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# Molecular characterization of sorghum (*Sorghum bicolor* L. Moench) germplasm by using SSR marker

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**Abstract**
Sorghum (*Sorghum bicolor* L. Moench) is self pollinating crop belongs to Poaceae family; it is fifth most important cereal crop in the world after wheat rice, maize and barley. Molecular markers are useful tools for assessing genetic diversity compared with morphology and pedigree information as they are not affected by environmental factors and reveal differences among genotypes at the DNA level. In the present investigation, the PCR amplification results of 15 SSR primers generated 525 bands. Out of these 327 bands were polymorphic and 198 bands were monomorphic among 33 germplasm. Highest polymorphism was recorded in SSR primers Xtxp248, Xtxp18, Xtxp69 Xtxp32 Xtxp88, Xtxp285, Xtxp10, Xtxp31, Xtxp75 (100% polymorphism) whereas Xcup11, Xtxp41, Xtxp40, Xtxp319, Xtxp228 and Xtxp312 produced monomorphic banding patterns. The average monomorphic band per SSR primer was 13.2 (37.71%) and also average polymorphic bands per SSR primer was 21.8 (62.28%).
The Polymorphism information content (PIC) value of SSR primers ranged from 0.46 to 0.88 with an average of 0.33. Xtxp32 was most informative having PIC value 0.88 whereas Xtxp18 found least informative having PIC value 0.46.The amplicon size was found different for each SSR primer. The amplicon size ranged from 75bp to 300 bp. Most of primer generated 200 to 300 bp fragment. Xtxp18 primer showed ~200-450 bp amplicon size in some germplasm.
The cluster analysis was performed using UPGMA method. The dendrogram was divided into two main clusters *i.e.* cluster I and cluster II. On the basis of cluster analysis the highest similarity *i.e.* 97% was observed amongst IS5148 and IS2187.
The polymorphism and similarity can be utilized for further breeding programme and varietal development using QTL character specific study.

**Keywords:** *Sorghum bicolor*, SSR, genotyping, cluster analysis

## Introduction
Sorghum [*Sorghum bicolor* (L.) Monech] is tropical monocot plant belongs to the family Poaceae and one of the most important crops in Africa, Asia and Latin America. It is one of the world's leading cereal crops, and 3rd most important cereal after wheat and rice in India. DNA marker-assisted breeding has become one of the most important applications of biotechnology in recent times. Molecular markers are being used worldwide to tag specific chromosome segments containing the desired gene (s) to be introgressed into the breeding lines. In this way, indirect selection with co-dominant molecular markers (like SSR) tightly linked to gene(s) controlling characters of interest improves accuracy of selection. DNA based marker-assisted selection (MAS) can supplement conventional breeding and therefore will become an integral part of the plant breeding practices in the coming years (Gupta *et al.* 2010).
Genetic diversity is important in sorghum breeding because it plays an important role in determining heterotic groups (Cheruiyot *et al.*, 2014) [2]. The genetic diversity of sorghum at global scale has been studied by using different types of molecular marker such as SSR, RAPD, and ISSR.
Amongst the different types of molecular markers, SSR are the most commonly used in applied breeding programme due to their hypervariable, co- dominant, robust and multiallelic nature (Ganapathy *et al.*, 2012) [5]. SSR are easily detected by polymerase chain reaction and also require small amount of DNA (Powell *et al.*, 1996) and are widely used for genetic diversity assessment in several cultivated crop species including sorghum (Dje *et al.*, 2000) [4]. SSR markers have been developed by different research groups in sorghum (Brown *et al.*, 1996; Taramino *et al.*, 1997; Schloss *et al.*, 2002, Ramu *et al.*, 2009) [1, 10, 9, 6].
Detection and analysis of genetic variation can help us to understand the molecular basis of various biological phenomena in plants. Knowledge of genetic variation is essential for genetic research, improvement in plant breeding strategies, conservation and effective utilization of genetic resources and intellectual property protection.

Molecular markers are useful tools for assessing genetic diversity compared with morphology and pedigree information as they are not affected by environmental factors and reveal differences among genotypes at the DNA level.

There has been a different promising line of sorghum (*Sorghum bicolor* L. Moench) which is used to generate high yielding hybrids. As the variation is the base for production of any hybrid, it was necessary to evaluate variation available in the promising lines of sorghum at molecular level. The purpose of this study was to screen promising lines of sorghum using SSR markers to evaluate inherent variation within the germplasm. Two shoot fly susceptible lines DJ6514 and SWARNA and shoot fly resistant line IS2205 (Choudhary *et al.*, 2013) [3] were also screened using SSR markers to observed putative variation in banding patterns within the check lines of shoot fly resistant and susceptible germplasm for further study.

## Materials and Methods

### Plant materials

A total 33 germplasm of sorghum (*Sorghum bicolor* L. Moench) collected from Sorghum Research Station, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani were used in this study. The selected germplasm are promising lines of sorghum used for breeding programme and varietal development. Total genomic DNA was extracted from 15 days old leaves, germinated in plastic plots containing sand, soil and compost manure with appropriate ratio (1:1:2) in green house.

### DNA Extraction

High quality genomic DNA was isolated from young and fresh leaves (6-8 days old) of sorghum using Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction method (Saghai-Maroof, 1984) [8] with some modifications.

### SSR PCR Programming

PCR amplification reactions were set in a 25 µl reaction volume. The different components of PCR were optimized to develop an appropriate DNA profile by employing SSR technique. Different concentrations of genomic DNA (25, 50, 75 ng per 25 µl reaction mixture), $MgCl_2$ (1.5, 2.5 and 3.5 mM) and *Taq* DNA polymerase (5 Unit/µl) were tried to obtain an optimum reaction mixture. The different concentrations of the primers (5, 10, 15, 20 and 25pM/µl) were also tried for the complete amplification of the genomic DNA. In case of optimization of primer annealing, the different temperatures (49, 50, $60^0$C.) were tried as the annealing temperature changes with primer being used.

A master mix in sterile double distilled water having all the above mentioned compounds in required quantities was prepared, while preparing the mix, buffer was added to the water followed by other components to avoid degradation of any of them and all operations were carried out on ice. Twenty five ng of genomic DNA from different samples were taken into individual 0.5 ml PCR tubes and master mix was added to them, final volume made was 25 µl. Amplifications were performed in a thermal cycler (Biometra) using the cyclic parameters.

**Table 1:** List of the primers used along with their sequences

| Sr. No. | Primer code | Primer sequence 5' to 3' | | $T_m$ | $T_A$ |
|---|---|---|---|---|---|
| 1. | *Xtxp10* | F | ATA CTA TCA AGA GGG GAG C | 54.7 | 55° C |
| | | R | AGT ACT AGCCAC ACG TCA C | 55.1 | |
| 2. | *Xtxp31* | F | TGC GAG GCT GCC CTA CTA G | 65.5 | 65° C |
| | | R | TGG ACG TAC CTA TTG GTG C | 61.0 | |
| 3. | *Xtxp32* | F | AGA AAT TCA CCA TGC TGC AG | 62.8 | 65° C |
| | | R | ACC TCA CAG GCC ATG TCG | 65.1 | |
| 4. | *Xtxp41* | F | TCT GGC CAT GAC TTA TCA C | 61.5 | 63° C |
| | | R | AAA TGG CGT AGA CTC CCT TG | 62.9 | |
| 5. | *Xtxp75* | F | CGA TGC CTC GAA AAA AAA ACG | 67.3 | 62° C |
| | | R | CCG ATC AGA GCG TGG CAG G | 71.7 | |
| 6. | *Xtxp88* | F | CGT GAA TCA GCG AGT GTT GG | 66.9 | 65° C |
| | | R | TGC GTA ATG TTC CTG CTC | 60.0 | |
| 7. | *Xtxp248* | F | GGG TGT CCA ATG TTG TCT GC | 65.5 | 64° C |
| | | R | GGC CGT TAC TGT CCC TTA CTC A | 66.1 | |
| 8. | *Xtxp285* | F | ATT TGA TTC TTC TTG CTT TGC CTT GT | 67.4 | 65° C |
| | | R | TTG TCA TTT CCC CCT TCT TTC TTT T | 67.2 | |
| 9. | *Xtxp69* | F | ACA CGC ATG GTT TGA CTG | 58.9 | 60° C |
| | | R | TTG ATA ATC TGA CGC AAC TG | 67.3 | |
| 10. | Xcup 11 | F | TAC CGC CAT GTC ATC ATC AG | 64.6 | 60° C |
| | | R | CGT ATC GCA AGC TGT GTT TG | 64.4 | |
| 11. | Xtxp18 | F | ACT GTC TAG AAC AAG CTG CG | 59.9 | 58° C |
| | | R | TTG CTC TAG CTA GGC ATT TC | 58.9 | |
| 12. | Xtxp228 | F | ACA GGT TGG CGA TGT TTC TCT | 64.8 | 61° C |
| | | R | TTC TTT TTC GAA TTC ATT CCT TTT | 62.6 | |
| 13. | Xtxp312 | F | CAG GAA AAT ACG ATC CGT GCC AAG T | 71.2 | 64° C |
| | | R | GTG AAC TAT TCG GAA GAA GTT TGG AGG AAA | 70.6 | |
| 14. | Xtxp319 | F | TAG ACA TCT GAA TTA AGG AGC | 55.2 | 60° C |
| | | R | CAT GCC CCT GAA AGA GA | 59.9 | |
| 15. | Xtxp40 | F | CAG CAA CTT GCA CTT GTC | 58.0 | 62° C |
| | | R | GGG AGC AAT TTG GCA CTA G | 62.2 | |

### Resolution of PCR product

Amplified PCR products were separated on 1.5% agarose gel (100V for 2.5 hr) using 100 bp DNA ladder (Banglore Genei Pvt. Ltd) as a molecular size standard. The gels stained with Ethidium bromide (10 mg/ml) were visualized and photographed under UV light in gel documentation system

(Alphaimager™ 2200).

**Data analysis**

Each amplification product was considered as SSR marker and was scored across all samples. Bands were scored as present (1) or absent (0). Molecular weight of the bands was estimated by using 1kb DNA ladder (MBI, Fermentas, U.K.) as standards. All amplifications were repeated at least twice and only reproducible bands were considered for analysis. The polymorphism information content (PIC) value was calculated as $PIC=\sum(1-P^2i)/n$ where $n$ is the number of band positions analyzed in the set of accession and $Pi$ is the frequency of $i^{th}$ pattern.

Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02, Rohlf, 1990) [7]. The SIMQUAL programme was used to calculate the DICE coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) based on DICE coefficient.

**Results**

Fifteen different sorghum specific SSR primers were used to screen 33 sorghum germplasm. Out of these 9 primers produced polymorphic band and remaining primers generated monomorphic band. The percentage of polymorphic band is higher than monomorphic band.

The PCR amplification results of 15 SSR primers generated 525 bands. Out of these 327 bands were polymorphic and 198 bands were monomorphic among 33 germplasm. In case of percent polymorphism, highest polymorphism was recorded in SSR primers Xtxp248, Xtxp18, Xtxp69 Xtxp32 Xtxp88, Xtxp285, Xtxp10, Xtxp31, Xtxp75, Fig.2 (100% polymorphism) whereas Xcup11, Xtxp41, Xtxp40, Xtxp319, Xtxp228 and Xtxp312 produced monomorphic banding patterns. The average monomorphic band per SSR primer was 13.2 (37.71%) and also average polymorphic bands per SSR primer was 21.8 (62.28%).

The Polymorphism information content (PIC) value of SSR primers ranged from 0.46 to 0.88 with an average 0.33. Xtxp32 was most informative having PIC value 0.88 whereas Xtxp18 found least informative having PIC value 0.46.

The amplicon size was found different on the basis of the SSR primer. The amplicon size ranged from 75bp to 300 bp. Most of primer generated 200 to 300 bp amplicon size. Xtxp18 primer showed ~400 -450 bp amplicon size in germplasm *viz*. IS33874, IS40199, IS 40290, PVR658, IS4496, IS2511, IS5148, IS24289, IS35733 and Solapur Dagade.

In the study conducted by Choudhary *et al.,* (2013) [3] shoot fly resistant and shoot susceptible sorghum genotypes were screened. In this study, 21 SSR primers were used out of these 21 primer 6 primer were amplified and produced 12 bands. Amongst total bands 5 bands were polymorphic showing 41.66 percent polymorphism. The most informative primer found was Xtxp88 showing 64 clear and reproducible amplicon. The similarity coefficient using all the bands was ranged from 0.58 to 1.00, whereas for polymorphic band, the value was ranged from 0.00 to 1.00.

Different sorghum specific SSR primers were used in our study to screen the 33 germplasm of sorghum. Many alleles were found similar in the different lines of sorghum using SSR primers. The PCR amplification of 15 SSR primers generated 525 bands in that 198 bands were monomorphic and 327 bands were polymorphic. High polymorphic result indicated a wide genetic base in sorghum germplasm and this genetic diversity may contribute to different morphological and physiological characters. The PIC value of SSR primers ranged from 0.46 to 0.88 with an average 0.33 Xtxp32 was most informative having value 0.88 whereas Xtxp18 found least informative having Polymorphism information content (PIC) value 0.46.

Genetic relationships between sorghum germplasm were determined on the basis of DICE similarity coefficient values. The similarity coefficient values obtained were presented in Table 2.

The value of similarity coefficient ranged from 0.47 to 0.97. The germplasm IS5148 showed highest similarity coefficient value with IS2187. The germplasm SPV570 showed lowest similarity coefficient value with IS26752. The average similarity coefficient value was 0.71.

The average similarity coefficient values ranged from 0.61-0.77. Average similarity coefficient value per germplasm were calculated which is given Table 2. The germplasm IS2511, IS40269 and PVRS 137 showed highest average similarity coefficient value (0.77) than other germplasm. It has indicted that the germplasm is genetically less diverse and the germplasm IS24878 showed that lowest average similarity coefficient value (0.61) has been found more diverse.

**Table 2:** Average similarity index of 33 sorghum germplasm

| Sr. No. | Germplasm Name | Average similarity value | Sr. No | Germplasm Name | Average similarity value |
|---|---|---|---|---|---|
| 1 | IS33874 | 0.72 | 17 | IS4882 | 0.74 |
| 2 | IS40199 | 0.74 | 18 | IS24878 | 0.61 |
| 3 | IS40290 | 0.72 | 19 | IS35733 | 0.73 |
| 4 | PVR658 | 0.73 | 20 | Solapur Dagade | 0.73 |
| 5 | Pop Sorghum 15 | 0.68 | 21 | IS26752 | 0.67 |
| 6 | IS4496 | 0.65 | 22 | IS27028 | 0.73 |
| 7 | IS2511 | 0.77 | 23 | IS25473 | 0.64 |
| 8 | IS5148 | 0.74 | 24 | IS27238 | 0.71 |
| 9 | IS30974 | 0.69 | 25 | IS4763 | 0.76 |
| 10 | IS2187 | 0.75 | 26 | PVRS 133 | 0.72 |
| 11 | IS17948 | 0.71 | 27 | PVRS 134 | 0.71 |
| 12 | IS40269 | 0.77 | 28 | PVRS 135 | 0.73 |
| 13 | SPV570 | 0.70 | 29 | PVRS 136 | 0.74 |
| 14 | IS4803 | 0.74 | 30 | PVRS 137 | 0.77 |
| 15 | IS24289 | 0.76 | 31 | IS 2205 | 0.75 |
| 16 | IS4482 | 0.66 | 32 | SWARNA | 0.66 |
| | | | 33 | DJ 6514 | 0.66 |
| | | | | Average | 0.71 |

The genetic diversity of the investigated accessions is high, distributed over groups and subgroups and exhibits a moderate level of association between genetic divergence.

Genetic diversity of sorghum is effectively investigated using SSR markers, which allow a more complete coverage of the genetic variation.

Sorghum specific SSR primers were selected to detect polymorphism in sorghum germplasm. Many alleles were similar in sorghum germplasm using SSR primers. The high polymorphic (100%) indicated wide genetic base in sorghum germplasm and genetic diversity may contribute to different morphological and physiological characters.

Similarity matrix was used to generate dendrogram through UPGMA cluster analysis by using NTSYS-pc. The dendrogram generated on the basis of SSR genotyping data was divided into two major cluster *viz*. cluster I and cluster II. Cluster I contained 28 germplasm and cluster II contained 5 germplasm (Table 2).

The major cluster I comprised 28 germplasm (IS33874, IS4482, IS40199, IS4882, IS40290, PVR658, IS2205, IS35733, Solapur Dagade, IS4763, PVRS137, IS27028, PVRS133, PVRS134, PVRS135, PVRS136, IS2511, IS40269, IS24289, IS4803, IS17948, IS27238, Pop Sorghum 15, IS5148, IS2187, SPV570, IS30974 and DJ6514) was further divided into 4 sub clusters *viz*. cluster IA, cluster IB, cluster IC and cluster ID with around 70% similarity with each other. The sub cluster IA comprised seven germplasm (IS33874, IS4482, IS40199, IS4882, IS40290, PVR658, and IS2205) with around 79% similarity with each other. Another sub cluster IB comprised 9 germplasm (IS35733, Solapur Dagade, IS4763, PVRS137, IS27028, PVRS133, PVRS134, PVRS135, and PVRS136) showing 81% similarity with each other. Cluster IC comprised 6 germplasm (IS2511, IS 40269, IS24289, IS4803, IS17948, and IS27238) having 78% similarity with each other. Cluster ID contained 6 germplasm (Pop Sorghum 15, IS5148, IS2187, SPV570, IS30974 and DJ6514) with an average similarity 72%.

The major cluster II comprised five germplasm (IS4496, IS26752, SWARNA, IS25473, and IS24878 with an average similarity 69%. The major cluster II was further divided in to two sub cluster *viz*. IIA and IIB. The IIA sub cluster comprised three germplasm (IS4496, IS26752, and SWARNA) with an average similarity was about 81%. Another sub cluster IIB comprised two germplasm (IS24878 and IS25473) having 82% similarity with each other.

Sorghum specific SSR primers were selected to detect polymorphism in sorghum germplasm. Many alleles were similar in sorghum germplasm using SSR primers. The high polymorphic (100%) indicated wide genetic base in sorghum germplasm and genetic diversity may contribute to different morphological and physiological characters.

The result revealed that SSR primers XtXp248 XtXp18, XtXp69 XtXp32 XtXp88, Xtxp31, Xtxp10, Xtxp75 and XtXp285 (100% polymorphism) were highly polymorphic. These primers can be used in diversity analysis of crops or sorghum germplasm.

Most of the SSR primer produced similar band in DJ6514 and SWARNA, these are shoot fly susceptible lines but IS2205 resistance line produced distinct band. This revealed that IS2205 possesses different amplicon than DJ6514 and SWARNA; this might be attributed to resistance and susceptibility.

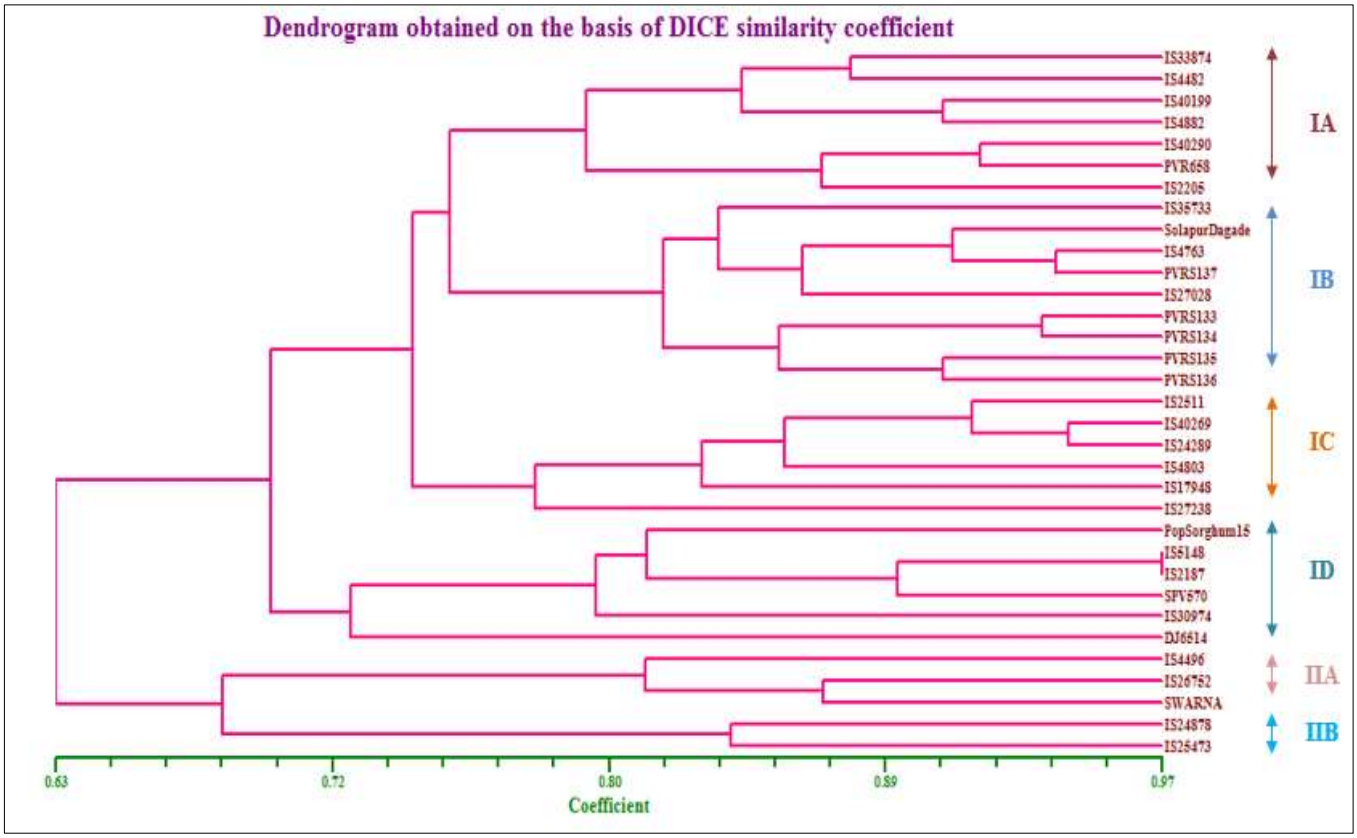


**Fig 1:** Dendrogram generated by UPGMA analysis based on SSR data showing relationship among 33 sorghum germplasm

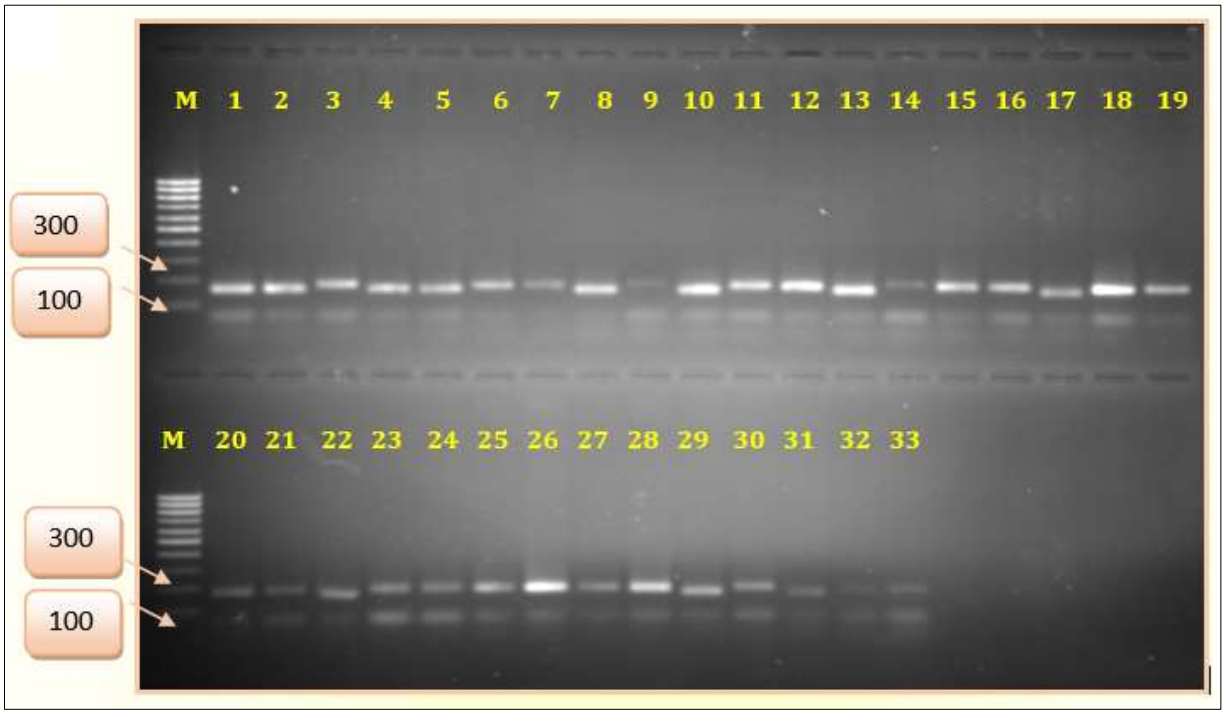


**Fig 2:** SSR profile of 33 cultivar of sorghum by using Xtxp75 primer lane M, (100 bp DNA ladder), lane 1-33 of sorghum as describing table no. 1st.

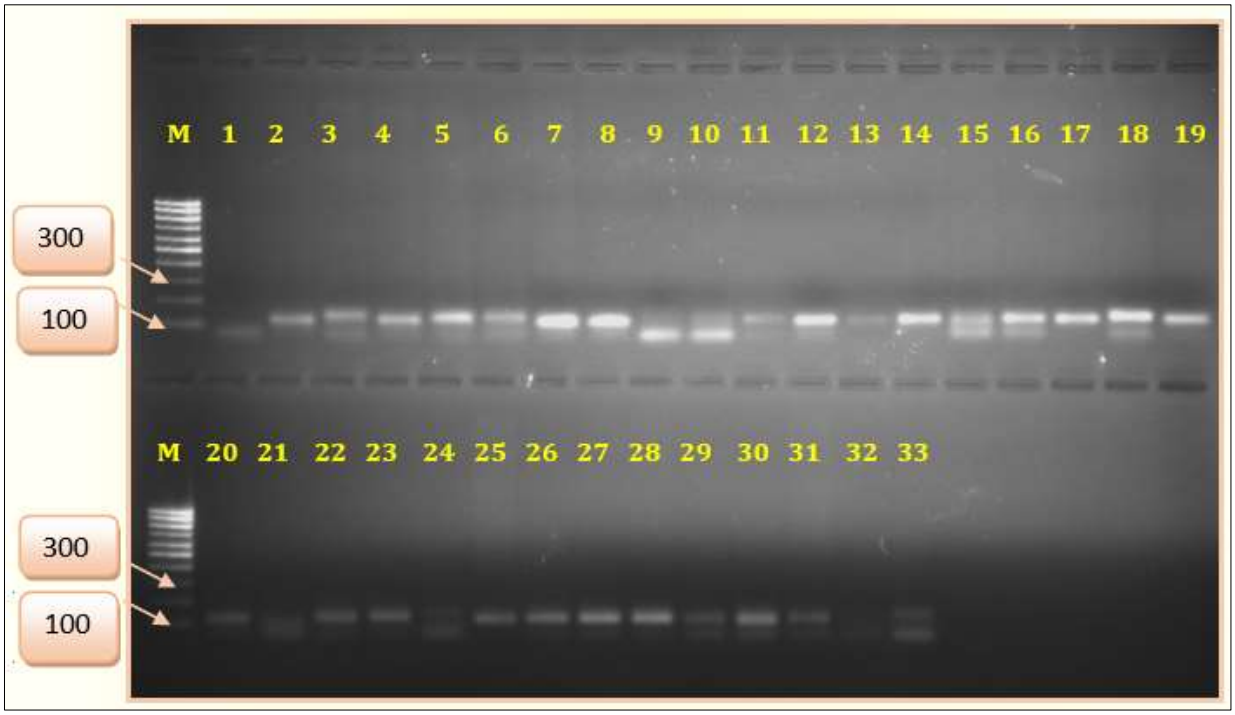


**Fig 3:** SSR profile of 33 cultivar of sorghum by using Xtxp88 primer lane M, (100 bp DNA ladder), lane 1-33 of sorghum as describing table no. 1st.

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