Vegetable Science (2021) 48 (1) : 95-101

Microsatellites mining in *Capsicum* genomes and development of SSR markers in *Capsicum baccatum*

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Received: October 2020/ Accepted: July 2021

Abstract

Capsicum species is versatile vegetable cum spice crop, which have high commercial value because of its appealing color and pungency. Crop breeding and improvement of chilli requires genomic resources. Computational mining of chromosome wise microsatellites was carried out using search criteria of minimum repeats of 12 for mono, 6 for di and 5 for tri, penta & hexa nucleotides and identified 130942, 271650 and 2,78,143 perfect SSRs in *C. baccatum* cv. PBC81, *C. annuum* cv. Zunala and in *C. chinense* cv PI159236 genomes, respectively. Of these perfect SSRs; 8999, 18060 and 18019 were in compound form in the three species genomes respectively. Overall density of SSRs/Mbp observed was 173.66, 149.39 and 137.55 in *C. baccatum*, *C. chinense* and *C. annuum*. From 130942 perfect SSRs, total 59510 primer pairs were designed in *C. baccatum* and 16 SSR markers were primarily selected to test the usability and validated in a set of eight genotypes consisting of four accessions of *C. annuum*, one accession each of *C. chinense* and *C. frutescens* and two accessions of *C. baccatum*. All the 16 SSR markers exhibited expected size of amplification product. This *C. baccatum* based SSRs will be useful in characterization of germplasm and marker assisted breeding

Key words: Genome, Microsatellites, Markers, *Capsicum*

Introduction

Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs) are short repeat motif (1-6 nucleotides long repeats) which shows high level of length polymorphism due to insertion or deletion or mutation of one or more repeat type. Usually these SSRs are isolated by preparing genomic libraries and screening them with probes. However recent advent of next generation sequencing technologies made the whole genome sequences available in public domain which can be mined for SSRs using the several available bioinformatics tools, which gives full picture of SSR frequency and distribution (Kumar et al. 2014). Various studies on SSR mining suggest that these are abundant across the genome and present in both coding and non-coding regions of DNA Sequences (Phumichai et al. 2015). Their abundance varies across the taxa and are abundant in non-coding regions of the DNA.

SSRs have been the most widely used molecular markers for genotyping because of their high reproducibility, multi allelic, co-dominant and high transferability nature across the related species (Mason 2015). SSR polymorphism is exhibited by the difference in the number of repeat motifs among the different individuals. These markers are highly useful and already being explored in several crops for various applications like DNA fingerprinting, linkage mapping (Garcia et al. 2006), QTL mapping (Arjun et al. 2018), gene tagging, diversity analysis and population genetic studies. SSRs are superior to SNP markers because SSRs can describe more information per locus than biallelic SNP markers (Xu et al. 2013).

Chilli (*Capsicum* spp.) (n=12) is majorly grown for its appealing color and pungency fruits. It has got wide applications in food and pharmaceutical industries. In *Capsicum* genus there are five domesticated species viz., *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens* and *Capsicum pubescens*) (Ashrafi et al. 2012). Genome sequence of *C. annuum* and *C. chinense* (3.48 Gb and 3.21Gb respectively) are available in public domain (Kim et al. 2017). Though several progresses have been made in chilli breeding, and a good number of varieties/hybrids are available, still the problems constraining the production are increasing due to several biotic and abiotic stresses. To solve the problems timely, marker assisting breeding plays

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a key role in accelerating the breeding process quickly. Several marker systems are being effectively used in chill breeding and SSRs are most preferred. Comprehensive study on characterization of SSRs in *Capsicum annuum* and *C. chinense* genomes has been done recently using the available genomic data (Cheng et al. 2015), however the mining and characterization of SSRs in newly available genome of *Capsicum baccatum* is not reported. The species of *Capsicum* genus belonging to *baccatum* species have high importance in breeding, mainly as it is the best resistant source for anthracnose fruit rot (Park et al. 2009), Powdery mildew (Ahn et al. 2018) and other diseases. *C. baccatum* is cross-incompatible with the *annuum* complex, however there are efforts being carried out globally to transfer these genes into *C. annuum* (Suwor et al. 2015). The development and characterization of SSR markers from the *baccatum* genome will help in breeding.

The advance in computation biology helps quick progress in developing markers and saves a lot of time and cost. Several publicly available SSR search and mining tools such as IMEx (Imperfect Microsatellite Extractor) Mudnuri et al. (2007), SciRoko (Kofler et al. 2007), MicroSAtellite identification tool (MISA) (Thie et al. 2003) and KRAIT (Lianming et al. 2017) are available to develop and characterize SSRs markers. Effective utilization of these tools helps in quickly characterize the larger genomes and develop customized markers to use in breeding programmes. The present work was carried out aiming at genome wide characterization of SSRs in *Capsicum* spp. (*C. annuum*, *C. chinense* and *C. baccatum*) whole genomes chromosomes and design SSR primers pair using *Capsicum baccatum* genome.

Materials and Methods

Data sources of genomic resources: Genomic sequences of three species of *Capsicum*- PBC-81 variety of *Capsicum baccatum*, Zunala variety of *Capsicum annuum and* PI159236 varieties of *Capsicum chinense* were retrieved from NCBI data base (https://www.ncbi.nlm.nih.gov/genome/) (Table 1).

In silico SSR mining and microsatellites Search criteria

KRAIT software: In this study, KRAIT (https://github.com/lmdu/krait under GPL2), a recently developed ultrafast tool for genome-wide survey of microsatellites and primer design (Lianming et al. 2017) was used. It is written in Python and can be run as a standalone desktop application on Windows, Linux or Mac systems without dependencies. The microsatellite search engine is written in C and compiled as Python modules for import into Krait. Krait accepts FASTA formatted files containing any sequences as input for microsatellites search and primer design. Furthermore, Krait can accept a gzip compressed FASTA file as input that is suitable for large genome analysis. Krait gives the larger data as excel format on perfect SSRs, imperfect SSRs, compound SSRs, VNTRs and the statistical report of the sequences. For genome wide SSRs mining in the three *Capsicum* spp. Nuclear genomes, FASTA sequences of specific genome was downloaded. SSR mining was done using KRAIT software (Lianming et al. 2017). A minimum of twelve for mononucleotide (mono-) repeats, six for di nucleotide (Di-) repeats, five for tri nucleotide (Tri-) repeats, five for tetra nucleotide (Penta -) repeats, five for penta nucleotide (Penta-) repeats, five for hexa nucleotide (Hexa-) repeats search criteria was used. A compound microsatellite was defined when the distance between two adjacent microsatellites was d”100bp. For mini satellites and microsatellites, VNTRs, parameter set as minimum motif length and maximum motif length is 7 and 30 respectively where the minimum repeats were set to be 2.To identify the imperfect microsatellites, the minimum seed length and seed repeats were set to be 8 and 3 respectively. The maximum consecutive edits (including substitution and indel) allowed were specified to 3. The penalty cost was set to 1 for mismatch and 2 for indel (gaps). The minimum required score for identified iSSR was set to be 10 and motif standardization level was set to be level 0 (No standard).

MISA software: It is the most commonly used software for identification of microsatellites from the sequences. The MIcro SAtellite Identification Tool (MISA) was developed by Thie et al. (2003). It is a perl based program and runs in the command prompt mode. It allows the identification and localization of perfect microsatellites as well as compound microsatellites which are interrupted by a certain number of bases. Since, MISA is a perl based program, the computer has to be installed with Active perl (http://www.perl.org/). Further, MISA allows interaction with primer3 (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) for primer designing. Same set of parameters mentioned above are used

Primers designing and validation: The SSRs loci identified mainly in *Capsicum baccatum* were used for primer designing, as there is no much information on SSR markers in this species is available. SSR primers were designed by considering the parameters such as length of flanking sequence (100bp), amplicon size (100-

250), length of primer sequence (18-27bp), GC content (40-60) and clamp (2), primer melting temperature (58-65°C), primer maximum end stability (99) and primer maximum poly X (0). 16 SSR primers were randomly selected across different chromosomes and were used to genotype eight chilli lines (two accessions of each *C. annuum, C. chinense, C. frutescence* and *C. baccatum,* respectively to validate their potential application.

DNA extraction and PCR analysis: The young and fresh terminal leaves were collected during cool hours of the day and used for genomic DNA extraction. The DNA was extracted through modified CTAB method (Doyle and Doyle 1990). The quality and quantity of isolated DNA was evaluated using agarose gel electrophoresis and spectrophotometer. The selected eight genotypes were screened with 16 SSR markers to check for amplification. PCR reaction was performed in 15 ìl reaction mixture containing 25 ng DNA/ ìl DNA, 1X *Taq* buffer, 0.25 mmol/l dNTPs, 0.15 mmol/l of each primer and 1.0 unit of *Taq* polymerase. The PCR was performed in eppendorf nexus gradient PCR system, with initial denaturation at94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 90 s, with final extension at72 °C for 5 min. The amplified PCR products were analyzed on 3 % agarose electrophoresis gel stained with ethidium bromideand the gel was visualized and photographed under UV transilluminator (Bio Glow, China).

Results and Discussion

The analysis has been done chromosome wise in the three *Capsicum spp.* and distribution of perfect, imperfect, VNTRs and compound SSRs was identified (Table. 2).

Chromosome wise characterization of SSRs in *C. baccatum*: Total 130942 perfect SSRs and 8999 types of compound SSRs were identified. The frequency of 31568, 61518, 32685, 3676, 536 and 959 number of mono, di, tri, tetra, penta and hexa-repeats, respectively were identified (Table 3 and Table 4). The compound SSRs ranged from 22 (Chr11) to 2267 (Chr7). Total perfect SSRs were found to be highest on Chr7 (31532) and lowest on Chr11 (551). The average relative density of SSRs per Mbp was found to be 137.55 ranging from 108.3 on Chr2 to 174.65 on Chr1. Krait output was cross checked by analyzing SSR motifs through MISA software and found 149553 numbers of SSRs markers (31589 mono, 61552 di, 32719 tri, 3722 tetra, 540 penta, 964 hexa) including 131086 simple SSRs and 18467 types of compound SSRs.

Table 3: Chromosome wise distribution of SSRs in *C. baccatum* cv PBC81

Chr no.	Perfect SSRs	Imperfect SSRs	Total	VNTRs	Compound SSRs
Chr 1	4022	23558	27580	1471	200
Chr 2	25212	166006	191218	12512	1697
Chr 3	718	4850	5568	368	36
Chr 4	2755	16716	19471	1128	194
Chr 5	3603	20256	23859	1186	182
Chr 6	5316	36079	41395	3059	404
Chr 7	31532	223637	255169	17375	2267
Chr 8	26886	187344	214230	14327	1801
Chr 9	1000	5178	6178	306	43
Chr 10	5519	35416	40935	2732	398
Chr 11	551	3165	3716	200	22
Chr 12	23828	174159	197987	13724	1755
Total	130942	896364	1027306	68388	8999

Chromosome wise characterization SSRs in *C. chinense*: The analysis of 12 chromosomes of *C. chinense* by Krait software identified total of 278143 perfect SSRs and 15849 were found to be in compound form. Microsatellites distribution shows the frequency of motif repeats in mono, di, tri, tetra, penta and hexa-repeats are 78870, 132639, 57749, 7301, 1482 and 662, respectively (Table 5). The compound SSRs ranged from 36 (Chr6) to 2403 (Chr3). Total perfect SSRs were found to be highest on Chr3 (38901) and lowest on Chr12 (712). The average relative density of SSRs per Mbp was found to be 149.39 ranging from 122.29 on Chromosome 10 to 197.96 on Chromosome 6. Similar output was obtained by analyzing SSR motifs through MISA software and found total 279366 SSRs containing 79551, mono, 132992 di, 57813 tri, 7418 tetra, 925 penta, 667 hexa SSR motifs along with 39988

Table 1: Genomic data of different *Capsicum spp.* used in the study

Species	Cultivar	Accession no.	Assembly level	Size (Gb)	GC%	Protein content
C.baccatum	PBC 81	GCA_002271885.1	Chromosome	3.21Gb	35.4251	35853
C. annuum	Zunala	GCA_000710875.1	Chromosome	3.48Gb	35.3818	40627
C. chinense	PI159236	GCA_002271895.2	Chromosome	3.07Gb	34.8664	34974

Table 2: Distribution of SSR types in *Capsicum* species

Species	Perfect SSRs	Imperfect SSRs	VNTRs	Compound SSRs
C. baccatum	130942	896364	68388	8999
C. annuum	271650	1806124	145412	18060
C. Chinense	278143	1863273	147967	18019

Table 4: Frequency of SSR motifs (1-6bp) in *Capsicum baccatum* cv PBC 81(Krait software)

Length (bp)	Chr	Mono	Di	Tri	Tetra	Penta	Hexa	Total	Compound	Relative abundance (SSR/Mbp)
18049528	1	1497	1578	812	95	14	26	4022	200	233.64
169143200	2	6293	11527	6341	734	109	208	25212	1697	154.1
4628671	3	175	343	173	22	0	5	718	36	159.65
16877874	4	881	1171	590	88	11	14	2755	194	168.91
14767715	5	1332	1413	732	87	22	17	3603	182	255.3
39143577	6	1294	2385	1409	162	24	42	5316	404	139.96
254917563	7	6744	15332	8194	908	122	232	31532	2267	127.26
210184557	8	6361	13133	6394	726	104	168	26886	1801	131.6
4424925	9	426	361	181	25	4	3	1000	43	235.53
35458989	10	1392	2354	1505	183	31	54	5519	398	161.21
2943109	11	208	224	98	15	4	2	551	22	194.36
200511653	12	4965	11697	6256	631	91	188	23828	1755	122.49
Total		31568	61518	32685	3676	536	959	130942	8999	173.6675

Table 5: Frequency of SSR motifs (1-6 bp) in *Capsicum chinense* cv PI159236 (Krait software)

Length (bp)	Chr	Mono	Di	Tri	Tetra	Penta	Hexa	Total	Compound	Relative Abundance (loci/Mbp)
241225625	1	10880	16306	7548	954	126	101	35915	2273	150.97
169927767	2	8768	11998	5576	741	89	69	27241	1695	162.88
275189702	3	11860	17769	8051	1009	119	93	38901	2403	143.3
9760878	4	791	689	327	33	6	1	1847	90	192.53
237150106	5	7912	14876	6435	814	107	74	30218	2086	128.8
4887987	6	429	316	183	18	2	1	949	36	197.96
234238532	7	8612	14768	6465	856	89	78	30868	1985	133.41
195914817	8	7374	12478	5095	632	85	52	25716	1361	132.8
258863286	9	8149	15950	6702	814	113	74	31802	211	124.3
183664776	10	5309	11416	4828	553	626	44	22216	1585	122.29
250851717	11	8518	15795	6408	845	118	74	31758	2079	128.1
4134258	12	268	278	131	32	2	1	712	45	175.37
Total		78870	132639	57749	7301	1482	662	278143	15849	149.3925

of compound SSRs in *Capsicum chinense cv.* PI159236.

Chromosome wise characterization SSRs in *C. annuum*: With the Krait tool, the analysis of 12 chromosomes of *C. annuum* identified total of 271650 perfect SSRs and 18010 were found to be in compound form (Table 6). The distribution of microsatellites of mono, Di, Tri, Tetra, Penta and hexa-repeats are 80196, 121825, 59549, 7762, 1045 and 1273 respectively. The compound SSRs ranged from 43 (Chr9) to 2449 (Chr3). Total perfect SSRs were found to be highest on Chr3 (38161) and lowest on Chr2 (614). The average relative density of SSRs per Mbp was found to be 137.55 ranging from 108.3 on Chromosome 2 to 174.65 on Chromosome 1. Krit output was cross checked by analyzing SSR motifs through MISA software and found total 271755 SSRs containing 80169 mono, 121800 di, 59569 tri, 7872 tetra, 1054 penta, 1291 hexa SSR motifs along with 38713 of compound SSRs in *Capsicum annuum* cv. Zunala

SSR markers development in *C. baccatum* and validation: In addition to the mining and characterization of SSRs, development of SSR markers for the identified SSR loci chromosome wise was carried out in *C. baccatum*. Total of around 59510 primer pairs were designed for the perfect SSR loci across 12 chromosomes with the parameters of PCR product size range was set to 100-250, primer sequence length was set to 18 and 27bp as minimum and maximum size respectively, GC content of maximum and minimum was set to be 60 and 40, GC clamp set as 2, primer melting temperature set was to the range of 58-65^{0}Celsius. The primer pairs for perfect SSR loci developed in the present study were 1696, 11261, 279, 1247, 1692, 2390, 14514, 12529, 456, 2400, 231, 10815 for Chromosomes 1 to 12 respectively.

To preliminarily test the usability of these SSR primers, a random set of 16 primer pairs for the SSR loci spanning across the chromosomes were selected and validated in a set of eight genotypes consisting of four accessions of *C. annuum*, one accession each of *C. chinense* and *C. frutescens* and two accessions of *C. baccatum*. All the 16 SSR markers got amplified at 55^0 C annealing temperature and exhibited expected size of amplification product. Out of 16 markers, 12 markers showed agarose gel based polymorphism, indicating there practical utility. Six SSR markers (cssr-5, cssr-44, cssr-16, cssr-2, cssr-36 and cssr-56) showed different allele for the *C. baccatum* genotypes.

SSRs are mainly identified by screening the cDNA or genomic libraries (Lioi et al. 2013), now a days with advent of next generation sequencing technologies enabling large scale whole genome sequencing and *de novo* assembly of genomes and transcriptome is helping fast discovery of markers. The progress in computation biology and bioinformatics tools accelerated the markers discovery, with the available bioinformatics software; SSRs can be easily mined from the large genomic data, which gives full picture of SSR frequency and distribution (Kumar et al. 2014). Further, there are tools like primer3 software which are integrated in to several SSRs search tools help in designing primers for the identified SSR loci enabling there use as molecular markers for practical applications in breeding.

Capsicum species is versatile vegetable cum spice, and have high export value, being extensively cultivated in India. The genome size of the *Capsicum* spp. is very large, approx. 3.2GB. Recently Cheng et al. (2015) did comprehensive characterization of SSRs in pepper genomes and identified an average of 868047.50, 45.50 and 30 SSR loci in nuclear, mitochondrial and chloroplast genomes, respectively. In the present study, we mainly focused on *C. baccatum*, another cultivated species which is cross-incompatible with *C. annuum*. The genome of *C. baccatum* cv. PBC81 was made available by Kim et al. (2017) and the cultivar PBC81 is well known for its high resistance to anthracnose fruit rot. The breeders have put effort in using this as resistant source in developing varieties/hybrids resistance to this disease. Though SSRs available in *annuum* are transferrable to *baccatum*, it necessities the development of SSR markers specific to *baccatum* for more efficient practical utility, as the earlier studies observed that there are chromosomal rearrangements among *annuum* and *baccatum* mainly on chr3, chr5 and chr9 (Kim et al. 2017).

In the present study, by Krait software total perfect SSRs identified are in 130942 in *C. baccatum*, 271,650 in *C. annuum* cv. Zunala and 2,78,143 in *C. chinense* cv. PI159236, whereas Cheng et al. (2015) identified 876,580 and 859,515 SSRs in Zunala and Chiltepin. The varied number of SSRs among our study and their study is because of Cheng et al. (2015) used minimum repeats of 10 for mononucleotides, 6 for dinucleotide, 4 for trinucleotide, tretranucelotide, pentanucelotide and hexanucleotide. Whereas we used search criteria of twelve for mononucleotide (mono) repeats, six for dinucleotide (Di) repeats, five for trinucleotide (Tri) repeats, five for tetranucleotide (Penta) repeats, five for pentanucleotide (Penta) repeats, five for hexanucleotide (Hexa) repeats. The minimum repeats have been increased in the present study and SSRs developed will have good PIC. Similarly Portis et al. (2018) used search criteria of minimum 15 repeats for mononucleotides, 8 of di, 3 of tri and 4 for tetra, penta and hexa nucleotides respsectively and found that perfect SSRs count were 277,513 in *C. baccatum*, 212,867 in *C. chinense* and 194,622 in *C. annuum*.

The SSRs identified in *C. baccatum* are 10,27,306 in which 130942 are perfect SSRs and 896364 are imperfect SSRs and 8999 were in compound form. There exists a difference among the content and distribution of SSRs in the three species studied. *C. annuum*. and *C. chinense* shown almost same pattern of content and distribution of SSRs, this is expected as *C. annuum* and *C. chinense* both are cross compatible and belong to *annuum* complex, whereas *C. baccatum* belongs to *baccatum* complex and cross incompatible with the *annuum* complex. The distribution and content of SSRs varied across the chromosomes in the all the three genomes of *Capsicum* spp. studied. As *bacctum* genome is not close proximate of C. annuum genome, the abundance of SSRs also varied among these two

Table 6: Frequency of SSR motifs(1-6bp) in *Capsicum annuum* cv Zunala (Krait software)

Length (bp)	Chr	Mono	Di	Tri	Tetra	Penta	Hexa	Total	Compound	Relative Abundance (loci/Mbp)
93487876	1	5560	6016	3453	444	69	72	15614	968	174.65
5799306	2	112	305	179	14	1	3	614	45	108.3
261510930	3	12202	16228	8341	1064	142	184	38161	2449	150.29
215701946	4	8309	12782	5761	799	93	112	27856	1817	132.59
217274494	5	7758	12816	6349	841	113	119	27996	1930	132.25
219521584	6	8554	13307	6461	749	109	134	29314	2003	136.79
222112641	7	7260	12232	6024	875	87	154	26632	1842	126.47
153299543	8	6218	9228	4075	553	82	88	20244	1202	135.23
5047871	9	281	288	171	15	0	0	755	43	154.53
205736368	10	7680	12458	6256	819	118	143	27474	1906	136.98
220335243	11	7621	12431	5774	709	93	112	26740	1783	127.54
229934170	12	8641	13734	6705	880	138	152	30250	2022	135.02
Total		80196	121825	59549	7762	1045	1273	271650	18010	137.5533

spp. Lee et al. (2016) also reported three translocations between chromosome 3 and chromosome 5, chromosome 3 and chromosome 9, and chromosome 1 and chr8 in *C. baccatum* and *C.annuum* genome, these might be one of the reasons for genetic barriers for wide hybridaization among these two spp. Further recently Assis et al. (2020) did comparative cytogenomic study and reported the *Capsicum* spp. showed diversity in genome composition and *C.annuum* is close proximation with *C.chinense* when compared to *C.baccatum.*

The SSR density (SSRs/Mbp) also varied across the chromosomes and among the genomes. Highest density was observed in *C. baccatum* (173.66) whereas *C. annuum* and *C. chinense* had density of 137.55 and 149.39 SSRs/Mbp. Though Capsicum genome is large to the tune of 3.2GB, the density of SSRs found to low compared to other *Solanaceous* crop spp. (Cheng et al. 2015) and other crops such as cucumber (654.55 SSRs/ Mbp) and rice (527.62 SSRs/Mbp).The SSRs are randomly distributed across the genomes and across the chromosomes of each genome in *Capsicum*, this clearly in agreement earlier study in other organisms (Morgante et al. 2002, Yu et al. 2017, PMD Base).

For further validation and to check the utility of these *C. baccatum* based SSR markers, 16 SSR markers (cSSRs) were selected for genotyping of the pepper genotypes set consisting of four accessions of *C. annuum*, one accession each of *C. chinense* and *C. frutescens* and two accessions of *C. baccatum*. All the 16 were successfully got amplified and produced the expected product band on 3% agarose gel. Out of 16 SSR markers genotyped, two markers showed amplification only in *C. baccatum* whereas no amplification observed in other species. Six SSR markers (cSSR-5, cSSR-44, cSSR-16, cSSR-2, cSSR-36, cSSR-56) showed different allele for the *C. baccatum* genotypes. These markers can be effectively used in DNA finger printing and crop improvement programmes.

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कैप्सिकम प्रजाति एक बहुमुखी सब्जी–मसाला फसल है जिसका अत्यधिक व्यवसायिक मूल्य है क्योंकि इनमें आकर्षक रंग तथा तीखापन होता है। मिर्च के प्रजनन एवं उन्नयन में जिनोमिक संसाधनों की आवश्यकता होती है। मिर्च में गुणसूत्रानुसार माइक्रोसैटेलाइट का कम्प्यूटर आधारित आंकड़ा जनन शोध कम से कम 12 बार एकल–6 बार द्वितीय तथा 35 बार तृतीय, पंचम व षट् न्यूक्लियोटाइड को दोहराया गया और 130942, 271650 व 2,78,143 पूर्ण एस एस आर *कैप्सिकम बकैटम* सीवी. पीबीसी–81, *कैप्सिकम एनम* सीवी. जुनाला व *कैप्सिकम चाइनेन्स* सीवी. पीआई–59236 जिनोम में पाया गया। उपयुक्त एसएसआर; 8999, 18060 एवं 18019 में संयुक्त प्रवृत्ति इन तीनों प्रजातियों में पाया गया। समस्त एसएसआर/एमबीपी 173.66, 149.39 व 137.55 *कैप्सिकम बकैटम, कैप्सिकम चाईनेन्स* तथा *कैप्सिकम एनम* में क्रमशः पाया गया। कुल 130942 पूर्ण एसएसआर मार्कर में कुल 59510 प्रायमर युगल अभिकल्प *कैप्सिकम बकैटम* तथा 16 एसएसआर मार्कर का चयन व्यहार्य के लिये किया गया व इसका मूल्यांकन एवं *कैटिसम फ्रूटीसेन्स* तथा दो प्रविष्टियों को *कैप्सिकम बकैटम* से लिया गया। सभी 16 एसएसआर मार्कर की अभिव्यक्ति आशातीत आकर के उपज विस्तारण में देखा। *कैप्सिकम बकैटम* में एसएसआर आधारित जननद्रव्यों के चरित्रीकृत तथा मार्कर आधारित प्रजनन में उपयोगी होगा।

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