International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 1, 2016, pp.-947-951.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

ASSESSMENT OF GENETIC DIVERSITY OF *PENNISETUM GLAUCUM* (L.) R. Br. AND *TRITICUM AESTIVUM* L. GENOTYPES THROUGH RAPD AND SSR MARKERS

CHAKRABORTY SUDESHNA*, SASIDHARAN N, TRIVEDI RUCHI, AHER BHUSHAN AND PATEL D.A.
Department of Seed Science and Technology, Anand Agricultural University, Anand 388110, Gujarat, India
*Corresponding Author: Email-schakraborty.bio@gmail.com

Received: December 17, 2015; Revised: December 30, 2015; Accepted: January 02, 2016

Abstract- The present work deals with the comparison of the level of information provided by RAPD and SSR marker systems for estimating genetic similarities in pearl millet and wheat genomes. For the present investigation sixteen pearl millet and fourteen wheat genotypes were used. The RAPD analysis of pearl millet and wheat genotypes produced 894 and 564 scorable bands respectively. The RAPD primer 22ES10G33 and OPN-7 revealed the highest PIC values of 0.96 and 0.97 for pearl millet and wheat respectively. As in the case of SSR primers CTM25, CTM27 and Xpsmp 2273 gave the highest PIC value of 0.84 for pearl millet and 0.88 for wheat genotypes by Xgwm153. In both the cases *i.e*, RAPD and SSR, the respective dendrograms developed revealed that the local varieties tend to group together, showing the efficiency of these marker systems in diversity analysis. However, although during the investigation, it was observed that RAPD markers are more efficient than SSR for revealing the latent diversity underlying these crop genotypes, it cannot be generalized in all instances.

Keywords- RAPD, SSR, PIC, Pearl millet, Wheat, Genetic diversity.

Citation: Chakraborty Sudeshna, et al., (2016) Assessment of Genetic Diversity of *Pennisetum Glaucum* (L.) R. Br. and *Triticum Aestivum* L. Genotypes through RAPD and SSR Markers. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 8, Issue 1, pp.-947-951.

Introduction

Our knowledge and understanding of the structure and behavior of cereal genomes has increased due to the development of the molecular techniques for genetic analysis. Recent progress in the development of new marker technologies allows for using them for various practical purposes. One of them is implementation of molecular markers in genetic identification in terms of cultivars, landraces, lines or clones. Based on DNA profiles of characterized objects, it is possible to determine their genetic similarity. The knowledge of genetic diversity on molecular level may be helpful in choosing the appropriate parents for breeding hybrids [2]. The molecular marker techniques have proved to a breakthrough in examining DNA sequence variation among the species in addition to introduce new and favorable traits from landraces and related grass species.

Genetic erosion and habitat destruction in modern agriculture has increased the importance of germplasm characterization of plant material. Thus, having information about genetic diversity of the gene pool would facilitate effective parent selection and shorten the breeding time [3]. The development in the agricultural research will help the future generation scientist to utilize it in a fruitful manner. One drawback of the new technologies developed today, such as molecular markers is their expensiveness in developing, however, once it reaches to a critical significant level proves to be a deeper, more flexible resource [1]. There are several methods to investigate the genetic variability among which DNA markers are more efficient and reliable [4]. The RAPD technology has proved its efficiency in DNA fingerprinting although does suffer from a certain lack of reproducibility due to mismatch annealing. Microsatellites (SSRs) occur frequently in most eukaryote genomes and can be very informative, multiallelic and reproducible and were suggested in order to overcome the limitations associated with RAPD and RFLP. The availability of suitable microsatellite markers developed for several species are pre requisite for better application of SSR markers in plants [5].

In the present study, we have selected two prominent members of the grass family, i.e. *Pennisetum glaucum* (L.) R. Br. (pearl millet or bajra) and *Triticum aestivum* L. (wheat). Both are among the very important cereal crops of the world, grown extensively for food and fodder [6]. *Pennisetum* sp are highly cross pollinated due to its protogynous nature (more than 85% outcrossing) diploid annual (2n=2x=14) with a large genome size (2450 Mbp). The *Triticum* sp on the other hand is highly self-pollinated. The former a genus with over 140 sp with the polybasic chromosome numbers, X=5, 7, 8 and 9. *P. glaucum* belongs to X=7 group (2n=14). *T. aestivum* is an allopolyploid with three genomes AA, BB and DD, which shows meiotic pairing characteristics due to suppression of homologous pairing. In the context of above mentioned facts, the present paper deals with the comparison of the level of information provided by RAPD and SSR marker systems for estimating genetic similarities in pearl millet and wheat genomes.

Materials and Methods

For the present work a total, of 16 genotypes of pearl millet (*P. glaucum*) and 14 genotypes of wheat *(T. aestivum*) were procured from Wheat Research Station, Arnej, Gujarat and Junagadh Agricultural University, Junagadh India respectively [Table-1]. DNA samples were extracted from these genotypes by standardized method of CTAB [2]. Twenty RAPD primers were used in the study for the diversity analysis whereas sixteen and seventeen SSR primers were utilized for pearl millet and wheat genotypes respectively [Table-2, 3 & 4].

RAPD-PCR reactions were cycled with an amplification condition of an initial denaturation step (4 min at 94ºC) followed by 45 cycles at 94ºC for 1 min, 37ºC for 1 min and 72ºC for 2 min terminating with a final extension at 72ºC for 10 min. PCR amplification products were separated by 1.5% agarose gel horizontal electrophoresis. The gels were stained with 0.1% ethidium bromide and photographed by using digital gel documentation and image analysis system (Syngene, UK).PCR conditions used for SSR amplification included initial denaturing step at 94ºC for 3 minutes followed by 34 cycles of 94ºC for 1 minute,

55ºC for 1 minute and 72ºC for 2 minute. In the last cycle, primer extension was done at 72ºC for 7 minutes. The amplified products were separately scored in binary form for each primer on the basis of presence or absence of band corresponding to each cultivar i.e., 1 for presence and 0 for absence of band. Software NTSYS-pc, version 2.0 was used for estimation of genetic relatedness among the genotypes using Jaccard's similarity coefficient and clustering was done with UPGMA.

Table-1 *List of Pearl Millet and Wheat Genotypes*

Sr. No.	Pearl Millet genotypes	Wheat genotypes
1.	GHB-526	GW 190
2.	GHB-558	GW 503
3.	GHB-538	GW 496
4.	GHB-719	GW 322
5.	GHB-757	GW 273
6.	GHB-732	GW 173
7.	ICMA-95222	GW 11
8.	ICMA-94555	GW 366
9.	ICMA-95444	Lok 1
10.	ICMA-92777	GW 1139
11.	ICMA-96222	GW 1255
12.	J-2372	GW 1
13.	J-2290	A 206
14.	J-2340	A-9-30-1
15.	J-2454	
16.	J-2467	

Table-2 *List of Pearl Millet RAPD and SSR Primers*

Sr. No.	RAPD	Sequence	PIC	SSR	SEQUENCE	PIC
1.	13ES10C24	GGCTCGTACC	0.89	CTM2	**F**:-GGTGATTAAAATCGAGGGTT **R**:- AGCAACTTGAGCAGCGG	0.25
2.	14ES10A25	GACCCCGGCA	0.92	CTM3	**F**:-GTCCATCGTCGCCGACGAA **R**:- GGATTTGCTAGTTGTGGGCT	0.45
3.	17ES10C28	GCCTCCTACC	0.84	CTM8	**F**:-GCTGCATCGGAGATAGGGAA **R**:- CTCAGCAAGCACGCTGCTCT	0.52
4.	22ES10G33	AGGCCCGATG	0.96	CTM10	**F**:-GAGGCAAAAGTGGAAGACAG **R**:- TTGATTCCCGGTTCTATCGA	0.82
5.	S-27	GAAACGGGTG	0.72	CTM12	**F**:GTTGCAAGCAGGAGTAGATCGA **R**:- CGCTCTGTAGGTTGAACTCCTT	0.37
6.	S-30	GTGATCGCAG	0.82	CTM21	**F**:-ATGCCTCCCACCCCACGTCG **R**:- CGTCGCACTAGCCACAGTCA	0.71
7.	S-68	TGGACCGGTG	0.74	CTM25	**F**:-GCGAAGTAGAACACCGCGCT **R**:- GCACTTCCTCCTCGCCGT	0.84
8.	OPA-4	AATCGGGCTG	0.91	CTM27	**F**:-GTTGCAAGCAGGAGTAGATCGA **R**:- CGCTCTGTAGGTTGAACTCCTT	0.84
9.	OPA-5	AGGGGTCTTG	0.62	CTM59	**F**:-TCCTCGACATCCTCCA **R**:- GACACCTCGTAGCACTCC	0.61
10.	OPA-6	GGTCCCTGAC	0.70	Xpsmp2248	**F**:-TCTGTTTGTTTGGGTCAGGTCCTTC **R**:CGAATACGTATGGAGAACTGCGCATC	0.12
11.	OPA-7	GAAACGGGTG	0.84	Xpsmp2229	**F**:CCACTACCTTCGTCTTCCTCCATTC **R**:- GTCCGTTCGGTTAGTTGTTGCC	0.22
12.	OPA-8	GTGACGTAGG	0.72	Xpsmp2089	**F**:-TTCGCCGCTGCTACATACTT **R**:- TGTGCATGTTGCTGGTCATT	0.63
13.	OPA-9	GGGTAACGCC	0.64	Xpsmp2273	**F**:AACCCCACCAGTAAGTTGTGCTGC **R**:- GATGACGACAAGACCTTCTCTCC	0.84
14.	OPA-13	CAGCACCCAC	0.60	PSMP2235	**F**:-ATAAGTGGACCCCATGCAGCAC **R**:-CGAAAGACTAGCAAAATTGCGCCTTC	0.19
15.	OPA-15	TTCCGAACCC	0.54	Xpsmp2249	**F**:-CAGTCTCTAACAAACAAACACGGC **R**:- GACAGCAACCAACTCCAAACTCCA	0.55
16.	OPA-18	AGGTGACCGT	0.93	Xpsmp2231	**F**:-TTGCCTGAAGACGTGCAATCGTCC **R**:- CTTAATGCGTCTAGAGAGTTAAGTTG	0.72
17.	OPK-19	CACAGGCGGA	0.84			
18.	OPN-7	CAGCCCAGAG	0.80			
19.	OPN-18	GGTGAGGTCA	0.72			
20.	OPW-16	CAGCCTACCA	0.82			

RAPD= Random Amplified Polymorphic DNA; SSR= Simple Sequence Repeat

Results and Discussion

In this study, we investigated genetic diversity of few pearl millet and wheat genotypes using two different marker systems *viz*. RAPD and SSR and compared relative efficiencies of both of them in exposing the levels of genetic diversity underlying among the different genotypes. Similar studies were earlier carried out on sorghum, where RAPD and SSR markers were compared using HRM analysis in determining genetic variation among selected sorghum genotypes. They revealed that the clustering of sorghum accessions using SSR markers highly corresponded with HRM analysis [7].

The genetic purity study of pearl millet genotypes was performed through RAPD and SSR primers [8]. The RAPD analysis of these genotypes produced 894 different scorable bands whereas SSR analysis showed 231 scorable bands. The dendrogram generated with RAPD primers had divided the genotypes into 2 main groups where in the hybrids *viz*., GHB-526, GHB-558, GHB-538, GHB-719, GHB757 and GHB-732 clustered together. It was clearly evident from the dendrogram that GHB-558 and GHB-538 shared a close lineage with each other [Fig-1]. For SSR analysis a total of 30 primers were used out of which 18 primers showed polymorphic results. The results depicted 220 different scorable bands in genotypes. Primer CTM-2 showed two bands of size 264 and 244 bp in two different genotypes i.e. GHB 719 and GHB 757 respectively. The primer CTM- 25 showed an amplified product size of 335 bp in the genotypes ICMA 95444 whereas 337 bp was observed in GHB719, 341 bp was observed in ICMA 95222 and ICMA 94555. The primer CTM-25 amplified product sizes of 337 bp, 341 bp and 335 bp with genotypes GHB 526, GHB 719, GHB 339, ICMA 95222, ICMA

94555 and ICMA 95444. Primer CTM-12 showed a specific band of size 376 bp with GHB- 732 [Fig-5]. In addition, primer CTM-21 showed an amplified band size of 444 bp and 422 bp with genotype GHB-538 [Fig-6]. The dendrogram generated by these primers have divided the genotypes into 2 different clusters. The cluster diagram clearly shows the relationship between GHB- 719 with it's A line ICMA 95222 [Fig-2]. The present result depicted that SSR and RAPD primers can be used to differentiate the hybrids from their parental genotypes. However, the SSR primers could be more efficient to identify hybrids due to their high specificity.

Table- 3 *List of Wheat RAPD and SSR Primers*

Sr. No.	RAPD Primer	Sequence	PIC	SSR Primer	SSR SEQUENCE	PIC
1.	13ES10C24	GGCTCGTACC	0.94	Xgwm153	F:-GATCTCGTCACCCGGAATTC R:-TGGTAGAGAAGGACGGAGAG	0.88
2.	14ES10A25	GACCCCGGCA	0.91	Xgwm609-2	F:-ACATTCTGTGTGGGGCC R:-GATCCCTCTCCGCTAGAAGC	0.37
3.	17ES10C28	GCCTCCTACC	0.96	Xgwm340-3B	F:-GCAATCTTTTTTCTGACCACG R:-ACGAGGCAAGAACACACTTG	0.13
4.	22ES10G33	AGGCCCGATG	0.77	Xgwm334-6A	F:-AATTTCAAAAGGAGAGAGA R:-AACATGTGTTTTTAGCTATC	0.82
5.	S-27	GAAACGGGTG	0.79	Xgdm872D	F:-CCCAAGCCCCAATCTCTCTCT R:-AATAATGTGGCAGACAGTCTTGG	0.53
6.	S-30	GTGATCGCAG	0.93	Xgdm88-4A	F:-AAGGACAAATCCCTGCATGA R:-TCCCACCTTTTTGCTGTAGA	0.84
7.	S-68	TGGACCGGTG	0.92	Xgwm93-2A	F:-GGAGGCATGGCTACATCCTTC R:-AAAAGCTGCTGGAGCATACA	0.5
8.	OPA-4	AATCGGGCTG	0.96	Xgdm-64-3B	F:-GTCTCTTGCGTACACAGGCC R:-CCGCTAGTGTTTGTGTTTG	0.62
9.	OPA-5	AGGGGTCTTG	0.84	Xgdm-86-2B	F:-GGCGCTCCATTCAATGG R:-GGTCACCCTCTCCCATCC	0.72
10.	OPA-6	GGTCCCTGAC	0.74	Xgdm-115-5D	F:-AAGGTAGGACGAGGGCATG R:-TTTCCATGTCCTATGCCCC	0.54
11.	OPA-7	GAAACGGGTG	0.82	xgdm-113-6B	F:-AAAATGCCCTTCCCAC R:-ACCCATCTGATATTTTGCGG	0.33
12.	OPA-8	GTGACGTAGG	0.84	Xgdm-93-2A	F:-GGAGGCATGGCTACATCCTTC R:-AAAAGCTGCTGGAGCATACA	0.42
13.	OPA-9	GGGTAACGCC	0.82	Xgdm-93-4B	F:-GGAGCATGGCTACATCCTTC R:-AAAAGCTGCTGGAGCATACA	0.82
14.	OPA-13	CAGCACCCAC	0.94	Xgdm -3-5D	F:-GTGTGATGTTTGAATACGCA R:-GTATCTCGGTGATGCAAGCAA	0.46
15.	OPA-15	TTCCGAACCC	0.95	Xgdm 88-4A	F:-AAGGACAAATCCCTGCATGA R:-TCCCACCTTTTTGCTGTAGA	0.34
16.	OPA-18	AGGTGACCGT	0.90	Xgdm-19-1D	F:-ATTGACAGCAGATGGCAGTG R:-GCGTTCGAGTGACTTCCAAT	0.34
17.	OPK-19	CACAGGCGGA	0.92	Xgdm87-2D	F:-CCCAAGCCCCAATCTCTCTCT R:-AATAATGTGGCAGACAGTCTTGG	0.64
18.	OPN-7	CAGCCCAGAG	0.97			
19.	OPN-18	GGTGAGGTCA	0.94			
20.	OPW-16	CAGCCTACCA	0.92			

RAPD= Random Amplified Polymorphic DNA; SSR= Simple Sequence Repeat

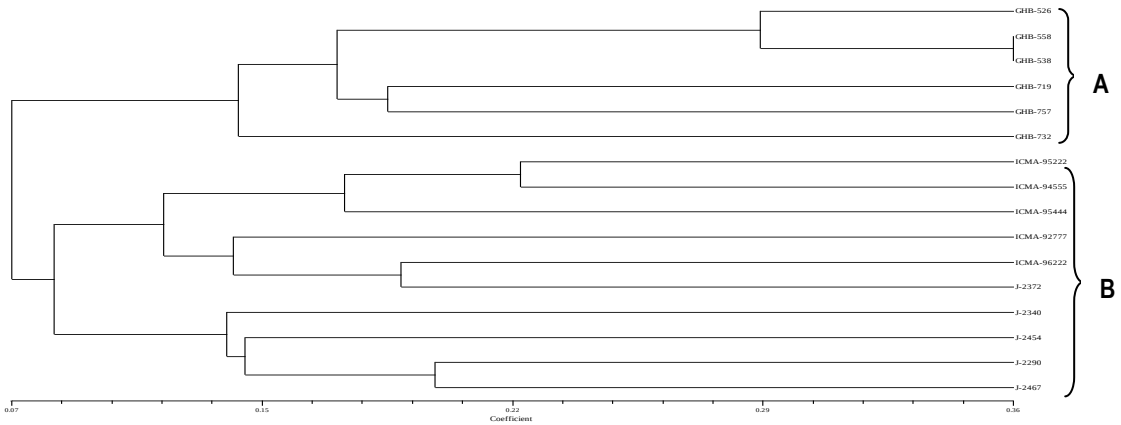

Fig-1 RAPD Dendrogram for Pearl Millet Genotypes

Cluster A=GHB-526, GHB-558, GHB-538, GHB-719, GHB-757, GHB-732

Cluster B=ICMA-95222, ICMA-94555, ICMA-95444, ICMA-92777, ICMA-96222, J-2372, J-2340, J-2454, J-2290, J-2467

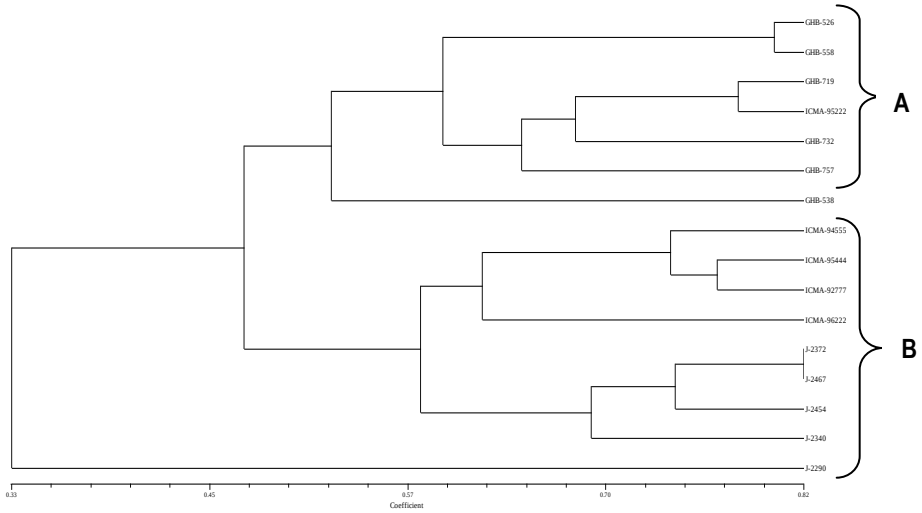

Fig-2 SSR Dendrogram for Pearl Millet Genotypes

Cluster A=GHB-526, GHB-558, GHB-719, ICMA-95222, GHB-732, GHB-757, GHB-538

Cluster B=ICMA-94555, ICMA-95444, ICMA-92777, ICMA-96222, J-2372, J-2467, J-2454, J-2340, J-2290

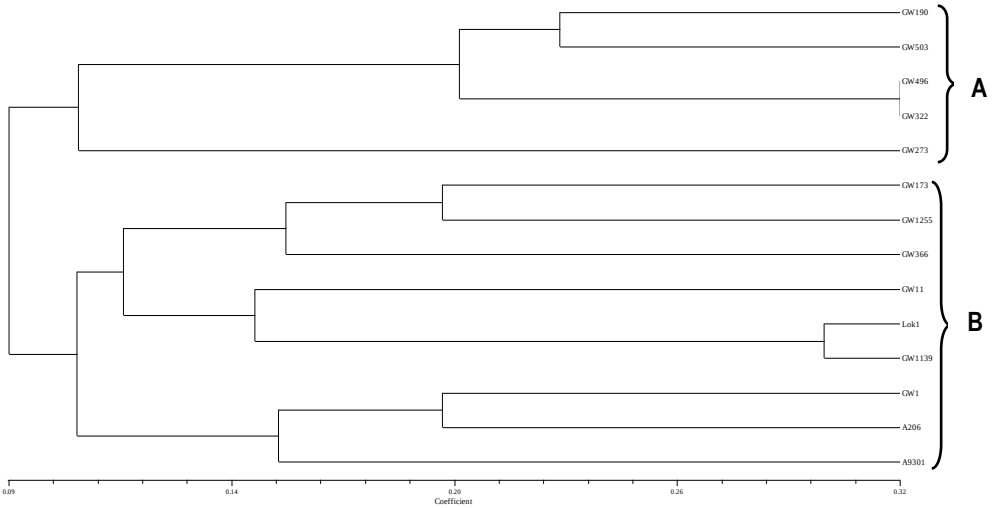

Fig-3 RAPD Dendrogram for Wheat Genotypes

Cluster A- GW190, GW503, GW496, GW322, GW273

Cluster B- GW173, GW1255, GW366, GW11, Lok1, GW1139, GW1, A206, A-9-30-1

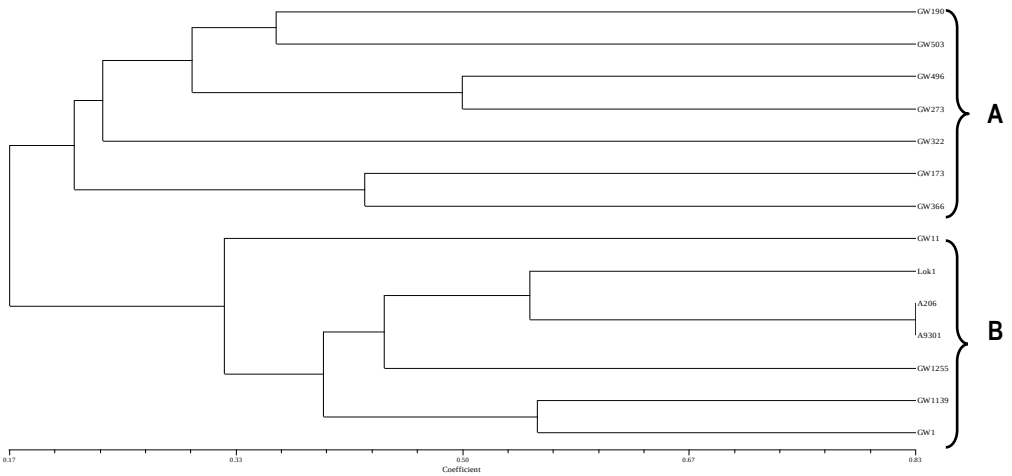

Fig-4 SSR Dendrogram for Wheat Genotypes

Cluster A- GW190, GW503, GW496, GW273, GW322, GW173, GW366

Cluster B- GW11, Lok1, A206, A-9-30-1, GW1255, GW1139, GW1

Fourteen different wheat genotypes were used for revealing the efficiency of different RAPD and SSR markers. The RAPD analysis produced 564 scorable bands. All fourteen genotypes were grouped into two different clusters i.e. A and B. The cluster A comprised of genotypes such as GW 190, GW 503, GW 496, GW 322 and GW 273, whereas others had grouped in cluster B [Fig-3]. In the SSR analysis of these genotypes, from 114 scorable bands, the genotypes grouped into two different clusters i.e. A which comprised of GW 190, GW 503, GW 496, GW 273, GW 322, GW 173, GW 366 and cluster B consisting of rest of the genotypes [Fig-4].

The PIC values obtained for both pearl millet and wheat genotypes showed a significant difference between RAPD and SSR primers. The highest PIC value was recorded in RAPD primers as compared to SSR primers used. The PIC analysis of the two markers revealed higher values in RAPD primers as compared to SSR. The highest PIC value of RAPD primer in pearl millet was recorded as 0.97 (OPN-7), while in wheat highest was recorded as 0.96 (22ES10G33) [Table-2]. In SSR analysis of these two crops, highest PIC value was recorded as 0.84 in primers, CTM-25, CTM-27 and Xpsmp 2273 for pearl millet [Table-2] while for wheat, highest PIC was recorded as 0.88 in the primer Xgwm 153 [Table-3].

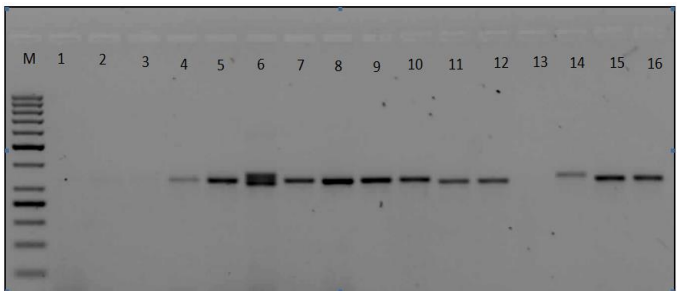

Fig-5 SSR Primer CTM 12 for Pearl Millet

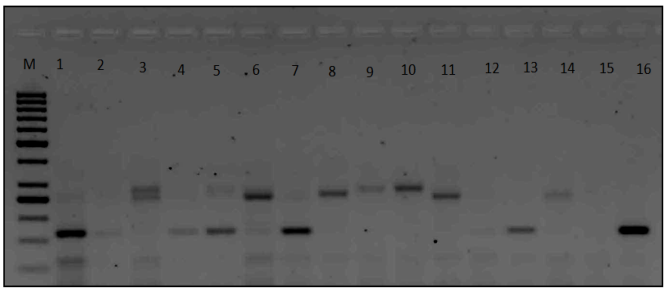

Fig-6 SSR Primer CTM 21 for Pearl Millet

In the above study, the results obtained from both techniques differed slightly which necessitates that in future, for study of genetic diversity, more than one marker systems should be employed for higher genetic resolution. Although it appears that the RAPD markers are more efficient than SSR for studying the diversity of these crops, it need not be a thumb rule in all instances. SSR markers due to their polymorphic potential, higher reproducibility, co-dominant mode of inheritance, recurrent occurrence and ubiquitous distribution in the genome, environmental neutral behavior, accessibility, rapid assay, cost effectiveness, high throughput and transferability across the related genera/species be more efficient as a tool for diversity assessment. Unfortunately, molecular marker systems invogue do not generally fulfill all these selection parameters. Therefore, molecular marker technologies involving second or third generation markers can be more reliable/ dependable tools for unraveling the mystery of gene alignments and associations.

Conflict of Interest: None declared

References

[1] Hash C.T. and Witcombe J.R. (2005) Pearl millet molecular marker research. *SAT* eJournal. 1(1), 1-8.

[2] Myskow B., Milczarski P. and Masojc, P. (2010) *Plant breeding and seed science*, 62, 107-115.

[3] Ahmadikhah A. (2008) *African Journal of Biotechnology*, 8(2), 323-327.

[4] Garcia A.A.F., Benchimol L.L., Barbosa A.M.M., Geraldi I.O., Souza Jr C.L. and Souza A.P. (2004) *Genetics and Molecular Biology*, 24(4), 579-588.

[5] Tonk F.A., Tosun M., Ilker E., Istipliler, D. and Tatar, O. (2014) *Bulgarian Journal of Agricultural Sciences*, 20(6), 1413-1420.

[6] Demuyakor B., Galyuon I., Kyereh S. and Ahmed M. (2013) *International Journal of Agriculture Sciences*, 5(1), 354-358.

[7] Mofokeng M.A., Watson G., Shimelis H. and Tongoona P. (2012) *African Journal of Biotechnology*, 11(2), 16697-16707.

[8] Zeb B., Khan I.A., Ali Shahid., Bacha S., Mumtaz S. and Swati Z.A. (2009) *African Journal of Biotechnology,* 8(17), 4016-4019.