POPULATION STRUCTURE AND GENETIC DIVERSITY OF TROPICAL MAIZE GERMPLASM REVEALS USING SSR MARKES

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Abstract:

Aim:

Tropical maize (*Zea mays L.,* 2n=20) is an important cereal crop of worldwide and India. In this study, we aimed to identify population structure and genetic diversity of 288 selected tropical inbred lines using 55 single sequence repeat molecular markers.

Methodology:

Initially, 288 maize genotypes used for population structure and genetic diversity using SSR molecular markers. The experiment was carried out at IIMR field during 2014 to 2016. The DNA was isolated by CTAB method. Purified and quantified for further use. 55 SSR markers were tested PCR amplification and each band was scored. These data were statistical analysis by STRUCTURE and NTSYS software.

Results:

SSR markers were amplified and shown high polymorphism. Out of 55 SSRs, five showed maximum amplification with expected size of ampli-con. All these SSR markers were select to detect high polymorphism. Five markers ware showed in various bands showing different types of alleles.

Discussion and conclusion:

Population structure were investigated using three complementary analysis methods the NTSYS and STRUCTURE, statistical analysis based on SSR molecular markers data. Selected 288 maize inbred lines were assigned into six sub-populations, which was in agreement with the assignments obtained by structure software clustering. It helped in maize breeding in India.

Key words: Genetic Diversity, tropical Maize, Inbred, Cluster Analysis, SSR marker,

Introduction:

Maize was competent about 9000 year ago first time in mexcio from tropical, *Zea mays* ssp., in India. Genetic diversity and between population structure tropical inbred lines and breeding element has extreme importance for maize breeding. With the application and popularization of maize inbred over the ago years, the frequent use of a few elite germplasm lines as maternal stock has led to a reduction in genetic diversity among maize breeding element in India. The establishment of exotic germplasm, using its sufficient genetic variation and good agronomic traits, is consequently imperative to solve the narrow genetic base for maize enhancement in India (Wen *et al*., 2012; Yong *et al*., 2013). However, it is necessary to make a extensive appraisal on the genetic diversity and population structure of tropical germplasm (Tarter *et al*., 2004; sarcevic *et al*., 2008; Zivanovic *et al*., 2012; Hoisington *et al*., 1994). SSR markers can engaged to consider levels of genetic diversity and population structure among maize inbred lines and breeding materials. Simple sequence repeats (SSRs), due to its abundant, highly polymorphic, genome individual, co-dominant in nature, have found application in analyses of population structure , genetic diversity gene mapping and assisted selection for maize improvement (Phumichai *et al*., 2012; Wende *et al.*, 2013; Semagn *et al*., 2014; Yang *et al*., 2013; Abakemal *et al*., 2015; Senior *et al*., 1996). Based on that, we aimed that 288 public sectors -selected lines were investigated using 55 SSR loci appropriated over the maize genome. Identify measurement the levels of population structure and genetic diversity (Garcia *et al*., 2004; Kostova *et al*., 2006).

Studies of population and genetic diversity across individuals have been stimulated by the amplification of DNA sequences using polymerase chain reaction (PCR) (Saiki et al., 1988). Various methods have been related in this regard: simple sequence repeat (SSR) (Tautz, 1989, Yao *et al.,* 2011), random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990), and amplified fragment length polymorphism (AFLP) (Zabeau and Vos, 1993). SSR is widely used in studies of tropical maize diversity attributable to its down cost and rapid detection of polymorphisms (Carvalho *et al*., 2004; Bruel *et al*., 2007). SSR features high intensity, discrimination and accuracy. Owed to their specificity and ease of detection, SSRs are five of the main markers used to genotype characterized the genetic resources of plants via the genotyping of germplasm selection and analysis of genetic diversity (Adeyemo *et al*., 2011; Terra *et al*., 2011; Lobonda *et al*., 2003). AFLP is a approximately optimal system because it allows the computation of genetic diversity crosswise individuals, species and populations (Laborda et al., 2005; Hartings et al., 2008, Eschholz *et al*., 2008). Of this technique reveals a large portion of the gene of any structure and permission for high-compress and high-quality genotypes. Described to Gerber et al. (2000), the deficiency of information attributable to the expression of the dominant marker is counter balance by the high number of polymorphic loci revealed by this method. The aims of the present study were to estimate the genetic diversity across 288 inbred maize line

from Indian by means of molecular genotyping using SSR, RAPD, and AFLP markers, cluster these tropical maize based on their index of establish possible correlations and genetic approximation with the collection locus (Moeller *et al*.,1999).

Material and method:

Plant material/ population and SSR markers

A total of 288 tropical maize genotype including indigenous, exotic accessions breeding lines and inbred lines from the public sectors were used for genetic diversity. These genotypes were taken from the germplasm collection maintained at IIMR, New Delhi, CIMMYT, AICRP and NBPGR. 55 SSRs markers were taken for polymorphism in genotypes.

SSR Markers and Genotyping

Genomic DNA was extracted from approximately 200 mg fresh leaf tissue using the cetyl tri-methyl ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984). 55 SSR primers, which were distributed evenly over the six maize chromosomes, were selected based on the information available in the Maize-GDB database (http://archive.maizegdb.org). PCR amplifications were carried out in 15 µl reaction volumes containing 20-30ng of2µL template DNA, 1µM each of 2.0µL primer, 5x 2.25 Taq-buffer, 0.05µL of 5 units µL-1 Taq DNA polymerase, 2.5µl dNTPs of 0.80µL, 25mM $Mgcl_2$ of 0.60 µl and dH_2O 7.30 µL. PCR protocols consisted of 32 cycles of 94 for 45s, an annealing temperature at either 45, 50, 55 or 60°C depending on the individual SSR primers for 45 s, and 72°C for 60s, and a final extension step of 72°C for 10 min. PCR products were analyzed by 3.5% Metaphor gel electrophoresis and visualized by blue dye staining with gel-doc.

Genetic Diversity Analysis

For each SSR locus, polymorphic bands were scored as one or in respected to presence or absence of the bands at the same mobility, respectively. Gene diversity (PIC) ware calculated for each marker using the formula: $PIC=1-\sum fi2$, where fi is the allele frequency for the i-th locus summed across all alleles for that locus.

(Liu et al., 2005) used the program Power-Marker v3.25 to calculate allele number, allele frequency, and genetic diversity of each locus as mentions.

Population Structure Analysis

The STRUCTURE v2.3.3 was used to assess the population structure of 288 maize inbred lines using the Bayesian model-based approach as described by (Pritchard *et al*., 2000). The number of subgroups (K), with each K repeated five times, was ranged from one to 12, with burn-in of 100,000 and run length of 100,000. The ad hoc criterion ΔK related to the second order rate of change in the log probability of data (LnP(D)) to determine the most probable K value was used (Evanno *et al*., 2005). Genetic diversity among the 288 maize inbred lines was examined; the data matrices of the genetic similarity were used to create the dendrogram using UPGMA with the computer structure software and NTSYS-pc v2.2 (Rohlf, F.J., 2009). Principal coordinate analysis (PCoA) was also used to identify relationships among 288 maize inbred lines.

Results and Data Analysis:

A subset of 55 randomly selected polymorphic SSRs marker was amplified in 288 genotypes of maize, which include tropical inbred line collection (which were collected from different states of India). From 0.33 to 0.99 the PIC value for these 55 polymorphic SSRs markers with respect to 288 genotypes. The average of polymorphism information content for the SSR markers was 0.57±0.19. Maximum PIC value was noticed for dupssr14 (0.99) followed by Phi 015 (0.96) and umc1232 (0.83), while the lowest PIC value (0.01) was found in Bnlg1092 (0.33), umc2208, and Phi 92. Majority of the SSRs (60%) showed >0.50 PIC value. There were only four SSRs which showed PIC value in ranges 0.83 to 0.99 (Table.1) The highest PIC value was observed in individual markers dupssr14 (0.99) indicated that this marker had shown high allelic variation among all. However, Lowest PIC value indicated the low allelic variation. The groups wise highest PIC value ware observed 0.57 in Bnlg252, while; lowest PIC value was found in-group Phi033 markers with value of 0.54 figure-2**.** Highly polymorphism of band in the present study were reported in primer dupssr14, *bnlg252, umc1232,umc1332, umc2303* (Fig. 2).

Table: 1. SSR Markers identified which have shown polymorphism (PIC>0.50) among all tropical maize lines

S.No	Markers	Forward Sequence	Reserves Sequence	Chromosome No	PIC Values
1	dupssr14	AGCAGGTACCACAATGGAG	GTGTACATCAAGGTCCAGATTT	8	0.99
2	umc1232	GGAATTACCACAACAAACTAAACTTGG	AGGCTCTAGCTACCTGGCTACGTT	4	0.69
3	umc1332	CCTCTTGCTTCCTCGTCATGTACT	AAGGAGCTGGAACATAAAACACCA	5	0.83
4	umc2303	AGAAGAAGGTGGAGGTCCAAGACT	CTGGTATCTGATCAGGGTGCG	5	0.83
5	bnlg252	CGTTCTCCGTACAGCACAGACCAACGT	CTCAGATGAACTCCTCAGCAGCTGTAGCCT	4	0.63

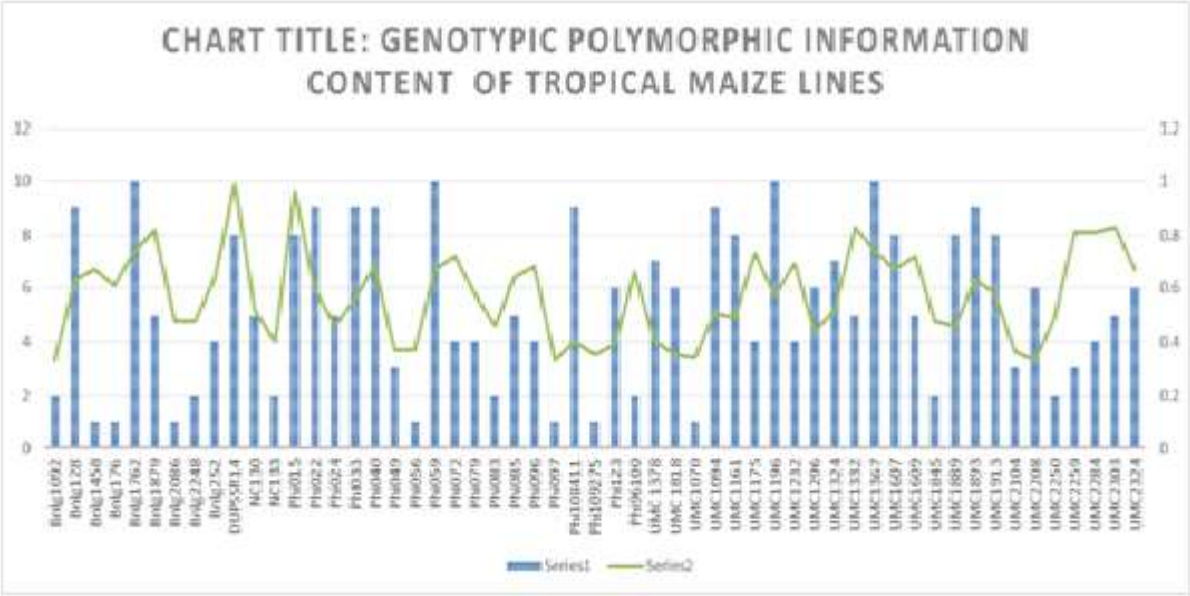


Figure: 1. SSR markers with high PIC value (> 0.99) Series 1 – chromosome number and series 2 – PIC value

Based on the maximum probability, 288 inbred lines were assigned into 6 subpopulations (Red, Green, Blue, yellow, Purple and Sky blue) (Fig.3). Red color included 64 tropical maize inbred lines closed to yellow color of 57 maize lines in genetic backgrounds. Green color had 24 inbred lines related to green color in genetic background. Blue color had included 22 inbred lines. Purple color had included 52 tropical maize inbred lines. Sky blue color had 38 inbred lines at 6 genetic backgrounds, (Table 2) which described as population structure subgroup as described by (Xie *et al*. (2008)

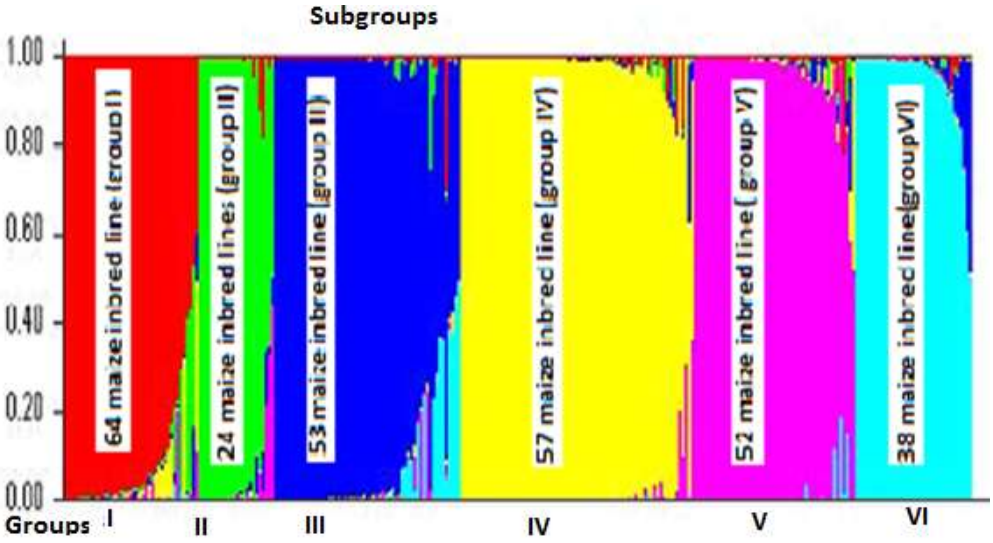


Figure: 2. Population structure of the 288 tropical maize inbred lines (K=6). Note: the vertical coordinate of represents the288 inbred line. The vertical coordinate of each subgroup indicates the coefficients for each individual along with colors.

The similarity coefficient among 288 maize inbred were lines ranged from 0.33 to 0.99 with average showing 0.60. While the similarity coefficient was 0.67, cluster analysis of 288 inbred lines cleared grouped into six subpopulations also clustering were consistent with their assignments using STRUCTURE software (Fig.3). and showed six subpopulations.

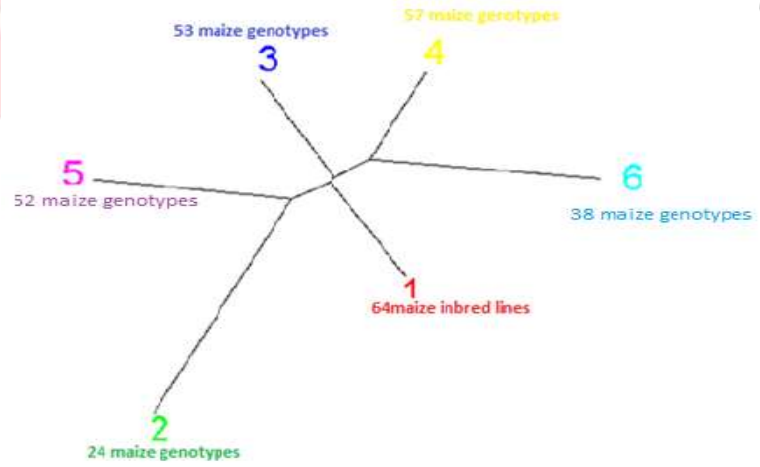
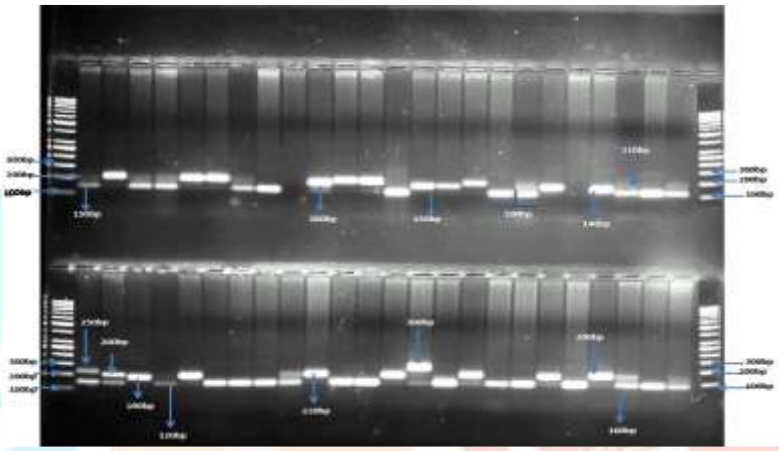


Figure: - 3. Dendrogram of 288 maize inbred lines using structure cluster analysis

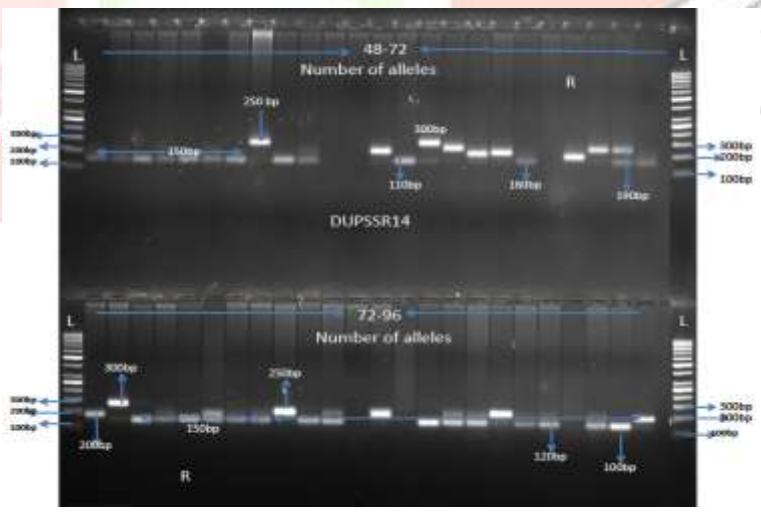
Table: 2.Cluster analysis of 288 maize genotypes along with grouped

Cluster Group	Maize Genotypes
I	CM 213, CML 141, CML 171, CML 37,CML 384, CML 111, CML 189BBB, IML12-55, CM202XE57, CM 210,CM 145, CM 212, CML 406, CM 138, DMRQPM 121, HKI 1105, CML 446BBB, CM 140, CML 493BBB,CML 548 W, CML 112BBB, CML 162, CML 248, IML12-52, IML12-116, CM 207, CML 542 W, CML 279, HKI 4C4B, IML12-161, CML 121, CML 12, CML 117-3-4, CM 149, CML 172, CM 400, CM 135, IML12-133, CML 336, CML 269, WX 484, IML12-74, CML 484BBB,CML 195, CML 24, CML 319, CML 278, CML 322, CML 271BBB, CML 218BBB, CML208BBB, CML180, CM 133, MAI-197, CML 22, CM 125, IML12-22, CML 170, ESM 113, IML12-135, CML 175, IML12-143, CML 207
II	IML12-220, IML 12-213, IML12-215, IML12-218, IML12-221, IML13-15, IML13-17, IML13-22, IML13-23, IML13-62, IML13-46, IML15-10, IML12-180, IML12-193, IML 13-84, IML12-195, IML12-170, IML12-212, IML 12-166, IML15-56, IML15-243, IML 15-65, IML15-48, IML15-60
III	CML 29, CML 327, CML 312, CML 556 W, LM 19, CML 295BBB, CML 420, CML 549 W, CML 557 W, LM 11, CML 550 W, CML 176, LM 5, LM 14 , LM 16, HKI 193-1, CML 304, CML 321, CML 142 X 150, LM 18, CML 282, CML 395, CML 559 W, CML 317, CML 409, HKI 488-1RG, CML 163, CML 554 W, HKI 1344, CML 422, CML 40BBB, LM 17, Bajim-08-27, CML 186, CML 551 Y, CML 494, HKI 193-2, CML 435, CML 227, CML 334, HKI 323, CML 27, CML 44, CML 451XE62, CML 23, CML 408, CML 452, CML 220, CML 55BB, CML 51, CML 202, HKI 1348-6-2, CM 108
IV	DML-26-2, DML-269, UMI 1201, DMRPE-6-4-B, DQL 1005, DQL-1001, DQL-779-1, DQL-609(WG)-1-4, DQL-593-4, DQL-784(O)-4-1, DML-49-1,UMI 1230, HKI 42050, DQL-610-12-4, DML-310, DQL-574-2, UMI 1200, DQL-653-2-4, DQL-609-5, DQL-785(seg)-1-8, DQL-1017-2, DQL-720-10-5, DQL-593-3, DQL-602-2, DML-346, DQL-653-5-1, DML-37-1, DML-242, DML-27-1, DQL-769(YELLOW)-6-3, DQL-716(Y)-1-3, DML-416, DMRQPM-103, DQL-676-16-3, DQL-785(seg)-1-1, V-373, DQL-790(PG)-2-4, DQL-594 (Spiral)-3, DQL-614-6, DQL-641-4-2, DQL-611-4-2, CM 120, DQL-653-3-1, DMRQPM-58, DML-241-1, DQL-609(dark purple)-1-3, DQL-74-1-4B, DMRQPM-03-102, DQL-659(YELLOW)-1-2, DQL-614-5-4, BML-45, DQL-685(Orange)-13-1, DQL-669-13-3, DML-221, DQL-780-2, BML 7, DQL-614-2-3
V	IML15-112, IML15-131, IML15-186, IML16-220, IML15-244, IML15-299, IML16-134, IML16-237, IML16-238, IML16-14, IML16-17, IML16-231, IML16-254, IML15-113, IML15-202, IML16-25, IML16-27, IML 16-208, IML16-210, IML15-280, IML16-157, IML15-268, IML16-28, IML16-143, IML15-288, IML16-269, IML16-108, IML16-205, IML15-269, IML16-193, IML15-97, IML 16-98, IML16-230, IML16-194, IML16-146, DML-165, DML-187-2, IML16-162, IML16-279, DML-106-1, IML16-185, IML16-188, IML16-282, IML16-183, IML15-69, DML-313, DML-187-1, DML-106, HKI 1352, MAI-105, HKI 1378, DQL-633-1-1
VI	DML-128, DML-104, DML-112, DML-16-2, DML-163-1, DML-170, DML-18-1, DML-181, DML-19, DQL-506-1, DQL-291-4, DML-127, DML-16, DML-193, DML-134B, DML-1, DML-119, DQL-297-1-3, DQL-565 (V)-6-2 (Orange), DQL-621 (Seg)-4-10, CLQRCYQ-107, DQL-506-12-2, DQL-626 (ORANGE)-2-3, DQL-295-1-1, DQL-565 (V)-5-2 (Orange), DQL-621(SEG)-1-7, DQL-299-1-1, CML 292, DQL-621 (Seg)-16-5, DQL-621-1-1A, DML-194, DQL-621 (Seg)-9-1, DQL-630-(ORANGE)-3-6, DQL-781-2, DQL-620-2-1, DML-196, DML-212A, BRASIL-117

55 SSR markers were analysed in the 228 maize inbred lines into six major groups. As inferred analysis by STRUCTURE software. Inbred lines in Red were mainly distributed in between 2 to 6 cluster group, green distributed in between 1 to 5 group, blue distributed in between 4 to 5 group, yellow distributed in between 3 to 6 group, purple distributed in between 2 to 3 group, sky blue distributed in between 1 to 4 group. Most individuals within Red and sky blue subpopulations were grouped more closely. The green color II group indicates higher genetic diversity subpopulations.



(a)



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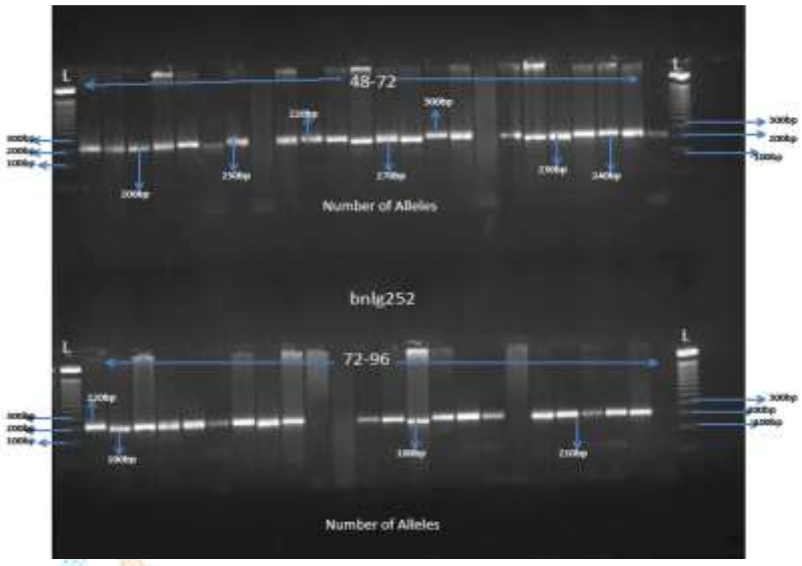

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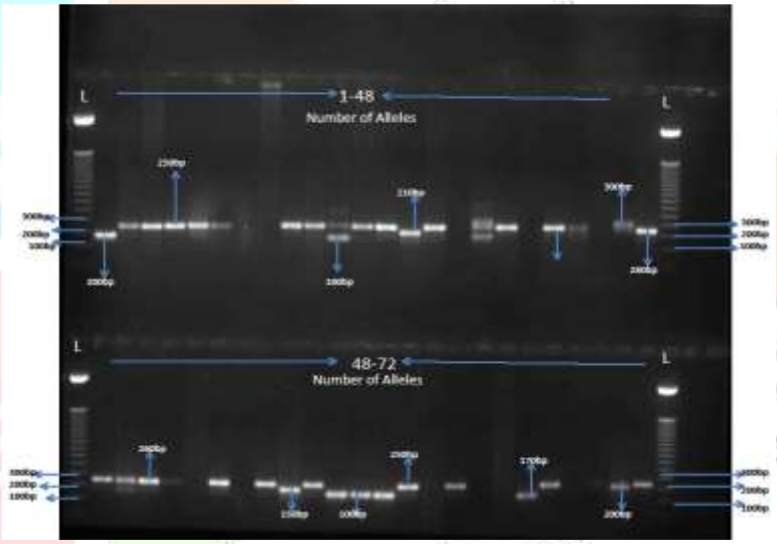

(d)

Figure: 4. SSR markers profiling for dupssr14 (a,b), (c) bnlg252 and (d) umc2303 have been depicted in gels with (marker 100 ladder L).

Discussion and Conclusion:

SSR markers, due to their abundance, co-dominance, and locus specificity, have been extensively used to assess diversity in tropical maize genotypes (Sarcevic *et al*., 2008). In the present study, all the 288 tested maize inbred lines were derived from public sector inbred selected lines. The average polymorphic information content (PIC) value was 0.33, which was lower than that in highest inbred lines with PIC over 0.97 (Wang *et al*., 2008; Xie *et al*., 2008). The number of alleles found in this study is also in agreement with other studies

(Wang *et al*., 2008; Park *et al*., 2015). (Wang *et al*. 2008) reported a total of 1,365 alleles with an average of 9.4 alleles per locus by screening 95 inbred lines using SSR markers. (Park *et al*. 2015) genotyped 174 maize inbred lines by 150 SSR markers and detected a total of 1082 alleles with an average of 7.21 alleles per locus. In our study, alleles were obtained at the genome level Chromosome 1 showed the lowest allele number (24 alleles) and chromosome 8 the highest (64 alleles). Therefore, we have thus determined that there is a higher-level genetic diversity in the 288 tropical maize-selected lines, which has the potential to enhance the genetic diversity of Indian and foreign maize breeding materials.

Population structure in the present study was also investigated using three complementary analysis methods NTSYS and STRUCTURE, statistical analysis based on SSR data. We selected maximum membership probability as the group criterion, 288 maize inbred lines were assigned into six sub-populations, which was in agreement with the assignments obtained by structure software clustering. Nevertheless, for the 288 tested maize inbred -selected lines, the pedigree information was not in accordance with their clustering. This finding can be partially explained by complex genetic background in tropical maize inbred line. Therefore, it is of significant importance to understand population structure and genetic diversity among inbred lines is for maize improvement. The allele frequencies, gene diversity and population structure obtained in the present study lead us to conclude that the 288 inbred lines derived from genetically diverse inbred maize lines contain extensive genetic variation and are a valuable resource for Indian maize breeding. It means that highest frequencies of polymorphism shown the susceptibility and survival in adverse condition.

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