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COMPARATIVE GENETIC ANALYSIS OF WILD FINGER MILLET ACCESSIONS USING FINGER MILLET AND MAIZE MICROSATELLITE MARKERS

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ABSTRACT

The genomic information available for finger millet is very scarce though the plant is a rich source of highly digestible proteins and dietary fibre with good amounts of soluble and insoluble fractions. In present study, 64 maize and finger millet genomic SSRs were used for cross transferability, identification of polymorphic markers and genetic diversity of finger millet, both cultivated and wild species. Out of 64 SSRs, only 43 (67%) were amplified across the finger millet genotypes. The PIC values of all the polymorphic loci across the 23 finger millet genotypes varied from 0.04 to 0.47 (average value 0.17). Based on the parameters of PIC values ≥ 0.26, gene diversity ≥0.43, inbreeding coefficient ≥ 0.60, the SSR loci UGEP33, UMC1858, UMC1805, and UMC2163 were found highly polymorphic. Comparison of SSR polymorphism in finger millet genotypes revealed that microsatellites of maize were more polymorphic and were able to identify more diversity in finger millet genotypes than the finger millet SSRs. Good correlations were found between genetic diversity analysis in differentiation of finger millet genotypes using finger millet and maize microsatellite markers. Dendrogram generated through UPGMA analysis grouped all the 23 genotypes clustered them into two major groups A and B with maximum similarity found between genotype pairs T552, T622 and T507, T74. The genotype pairs T671 and T760; T187 and T 631, T824 showed lowest similarity in finger millet which could be selected for hybrid plant production. The present study enriched the finger millet genomics by identifying suitable polymorphic markers of maize and finger millet, which can be used for diversity analysis, cultivar identification and QTL mapping studies.

Key words: Finger millet, SSR markers, genetic analysis, maize microsatellites

INTRODUCTION

Millets are minor cereals belonging to the grass family Poaceae. They are small seeded annual cereal grasses, many of which are adapted to tropical and arid climates and are characterized by their short duration and ability to survive under conditions of moisture stress and low soil fertility. Finger millet, *Eleusine coracona* (L.) Gaertn., is a tetraploid (2n = 4x = 36) belonging to subfamily Chloridodeae of family Poaceae. It is cultivated mainly in East Africa and Southern India. The first India variety was released in 1976. India has become a secondary center of diversity for finger millet. The knowledge of patterns of genetic diversity among the available genotypes is of great significance in finger millet breeding programs in order to maximize heterosis in hybrid

combinations and to maintain diversity of breeding lines. In addition, local inbred lines are useful for the development of breeding materials adapted to the local environment and for the exploitation and introduction of other genotypes. The morphological characters often do not reliably portray genetic relationships due to environmental interactions.

Molecular markers have been applied for the quantification of genetic diversity, genotype identification, delineation and marker assisted selection. Assessment of the extent of genetic variation in millet has been carried out based on restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) (Babu *et al.,* 2007) and simple sequence repeat (SSR) (Kumar *et al.,* 2013; Babu *et al.,* 2014a;). Among these, PCR based SSRs have been widely used in molecular characterization of finger millet germplasm and genetic diversity among genotypes via automated systems. SSR analysis presents potential advantages of reliability, reproducibility, discrimination, standardization and cost-effectiveness over other marker technologies. Since, the development of SSRs is a costly affair involving high cost of library screening and clone sequencing, the alternative strategy is to identify the best suitable genomic SSR markers through transferability from the related close species like maize. The present study is the first kind of report for identification of best suitable polymorphic genomic SSRs through cross transferability of maize genomic SSR markers. With this aim, the present study was conducted on a set of 23 finger millet genotypes to assess cross transferability of maize genomic SSR markers into finger millet and identify polymorphic markers, as well as compare the genetic analysis of finger millet and maize genomic SSR markers and analyze genetic diversity in finger millet genotypes using polymorphic finger millet and maize genomic SSRs.

MATERIALS AND METHODS

Plant materials and DNA extraction

Twenty-three finger millet genotypes were taken to find transferable markers and polymorphic markers. The 23 genotypes used for molecular characterization and diversity analysis consisted of both wild and local accessions. The genotypes used were T9, T74, T507, T367, T296, T685, T422, T6, T187, T581, T65, T99, T593, T774, T595, T631, T824, T671, T401, T760, T552, T622, and T100. The genomic DNA of different accessions was isolated by standard method (Murray and Thomson, 1980), quantified and analyzed on agarose gel electrophoresis (Maniatis *et al.,* 1989).

Cross amplification of maize genomic SSR markers

Cross-species amplification of finger millet genotypes was done by using 50 maize genomic SSR markers. The maize genomic SSRs were obtained from maize gdb website; *www.maizegdb.org*.

SSR amplification and detection

The polymerase chain reactions (PCR) were performed in 20 µL reaction volume containing 2 µL 10X buffer having 15 mM $MgCl_2$, 0.2 µM each forward and reverse primer, 2 µL 2 mM dNTPs, 0.2 µL 1 U of *Taq* DNA polymerase (Invitrogen, USA), and about 25 ng template DNA. The PCR amplification protocol was standardized for genomic SSRs. The reaction conditions for maize genomic SSRs were: initial denaturation of 4 min at 95°C followed by 40 cycles of 30 sec at 94°C, 30 sec of annealing temperature 52°C, extension of 1.0 min at 72°C, with a final extension of 7 min at 72°C and hold at 4°C. The electrophoresis was held at 100 volts for 3 h at room temperature. The reaction conditions for finger millet genomic SSRs were followed as per Dida *et al.* (2007). Gels were stained with ethidium bromide and visualized using Bio Imaging System (SynGene, USA).

Data analysis

The data set of SSR loci on finger millet genotypes were used for diversity analysis using Power Marker V3.0 (Liu and Muse, 2005) for estimating the polymorphism information content (PIC),

gene diversity, allele frequency and most frequent and rare alleles. Unweighted pair group method (UPGMA) was used to generate the tree using the CS Chord, 1967 frequency matrix.

RESULTS AND DISCUSSION

Cross transferability of maize genomic SSRs

Finger millet genomics is lagging far behind of major cereal crops like rice, maize and wheat and small millets like foxtail millet. Till now very a few reports available on the transferability of EST based SSR markers in finger millet species (Kumari *et al.,* 2013). However, in present study, a set of50 maize genomic SSR markers were used to amplify 23 finger millet genotypes. Out of 50 SSRs, only 29 (58%) were amplified across the finger millet genotypes. Only these 29 SSRs were considered for molecular characterization. The high amount of transfer- ability between maize and finger millet was a very good sign of utilizing the genomic SSRs of maize in finger millet genomics applications. The high amount of transferability was similar to the earlier reports with EST-SSRs (Nirgude *et al.,* 2014), SSRs (Shambhavi *et al.,* 2015). Rajput *et al.* (2014) used genomic SSRs of switch grass into Proso millet and found 62% transferability. Yadav *et al.* (2008) studied the transfer-ability of sorghum, rice and wheat SSR markers into pearl millet and found that sorghum SSR markers were much high transferable than others. The present study revealed that finger millet researchers can directly use the reported poly-morphic markers directly in genomic studies. The polymorphic SSRs with their chromosome number and repeat markers directly in their motif were:

Maize markers: Umc1066, Umc2364, Bnlg2123, Umc 2030, Bnlg1267, Umc1230, Umc2071, Mmc0231, Mmc0371, Dupssr23, Umc1030, Umc1805, Phi026, Umc1407, Umc2101, Mmc0081, Umc1148, Umc1075, Umc1161, Bnlg1325, Mmc082, Umc1012, Bnlg2323, Umc1858, Bnlg1600, Umc1941, Umc1155, Umc1052, Umc3228, Phi101049, Phi 114, Bnlg1904, Umc1077, Umc2163, Umc2206, Umc1804, Umc1799, Bnlg1346, Phi057, Dupssr 29, Umc1054, Umc1506, Phi112, Umc1279, Umc2136 and Umc1904.

Finger millet markers: UGEP 33, UGEP 57, UGEP 27, UGEP 22, FM10, UGEP 52, FM6, U107, UGEP 26, UGEP 21, U104, UGEP 90, F.M1, U3, UGEP 110, FM5, UGEP 76 and UGEP 11.

Table 1: The polymorphism details such as allele number, PIC, gene diversity and heterozygosity using maize SSRs

Marker	Major allele frequency	Allele number	Gene diversity	Hetero-zygosity	PIC
UMC1230	0.93	2.00	0.12		0.11
BNLG1267	0.68	2.00	0.44	0.13	0.34
UMC2071	0.91	2.00	0.16	0.17	0.15
MMC0081	1.00	1.00	0.00	0.00	0.00
UMC1012	0.50	2.00	0.50	1.00	0.38
UMC2101	1.00	1.00	0.00	0.00	0.00
DUPSSR23	0.89	3.00	0.20	0.04	0.19
MMC0371	0.50	2.00	0.50	1.00	0.38
UMC1075	1.00	1.00	0.00	0.00	0.00
UMC1052	1.00	1.00	0.00	0.00	0.00
UMC1941	0.96	2.00	0.08	0.09	0.08
BNLG1600	0.96	3.00	0.08	0.09	0.08
UMC1805	0.72	2.00	0.40	0.56	0.32
UMC2364	0.98	2.00	0.05	0.05	0.05
UMC1407	0.98	2.00	0.04	0.04	0.04
UMC1858	0.61	2.00	0.48	0.78	0.36
UMC1804	0.52	3.00	0.57	0.70	0.48
UMC2163	0.80	2.00	0.31	0.39	0.27
UMC1054	1.00	1.00	0.00	0.00	0.00
UMC1506	0.50	2.00	0.50	1.00	0.38
PHI026	0.98	2.00	0.05	0.05	0.05
BNLG1325	0.50	2.00	0.50	1.00	0.38
UMC1030	1.00	1.00	0.00	0.00	0.00
BNLG1346	0.55	3.00	0.55	0.14	0.46
MMC0282	0.50	2.00	0.50	1.00	0.38
PHI114	1.00	1.00	0.00	0.00	0.00
UMC1161	1.00	1.00	0.00	0.00	0.00
DUPSSR19	0.94	2.00	0.10	0.11	0.10
BNLG1839	1.00	1.00	0.00	0.00	0.00
Mean		1.83	0.21	0.30	0.17

Table 2: The polymorphism details such as allele number, PIC, gene diversity and heterozygosity using finger millet SSRs

Markers	Major allele frequency	Allele number	Gene diversity	Hetero-zygosity	PIC
UGEP52	0.50	2.00	0.50	1.00	0.38
UGEP90	1.00	1.00	0.00	0.00	0.00
UGEP26	0.88	2.00	0.21	0.24	0.19
FM6	0.88	3.00	0.21	0.12	0.20
UGEP104	1.00	1.00	0.00	0.00	0.00
UGEP110	0.50	2.00	0.50	1.00	0.38
UGEP57	1.00	1.00	0.00	0.00	0.00
UGEP33	0.59	2.00	0.48	0.13	0.37
UGEP27	1.00	1.00	0.00	0.00	0.00
UGEP22	1.00	1.00	0.00	0.00	0.00
UGEP21	0.50	2.00	0.50	1.00	0.38
UGEP3	0.50	2.00	0.50	1.00	0.38
UGEP76	1.00	1.00	0.00	0.00	0.00
UGEP11	1.00	1.00	0.00	0.00	0.00
Mean	-	1.57	0.21	0.32	0.16

These results showed that there might be high similarity existed between maize and finger millet genome.

Out of the 29 amplified markers, 21 (72%) markers were polymorphic between the genotypes. The genomic DNA of 23 finger millet accessions were amplified using 29 SSR markers and yielded 53 scorable alleles, of which 45 alleles were polymorphic and 8 monomorphic. The number of alleles generated with polymorphic primers ranged from 2 to 3 among the finger millet accessions at an average of 1.8 alleles per locus. The SSR locus DUPSSR23, BNLG1600, UMC1804 and BNLG1346 had maximum number of allele. This high amount of polymorphism is useful in diversity studies of finger millet germplasm. The gel pattern showed polymorphism among the genotypes with maize SSR UMC2071 (Fig. 1a). These polymorphic markers can be effectively used in genotyping mapping populations for the construction of linkage maps, diversity studies, for generation of comparative maps between maize and finger millets and identification of QTLs. Such a high amount of polymorphism has not been reported by using finger millet genomic SSRs in finger millet germplasms (Babu *et al.,* 2014a; Bharathi, 2011). In our earlier studies on finger millet, only 21 SSR loci out of 46 were found polymorphic and rest 25 (54%) were monomorphic SSR loci (Babu *et al.,* 2014a). Similarly, Bharathi (2011) has reported that out of 30 genomic SSRs studied 20 were polymorphic.

The PIC values of all the polymorphic loci across the 23 finger millet genotypes varied from 0.04 to 0.48 (average: 0.17). The PIC values observed in present study were lower in comparison to our earlier reports (Babu *et al.,* 2014b) and those by Bharathi (2011) wherein the PIC range of 0.196 to 0.834 has been reported. The PIC values obtained indicated that SSR markers were highly potential in identifying the genetic relationships. Out of the 21 primers UMC1804 and BNLG 1346 primers showed highest PIC values of 0.48 and 0.46, respectively; while the lowest values were observed in primer UMC1407 (0.041). The SSR identified in present study were polymorphic by using the maize SSRs than the earlier studies (Nirgude *et al.,* 2014). The SSR loci which had more PIC value also generated more number of alleles. For example, the SSR marker UMC1804 had high PIC values and also generated more number of alleles (3). In earlier reports, we observed that rice genomic SSRs were more polymorphic than EST based SSRs and hence the polymorphic SSRs obtained in present study can be used for genomic applications of finger millet. Major alleles with highest frequency were observed for locus UMC1407 (98%). Based on SSR analysis by considering PIC values ≥0.26, gene diversity ≥0.43 and inbreeding coefficient ≥0.60, the SSR loci UGEP33, UMC1858, UMC1805 and UMC2163 were identified to be highly polymorphic.

Gene diversity (*He*) was in the range of 0.04 (UMC2364) – 0.56 (UMC1804) with average value of 0.22. In earlier reports, we found *He* in the range of 0.208 to 0.726 with an average value of 0.487 (Babu *et al.,* 2014b). However, Nirgude *et al.* (2014) based on EST-SSRs found very less gene diversity (0.024-0.327). The expected heterozygosity was highest with SSR marker UMC1804

Table 3: Comparison of polymorphism parameters of finger millet and Maize microsatellites in finger millet genotypes

Genetic polymorphism parameters	Maize	Finger millet
Total markers used for amplification	50	14
Total amplified markers	29 (58%)	14 (100%)
Total polymorphic markers	21 (73%)	7 (50%)
Percentage of polymorphism	73%	50%
Minimum allele number	1.00	1.00
Maximum allele number	3.00	3.00
Mean allele number	1.83	1.6
Minimum gene diversity	0.00	0.21
Maximum gene diversity	0.77	0.50
Mean gene diversity	0.21	0.21
Minimum heterozygosity	0.00	0.12
Maximum heterozygosity	1.00	1.00
Mean heterozygosity	0.30	0.32
Minimum PIC	0.25	0.19
Maximum PIC	0.73	0.38
Mean PIC	0.16	0.17

(0.56) across the 23 finger millet genotypes, followed by BNLG1346 (0.55). The heterozygosity (*Ho*) was in the range of 0.041 to 1.00 (average 0.30) which showed wide heterozygosity in finger millet genotypes. The observed heterozygosity was highest in SSR markers MMC0282, UMC1012, MMC0371, UMC1506 and BNLG1325 (1.00) (Table 2). The heterozygosity observed in the selected finger millet genotypes was in congruence with the earlier reports in finger millet (Babu *et al.*, 2014c; Bharathi, 2011). The high amount of heterozygosity observed may also be due to wild finger millet genotypes used in present study and because of mutational rate exhibited in some of the SSR markers (Udupa and Baum, 2001). As a result, many markers which displayed heterozygous nature have a large number of SSR units (Dje *et al.*, 2000). Rare and unique alleles were also present in the genotypes. In addition to the total number of alleles and unique alleles, the number of rare alleles obtained indicated diversity at a given SSR locus. Major alleles with highest frequency were observed for the locus UMC2364, and UMC1407.

Genetic variation of finger millet SSR markers on finger millet genotypes

Out of the 14 amplified markers, 50% markers were found polymorphic across the genotypes. These 14 SSRs were used for polymorphic studies among the 23 finger millet genotypes and yielded 22 scorable alleles with a mean of 1.6 alleles per locus. The number of alleles generated ranged from 2 to 3 among the finger millet genotypes studied. The banding pattern of finger millet genotypes using SSR marker UGEP110 is given in Fig. 1b. The SSR marker FM6 only contained

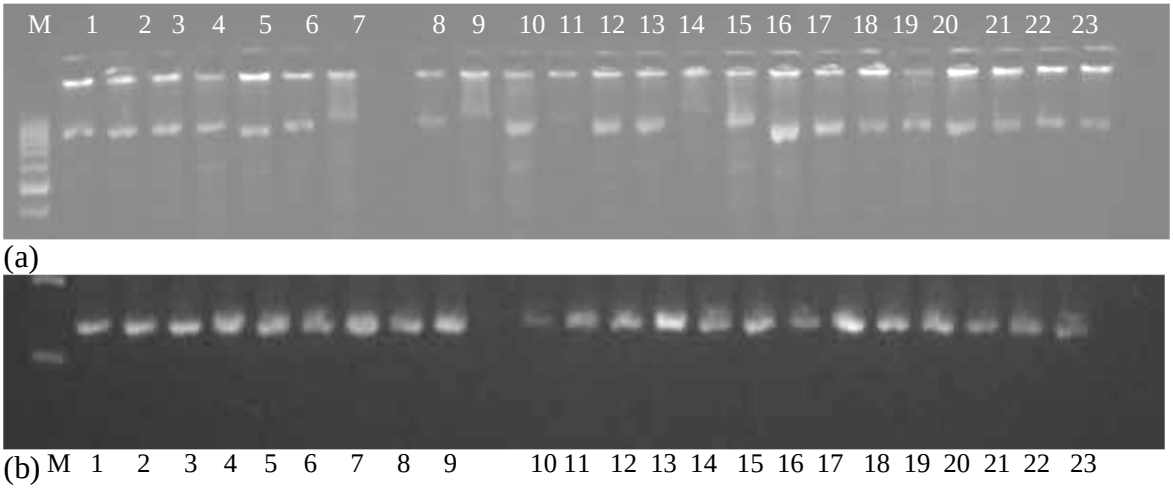

Fig. 1: The banding pattern of 23 finger millet genotypes using maize primer UMC2071 (1a), and finger millet primer UGEP110 (1b), M-100bp ladder; Lanes 1-23 represents finger millet genotype)

maximum number of 3 alleles (Table 1). Bharathi (2011) studied a large set of finger millet genotypes and found alleles from 7 to 25 with an average of 11.55 alleles per locus and attributed it to a very large collection of genotypes used in their study (nearly 900). Babu *et al.* (2014a) found the number of alleles generated with polymorphic loci ranged from 2-5 among the finger millet genotypes. The PIC values of all the polymorphic loci across 23 finger millet genotypes varied from 0.19 to 0.38 (average: 0.16). These values were lower than earlier reports of 0.196 to 0.834 (Babu *et al.*, 2014a; Bharathi 2011). Out of the 7 polymorphic primers, four (UGEP52, UGEP110, UGEP21 and UGEP3) had high PIC values (0.38) while lowest value was observed in primer UGEP26 (0.18). Major alleles with highest frequency were observed for locus UGEP52, UGEP110 and UGEP3. Based on above SSR analysis by considering the parameters of PIC values ≥0.3 and gene diversity ≥0.5, the SSR loci UGEP52, UGEP110, UGEP21 and UGEP3were highly polymorphic.

Gene diversity (*He*) was in found range of 0.21 (UGEP26) – 0.50, with average value of 0.21. Babu *et al.* (2014b) found *He* in the range of 0.208 to 0.726 with average value of 0.487. This value is higher than earlier report based on RAPD markers in finger millet (0.330) (Babu *et al.*, 2007) and based on EST-SSRs Nirgude *et al.* (2014) found very less gene diversity (0.024-0.327). The expected heterozygosity was highest with SSR markers UGEP52, UGEP110, UGEP21 and UGEP3 (0.50) across the 23 finger millet genotypes. However, lowest gene diversity was found in SSR marker UGEP26 (0.21). The heterozygosity (*Ho*) was in the range of 0.12 to 1.00 (mean value: 0.32, this showed that a wide range of heterozygosity was present in finger millet genotypes. The observed heterozygosity was highest in SSR markers UGEP52, UGEP110, UGEP21 and UGEP3 (1.00) (Table 3). The heterozygosity observed among the selected finger millet genotypes was in congruence with earlier reports in finger millet (Babu *et al.*, 2014a; Bharathi, 2011). However, lowest heterozygosity (0.12) was found in FM6 marker.

Comparison of polymorphism of maize and finger millet SSRs

The comparison of maize and finger millet SSR polymorphism on finger millet genotypes revealed that maize microsatellites were highly transferable, more polymorphic and also able to differentiate and identify more diversity in finger millet genotypes than finger millet microsatellite markers (Table 3). High transferability existed between finger millet and maize may be due to their physiological relationships since both were C4 crop plants. The study also revealed that maize genome sequence can be used as reference map for the identification of homologs and orthologs for traits identified as syntenic between maize and finger millet crops. The polymorphism information content generated from maize SSRs was more than finger millet SSR markers. The results showed that maize crop is more correlated to finger millet at molecular level.

Diversity analysis based on maize and finger millet SSRs

The genetic diversity analysis among 23 finger millet genotypes consisting of both land races and cultivated genotypes, were done using 64 genomic SSRs of finger millet and maize. The dendrogram with 64 SSRs was generated through UPGMA analysis of Power Marker V3.25 software. These 64 SSR markers grouped the 23 finger millet genotypes into 2 major clusters (A, and B) based on the UPGMA analysis of Power Marker V3.25 software (Fig. 2). The clustering of finger millet genotypes was largely based on their geographical origin. The cluster A comprised of genotypes T99, T100, T367, T595, T774 and T422. Cluster B comprised of genotypes T65, T631, T671, T581, T9, T760, T593, T401, T685, T296, T524, T187, T74, T507, T622, and T552. The maximum similarity was found between the genotype pairs T552, T622 and T507, T74. The results on similarity were also substantiated by the dendrogram generated using software power marker. The dendrogram was constructed by using both NTSYSpc2.11 and power marker software (rotted and rectangular), and both were similar. This clustering may be due that same races or parents may be involved in breeding of these genotypes. Similarly, Dida *et al.* (2008) analyzed a set of 79 finger millet accessions using 45 SSR markers and were able to differentiate into two phylogenetic groups according to their geographic origin based on the power marker analysis.

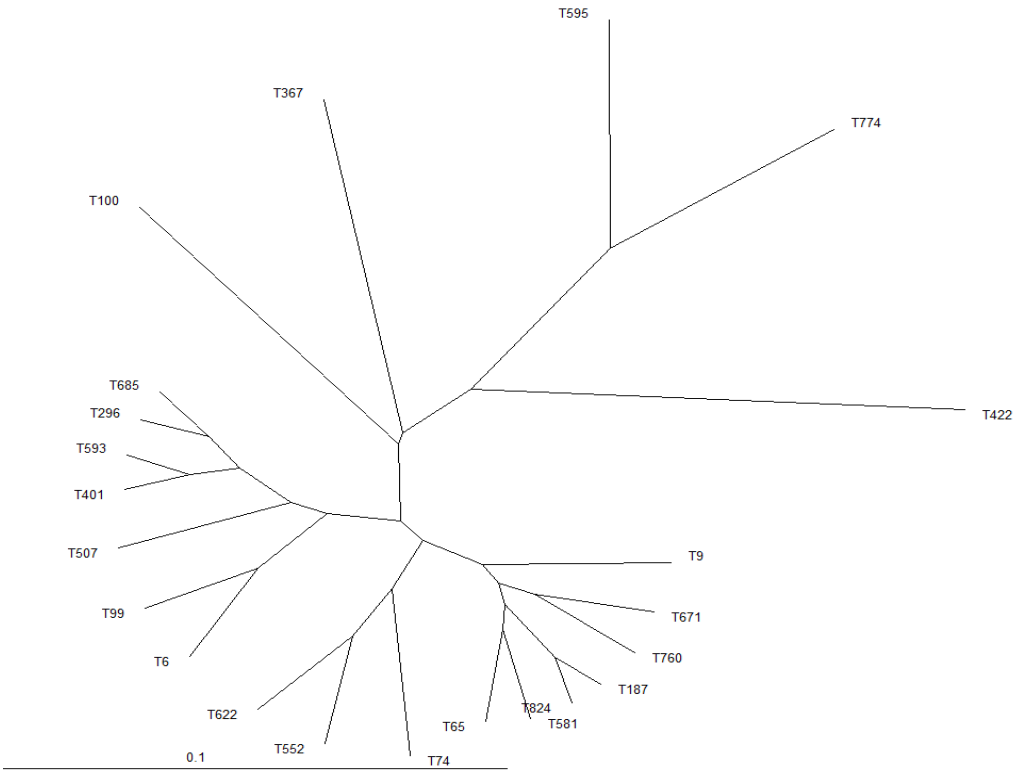

Fig. 2: The rooted dendrogram generated from the UPGMA analysis among the finger millet genotypes using combination of maize and finger millet SSRs

Conclusion: Genomic information on finger millet is scanty, so the present study enriched the finger millet genomics by identifying the suitable polymorphic markers of maize and finger millet, which can be used for diversity analysis, cultivar identification and QTL mapping. Maize microsatellites were highly transferable, more polymorphic and able to differentiate and identify more diversity in barnyard millet genotypes than finger millet microsatellite markers.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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