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Analysis of Genetic Relationships of Pearl Millet (*Pennisetum glaucum* L.) Landraces from Zimbabwe, Using Microsatellites

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Abstract: Pearl millet landraces collected from two districts of Zimbabwe, Nyanga North and Tsholotsho were analysed to assess genetic relationships based on ten microsatellite primers and indigenous farmer given names. Analysis was done by polyacrylamide gel electrophoresis stained with ethidium bromide. Simple matching coefficients were compared and the genetic relationships between genotypes were clarified on dendrograms by unweighted pair-group averages (UPGMA). Two polymorphic primers (PSMP2008 and PSMP2013) were able to detect some level of polymorphism at DNA level clustering the landraces into four major clusters joined at 64% similarity level. Sixteen accessions from Nyanga were identical to those from Tsholotsho. Fourteen accessions from Nyanga and 18 from Tsholotsho were identical to at least one genotype from the same district. Despite the same local names, genotypes were scattered throughout the clusters suggesting either poor discrimination by the primers or marked genetic differences. The Simpson Index of diversity were almost the same at 0.690 and 0.700 for Nyanga and Tsholotsho respectively. In conclusion, our results show the potential of microsatellites in studying diversity in pearl millet and show marked duplication of the germplasm both in genetic relationships and local names. However, the study provides a strong background for further analysis of the germplasm.

Key words: Pearl millet, landraces, microsatellites, genetic diversity

INTRODUCTION

Pearl millet (*Pennisetum glaucum* L.) is ranked seventh most important cereal crop in the world (FAOSTAT, 2004) and a staple food for people situated in the semi-arid areas where environmental stress such as drought, high temperature and poor soil nutrient availability are endemic. Besides the use as a grain, fodder, construction material, brooms and syrup, pearl millet has great potential for making bioethanol, an area that has not been adequately explored. Given enough research, pearl millet has the potential of playing a leading role in hunger alleviation in drier areas of Africa (O'Kennedy and Girgi, 2007) especially under the ever changing and unpredictable climate in Sub-Saharan Africa.

Zimbabwe's pearl millet yields are relatively low at about 500 kg ha^{-1} (FAOSTAT, 2004), a yield far lower than the global average of 780 kg ha^{-1}. This is partly because of farmers dependence on uncharacterized, low producing but well-adapted landraces. Despite their low yield, other factors such as prevalence of indigenous knowledge and perceptions about storability, nutritional value and resistance to both biotic and abiotic stress, make landraces very good candidates for communal agriculture. Farmers usually grow mixtures of landraces, maturing at different times, to meet

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intermediate food requirements (Rao and Mushonga, 1987). While communal agriculture is dependent on the rich agrobiodiversity of landraces, relatively little attention has been accorded to the conservation of this genetic diversity in communal areas. Moreover, the data is poorly documented especially for collections from Zimbabwe. Nevertheless, there is a growing realization of the importance of information of genetic diversity millets.

Several methods have been used to study pearl millet genetic diversity including isozymes (Tostain, 1992, 1994), AFLP markers (Vom Brocke *et al*., 2003), RFLP markers (Bhattacharjee *et al*., 2002), SSCP-SNP (Bertin *et al*., 2005) and recently microsatellites (Mariac *et al*., 2006). Microsatellites are the marker of choice for practical breeding applications particularly in developing countries. They are highly polymorphic, informative and co-dominant markers which are often broadly applicable since loci are frequently conserved between related species and sometimes genera. In the current study we used microsatellites to analyse genetic relationships among pearl millet landraces collected from Zimbabwe's communal areas.

MATERIALS AND METHODS

The study was carried out using 47 landraces of pearl millet that had been collected by the National Genebank of Zimbabwe from two districts of that nation, Tsholotsho and Nyanga North in 1998 (Mafa, 1999) (Table 1). The two districts are in almost opposite ends of Zimbabwe and isolated from each other by climates that are not very suitable for pearl millet and their climates are significantly distinct (Table 2), sample collection was as described earlier (Chakauya *et al*., 2006). DNA was extracted by a modified cetyltrimethylammonium bromide (CTAB) method as described by Saghai-Maroof *et al*. (1984). Fresh leaf material was taken from 3 to 4 week old plants grown in a greenhouse. Five to eight plants of each accession were sampled and the material bulked.

For microsatellite analysis, ten primers (Qi *et al*., 2004, Table 3) provided by John Innes Centre were used. PCR reactions were performed in 25 µL-volumes containing 30 ng template DNA, 1x PCR Buffer (50 mM KCl, 10 mm Tris-HCl, (pH 8.3), 1.5 mm $MgCl_2$), 0.25 mm deoxynucleotide triphosphates, 4.13 to 5.68 pmoles (30 ng) of each primer (Table 3) and 1.2 units Taq polymerase. Temperature cycling was performed on a GeneAmp PCR system 9700 (Perkin-Elmer) programmed to run 4 min at 94°C, followed by 35 cycles of 94°C for 1 min, 1 min at annealing temperature (Table 3) and 72°C for 1 min. The final cycle was identical to the above but had a final elongation of 10 min at 72°C. PCR products were separated by electrophoresis using 1xTBE on 8% PAGE, stained with ethidium bromide and band sizes estimated with a 100 base pair ladder (Roche Diagnostics).

Gels were scored manually with each polymorphic band being treated as a unit character and scoring was for the presence (1) or absence (0) of a band. Two criteria were used for scoring bands: the band being scored had to stain strongly and there had to be an unambiguous difference between the allelic states of the band being scored (i.e.presence or absence of a band). The statistical analyses were carried out in the R statistics environment version 1.7.1 (Venables *et al*., 2003). The degree of similarity between collections was calculated using the implementation of the simple matching coefficient in the R function daisy of the package cluster version 1.7.4 (Struyf *et al*., 1996). Hierarchical clustering was done with the R function hclust in the package mva, version 1.7.1, using the unweighted pair-group method of arithmetic averages (UPGMA). The Kelley-Gardner-Sutcliffe penalty function for a hierarchical cluster tree was calculated using the function kg in the package maptree (Kelley *et al*., 1996) to suggest a number of clusters in the dataset.

Table 1: Collection identities, farmer-given local names and origin of pearl millet landraces analysed for microsatellite polymorphisms

Accession No.	Farmer-given name	Village	District
1344	Nyagushe	Samakande	Nyanga North
1447	Nyagushe	Samakande	Nyanga North
1354	Mudhambure	Chibvende	Nyanga North
1374	Unspecified	Karikoga	Nyanga North
1375	Nyagushe	Karikoga	Nyanga North
1376	Mudhambure	Karikoga	Nyanga North
1382	Mudhambure	Mangezi	Nyanga North
1386	Nyagushe	Mangezi	Nyanga North
1396	Mudhambure	Mangezi	Nyanga North
1408	Mudhambure	Mangezi	Nyanga North
1418	Mudhambure	Mangezi	Nyanga North
1422	Nyagushe	Mangezi	Nyanga North
1423	Mudhambure	Mangezi	Nyanga North
1435	Mudhambure	Kamunhukamwe	Nyanga North
1440	Mudhambure	Kamunhukamwe	Nyanga North
1443	Mudhambure	Kamunhukamwe	Nyanga North
1462	Mudhambure	Renzva	Nyanga North
1466	Mudhambure	Renzva	Nyanga North
1500	PMV-3	Siyabandela	Tsholotsho
1501	Halale	Siyabandela	Tsholotsho
1506	Halale	Siyabandela	Tsholotsho
1515	Tsholotsho-bearded	Siyabandela	Tsholotsho
1524	PMV-3	Siyabandela	Tsholotsho
1544	Isifumbata	Sizanani	Tsholotsho
1548	Halale	Sizanani	Tsholotsho
549	Tsholotsho-bearded	Sizanani	Tsholotsho
1562	Halale	Sizanani	Tsholotsho
1563	Nyauthi-Halale	Siyazama	Tsholotsho
1564	Isifumbata	Siyazama	Tsholotsho
1570	Isigumu	Siyazama	Tsholotsho
1571	Halale	Siyazama	Tsholotsho
1579	Halale	Siyazama	Tsholotsho
1581	Isigumu	Siyazama	Tsholotsho
1584	Isigumu	Siyazama	Tsholotsho
1587	Isigumu	Siyazama	Tsholotsho
1596	Halale	Siyazama	Tsholotsho
1606	Halale	Siyazama	Tsholotsho
1624	Halale	Phakamani	Tsholotsho
1626	Isifumbata	Phakamani	Tsholotsho
1630	Halale	Phakamani	Nyanga North
1633	Halale	Phakamani	Tsholotsho
1636	Halale	Phakamani	Tsholotsho
1642	Isifumbata	Phakamani	Tsholotsho
1643	Tsholotsho-bearded	Phakamani	Tsholotsho
1644	Tsholotsho-bearded	Phakamani	Tsholotsho
1647	Halale	Phakamani	Tsholotsho
1650	Isifumbata	Phakamani	Tsholotsho

RESULTS AND DISCUSSION

Out of the ten primers screened for the ability to amplify and detect polymorphism, four did not produce amplification products (Table 3), four were monomorphic (PSMP2019, PSMP2059, PSMP2056 and PSMP2069), while two primers (PSMP2008, PSMP2013) were polymorphic with 2-4 bands ranging between 150 and 220 base pairs (Fig. 1). The two polymorphic primers were used for cluster analysis. The hierarchical clustering of the accessions grouped into four hierarchical clusters (Fig. 2) joined at 64% similarity level. The Kelly-Gardener-Sutcliffe penalty function minimized at four clusters which form the basis of all the analysis below. With the exception of the last cluster with predominantly material from Tsholotsho, the accessions from the two districts showed almost similar

Table 2: Comparison of monthly rainfall and mean temperature in Nyanga North and Tsholotsho showing the substantial differences that exist between the two sites

	Rainfall (mm)		Temperature (°C)	
Months	Tsholostho	Nyanga North	Tsholotsho	Nyanga North
Jan	370	146	24.0	22.0
Feb	320	131	23.0	21.0
Mar	221	189	23.0	21.0
Apr	100	33	22.0	21.0
May	42	3	19.0	19.0
Jun	32	0	16.0	18.0
Jul	11	0	18.0	18.0
Aug	15	0	19.0	18.5
Sept	10	0	23.5	21.6
Oct	55	4	26.0	23.9
Nov	128	54	25.0	25.0
Dec	346	149	25.8	24.5

Table 3: Characteristics of pearl millet microsatellite primers used in the study

Marker	Primer sequence	PCR product	Linkage group	Annealing temperature (Tm)	No. of amplicons
PSMP2001	CATGAAGCCAATTAGGTCTCACCAT CTGACTTGTTCTTATCC	304	5	61	0
PSMP2006	GACTTATAGTCACTGGGAAAGCTC GCTTTAATAACTTTGTGCGTATT	256	3	52	0
PSMP2008	GATCATGTTGTCATGAATCACCACA CTACACCTACATACGCTCC	238	4	61	2
PSMP2013	GTAACCCACTAACCCTTACCGTCGC ACAGAAAAAGAATAG	153	7	61	4
PSMP2018	CGCAAGACATTTTAGTATCACCACA GTCATCCTCAGTCGTCC	203	6	61	0
PSMP2019	TGTGCCACAGCTTGTTCCTCCAAGC AGCCAGTTCCTCATC	260	7	61	1
PSMP2056	ACCTGTAGCTTCAAAATTCAAAAAAA TTCAGTGTGATTTCGATGTTGC	213	3	61	1
PSMP2059	GGGGAGATGAGAAAACACAATCAC TCGAGAGAGGAACCTGATCCTAA	119	2	61	1
PSMP2066	ATATTAGAGCATTGCATCGCGCATA GCAGCATACAGCAGCAACTAA	267	2	61	0
PSMP2069	CCCATCTGAAATCTGGCTGAGAAC CGTGTTCGTACAAGGTTTTGC	225	1	61	1

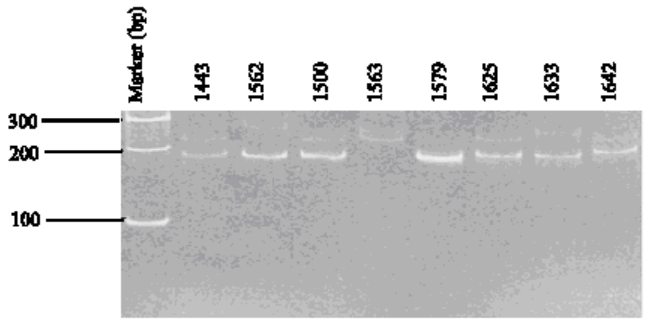

Fig. 1: Polymorphism analysis of marker PSMP2013 against a panel of eight pearl miller lines

evenness in distribution among the clusters. This is shown by the similar Simpson index of diversity of 0.690 and 0.700 for Nyanga and Tsholotsho. This index is interpreted as the probability that a second accession sampled will belong to a different cluster to the first.

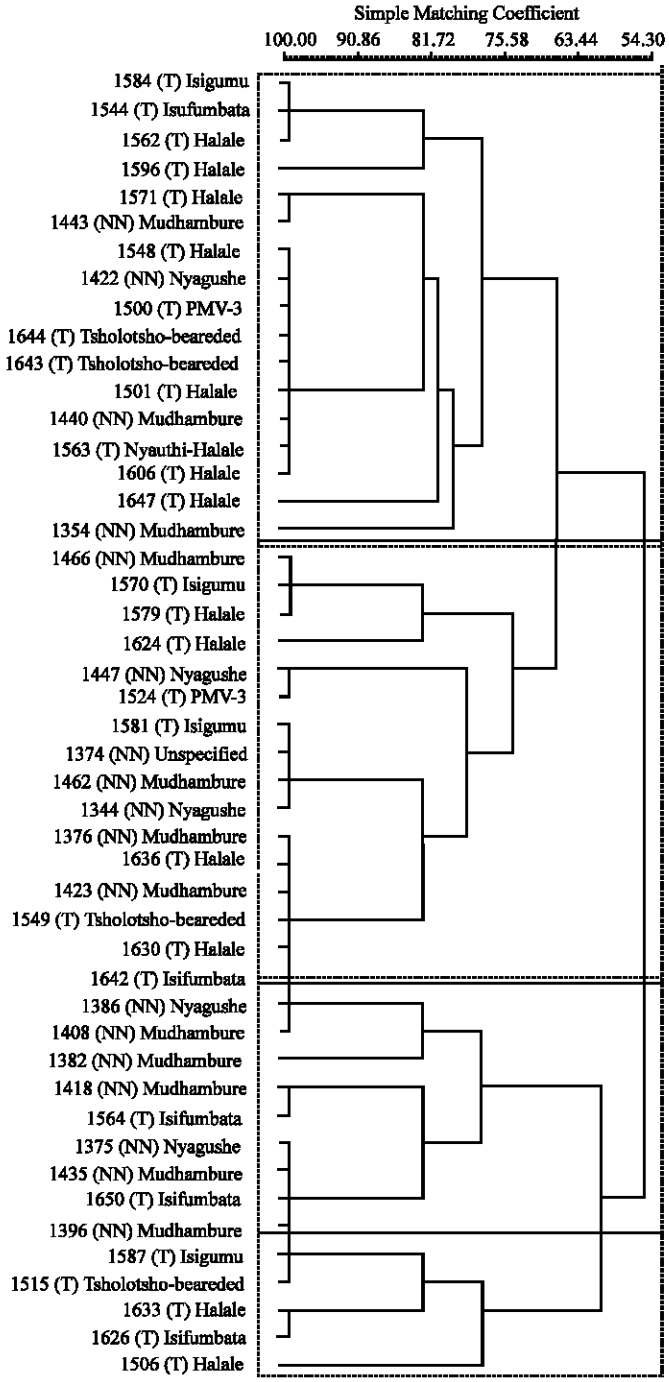

Fig. 2: Hierarchical clustering of Pearl millet collections from Zimbabwe based on a simple matching co-efficient and unweighted pair-group method of arithmetic averages (UPGMA). The labels are from the left to right, accession number and farmer-given names

A total of nine local farmer given names were recorded for the 47 accessions used, including PMV-3 a product of the national pearl millet breeding program in Zimbabwe. *Halale* was the most common name used for more than half the accessions from Tsholotsho, followed by *Mudhambure* with 12 accessions. *Nyagushe* and *Isifumbata* had four accessions, *Isigumu* and Tsholotsho-bearded with four, PMV-3 for two accessions while *Nyauthi-Halale* and an unspecified genotype with one accession each. Interestingly, the local names were used for more than one collection often fell in different clusters based on microsatellite classification. Eighteen accessions from Tsholotsho were identical to at least one accession from the same location compared to 14 for accessions from Nyanga. Moreover, 16 accessions from Nyanga were identical to the material from Tsholotsho. This observation suggests either poor discrimination by the primers used for the study or an indication of marked genetic differences between the genotypes.

CONCLUSION

Zimbabwe has a rich source of pearl millet germplasm which might play a major role in breeding programs to address current challenges of food security, biotic and abiotic stress and biofuel demands. This study showed that the two polymorphic primers (PSMP2008 and PSMP2013) could detect some level of polymorphism among the germplasm analyzed. The utility of microsatellites in pearl millet diversity studies has already been shown elsewhere (Mariac *et al*., 2006; Qi *et al*., 2004). Our results from microsatellite analysis contrast with preliminary agromorphological characterization data results of the same germplasm that showed variability plant height, head shape, maturity levels and size of awns for most lines (Mafa, 1999). This variability does not however seem evident in the molecular data. Although, further analysis of the germplasm is needed in order to make firm conclusions, some interesting inferences can be made from the data. The limited number of polymorphic primers reduced the discrimination power of the microsatellites resulting in the tendency to classify accessions with different names as identical and vice versa. Farmer-given local names are generally not unique indicating complexity in farmer classification as described earlier (Chakauya *et al*., 2006) and difficulty in using it for sampling. For further investigations more polymorphic primers would need to be used to make a more informed inferences on the germplasm.

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