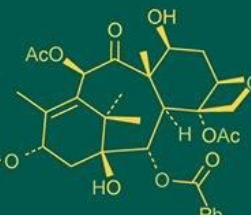
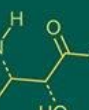
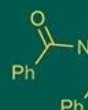


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KV Lakshmi

Department of Vegetable
Science, SKLTSHU, College of
Horticulture, Rajendranagar,
Hyderabad, Telangana, India

P Saidaiah

Assistant Professor,
Department of Genetics and
Plant Breeding, SKLTSHU,
College of Horticulture,
Rajendranagar, Hyderabad,
Telangana, India

M Padma

Professor (Hort.), Dean of P.G.
Studies and Controller of
Examinations (Retd.),
SKLTSHU, Rajendranagar,
Hyderabad, Telangana, India

SR Pandravada

Principal Scientist (Retd.),
NBPG Regional Station,
Rajendranagar, Hyderabad,
Telangana, India

Corresponding Author:**KV Lakshmi**

Department of Vegetable
Science, SKLTSHU, College of
Horticulture, Rajendranagar,
Hyderabad, Telangana, India

SSR based molecular profiling in chilli (*Capsicum annuum* L.)

KV Lakshmi, P Saidaiah, M Padma and SR Pandravada

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Abstract

Augmenting the plant genetic resources and exploiting them in various crop improvement programmes finds germplasm characterisation to be indispensable. Chilli, being a member of the family Solanaceae with genus *Capsicum*, owns abundant genetic diversity. The current investigation was intended to describe the genetic divergence in 40 accessions of Chilli (*Capsicum annuum* L.). A total of 32 simple sequence repeat (SSR) markers were assessed to generate binary data profiles; out of these, 29 markers were informative with scorable and reproducible banding patterns. 100% polymorphism was accomplished across the genotypes with these 29 markers. The polymorphic information content value among the genotypes varied from 0.19 to 0.98. A dendrogram constructed by the Unweighted Pair Group Method with Arithmetic Averages method categorised 40 genotypes into 4 major clusters. Hirkani and SRS 13578 showed the highest similarity (89%). NIC 19967 and SR 6514 were found to be the least similar or highly divergent. Therefore, the present research findings unveil characterisation and differentiation of chilli by describing genetic variation amongst the 40 chilli genotypes. This may further be advantageous for genome mapping, marker-assisted selection, crop improvement, and to develop new plant varieties in chilli.

Keywords: Chilli, *Capsicum annuum*, genetic diversity, SSR markers, cluster analysis

Introduction

Chilli (*Capsicum annuum* L.), a member of the family Solanaceae with chromosome number 24, is one of the widely cultivated crops throughout the world. It originated from South and Central America, where it is still under cultivation (Pickersgill, 1997) with the secondary centers in Gautemala and Bulgaria. Brazil is the major centre of diversity where representatives at all cited levels are found. Chilli was introduced into India from Brazil in 16th century by Portuguese.

Chilli is used as culinary supplement, vegetable, spice, condiment, medicine and as an ornamental plant. Therefore, it is indispensable to the Indian and many African and South American people. Dry chilli, powdered or ground into paste and even green chillies are used everyday in Indian kitchens for curries, sambar, rasam, pickles, sauce, soups, salads and other savory dishes to impart colour, pungency and flavour to the food stuff. Capsaicin is the pungent principle in chilli which produces a highly irritating vapour on heating. Chilli is also called as hot pepper because of its pungency. This volatile acrid crystalline compound present in the pericarp and placenta of the fruit has immense therapeutic and prophylactic uses in allopathic and ayurvedic medications. Chillies are medicinally found to be used in treatment of constipation, indigestion, cold and as pain killer. It is used in tear gas preparation by the security agencies. A non-conventional use of chilli is its use in self defence sprays which is gaining popularity in USA. The capsicum oleoresin present in the spray released at ultra high emission rate temporarily immobilizes the attacker. Moreover, capsaicin has shown great potential as chemo preventive agent against cancer disease. Green chilli is rich in rutin (Used in pharmaceutical industries). Red colour in chilli is due to capsanthin and capsorubin. The colour extracts find use as colour additives in food industry, poultry and prawn feed industry. In food and beverage industries, chilli is being used in the form of oleoresin which permits better distribution of colour and flavour in food. Chilli is rich in vitamin-A and C than tomato (Vitamin-A: 870IU/100 g and Vitamin-C: 321 mg/100 g). Chilli is sometimes referred as capsule of vitamin C because of rich vitamin C content in the fruit.

Various chilli accessions were developed by different breeding and crop improvement methods. The presence of genetically divergent chilli genotypes made it an impactful source for the introduction of new characteristics but most of them are uninvestigated. Collection of this germplasm and their appropriate characterization and classification is a tenacious need for the improvement of these genotypes. Subjective classification of the chilli germplasm based on the reported morphological data is tedious and also confusing (Costa *et al.*, 2006) [4, 5]. Inadequacy of polymorphism at morphological level limits the differentiation of genotypes in addition to this, they are effected by the environmental fluctuations. This once again hinders the crop improvement. Molecular markers combats these complications and acts as a usefull tool for genotypes identification, organization, and evaluation. DNA markers are widely used and are not affected by the different growth stages and the environment, due to which they are usually preferred than morphological and biochemical markers. Various molecular markers like restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs) have been advanced for chilli. Among these molecular markers, SSRs stand for highly polymorphic, co-dominant, reproducible and multi allelic type of variation (Beecher *et al.*, 2000) and are being employed for genome mapping, gene tagging, and estimation of genetic diversity, variety identification, and marker-assisted selection. Hence, in order to devise a sound breeding and crop improvement plan, the present experiment was conducted to determine gentic diversity in forty chilli genotypes using SSR markers.

Material and Methods

Experimental site

The experiment was conducted in Biotechnology laboratory, SKLTSHU, College of Horticulture, Rajendranagar, Hyderabad-500030, Telangana, India.

Plant material

The experimental material comprising of 40 chilli genotypes (Table 1), obtained from NBPGR Regional Station, Rajendranagar, Hyderabad and few local varieties collected from Warangal district of Telangana state, few released varieties from RARS, Lam, Guntur, Andhra Pradesh and AVRDC Taiwan. The details of seed germplasm are presented in Table 1. The genotypes were grown during *Kharif*, 2018 at PG Research Block, Department of Vegetable Science, Sri Konda Laxman Telangana State Horticultural University, College of Horticulture, Rajendranagar, Hyderabad-500030, Telangana.

D.N.A isolation

Approximately 100 mg of fresh young leaves from each accession of chilli were collected. DNA extraction was done by modified C-TAB method. The quality of DNA was checked using 1% Agarose gel electrophoresis and quantity

of DNA was determined with a biophotometer (Eppendorf, India). These samples were diluted to 25 ng/ul for SSR analysis and stored at -20 °C until use.

SSR analysis

Total thirty two SSR markers (Table 2) were tested for PCR amplification. Each primer was amplified according to it's specific annealing temperature. PCR amplified products were subjected to horizontal gel electrophoresis using 3% Agarose gel in 1X TBE buffer at 80V using (Cleaver Scientifics). The ethidium bromide stained gels were documented using gel documentation unit.

Data analysis

Clear amplified fragments of RAPD markers were scored as present (1) or absent (0) and each characteristic state as taken as independent allele. All the polymorphic and no polymorphic bands were calculated for each individual primer and the polymorphic information content (PIC) value was calculated using following formula developed by Roldan-Ruiz *et al.* (2000)

$$PIC = 2fi(1-fi)$$

fi = frequency of bands present

1-fi = frequency of bands absent.

The PIC values may range from 0 indicates monomorphic to 1, very highly discriminative with many alleles in equal frequencies. NTSYS-PC program (Rohlf 1998) was used to construct the clustering dendrogram based on the genetic distance matrix. The Unweighted Pair Group Method Arithmetic Mean (UPGMA) was used for cluster analysis.

Results and Discussion

Genetic diversity analysis of 40 chill genotypes was carried out using 32 Simple Sequence Repeats (SSRs) selected from the linkage map of chilli genome covering all the 12 chromosomes to study the polymorphism, diversity and similarity. Results obtained from the study have been presented below under the following headings.

PCR analysis of the 40 genotypes using 32 SSRs ensued distinct polymorphism with 29 markers and monomorphism with 3 markers. Using the allelic data of 29 polymorphic markers, binary matrix data was prepared. A total of 110 alleles were observed and the number of alleles varied from 1 (monomorphic) to 12 with an average number of alleles per locus being 3.44. The mean number of alleles per locus furnishes supportive knowledge of polymorphism and adequate information to co-dominant markers (IPGRI, 1999). Highest number of alleles were observed with HpmsE 074 (Fig.1) and the same marker was used by Nanda *et al.*, (2016) for tagging genomic regions controlling anthracnose resistance and reported polymorphic to F₂ mapping population parents (PBC 80 and SB 1) in *Capsicum baccatum* followed by HpmsE 022 (10) and HpmsE 114 (10).

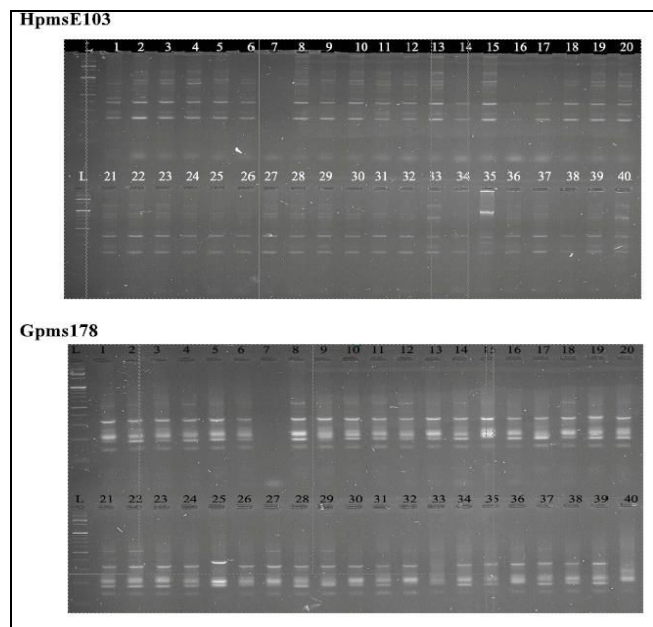


Fig 1: Banding pattern of the SSR markers HpmsE103 and Gpms178 in 40 chilli genotypes

Table 1: 40 genotypes of chilli used for analysing using SSR markers

Tr. No.	Genotype	Source
T ₁	SR-6514	NBPGR, Hyderabad
T ₂	IC-528454	NBPGR, Hyderabad
T ₃	IC-255896	NBPGR, Hyderabad
T ₄	AAT-2	NBPGR, Hyderabad
T ₅	SINDHUR	RARS, Lam, Guntur, AP
T ₆	NIC-23923	NBPGR, Hyderabad
T ₇	WARANGAL CHAPATA	Landrace, Warangal, Telangana
T ₈	PSR-7075	NBPGR, Hyderabad
T ₉	HIRKANI	PDKV, Akola
T ₁₀	IC-572469	NBPGR, Hyderabad
T ₁₁	SD-5335	NBPGR, Hyderabad
T ₁₂	ST-13874	NBPGR, Hyderabad
T ₁₃	IC-214966	NBPGR, Hyderabad
T ₁₄	PSR-13944	NBPGR, Hyderabad
T ₁₅	PSR-7074	NBPGR, Hyderabad
T ₁₆	EC-402101	NBPGR, Hyderabad
T ₁₇	PSR-13968	NBPGR, Hyderabad
T ₁₈	SRS-13578	NBPGR, Hyderabad
T ₁₉	EC-391087	NBPGR, Hyderabad
T ₂₀	AVPP-9813	AVRDC, Taiwan
T ₂₁	LCA-620	RARS, Lam, Guntur, AP
T ₂₂	LCA-625	RARS, Lam, Guntur, AP
T ₂₃	LCA-334	RARS, Lam, Guntur, AP
T ₂₄	IC-572480	NBPGR, Hyderabad
T ₂₅	EC-399565	NBPGR, Hyderabad
T ₂₆	EC-399574	NBPGR, Hyderabad
T ₂₇	EC-376868	NBPGR, Hyderabad
T ₂₈	EC-391083	NBPGR, Hyderabad
T ₂₉	EC-402109	NBPGR, Hyderabad
T ₃₀	EC-399567	NBPGR, Hyderabad
T ₃₁	PSR-13976	NBPGR, Hyderabad
T ₃₂	NIC-19969	NBPGR, Hyderabad
T ₃₃	ST-13837	NBPGR, Hyderabad
T ₃₄	NIC-19967	NBPGR, Hyderabad
T ₃₅	PBC-80	NBPGR, Hyderabad
T ₃₆	EC-399572	NBPGR, Hyderabad
T ₃₇	SR-3429	NBPGR, Hyderabad
T ₃₈	EC-399575	NBPGR, Hyderabad
T ₃₉	EC-401105	NBPGR, Hyderabad
T ₄₀	ST-13875	NBPGR, Hyderabad

Polymorphic Information Content (PIC)

Genetic diversity among the genotypes was assessed on the basis of Polymorphic information content (PIC) value. PIC values among the SSRs varied widely from 0.19 to 0.98 with an average of 0.50. The observations of the present study suggests polymorphism in the genotypes which is reflected by the PIC values. Highest PIC value of 0.98 was observed with Gpms165, followed by HpmsE015 (0.90), HpmsE140 (0.81) implying their further use in genetic variability studies of chilli germplasm. Whereas lowest PIC value of 0.19 was observed with HpmsE 096 marker. PIC value has given out that Gpms165 is contemplated as the prudent marker for 40 chilli germplasm followed by HpmsE015 and HpmsE140 (Fig.2). While the primer HpmsE 096 can be judged as the least powerful marker. Higher PIC values might be result of distantly related genotypes and vice versa. Studies conducted by Senior *et al.*, 1998 revealed that the highest PIC values are exhibited by those markers whose average number of alleles per loci are at identical frequencies. The observed average PIC value is higher than the earlier reported PIC values of diversity studies in chilli by Afsana sharmin *et al.*, 2018 (0.371); Nanayakkara *et al.*, 2018 (.46). However, it was observed to be lower than the PIC values outlined by Yong sham *et al.*, 2005 (0.53); Dhaliwal *et al.*, 2014 (0.59); Buso *et al.*, 2016 (.60) and Afsana sharmin *et al.*, 2018(.57). Contrast in the genetic composition of screened populations and a number of molecular techniques used could be the cause of diverse degrees of polymorphism in chilli as investigated by various researchers. PIC values of all the genotypes are listed in the Table 2.

Cluster analysis

The binary matrix data derived from the 32 SSR markers analysis was used for diversity assessment. Jaccard's similarity coefficient values were calculated for 40 chilli accessions using NTSYS-pc version 2.02e (Rohlf, 1998) and subsequently the dendrogram was constructed by UPGMA (Unweighted pair group method with arithmetic mean) method by using the allelic data. 40 chilli genotypes were grouped into 4 clusters in the dendrogram (Fig 3, Table 4) i.e., cluster A, cluster B, cluster C and cluster D. Clustering of the genotypes is depicted in Figure 4.9. Cluster A is comprised of 13 genotypes *viz.*, SR 6514, IC 255896, PSR 7075, IC 572469, NIC 23923, Sindhur, Hirkani, SRS 13578, AAT 2, SD 5335, ST 13874, IC 528454 and PSR 7074. Cluster B is also with 13 genotypes namely., LCA 620, LCA 625, LCA 334, IC 572480, EC 399567, EC 399565, EC 391087, EC 401105, EC 391083, EC 402109, EC 399572, EC 399574, EC 378688. Cluster C has 13 genotypes *viz.*, AVPP 9813, SR 3429, PSR 13976, NIC 19969, Warangal Chapata, PSR 13944, EC 402101, PSR 13968, EC 399575, ST 13837, ST 13875 and PBC 80 and cluster D is with only one genotype NIC 19967. Highest similarity (89%) was observed between Hirkani and SRS 13578, which are placed in cluster A. The cluster of these two genotypes (Hirkani and SRS 13578) was also observed to be closely related to the yield check Sindhur (84%). Presenting such a close similarity with Sindhur, the two genotypes Hirkani and SR 13578 appears to be promising alternatives. The genotypes EC 391083 and EC 402109 were also found to be very closely related (88.5%). Cluster D, which is comprised of only one genotype NIC 19967 is presenting least similarity (50%) with rest of the genotypes

in the 3 clusters. The same pace is followed by the genotypes ST 13837, ST 13875 and PBC 80 of cluster C showing a similarity of 65% with rest of the genotypes which might be due to its distinctiveness from other genotypes at gene level. Comparable results were reported by Afsana *et al.*, 2018. In this study, the dendrogram analysis revealed that, those genotypes which are genetically

similar, clustered with one accord. Clustering based on morphological traits exhibited some level of similarity to the SSR based dendrogram. It can be detailed that, as SSR markers chiefly quantify genetic variation, to a greater extent in non-coding sequences, which perhaps do not have a considerable influence on the genotype's morphology.

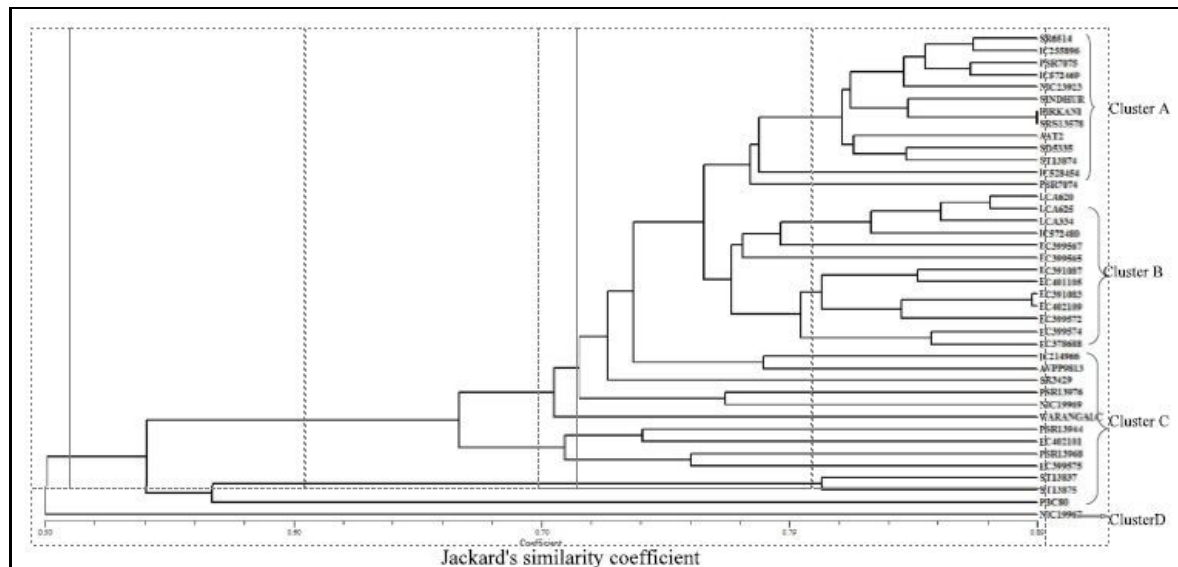


Fig 2: Cluster analysis of 40 genotypes of chilli based on UPGMA based on 32 SSR primers.

Conclusion

SSR markers are assessed to be the most powerful tools for the approximation of genetic diversity and similarity among the studied chilli genotypes. Out of 40 genotypes, two genotypes NIC 19967 was found to be the most diverse, grouping separately with lowest similarity, followed by PBC 80, ST 13837 and ST 13875 and two highly similar genotypes (Hirkani and SRS 13578) were identified which can be deployed in chilli breeding and crop improvement programmes. Those genotypes which showed highest genetic variation can be used as parental lines for breeding to improve chilli varieties. The present effort is a preparatory investigation to distinguish and notice the genetic variation of chilli genotypes under study and had few shortcomings like restricted number of genotypes taken for the study besides the less number of primers used. The results manifested in the current study can be exploited as a guideline for progressing mapping population, advancing marker assisted selection (MAS) and crop improvement of chilli germplasm.

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