = biological process; CC = cellular component; MF = molecular function.

Watermelon (97103) v1 genome used in the previous study, whereas the updated Watermelon (97103) v2 genome was used in the present study.

Plant hormones, such as auxin, cytokinin, and strigolactone, play pivotal roles in the development of lateral branches in plants, with auxin having a specific role in mediating the formation of axillary meristems (Beveridge et al. 2000; Domagalska and Leyser 2011; Sauer et al. 2006). Hormone-related genes associated with lateral branch development have been identified in *Arabidopsis thaliana*, rice (*Oryza sativa*), and pea (*Pisum sativum*). These genes mainly encode auxin transporters, threonine/serine kinases, and cytochrome P450 (Wang et al. 2018). In the present study, eight candidate genes were identified through gene annotation and KEGG and GO enrichment analyses, including the cytochrome P450 gene *Cla97C04G076340* with potential implications in watermelon lateral branch development.

Transcription factors from the TCP and GRAS families can inhibit the growth of lateral branches. Genes that encode TCP transcription factors such as *TB1* in maize (*Zea mays*) and rice and *BRANCHED1* (*BRC1*) in *A. thaliana*, pea, and tomato (*Solanum lycopersicum*) are specifically expressed in axils. Overexpression of these genes suppressed lateral branch formation, whereas their deletion increased lateral branching (Aguilar-Martínez et al. 2007; Braun et al. 2012; González-Grandío et al. 2013; Hubbard et al. 2002; Martín-Trillo et al. 2011; Minakuchi et al. 2010). Shen et al. (2019) revealed that expression of the lateral branch regulator gene *CsBRC1* (*BRANCHED1*) increased during the domestication of cucumber (*Cucumis sativus*) from wild-type plants with lateral branches to cultivated plants without lateral branches. The increased expression of *CsBRC1* directly inhibited the expression of the auxin transporter gene *PIN3* in lateral branches, leading to an excessive accumulation of auxin and subsequent inhibition of lateral branch growth and development. Furthermore, through homologous alignment and cloning, Shen et al. (2021) identified *CsBRC1-like*, a homologous gene of *CsBRC1*. The *A. thaliana brc1* mutant overexpressing *CsBRC1-like* showed a reduced number of branches. However, *CsBRC1-like*-RNAi cucumber plants showed alterations in the shape of true leaves without affecting the branches. In the present study, the candidate region controlling the branchless phenotype in watermelon hosted the TCP transcription factor gene *Cla97C04G075950*. Unlike the expression of the aforementioned TCP transcription factor genes, *Cla97C04G075950* exhibited significantly higher expression in the axils of multibranched plants than in those of branchless plants. The function of this gene in watermelon lateral branch development necessitates confirmation through transgenic studies.

The *LATERAL SUPPRESSOR* (*LAS*/*LS*) gene in *A. thaliana* and tomato and the *MONOCLUM* (*MOC1*) gene in rice encode regulatory proteins that belong to the GRAS family, and these genes have been reported to inhibit lateral branching (Greb et al. 2003; Li et al. 2003; Schumacher et al. 1999). Studies conducted by Yuan et al. (2010) and Jiang et al. (2023) showed that the *LATERAL SUPPRESSOR* gene in cucumber and watermelon could inhibit the formation of lateral branches. However, through the BSA-seq analysis, we did not identify a homologous gene of *LATERAL SUPPRESSOR* in the candidate region governing the branchless phenotype. This discrepancy might be attributed to the variations in lateral branching control genes among different watermelon cultivars, or it could be indicative of lateral branching being regulated by intricate transcriptional

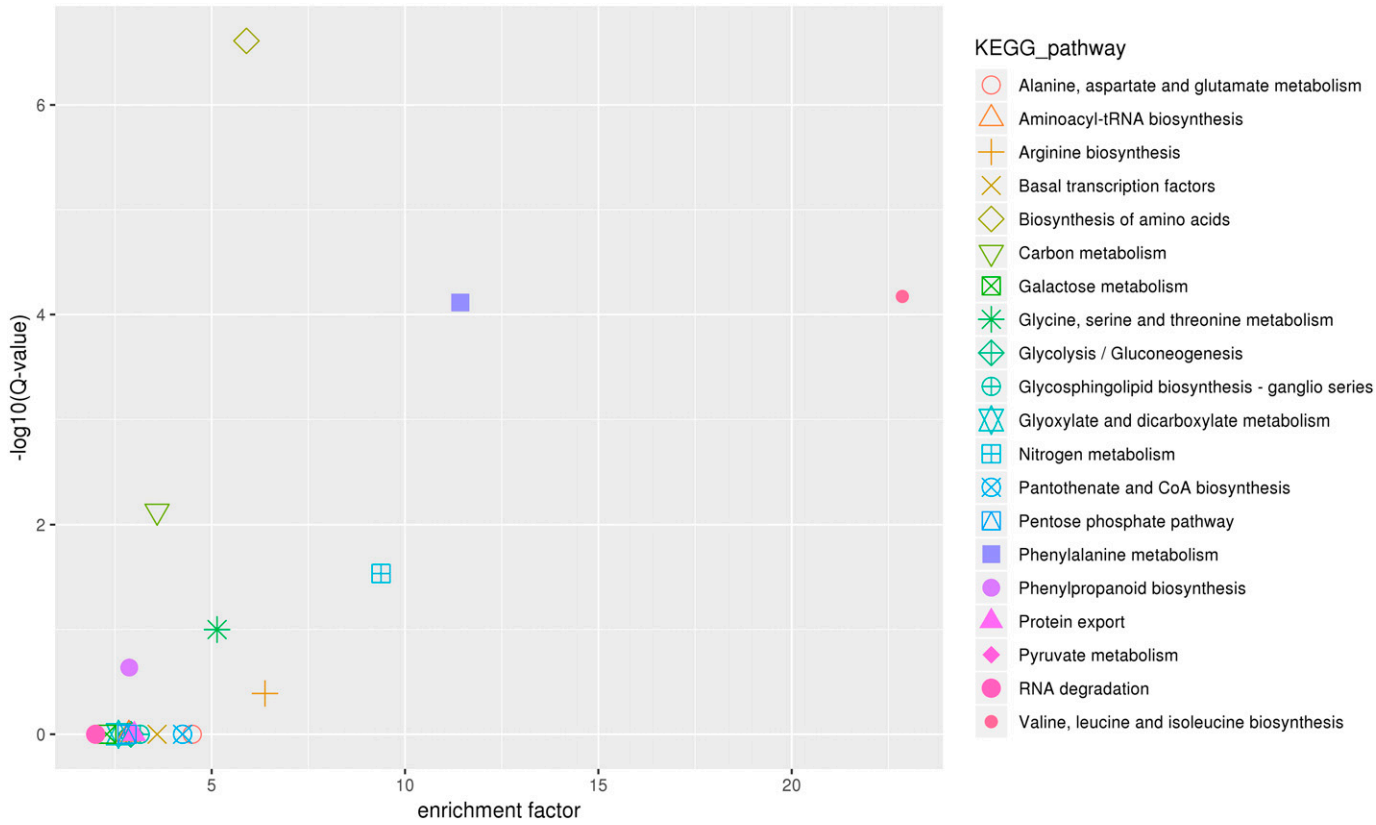

Fig. 6. Pathway enrichment analysis of genes in candidate regions. The horizontal coordinate is the enrichment factor of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The vertical coordinate is the Q-value (statistical significance of enrichment). The lower the Q-value, the higher the degree of enrichment.

processes during which *LATERAL SUPPRESSOR* may not be the predominant gene in the specific watermelon cultivar undergoing study.

Moreover, Dou et al. (2022) showed that the gene *Cla018392* on chromosome 4 encoding TERMINAL FLOWER 1 protein controlled the branchless phenotype in watermelon. The expression of this gene was significantly reduced in the axils of watermelon plants lacking lateral branches, and its ectopic expression in *A. thaliana* resulted in an increased number of lateral branches. In the current study, we identified a candidate gene, *Cla97C04G076830*, that also encoded TERMINAL FLOWER 1. In contrast to the finding of Dou et al. (2022), the expression of *Cla97C04G076830* was significantly lower in the axils of multi-branched plants compared with that of branchless plants. The function of this gene in watermelon lateral branch development needs to be validated through additional transgenic studies.

Conclusion

The branchless phenotype in watermelon was controlled by a single recessive gene. The BSA-seq analysis yielded 64,295,076 bp to 81,658,958 bp clean reads with sufficient genome coverage and high quality. Through SNP/InDel association analyses, the candidate genes were confined to a 2.01-Mb region (22,958,925 bp–24,971,894 bp) containing 182 annotated genes on chromosome 4. The KEGG and GO enrichment analyses revealed that these candidate genes were associated with 10 cellular components, 10 molecular functions, and 12 biological processes. These genes were primarily involved in amino acid biosynthesis, amino acid metabolism, carbon metabolism, nitrogen metabolism, phenylpropanoid biosynthesis, and phenylalanine metabolism. The following eight candidate genes governing the branchless phenotype in watermelon were identified: *Cla97C04G076340*,

Table 7. Candidate gene annotation.

Gene ID	Location	Size (bp)	Nonsynonymous	Frame shift	Gene description
Cla97C04G076340	Chr04:23909601 to 23915211	5611	0	0	Cytochrome P450
Cla97C04G075820	Chr04:23307529 to 23308662	1134	1	0	Phenylalanine ammonia-lyase
Cla97C04G076060	Chr04:23474690 to 23475088	399	1	0	S-adenosylmethionine synthase
Cla97C04G076250	Chr04:23629200 to 23640734	11535	3	1	Glutamate-ammonia ligase-like protein
Cla97C04G076280	Chr04:23654017 to 23660027	6011	3	0	Glutamine synthetase
Cla97C04G076380	Chr04:23952226 to 23955095	2870	3	1	Threonine dehydratase
Cla97C04G076830	Chr04:24395125 to 24396489	1365	1	0	Terminal flower
Cla97C04G075950	Chr04:23396428 to 23397178	751	0	0	TCP transcription factor

ID = identification; TCP = Teosinte branched1/Cincinnata/proliferating cell factor.

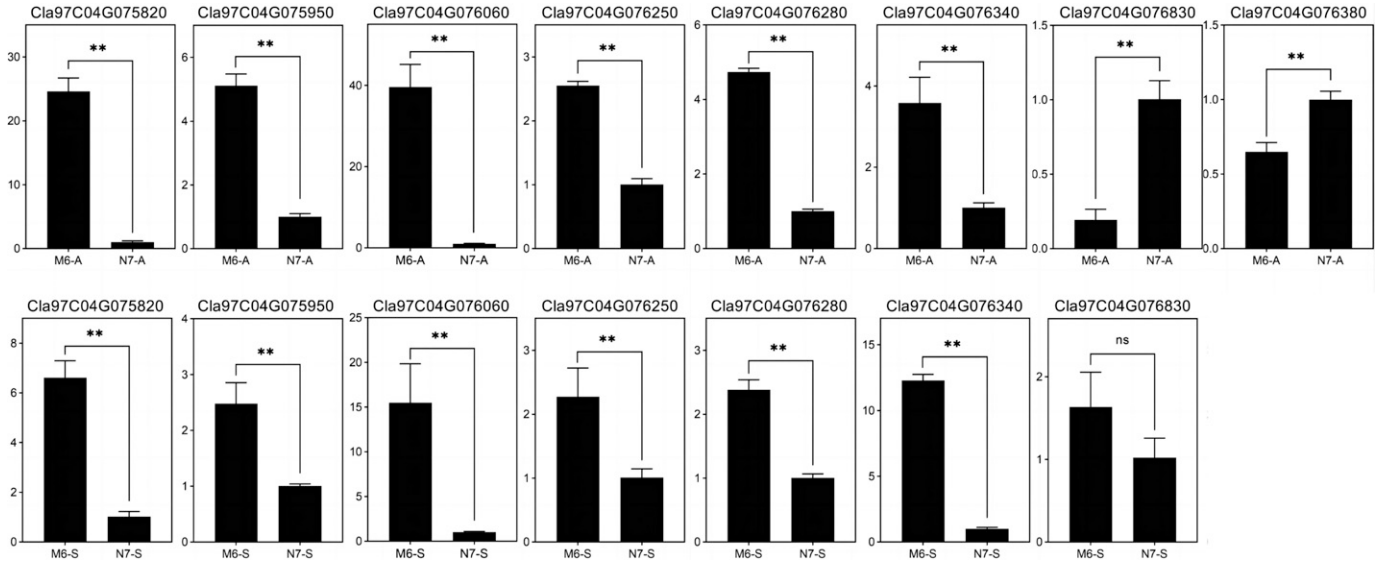

Fig. 7. Expression of candidate genes in M6 and N7 watermelon. ns = the difference did not reach a significant level. ** $P < 0.01$ reached a significant level. Error bars represent the *SE* of three independent biological replicates.

Cla97C04G075820, *Cla97C04G076060*, *Cla97C04G076250*, *Cla97C04G076280*, *Cla97C04G076380*, *Cla97C04G076830*, and *Cla97C04G075950*. These genes were identified as primarily participating in amino acid biosynthesis and catabolism, TCP transcription factor activity, and regulation of flower development. Twelve exonic SNPs with nonsynonymous mutations and two InDels with frame shift mutations were detected in these genes.

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