



Whole genome SNP identification and validation in *Cucumis melo* L. cultivars using genome resequencing approach

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Abstract

Identification of polymorphic loci linked to specific trait variations is an important objective of crop genomics studies. We used next generation sequencing techniques (Illumina and Ion torrent) for whole genome resequencing of two melon (*Cucumis melo* L.) cultivars, Durgapur Madhu (DM) and BS25. After filtering the raw reads, 92% good quality reads obtained were mapped on to the reference melon genome DHL92. A total of 1,709,383 single nucleotide polymorphisms (SNPs-981,560 in DM and 727,823 in BS25) and 433,459 insertion/deletions (InDels; 199,972 in DM and 233,487 in BS25) were identified. Among these 46.56% and 46.07% non-synonymous and 53.43% and 53.92% synonymous SNPs were detected in DM and BS25 cultivars respectively. Based on gene ontology classification, 11,666 annotated genes were assigned to 53 GO terms categorised as related to biological processes (23), molecular functions (16) and cellular components (14). Homology search against *Arabidopsis* showed SNPs containing sequence similarity with *CLCs* and *AHK1* gene in chromosome 2 and 4 of melon and were related to drought and salt stress traits. The SNPs and InDels identified would facilitate the study of economically important traits through mapping and construction of high density linkage maps since the two cultivars resequenced have contrasting response to water stress. These markers can be effectively used to design SNP markers for large scale genotyping and their validation in diverse mapping populations that will accelerate marker assisted breeding in melons.

Key words: Whole genome resequencing, melons, single nucleotide polymorphism, InDels, next generation sequencing

Introduction

Melon (*Cucumis melo* L.) is an important diploid ($2n=2x=24$) horticultural crop from the family

Cucurbitaceae. The melon cultivars exhibit high variability for fruit traits like size, taste, color and nutrient composition (Menon and Rao 2012). Melon has excellent antioxidant potential and is used in the treatment of diabetes and respiratory problems (Kaur and Arora 2011). Muskmelon has been acknowledged as an important model plant for studying biological processes such as phloem transport, ripening of fruits (Pech et al. 2008) and analyses of quantitative trait loci (QTL) for fruit morphological traits like length, shape and girth (Diaz et al. 2014). Variety improvement programs aimed at incorporation of genes for disease resistance, quality traits and QTLs for yield related attributes and stress tolerance require robust molecular markers to enable gene tagging. Availability of good quality, assembled whole genome sequence with high coverage greatly facilitates marker discovery studies. Single nucleotide polymorphisms (SNPs) are recognised as good candidates for development of molecular markers, because they have been shown to represent common polymorphism in genomes of different organisms (Ganal et al. 2009). Next generation sequencing (NGS) approaches have been used for SNP identification in a number of crops (Xu and Bai 2015). Whole genome resequencing is an efficient approach for identification of SNPs in comparison to other methods (Ganal et al. 2009) since optimum genome coverage ensures detection of common polymorphic regions in high numbers thereby avoiding the need to impute missing data. The genome resequencing process has been utilized for identification of variations in copy number of SNPs, InDels and QTL mapping in melons (Xu and Bai 2015),

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though there is much validation work that needs to be done.

Whole genome assembly of melon cultivar, DHL92 (doubled haploid) reported earlier (Garcia-Mas et al. 2012) was used as a reference genome in this study. In earlier reports, a number of variants (SNPs and InDels) located on chromosomes with genes for powdery mildew response were identified and physical map associated with the disease was constructed (Natarajan et al. 2016; Li et al. 2017). Fruit flavour contributing traits were studied in *C. melo* and SNPs and specific length amplified fragment (SLAF) markers were developed by genome resequencing (Zhang et al. 2016). Fruit flesh color traits related SNPs were also reported using NGS in melons (Sim et al. 2018). The scaffold genome assembly of DHL92 was constructed by using resequencing data of the parental lines, and targeted SNP selection (Argyris et al. 2015). Cleaved amplified polymorphism sequence (CAPS) markers were developed from identified SNPs by melon genome resequencing. Fruit quality contributing traits (length, weight, brix, shape) related QTLs were also reported (Amanullah et al. 2018). A set of high-density markers were generated using GBS approach to construct a high-quality genetic map and to identify QTLs controlling fruit traits in melon (Chang et al. 2017). However, detailed analyses of the association between markers and abiotic stress tolerance traits have not been reported in melon till date (Pandey et al. 2013; 2016).

Moisture stress tolerance is an important trait for incorporation in cultivar improvement programs considering the increasing scarcity of irrigation water for melon cultivation. The present study was hence planned to identify the SNPs and InDels associated with moisture stress tolerance so that the markers identified could be useful in breeding stress tolerant varieties of melons. The markers identified in the present study can be validated through high density linkage mapping and map based cloning of genes controlling QTLs for drought stress tolerance in melon.

Materials and methods

Plant material

Two cultivars with contrasting response to drought tolerance were selected for the analyses (Pandey et al. 2013, 2016). Durgapur Madhu (DM) showed good general combining ability (gca) for the number of fruits and percent soluble solids (TSS) (Randhawa and Singh 1990; Choudhary et al. 2006). The cultivar BS25 has

high drought tolerant efficiency (DTE %) in the field (Pandey et al. 2013, 2016). The cultivars were sown during spring season in the greenhouse at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, under rain-out shelter conditions and fresh leaves were harvested for DNA extraction.

DNA extraction and whole genome resequencing

DNA was isolated from leaves of two week old seedlings using Qiagen DNeasy Plant Mini Kit (Cat no. 69106). The quality of purified DNA was checked on 0.8% agarose gel and quantified by Qubit 2.0 Fluorometer (Invitrogen).

The two samples were sequenced in triplicate (one run in Miseq, two runs in Ion Torrent PGM). For Miseq analysis, the libraries of 350bp size fragments were prepared using TruSeq DNA PCR- Free kit (cat. no. 15036187 Rev. B) following the manufacturer's protocol (Illumina Technologies). Library quality was determined using Bioanalyzer 2100 (Agilent Technologies) and quantified with qPCR in 480 II Light Cycler (Roche) using KAPA library quantification kit (cat no. KK4824). The paired-end (PE) libraries were sequenced in Miseq to generate 300 base long reads.

For sequencing in Ion Torrent, DNA libraries of 450 bases length fragments were constructed using the Ion Shear Plus Reagent Kit (Life Technologies) according to the user guide (cat. no.4471248, Revision C.0) with minor modification (shearing time 80 seconds). Libraries were then subjected to emulsion PCR in the Ion OneTouch 2 (OT2) using Ion PGM™ Template OT2 400 Kit (Cat. no. 4479878, Revision 3.0). The Ion PGM Sequencing Kit was used to sequence the libraries on PGM using 318v2 chip.

Mapping reads and identification of SNPs and InDels

The melon genome assembly v3.5.1 pseudomolecule (CM3.5.1_pseudomol.fa) from MELONOMICS (<http://melonomics.net>, v3.5.1) was used as the reference assembly for alignment of sequenced reads. The sequences generated in the present study were passed through quality control and filtered reads from each melon cultivar were mapped on to the melon reference genome using CLC Genomics Workbench (v9.5.3) with default parameters. The raw reads of less than 30 base pair length and poor quality scores (Q<20 for Ion Torrent reads and Q<30 for Illumina reads) were deleted. To map the filtered reads, length fractions of 0.5 and similarity parameter of 0.9 were used. SNPs

were detected using CLC Genomic Workbench with the following criteria: minimum coverage 10 and minimum frequency of 20%. The SNPs and InDels identified were filtered based on stringent parameters such as, minimum 90% variant frequency, minimum read depth 10 and the average base quality of SNP base >30 (Jain et al. 2014). Distribution and frequency of DNA polymorphisms were calculated for all the chromosomes using Circos software (Krzywinski et al. 2009).

Annotation of SNPs and InDels

The filtered SNPs and InDels files (vcf variant call format) were annotated based on their location in the genome and categorized according to the effects of these variations and functional categories using single nucleotide polymorphism effect predictor (SnEff, v4.3). The SnEff database was created using the annotation file (gff3) of melon genome sequence. The SNPs were annotated accordingly into categorized functional class (nonsense, silent and missense) and effect (low, moderate and high). The output files from these analyses (HTML and annotation vcf files) were further used for functional classification of variants. Putative functions of the SNP containing genes were predicted using BLASTP search. Blast2GO (Conesa et al. 2005) was performed for gene ontology annotation and functional categorization and the results were studied with the help of WEGO representation (Web Gene Ontology Annotation Plot) (Ye et al. 2006).

Validation of SNPs

For validation, we randomly selected 2-5 SNPs from each chromosome amounting to a total of 36 SNPs. These SNPs with their corresponding flanking sequences of 400 base pair were sorted, and primer pairs were designed using the software Primer 3 (Supplementary Table S1). A total of 50 SNP primer pairs were designed and synthesized. Polymerase chain reaction (PCR) amplification was done in Eppendorf Master Cycler in a reaction mixture (25 μ L) containing 20 ng template DNA, 1X buffer, 0.2 mM dNTPs, 1.0 μ M primers, 2.0 mM MgCl₂ and 0.5 units Taq polymerase (Thermo Scientific). The amplified product was purified using AxyPrep PCR cleanup kit (Axygen, USA) and sequenced by Sanger sequencing method. The DM and BS25 cultivars were used for validation along with reference melon genome. After sequencing, sequences of amplified products of DM and BS25 cultivars were aligned to the reference melon genome using JAL view software (www.jalview.org),

followed by standalone blastx by making a database using stress related protein sequences, and downloaded from TAIR (www.arabidopsis.org) to find out the sequence homology.

Results and discussion

Genome resequencing and mapping reads

Whole genome resequencing was performed for the cultivars DM and BS25 using Illumina Miseq and Ion torrent PGM platform. A total of 36,253,768 and 34,571,396 high quality reads respectively were obtained for the two cultivars. The sequencing data of both the platforms (Illumina Miseq and Ion Torrent PGM) have been submitted in the Sequence Read Archive (SRA) database for both DM and BS25 cultivars at NCBI as SRA accession: SRP131944. In total, 92.52% reads in DM and 92.96% in BS25 were mapped on to the reference genome. The mapping statistics of each cultivar is summarized in Table 1.

Table 1. Statistics of re-sequenced reads mapped onto reference genome

Melon cultivars	DM	BS25
Raw reads	38,262,935	36,253,758
High quality reads	36,253,768	34,571,396
Mapped reads	33,540,502	32,136,981
Unmapped reads	2,713,266	2,434,415

Identification and characterization of SNPs and InDels

Variants annotation tool SnEff was used to detect SNPs and InDels from DM and BS25 by comparing the cultivar sequences with the reference genome. A total number of variants identified were 981,560 and 727,823 SNPs in DM and BS25 respectively; and for InDels 199,972 (45,996 insertion and 153,976 deletion) and 233,487 (34,277 insertion and 199,210 deletion) in DM and BS25 respectively. The false positive SNPs and InDels were reduced using stringent parameters. These variants were screened at minimal read depth 10 with Q30 base quality and more than 90% polymorphism call rate (Jain et al. 2014). The variant rates of SNPs were, one per 414 bases in DM and one per 559 bases in BS25. The occurrence rate of InDels was one per 2,034 bases in DM and one per 1,742 bases in BS25. The number of transitions (Ts) and transversions (Tv) in both the cultivars were

calculated individually by comparison with reference melon genome as shown in Table 2. SNPs are increasingly being used in crop improvement and genetics because of their cost effectiveness, abundance and high-throughput genotyping. The variations in SNPs among various cultivars are proving to be the major determinants of phenotypic and genotypic differences (Jain et al. 2014; Zhang et al. 2016). Hence, the SNPs identified here have application in determination of population structure and analysis of linkage disequilibrium.

Table 2. Number of transitions and transversions in both the cultivars (DM and BS25) were individually calculated with reference melon genome

Type	DM	BS25
Transitions	680,279	505,467
Transversions	301,281	222,356
Ts/Tv ratio	2.258	2.273

Distribution of SNPs and InDels across chromosomes

The melon reference genome has 12 Pseudo chromosomes (Chr.) numbered 1 to 12 and chr.0 which includes non-anchored scaffolds. The Chr.4 of DM was found to harbor highest number of SNPs (132,101). Contrastingly, Chr.11 of BS25 had the highest number of SNPs (107,171). The lowest number of SNPs was found in Chr.9 of both the cultivars (40,631 and 15,833). The InDels were distributed in higher numbers in Chr.4 and Chr.7 in DM and BS25 respectively. The rate of recombination was high in chromosomes at extreme positions and was suppressed in central regions, which is as per expectations based on genetic studies indicating low recombination rates in the vicinity of centromeric regions and higher rates away from centromere. Low rate of recombination in a chromosome results in low frequency of genetic variations. In addition, marker density would also be reduced in the genome (Diaz et al. 2015; Sanseverino et al. 2015). The overall SNP variations were higher in DM as compared to BS25 while higher InDels were observed in BS25 (Table 3) despite comparable genome coverage in terms of total reads in both the cultivars.

The variants frequencies identified among the chromosomes have been depicted using Circos in Fig. 1 (I, II). This study indicated unequal distribution of

Table 3. Distribution of SNPs and InDels in chromosomes of both the cultivars (DM and BS25) were individually compared with reference melon genome

Chromosome	SNPs		InDels	
	DM	BS25	DM	BS25
0	21,908	16,484	3,286	2,854
1	44,398	35,286	8,501	8,129
2	60,643	41,884	8,474	6,406
3	89,499	71,913	14,023	8,181
4	132,101	102,928	81,183	12,159
5	64,898	50,383	8,391	7,067
6	88,424	60,332	12,116	9,486
7	98,710	77,686	11,808	75,810
8	54,140	32,278	7,944	6,270
9	40,631	15,833	6,928	71,093
10	70,400	48,560	8,620	6,135
11	120,406	107,171	13,276	11,565
12	95,402	67,085	15,422	8,332
Total	981,560	727,823	199,972	233,487

variants across the chromosomes. We identified 31 hotspots (>800 SNPs per 100kb) in DM and 19 hotspots in BS25. The chromosome 11 of both cultivars anchored highest number of SNPs (1416 in DM and 1257 in BS25). The cultivar DM had 1,049 high density and 853 low density SNPs while BS25 had 631 high density and 1103 low density SNPs. Similarly, 61 InDels regions in DM and 20 in BS25 were observed to be hotspots (>80 per100kb chromosomal region). Chromosome 11 of DM (118) and chromosome 6 of BS25 (101 InDels) had highest number of InDels. Further, DM had 1252 regions with high density InDels (>40) and 146 regions with low density InDels (dd3) while in BS25 726 regions with high density and <3 regions with low density were observed. The results are in agreement with earlier reports that chromosome size is directly proportional to the density of SNPs and InDels in the genome (Krzywinski et al. 2009; Jain et al. 2014).

Distribution of SNPs and InDels across different genomic regions and annotation of sequences carrying SNPs and InDels

The annotation of the SNPs and InDels was done to study their distribution in exonic, intronic and intergenic regions of the genome (Fig. 2). The SNPs and InDels

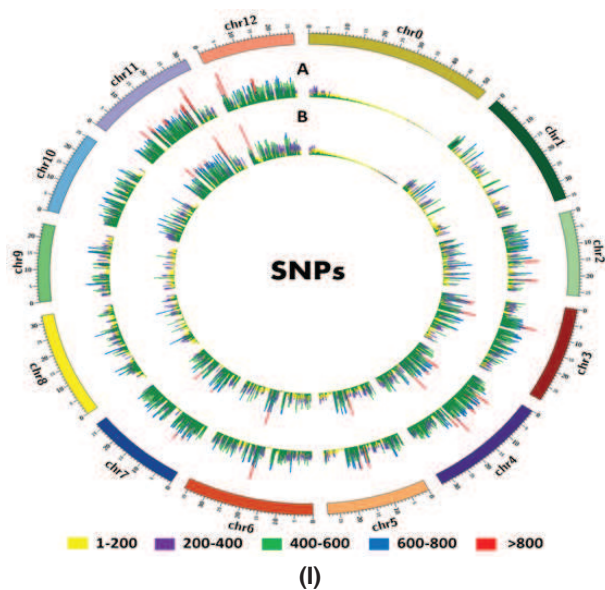


Fig. 1. The circos plot of identified (I) SNPs and (II) InDels frequency in all chromosome of melon. DM (A, outer ring) and BS25 (B, inner ring)

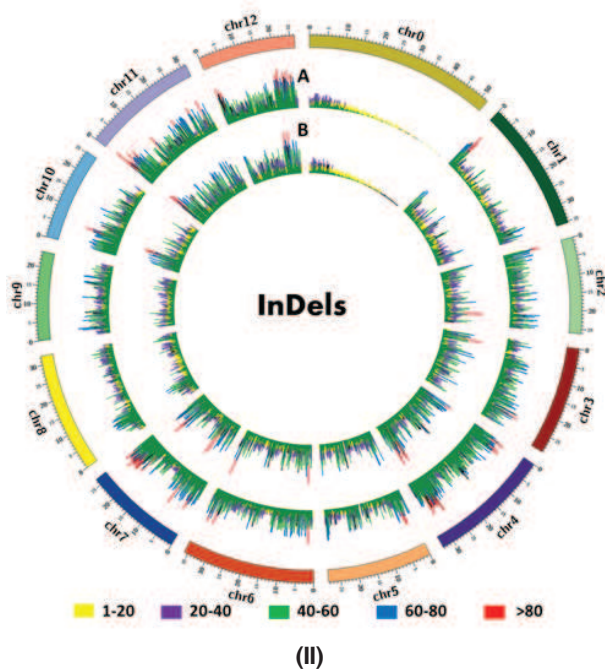


Fig. 2. Annotation of SNPs and InDels in melon cultivars DM and BS25 showing distribution of SNPs and InDels in the intergenic region, upstream, downstream, exonic, intronic, UTR_3' and 5' region

were categorized into four classes, namely, high, low, moderate and modifier effect (Table 4), using SnpEff (Cingolani et al. 2012). The functional classification of

variants has been shown in Table 5. On the basis of functional classification of coding SNPs, approximately 46% non-synonymous (missense and non-sense) variations was found in the cultivars which indicated changes in codon of the amino acid and remaining 54% variations were synonymous (Table 5). Downstream and upstream area of the genic region contained a large number of InDels. It is predictable because of the reduced number of conserved sequences in them as compared to coding regions (Jain et al. 2014). In recent years, a number of studies have been reported on SNPs of melon with different objectives (Deleu et al. 2009; Blanca et al. 2011, 2012; Sanseverino et al. 2015; Argyris et al. 2015; Nimmakayala et al. 2016; Natarajan et al. 2016; Li et al. 2017; Chang et al. 2017). In our analyses, the melon cultivars showed low sequence conservation due to the higher number of InDels in the non-coding regions. On the basis of effects of total coding and non-coding SNPs, average 98% SNPs were categorized as sequence modifier (affecting non-coding region) and rest 2% into other categories as high, low, moderate (Table 4). The results are in conformity with earlier findings in melon (Natarajan et al. 2016) and mungbean (Yadav et al. 2015). The InDels were the cause for frame shift mutations (Causse et al. 2013). Based on the BLASTP search against the melonomics database, 27638 and 28787 protein sequences were identified from DM and BS25 cultivars respectively. A cutoff e-value of $1e-5$ resulted in identification of 6550 and 6774 significant blast hits out of which we could annotate 5737 and 5929 genes in the cultivars DM and BS25 respectively. In GO annotation, 11,666 annotated genes were assigned to 53 GO terms (Fig. 3), which were classified into three categories: biological processes, molecular functions and cellular components. Among biological processes, “cellular process” represented the most abundant subcategory followed by “metabolic process” and “biological regulation”. Within the molecular function category, genes were assigned to binding, catalytic activity, transporter activity and molecular transducer activity. Under the cellular components category, the major GO terms were cell and cell part. A relatively large number of genes belonged to the basic functions of reproduction and pigmentation (Supplementary Table S2).

We also analyzed the significant blast hits to identify common as well as unique locus. We found 137 (Supplementary Table S3) and 325 (Supplementary Table S4) unique and 6366 common locus in DM and

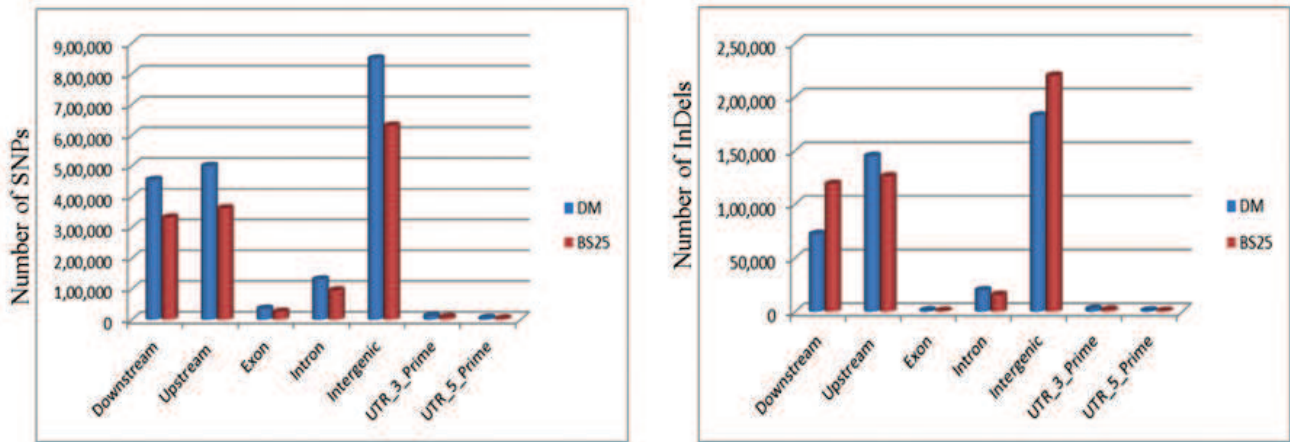


Fig. 3. WEGO presentation of gene ontology categories from resequenced genomes of DM and BS25 cultivars showing biological process, molecular function and cellular component

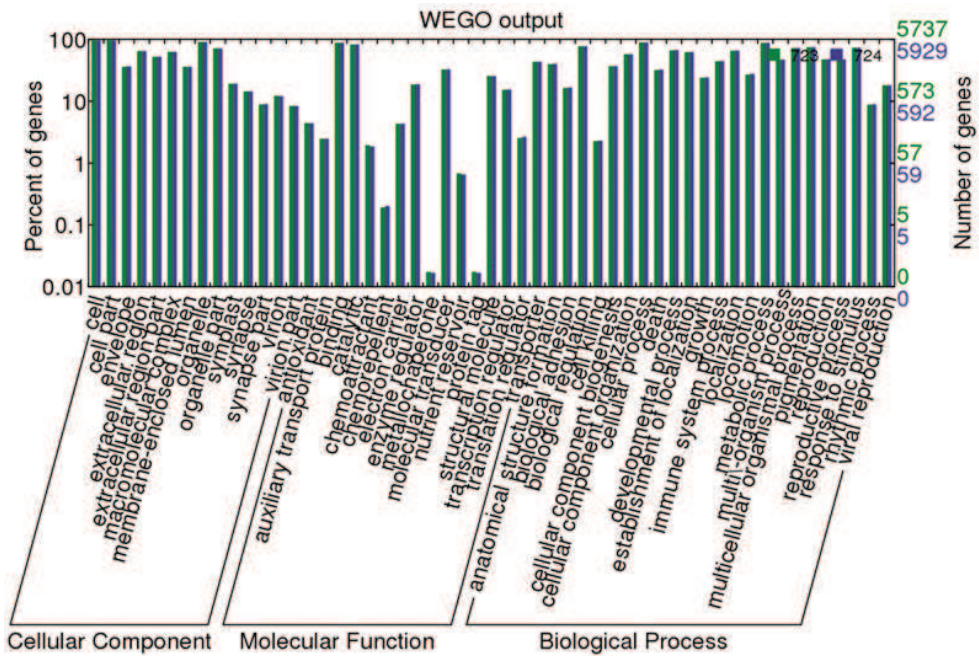


Fig. 4. Multiple sequence alignment of sequences amplified using primer designed from melon cultivar DHL92 genome as reference. SNP position is indicated with arrow

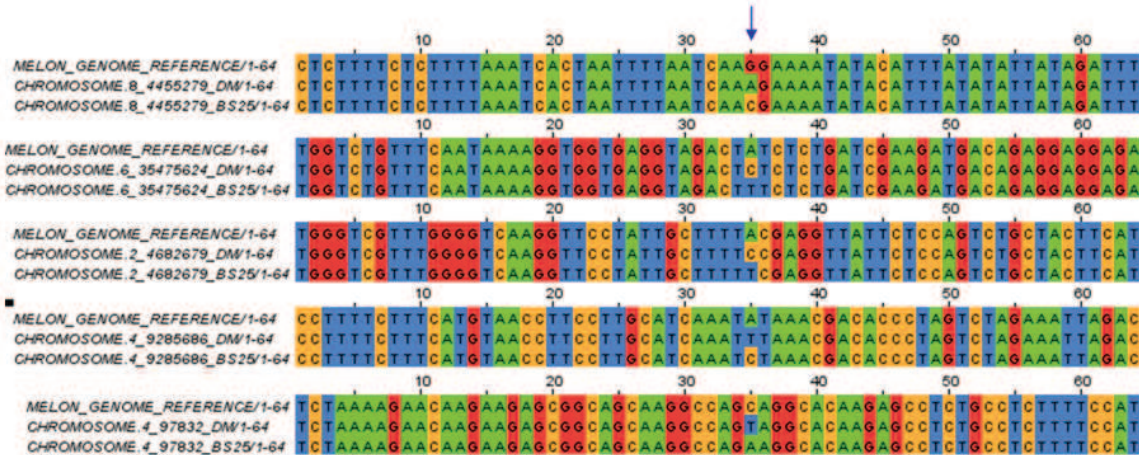


Table 4. Classification of SNPs and InDels effects in melon accessions

Type	SNPs		InDels	
	DM	BS25	DM	BS25
High	0.022%	0.021%	0.192%	0.146%
Low	1.048%	1.038%	0.086%	0.058%
Moderate	0.762%	0.752%	0.056%	0.035%
Modifier	98.166%	98.189%	99.666%	99.761%

Table 5. SNPs functional classification in melon cultivars

Type	DM	BS25
Missense	45.841%	45.465%
Nonsense	0.726%	0.61%
Silent	53.433%	53.925%
Missense/silent ratio	0.8579	0.8431

BS25 respectively. On the basis of putative functions assigned by Blast search we found two stress response loci, MELO3C022081P1 (stress-induced protein KIN2-like *Cucumis melo*) and MELO3C006838P1 (zinc finger A20 and AN1 domain-containing stress-associated protein 10-like [*Cucumis melo*]) to be unique to BS25, however, this finding requires further validation through functional analyses. Among the total SNPs in DM and BS25 cultivars, we have found that only 1775 SNPs were different in these cultivars in relation to the reference cultivar. The putative function related to these SNPs was assigned from the blast results. The functional changes in the genome might have occurred due to various biotic and abiotic factors (Dixit et al. 2014).

Validation of SNPs

A selected set of 36 SNPs identified in the resequenced genomes were validated by PCR amplification using primers designed from flanking sequences followed by their sequencing (Fig. 4). Homology search (Blastx, stand-alone) for the SNP regions along with flanking sequences was carried out with default parameters (Sudakara et al. 2017) against the *Arabidopsis* stress specific protein sequences from TAIR (The *Arabidopsis* Information Resource). *Arabidopsis thaliana* has two genes, *CLCc* (AT5G49890) and *AHK1* (AT2G17820) which regulate phenotypic effects of tolerance to drought and salt stress. The *CLCc* gene regulates stomatal movement and is useful in salt stress tolerance (Jossier et al.

2010). *AHK1* gene is a positive regulator of salt and drought stress responses and abscisic acid pathways (Phan Tran et al. 2007). These two genes showed sequence similarity with the identified SNP containing sequences. The homology search resulted in localization of *CLCc* and *AHK1* SNP flanking sequences to the chromosomes 2 and 4 at the positions 4682679 and 97832 respectively. These sequences showed maximum similarity with *CLCc* and *AHK1* genes suggesting that these variations present in intergenic areas of the melon genome are possibly associated with drought stress. Natural selection of these SNPs during the course of evolution might have led to greater stress tolerance of BS25 (Dixit et al. 2014). The two candidate SNPs reported in the present study require further validation by genotyping a number of drought tolerant and susceptible genotypes of melon in addition to analysis of gene function. DM and BS25 were identified as drought susceptible and tolerant cultivars based on field screening and characterization earlier (Pandey et al. 2013; 2016). Genome wide identification of SNPs from two important melon cultivars generated useful information for downstream studies. The data generated could be further utilized for gene tagging, high density SNP genotyping, association and linkage mapping, variety identification and marker assisted selection related to moisture stress in melon.

Authors' contribution

Conceptualization of research (KVB, SK); Designing of the experiments (KVB, SK, ABG); Contribution of experimental materials (KVB); Execution of field/lab experiments and data collection (SK, SS, BT, PB); Analysis of data and interpretation (SK, PB, NT, KVB); Preparation of manuscript (SK, SV, PB, RK).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. The list of the primer for SNP validation in both the cultivars (DM and BS25 sample)

Chromosome	SNP_ Position	Refere- nce Base	DM, BS25 base	Seq_Forward_primer (5'-3')	StartLength	tm	GC%	Product size	Seq_Reverse_primer (3'-5')	Start Length	tm	GC%		
1	14726754	A	C, G	GGGTGTATATGTTTTGTTATCTTCT	18	25	55.1	32.0	304	CTTGCTCGCCTAGTGAAC	321	18	55.4	55.6
	408088	G	A, C	ATATCGAGGGAAAGTCAAGG	77	20	54.2	45.0	250	GTAAGGAATTGAAGTCGGGT	326	20	55.1	45.0
2	14730502	C	A, T	GCCCTAAACCCCTAATTCAT	73	20	54.7	45.0	300	ACTCTGACGAATTGACACAT	372	20	54.7	40.0
	4682679	A	C, T	GCTGCCTTAGGTACATTCTT	78	20	55.1	45.0	266	ACGTCATGGTCAATATCAGT	343	20	54.1	40.0
3	14576794	G	C, T	CACCTTCATCTCGTTATGA	40	20	54.9	45.0	300	AGAGGTTGTGGTAGAGGAA	339	19	54.4	47.4
	17544543	G	T, C	CATCAAAACACCAATAGACGT	58	21	54.5	38.1	284	AGAAGGTTGACATTAGGCAA	341	20	54.5	40.0
	9114190	T	A, G	AGCAGACATAATTCTTGGTGT	57	21	55.2	38.1	293	CCCGGTGGTATCTATCAGAA	349	20	55.8	50.0
4	31659681	A	T, C	AGTCTAACACCCACTCCTAA	6	20	54.7	45.0	330	TAGTTGAGTTGTGTGCATGT	335	20	55.2	40.0
	16870558	C	A, G	ACAGTTGAGATCCTTTAGTC	77	21	54.8	42.0	247	CTTGGTTTAAAGTCAATTTGGTT	323	23	54.2	30.0
	9285686	A	T, C	CCTACCTCAAACGTTTAGCT	66	20	55.1	45.0	269	GTTTCGTGCAAGGATTCATT	334	20	54.8	40.0
	9152128	C	A, T	GGAAACAGAGCAAGGATTCT	14	20	55.3	45.0	383	GTTGTACTTTACCTGTGATTTGT	396	23	55.1	34.8
	97832	C	T, A	CGTCCAATCATCTAAATCCG	59	20	54.0	45.0	299	GGGGATAGGTTTGAATGTCT	357	20	54.8	45.0
5	14243747	T	A, C	CTGATTCCTCTAACCGATGG	67	20	55.0	50.0	284	TAACGTGATGAAAGCAATGG	350	20	54.3	40.0
	10509509	A	T, C	GGATACGAGACTATTCATGC	80	20	53.1	45.0	247	GGATCATCCGTTTTAACTTG	326	20	52.2	40.0
	23082131	C	T, A	ATTTGTGTTGGAATGAAGCC	60	20	54.7	40.0	280	GCAGTAATAGAGAGTGGACC	339	20	54.7	50.0
6	7161229	A	G, C	GGTTTTGCCTATTGATGGATG	54	21	55.2	42.9	300	CTCTATCTCGCCAAATCAA	353	20	54.9	45.0
	35475624	A	C, T	TGGGTTCAACTATTTCCCTCTC	49	21	54.9	42.9	294	TTCTCAACTACTTCCATGCC	342	20	55.0	45.0
	19529122	G	A, T	CAAGGACGTTCTTCTTCG	78	18	53.1	50.0	243	AAAGAATTTGAGAGAGGCTT	320	20	52.3	35.0
	27795395	A	C, T	TGTTTTGTCCTTGAGTGGTA	79	20	54.8	40.0	249	AGATCCCTAATGCTATGTGTT	327	21	54.0	38.0
8	4455279	G	A, C	TCTTAATCTCCTTACCTCAACC	78	22	54.6	40.9	263	CGTGTGAAAGTCCAGATCTT	340	20	55.4	45.0
	24433675	G	T, C	GCATCTTAGCTAACATTAACGC	78	22	55.9	40.9	247	TTGGGTTTCATCTTCAATTTTGG	324	22	55.4	36.4
	29157432	T	G, A	TGATTTACTCAACAGACGCC	76	20	55.4	45.0	295	GTTGTCAAAGGTAGGGCTAG	370	20	55.7	50.0
	11345681	C	T, G	GTCTATTAGCACTCCCAGTAG	79	21	54.6	47.6	250	AAAGACTCGCCTAGAATGTC	328	20	54.9	45.0
	10031669	G	C, A	GCACCTCCCCTAAAATATGA	75	20	54.4	45.0	249	AGATTGTAGCGAGAGTAAAGT	323	21	54.1	38.0
9	8007580	T	C, A	AGTTCAACGACATTCTCCA	66	20	54.9	40.0	281	GTCCCAAAGTCATTTAACC	346	21	55.0	42.9
	12891269	G	A, C	GGAATTGCTGGATAAGGGAT	75	20	54.8	45.0	248	CCTCGTCCTTAATTCTCAACT	322	21	55.1	42.9
10	3727215	C	A, T	CCAGGGGAGATATGTTTAAT	1	20	51.6	40.0	376	CCTTTACATTTTCCGTGAC	376	20	52.9	40.0
	18691464	T	G, C	GTTGTAGTTGTAGAGTGTCCA	56	21	54.9	42.9	300	CCAAAACCTGAGTGCCTAA	355	20	55.8	45.0
	12015207	T	C, A	TCAATTGCATGATCGAAAGT	74	20	53.5	35.0	249	CCCTACCTATCTTCGGACTA	322	20	54.5	50.0
	7891200	A	G, C	GGATACAACAGCGGAAAA	44	18	52.6	44.4	287	ATGATAGAGGTCGTTTGTCT	330	20	53.5	40.0
	6417844	A	C, G	TGCCTCCGATCAATCTTAAA	49	20	54.4	40.0	296	AGGACAAAGCTGAAATGTTT	344	20	53.6	35.0
11	9897095	T	C, A	CTCTCTAAATGTTGTTCCACAC	67	22	55.6	40.9	294	TTGGGCATAGCTTATTCAGG	360	20	55.2	45.0
	29639574	C	G, T	GTCTTTGACTCATTCTCTCCT	44	21	54.7	42.9	299	TCACTTGACCTTCATCTGTT	342	20	54.2	40.0
12	1688529	T	C, A	CTCTTTCCCTCCCTAAAACC	80	20	55.0	50.0	247	ACCACCTCGATCACTTCTTA	326	20	55.6	45.0
	820911	A	T, G	TTTCCAAAACGAAGAAAGC	8	20	55.0	40.0	368	ACAATCTTGAATTCGATGCG	375	20	54.8	40.0
0	974	G	T, A	TTAGTTATAAGTTTCCACCA	77	20	48.8	30.0	247	CTTGCTATCCTAGTATTAGAA	323	21	49.2	33.3

Supplementary Table S2. Gene ontology classification of resequenced genomes of DM and BS25 melon cultivars

S.No.	Gene number (DM: BS25)	Percentage	P-value	GO id	Putative function
Cellular component					
1	3716:3847	(64.8:64.9)	[0.899]	GO:0005576	extracellular region
2	5520:5708	(96.2:96.3)	[0.876]	GO:0005623	cell
3	703:722	(12.3:12.2)	[0.900]	GO:0019012	virion
4	2071:2143	(36.1:36.1)	[0.959]	GO:0031974	membrane-enclosed lumen
5	2065:2175	(36.0:36.7)	[0.439]	GO:0031975	envelope
6	3607:3735	(62.9:63.0)	[0.891]	GO:0032991	macromolecular complex
7	5214:5399	(90.9:91.1)	[0.739]	GO:0043226	organelle
8	3007:3128	(52.4:52.8)	[0.710]	GO:0044421	extracellular region part
9	4107:4255	(71.6:71.8)	[0.831]	GO:0044422	organelle part
10	482:499	(8.4:8.4)	[0.977]	GO:0044423	virion part
11	509:535	(8.9:9.0)	[0.775]	GO:0044456	synapse part
12	5519:5707	(96.2:96.3)	[0.875]	GO:0044464	cell part
13	827:857	(14.4:14.5)	[0.952]	GO:0045202	Synapse
14	1100:1143	(19.2:19.3)	[0.886]	GO:0055044	symplast
Biological process					
1	2118:2198	(36.9:37.1)	[0.863]	GO:0044085	cellular component biogenesis
2	3817:3974	(66.5:67.0)	[0.572]	GO:0032502	developmental process
3	2722:2841	(47.4:47.9)	[0.611]	GO:0000003	reproduction
4	3308:3410	(57.7:57.5)	[0.873]	GO:0016043	cellular component organization
5	1831:1931	(31.9:32.6)	[0.451]	GO:0016265	death
6	2695:2811	(47.0:47.4)	[0.638]	GO:0022414	reproductive process
7	2546:2655	(44.4:44.8)	[0.663]	GO:0002376	immune system process
8	4207:4358	(73.3:73.5)	[0.833]	GO:0050896	response to stimulus
9	4142:4307	(72.2:72.6)	[0.591]	GO:0032501	multicellular organismal process
10	2287:2384	(39.9:40.2)	[0.704]	GO:0010926	anatomical structure formation
11	2682:2792	(46.7:47.1)	[0.712]	GO:0051704	multi\>-organism process
12	3565:3710	(62.1:62.6)	[0.629]	GO:0051234	establishment of localization
13	941:987	(16.4:16.6)	[0.722]	GO:0022610	biological adhesion
14	5044:5218	(87.9:88.0)	[0.884]	GO:0008152	metabolic process
15	1036:1072	(18.1:18.1)	[0.975]	GO:0016032	viral reproduction
16	504:529	(8.8:8.9)	[0.794]	GO:0048511	rhythmic process
17	4289:4448	(74.8:75.0)	[0.745]	GO:0043473	pigmentation
18	1551:1644	(27.0:27.7)	[0.401]	GO:0040011	locomotion
19	3755:3908	(65.5:65.9)	[0.600]	GO:0051179	localization
20	1372:1441	(23.9:24.3)	[0.623]	GO:0040007	growth
21	5130:5302	(89.4:89.4)	[0.993]	GO:0009987	cellular process
22	130:137	(2.3:2.3)	[0.872]	GO:0001906	cell killing
23	4445:4608	(77.5:77.7)	[0.756]	GO:0065007	biological regulation

Molecular function

1	4758:4924	(82.9:83.0)	[0.870]	GO:0003824	catalytic activity
2	1470:1517	(25.6:25.6)	[0.963]	GO:0005198	structural molecule activity
3	2495:2593	(43.5:43.7)	[0.790]	GO:0005215	transporter activity
4	5062:5239	(88.2:88.4)	[0.830]	GO:0005488	binding
5	248:258	(4.3:4.4)	[0.939]	GO:0009055	electron carrier activity
6	142:147	(2.5:2.5)	[0.988]	GO:0015457	auxiliary transport protein activity
7	254:262	(4.4:4.4)	[0.982]	GO:0016209	antioxidant activity
8	1:1	(0.0:0.0)	[M]	GO:0016530	metallochaperone activity
9	1070:1117	(18.7:18.8)	[0.794]	GO:0030234	enzyme regulator activity
10	881:918	(15.4:15.5)	[0.850]	GO:0030528	transcription regulator activity
11	1:1	(0.0:0.0)	[M]	GO:0031386	protein tag
12	112:110	(2.0:1.9)	[0.702]	GO:0042056	chemoattractant activity
13	146:158	(2.5:2.7)	[0.684]	GO:0045182	translation regulator activity
14	11:12	(0.2:0.2)	[0.897]	GO:0045499	chemorepellent activity
15	39:39	(0.7:0.7)	[0.884]	GO:0045735	nutrient reservoir activity
16	1870:1949	(32.6:32.9)	[0.750]	GO:0060089	molecular transducer activity

Total GO terms in three ontologies: 53

Supplementary Table S3. Data showing annotation of identified unique SNPs with their locus ids and positions in chromosome of DM cultivars in comparison to BS25 cultivars and reference melon genome

Locus_ids	Chromosome	Position base	Reference sample base	DM	SnEff_annotation	Blast_annotation
MELO3C012512P1	chr1	17846240	C	T	upstream_gene_variant MODIFIER	protein_RADIALIS-like_2_[Cucumis_melo]
MELO3C012590P1	chr1	19623216	T	C	intron_variant MODIFIER	myb_family_transcription_factor_APL-like_isoform_X2_[Cucumis_melo]
MELO3C013169P1	chr1	12953274	G	A	synonymous_variant LOW	cingulin-like_[Cucumis_melo]
MELO3C013393P1	chr1	16416136	G	T	missense_variant MODERATE	auxin-induced_protein_15A-like_[Cucumis_melo]
MELO3C013432P1	chr1	16956382	A	C	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103490416_[Cucumis_melo]
MELO3C015780P1	chr1	28644335	C	G	missense_variant MODERATE	calcium-transporting_ATPase_12,_plasma_membrane-type-like_[Cucumis_melo]
MELO3C015818P1	chr1	29341556	T	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103492656_[Cucumis_melo]
MELO3C015869P1	chr1	29970373	T	C	downstream_gene_variant MODIFIER	BSD_domain-containing_protein_1-A_[Cucumis_melo]
MELO3C015943P1	chr1	30633826	C	T	synonymous_variant LOW	zinc_finger_protein_6-like_[Cucumis_melo]
MELO3C018773P1	chr1	2721119	C	T	upstream_gene_variant MODIFIER	transcription_factor_MYB39_[Cucumis_melo]
MELO3C018828P1	chr1	3175486	A	T	upstream_gene_variant MODIFIER	putative_pentatricopeptide_repeat-containing_protein_At1g10330_[Cucumis_melo]
MELO3C021012P1	chr1	24351046	G	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103497541_isoform_X2_[Cucumis_melo]
MELO3C021096P1	chr1	26106985	A	G	downstream_gene_variant MODIFIER	transcription_initiation_factor_TFIID_subunit_1_isoform_X1_[Cucumis_melo]
MELO3C024067P1	chr1	7993266	T	C	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103490375_[Cucumis_melo]
MELO3C024107P1	chr1	9316615	T	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103500429_isoform_X1_[Cucumis_melo]
MELO3C024111P1	chr1	9394988	G	A	upstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At5g65560-like_[Cucumis_melo]
MELO3C024319P1	chr1	34312184	T	C	upstream_gene_variant MODIFIER	GDSL_esterase/lipase_1-like_[Cucumis_melo]
MELO3C024404P1	chr1	34982307	C	T	upstream_gene_variant MODIFIER	uncharacterized_protein_At1g51745-like_[Cucumis_melo]

MELO3C024956P1	chr1	11147133	T	C	upstream_gene_variant MODIFIER	uncharacterized_protein_ LOC103492066_[Cucumis_melo]
MELO3C010056P1	chr2	11957193	T	A	downstream_gene_variant MODIFIER	uncharacterized_protein_ LOC103487228_[Cucumis_melo]
MELO3C010093P1	chr2	12781903	A	C	downstream_gene_variant MODIFIER	uncharacterized_protein_ LOC103490375_[Cucumis_melo]
MELO3C010156P1	chr2	14058084	A	T	upstream_gene_variant MODIFIER	exportin-4_isoform_X1_[Cucumis_melo]
MELO3C015054P1	chr2	8514185	G	T	upstream_gene_variant MODIFIER	DNA_ligase_1-like_[Cucumis_melo]
MELO3C015142P1	chr2	6550313	G	C	downstream_gene_variant MODIFIER	isoleucine_N-monooxygenase_1- like_[Cucumis_melo]
MELO3C015284P1	chr2	557152	C	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103492137_ isoform_X2_[Cucumis_melo]
MELO3C015577P1	chr2	3008901	C	T	upstream_gene_variant MODIFIER	calpain-type_cysteine_ protease_DEK1_[Cucumis_melo]
MELO3C017377P1	chr2	22970549	C	A	missense_variant&splice_region_ variant MODERATE	phragmoplast_orienting_ kinesin_2_[Cucumis_melo]
MELO3C017435P1	chr2	22413139	C	T	upstream_gene_variant MODIFIER	aspartic_proteinase_CDR1-like_[Cucumis_melo]
MELO3C017448P1	chr2	22201867	T	A	upstream_gene_variant MODIFIER	putative_glucose-6-phosphate_1- epimerase_[Cucumis_melo]
MELO3C017470P1	chr2	21909619	T	C	upstream_gene_variant MODIFIER	uncharacterized_protein_ LOC103494272_[Cucumis_melo]
MELO3C017510P1	chr2	21449581	A	C	upstream_gene_variant MODIFIER	glutamate_receptor_2.7-like_[Cucumis_melo]
MELO3C025326P1	chr2	18541438	A	C	upstream_gene_variant MODIFIER	probable_sulfate_transporter_ 3.5_[Cucumis_melo]
MELO3C008072P1	chr3	108761	G	A	downstream_gene_variant MODIFIER	Fanconi_anemia_group_M_protein_ isoform_X2_[Cucumis_melo]
MELO3C008074P1	chr3	145462	A	G	downstream_gene_variant MODIFIER	Fanconi_anemia_group_M_protein_ isoform_X4_[Cucumis_melo]
MELO3C008127P1	chr3	777878	T	C	downstream_gene_variant MODIFIER	protein_disulfide-isomerase_5- 2_isoform_X1_[Cucumis_melo]
MELO3C008271P1	chr3	2785650	A	G	downstream_gene_variant MODIFIER	uncharacterized_protein_At1g04910- like_[Cucumis_melo]
MELO3C008279P1	chr3	2965818	T	C	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103503225_ [Cucumis_melo]
MELO3C010752P1	chr3	28822007	C	A	downstream_gene_variant MODIFIER	LOB_domain-containing_protein_22- like_[Cucumis_melo]
MELO3C010862P1	chr3	28066499	G	A	splice_region_variant&intron_variant LOW	UDP-glucuronate:xylan_alpha- glucuronosyltransferase_2_isoform_ X1_[Cucumis_melo]

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MELO3C010988P1	chr3	27267282	G	A	upstream_gene_variant MODIFIER	kinesin-like_protein_NACK1_[Cucumis_melo]
MELO3C011134P1	chr3	26151830	C	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103488175_[Cucumis_melo]
MELO3C011202P1	chr3	25614367	T	A	downstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At5g24830_isoform_X1_[Cucumis_melo]
MELO3C019945P1	chr3	20575248	C	T	upstream_gene_variant MODIFIER	exocyst_complex_component_SEC6_[Cucumis_melo]
MELO3C003353P1	chr4	509630	G	A	missense_variant MODERATE	uncharacterized_protein_LOC103503494_isoform_X1_[Cucumis_melo]
MELO3C012886P1	chr4	13935195	T	G	missense_variant MODERATE	legumin_A-like_[Cucumis_melo]
MELO3C013067P1	chr4	17029185	G	A	upstream_gene_variant MODIFIER	ankyrin_repeat-containing_protein_At5g02620-like_[Cucumis_melo]
MELO3C003972P1	chr5	20823723	T	C	upstream_gene_variant MODIFIER	DNA_ligase_4_isoform_X3_[Cucumis_melo]
MELO3C003995P1	chr5	21326188	G	T	missense_variant MODERATE	putative_inactive_cadmium/zinc-transporting_ATPase_HMA3_isoform_X2_[Cucumis_melo]
MELO3C004290P1	chr5	25089825	C	T	missense_variant MODERATE	TMV_resistance_protein_N-like_isoform_X2_[Cucumis_melo]
MELO3C004292P1	chr5	25132098	C	G	upstream_gene_variant MODIFIER	TMV_resistance_protein_N-like_isoform_X1_[Cucumis_melo]
MELO3C004348P1	chr5	25757839	A	G	downstream_gene_variant MODIFIER	protein_HASTY_1_isoform_X2_[Cucumis_melo]
MELO3C004433P1	chr5	26454198	G	A	synonymous_variant LOW	calcium_uptake_protein_1_mitochondrial-like_isoform_X2_[Cucumis_melo]
MELO3C004489P1	chr5	26950827	C	A	synonymous_variant LOW	two-component_response_regulator_ARR12-like_[Cucumis_melo]
MELO3C004602P1	chr5	27885307	G	A	upstream_gene_variant MODIFIER	leucine-rich_repeat_extensin-like_protein_4_[Cucumis_melo]
MELO3C014243P1	chr5	4864546	T	A	upstream_gene_variant MODIFIER	aquaporin_PIP2-2-like_[Cucumis_melo]
MELO3C014616P1	chr5	914207	A	C	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103491531_[Cucumis_melo]
MELO3C014627P1	chr5	855207	C	T	upstream_gene_variant MODIFIER	linoleate_13S-lipoxygenase_2-1_chloroplast-like_[Cucumis_melo]
MELO3C014705P1	chr5	280666	C	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103491613_[Cucumis_melo]
MELO3C020462P1	chr5	8180950	G	A	downstream_gene_variant MODIFIER	RING_finger_protein_165-like_[Cucumis_melo]
MELO3C005960P1	chr6	227011	A	G	downstream_gene_variant MODIFIER	zinc_finger_protein_ZAT9_[Cucumis_melo]

MELO3C006531P1	chr6	3952636	A	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103483710_[Cucumis_melo]_
MELO3C006709P1	chr6	5258964	G	A	upstream_gene_variant MODIFIER	bromodomain_and_WD_repeat-containing_protein_1_isoform_X1_[Cucumis_melo]
MELO3C006736P1	chr6	5544630	T	C	upstream_gene_variant MODIFIER	probable_beta-1,3-galactosyltransferase_16_[Cucumis_melo]
MELO3C006739P1	chr6	5594514	G	T	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103483912_[Cucumis_melo]_
MELO3C006884P1	chr6	6962620	T	C	upstream_gene_variant MODIFIER	protein_IQ-DOMAIN_14_[Cucumis_melo]
MELO3C008502P1	chr6	8668439	T	A	missense_variant MODERATE	protein_RKD4_[Cucumis_melo]_
MELO3C013737P1	chr6	35594631	C	T	synonymous_variant LOW	uncharacterized_protein_LOC103490660_[Cucumis_melo]
MELO3C013795P1	chr6	35154087	T	G	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103490722_[Cucumis_melo]
MELO3C014144P1	chr6	31288929	G	T	upstream_gene_variant MODIFIER	pirin-like_protein_At1g50590_[Cucumis_melo]
MELO3C014145P1	chr6	31288798	C	T	synonymous_variant LOW	pirin-like_protein_At1g50590_[Cucumis_melo]
MELO3C014745P1	chr6	18613849	T	A	upstream_gene_variant MODIFIER	transportin-3_isoform_X1_[Cucumis_melo]
MELO3C014779P1	chr6	19615194	T	C	upstream_gene_variant MODIFIER	histone-lysine_N-methyltransferase,_H3_lysine-9_specific_SUVH4_[Cucumis_melo]_
MELO3C016472P1	chr6	25442202	A	C	missense_variant MODERATE	GDSL_esterase/lipase_At1g71691-like_[Cucumis_melo]
MELO3C019399P1	chr6	9088712	A	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103496212_[Cucumis_melo]
MELO3C023770P1	chr6	17468448	C	T	downstream_gene_variant MODIFIER	LOW_QUALITY_PROTEIN: transcription_factor_AS1-like_[Cucumis_melo]
MELO3C023796P1	chr6	16778466	C	T	downstream_gene_variant MODIFIER	cytokinin_hydroxylase-like_[Cucumis_melo]
MELO3C025412P1	chr6	29914050	G	A	upstream_gene_variant MODIFIER	probable_tetraacyldisaccharide_4'-kinase,_mitochondrial_[Cucumis_melo]
MELO3C016064P1	chr7	18353616	T	C	upstream_gene_variant MODIFIER	nipped-B-like_protein_isoform_X1_[Cucumis_melo]
MELO3C016857P1	chr7	1569222	G	T	upstream_gene_variant MODIFIER	probable_protein_phosphatase_2C_72_isoform_X1_[Cucumis_melo]
MELO3C017055P1	chr7	203385	C	T	upstream_gene_variant MODIFIER	probable_inorganic_phosphate_transporter_1-9_[Cucumis_melo]
MELO3C017556P1	chr7	22888340	T	C	upstream_gene_variant MODIFIER	F-box_protein_At5g49610-like_[Cucumis_melo]
MELO3C017969P1	chr7	26204597	A	G	upstream_gene_variant MODIFIER	alkylated_DNA_repair_protein_alkB_homolog_8_isoform_X1_[Cucumis_melo]

MELO3C017990P1	chr7	26306919	T	A	missense_variant MODERATE	uncharacterized_protein_LOC103494799_[Cucumis_melo]
MELO3C025623P1	chr7	4867776	T	C	downstream_gene_variant MODIFIER	transcription_factor_RF2b-like_[Cucumis_melo]
MELO3C025668P1	chr7	4320223	A	G	downstream_gene_variant MODIFIER	probable_leucine-rich_repeat_receptor-like_serine/threonine-protein_kinase_At3g14840_isoform_X1_[Cucumis_melo]
MELO3C007223P1	chr8	1590740	G	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103484460_[Cucumis_melo]
MELO3C007522P1	chr8	3346298	A	T	synonymous_variant LOW	uncharacterized_protein_LOC103484737_isoform_X1_[Cucumis_melo]
MELO3C007594P1	chr8	3954462	A	G	downstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At4g37380,_chloroplastic_[Cucumis_melo]
MELO3C007638P1	chr8	4291937	A	T	upstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At5g67570,_chloroplastic_[Cucumis_melo]
MELO3C007797P1	chr8	5418210	G	A	missense_variant MODERATE	cytochrome_P450_CYP736A12-like_[Cucumis_melo]
MELO3C008980P1	chr8	20050908	C	T	downstream_gene_variant MODIFIER	RING_finger_protein_165-like_[Cucumis_melo]
MELO3C019062P1	chr8	10866158	A	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103493407_[Cucumis_melo]
MELO3C020732P1	chr8	9619384	C	T	missense_variant MODERATE	pentatricopeptide_repeat-containing_protein_At4g35130,_chloroplastic-like_[Cucumis_melo]
MELO3C024561P1	chr8	8175499	A	G	downstream_gene_variant MODIFIER	NAC_domain-containing_protein_90-like_[Cucumis_melo]
MELO3C005450P1	chr9	20551702	A	G	downstream_gene_variant MODIFIER	leucine-rich_repeat_receptor_protein_kinase_EXS-like_[Cucumis_melo]
MELO3C005459P1	chr9	20629585	A	T	upstream_gene_variant MODIFIER	pectinesterase_2-like_[Cucumis_melo]
MELO3C005470P1	chr9	20684158	C	T	synonymous_variant LOW	tripeptidyl-peptidase_2_isoform_X1_[Cucumis_melo]
MELO3C005546P1	chr9	21198276	A	G	upstream_gene_variant MODIFIER	lipid_transfer_protein_EARLI_1-like_[Cucumis_melo]
MELO3C005548P1	chr9	21202908	C	T	synonymous_variant LOW	pEARLI1-like_lipid_transfer_protein_1_[Cucumis_melo]
MELO3C005550P1	chr9	21208201	T	C	missense_variant MODERATE	pEARLI1-like_lipid_transfer_protein_1_[Cucumis_melo]
MELO3C011758P1	chr10	5095631	G	T	upstream_gene_variant MODIFIER	GEM-like_protein_4_[Cucumis_melo]
MELO3C011871P1	chr10	4222477	C	T	downstream_gene_variant MODIFIER	50S_ribosomal_protein_L12,_chloroplastic_[Cucumis_melo]

MELO3C011994P1	chr10	3408015	G	A	downstream_gene_variant MODIFIER	transcription_factor_ABORTED_MICROSPORES_isoform_X1_[Cucumis_melo]_
MELO3C012157P1	chr10	2305770	C	A	downstream_gene_variant MODIFIER	cell_division_control_protein_48_homolog_B_[Cucumis_melo]_
MELO3C012186P1	chr10	2123851	C	T	upstream_gene_variant MODIFIER	protein_NSP-INTERACTING_KINASE_1_isoform_X2_[Cucumis_melo]
MELO3C022590P1	chr10	15758692	A	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103499108_[Cucumis_melo]
MELO3C026667P1	chr10	22590509	T	C	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103499323_isoform_X1_[Cucumis_melo]_
MELO3C026668P1	chr10	22601090	G	A	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103493225_[Cucumis_melo]
MELO3C013609P1	chr11	18132711	C	T	upstream_gene_variant MODIFIER	N-alpha-acetyltransferase_30-like_[Cucumis_melo]_
MELO3C019278P1	chr11	10554703	G	A	upstream_gene_variant MODIFIER	tetraspanin-8-like_[Cucumis_melo]
MELO3C019697P1	chr11	23260250	T	G	upstream_gene_variant MODIFIER	elongation_factor_2_[Cucumis_melo]
MELO3C020914P1	chr11	2824307	A	C	missense_variant MODERATE	B3_domain-containing_transcription_factor_VRN1-like_[Cucumis_melo]
MELO3C020936P1	chr11	2691631	T	G	missense_variant MODERATE	patatin-like_protein_2_isoform_X1_[Cucumis_melo]
MELO3C020973P1	chr11	2309825	A	G	upstream_gene_variant MODIFIER	ubiquitin_carboxyl-terminal_hydrolase_27_[Cucumis_melo]
MELO3C021399P1	chr11	26841438	A	C	upstream_gene_variant MODIFIER	DUF21_domain-containing_protein_At4g14240-like_[Cucumis_melo]
MELO3C025692P1	chr11	24945053	A	G	upstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At3g18110,_chloroplastic_[Cucumis_melo]_
MELO3C025719P1	chr11	25340522	C	T	missense_variant MODERATE	transcriptional_regulator_ATRX_homolog_[Cucumis_melo]
MELO3C025955P1	chr11	13667431	G	A	synonymous_variant LOW	histone-lysine_N-methyltransferase_ASHR1_isoform_X4_[Cucumis_melo]
MELO3C026962P1	chr11	26188310	T	G	upstream_gene_variant MODIFIER	LOW_QUALITY_PROTEIN:_serine_carboxypeptidase-like_51_[Cucumis_melo]
MELO3C004990P1	chr12	4950139	C	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103502605_[Cucumis_melo]
MELO3C000029P1	chr0	8944531	C	A	upstream_gene_variant MODIFIER	putative_DNA-binding_protein_ESCAROLA_[Cucumis_melo]_
MELO3C000273P1	chr0	12312629	A	G	upstream_gene_variant MODIFIER	probable_prefoldin_subunit_5_[Cucumis_melo]

MELO3C000762P1	chr0	18913747	A	G	missense_variant MODERATE	proteasome_subunit_beta_type-3-like,_partial_[Cucumis_melo]	x
MELO3C001027P1	chr0	22150941	A	G	upstream_gene_variant MODIFIER	40S_ribosomal_protein_S13-like,_partial_[Cucumis_melo]	
MELO3C001058P1	chr0	22918942	G	A	synonymous_variant LOW	linoleate_9S-lipoxygenase_6-like_[Cucumis_melo]	
MELO3C001235P1	chr0	25629590	C	T	synonymous_variant LOW	protein_TRANSPARENT_TESTA_12-like,_partial_[Cucumis_melo]	
MELO3C001378P1	chr0	28406499	C	G	upstream_gene_variant MODIFIER	probable_serine/threonine-protein_kinase_At1g09600_isoform_X1_[Cucumis_melo]	
MELO3C001427P1	chr0	28908734	T	C	upstream_gene_variant MODIFIER	L-ascorbate_oxidase_homolog_[Cucumis_melo]	
MELO3C001500P1	chr0	29945791	G	A	synonymous_variant LOW	beta-amyrin_synthase-like_isoform_X1_[Cucumis_melo]	
MELO3C001556P1	chr0	31004693	G	A	missense_variant MODERATE	U3_small_nucleolar_ribonucleoprotein_protein_IMP3_[Cucumis_melo]	
MELO3C001637P1	chr0	32668917	T	A	downstream_gene_variant MODIFIER	putative_B3_domain-containing_protein_At2g27410_[Cucumis_melo]	
MELO3C001671P1	chr0	33420706	G	A	missense_variant MODERATE	TMV_resistance_protein_N-like_[Cucumis_melo]	
MELO3C001738P1	chr0	35074568	A	G	intergenic_region MODIFIER	nipped-B-like_protein_isoform_X3_[Cucumis_melo]	
MELO3C027132P1	chr0	4106979	T	G	upstream_gene_variant MODIFIER	putative_disease_resistance_protein_RGA4_[Cucumis_melo]	
MELO3C027303P1	chr0	7025304	T	C	upstream_gene_variant MODIFIER	probable_pectinesterase/pectinesterase_inhibitor_47,_partial_[Cucumis_melo]	
MELO3C027337P1	chr0	7575187	A	C	splice_region_variant&intron_variant LOW	uncharacterized_protein_LOC103503249_[Cucumis_melo]	
MELO3C027407P1	chr0	8352000	G	A	synonymous_variant LOW	regulatory-associated_protein_of_TOR_2-like_[Cucumis_melo]	

Supplementary Table S4. Data showing annotation of identified unique SNPs with their locus ids and positions in chromosome of BS25 cultivars in comparison to DM cultivars and reference melon genome

Locus_ids	Chromo-Position	Reference base	BS25 sample base	SnEff_annotation	Blast_annotation
MELO3C012632P1	chr120512987		G	upstream_gene_variant MODIFIER	callose_synthase_7_[Cucumis_melo]
MELO3C013151P1	chr112599342	C	G	upstream_gene_variant MODIFIER	mitogen-activated_protein_kinase_kinase_kinase_2_[Cucumis_melo]
MELO3C015819P1	chr129348142	T	C	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103492658_[Cucumis_melo]
MELO3C015957P1	chr130710090	C	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103492784_[Cucumis_melo]
MELO3C015974P1	chr130891269	T	C	synonymous_variant LOW	uncharacterized_protein_LOC103504024_[Cucumis_melo]
MELO3C016037P1	chr131399044	A	C	missense_variant MODERATE	potassium_transporter_5-like_[Cucumis_melo]
MELO3C018714P1	chr12309301	G	T	upstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At2g41080_[Cucumis_melo]
MELO3C018719P1	chr12350991	G	A	synonymous_variant LOW	peroxidase_P7-like_[Cucumis_melo]
MELO3C018743P1	chr12542041	T	C	upstream_gene_variant MODIFIER	expansin-B3_[Cucumis_melo]
MELO3C018745P1	chr12560309	C	T	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103490509_[Cucumis_melo]
MELO3C018751P1	chr12594545	T	G	upstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At1g03560_mitochondrial_[Cucumis_melo]
MELO3C018755P1	chr12608938	A	G	synonymous_variant LOW	uncharacterized_protein_LOC103495530_[Cucumis_melo]
MELO3C018759P1	chr12637008	C	T	downstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At1g03540_[Cucumis_melo]
MELO3C018776P1	chr12743107	C	T	missense_variant MODERATE	transcription_factor_WER-like_[Cucumis_melo]
MELO3C018779P1	chr12769501	C	A	downstream_gene_variant MODIFIER	5'_exonuclease_Apollo_[Cucumis_melo]
MELO3C018787P1	chr12822917	A	G	upstream_gene_variant MODIFIER	geranylgeranyl_transferase_type-2_subunit_beta_[Cucumis_melo]
MELO3C018797P1	chr12877901	A	G	synonymous_variant LOW	cysteine-rich_repeat_secretory_protein_38-like_[Cucumis_melo]
MELO3C018800P1	chr12891367	T	A	missense_variant MODERATE	uncharacterized_protein_LOC103495576_[Cucumis_melo]
MELO3C021136P1	chr126967221	T	A	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103486195_[Cucumis_melo]

MELO3C023365P1	chr133602422	C	T	downstream_gene_variant MODIFIER	probable_receptor-like_protein_kinase_At2g21480_[Cucumis_melo]
MELO3C023382P1	chr133457491	G	A	upstream_gene_variant MODIFIER	pleiotropic_drug_resistance_protein_1-like_[Cucumis_melo]
MELO3C023386P1	chr133438413	C	A	upstream_gene_variant MODIFIER	pentatricopeptide_repeat-containing_protein_At1g15510,_chloroplastic_[Cucumis_melo]
MELO3C023392P1	chr133400798	A	G	missense_variant MODERATE	G-type_lectin_S-receptor-like_serine/threonine-protein_kinase_At1g67520_[Cucumis_melo]
MELO3C023399P1	chr133372825	T	C	synonymous_variant LOW	late_embryogenesis_abundant_protein_D-7_[Cucumis_melo]
MELO3C023462P1	chr133045432	T	C	synonymous_variant LOW	zinc_finger_protein_1-like_[Cucumis_melo]
MELO3C023465P1	chr133015234	G	T	downstream_gene_variant MODIFIER	cinnamoyl-CoA_reductase_1-like_[Cucumis_melo]
MELO3C023487P1	chr132821442	T	G	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103499862_[Cucumis_melo]
MELO3C024198P1	chr14072802	G	T	missense_variant MODERATE	receptor-like_protein_12_[Cucumis_melo]
MELO3C024231P1	chr133743663	T	C	upstream_gene_variant MODIFIER	regulator_of_telomere_elongation_helicase_1_isoform_X1_[Cucumis_melo]
MELO3C024265P1	chr133920266	C	T	upstream_gene_variant MODIFIER	zinc_phosphodiesterase_ELAC_protein_2-like_[Cucumis_melo]
MELO3C024282P1	chr134040715	T	G	synonymous_variant LOW	uncharacterized_protein_LOC103500617_[Cucumis_melo]
MELO3C024321P1	chr134332403	G	A	upstream_gene_variant MODIFIER	phosphoinositide_phosphatase_SAC2-like_[Cucumis_melo]
MELO3C024384P1	chr134829764	G	T	downstream_gene_variant MODIFIER	beta-fructofuranosidase,_insoluble_isoenzyme_CWINV1-like,_partial_[Cucumis_melo]
MELO3C024398P1	chr134935208	T	A	upstream_gene_variant MODIFIER	ABC_transporter_C_family_member_8_[Cucumis_melo]
MELO3C024418P1	chr135058239	G	A	synonymous_variant LOW	putative_leucine-rich_repeat_receptor-like_protein_kinase_At2g19210_[Cucumis_melo]
MELO3C024427P1	chr135104965	G	A	upstream_gene_variant MODIFIER	putative_leucine-rich_repeat_receptor-like_protein_kinase_At2g19210_[Cucumis_melo]
MELO3C024428P1	chr135111339	T	C	upstream_gene_variant MODIFIER	probable_LRR_receptor-like_serine/threonine-protein_kinase_At1g05700_isoform_X4_[Cucumis_melo]
MELO3C024429P1	chr135116868	A	T	upstream_gene_variant MODIFIER	probable_LRR_receptor-like_serine/threonine-protein_kinase_At1g05700_isoform_X2_[Cucumis_melo]
MELO3C024912P1	chr110320180	T	A	upstream_gene_variant MODIFIER	probable_leucine-rich_repeat_receptor-like_protein_kinase_At1g68400_isoform_X1_[Cucumis_melo]

MELO3C024947P1	chr110959564	A	T	missense_variant MODERATE	uncharacterized_protein_LOC103501253_ isoform_X2_[Cucumis_melo]
MELO3C010042P1	chr211639615	A	T	upstream_gene_variant MODIFIER	endoribonuclease_Dicer_homolog_2_ isoform_X1_[Cucumis_melo]
MELO3C010157P1	chr214090237	T	C	upstream_gene_variant MODIFIER	exportin-4_ isoform_X1_[Cucumis_melo]
MELO3C014987P1	chr29835336	C	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103486002_ [Cucumis_melo]
MELO3C015069P1	chr28036337	C	G	synonymous_variant LOW	aluminum-activated_malate_transporter_2- like_[Cucumis_melo]
MELO3C015092P1	chr27592397	A	T	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103487886_ [Cucumis_melo]
MELO3C015104P1	chr27390145	A	G	upstream_gene_variant MODIFIER	histone_deacetylase_15_ isoform_X4_[Cucumis_melo]
MELO3C015153P1	chr26295350	A	T	downstream_gene_variant MODIFIER	pseudouridine-metabolizing_bifunctional_ protein_C1861.05_ isoform_X1_[Cucumis_melo]
MELO3C015159P1	chr26216781	T	C	upstream_gene_variant MODIFIER	nuclear_cap-binding_protein_subunit_1_[Cucumis_melo]
MELO3C015315P1	chr2_728290	T	A	upstream_gene_variant MODIFIER	zinc_finger_protein_MAGPIE-like_[Cucumis_melo]
MELO3C015356P1	chr2_996034	T	C	upstream_gene_variant MODIFIER	protein_CUP-SHAPED_COTYLEDON_3- like_[Cucumis_melo]
MELO3C015392P1	chr21298407	A	C	upstream_gene_variant MODIFIER	LOW_QUALITY_PROTEIN:_protein_MON2_homolog_ [Cucumis_melo]
MELO3C015393P1	chr21312146	A	G	upstream_gene_variant MODIFIER	LOW_QUALITY_PROTEIN:_protein_MON2_homolog_ [Cucumis_melo]
MELO3C015394P1	chr21316934	T	G	upstream_gene_variant MODIFIER	LOW_QUALITY_PROTEIN:_protein_MON2_homolog_ [Cucumis_melo]
MELO3C015498P1	chr22328372	T	C	upstream_gene_variant MODIFIER	LOW_QUALITY_PROTEIN:_ras-associated_and_ pleckstrin_homology_domains-containing_protein_1_ [Cucumis_melo]
MELO3C015501P1	chr22343288	G	A	upstream_gene_variant MODIFIER	myosin-17-like_[Cucumis_melo]
MELO3C015516P1	chr22492860	T	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103492366_ [Cucumis_melo]
MELO3C015547P1	chr22722822	A	C	upstream_gene_variant MODIFIER	3-oxoacyl-[acyl-carrier-protein]_synthase_II,_chloroplastic_ [Cucumis_melo]
MELO3C015551P1	chr22757527	A	G	downstream_gene_variant MODIFIER	probable_flavin-containing_monooxygenase_1_ [Cucumis_melo]
MELO3C015579P1	chr23034823	C	T	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103492424_ isoform_X2_ [Cucumis_melo]

MELO3C017101P1	chr225081462	A	G	synonymous_variant LOW	probable_cyclic_nucleotide-gated_ion_channel_16_[Cucumis_melo]
MELO3C017102P1	chr225074143	T	C	downstream_gene_variant MODIFIER	spermidine_synthase-like_isoform_X3_[Cucumis_melo]_
MELO3C017129P1	chr224896969	A	G	synonymous_variant LOW	chalcone_synthase_[Cucumis_melo]
MELO3C017143P1	chr224802452	C	G	upstream_gene_variant MODIFIER	transcription_factor_MYB24-like_[Cucumis_melo]
MELO3C017148P1	chr224759902	G	A	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103493964_isoform_X1_[Cucumis_melo]
MELO3C017157P1	chr224696466	A	T	missense_variant MODERATE	probable_WRKY_transcription_factor_61_isoform_X1_[Cucumis_melo]
MELO3C017161P1	chr224676740	C	T	upstream_gene_variant MODIFIER	aldose_1-epimerase-like_[Cucumis_melo]
MELO3C017210P1	chr224343985	T	G	upstream_gene_variant MODIFIER	zinc_finger_protein_ZAT1-like_[Cucumis_melo]
MELO3C017215P1	chr224308864	G	T	upstream_gene_variant MODIFIER	putative_pre-mRNA-splicing_factor_ATP-dependent_RNA_helicase_DHX16_[Cucumis_melo]_
MELO3C017228P1	chr224218873	T	C	downstream_gene_variant MODIFIER	replication_protein_A_70_kDa_DNA-binding_subunit_B_[Cucumis_melo]
MELO3C017230P1	chr224196627	C	T	synonymous_variant LOW	transcription_factor_TCP5_[Cucumis_melo]_
MELO3C017248P1	chr224058443	T	C	upstream_gene_variant MODIFIER	putative_ATP-dependent_helicase_hrq1_isoform_X1_[Cucumis_melo]
MELO3C017249P1	chr224060389	C	T	downstream_gene_variant MODIFIER	putative_ATP-dependent_helicase_hrq1_isoform_X3_[Cucumis_melo]
MELO3C017250P1	chr224049255	A	T	upstream_gene_variant MODIFIER	putative_ATP-dependent_helicase_hrq1_isoform_X3_[Cucumis_melo]
MELO3C017271P1	chr223885857	T	G	upstream_gene_variant MODIFIER	probable_acyl-activating_enzyme_17,_peroxisomal_[Cucumis_melo]
MELO3C017299P1	chr223681996	T	G	upstream_gene_variant MODIFIER	protein_YLS3_[Cucumis_melo]
MELO3C017329P1	chr223425224	C	A	missense_variant MODERATE	uncharacterized_protein_LOC103494148_[Cucumis_melo]
MELO3C017335P1	chr223340742	A	T	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103487886_[Cucumis_melo]
MELO3C026180P1	chr226012304	T	A	upstream_gene_variant MODIFIER	NAC_domain-containing_protein_18-like_[Cucumis_melo]
MELO3C026181P1	chr226010627	G	A	synonymous_variant LOW	exosome_complex_component_RRP4-like_[Cucumis_melo]
MELO3C026217P1	chr225790014	A	G	synonymous_variant LOW	callose_synthase_10_[Cucumis_melo]_
MELO3C026218P1	chr225784881	T	G	synonymous_variant LOW	callose_synthase_10_[Cucumis_melo]_
MELO3C026236P1	chr225663653	T	A	missense_variant MODERATE	vinorine_synthase-like_[Cucumis_melo]
MELO3C026276P1	chr225425909	C	T	downstream_gene_variant MODIFIER	short-chain_dehydrogenase_TIC_32,_chloroplastic_[Cucumis_melo]

MELO3C026289P1	chr225323546	C	T	downstream_gene_variant MODIFIER	homeobox_protein_knotted-1-like_2_[Cucumis_melo]
MELO3C026641P1	chr217311153	T	C	intergenic_region MODIFIER	pentatricopeptide_repeat-containing_protein_At1g08070_ isoform_X1_[Cucumis_melo]
MELO3C008234P1	chr32148152	T	C	synonymous_variant LOW	uncharacterized_protein_LOC103485521_[Cucumis_melo]
MELO3C010790P1	chr328515761	T	A	downstream_gene_variant MODIFIER	V-type_proton_ATPase_subunit_G3_[Cucumis_melo]
MELO3C010889P1	chr327920325	G	A	upstream_gene_variant MODIFIER	patatin-like_protein_2_[Cucumis_melo]
MELO3C010907P1	chr327809566	T	C	synonymous_variant LOW	vegetative_incompatibility_protein_HET-E-1- like_[Cucumis_melo]
MELO3C010915P1	chr327772429	A	T	upstream_gene_variant MODIFIER	probable_galacturonosyltransferase- like_1_[Cucumis_melo]
MELO3C010932P1	chr327684664	A	G	downstream_gene_variant MODIFIER	uncharacterized_protein_LOC103487956_[Cucumis_melo]
MELO3C010969P1	chr327439136	G	C	splice_region_variant&synonymous_ variant LOW	uncharacterized_protein_LOC103487998_[Cucumis_melo]
MELO3C011003P1	chr327137432	G	T	upstream_gene_variant MODIFIER	transcription_factor_E2FC_isoform_X3_[Cucumis_melo]
MELO3C011004P1	chr327133244	A	C	upstream_gene_variant MODIFIER	transcription_factor_E2FC_isoform_X3_[Cucumis_melo]
MELO3C011042P1	chr326891685	T	C	synonymous_variant LOW	putative_uncharacterized_protein_FLJ22184_[Cucumis_melo]
MELO3C011061P1	chr326764757	G	A	upstream_gene_variant MODIFIER	ovarian_cancer-associated_gene_2_protein_homolog_[Cucumis_melo]
MELO3C011126P1	chr326213555	A	G	downstream_gene_variant MODIFIER	poly(A)_polymerase_pla1_isoform_X3_[Cucumis_melo]
MELO3C011148P1	chr326027164	G	A	synonymous_variant LOW	protein_NRT1/PTR_FAMILY_1.2-like_[Cucumis_melo]
MELO3C011156P1	chr325953963	A	G	upstream_gene_variant MODIFIER	myb-related_protein_3R-1_[Cucumis_melo]
MELO3C011164P1	chr325857100	G	A	downstream_gene_variant MODIFIER	transcription_factor_JUNGBRUNNEN_1_[Cucumis_melo]
MELO3C011175P1	chr325787535	C	T	downstream_gene_variant MODIFIER	probable_LRR_receptor-like_serine/threonine- protein_kinase_At4g31250_[Cucumis_melo]
MELO3C011223P1	chr325402415	C	T	upstream_gene_variant MODIFIER	uncharacterized_protein_LOC103488267_[Cucumis_melo]
MELO3C011224P1	chr325394263	T	G	upstream_gene_variant MODIFIER	pleiotropic_drug_resistance_protein_1- like_[Cucumis_melo]
MELO3C011326P1	chr324642972	T	A	upstream_gene_variant MODIFIER	glutamate_receptor_2.1-like_isoform_X1_[Cucumis_melo]
MELO3C011344P1	chr324517926	G	A	synonymous_variant LOW	uncharacterized_protein_LOC103488388_[Cucumis_melo]