CHAPTER-IV

RESULTS AND DISCUSSION

Apium graveolens L. (celery) is a spice plant which has enormous medicinal properties. Limited efforts have been made towards the genome and development of molecular markers and their use in genetic/genomic studies in *A. graveolens.* Therefore, the present investigation was undertaken to sequence the genome and to develop SSRs from genomic libraries and thereafter to validate SSR.

The present investigation on "***De novo* Sequencing of Ancient Seed Spice Celery (*Apium graveolens* L.) and Development of Microsatellites"** is presented here. The experiment was conducted at Department of Biotechnology and Food Testing Laboratory, J.A.U. Junagadh. The experimental material was procured from the National Research Centre on Seed Spices, Ajmer (Rajasthan), India. The results obtained in the present investigation are presented and discussed in this chapter.

4.1 DETERMINATION OF GENOME SIZE BY FLOW CYTOMETER

In the present study, for the measurement of genome size, flow cytometer (Accuri C6) was used. The young leaves of celery were utilized in flow cytometry study, in which many intercalating dyes were used. But here (PI) propidium iodide dye was used and the details of the procedure are described in chapter 3. After completing the procedure, flow cytometer gave a peak position. Using the following equation, C value and genome size was calculated.

$$\text{Sample 2C value(DNA pg or Mbp)} = \text{Reference 2C value} \times \frac{\text{sample 2C mean peak position}}{\text{reference 2C mean peak position}}$$

Reference 2C value = 1.51 (*Cartherenceous roseus*)

Sample peak position = 2,074,667 pg (Figure 4.1)

Reference peak position= 388,181 pg (Figure 4.2)

Sample 2C value = 8.07 pg

Sample C value = 4.03 pg

After the calculation, sample C value was 4.03 pg. Here 2C is mentioned because celery is a diploid plant. Sample C value was multiplied with 978 Mb which is a constant for all samples or plants.

Sample genome size = 4.03 X 978 Mb = 3946.23Mb ~ 3.94 Gb

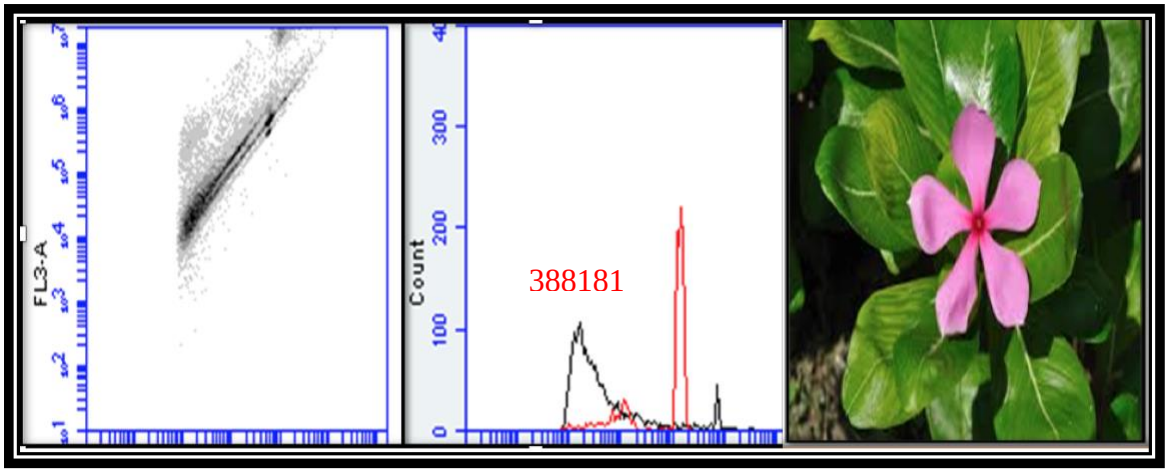

Figure 4.1: Scatter plot and pick position of *Cartherenceous roseus* generated by accuri C6 flow cytometer (as a reference plant)

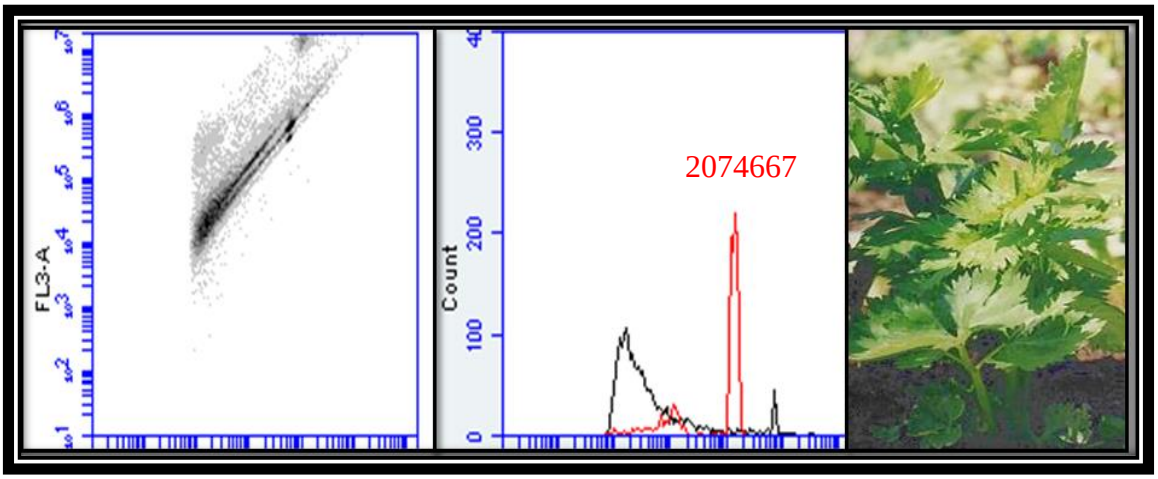

Figure 4.2: Scatter plot and pick position of *Apium graveolens* L. generated by accuri C6 flow cytometer

Wang *et al.* (2014) determined the DNA content of sesame. Sesame samples and reference material were analyzed on an EPICS Elite ESP cytometer (Beckman- Coulter, Hialeah, FL, USA) with an air-cooled argon laser at 488 nm using 20 mW. Salmon erythrocytes (2.16 pg/1C) were used as internal biological reference materials. Nuclear DNA content (in picograms) of sesame samples was estimated according to the following equation: 1C nuclear DNA content = (1C reference in picograms × Peak mean of sesame)/(Peak mean of reference). The number of base pairs per haploid genome was calculated based on the equivalent of 1 pg DNA = 978 Mb. As a result, the C-value of sesame was estimated to be 0.37 pg/1C and its genome size was estimated to be approximately 362 Mb.

4.2 ISOLATION OF PURE GENOMIC DNA

Pure genomic DNA from celery was isolated manually by plant DNA isolation method (CTAB) and even using the kit. Both the method details are described in chapter 3.

Concentration of DNA was measured (Table 4.1) in Qubit® Fluorometer using ds DNA assay kit.

Table 4.1 Concentration of Genomic DNA

No. of sample duplication	Sample name	DNA(ng/µl)
1.	Celery Seedling (8-10 DAG)	11.3
2.	Celery Seedling (8-10 DAG)	13.0

4.3 CONSTRUCTION OF LIBRARY

In the present review, fragment library was constructed for Ion S5 sequencing platform.

4.3.1 Construction of Fragment library

The genomic DNA was sheared by Ion Shear™ plus Enzyme Mix II. Reaction time was kept 5-6 minutes to obtain 350-450 bp fragment size. After the fragmentation library was purified and preceded to size selection.

4.3.2 Assessment of the yield and size distribution of amplified DNA

The amplified library was loaded on the 2% agarose E-gel for the estimation size distribution and conformation of amplify fragment library. Fragmentation of genomic DNA showed in 3rd well of gel, while M is 50bp ladder (Figure 4.3).

4.3.3 Assessment of yield of final library

Qubit® Fluorometer was used to measure the concentration of amplified fragment library (Table 4.2). The final library concentration was 3.67 and 1.72 µg/ml in respect to total run.

Table 4.2: Concentration of amplified fragment library

No. of performed run	Sample name	Final Library (µg/ml)
1.	Fragment library	3.67
2.	Fragment library	1.72

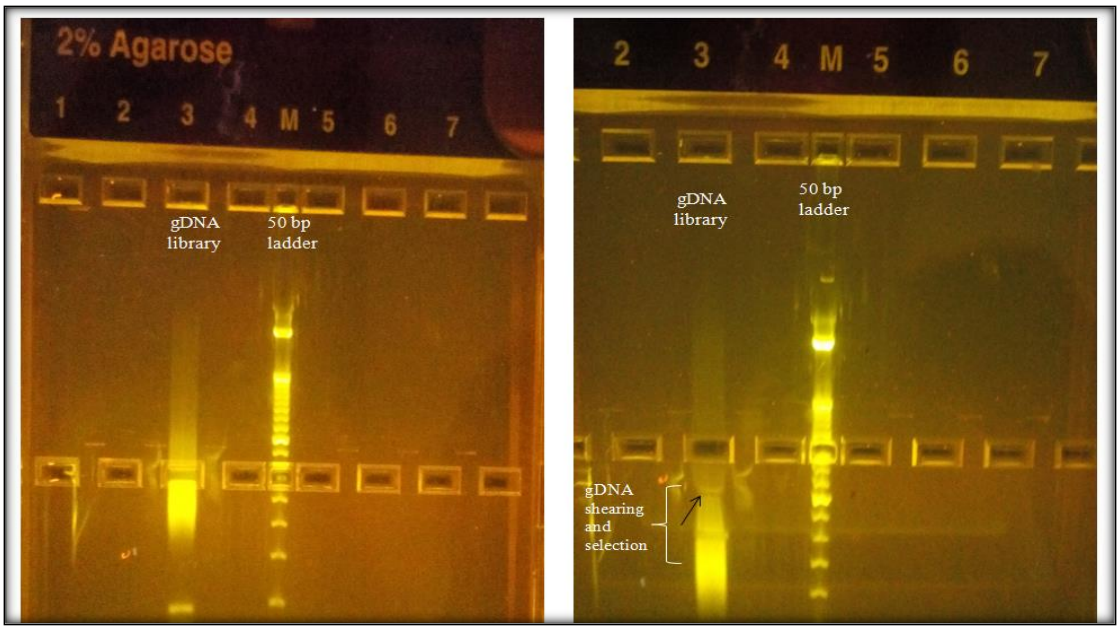

Figure 4.3: 400 base read library gel

4.3.4 Calculation of dilution of final library

The following conversion formula was used for the dilution of the final library.

Conversion formula of pg to pmol =

$$\frac{1}{660 \text{ X N}} \text{x} \frac{\text{DNA Conc. (pg)}}{1} \text{x} \frac{10^6}{1} = \text{pmol DNA}$$

pg= Concentration of amplified library using Qubit® Fluorometer

N= Number of base (Read length).

The concentration of final library was measured in pmol using the above formula. Read length and concentration of DNA library is given in Table 4.3. The concentration of the all samples was adjusted to 100 pmol by dilution with nuclease-free water.

Table 4.3: Read length and pmol concentration of Amplified library

No. of performed run	Library conc^{n} pg/µl	Read length (bp)	Total pmol of DNA library
1.	3670	400	13901
2.	1720	200	13030

4.3.5 Emulsion based clonal amplification and NGS sequencing run report

For *in vitro* amplification of the single stranded library of quantitated DNA fragments, the emulsion (emPCR) was performed, further, to generate a library of clonally amplified, bead-immobilized, single-stranded DNA fragments representative of the entire DNA by using OT2 emPCR Kit. Total volume was subjected for enrichment and after enrichment to the emPCR for clonally amplified bead ligated DNA fragments. Sequencing primers were added to each tube.

4.4 GENOME SEQUENCING

4.4.1 Ion torrent (Ion S5)

After the completion of enrichment, ISPs were loaded on each new Ion 530 and Ion 540 chip for sequencing. Two runs were performed of Ion S5. 6.01 Gb data was generated during the first run of sample. ISPs loading (Figure 4.4) was 92% on the Ion 530^{TM} chip and 19,533,024 bp were the total reads generated after the removal of polyclonal (25%) and low quality data (23%), with mean length 308 bp. The percentage of usable reads was 58%. Second run was carried out in Ion 540 ^{TM} chip and 19.6 Gb data was generated in total. This run was preceded by using barcodes for different samples, out which celery was barcoded with IonXpress_001 barcode name. ISPs loading (Figure 4.5) was 90% on the chip. The total number of reads generated were 99,125,389 bp after removing polyclonal (18%) and low quality data (7%), with mean length 198 bp. For celery genome, total of 3.3 Gb data was generated and there were 16,179,010 numbers of reads with 209 bp mean read length. The statistical data of the two runs is shown in Table 4.4.

Table 4.4: Statistical data of run on Ion S5

Sr. no.	Parameter	1st run	2nd run
1	Total number of reads	19,533,024	99,125,389
2	Total bases (Gb)	6.01	3.3
3	Mean length (bp)	308	209
4.	ISP Loading (%)	92	90
5.	Usable reads (%)	58	75

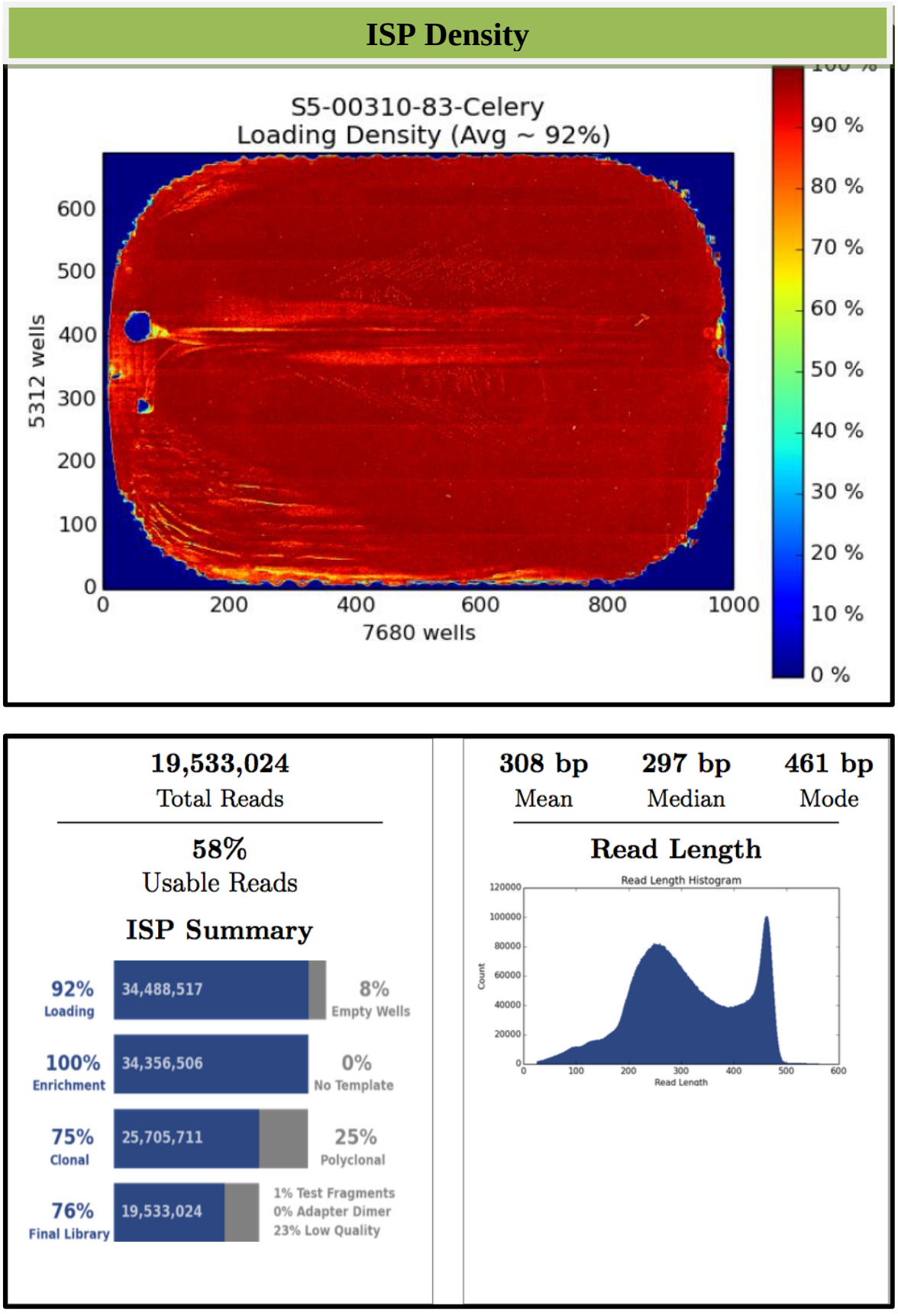

Figure 4.4: ISP loading summary of celery (1st Run)

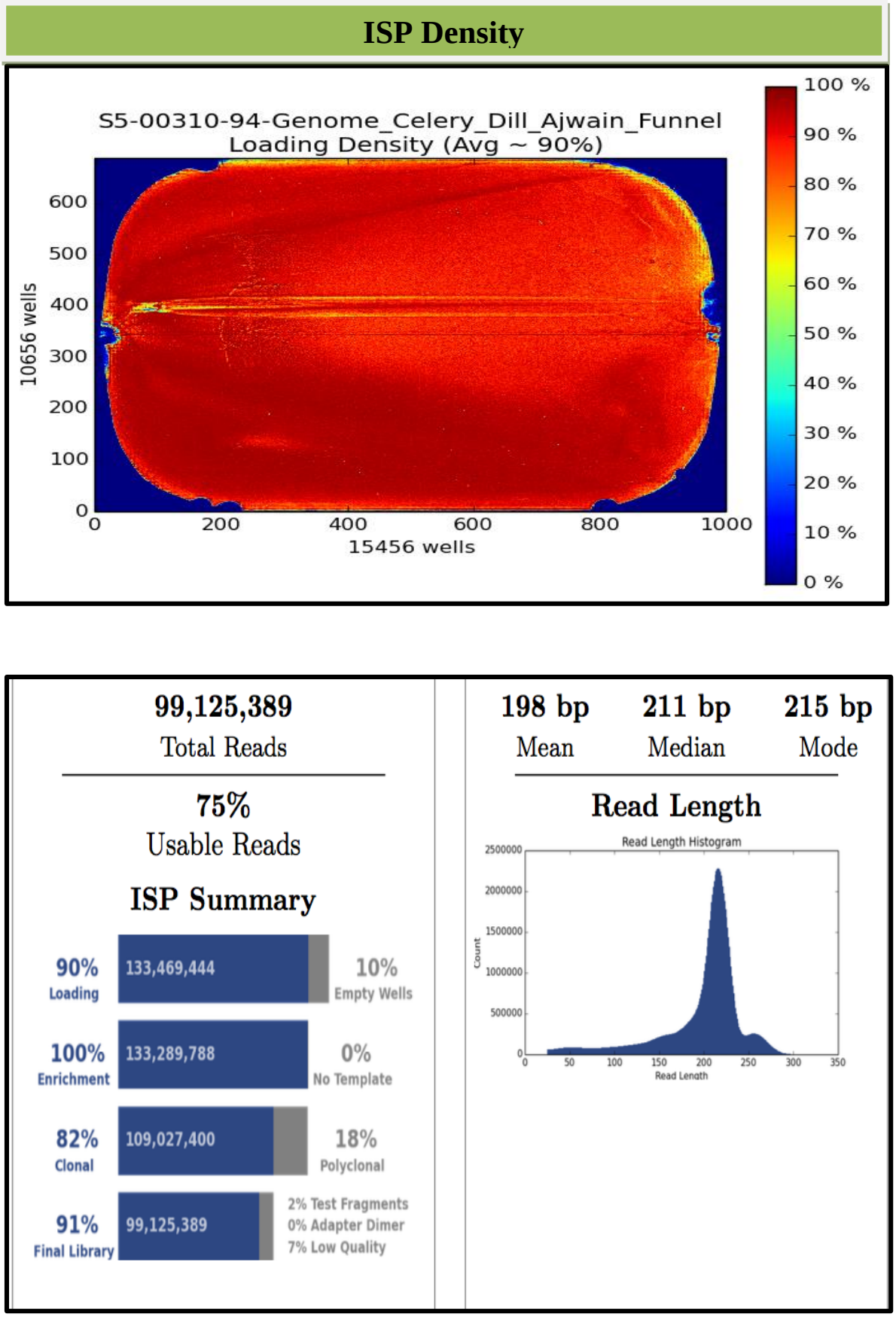

Figure 4.5: ISP loading summary of celery (2nd Run)

4.4.2 Total run (raw data) details of sequencing:

Next generation sequencing of Ajmer Celery-1 was carried out using Ion torrent (Ion S5) sequencing system. Two runs were carried out in this research study. The data produced after the first run was 6.01 Gb. The second run was preceded by using barcodes for specific samples and that generated 3.3 Gb data. Details of the run are given in Table 4.5.

Table 4.5: Genome sequencing run details of celery

Run No.	Type of run	Sequencing platform	Raw Data generated	Use of Data
1	Genome sequencing	Ion Torrent (Ion S5)	6.01 Gb	Comparative genome analysis. Identification of genes.
2	Genome sequencing	Ion Torrent (Ion S5)	3.3 Gb	Comparative genome analysis. Identification of genes.

4.5 EXPERIMENTAL ANALYSIS

4.5.1 Analysis Workflow

After the completion of each sample run, the raw sequence data were collected. These data were further processed for quality assessment and processing step, which comprised of the removal of adapter sequences and trimming of the sequences on quality bases. Trimmed sequences were then processed for *de novo* mapping. *De novo* assembly was carried out using the CLC work bench (Version 9.5.4). Blast2GO and KEGG pathways were analyzed alongwith SSRs identification and primer design.

4.5.2 Quality analysis using CLC Genomics Workbench

Raw data (reads) generated from sequencing (NGS) were assessed with the help of CLC Genomics Workbench v9.5.4, in which per-sequence coverage analysis was carried out. The total sequences were 27,361,042 bp (combined 2 runs) and total nucleotides were 5,412,056,746 bp.

4.5.2.1 Distribution of sequence lengths

The x-axis depicts the sequence length in base-pairs and y-axis depicts the number of sequences featuring a particular length normalized to the total number of sequences. The graph revealed the number of sequences that peaked (above 15%) between 200 to 225 bp (Figure 4.6).

4.5.2.2 Distribution of GC content

The GC-content of a sequence is calculated as the number of G and C bases compared to all bases (including ambiguous bases). Average GC content was 35%, while maximum GC content was 38% at relative sequence about 6% (Figure 4.7).

4.5.2.3 Quality Distribution

It is the distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities. The x-axis depicts the PHRED score. A PHRED quality score is a measure of the quality of the identification of the nucleobases generated by automated DNA sequencing. The PHRED score was 25 at 23% (Figure 4.8).

4.5.2.4 Coverage

Coverage is the number of sequences that support (cover) the individual base positions. As far as untrimmed data is concerned, the coverage of per base position was 100% at level of about 240 bp read length (Figure 4.9).

4.5.2.5 Nucleotide contributions

This graph describes the coverage for the four DNA nucleotides with ambiguous bases. The four (A/T/G/C) nucleotides were observed between 10-30% at level of base position at 360 bp. The ambiguous nucleotide distribution was zero (Figure 4.10).

4.6 QUALITY FILTERING OF GENOME SEQUENCING DATA (TRIM REPORT)

CLC Genomics Workbench v9.5.4 was used to generate trim report of reads by following the described parameters: (1) removal of low quality sequence (limit = 0.05), (2) removal of ambiguous nucleotides: maximal 2 nucleotides allowed, (3) removal of terminal nucleotides: 1 nucleotide from the 5' end and 1 nucleotide from the 3' end and (4) removal of sequences on length: minimum length 20 nucleotides.

Initially, the report which was generated after the sequencing of the celery genome comprised of 27,361,042 bp with 197.8 bp as the average read length. Later, trimming process generated a trim report describing the number of reads to be 27,218,172 bp with average read length of 194.4 bp (Table 4.6). The read length distribution graph depicted the trim report. The blue line showed before trimming reads while the black line represented the after trimming reads (Figure 4.11).

Table 4.6: Before/after trimming report of reads

Sr. No.	Performed run	Before Trimming (bp)		After Trimming (bp)		Percentage of sequence remain after trimmed
		Number of reads	Average read length	Number of reads	Average read length	
1.	Celery	27,361,042	197.8	27,218,172	194.4	99.48

4.7 *DE NOVO* ASSEMBLY OF THE RAW DATA USING CLC GENOMICS WORKBENCH

4.7.1 *De Novo* Assembly of Ion S5 data

After reads filtering, 4.4 Gb of high-quality data was generated. *De novo* assembly of celery was carried out with the help of CLC genomics workbench (Version 9.5.4) *de novo* Assembler. Data generated after assembly is given in (Table 4.7). The *de novo* assembly of 27,218,172 reads yielded assembled reads of 441,399,737 from celery sample with 827,971 number of contigs. This comprised of 32.9% adenine, 17.2% cytosine, 17.1% guanine and 32.9% thymine (Table 4.8). The minimum, maximum and average length of contigs were 200, 15978 and 533 respectively. N25, N50 and N75 of contigs were found to be 1014 bp, 591 bp, and 395 bp respectively. N50 contig size is a value such that at least half of the genome is contained in contigs of that size larger. N25 and N75 are the equivalent values at which 25% and 75% of the genome is covered by contigs of that size larger.

Table 4.7: Assembly data generated from genome sequencing of Ajmer Celery-1

Sr. No	Total sequences	Number of Contigs	Minimum contig length	Maximum contig length	Average length	N25	N50	N75
Contig measurements								
1.	441,399,737	827,971	200	15,978	533	1014	591	395

Table 4.8: Nucleotide distribution of assembled data

Sr. No.	Nucleotide	Count	Frequency
1.	Adenine (A)	145,161,946	32.9%
2.	Cytosine (C)	75,758,881	17.2%
3.	Guanine (G)	75,381,472	17.1%
4.	Thymine (G)	145,097,438	32.9%

Schmutz *et al.* (2010) studied genome sequence of the palaeopolyploid soybean. Construction of 6.5x scaffold assemblies was carried out using the Arachne assembler. In order to assess sequencing, assembly characteristics and extent of marker anchoring, provisional scaffold assemblies were constructed at the stage of 6.5x WGS coverage. The 6.5x assembly consisted of 993,511,522 bases in 3,119 scaffolds. Most of the sequence was contained in large scaffolds. The N50 was 6.5 Mb, and 97.6% of the sequence was in 364 scaffolds larger than 100 kb.

4.8 NCBI SEQUENCE SUBMISSION WITH ACCESSION NUMBER

The data of whole genome have been submitted in National Centre for Biotechnology Information (NCBI) with Data submission portal under Bioproject (PRJNA384920) and Biosample (SAMN06854046) of *Apium graveolens* L. and Bioproject submission ID is SUB2617482 and Sequence Read Archive is SRS2154565.

4.9 BLAST2GO ANALYSIS OF GENOME SEQUENCING DATA

Blast2GO is a bioinformatical tool for functional annotation of (novel) sequences and the analysis of annotated data. It is suitable platform for functional genomics research in non-model species (Conesa *et al.,* 2005). The procedure carried out for the analysis of genome sequening of Celery (Ajmer Celery-1) via Blast2GO platform is described as under.

4.9.1 Functional annotation of *de novo* genome assembly of celery sequence using CLC workbench

In *de* novo assembly, 827,971 number of contigs were generated. Somehow, it is a tedious job to perform annotation 827,971 contigs. Therefore annotation for only selected contigs was carried out. Contigs with size more than 5000 bp were filtered out and used for the annotation (375 sequences). Blast2GO tool was utilized for the functional annotation and validation of these sequences.

4.9.2 Analysis Progress of Blast2GO result

Total 375 sequences were functionally annotated out of which 374 showed positive InterProScan and 355 got Blast hits. 351 and 290 sequences were mapped and annotated respectively (Figure 4.12).

4.9.3 Data distribution of Blast2GO result

In data distribution graph of celery, the 375 assembled sequences were used for analysis. 22 sequences were blasted without hits and 5 with blast hits. 60 sequences were analyzed with mapping and 289 sequences were observed with Gene Ontology (GO) annotation (Figure 4.13).

4.9.4 Species distribution of Blast2GO result

The assembled samples showed highest similarity with UDP-forming with 74 Blast hits followed by Protein-PII with 73 Blast hits. A maximum number of blast hits of approximately 320 were found in others species (Figure 4.14).

4.9.5 Top-Hit species distribution of Blast2GO result

Result of Top-Hit species distribution showed equal similarity with ADP-ribose, Cauliflower mosaic virus, ADP-forming, Phalaenopsis aphrodite subsp. Formosana, Manihot esculenta, marchantia polymorpha, UDP-forming, Panax ginseng and Ostreid herpesvius 1 with one Blast hit (Figure 4.15).

4.9.6 Sequences similarity distribution during Blast2GO result

No hits were observed upto 32 sequences in graph generated of the percent similarity distribution of sequences during Blast2Go. Hits were generated after 32 sequences with a maximum peak of about 1400 hits (Figure 4.16).

4.9.7 E-value distribution during Blast2GO result

E-value describes the number of hits expected while searching a database of particular size; the lower the E-value, more is the significant match. It is evident that with the increasing E-value, the number of hits is decreased. At 1E-01 the highest hits obtained is 3900. Negligible hits were seen after 1E-100 (Figure 4.17).

4.9.8 High scoring segment pair (HSP) per Hits distribution

High scoring segment pair (HSP) distribution indicated that 35,900 hits distribution was found between hits 0 to 2 (Figure 4.18).

4.9.9 Mapping database distribution

Mapping database distribution of celery genome showed highest similarity with UniprotKB (375,000 sequences) followed by Saccharomyces Genome Database (SGD) (85,000 sequences) and The Arabidopsis Information Resources (TAIR) (75,000 sequences) (Figure 4.19).

4.9.10 Evidence code distribution of Blast hits

The evidence code distribution of Blast hits graph presents total assembled sequences (375). Out of those sequences, the majority of sequences were grouped in to Inferred from Electronic Annotation (IEA) (more than 400,000 Hits) evidence code followed by Inferred from sequence or Structural Similarity (ISS) with 40,000 Hits and Inferred from Direct Assay (IDA) with 20,000 Hits (Figure 4.20).

4.9.11 Evidence code distribution of sequences

Evidence code distribution of sequences was found to be distributed in nineteen databases among which IEA had 350 sequences and IDA had 321 sequences while from Inferred Mutant Phenotype (IMP) database it was 272 sequences (Figure 4.21).

4.9.12 Annotation distribution

Annotation distribution of GO graph ranged from 0 to 90. 0-50 GOs were obtained for various sequences. Very fewer sequences were seen with GOs after 100 score (Figure 4.22).

4.10 GENE ONTOLOGY (GO) IDs AND SEQUENCE DISTRIBUTION

Gene ontology is mainly divided into three groups: biological process, cellular components and molecular function. Total 5032 GO IDs were found which were grouped into biological process, cellular components and molecular function (Figure 4.23). During gene ontology direct GO count were generated. In biological processes very few sequences were showing maximum GO count to DNA metabolic process, nucleic and metabolic process, response to salt stress (Figure 4.24). In cellular component maximum sequences were having maximum GO count which respond to cytosol, nucleus and plasma membrane (Figure 4.25). In molecular function maximum sequences from total sequences respond to hydrolase activity showing maximum GO count followed by protein binding, organic cycling compound binding, heterocyclic compound binding and ATP binding (Figure 4.26).

4.10.1 Biological process

Biological processes like, biological regulation, cellular component organization, cellular process, metabolic process, reproduction, growth and response to stimulus processes were observed. In the pie chart of biological process at graph level 3 indicated many information about biological process. Out of total sequences, 238, 160 and 133 sequences were responsible for the cellular metabolic process, single organism cellular process and response to stress respectively (Figure 4.27).

4.10.2 Cellular component

In the cellular component cell, cell part, organelle, envelope, extracellular region, extracellular region part, macromolecular complex, organelle parts such type process take place. In the pie chart of cellular component indicated so many sequences. Out of that 210, 176 and 164 responsible for intracellular membrane bounded organelle, cytoplasm and cytoplasmic part (Figure 4.28).

4.10.3 Molecular function

Transferase activity, nucleic acid binding, nucleotide binding, cation binding hydrolase activity were showed in the pie chart (Figure 4.29). Minimum sequences (30) were found for receptor binding.

4.11 INTERPROSCAN RESULTS

Sequence similarity was also tested based on the protein domain conserved region through InterProScan. 320 sequences showed positive InterPro result while 50 sequences did not show any InterPro results and 130 sequences was scanned with GOs (Figure 4.30).

InterProScan is a widely used tool for protein and nucleotide sequence analysis. Rather than just simply supplying a convenient way of searching disparate analytical signatures from different source databases, it also allows users to obtain a better overview of what the results mean by adding valuable information from the InterPro database. InterProScan results provide additional annotation of genome based on protein structure and functional information.

4.12 ENZYME CLASS DISTRIBUTION

The assembled sequences of celery were divided into six main classes of enzymes. Among all the sequences, 22 sequences were grouped into Oxidoreductases class followed by Transferases class (66 sequences), Hydrolases class (95 sequences), Lyases

class (17 sequences) Isomerases class (08 sequences) and Ligases class (07 sequences) (Figure 4.31).

4.13 KEGG PATHWAY ANALYSES AND ENZYME MAPPED

Kyoto Encyclopedia of Genes and Genomes (KEGG) is a bioinformatics resource for understanding biological function from a genomic perspective. It is a multispecies, integrated resource consisting of genomic, chemical and network information with cross-references to numerous outside databases and containing a complete set of building blocks (genes and molecules) and wiring diagrams (biological pathways) to represent cellular functions. In present study KEGG analysis gave 76 pathways (Table 4.9).

Tao *et al.* (2011) studied the expression sequence tags from a full-length-enriched cDNA library of developing sesame seeds**.** 18,549 uniESTs were successfully annotated with Gene ontology (GO) terms using Blast2GO program. An additional 3,079 sequences were then annotated using InterProScan program. Overall, a total of 21,628 uniESTs were annotated with 111,600 GO terms distributing among the three main GO categories. 9,347 tentative unique genes (TUGs) representing 21,628 uniESTs across the various GO terms were examined with WEGO. Under the category biological process, subcategories "cellular process" and "metabolic process" accounted for approximately 49.5% and 46.2% of the annotations for the TUGs respectively. In correspondence to these processes, in the main category Molecular Function, 41.8% of the TUGs annotations were grouped into the subcategory "binding" and 40.2% in the subcategory "catalytic activity".

Table 4.9: A list of Encyclopedia of Genes and Genomes (KEGG) Pathway with enzyme ID and sequences number

Sr. No.	Pathway	SP	SE	Enzyme ID
1	Caprolactam degradation	2	2	EC: 1.14.15.3 EC:1.1.1.2
2	Thiamine metabolism	30	2	EC: 3.6.1.15 EC: 2.7.4.3
3	Glycosaminoglycan degradation	1	1	EC: 3.2.1.23
4	Arginine and proline metabolism	2	2	EC: 1.2.1.3 EC: 2.6.1.1
5	Valine, leucine and isoleucine biosynthesis	2	3	EC: 3.1.2.4 EC:1.2.3.1
6	Sulfur metabolism	1	1	EC: 2.5.1.47
7	Carbon fixation pathways in prokaryotes	2	2	EC:4.1.1.31 EC: 6.2.1.5
8	Streptomycin biosynthesis	1	1	EC: 5.5.1.4
9	Amino sugar and nucleotide sugar metabolism	1	1	EC: 5.1.3.18
10	Methane metabolism	2	2	EC: 6.2.1.9 EC: 4.1.1.31
11	Glutathione metabolism	3	5	EC: 2.3.2.2 EC: 6.3.2.2 EC: 3.4.19.13 EC: 1.11.1.12 EC:1.11.1.9
12	Geraniol degradation	1	1	EC: 1.1.1.183
13	Carbon fixation in photosynthetic organisms	3	3	EC: 4.1.1.31 EC: 2.6.1.1 EC: 5.3.1.1
14	Lysine degradation	4	2	EC: 2.1.1.43
15	Carotenoid biosynthesis	1	1	EC: 1.2.3.14
16	Limonene and pinene degradation	1	1	EC: 1.2.1.3
17	Cysteine and methionine metabolism	3	6	EC: 4.4.1.1 EC: 2.6.1.1 EC: 2.5.1.47 EC: 2.6.1.57 EC:4.4.1.14 EC: 6.3.2.2
18	Terpenoid backbone biosynthesis	2	2	EC: 11.1.2.67 EC: 4.6.1.12
19	Cutin, suberine and wax biosynthesis	1	1	EC: 1.2.1.50
20	Fatty acid degradation	2	2	EC: 1.14.15.3
21	Porphyrin and chlorophyll metabolism	1	1	EC: 1.16.3.1
22	Starch and sucrose metabolism	9	8	EC: 3.2.1.48 EC: 3.2.1.4 EC: 2.4.1.12 EC: 2.4.1.34 EC: 2.4.1.13 EC: 3.2.1.21 EC: 3.2.1.26 EC: 3.2.1.68
23	C5-Branched dibasic acid metabolism	1	1	EC: 6.2.1.5
24	Lysine biosynthesis	2	2	EC: 2.6.1.57 EC: 3.5.1.47

25	Cyanoamino acid metabolism	2	2	EC: 2.3.2.2 EC: 3.2.1.21 EC:
26	Steroid biosynthesis	3	3	EC: 5.5.1.9 EC: 5.4.99.7
27	Glycine, serine and threonine metabolism	2	2	EC: 4.4.1.1 EC: 4.1.2.5
28	Fatty acid elongation	1	1	EC: 1.3.1.38
29	Alanine, aspartate and glutamate metabolism	1	1	EC: 2.6.1.1
30	Ascorbate and aldarate metabolism	2	2	EC: 5.1.3.18 EC: 1.2.1.3
31	Galactose metabolism	2	2	EC: 3.2.1.23
32	Fructose and mannose metabolism	1	1	EC: 5.3.1.1
33	Propanoate metabolism	2	3	EC: 3.1.2.4 EC: 6.2.1.4 EC: 6.2.1.5
34	Phosphonate and phosphinate metabolism	1	1	EC: 2.7.7.15
35	Pyrimidine metabolism	9	6	EC: 2.7.7.8 EC: 2.7.7.6 EC:2.7.4.6 EC: 2.7.4.14 EC: 2.7.4.22 EC: 2.7.1.48
36	Pentose and glucuronate interconversions	4	3	EC: 3.1.1.11 EC:3.2.1.15 EC:1.1.1.2
37	Retinol metabolism	2	2	EC: 1.2.3.1
38	Glyoxylate and dicarboxylate metabolism	2	2	EC: 6.2.1.9 EC:1.11.1.6
39	Taurine and hypotaurine metabolism	1	1	EC: 2.3.2.2
40	Arachidonic acid metabolism	2	2	EC: 1.11.1.9
41	Caffeine metabolism	1	1	EC: 1.17.3.2
42	Purine metabolism	49	10	EC: 4.6.1.1 EC:2.7.4.6 EC: 2.7.7.8 EC: 1.17.1.4 EC: 1.17.3.2
43	Drug metabolism - other enzymes	5	3	EC: 3.1.1.1 EC: 2.7.1.48 EC: 1.17.3.2
45	Aminobenzoate degradation	6	3	EC: 1.2.1.28 EC: 3.1.3.41 EC: 3.1.3.2
46	Drug metabolism - cytochrome P450	1	1	EC: 1.2.3.1
47	InsECt hormone biosynthesis	1	1	EC: 1.2.1.3
48	Chloroalkane and chloroalkene degradation	1	1	EC: 1.2.1.3
49	Toluene degradation	1	1	EC: 1.2.1.28
50	Xylene degradation	1	1	EC: 1.2.1.28
51	Pyruvate metabolism	2	2	EC: 4.1.1.31
52	Arginine biosynthesis	2	2	EC: 2.6.1.1 EC: 3.5.1.14
53	Tryptophan metabolism	3	5	EC: 1.2.1.3 EC: 1.2.3.1 EC: 2.4.1.195 EC: 1.11.1.6 EC: 1.2.3.7
54	Citrate cycle (TCA cycle)	1	2	EC: 6.2.1.4 EC:6.2.1.5

55	beta-Alanine metabolism	2	2	EC: 1.2.1.3 EC: 3.1.2.4
56	Glycolysis / Gluconeogenesis	3	3	EC: 5.3.1.1 EC:1.1.1.2
57	Tropane, piperidine and pyridine biosynthesis	1	2	EC: 2.6.1.1 EC: 2.6.1.57
58	Glycosphingolipid biosynthesis - ganglio series	1	1	EC: 3.2.1.23
59	Sphingolipid metabolism	2	2	EC: 3.1.3.4 EC:3.2.1.23
60	Nicotinate and nicotinamide metabolism	2	2	EC: 1.2.3.1 EC: 2.7.1.23
61	Ether lipid metabolism	1	1	EC: 3.1.3.4
62	Glycerophospholipid metabolism	3	3	EC: 3.1.3.4 EC: 2.7.7.15
63	Novobiocin biosynthesis	1	2	EC: 2.6.1.1 EC: 2.6.1.57
64	Phenylalanine, tyrosine and tryptophan biosynthesis	1	2	EC: 2.6.1.57 EC: 2.6.1.1
65	Inositol phosphate metabolism	3	3	EC: 5.5.1.4 EC: 5.3.1.1
66	Glycerolipid metabolism	3	3	EC: 1.2.1.3 EC: 1.1.1.2 EC:3.1.3.4
67	Phenylalanine metabolism	1	2	EC: 2.6.1.1 EC: 2.6.1.57
68	Isoquinoline alkaloid biosynthesis	1	2	EC: 2.6.1.1 EC: 2.6.1.57
69	Vitamin B6 metabolism	1	1	EC: 1.2.3.1
70	Tyrosine metabolism	2	3	EC: 2.6.1.1 EC: 2.6.1.57 EC: 1.2.3.1
71	Flavone and flavonol biosynthesis	1	3	EC: 2.4.1.91 EC:2.4.1.239 EC: 2.4.1.159
72	Isoflavonoid biosynthesis	3	1	EC: 2.4.1.170
73	Anthocyanin biosynthesis	2	1	EC: 2.4.1.115
74	Phenylpropanoid biosynthesis	8	6	EC: 1.1.1.195 EC:1.11.1.7 EC:3.2.1.21 EC: 2.4.1.111 EC: 2.4.1.128 EC: 2.4.1.120
75	Riboflavin metabolism	2	1	EC: 3.1.3.2
76	Histidine metabolism	1	1	EC: 1.2.1.3

4.14 OVERVIEW OF SSR MARKERS DEVELOPMENT USING NEXT GENERATION SEQUENCING TECHNOLOGY (BatchPrimer3, V 1.0)

A superior alternative to the conventional methods used for developing SSR markers is provided by high throughput sequencing technology along with bioinformatics tools (Abdelkrim *et al.,* 2009). With the arrival of next generation sequencing (NGS) platforms, large volumes of sequencing data are being generated that could be screened with the aid of bioinformatics tools for identifying microsatellite repeats. This avoids the construction of microsatellite-enriched DNA libraries and provides a rapid approach for the large-scale generation of microsatellite loci. The recent availability of massively parallel sequencing (MPS) facilitated the sequencing of microsatellite-enriched genomic libraries in multiplex pools, thereby reducing sample preparation and sequencing costs (Jennings *et al.*,2011) reduction in sequencing costs will further enable easier, cheaper and rapid identification of microsatellite markers in future. The pyrosequencing technique has been applied for the generation of microsatellite. Using these various approaches, a large number of genomic SSR markers have been developed in several economically important crops. However, comparing the efficiency of SSR isolation protocols is generally difficult due to the difference in search criteria used for identifying SSRs and other variance factors existing among different laboratories and researchers (Techen *et al.,* 2010). High redundancy, lack of SSRs in majority of sequenced clones and varying enrichment efficiency (observed when the same protocol is applied to the members of same or different genus/species) are some of the inherent problems associated with the improved protocols. The other factors that influence frequencies of SSR include variation in sampling regions of the genome used for SSR detection.

4.14.1 SSR marker identification and primer design using BatchPrimer3, V 1.0 in *Apium Graveolens* L. (Ajmer Celery-1)

SSR markers are co-dominant markers and are widely used for high-throughput genotyping and map construction. They are advantageous due to high abundance, random distribution within the genome, high polymorphism information content (PIC) and stable co-dominance. The reproducibility, co-dominance, relative abundance and complete genome coverage of SSR markers have made them one of the most useful tools for detecting genetic diversity, genetic linkage mapping, association mapping and evolution analysis. Genomic SSRs and expressed sequence

tag (EST)-SSRs, which are considered complementary to plant genome mapping. During this study a few SSRs were developed and used to detect genetic diversity for celery.

The SSR primers were designed using BatchPrimer3 (version 1.0) online software. These SSR markers will useful to study of genetic diversity, genetic linkage mapping and evolution analysis. For the identification of SSRs, FASTA file of 375 contigs were used.

Total 506 primers met the following parameters: 100–300 final product length (optimal 200 bp), primer size from 19 to 23 bp (optimal 21 bp) and GC content 30%–70% (optimal 50%); the annealing temperature was set at 50–60 °C (optimal 55 °C).

Wei *et al.* (2014) carried out a whole genome survey to develop simple sequence repeat (SSR) markers and to detect the genetic diversity of sesame germplasm. From the initial assembled sesame genome, 23,438 SSRs (≥5 repeats) were identified. The Perl script MIcroSAtelitte (MISA) was used to identify microsatellites in sesame genomes. To identify the presence of SSRs, only two to six nucleotide motifs were considered, and the minimum repeat unit was defined as six reiterations for dinucleotides and five reiterations for other repeat units. Mononucleotide repeats and complex SSR types were excluded from the study since distinguishing genuine mononucleotide repeats from polyadenylation products and single nucleotide stretch errors generated by sequencing was difficult.

4.15 VALIDATION OF SSR MARKERS

In order to validate the SSR primers in 3 different genotypes of celery (Ajmer Celery-1, Amy vishnagar and Tall Utah), 25 SSRs were selected manually. Criteria used to select the SSR primers for validation purpose was based on the melting temperature (Tm) and GC content (GC%) of the primers. Those primers were selected which were having melting temperature above 55ºC and GC content (GC%) was above 30%. Details of primers of celery are given in Table 4.10.

Total 100 primers were used, 25 each from Celery, Ajwain (Table 4.11), Fennel (Table 4.12) and Dill (Table 4.13). Ajwain, Fennel and Dill are the crops from Apiaceae family. So cross-species primers were used for the validation process. Initially, all the 100 primers were screened at different annealing temperature and 68 primers showed DNA amplification. Further, these primers from CEL series, AJ series, FEN series and DILL series were collectively used for validation.

Table 4.10: List of 25 SSR primers of celery (Ajmer Celery-1)

Sr. No.	PN	Sequence ID	O	PL	PS (bp)	Tm (°C)	GC%	Primer Sequence	SSR
1	CEL1	Celery_D_(single)_trimmed _contig_list_contig_5	F	21	217	54.6	42.86	ATGTCACCTTATCTCTCCACA	TATATATATATA
	CEL1R	Celery_D_(single)_trimmed _contig_list_contig_5	R	21		54.87	33.33	AAAGTGGAATGGAAATAAACC	
2	CEL2	Celery_D_(single)_trimmed _contig_list_contig_18	F	22	150	55.52	36.36	GTTAACGAAATATGGACAAAGG	GCTCGGCTCGGCTCG
	CEL2R	Celery_D_(single)_trimmed _contig_list_contig_18	R	22		54.82	40.91	CTTTGTACTCTTCTGCCTTCTT	
3	CEL3	Celery_D_(single)_trimmed _contig_list_contig_7	F	21	154	55.13	52.38	ACTCTGTCTCTCTCGCTCTCT	TCTCTCTCTCTC
	CEL3R	Celery_D_(single)_trimmed _contig_list_contig_7	R	21		54.96	42.86	TAGGCCACCTACTTCATCATA	
4	CEL4	Celery_D_(single)_trimmed _contig_list_contig_7	F	21	154	56.96	42.86	TAAATGAAGAGTTGGCCTCAC	AGTTAAGTTAAGTTA
	CEL4R	Celery_D_(single)_trimmed _contig_list_contig_7	R	21		54.44	38.1	CAATCAACTCATTACCACACA	
5	CEL5	Celery_D_(single)_trimmed _contig_list_contig_97	F	21	178	55.26	47.62	CTTTCTGGGCCTGTAGATACT	AAAGAAAGAAAG
	CEL5R	Celery_D_(single)_trimmed _contig_list_contig_97	R	21		55.42	42.86	CTGCAGGTAGATGTTTTGTGT	
6	CEL6	Celery_D_(single)_trimmed _contig_list_contig_33	F	21	152	54.72	52.38	GGTACAGGTGATACTGCAGAC	TCAATCAATCAA
	CEL6R	Celery_D_(single)_trimmed	R	21		55.04	42.86	AGAGCATGTCTTGTACTGGAA	

		_contig_list_contig_33							
7	CEL7	Celery_D_(single)_trimmed _contig_list_contig_25	F	20	143	54.67	40	GCACGTAATCACATCAAGAA	AGAGAGAGAGAGAG AG
	CEL7R	Celery_D_(single)_trimmed _contig_list_contig_25	R	21		55.16	52.38	CTCTGCCTAACACTCGTACAC	
8	CEL8	Celery_D_(single)_trimmed _contig_list_contig_235	F	22	158	54.98	45.45	GCTTAGCTCTACATCATTACCC	GAAAGAAAGAAA
	CEL8R	Celery_D_(single)_trimmed _contig_list_contig_235	R	22		55.18	40.91	ACTTCACTGTCAAGGTTCTTTC	
9	CEL9	Celery_D_(single)_trimmed _contig_list_contig_231	F	22	157	54.73	40.91	AGTAGCAGGGTATTGACAAGAT	CACACACACACACA
	CEL9R	Celery_D_(single)_trimmed _contig_list_contig_231	R	22		54.47	40.91	CAGTAACCATAACAAAGCTGAG	
10	CEL10	Celery_D_(single)_trimmed _contig_list_contig_70	F	21	117	55.00	42.86	TAGGAAGCTAACCCTTTCTGT	ACACACACACACAC AC
	CEL10R	Celery_D_(single)_trimmed _contig_list_contig_70	R	21		55.49	52.38	GCATAGTTCCCTACCAGACTC	
11	CEL11	Celery_D_(single)_trimmed _contig_list_contig_323	F	21	150	54.88	42.86	GCCTCAAGATGTTGTGTTTAC	AAATAAATAAATAA AT
	CEL11R	Celery_D_(single)_trimmed _contig_list_contig_323	R	20		55.14	50	CGCTCACTAGTGCAGATACA	
12	CEL12	Celery_D_(single)_trimmed _contig_list_contig_288	F	21	146	55.24	52.38	CCACTGTCCTTCTCTCTCTCT	CTCTCTCTCTCT
	CEL12R	Celery_D_(single)_trimmed _contig_list_contig_288	R	21		54.76	52.38	GAGTGGAGAGGTGTGTGTAAG	
13	CEL13	Celery_D_(single)_trimmed _contig_list_contig_273	F	21	149	54.49	52.38	AAGAGACTAGGAGAGCACCTC	AGAAAGAAAGAA
	CEL13R	Celery_D_(single)_trimmed _contig_list_contig_273	R	21		54.1	42.86	GTGTCCGTTGATTAATAGCTC	

14	CEL14	Celery_D_(single)_trimmed _contig_list_contig_284	F	21	190	54.4	42.86	GTGCGTGTATTGTATGTGTGT	GAGAGAGAGAGAGA
	CEL14R	Celery_D_(single)_trimmed _contig_list_contig_284	R	21		55.54	52.38	GGGTTGGAGTCTTCTACAGAG	
15	CEL15	Celery_D_(single)_trimmed _contig_list_contig_216	F	20	150	54.68	40	CATGAGCATTTTAGGTGTCA	GGTGGTGGTGGT
	CEL15R	Celery_D_(single)_trimmed _contig_list_contig_216	R	20		54.35	50	GTCCTGGGATATCTCTGCTA	
16	CEL16	Celery_D_(single)_trimmed _contig_list_contig_206	F	21	224	54.86	42.86	AGCCTAGCTCCTTTATTTCTG	ACAGACAGACAG
	CEL16R	Celery_D_(single)_trimmed _contig_list_contig_206	R	22		55.75	50	GGAGCACTCTTGAGTACTATGG	
17	CEL17	Celery_D_(single)_trimmed _contig_list_contig_125	F	21	122	50.74	52.38	CTCTCTCTGTCTCTCTCTCTG	TCTCTCTCTCTCTCTC TC
	CEL17R	Celery_D_(single)_trimmed _contig_list_contig_125	R	20		54.67	40	GCACGTAATCACATCAAGAA	
18	CEL18	Celery_D_(single)_trimmed _contig_list_contig_125	F	22	180	54.14	54.55	CTGTCTCTCTCTCTCTCTCTCC	TCTCTCTCTCTCTCTC
	CEL18R	Celery_D_(single)_trimmed _contig_list_contig_125	R	20		54.89	40	AGAGTTAAGCCTTGCATTTG	
19	CEL19	Celery_D_(single)_trimmed _contig_list_contig_159	F	21	126	55.25	42.86	ACGTCAGTTGGACAAATACTG	ATATATATATAT
	CEL19R	Celery_D_(single)_trimmed _contig_list_contig_159	R	21		54.94	52.38	CCAGGAGAGTACTGTTGTCAG	
20	CEL20	Celery_D_(single)_trimmed _contig_list_contig_159	F	21	132	54.94	52.38	CCTGACAACAGTACTCTCCTG	ACAACAACAACA
	CEL20R	Celery_D_(single)_trimmed _contig_list_contig_159	R	21		54.26	42.86	GCTGTATAATTCGTTCTCGTC	
21	CEL21	Celery_D_(single)_trimmed	F	22	150	54.77	40.91	GAGGTTAACTGAAAGAGGCTTA	GAGAGAGAGAGAGA

		_contig_list_contig_160							GAGAGA
	CEL21R	Celery_D_(single)_trimmed _contig_list_contig_160	R	21		54.48	52.38	TCCTCCTCTCTCTTCTCTCTC	
22	CEL22	Celery_D_(single)_trimmed _contig_list_contig_25	F	22	147	55.31	50	CAAAGAGAGAGAGAGAGAGACG	GAGAGAGAGAGAGA GAGAGAGAGAGAGA GAGAGAGA
	CEL22R	Celery_D_(single)_trimmed _contig_list_contig_25	R	22		54.96	50	GTACTACAACTCCGCCTAAGTC	
23	CEL23	Celery_D_(single)_trimmed _contig_list_contig_31	F	22	190	54.55	40.91	TAATAGAAAGAGGAAGGACCAG	GATGATGATGAT
	CEL23R	Celery_D_(single)_trimmed _contig_list_contig_31	R	22		55.44	45.45	CTGTGGTTAGAGTGGGAAATAC	
24	CEL24	Celery_D_(single)_trimmed _contig_list_contig_13	F	22	149	55.1	45.45	GTGGGTACTGTAGTGACATGAA	GCCTGCCTGCCT
	CEL24R	Celery_D_(single)_trimmed _contig_list_contig_13	R	22		55.21	40.91	CATTCTAGGCAGGAATGACTAT	
25	CEL25	Celery_D_(single)_trimmed _contig_list_contig_7	F	22	144	55.1	54.55	GCTCTCTCACTCTCTCTGTCTC	TCTCTCTCTCTC
	CEL25R	Celery_D_(single)_trimmed _contig_list_contig_7	R	22		54.84	45.45	CTATTAGGCCACCTACTTCATC	

Note:- **PN:** Primer name, **O**: Orientation, **PL**: Primer length, **Tm**: Melting temperature, **PS:** Product size

Table 4.11: List of 25 SSR primers of Ajwain (Gujarat AJ-2)

Sr. No.	PN	Sequence ID	O	PS	Len	Tm (ºC)	GC%	Sequence
1	AJ1	Ajwain_trimmed_contig_370	F	162	22	60.3	50	GATGATAGTGGGGTGATGTGGT
	AJ1R	Ajwain_trimmed_contig 370	R		22	60.3	50	CTGGAGTCGGTGGAATTAGAAG
2	AJ2	Ajwain_trimmed_contig_396	F	177	18	53.7	50	TTGGTTGCGGATTTGGAC
	AJ2R	Ajwain_trimmed_contig_396	R		22	60.3	50	GTTGAGGGAGGTTTGAAGGACT
3	AJ3	Ajwain_trimmed_contig_369	F	196	20	57.3	50	TGTCAGCAAGCCATGTCACT
	AJ3R	Ajwain_trimmed_contig_369	R		22	60.3	50	TTGTACCACTCTCGCAGATGTC
4	AJ4	Ajwain_trimmed_contig_249	F	204	22	60.3	50	ACCTCCCCAGCTTGAGTAATCT
	AJ4R	Ajwain_trimmed_contig_249	R		22	60.3	50	GGCTCGAAACACTAGCACAGTT
5	AJ5	Ajwain_trimmed_contig_266	F	169	22	57.3	50	CCGTGCAAAGGACTTCAAAC
	AJ5R	Ajwain_trimmed_contig_266	R		20	57.3	50	AAAGGACACCTACGGCATCA
6	AJ6	Ajwain_trimmed_contig_340	F	198	22	60.3	50	GAACTAGGCCCTACAATGTTGC
	AJ6R	Ajwain_trimmed_contig_340	R		22	60.3	50	CACTGGTCGCTTCTCTAATGGT
7	AJ7	Ajwain_trimmed_contig_344	F	180	22	60.3	50	GGTCCTGTTTCGTCGTAATCTG
	AJ7R	Ajwain_trimmed_contig_344	R		18	53.7	50	TTGCAGTCGCATTGGCTA
8	AJ8	Ajwain_trimmed_contig_335	F	129	22	60.3	50	CAAGCTTGTATGAGCGTAGCTG
	AJ8R	Ajwain_trimmed_contig_335	R		22	60.3	50	CAGGAAGGAGATCAGAAACTGC

9	AJ9	Ajwain_trimmed_contig_324	F	197	20	57.3	50	CGCAAAGAACGAGACCATCT
	AJ9R	Ajwain_trimmed_contig_324	R		22	60.3	50	AACCCTGACACTCCAATCACTC
10	AJ10	Ajwain_trimmed_contig_293	F	221	22	60.3	50	AGGTGCTCCTGAACATCTGAAC
	AJ10R	Ajwain_trimmed_contig_293	R		22	60.3	50	ATGTGGGTCGATACATCTCTCC
11	AJ11	Ajwain_trimmed_contig_323	F	229	20	57.3	50	GAGCAGTTTCGCGTTTGTCT
	AJ11R	Ajwain_trimmed_contig_323	R		22	60.3	50	ATCCTCCTTTGGTAGGTGGAGT
12	AJ12	Ajwain_trimmed_contig_259	F	258	22	60.3	50	GGTGGGTGTGTGAGTGTGTTTA
	AJ12R	Ajwain_trimmed_contig_259	R		22	60.3	50	CGGTTAGTCTGGGCTTTATGTC
13	AJ13	Ajwain_trimmed_contig_188	F	242	22	60.3	50	GAGGTAGAGGGTTTATGCCACA
	AJ13R	Ajwain_trimmed_contig_188	R		22	60.3	50	CTATTGTACGGGGTTTGCTCAG
14	AJ14	Ajwain_trimmed_contig_228	F	197	18	53.7	50	CGCCCTTCATTTCCATTG
	AJ14R	Ajwain_trimmed_contig_228	R		22	60.3	50	CAGTGCTGCTTCAAGTTCAGAG
15	AJ15	Ajwain_trimmed_contig_230	F	184	22	60.3	50	GTTGGTGAGGGAGCAAGAAGTA
	AJ15R	Ajwain_trimmed_contig_230	R		22	60.3	50	GGAGGGAGGAAAAGAGAGATTG
16	AJ16	Ajwain_trimmed_contig_244	F	247	22	60.3	50	AATACCATGACTCGCCTACTGG
	AJ16R	Ajwain_trimmed_contig_244	R		20	57.3	50	AAACCCGTGTAGACGGACAA
17	AJ17	Ajwain_trimmed_contig_247	F	219	20	57.3	50	CAGGATGAGGGAATCAAACG

	AJ17R	Ajwain_trimmed_contig_247	R		22	60.3	50	CACTGGTAAAAGGAGGGAGACA
18	AJ18	Ajwain_trimmed_contig_286	F	227	22	60.3	50	GAAGGGGTGTGACAAGGACTAA
	AJ18R	Ajwain_trimmed_contig_286	R		22	60.3	50	ATATCAAGCTGGAGGAGCACTG
19	AJ19	Ajwain_trimmed_contig_308	F	196	22	60.3	50	GATTTCCCAGAAGATCCTCCTC
	AJ19R	Ajwain_trimmed_contig_308	R		22	60.3	50	TCACTCACTAGTGCGCTCAGAT
20	AJ20	Ajwain_trimmed_contig_311	F	185	22	60.3	50	CCTTGTAACCAGACCCACTGTT
	AJ20R	Ajwain_trimmed_contig_311	R		20	57.3	50	GAGCAATCGGCCGTATCTTA
21	AJ21	Ajwain_trimmed_contig_314	F	204	20	57.3	50	GTGGCAAGGGTGTCAAAAAG
	AJ21R	Ajwain_trimmed_contig_314	R		22	60.3	50	GGGATCGGATGACTGGATACTA
22	AJ22	Ajwain_trimmed_contig_336	F	216	22	60.3	50	AACAACTACCCCCTGAATCTCC
	AJ22R	Ajwain_trimmed_contig_336	R		22	60.3	50	TATCCGAGTCACGGTTAGAAGC
23	AJ23	Ajwain_trimmed_contig_384	F	169	22	60.3	50	GTACGGGGAAGACAATGAAGTG
	AJ23R	Ajwain_trimmed_contig_384	R		22	60.3	50	GGAGGGAACATGTCAACTCTGT
24	AJ24	Ajwain_trimmed_contig_399	F	194	20	57.3	50	GTGTATGCATATGCGCCTCA
	AJ24R	Ajwain_trimmed_contig_399	R		22	60.3	50	CTTCGGTCCACCTTTACTTGTG
25	AJ25	Ajwain_trimmed_contig_401	F	231	22	60.3	50	AAAGGGTCTAGACGGTGGTTCT
	AJ25R	Ajwain_trimmed_contig_401	R		20	57.3	50	AGATCGATGGTGGTGGTTGT

Note:-**PN:** Primer name, **O**: Orientation, **PS**: Product size, **Tm**: Melting temperature

Table 4.12: List of 25 SSR primers of Fennel (Gujarat Fennel-2)

Sr. No.	PN	Sequence ID	O	Len	PS (bp)	Tm (ºC)	GC%	Sequence
1	FEN10	Fen_trimmed_contig_10	F	20	203	55.2	40	GGTTTTGTGTTTACCCTTGA
	FEN10R		R	20		56.65	50	TTACCCTTGAGGTACACGAC
2	FEN119	Fen_trimmed_contig_119	F	22	162	55.67	42.86	ATGTCAGCAGCACTAAACAAG
	FEN119R		R	22		56.43	50	GACAGAGAGAGAGATGTGTGTG
3	FEN96	Fen_trimmed_contig_496	F	20	153	55.03	36.36	AAGCTACCTCAACAATTTCACT
	FEN96R		R	22		56.33	50	GAGTGGAAGAGACTATGGAGAG
4	FEN503	Fen_trimmed_contig_450	F	22	142	56.4	50	GTGAGTATCCCCACTAGACAGT
	FEN503R		R	22		55.44	40.91	AAGATCCTCCTCCTCTACATTT
5	FEN4003	Fen_trimmed_contig_903	F	22	130	56.66	50	ACCTGAACCAGAGAACAGTG
	FEN4003R		R	22		55.16	36.36	ACATTTCCGAGGCTTATCTAAT
6	FEN4508	Fen_trimmed_contig_1011	F	22	150	58.19	50	AGAAAGTCGTCTAGGGCTTC
	FEN4508R		R	22		54.89	31.82	ATTTAATATAAAAGGCGCCAAC

7	FEN4510	Fen_trimmed_contig_1158	F	22	171	56.88	54.55	ACATGATGTGATCATTTGAAAC
	FEN4510R		R	22		56.58	52.38	ATACAGGAGGATGTACAAGAGG
8	FEN5003	Fen_trimmed_contig_1589	F	22	159	58.12	61.11	TCCTTTTGATGGTATGTGTTTA
	FEN5003R		R	22		57.89	31.82	AATTGGTGTATCCAAATCATCT
9	FEN6003	Fen_trimmed_contig_1269	F	22	144	56.37	61.11	CATGATATTCATCATCATCGTC
	FEN6003R		R	22		56.33	52.38	ACTATGTGACAAACTGCAAAAA
10	FEN7002	Fen_trimmed_contig_1987	F	22	122	56.25	50	TCGACTTCGATGTTGTATTTTA
	FEN7002R		R	22		56.15	50	AATACAACTAGTGGTCCCAAAA
11	FEN7011	Fen_trimmed_contig_2074	F	23	141	56.02	50	TTTCAGTGTAGTGTTTGGTCAT
	FEN7011R		R	21		55.95	50	CATGGATAACTTAAAGTGCAAA
12	FEN7015	Fen_trimmed_contig_2158	F	22	129	55.9	50	TGACTGTACAATTCTTGTTGGT
	FEN7015R		R	22		55.87	47.62	AGTTTCCATCTTGACATTCTTC
13	FEN8006	Fen_trimmed_contig_2645	F	23	152	57.76	54.55	TCTTGAGTTCTCTCTTTTCTCTC
	FEN8006R		R	22		57.23	52.63	TATGTCTGTAGCATGGGTAAAA
14	FEN9004	Fen_trimmed_contig_2713	F	23	160	65.69	66.67	CATATTTGCTCTCCACAATAAA
	FEN9004R		R	22		57.34	57.14	TCTCTCAGGTCTCTGTCTCTCT

15	FEN9508	Fen_trimmed_contig_2901	F	22	156	56.84	55.56	AATACAGAGAGAGAGAGAAGG
	FEN9508R		R	22		56.59	52.38	GTTTCATTCACACCCTTTTAGT
16	FEN9510	Fen_trimmed_contig_2987	F	22	130	56.31	50	TATGACTCGTTTTGATGTTGAT
	FEN9510R		R	22		56.21	50	ATCGCAATCTAACAAATTCTGA
17	FEN9511	Fen_trimmed_contig_3006	F	22	145	56.16	50	GTTGGTTGATTTTTGTTATCG
	FEN9511R		R	22		56.16	50	CGGTAAACTTGGTAGAAGGATA
18	FEN9513	Fen_trimmed_contig_3074	F	22	132	56.13	50	TTATAGTACTTTCGCCTGAAGC
	FEN9513R		R	22		56.12	50	TAGTGAAGAGTGCTTGTAGTGG
19	FEN10013	Fen_trimmed_contig_3105	F	22	167	57.09	54.55	TGTGATTTCAATCTACTTACCG
	FEN10013R		R	22		57.02	54.55	GTGCTGCTGTTCATTTACTTTA
20	FEN10016	Fen_trimmed_contig_3186	F	22	171	56.81	52.17	CACCCAACCCGATGTATAA
	FEN10016R		R	22		56.74	50	GAGTGAGCGAGAGATACAAAAT
21	FEN10054	Fen_trimmed_contig_3210	F	22	135	56.74	50	GAACATGGTTTGGAATGTTTAT
	FEN10054R		R	22		56.7	50	GAACACTACATCCAGTCCCTAC
22	FEN21343	Fen_trimmed_contig_3248	F	22	158	56.87	52.63	CTGTATATCTTGTTGCGGACTA

	FEN21343R		R	18		56.56	52.38	AGGAAAGGAAACAGTTGAGTC
23	FEN23028	Fen_trimmed_contig_3256	F	22	169	56.42	50	GCTACAAAGTTTCACTGGTTTT
	FEN23028R		R	22		56.24	50	TGGCTAGTCTACAAATGAACAA
24	FEN23348	Fen_trimmed_contig_3610	F	22	138	57.48	50	CTATAATTGTTTGGGCTAGAAAA
	FEN23348R		R	22		56.46	50	TAAAGTAGGGAAGACCTGAAAA
25	FEN4508	Fen_trimmed_contig_4508	F	22	145	55.55	50	TCGACTTCGATGTTGTATTTTA
	FEN4508R		R	22		56.55	50	AATACAACTAGTGGTCCCAAAA

Note:-**PN:** Primer name, **O**: Orientation, **PS**: Product size, **Tm**: Melting temperature

Table 4.13: List of 25 SSR primers of dill (Gujarat Dill-1)

Sr. No.	PN	Sequence ID	O	Len	PS (bp)	Tm (ºC)	GC %	Sequence
1	DILL1	Dill_D_(single)_trimmed_contig_1	F	22	170	54.76	40.91	CGTATAGAATGCAAGCACTAAG
	DILL1R	Dill_D_(single)_trimmed_contig_1	R	22		54.84	45.45	TACATAGCGTGTATGGCTACTC
2	DILL2	Dill_D_(single)_trimmed_contig_6	F	22	138	54.57	36.36	GTCTCACATTCAAGTCTTTCAA
	DILL2R	Dill_D_(single)_trimmed_contig_6	R	22		55	45.45	TCTAATGGATAGGACAGAGGTC
3	DILL3	Dill_D_(single)_trimmed_contig_6	F	22	144	55.01	36.36	GGGGTCGTAATTCTGATATAAA
	DILL3R	Dill_D_(single)_trimmed_contig_6	R	22		55.1	40.91	GAGAAAGGACAGAAAGACACAT
4	DILL4	Dill_D_(single)_trimmed_contig_8	F	22	151	54.19	45.45	CAATCAGTGAACTAGAGAGTGG

	DILL4R	Dill_D_(single)_trimmed_contig_8	R	22		55.07	31.82	TAAAGCAATTCCCCTTATCTTA
5	DILL5	Dill_D_(single)_trimmed_contig_10	F	22	162	55.68	36.36	CATTCTCAAGAACCAAAAAGAC
	DILL5R	Dill_D_(single)_trimmed_contig_10	R	22		55.02	45.45	ACTACTGTGAGCGGTCTAAACT
6	DILL6	Dill_D_(single)_trimmed_contig_10	F	22	146	55.16	40.91	AGTCAATCAAGAAGGTGGTAAG
	DILL6R	Dill_D_(single)_trimmed_contig_10	R	21		55.02	33.33	GCATCGCAACTAATAGAAAAA
7	DILL7	Dill_D_(single)_trimmed_contig_11	F	22	168	54.99	45.45	CTAAGGCACATACGTCACATAC
	DILL7R	Dill_D_(single)_trimmed_contig_11	R	21		54.88	33.33	AAGATGGAGCAAATTCTCTTT
8	DILL8	Dill_D_(single)_trimmed_contig_11	F	21	183	54.88	33.33	AAAGAGAATTTGCTCCATCTT
	DILL8R	Dill_D_(single)_trimmed_contig_11	R	20		54.35	50	GAGAACACATACGCACTACG
9	DILL9	Dill_D_(single)_trimmed_contig_12	F	22	146	54.78	31.82	TTCACATCTCTCAATCAAATGT
	DILL9R	Dill_D_(single)_trimmed_contig_12	R	22		55.16	40.91	CTTCTAGTGTTGGGAAAGTCAT
10	DILL10	Dill_D_(single)_trimmed_contig_12	F	22	150	55.62	40.91	TTTACACTTTACCGAAGAGAGC
	DILL10R	Dill_D_(single)_trimmed_contig_12	R	22		54.9	31.82	TTCAAGTATGGATCATTTCTCA
11	DILL11	Dill_D_(single)_trimmed_contig_13	F	22	150	54.88	45.45	CTATAAGTCTAAGAGCCGGAGA
	DILL11R	Dill_D_(single)_trimmed_contig_13	R	22		55.68	31.82	TAATCCCATTCATTACAAAACC
12	DILL12	Dill_D_(single)_trimmed_contig_18	F	22	143	55.04	45.45	GTAAGGTTCTGAAAGAGACTCG
	DILL12R	Dill_D_(single)_trimmed_contig_18	R	22		54.57	40.91	AGCTAGTTGAAGAATGGTTCTC
13	DILL13	Dill_D_(single)_trimmed_contig_23	F	22	153	55.33	31.82	TTTAATAGCAAGAAAAGGCTGT
	DILL13R	Dill_D_(single)_trimmed_contig_23	R	22		55.84	40.91	GGTATGGCAAAAGCAATAGTAG
14	DILL14	Dill_D_(single)_trimmed_contig_23	F	22	155	54.91	40.91	CCCTTTTTCACTAAAGAGTCAC
	DILL14R	Dill_D_(single)_trimmed_contig_23	R	22		56.15	40.91	ACGGCTGGTTCTATTTACCTAT
15	DILL15	Dill_D_(single)_trimmed_contig_1	F	22	170	54.76	40.91	CGTATAGAATGCAAGCACTAAG
	DILL15R	Dill_D_(single)_trimmed_contig_1	R	22		54.84	45.45	TACATAGCGTGTATGGCTACTC
16	DILL16	Dill_D_(single)_trimmed_contig_6	F	22	144	55.01	36.36	GGGGTCGTAATTCTGATATAAA

	DILL16R	Dill_D_(single)_trimmed_contig_6	R	22		55.1	40.91	GAGAAAGGACAGAAAGACACAT
17	DILL17	Dill_D_(single)_trimmed_contig_8	F	22	151	54.19	45.45	CAATCAGTGAACTAGAGAGTGG
	DILL17R	Dill_D_(single)_trimmed_contig_8	R	22		55.07	31.82	TAAAGCAATTCCCCTTATCTTA
18	DILL18	Dill_D_(single)_trimmed_contig_10	F	22	162	55.68	36.36	CATTCTCAAGAACCAAAAAGAC
	DILL18R	Dill_D_(single)_trimmed_contig_10	R	22		55.02	45.45	ACTACTGTGAGCGGTCTAAACT
19	DILL19	Dill_D_(single)_trimmed_contig_10	F	22	146	55.16	40.91	AGTCAATCAAGAAGGTGGTAAG
	DILL19R	Dill_D_(single)_trimmed_contig_10	R	21		55.02	33.33	GCATCGCAACTAATAGAAAAA
20	DILL20	Dill_D_(single)_trimmed_contig_11	F	21	183	54.88	33.33	AAAGAGAATTTGCTCCATCTT
	DILL20R	Dill_D_(single)_trimmed_contig_11	R	20		54.35	50	GAGAACACATACGCACTACG
21	DILL21	Dill_D_(single)_trimmed_contig_12	F	22	150	55.62	40.91	TTTACACTTTACCGAAGAGAGC
	DILL21R	Dill_D_(single)_trimmed_contig_12	R	22		54.9	31.82	TTCAAGTATGGATCATTTCTCA
22	DILL22	Dill_D_(single)_trimmed_contig_13	F	22	150	54.88	45.45	CTATAAGTCTAAGAGCCGGAGA
	DILL22R	Dill_D_(single)_trimmed_contig_13	R	22		55.68	31.82	TAATCCCATTCATTACAAAACC
23	DILL23	Dill_D_(single)_trimmed_contig_18	F	22	143	55.04	45.45	GTAAGGTTCTGAAAGAGACTCG
	DILL23R	Dill_D_(single)_trimmed_contig_18	R	22		54.57	40.91	AGCTAGTTGAAGAATGGTTCTC
24	DILL24	Dill_D_(single)_trimmed_contig_20	F	22	150	54.25	40.91	ACTAGCTTCTCTCTTTGCTTTC
	DILL24R	Dill_D_(single)_trimmed_contig_20	R	22		54.87	36.36	AGTCATCTTTTGTTCGAAGTCT
25	DILL25	Dill_D_(single)_trimmed_contig_23	F	22	155	54.91	40.91	CCCTTTTTCACTAAAGAGTCAC
	DILL25R	Dill_D_(single)_trimmed_contig_23	R	22		56.15	40.91	ACGGCTGGTTCTATTTACCTAT

Note:-**PN:** Primer name, **O**: Orientation, **PS**: Product size, **Tm**: Melting temperature

4.16 POLYMORPHISM PATTERN OF SSRs

68 SSRs primers were amplified and gave a total of 101 bands. The SSR primer FEN5003 and FEN6003 produced maximum number of 3 bands, while CEL2, CEL4, CEL6, CEL8, CEL14, CEL19 and others produced minimum number of 1 band. Out of 101 bands, 43 bands were polymorphic with an average of 0.63 bands per primer and 58 bands were monomorphic. Among the 43 polymorphic bands, 29 bands were sheared polymorphic while 14 bands were unique-polymorphic (Table 4.14). The amplified fragments ranged from 90-1272 bp. The largest amplicone of 1272 bp was amplified by SSR primer AJ22 and smallest fragment of 90 bp was found with SSR primer CELL18 and DILL7.

The percent polymorphism obtained for SSR primers were ranged from 0 % to 100% with an average value of 43.13% per primer. The polymorphic information content (PIC) was calculated for each primer and it was ranged from 0.37 to 0.66, with an average value of 0.19 for each primer. The SSR primer index (SPI) differed from 0.74 to 1.98 with an average value of 0.45 (Table 4.14).

The agarose gel electrophoresis was used to separate amplified product of SSR primers. The performance of individual primer to amplify genomic DNA of 3 celery genotypes is discussed as under.

Table 4.14: Size, number of amplified bands, percent polymorphism and PIC obtained by SSR primers

Sr. No.	SSR Primer	Band Size (bp)	Total No. of Band (A)	Polymorphic Bands (B)			Mono-Morphic Band	% Poly-Morphism (B/A)	PIC	SPI
				S	U	T				
1	CEL1	147	1	0	0	0	1	0	0	0
2	CEL2	95-125	2	1	0	1	1	50.00	0.48	0.96
3	CEL3	140	1	0	0	0	1	0	0	0
4	CEL4	173-240	2	1	0	1	1	50.00	0.48	0.96
5	CEL6	101	1	1	0	1	0	100.00	0	0
6	CEL7	125	1	0	1	1	0	100.00	0	0
7	CEL8	436-709	2	1	0	1	1	50.00	0.48	0.96
8	CEL9	132	1	0	0	0	1	0	0	0
9	CEL10	377-1030	3	0	0	0	3	0	0.66	1.98
10	CEL11	154	1	0	0	0	1	0	0	0
11	CEL12	194	1	0	1	1	0	100.00	0	0
12	CEL13	162	1	0	0	0	1	0	0	0
13	CEL14	204-278	2	1	0	1	1	50.00	0.48	0.96
14	CEL15	121	1	0	0	0	1	0	0	0
15	CEL16	242	1	0	0	0	1	0	0	0
16	CEL17	172-270	2	0	0	0	2	0	0.50	0.96
17	CEL18	90-125	2	0	1	1	1	50.00	0.60	1.2
18	CEL19	298	1	1	0	1	0	100.00	0	0
19	CEL22	219	1	1	0	1	0	100.00	0	0
20	CEL24	256	1	1	0	1	0	100.00	0	0
21	AJ1	164	1	0	0	0	1	0	0	0
22	AJ2	147	1	0	0	0	1	0	0	0

23	AJ3	371	1	0	0	0	1	0	0	0
24	AJ5	391	1	0	0	0	1	0	0	0
25	AJ8	332-709	2	0	0	0	2	0	0.50	1
26	AJ9	282-749	2	0	0	0	2	0	0.50	1
27	AJ13	106-603	3	1	0	1	2	33.33	0.65	1.95
28	AJ14	227-268	2	0	0	0	2	0	0.50	1
29	AJ15	147-256	2	0	0	0	2	0	0.50	1
30	AJ16	229	1	0	0	0	1	0	0	0
31	AJ17	271	1	0	0	0	1	0	0	0
32	AJ19	147	1	0	0	0	1	0	0	0
33	AJ20	106	1	1	0	1	0	100.00	0	0
34	AJ22	492-1272	2	0	0	0	2	0	0.50	0
35	AJ25	977	1	1	0	1	0	100.00	0	0
36	DILL1	138	1	0	1	1	0	100.00	0	0
37	DILL2	101	1	0	0	0	1	0	0	0
38	DILL4	118	1	1	0	1	0	100.00	0	0
39	DILL5	124	1	1	0	1	0	100.00	0	0
40	DILL6	112	1	0	0	0	1	0	0	0
41	DILL7	90	1	1	0	1	0	100.00	0	0
42	DILL8	246	1	1	0	1	0	100.00	0	0
43	DILL9	147	1	1	0	1	0	100.00	0	0
44	DILL10	146	1	0	0	0	1	0	0	0
45	DILL11	124	1	0	1	1	0	100.00	0	0
46	DILL12	131	1	0	1	1	0	100.00	0	0
47	DILL13	146	1	0	1	1	0	100.00	0	0
48	DILL14	154	1	1	0	1	0	100.00	0	0
49	DILL15	124	1	0	1	1	0	100.00	0	0
50	DILL17	246-493	2	1	0	1	1	50.00	0.48	0.96

51	DILL18	144	1	0	0	0	1	0	0	0
52	DILL19	224	1	1	0	1	0	100.00	0	0
53	DILL20	181	1	0	1	1	0	100.00	0	0
54	DILL21	138	1	1	0	1	0	100.00	0	0
55	DILL22	131	1	0	0	0	1	0	0	0
56	DILL23	237	1	0	0	0	1	0	0	0
57	DILL24	291-747	3	0	0	0	3	0	0.66	1.98
58	DILL25	101	1	0	0	0	1	0	0	0
59	FEN10	227-370	2	0	0	0	2	0	0.50	1
60	FEN96	370 -571	2	1	1	2	0	100.00	0.37	0.74
61	FEN119	164	1	0	0	0	1	0	0	0
62	FEN4003	106-513	3	1	0	1	2	33.33	0.65	1.95
63	FEN4510	100-530	3	1	1	2	1	66.66	0.61	1.83
64	FEN5003	364-1067	3	3	0	3	0	100.00	0.66	1.98
65	FEN6003	212-1126	3	2	1	3	0	100.00	0.64	1.92
66	FEN7011	266-376	2	0	0	0	2	0	0.50	1
67	FEN8006	466-1030	3	0	1	1	2	33.33	0.61	1.83
68	FEN9511	442-879	3	1	1	2	1	66.66	0.61	1.83
	TOTAL		101	29	14	43	58	-	-	-
	AVERAGE					0.6	0.85	43.13	0.19	0.45

S = Sheared; U = Unique**; T =** Total Polymorphic Bands; **PIC** = Polymorphism information content; **SPI** = SSR Primer Index = Number of Bands X PIC

CEL1

The primer CEL1 amplified one fragment with size 147 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL2

The primer CEL2 amplified two fragments with size ranging from 95-125 bp having one monomorphic and one sheared fragment with 50% polymorphism and PIC value 0.48 (Table 4.14). This primer did not produce any unique band.

CEL3

The primer CEL3 amplified one fragment with size 140 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL4

The primer CEL4 amplified two fragments with size ranging from 173-240 bp having one monomorphic and one sheared fragment with 50% polymorphism and PIC value 0.48 (Table 4.14). This primer did not produce any unique band.

CEL6

The primer CEL6 amplified one fragment with size 101 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

CEL7

The primer CEL7 amplified one fragment with size 125 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

CEL8

The primer CEL8 amplified two fragments with size ranging from 436-709 bp having one monomorphic and one sheared fragment with 50% polymorphism and PIC value 0.48 (Table 4.14). This primer did not produce any unique band.

CEL9

The primer CEL9 amplified one fragment with size 132 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL10

The primer CEL10 amplified three fragments with size ranging from 377-1030 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.66 (Table 4.14). This primer did not produce any unique or sheared band.

CEL11

The primer CEL11 amplified one fragment with size 154 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL12

The primer CEL12 amplified one fragment with size 194 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

CEL13

The primer CEL13 amplified one fragment with size 162 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL14

The primer CEL14 amplified two fragments with size ranging from 204-278 bp having one monomorphic and one sheared fragment having 50% polymorphism and PIC value 0.48 (Table 4.14). This primer did not produce any unique band.

CEL15

The primer CEL15 amplified one fragment with size 121 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL16

The primer CEL16 amplified one fragment with size 242 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

CEL17

The primer CEL17 amplified two fragments with size ranging from 172-270 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

CEL18

The primer CEL18 amplified two fragments with size ranging from 90-125 bp with one monomorphic fragment and one unique fragment having 50% polymorphism and PIC value 0.6 (Table 4.14). This primer did not produce any sheared band.

CEL19

The primer CEL19 amplified one fragment with size 298 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

CEL22

The primer CEL22 amplified one fragment with size 219 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

CEL24

The primer CEL24 amplified one fragment with size 256 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

AJ1

The primer AJ1 amplified only one fragment with size 164 bp which was monomorphic having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ2

The primer AJ2 amplified one fragment with size 147 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ3

The primer AJ3 amplified one fragment with size 371 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ5

The primer AJ5 amplified one fragment with size 391 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ8

The primer AJ8 amplified two fragments with size ranging from 332-709 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

AJ9

The primer AJ9 amplified two fragments with size ranging from 282-749 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

AJ13

The primer AJ13 amplified three fragments with size ranging from 106-603 bp which produced one sheared fragment and two monomorphic fragments having 33.33% polymorphism and PIC value 0.65 (Table 4.14). This primer did not produce any unique band.

AJ14

The primer AJ14 amplified two fragments with size ranging from 227-268 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

AJ15

The primer AJ15 amplified two fragments with size ranging from 147-256 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

AJ16

The primer AJ16 amplified one fragment with size 229 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ17

The primer AJ17 amplified one fragment with size 271 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ19

The primer AJ19 amplified one fragment with size 147 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

AJ20

The primer AJ20 amplified one fragment with size 106 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

AJ22

The primer AJ22 amplified two fragments with size ranging from 492-1272 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

AJ25

The primer AJ25 amplified one fragment with size 977 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL1

The primer DILL1 amplified one fragment with size 138 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

DILL2

The primer DILL2 amplified one fragment with size 101 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

DILL4

The primer DILL4 amplified one fragment with size 118 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL5

The primer DILL5 amplified one fragment with size 124 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL6

The primer DILL6 amplified one fragment with size 112 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

DILL7

The primer DILL7 amplified one fragment with size 90 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL8

The primer DILL8 amplified one fragment with size 246 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL9

The primer DILL9 amplified one fragment with size 147 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL10

The primer DILL10 amplified one fragment with size 146 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

DILL11

The primer DILL11 amplified one fragment with size 124 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

DILL12

The primer DILL12 amplified one fragment with size 131 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

DILL13

The primer DILL13 amplified one fragment with size 146 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

DILL14

The primer DILL14 amplified one fragment with size 154 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL15

The primer DILL15 amplified one fragment with size 124 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

DILL17

The primer DILL17 amplified two fragments with size ranging from 246-493 bp having one monomorphic and one sheared fragment with 50% polymorphism and PIC value 0.48 (Table 4.14). This primer did not produce any unique band.

DILL18

The primer DILL18 amplified one fragment with size 144 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

DILL19

The primer DILL19 amplified one fragment with size 224 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL20

The primer DILL20 amplified one fragment with size 181 bp which was polymorphic unique fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any sheared band.

DILL21

The primer DILL21 amplified one fragment with size 138 bp which was polymorphic sheared fragment having 100% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique band.

DILL22

The primer DILL22 amplified one fragment with size 131 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

DILL23

The primer DILL23 amplified one fragment with size 237 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

DILL24

The primer DILL24 amplified three fragments ranging from size 291-747 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.66 (Table 4.14). This primer did not produce any unique or sheared band.

DILL25

The primer DILL25 amplified one fragment with size 101 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

FEN10

The primer FEN10 amplified two fragments with size ranging from 227-370 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

FEN96

The primer FEN96 amplified two fragments with size ranging from 370-571 bp which had one unique fragment and one sheared fragment having 100% polymorphism and PIC value 0.37 (Table 4.14). This primer did not produce any monomorphic band.

FEN119

The primer FEN119 amplified one fragment with size 164 bp which was monomorphic fragment having 0% polymorphism and PIC value 0 (Table 4.14). This primer did not produce any unique or sheared band.

FEN4003

The primer FEN4003 amplified three fragments with size ranging from 106-513 bp having two monomorphic and one sheared fragment with 33.33% polymorphism and PIC value 0.65 (Table 4.14). This primer did not produce any unique band.

FEN4510

The primer FEN4510 amplified three fragments with size ranging from 100-530 bp containing one unique, one sheared and one monomorphic fragment having 66.6% polymorphism and PIC value 0.61 (Table 4.14).

FEN5003

The primer FEN5003 amplified three fragments with size ranging from 364-1067 bp which were polymorphic sheared fragments having 100% polymorphism and PIC value 0.66 (Table 4.14). This primer did not produce any unique band.

FEN6003

The primer FEN6003 amplified three fragments with size ranging from 212-1126 bp containing one unique and two sheared fragments having 66.6% polymorphism and PIC value 0.64 (Table 4.14). This primer did not produce any monomorphic band.

FEN7011

The primer FEN7011 amplified two fragments with size ranging from 266-376 bp which were monomorphic fragments having 0% polymorphism and PIC value 0.5 (Table 4.14). This primer did not produce any unique or sheared band.

FEN8006

The primer FEN8006 amplified three fragments with size ranging from 466-1030 bp having two monomorphic and one unique fragment with 33.33% polymorphism and PIC value 0.61 (Table 4.14). This primer did not produce any sheared band.

FEN9511

The primer FEN9511 amplified three fragments with size ranging from 442-879 bp containing one unique, one sheared and one monomorphic fragment having 66.6% polymorphism and PIC value 0.61 (Table 4.14).

Bosamia *et al.* (2015) developed stress relevant EST SSR markers and validated in 11 different genotypes of peanut. Total of 2456 EST-SSR novel primer pairs were designed, out of which 366 unigenes having relevance to various stresses and other functions, were PCR validated using a set of 11 diverse peanut genotypes. Of these, 340 (92.62%) primer pairs yielded clear and scorable PCR products and 39 (10.66%) primer pairs exhibited polymorphisms. Overall, the number of alleles per marker ranged from 1-12 with an average of 3.77 and the PIC ranged from 0.028 to 0.375 with an average of 0.325.

In present study, among 100 primer pairs, 68 were amplified, which illustrated the suitability of *in silico* primer designing criteria employed for primer designing (Table 4.14). Out of all the synthesized primer-pairs, 43 primers displayed polymorphism within 3 selected celery genotypes.