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# Estimation of polymorphic contents and molecular diversity of chilli genotypes using SSR markers

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**Abstract**
The enormous wealth of chilli (*Capsicum annuum* L.) genetic diversity extended the success of crop improvement. The molecular diversity of Indian chilli genotypes were assessed using simple sequence–repeats–markers. Nine among forty-three SSR markers were showed the polymorphism ranged from 2 to 16, and an average of 6.9 alleles in the locus, the Hpms1-1c marker recorded the highest alleles (16 alleles per locus). The polymorphic information content (PIC) varied with 0.28 to 0.89 by an average of 0.73. Molecular diversity of 37 genotypes assigned by cluster analysis, using Darwin cluster software indicated that SSR markers grouped into 3 major clusters with 2 and 3 sub-clusters, and genetic variability for SSR markers revealed in the chilli genotypes. Thus, these SSRs are powerful aids in the estimation of molecular diversity and fingerprinting of chilli genotypes. Thus, the results appeal to potential applications SSRs in the breeding, and genetic improvement of chillies.

**Keywords:** *Capsicum annuum* L., Cluster analysis, molecular characterization, polymorphism, SSR

## Introduction
Chilli (*Capsicum annuum* L.) fitted in the family Solanaceae of genus *Capsicum* includes 30 species, five species being in domestication and under cultivation are *Capsicum annuum* L., *C. pubescens* L., *C. chinense* L., *C. frutescens* L., and *C. baccatum* L. (Bosland and Votava, 2000; Mimura *et al.* 2012) [1, 9]. Among these species, the *Capsicum annuum* L. is known as chilli, it is an economically important crop, hence cultivating commercial across India as well as the rest of the world. It is being grown broadly all over the states of India in 0.77 mha, production of 1.49 mt, and 1.9 t/ha productivity. Thus, India is the world's largest chillies producer, consumer, and exporter (NHB 2017) [11]. Phenotypic and genotypic traits were used to distinguish the chilli genotypes, thus classify them into different groups based on similar or dissimilarity index. Info of genetic similarity has vital importance in plant breeding, utmost genetic distances datasets of genotypes revealed to be having highest genetic diversity among the genotypes, where the cultivars with narrow genetic base must aim to enrich the germplasms by regional collections, and plant introductions from across the world (Geleta *et al.* 2005) [3]. The germplasm categorization is a fundamental tread of a breeding program to assort the parental population. Although morphological characterization in chillies is a simple method of finding the differences among the genotypes, and which are highly influenced by factors of environment, cause nonprecise differentiation among the narrow base population. The population's genetic status is the subject of practical utility. Hence, detection of variability among population must be employed by a quick, simple, robust and reliable molecular markers are ultimate to assort the genotypes within and among the population on a genetic level (Gilbert *et al.* 1999) [4]. The genetic variability sum is essential to adapt and exist in varied environments, thus their assessment by molecular markers is essential to know the population genetic configuration for strategic conservation of germplasm (Se-Jong *et al.* 2012) [14].
The molecular markers are powerful tools, the complement to phenotypic screening varied with environmental influence, thus genetic diversity within the genotype, among the inbred lines assessed by amplified fragment length polymorphisms (AFLP) and random amplified polymorphic–DNA (RAPD) markers (Lefebvre *et al.* 2001) [7], the restriction fragment length polymorphisms (RFLP), RAPDs, AFLP and simple–sequence–repeats (SSR) markers were used in genetic diversity study of *Capsicum* species, these markers offer varied hierarchy info cater the management of germplasm and crop improvement (Tam *et al.* 2005) [15]. Among several markers, the SSRs are co-dominant, readily polymorphic, simple, rapid to use, and reliable in results upon genetic diversity examination in the pepper of Bulgaria landraces (Se-Jong *et al.* 2012) [14].

RAPD markers have a problem of reproducibility and RFLP, AFLP, CAPS marker have the problem of laborious procedures, thus these marker applications have been replaced by SSR markers, due to cost-benefit, throughput, efficiency, reproducibility, co-dominance, multi-allelic, and sensitivity of SSR markers are exceptionally outstanding in genetic diversity studies due to widespread in pepper genome, therefore elucidation of genetic matter differences in between, and among the population, breeding lines, and germplasm (Mimura *et al.* 2012) [9]. Therefore, molecular markers validations are inevitable and superior over the morphological characterization of genotypes and are independent of environmental factors. Thus, the study aimed to estimate the polymorphic info and genetic divergence of chilli genotypes using SSR markers.

## Material and Methods

### Experimental site and germplasm resources

The trial was conducted during the *kharif* season of 2016-2017 at the University of Horticultural Sciences (UHS), horticulture research and extension station (HRES), Devihosur, Haveri, District, Karnataka State of India. Experimental site situated in Northern transitional agro-climatic zone (Zone-8), North latitude of 14°47', and East longitudinal of 75°21' at 563 meters above mean–sea–level (MSL). Thirty-seven genotypes were used as plant materials (Table 1). The genotypes were grown in randomized–complete–block–design (RCBD) with two replications, and twenty plants per treatment, seed were sown on 1st June 2016 in 98 cell pro-tray and seedling were transplanted apart 60 × 60 cm on 8th July 2016 to the experimental field, and the experiment was managed as the regular agronomic practices set by UHS package of practice (2013) [16].

**Table 1:** List of chilli genotypes used in the experiment and their source of collection

| Sl. No. | Genotype name | Origin / Source |
|---|---|---|
| 1 | ST-01 | UHS, HRES, Devihosur |
| 2 | ST-02 | UHS, HRES, Devihosur |
| 3 | ST-03 | UHS, HRES, Devihosur |
| 4 | ST-04 | UHS, HRES, Devihosur |
| 5 | ST-05 | UHS, HRES, Devihosur |
| 6 | ST-06 | UHS, HRES, Devihosur |
| 7 | ST-07 | UHS, HRES, Devihosur |
| 8 | ST-08 | UHS, HRES, Devihosur |
| 9 | ST-09 | UHS, HRES, Devihosur |
| 10 | ST-10 | UHS, HRES, Devihosur |
| 11 | ST-11 | UHS, HRES, Devihosur |
| 12 | ST-12 | UHS, HRES, Devihosur |
| 13 | ST-13 | UHS, HRES, Devihosur |
| 14 | ST-14 | UHS, HRES, Devihosur |
| 15 | ST-15 | UHS, HRES, Devihosur |
| 16 | ST-16 | UHS, HRES, Devihosur |
| 17 | ST-17 | UHS, HRES, Devihosur |
| 18 | ST-18 | UHS, HRES, Devihosur |
| 19 | ST-19 | UHS, HRES, Devihosur |
| 20 | ST-20 | UHS, HRES, Devihosur |
| 21 | ST-21 | UHS, HRES, Devihosur |
| 22 | ST-22 | UHS, HRES, Devihosur |
| 23 | ST-23 | UHS, HRES, Devihosur |
| 24 | ST-24 | UHS, HRES, Devihosur |
| 25 | ST-25 | UHS, HRES, Devihosur |
| 26 | ST-26 | UHS, HRES, Devihosur |
| 27 | ST-27 | UHS, HRES, Devihosur |
| 28 | ST-28 | UHS, HRES, Devihosur |
| 29 | ST-29 | UHS, HRES, Devihosur |
| 30 | ST-30 | UHS, HRES, Devihosur |
| 31 | ST-31 | UHS, HRES, Devihosur |
| 32 | ST-32 | UHS, HRES, Devihosur |
| 33 | ST-33 | UHS, HRES, Devihosur |
| 34 | ST-34 | UHS, HRES, Devihosur |
| 35 | ST-35 | UHS, HRES, Devihosur |
| 36 | ST-36 | UHS, HRES, Devihosur |
| 37 | ST-37 | UHS, HRES, Devihosur |

### Isolation of genomic DNA from chilli genotypes

Genomic DNA of chilli genotypes was extracted from fresh young leaf at 3 to 4 true leaf stage of seedling, 2g leaf tissue was used from each genotype for DNA extraction using cetyl-trimethyl-ammonium-bromide buffer (CTAB) method with some minor modification (Sambrook *et al.* 2001) [13], The concentration of DNA was verified by spectro-photometrically on Nano-Drop 2000 (Thermo Scientific, USA) and the quality extracted DNA was visualized in 1% agarose gel for quality and10ng/µl of DNA dilution was used as a template in PCR reaction.

### Analysis of genotypes by SSR markers

Forty-three SSRs (Table 2) used for categorization of thirty-seven chilli genotypes by genetically (Table 1). DNA amplification was performed using ABI thermal cycler, the PCR of 10 µl reaction mixture containing 1µl template DNA, 0.25µl each forward primers and reverse primers, 0.2µl *Taq*

DNA polymerase, 1.0µl *Taq* buffer with $MgCl_2$, 1µl dNTPs and 6.3µl Sterile $H_2O$.

The SSRs markers were amplified with PCR reaction conditions of initial denaturation for 3 minutes at 94 °C, final denaturation for 30 seconds at 94 °C for, annealed for 30 seconds at 60-65 °C, an extension for 30 seconds at 72 °C, the final extension for 5 minutes at 72 °C and finally stored at 4 °C. The PCR products visualized on 1.5% agarose gel containing 2µl ethidium bromide in 1× TBE buffer at 50 V for 3 hours. The Bromophenol blue dye migrated upon completion of the gel electrophoresis cycle, the amplicons were visualized under ultra-violet light on trans-illuminator, SSR bands were observed, and then PCR product was docked by gel documentation system.

**Table 2:** List of SSR primers used in molecular characterization of chilli genotypes

| Sl. No. | Markers | Forward sequence 5' to 3' | Reverse sequence 3' to 5' |
|---|---|---|---|
| 1 | Hpms 1–1c | TCAACCCAATATTAAGGTCACTTCC | CCAGGCGGGGATTGTAGATG |
| 2 | Hpms 1–5 | CCAAACGAACCGATGAACACTC | GACAATGTTGAAAAAGGTGGAAGAC |
| 3 | Hpms 1–6 | TCCATAACTTCACCCATGAGTATGA | GCAACACCCACATTCCCTTCTC |
| 4 | Hpms 1–62 | CATGAGGTCTCGCATGATTTCAC | GGAGAAGGACCATGTACTGCAGAG |
| 5 | Hpms 1-111 | AAGCTTATCCCTTTCAAATATAA | ATATCTCACGTATTGCGGATTCTT |
| 6 | Hpms 1–143 | AATGCTGAGCTGGCAAGGAAAG | TGAAGGCAGTAGGTGGGGAGTG |
| 7 | Hpms 1–145 | AGCTTGTGTCATAATCTTGAAAAACTC | TGAAAAGACGATTTTGTCTAATGCG |
| 8 | Hpms 1–148 | GGCGGAGAAGAACTAGACGATTAGC | CCACCCAATCCACATAGACG |
| 9 | Hpms 1–168 | GCCCCGATCAATGAATTTCAAC | TGATTTTTGGGTGGAGAGAAAACC |
| 10 | Hpms 1–172 | GGGTTTGCATGATCTAAGCATTTT | CGCTGGAATGCATTGTCAAAGA |
| 11 | Hpms 1–173 | TGCTGGGAAAGATCTCAAAAGG | ATCAAGGAAGCAAACCAATGC |
| 12 | Hpms 1–214 | TGCGAGTACCGAGTTCTTTCTAG | GGCAGTCCTGGGACAACTCG |
| 13 | Hpms 1–274 | TCCCAGACCCCTCGTGATAG | TCCTGCTCCTTCCACAACTG |
| 14 | Hpms 2–2h | GCAAGGATGCTTAGTTGGGTGTC | TCCCAAAATTACCTTGCAGCAC |
| 15 | Hpms 2–13 | TCACCTCATAAGGGCTTATCAATC | TCCTTAACCTTACGAAACCTTGG |
| 16 | Hpms 2–21 | TTTTTCAATTGATGCATGACCGATA | CATGTCATTTTGTCATTGATTTGG |
| 17 | Hpms 2–24 | TCGTATTGGCTTGTGATTTACCG | TTGAATCGAATACCCGCAGGAG |
| 18 | HpmsAT2–14 | TTTAGGGTTTCCAACTCTTCTTCC | CTAACCCCACCAAGCAAAACAC |
| 19 | HpmsCaSIG19 | CATGAATTTCGTCTTGAAGGTCCC | AAGGGTGTATCGTACGCAGCCTTA |
| 20 | CAN130829 | GCTAATTACTTGCTCCGTTTTG | AATGGGGGAGTTTGTTTTGG |
| 21 | AF244121 | TACCTCCTCGCCAATCCTTCTG | TTGAAAGTTCTTTCCATGACAACC |
| 22 | CAN010950 | GATTTTGGTGGCAGAAGAATTGG | TGCACTTTCGAAGCAAACAAACC |
| 23 | AF039662 | CCCCCTCGTCTCTCTTTATTT | TTGCAAATCTTTTGTCAATTTTT |
| 24 | CM0005 | CATGACCACCATGAGGATA | GATAGCCACGAGCATAGTATT |
| 25 | CAMS 072 | CCCGCGAAATCAAGGTAAT | AAAGCTATTGCTACTGGGTTCG |
| 26 | CAMS 647 | CGGATTCGGTTGAGTCGATA | GTGCTTTGGTTCGGTCTTTC |
| 27 | CAMS 194 | TCATGGAAAATTAACAACGCATA | GGGGGTTGGAGAAGAAAGTT |
| 28 | AVRDC PP 166 | GCACGAGGCTTCATGTCA | GCAGCACTGATCGACAAACT |
| 29 | AVRDC PP 167 | TCATCTTACACGGCTTGCTC | AGCTCCTCAACTGCCTTTTA |
| 30 | AVRDC PP 154 | CTTCCTAGCCACACACCTCA | GAGCCCAAAATTCAACCAGT |
| 31 | AVRDC PP 65 | GTGAGGCCGAGAATGAAGAT | AACGACCATGTGTGGTTGA |
| 32 | AVRDC PP 3 | CTCGATGACTTGATCGTGA | CTTGCATTGTGAGGTCACTG |
| 33 | AVRDC PP 17 | CTACTACCGCTCCTGCTCCT | AGCTTCTGCTTTTGGTTCGT |
| 34 | AVRDC PP 18 | GCTAGGCTTGATCCTTCACC | CGCTTGAAATCATGCTCACT |
| 35 | AVRDC PP 24 | AAAGCATGAAATCACCCTCC | CGGCAAGAAGATGAAAGTCA |
| 36 | AVRDC PP 32 | ATGGAGGATTACCTCGCAAC | CATGATGACCATCCATCCAT |
| 37 | AVRDC PP 205 | AACCCCTTCAAACTTGTTGC | GGGGGTTCGAAGTAGATGAA |
| 38 | AVRDC PP 157 | GAATTAGCTGCAACCCAACA | GATTTGTGATGCCACCAGAC |
| 39 | AVRDC PP 239 | CAAATGCTGCCACTCACTTT | ACAACAAGGGGTGTTTCCTC |
| 40 | CAMS 679 | TTTGCATGTTTTACCCATTCC | CCCCAAAAATTTTCCCTCAT |
| 41 | CAMS-117 | TTGTGGAGGAAACAAGCAAA | CCTCAGCCCAGGAGACATAA |
| 42 | CAMS 647 | CGGATTCGGTTGAGTCGATA | GTGCTTTGGTTCGGTCTTTC |
| 43 | CAMS 864 | CTGTTGTGGAAGAAGAGGACA | GCTTCTTTTCAACCTCCTCCT |

### Genetic analysis of genotypes using SSR data

The alleles per locus were used for statistical assay to find out the major allele frequency, gene diversity (GD), and polymorphism–information–content (PIC) values were determined using Power Marker V.3·0 genetic marker data analysis software. The allele frequency data in binary format (presence of allele as 1, and absence of allele as 0) was exported for data analysis with NTSYS-PC (Numerical Taxonomy and Multiware Analysis System), the cluster analysis was performed employing Darwin's procedure of NTSYS-PC via unweighted pair-group method using arithmetic average (UPGMA) and developed the dendrograms

## Results

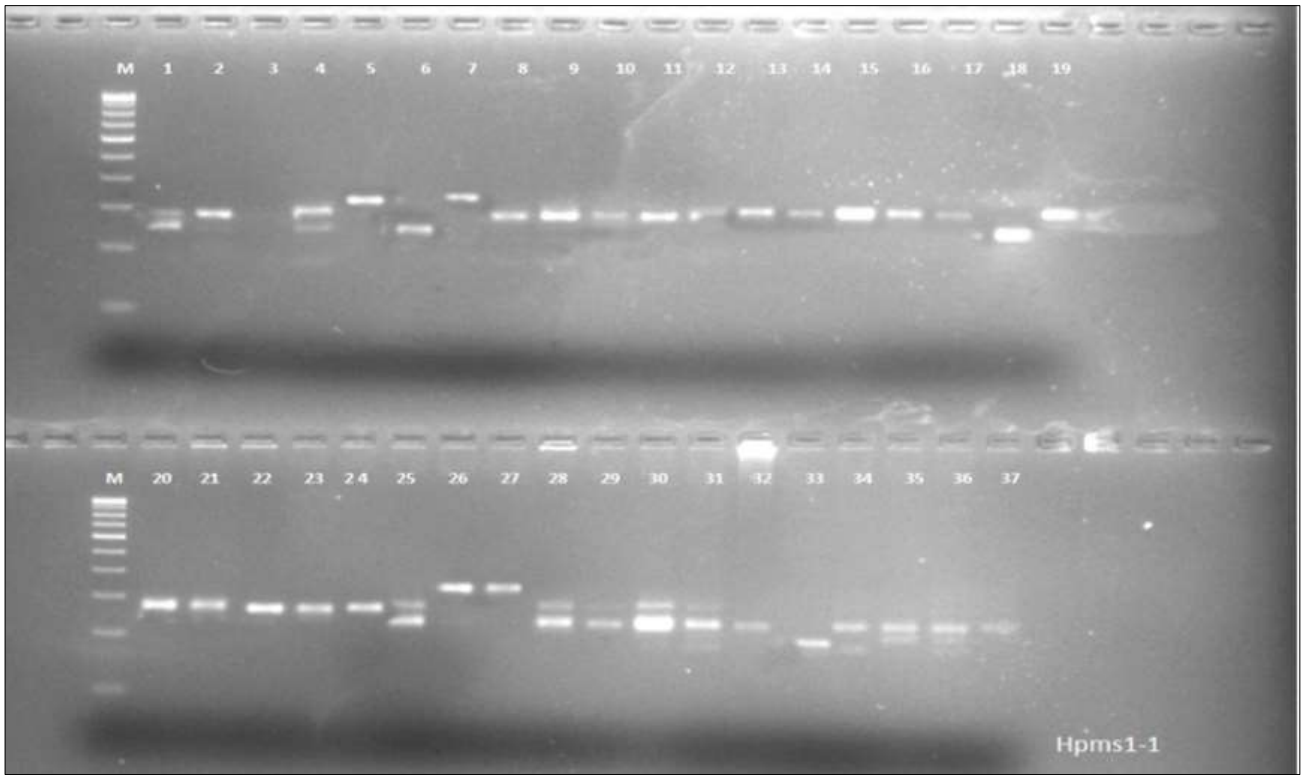
### Screening of chilli genotypes for polymorphic alleles, gene diversity, and PIC through SSR markers

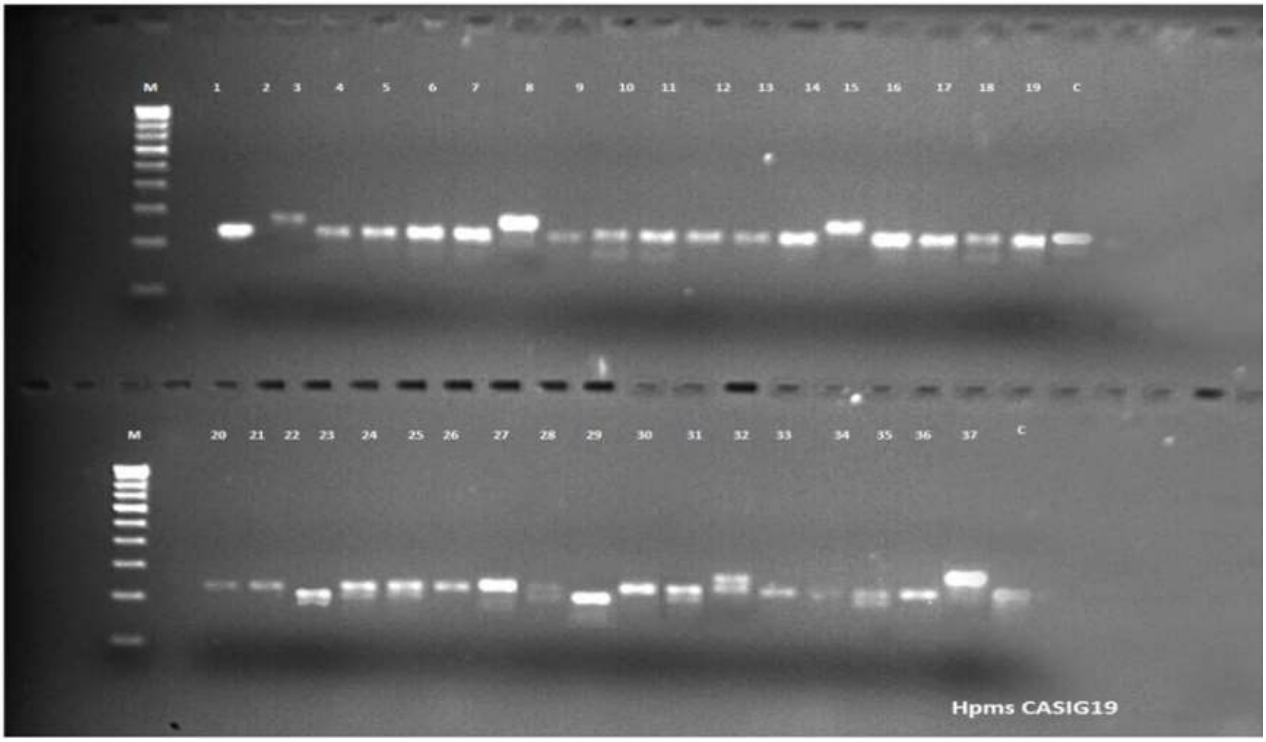
The thirty-seven chilli genotypes were analyzed by forty-three SSR markers for polymorphisms (Table 3), nine primers (Hpms1-1c, HpmsCaSIG19, CAMS647, CM0005, AVRDC PP18, Hpms1-173, AVRDC PP 24, Hpms1-172, AVRDC PP 205,) were found be polymorphic and five (CAN130829, AF224121, CAN010950, AFO39662 and CAMS 117) are monomorphic and remaining markers could not amply. Furthermore, the nine SSR primer pairs polymorphic markers were deployed for genetic diversity estimation through

genotyping of thirty-seven genotypes, these markers showed reproducible and distinct polymorphic amplicons by the number of alleles per locus, which was varied with 2 to 16 alleles per locus with an average of 6.9. The highest numbers of alleles (16) were detected in primer Hpms1-1c followed by primer HpmsCaSIG19, CAMS647, and CM0005. Gene diversity has been ranged from 0.34 to 0.9. The highest gene diversity (0.9) recorded by Hpms1-1c and HpmsCaSIG19 markers followed by CAMS647, CM0005, and AVRDC PP18 primers. The mean gene diversity of 0.69 had noted among the genotypes, based on the PIC value the Hpms1-1c is the best marker for genotyping of thirty-seven chilli genotypes followed by HpmsCaSIG19 and CAMS647 (Table 2). The PIC has a discrimination power of marker genotypes, on basis of alleles on locus and relative allelic frequencies, lower PIC resultant of closely related genotypes, and vice versa (Dhaliwal *et al.* 2014) [2]. The *Capsicum* SSRs highly informative markers for genetic analysis and SSRs noted a high PIC value of 0.76 (Lee *et al.* 2004) [6]. The SSR markers are more reliable due to highly consistent profiles, complementary information of polymorphism and co-dominance, SSRs revealed mean 2.9 alleles per locus and the mean 0.46 PIC value among 14 lines of within *C. annuum* (Minamiyama *et al.* 2006) [10]. An average value of polymorphic information contents was noted 0.33 in chillis, PIC ranged from 0.06 to 0.63 revealed by SSR markers (Se-Jong *et al.* 2012) [14]. The 40 SSR polymorphic primers among 32 pepper germplasm revealed an average of 3.05 loci, showed the practicability of SSR primers in the genetic analysis of pepper (Meng *et al.* 2017) [8], thus, these high polymorphic SSR markers are useful for MAS in chilli breeding upon linking with the marker-trait association (Supplimentary Figures)

**Table 3:** Molecular diversity data obtained across 9 SSR loci for 37 genotypes

| Markers | Major allele frequency | Gene diversity | Alleles per locus | PIC |
|---|---|---|---|---|
| Hpms1-1c | 0.20 | 0.90 | 16.0 | 0.89 |
| HpmsCaSIG19 | 0.19 | 0.90 | 13.0 | 0.89 |
| CAMS647 | 0.22 | 0.85 | 09.0 | 0.83 |
| CM0005 | 0.38 | 0.78 | 09.0 | 0.75 |
| AVRDC PP18 | 0.38 | 0.72 | 07.0 | 0.68 |
| Hpms1-173 | 0.24 | 0.81 | 08.0 | 0.79 |
| AVRDC PP 24 | 0.26 | 0.86 | 10.0 | 0.84 |
| Hpms1-172 | 0.32 | 0.78 | 07.0 | 0.75 |
| AVRDC PP 205 | 0.32 | 0.79 | 08.0 | 0.76 |
| CAN130829 | 0.65 | 0.46 | 02.0 | 0.35 |
| AF224121 | 0.65 | 0.46 | 02.0 | 0.35 |
| CAN010950 | 0.65 | 0.46 | 02.0 | 0.35 |
| AFO39662 | 0.54 | 0.50 | 02.0 | 0.37 |
| CAMS 117 | 0.78 | 0.34 | 02.0 | 0.28 |
| Mean | 0.41 | 0.69 | 06.9 | 0.63 |

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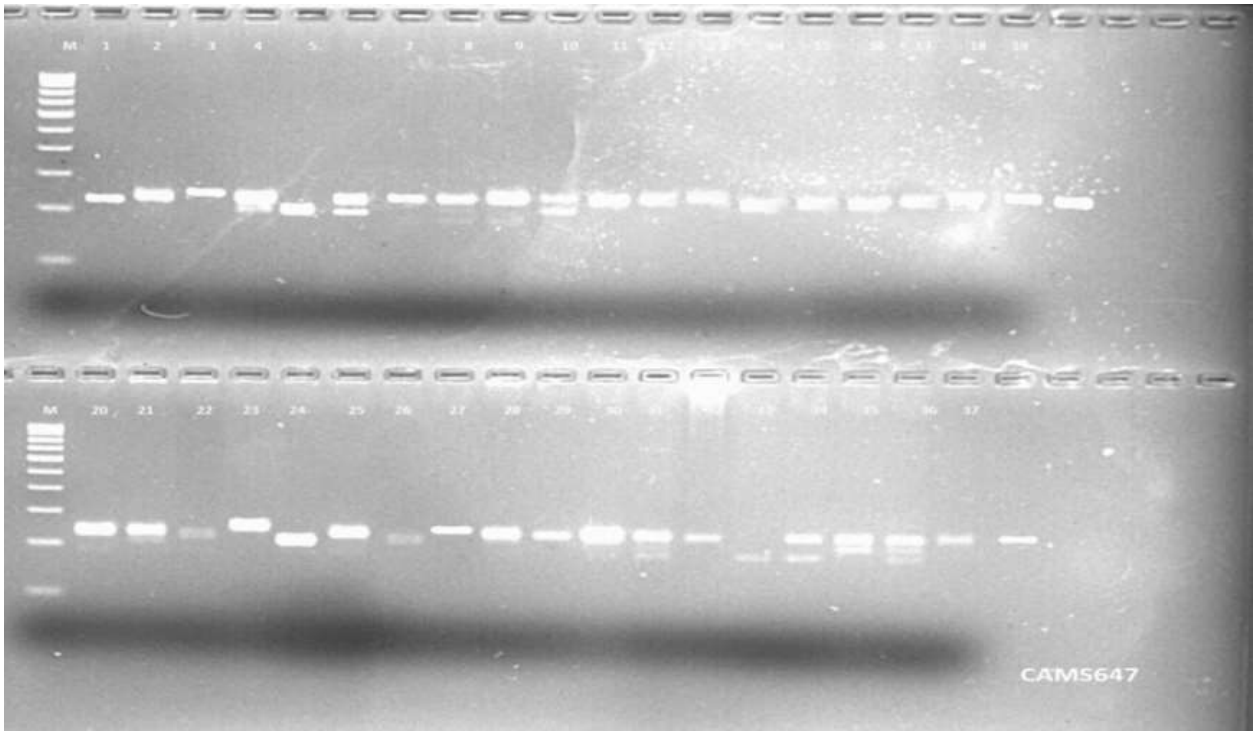
Hpms CASIG19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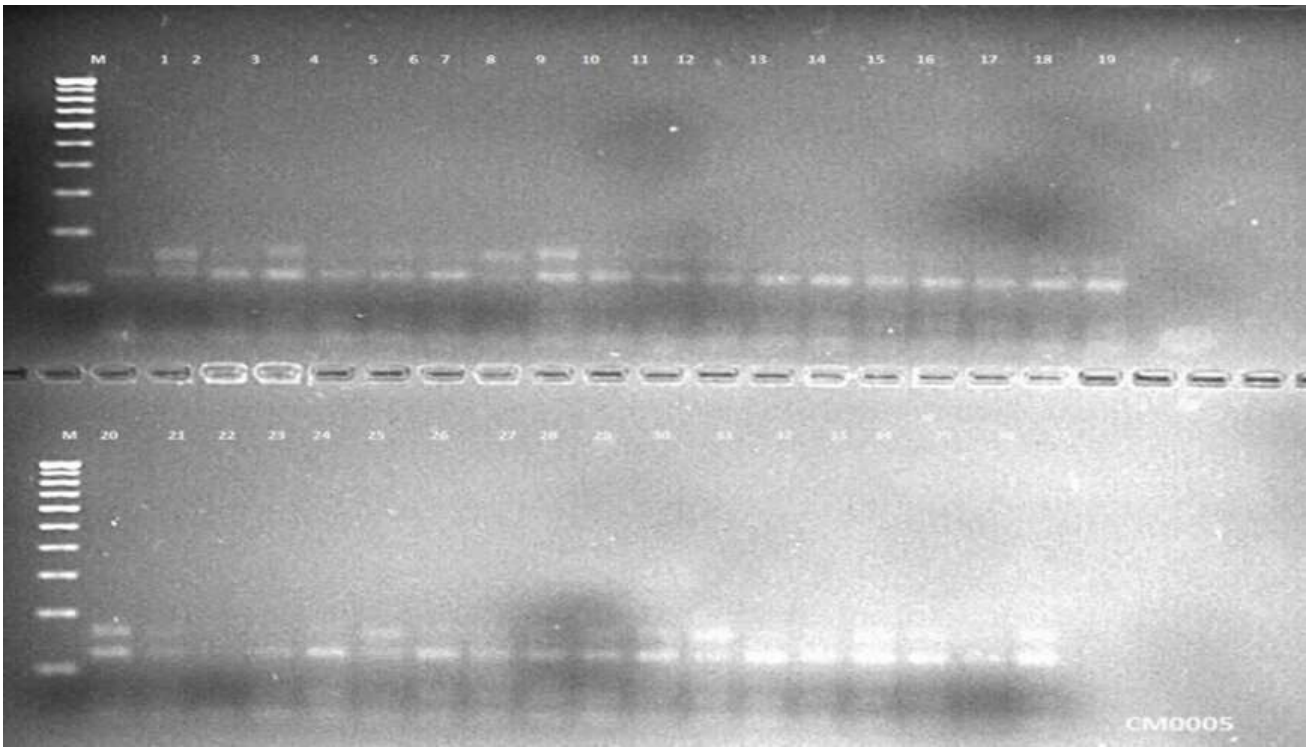
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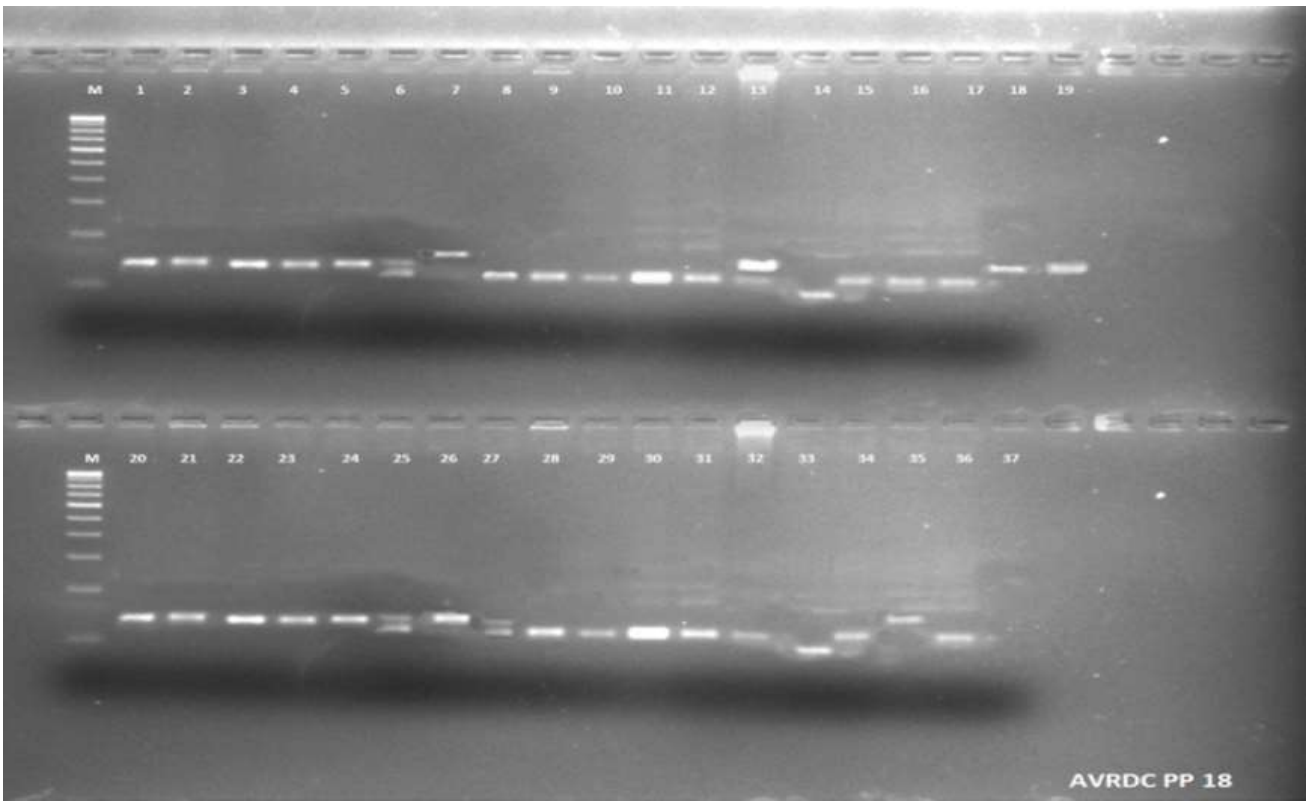
CM0005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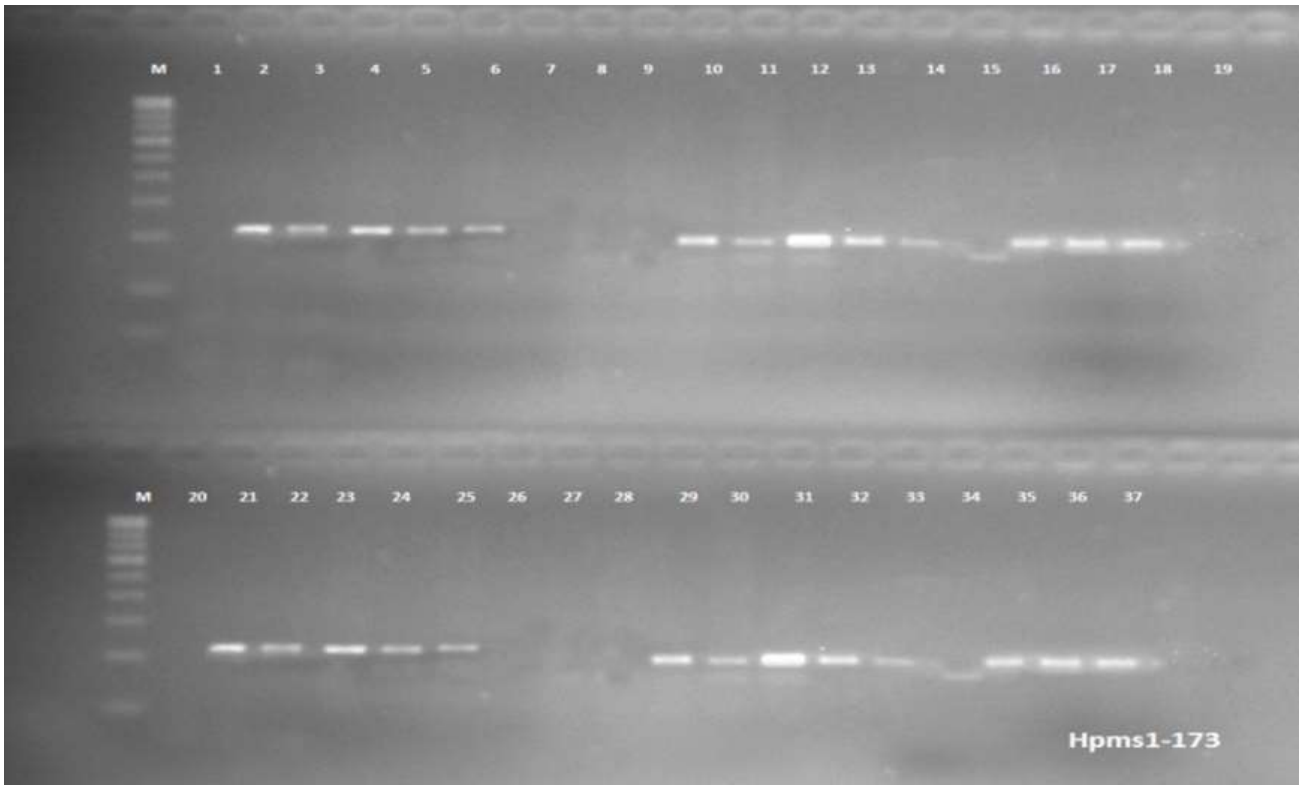
AVRDC PP 18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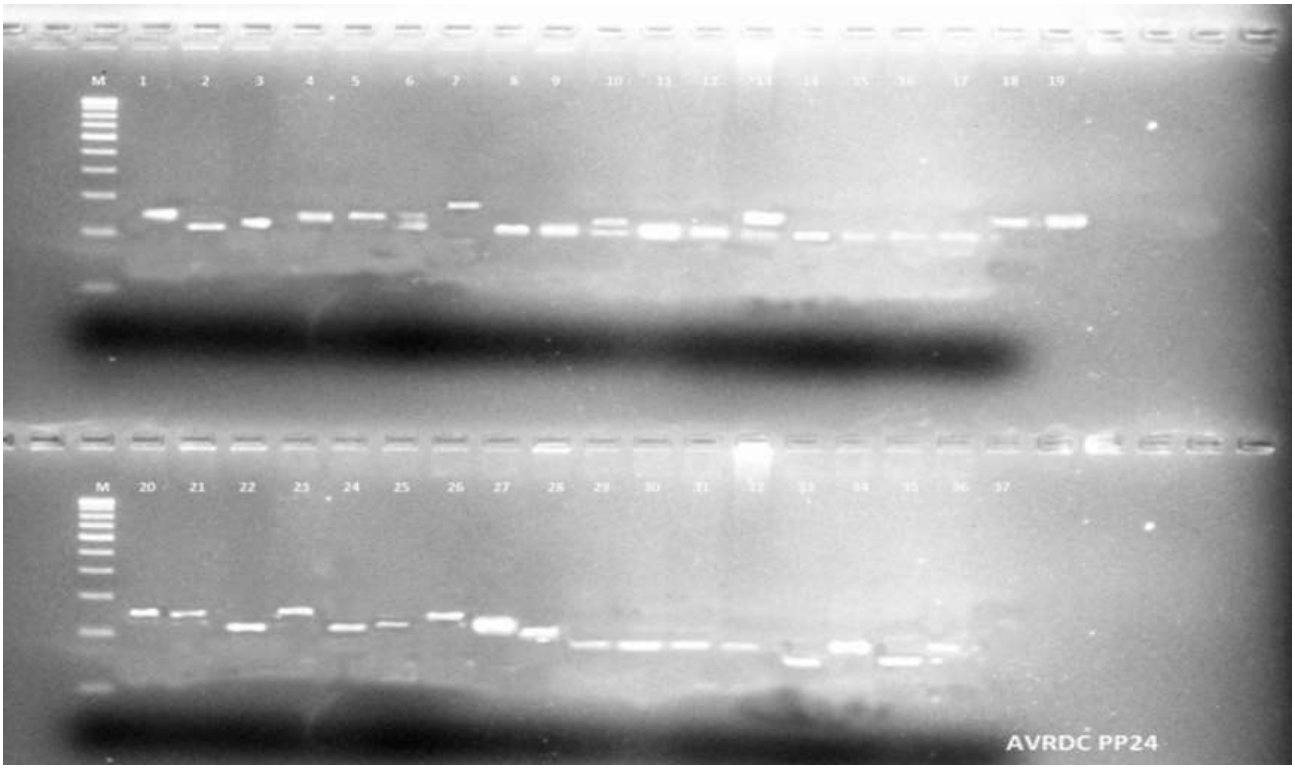
Hpms1-173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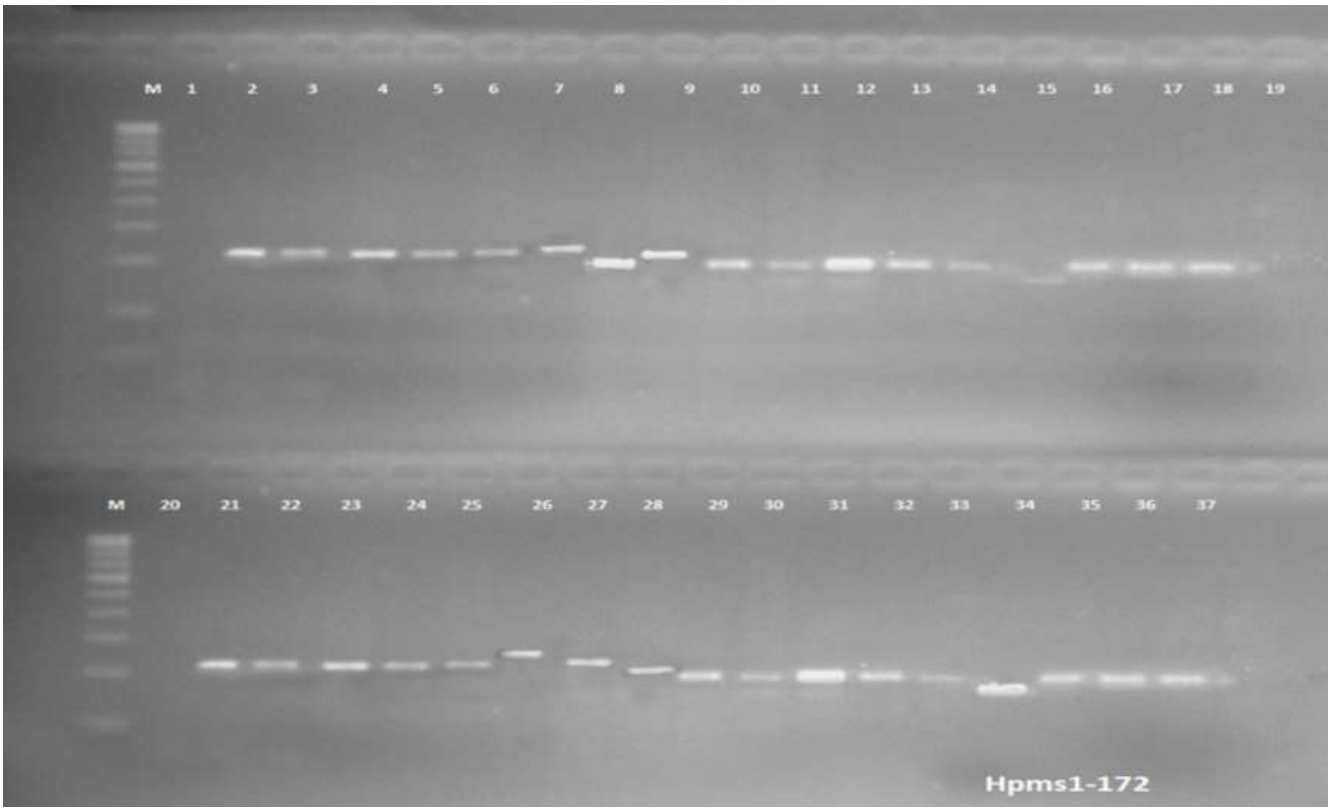
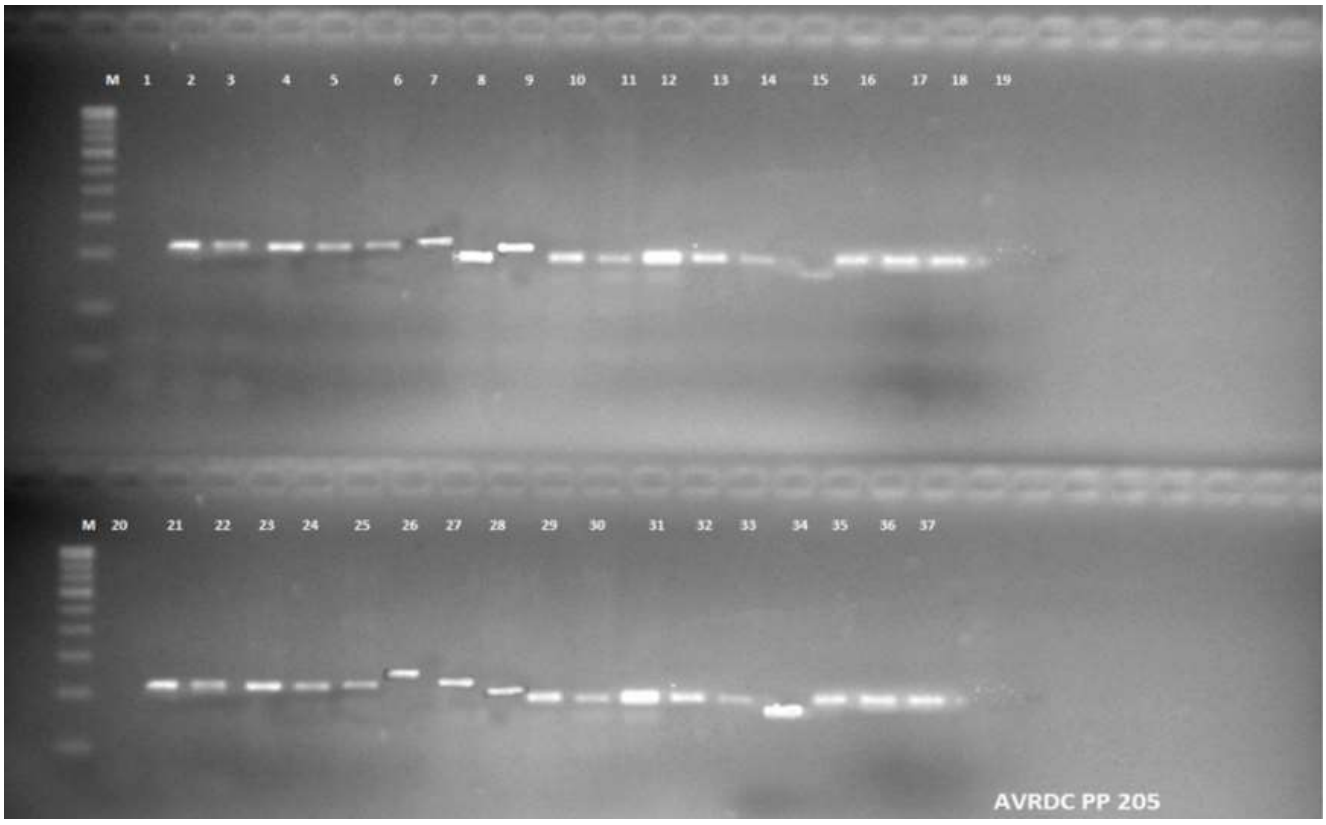
AVRDC PP24




**Supplementary Figures:** Primers Hpms1-1c, HpmsCaSIG19, CAMS647, CM0005, AVRDC PP18, Hpms1-173, AVRDC PP 24, Hpms1-172, AVRDC PP 205

**Molecular analyses of genetic diversity among chilli genotypes**

Cluster analysis of thirty-seven genotypes deployed into three major clusters (Fig. 1) using Darwin software (10,000 bootstraps). The cluster-I had two genotypes ST-23 and ST-27. Cluster II had two sub-clusters, sub-clusters 2.1 has 6 genotypes and sub-clusters 2.2 have 12 genotypes. Cluster-III also has two sub-clusters, sub-cluster 3.1 has only 2 genotypes and sub-cluster 3.1 has 15 genotypes. The highest and lowest genetic identity indicates the existence of genetic diversity among thirty-seven chilli genotypes. The highest genetic distance among genotypes reveals the high divergence (Hossain *et al.* 2014) [5]. Thus, the study indicated genotypes have shown the highest divergences are used as parental breeding lines in the improvement of chilli genotypes. Diversity study of 64 Indian chillies with 50 SSR markers

analysis allowed grouping of nine clusters, thus the divergent genotypes can be used for genetic improvement (Dhaliwal *et al.* 2014) [2]. The 22 chilli germplasm grouped by UPGMA (Nei's genetic distance) into 3 clusters, the maximum genetic distance indicates the varieties of different origin, thus could be exploited in breeding and improvement of chillies (Hossain *et al.* 2014) [5]. The 27 polymorphic primers have a mean of 2.78 alleles per locus, PIC values varied 0.39 (AVRDC PP 138) to 0.78 (AVRDC PP 18), with mean 0.59 value, the high PIC values of primers has most informative thus used to characterize accessions (Dhaliwal *et al.* 2014) [2]. Molecular analyses reveal the precise genetic diversity among landraces, and the presence of specific alleles, which were not previously detected in other Spanish pepper landraces (Rivera *et al.*, 2016) [12]. Furthermore for accuracy and confirmation of molecular diversity results were necessary to associate with phenotypic data.

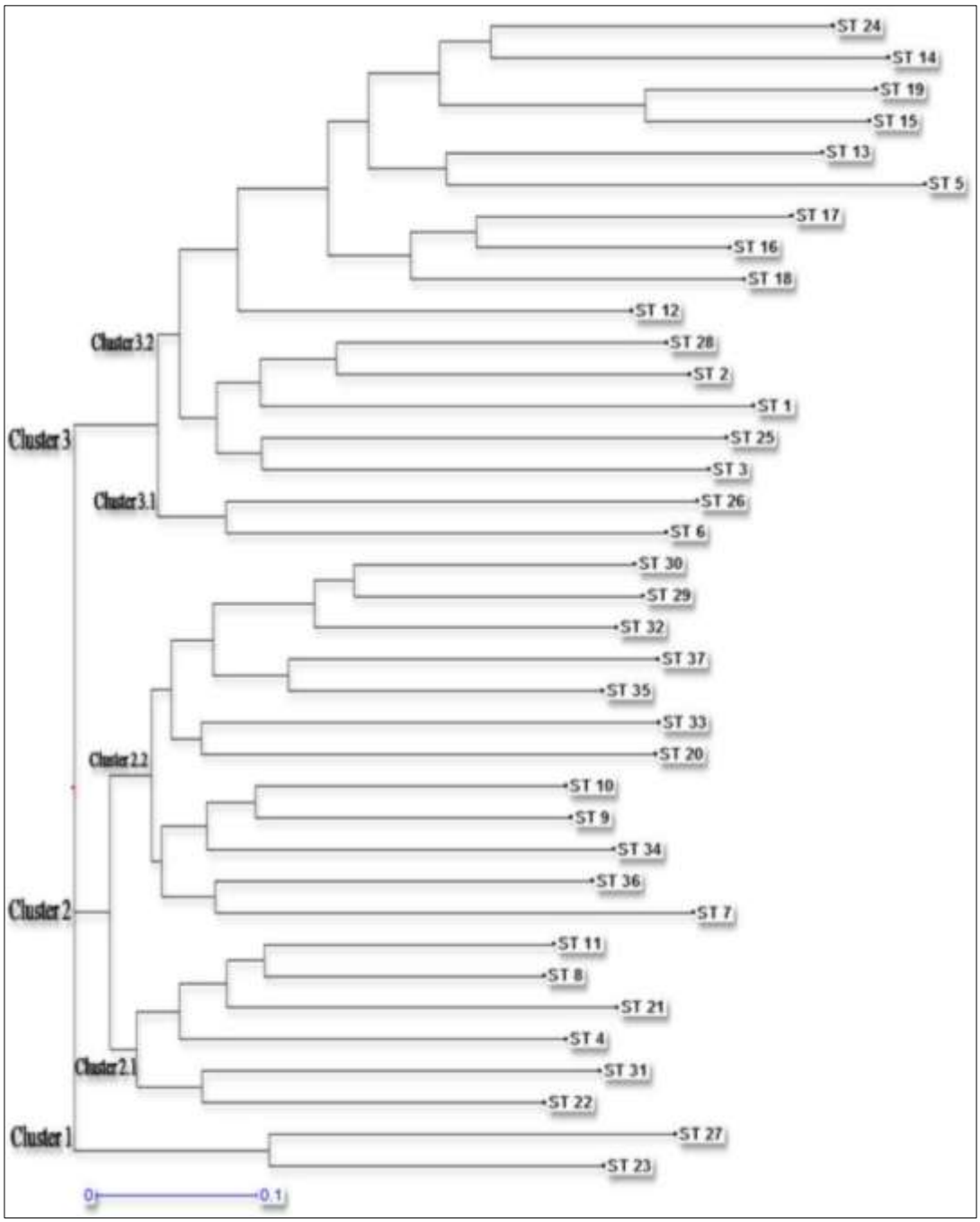



**Fig 1:** Dendrogram of 37 chilli genotypes revealed by UPGMA cluster analysis based on SSR markers

## Conclusion

SSRs are powerful aids for estimating genetic similarities and dissimilarities and genetic diversity. High values of PIC with maximum alleles could be exploited in fingerprinting for genotypes, and MAS of genotype upon trait linkage. The genetic diversity analysis has shown the existence of variability at a genetic level among genotypes, thus these genotypes could be used as a source for breeding and genetic improvement of chillis.

## References

1. Bosland PW, Votava EJ. Peppers: Vegetable and Spice Capsicums. UK, London, CABI Publishing 2000, 230.
2. Dhaliwal MS, Yadav A, Jindal SK. Molecular characterization and diversity analysis in chilli pepper using simple sequence repeats (SSR) markers. African J Biotech 2014;13(31):3137-3143. https://doi.org/10.5897/AJB2014.13695

3. Geleta L, Labuschagne M, Viljoen C. Genetic Variability in Pepper (*Capsicum annuum* L.) Estimated by Morphological Data and Amplified Fragment Length Polymorphism Markers. Biodivers Conserv 2005;14:2361-2375. https://doi.org/10.1007/s10531-004-1669-9
4. Gilbert JE, Lewis RV, Wilkinson MJ, Caligari PDS. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theor Appl Genet 1999;98:1125-1131. https://doi.org/10.1007/s001220051176
5. Hossain SM, Habiba U, bhuyan SI, Haque MS, Begum S, Hossain DM. DNA Fingerprinting and genetic diversity analysis of chilli germplasm using microsatellite markers. Biotech 2014;13(4):174-180. https://doi.org/10.3923/biotech.2014.174.180
6. Lee JM, Nahm SH, Kim YM, Kim BD. Characterization and molecular genetic mapping of microsatellite loci in pepper. Theor Appl Genet 2004;108:619-627. https://doi.org/10.1007/s00122-003-1467-x
7. Lefebvre V, Goffinet B, Chauvet JC, Caromel B, Signoret P, Brand R *et al*. Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotypic data. Theor Appl Genet 2001;102:741-750. https://doi.org/10.1007/s001220051705
8. Meng CY, Wei XC, Zhao YY, Yuan YX, Yang SJ, Wang ZY *et al.* Genetic diversity analysis of *Capsicum* genus by SSR markers, Molecular Plant Breeding 2017;8(8):70-78 DOI: 10.5376/mpb.2017.08.008
9. Mimura Y, Inoue T, Minamiyama Y, Kubo N. An SSR-based genetic map of pepper (*Capsicum annuum* L.) serves as an anchor for the alignment of major pepper maps. Breed Sci 2012;62(1):93-98. https://doi.org/10.1270/jsbbs.62.93
10. Minamiyama Y, Tsuro M, Hirai M. An SSR-based linkage map of *Capsicum annuum*. Mol Breed 2006;18:157-169. https://doi.org/10.1007/s11032-006-9024-3
11. NHB. National Horticulture Board, Horticultural Statistics at a Glance 2017, 80-476.
12. Rivera A, Monteagudo AB, Igartua E, Taboada A, García-Ulloa A, Pomar F *et al.* Assessing genetic and phenotypic diversity in pepper (*Capsicum annuum* L.) landraces from North-West Spain, Sci Hort 2016;203:1-11. https://doi.org/10.1016/j.scienta.2016.03.006
13. Sambrook J, Russel D. Molecular Cloning: A Laboratory Manual, 3rd Edn. Cold Spring Harbor, N.Y, USA, Cold Spring Harbor Laboratory Press 2001.
14. Se-Jong Oh, Song JY, Lee J, Lee GA, Ko HC, Stoilova T *et al.* Evaluation of genetic diversity of red pepper landraces (*Capsicum annuum* L.) from Bulgaria using SSR markers. Korean J Int Agriculture 2012;24(5):547-549. DOI: 10.12719/KSIA.2012.24.5.547
15. Tam SM, Mhiri C, Vogelaar A, Kerkveld M, Pearce RS, Grandbastien MA. Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. Theor Appl Genet 2005;110:819-831. https://doi.org/10.1007/s00122-004-1837-z
16. UHS. University of Horticultural Sciences, Bagalkot, Karnataka. Package of practice: onion, 2013. http://uhsbagalkot.in/documents/Horticulture_POP[1].pdf Assessed 1st April 2016.