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Study of Genetic Diversity of Bhut Jolokia Germplasm in North East India by SSR Markers and Morphology

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ABSTRACT

North Eastern states are home to the genetic variability where several inter-specific hybrids/derivatives were originated, among which 'Bhut Jolokia' (Capsicum chinense Jacq.) is one of the world's hottest peppers. This study undertaken to determine the distinctiveness of Bhut Jolokia within the germplasm. About 30 Bhut Jolokia genotypes were collected from different regions of North East India, mainly from Assam, Nagaland and Manipur based on fruit morphological characters and their genetic diversity studied by using simple sequence repeats (SSR) markers. Ten simple sequence repeats (SSR) markers were used against the 30 genotypes to study the genetic diversity. Results revealed 100 per cent polymorphism in 9 out of 10 markers and polymorphic information content (PIC) ranging from 0.11(HpmsE014, HpmsE026) to 0.37 (CAeMS009) were recorded.

Key words: *Bhut Jolokia; IBPGR; SSR markers; Genetic diversity; North eastern states.*

INTRODUCTION

One of the earliest domesticated plant genera is believed to be the genus *Capsicum*[2]. These are popular worldwide due to sensory attributes of color, pungency and flavor[11]. India is the largest producer of chillies in the world. India is known to be a 'land of diversity' due to which provides varied congenial climatic conditions for different crops. Among which *Capsicum* genotypes have greater diversity especially in the North eastern region of India. The unique climatic conditions with high humidity given rise to a natural inter-specific hybrid, 'Bhut Jolokia'[6]. Presently Bhut Jolokia is recognized as the 7th hottest chilli in the world having a scoville heat unit of 1,041,427. It is locally know as Bhut Jolokia (Ghost chilli) or Bih Jolokia (poison chilli) in Assam, Naga Jolokia in Nagaland and Oo-morok (tree chilli) in Manipur. Taxonomic position of Bhut Jolokia was confirmed by RAPD analysis and found to be a natural hybrid between *Capsicum fruitescence* and *C. chinense* [3]. Very limited documentation is available on genetic resources of chilli landraces in North East India which is regarded as the secondary origin for chilli. This study made an attempt to know the existence of any new genotypes within the Bhut Jolokia germplasm of North Eastern states especially in Assam, Nagaland and Manipur.

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MATERIALS AND METHODS

Thirty different genotypes of Bhut Jolokia were collected from 3 north eastern states *viz.*, Assam, Nagaland and Manipur. Selection of genotypes was done based on plant growth habit and fruit characteristics[7] like colour, shape, size *etc.*,and designated as BJ001-BJ030. A suitable and uniform well leveled upland site was selected for the field experiment. The climatic conditions of Jorhat were cool winter and humid with hot and dry summer.

Raising of seedlings

Seeds of the selected fruits were extracted and washed in clean water to remove the unwanted materials from it, thereafter they were soaked in potassium nitrate (0.3%) over night and kept under shade for drying. The seeds were then sown in plastic trays filled with coco peat. Germination of seedlings took place at 10-15 days after sowing. Each pit was filled up with the top soil along with 10 kg FYM, 4 kg of urea (half of the dose), 10 kg of SSP and 2.5 kg of MOP was applied to the soil at recommended dose and mixed thoroughly one month before planting. After one week the seedlings at 6-8 leaves stage were transplanted in the plots of the main field with the spacing of 75cm x 75cm. One fourth dose of remaining (half dose) urea was applied at 30 days after transplanting and another one fourth dose at 60 days after transplanting.

Phenotyping

Morphological data were taken according to the *Capsicum* descriptors given by International Board of Plant Genetic Resources[7] *viz.*, Fruit color, fruit shape, fruit length and diameter.

Genomic DNA extraction and PCR analysis

Healthy leaves were collected from each genotype at 20 days of after transplanting. Extraction of genomic DNA was done from the leaf samples by grinding and followed by extraction method[5] with slight modifications. A total of 10 SSR markers were used as genome- wide basis. Estimated quantity of genomic DNA by spectrophotometer analysis as and quality of genomic DNA was confirmed by 0.8 per cent agarose gel. PCR conditions were Initial denaturation 94°C for 5 min, Denaturation 94°C for 1 min, Annealing Tm ± 2°C for 1 min, Extension 72°C for 1 min for 35 cycles, Final extension 72°C for 7 min and Holding 4°C for infinity.

The annealing temperature of a primer is dependent on melting temperature (Tm) of the primer, which has been calculated by using following formula

$$Tm = 4(G+C) + 2(A+T)\ °C$$

An annealing temperature ± 2 °C of the Tm for a specific primer was used in the study.

Gel electrophoresis and documentation

Agarose gel of 3 per cent strength was prepared by melting 9 g of agarose in a total volume of 300ml 1X TBE by heating and 15µl of ethidium bromide (10mg/ml) was added, the solution was cooled down to 50°C. The solution was poured into the casting tray. After one hour of solidification, combs were removed and then mounted on a gel tank containing 1000ml of 1X TBE. To each PCR tube containing the amplified PCR products, 2µl of 6X loading buffer was added. Ten µl of each PCR product was loaded onto the wells of the gel. Gel was run till bromophenol reached the end of the gel. The photograph of the gel was digitally documented in Gel documentation system (Alpha Innotech, USA).

Scoring of SSR data

The molecular weight of PCR products, obtained for each marker was designated, based on a known ladder. During band scoring, faint bands and bands with smeared background were avoided and only intense bands were scored on the basis of presence and absence of an amplified particular DNA fragment. If a product was present in a certain genotype, it was designated as '1' and if absent; it was designated as '0'.

Data analysis

Phenotypic data were analysed in MS- EXCEL and SPSS version 15.0. The SSR data were analysed by using the software package, NTSYS-pc Version 2.1[12].

Level of polymorphism

The binary data generated by SSR analysis were used to calculate per cent polymorphism by dividing amplified polymorphic fragment by total number of bands observed. Polymorphism information content (PIC) was evaluated by using the formula[1]

$$PIC=1-\sum_{J=1} P\,ij^2$$

Where, $P_{ij}$is the frequency of j^{th} allele or band for i^{th} marker and summation extends over n alleles or bands.

Genetic similarity analysis

Genetic relatedness among the genotypes was computed by using the Jaccard's coefficient of similarity using SIMQUAL module of NTSYS-pc. The pair wise genetic similarity index was calculated as per Jaccard's coefficient of similarity [8] and is given below:

$$F = \frac{N_{AB1}}{(N_T - N_{AB0})}$$

Where, F = Similarity index

N_{AB1} =Number of bands present(Scored 1) in both accessions A and B

N_{AB0} = Number of bands present in all test entries but not present in accessionA or B

N_T = Total number of bands scored in the study

Cluster analysis

The degree of genetic relationship among the studied BhutJolokia genotypes as revealed by Jaccard's coefficient of similarity was represented through cluster analysis using the algorithm of "Unweighted Pair Group Method with Arithmetic Average"(UPGMA), by feeding similarity matrix as input data. In UPGMA, averaging of distance is based on the total number of taxa in the clusters. In other words, if cluster i contains T_i taxa and cluster j contains T_j taxa, then

$$d_{ku} = \frac{Tidki + Tjdkj}{T_i + T_j}$$

The graphical representation of genetic relationship among the genotypes was done in the form of dendrogram. Clustering of the population was also done based on the Nei's genetic distance.

RESULTS AND DISCUSSION

Phenotypic diversity

Phenotyping of Bhut Jolokia germplasm were classified based on the *Capsicum* descriptors[7]. Plant growth habit in germplasm was categorized as prostate, intermediate and erect growth. Whereas stem and leaf pubescence, branching habit and leaf densities were categorized as sparse, intermediate and dense. Leaf descriptors such color *viz.*, light green, green, dark green and leaf shapes *viz.*, deltoid, ovate and lanceolate shapes in the germplasm were categorized (Table 3). Fruit descriptors like fruit color, fruit shape, fruit length, fruit diameter and fruit shape at blossom end were recorded (Table 4). All the observations were recorded when 50 per cent of plants beared ripe fruits.

Genotypic diversity PCR was performed using 10 SSR markers, out of which 9 were found polymorphicand C2_AT3G4460 was found to be monomorphic. Polymorphic data of SSR markers were used for further analysis.

Level of polymorphism

The polymorphic SSR data was analyzed to calculate polymorphism information content (PIC) of each marker. Higher the PIC value of a marker, greater is the potential of the marker to detect polymorphism, describes in relation to the frequency of each marker allele. The SSR marker CAeMS009had the highest PIC value of 0.3739, indicating the robustness in differentiating the germplasm under study and lowest PIC value of 0.1167 was recorded in HpmsE014 and HpmsE026 (Table 5).

Analysis of genetic diversity in Bhut Jolokia germplasm

The Jaccard's similarity coefficient, based on pair- wise similarity of SSR data ranged from 0.077 to 1.000. The dendrogram obtained based on SSR data using UPGMA, revealed three major clusters (Fig.1). Jaccard's

similarity coefficient matrix revealed that maximum similarity was observed between the genotypes BJ003, BJ004, BJ005 and BJ024, BJ026 in cluster 'A'. In cluster 'B' observed maximum similarity between the genotypes BJ010, BJ014; BJ011, BJ013, BJ016, BJ020 and BJ017, BJ018, BJ029. The genotypes BJ023 and BJ028 were showed maximum variation with other genotypes (Table 6).

Table 1: List of Bhut Jolokia genotypes used in this study

Accession No.	Village	District	State
BJ001	Alengmora	Jorhat	Assam
BJ002	Alengmora	Jorhat	Assam
BJ003	Namdeuri	Jorhat	Assam
BJ004	Hatigarh	Jorhat	Assam
BJ005	Alengmora	Jorhat	Assam
BJ006	Silonijan	Golaghat	Assam
BJ007	Silonijan	Golaghat	Assam
BJ008	Silonijan	Golaghat	Assam
BJ009	Senapati	Senapati	Manipur
BJ010	Dikoi	Dimapur	Nagaland
BJ011	Dikoi	Dimapur	Nagaland
BJ012	Sukori	Dimapur	Nagaland
BJ013	Senjum	Dimapur	Nagaland
BJ014	Tipomia	Jorhat	Assam
BJ015	Borbhula	Jorhat	Assam
BJ016	Dhekiajuli	Jorhat	Assam
BJ017	Harupathar	Golaghat	Assam
BJ018	Borpathar	Golaghat	Assam
BJ019	Ungma	Mokukchung	Nagaland
BJ020	Longjung	Mokukchung	Nagaland
BJ021	Longjung	Mokukchung	Nagaland
BJ022	Jorhat	Jorhat	Assam
BJ023	Jorhat	Jorhat	Assam
BJ024	Jorhat	Jorhat	Assam
BJ025	Dibrugarh	Dibrugarh	Assam
BJ026	Khwang	Dibrugarh	Assam
BJ027	Dolonikher	Dibrugarh	Assam
BJ028	AAU-1	Jorhat	Assam
BJ029	AAU-2	Jorhat	Assam
BJ030	AAU-3	Jorhat	Assam

Table 2: List of SSR markers used in the study

Sl. No.	Marker name	Chromosome location	Forward primer sequence	Reverse primer sequence	Annealing temperature	Reference
1	CAMS 451	8	TGCATTGGTGGGCTAACATA	GCTCTTGACACAACCCCAAT	46	[10]
2	CAMS 368	9	GAGTGGATAAGCAAGGACGTTT	TTTGCTTCCCTTTTTGCTTC	45.5	[9]
3	CAeMS 009	10	ACGCACCAACGAATATCTATCTCA	GTTTCCGTCCAGATCTACTTTTCCGC	52	[9]
4	CAMS 032	7	TGCCACATAGGTTGGCTTTC	CAAAGCCAATGCACATAATCA	45.5	[9]
5	CAMS 215	7	CGTGGGTGGTCTAGGATGAT	GCTGGCAAGTCACTCTGGAT	49	[10]
6	HpmsE014	6	CTTTGGAACATTTCTTTGGGGG	GCGGACGTAGCAGTAGGTTTGG	51	[9]
7	HpmsE026	1	CCAAAGTCCATCGACGTCTCAA	ATCAAATGGCAAACCAGGAGGA	49	[13]
8	HpmsE047	2	AACCCGTGTTCAATCCCCAAAT	TGGCCATACCACCAGCAGTAGA	50	[9]
9	HpmsE070	11	CACTCGTTATATTTTTCTGTCTCG	GTGAATATATCCGACCCTGTTT	46.5	[9]
10	C2_At3g44600	11	TCCTTTATACCGACTTGAAGCTATTG	AGATTCTATGTTTCTTGAAAGCACAGC	50	[9]

Table 3: Morphological characteristics of Bhut Jolokia germplasm

Accession N	Stem pubescence	Plant growth habit	Branching habit	Leaf density	Leaf shape	Leaf pubescence	Leaf colour
BJ001	Intermediate	Erect	Intermediate	Dense	Ovate	Sparse	Dark green
BJ002	Sparse	Intermediate	Intermediate	Dense	Ovate	Sparse	Light green
BJ003	Sparse	Intermediate	Intermediate	Dense	Ovate	Intermediate	Green
BJ004	Intermediate	Intermediate	Intermediate	Intermediate	deltoid	Sparse	Dark green
BJ005	Sparse	Intermediate	Intermediate	Dense	Deltoid	Sparse	Green
BJ006	Sparse	Intermediate	Intermediate	Intermediate	Ovate	Sparse	Green
BJ007	Intermediate	Intermediate	Intermediate	Dense	Ovate	Sparse	Dark green
BJ008	Sparse	Intermediate	Intermediate	Intermediate	ovate	Sparse	Light green
BJ009	Intermediate	Prostate	Intermediate	Dense	Ovate	Intermediate	Dark green
BJ010	Intermediate	Erect	Intermediate	Dense	Ovate	Sparse	Light green
BJ011	Sparse	Erect	Intermediate	Dense	Ovate	Sparse	Light green
BJ012	Intermediate	Erect	Intermediate	Dense	lanceolate	Sparse	Dark green
BJ013	Intermediate	Erect	Intermediate	Sparse	Ovate	Sparse	Green
BJ014	Sparse	Erect	Intermediate	Dense	Deltoid	Sparse	Dark green
BJ015	Intermediate	Erect	Intermediate	Sparse	Ovate	Intermediate	Dark green
BJ016	Intermediate	Erect	Intermediate	Dense	Lanceolate	Intermediate	Dark green
BJ017	Intermediate	Erect	Intermediate	Dense	Ovate	Intermediate	Dark green
BJ018	Intermediate	Erect	Intermediate	Dense	Deltoid	Intermediate	Dark green
BJ019	Sparse	Erect	Intermediate	Dense	Deltoid	Sparse	Dark green
BJ020	Intermediate	Erect	Intermediate	Sparse	Ovate	Sparse	Green
BJ021	Sparse	Erect	Intermediate	Dense	Ovate	Sparse	Green
BJ022	Intermediate	Prostate	Dense	Dense	Lanceolate	Intermediate	Dark green
BJ023	Intermediate	Prostate	Intermediate	Dense	Ovate	Sparse	Green
BJ024	Intermediate	Prostate	Intermediate	Dense	Dense	Sparse	Dark green
BJ025	Intermediate	Erect	Intermediate	Dense	Ovate	Sparse	Green
BJ026	Sparse	Erect	Intermediate	Dense	Ovate	Sparse	Green
BJ027	Intermediate	Erect	Intermediate	Dense	Ovate	Intermediate	Dark green
BJ028	Sparse	Erect	Intermediate	Dense	Ovate	Sparse	Green
BJ029	Sparse	Prostate	Intermediate	Intermediate	Ovate	Sparse	Dark green
BJ030	Dense	Intermediate	Intermediate	Dense	Ovate	Intermediate	green

Table 4: Differences in Fruit characters

Accession number	Fruit Characters		Fruit length (cm)	Fruit diameter (cm)
BJOO1	Red , blunt blossom end	5.20	2.22	
BJOO2	Red , pointed blossom end	6.00	2.46	
BJOO3	Red , blunt blossom end	5.66	1.50	
BJOO4	Orange , small size, blunt blossom end	4.33	1.77	
BJOO5	Chocolate, elongate , pointed blossom end	7.00	2.97	
BJOO6	Red , blunt blossom end	5.70	3.13	
BJOO7	Red , sunken and pointed blossom end	6.20	2.80	
BJOO8	Orange red, pointed blossom end	6.35	2.78	
BJOO9	Red, pointed blossom end	6.83	2.97	
BJO10	Chocolate, pointed blossom end	6.50	2.63	
BJO11	Red, sunken and pointed blossom end	5.00	3.08	
BJO12	Red, sunken and pointed blossom end	4.83	2.69	
BJO13	Dark red, sunken and pointed blossom end	5.50	2.35	
BJO14	Dark red, sunken and pointed blossom end	5.00	2.68	
BJO15	Dark red, pointed blossom end	5.60	3.13	
BJO16	Orange, pointed blossom end	5.73	2.58	
BJO17	Dark red, pointed blossom end	6.10	2.92	
BJO18	Dark red, sunken blossom end	5.73	2.59	
BJO19	Dark red, sunken blossom end	4.26	2.45	
BJO20	Red ,pointed blossom end	6.00	2.22	
BJO21	Red , sunken and pointed blossom end	5.40	2.73	
BJO22	Dark red, pointed blossom end	5.76	2.27	
BJO23	Red , pointed blossom end	7.46	2.07	
BJO24	Red , sunken blossom end	5.76	2.89	
BJO25	Dark red, small size, blunt blossom end	3.40	1.64	
BJO26	Red , small size, sunken and pointed end	3.90	2.46	
BJO27	Orange, pointed blossom end	5.93	3.03	
BJO28	Red, elongate , pointed blossom end	7.13	2.71	
BJO29	Yellowish green, sunken and pointed blossom end	4.40	2.78	
BJO30	Red, blunt blossom end	4.66	2.62	

Table 5: Fidelity of markers in distinguishing germplasm under study

Sl.No	Marker name	Expected product size (in base pairs)	Per cent polymorphism	PIC
1	CAMS 451	233	100	0.3576
2	CAMS 368	206, 180	100	0.3698
3	CAeMS 009	206	100	0.3739
4	CAMS 032	233	100	0.1278
5	CAMS 215	220	100	0.3613
6	HpmsE014	106	100	0.1167
7	HpmsE026	222	100	0.1167
8	HpmsE047	260	100	0.2938
9	HpmsE070	215	100	0.2044
10	C2_At3g44600	500, 530	0	-

Table 6: Jaccard similarity matrix with 30 Bhut Jolokia genotypes

	BJ001	BJ002	BJ003	BJ004	BJ005	BJ006	BJ007	BJ008	BJ009	BJ010	BJ011	BJ012	BJ013	BJ014	BJ015	BJ016	BJ017	BJ018	BJ019	BJ020	BJ021	BJ022	BJ023	BJ024	BJ025	BJ026	BJ027	BJ028	BJ029	BJ030
BJ001	1.000																													
BJ002	0.250	1.000																												
BJ003	0.800	0.250	1.000																											
BJ004	0.800	0.250	1.000	1.000																										
BJ005	0.800	0.250	1.000	1.000	1.000																									
BJ006	0.455	0.300	0.455	0.455	0.455	1.000																								
BJ007	0.231	0.300	0.333	0.333	0.333	0.400	1.000																							
BJ008	0.385	0.250	0.500	0.500	0.500	0.333	0.231	1.000																						
BJ009	0.636	0.500	0.500	0.500	0.500	0.455	0.455	0.385	1.000																					
BJ010	0.417	0.400	0.308	0.308	0.308	0.500	0.500	0.214	0.700	1.000																				
BJ011	0.385	0.364	0.286	0.286	0.286	0.600	0.455	0.200	0.636	0.889	1.000																			
BJ012	0.333	0.444	0.455	0.455	0.455	0.556	0.750	0.333	0.600	0.667	0.600	1.000																		
BJ013	0.385	0.364	0.286	0.286	0.286	0.600	0.455	0.200	0.636	0.889	1.000	0.600	1.000																	
BJ014	0.417	0.400	0.308	0.308	0.308	0.500	0.500	0.214	0.700	1.000	0.889	0.667	0.889	1.000																
BJ015	0.455	0.444	0.333	0.333	0.333	0.556	0.556	0.231	0.778	0.875	0.778	0.750	0.778	0.875	1.000															
BJ016	0.385	0.364	0.286	0.286	0.286	0.600	0.455	0.200	0.636	0.889	1.000	0.600	1.000	0.889	0.778	1.000														
BJ017	0.500	0.250	0.385	0.385	0.385	0.455	0.333	0.125	0.500	0.700	0.800	0.455	0.800	0.700	0.600	0.800	1.000													
BJ018	0.500	0.250	0.385	0.385	0.385	0.455	0.333	0.125	0.500	0.700	0.800	0.455	0.800	0.700	0.600	0.800	1.000	1.000												
BJ019	0.500	0.250	0.385	0.385	0.385	0.455	0.333	0.125	0.500	0.700	0.800	0.455	0.800	0.700	0.600	0.800	1.000	1.000	1.000											
BJ020	0.385	0.364	0.286	0.286	0.286	0.600	0.455	0.200	0.636	0.889	1.000	0.600	1.000	0.889	0.778	1.000	0.800	0.800	0.800	1.000										
BJ021	0.636	0.250	0.500	0.500	0.500	0.600	0.333	0.385	0.636	0.700	0.636	0.455	0.636	0.700	0.600	0.636	0.500	0.500	0.500	0.636	1.000									
BJ022	0.500	0.364	0.385	0.385	0.385	0.455	0.455	0.286	0.800	0.889	0.800	0.600	0.800	0.889	0.778	0.800	0.636	0.636	0.636	0.800	0.800	1.000								
BJ023	0.231	0.182	0.231	0.231	0.231	0.400	0.400	0.333	0.231	0.364	0.333	0.273	0.333	0.364	0.273	0.333	0.231	0.231	0.231	0.333	0.455	0.333	1.000							
BJ024	0.500	0.250	0.636	0.636	0.636	0.600	0.455	0.500	0.500	0.545	0.500	0.600	0.500	0.545	0.455	0.500	0.385	0.385	0.385	0.500	0.800	0.636	0.455	1.000						
BJ025	0.385	0.364	0.500	0.500	0.500	0.455	0.600	0.385	0.636	0.700	0.636	0.778	0.636	0.700	0.600	0.636	0.500	0.500	0.500	0.636	0.636	0.800	0.333	0.800	1.000					
BJ026	0.500	0.250	0.636	0.636	0.636	0.600	0.455	0.500	0.500	0.545	0.500	0.600	0.500	0.545	0.455	0.500	0.385	0.385	0.385	0.500	0.800	0.636	0.455	1.000	0.800	1.000				
BJ027	0.636	0.500	0.500	0.500	0.500	0.455	0.231	0.385	0.636	0.417	0.385	0.333	0.385	0.417	0.455	0.385	0.286	0.286	0.286	0.385	0.636	0.500	0.231	0.500	0.385	0.500	1.000			
BJ028	0.231	0.300	0.143	0.143	0.143	0.273	0.077	0.333	0.231	0.364	0.333	0.167	0.333	0.364	0.273	0.333	0.231	0.231	0.231	0.333	0.455	0.333	0.273	0.333	0.231	0.333	0.455	1.000		
BJ029	0.636	0.364	0.800	0.800	0.800	0.600	0.455	0.636	0.636	0.417	0.385	0.600	0.385	0.417	0.455	0.385	0.286	0.286	0.286	0.385	0.636	0.500	0.333	0.800	0.636	0.800	0.636	0.231	1.000	
BJ030	0.417	0.556	0.308	0.308	0.308	0.500	0.154	0.308	0.417	0.333	0.417	0.250	0.417	0.333	0.364	0.417	0.308	0.308	0.308	0.417	0.417	0.308	0.250	0.308	0.214	0.308	0.700	0.500	0.417	1.000

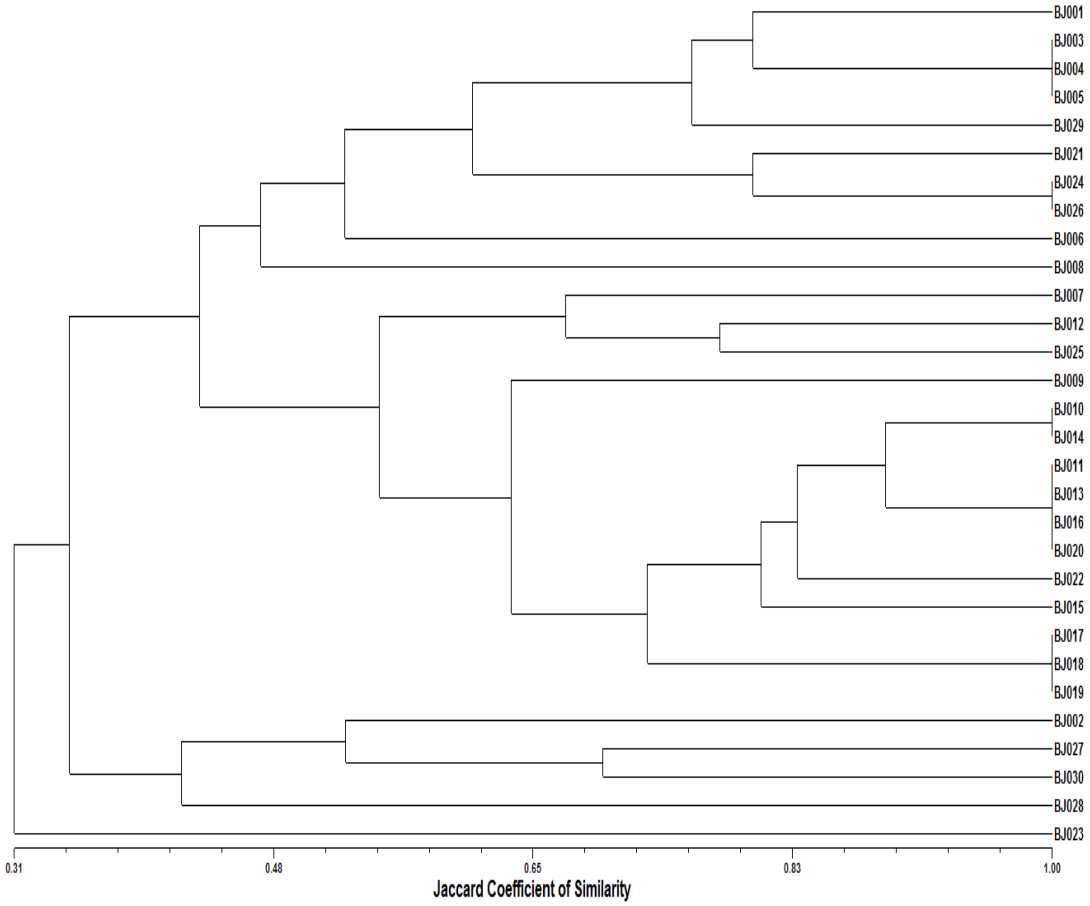

Fig. 1: Genetic relatedness among 30 Bhut Jolokia genotypes using Jaccard's coefficient analysis

DISCUSSION

About 30 Bhut Jolokia genotypes were collected from different regions of North Eastern India, mainly from Assam, Nagaland and Manipur based on fruit morphological characters[7] and their genetic diversity studied by using simple sequence repeats (SSR) markers. Ten simple sequence repeats (SSR) markers were used against the 30 genotypes to study the genetic diversity. Results revealed 100 per cent polymorphism in 9 out of 10 markers and polymorphic information content (PIC) ranging from 0.11(HpmsE014, HpmsE026) to 0.37 (CAeMS009) were recorded. Similar work was done with 64 chilli pepper accessions with fifty SSR markers. Twenty seven polymorphic primers amplified a total of 75 alleles with an average of 2.78 alleles per locus. The polymorphic information content (PIC) values ranged from 0.39 (AVRDC PP 138) to 0.78 (AVRDC PP 18), with an average of 0.59[4].

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