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Assessment of genetic diversity among 60 sorghum accessions in Ghana using microsatellites

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ABSTRACT

The study was carried out to assess genetic diversity among sixty sorghum accessions from the national germplasm collection using microsatellite markers. Genetic diversity and relationship among the sixty accessions were evaluated using 24 microsatellites. The 24 markers generated 64 alleles; the mean number of alleles was 2.773, indicating a medium range of diversity among the sixty sorghum accessions compared to other genetic diversity studies in sorghum using microsatellites, the average polymorphic information content (PIC) (0.575) gave an indication that the microsatellites are informative. Microsatellites cluster analysis resolved the sixty sorghum accessions into three major clusters. The percent similarity between the sorghum accessions ranged from 56% to 89%. Most of accessions clustered according to geographical site of the collection. Heterozygosity in the sixty sorghum accessions was very low .Observed heterozygosity ranged from 0 to 0.0333 as against the expected heterozygosity of 0.4263 to 0.7708.

Introduction

Sorghum (*Sorghum Bicolor* (L.) Moench) is an important cereal crop in many tropical and subtropical areas of the world, especially in the drier Savannah zones, where it thrives best and is used in a variety of food preparations. Its drought tolerance and water use efficiency makes it the most preferred cereal in areas where the rainfall is erratic and insufficient (Kudajie, 2005).Morphological characterization is an important measure of assessing genetic diversity in crops. However, information from morphological variation does not reliably reflect the real genetic variation because of genotype-environment interactions. On the other hand molecular markers are not influenced by environmental factors; they reflect genetic similarities and differences without environmental interaction (Bohn *et al.*, 1999).

Many researchers used different kinds of molecular marker techniques such as Restriction Fragment Length Polymorphism (RFLPs) (Ahnert *et al.*, 1996), Random Amplified Polymorphic DNA (RAPDs) (Agrama and Tuinstra, 2003), Amplified Fragment Length Polymorphism (AFLPs) (Menz *et al.*, 2004) and Microsatellites (SSRs) (Ali *et al.*, 2008) in assessing genetic diversity in sorghum. Microsatellites markers are becoming more popular for diversity studies in sorghum because of their repeatability, simplicity, and amenable to automation by Polymerase Chain Reaction. Also, their multiallelic nature makes these markers more informative than most other marker types. (Mondini *et al.*, 2009)

The national collection for sorghum in Ghana holds many accessions with no information on their diversity. Limited genetic information is therefore available to establish the identity of these accessions in the national collection and develop a national core collection for sorghum.

The objective of the study was to estimate the genetic diversity among sixty sorghum accessions assembled from the national collection using microsatellites markers.

Materials and methods

Genetic materials

Sixty sorghum accessions were accessed from the sorghum germplasm collection currently held at the Savanna Agriculture Research Institute (SARI) for the study (Table 1.0). The Sorghum accessions used comprised 23 from Upper East Region (UER); 7 from Northern Region (NR); 21 from Nyakpala and 11 Sorghum introductions (SI) from Mali (SARI-MALI) and Burkina Faso (SARI-BURKINA FASO) adapted to Ghana.

DNA Extraction

Leaves from two-week old seedlings were harvested and bulked (3-5) per accession for Genomic DNA extraction using the CTAB method described by Murray and Thompson (1980) with some modification. Extraction buffer was composed of 2% CTAB, 50Mm Tris-HCI pH 8.0, 10Mm EDTA, 0.7M NaCl, 0.1% SDS, 0.1mg/ml Proteinase K, 2% insoluble PVP and 2% 2-mercaptoethanol. Chloroform extraction was to remove cellular debris and proteins by using chloroform-isoamyl alcohol (24:1 v/v), DNA was precipitated by addition of 2-isopropanol and the precipitate was washed twice in 70% ethanol. The final precipitate was dissolved in 50 ul of 1/10 TAE solution containing RNase A, incubated at 20^0C overnight, and stored at 4^0C. The quality of the DNA was verified on a 1% agarose gel and diluted to a working concentration of 5 ng/ul.

Selection of the Primers

A total of forty six microsatellites primer pairs were screened for their ability to detect polymorphisms on some of the sorghum accessions. Twenty four microsatellite primer pairs which showed clear banding patterns were selected for DNA fingerprinting of the sixty accessions. The primer pairs chosen have been used in other sorghum genetic diversity studies (Ali *et al.*, 2008, and Pei *et al.*, 2010) and were selected from published sorghum linkage maps (Brown *et al.*, 1996; Taramino *et al.*, 1997; Kong *et al.*, 2000; Schloss *et al.*, 2002; Bhattramakki *et*

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al., 2000). The list of the twenty four microsatellites markers and their primer sequences are presented in Table 2.

Polymerase Chain Reaction and Gel electrophoresis

PCR amplification conditions were: 2 minutes initial denaturation at 95 ºC, 30 cycles, each for a minutes at 95 ºC denaturation, 1 minutes at 60 ºC (Melting temperature), 1 minutes elongation at 72 ºC followed by a final elongation at 72 ºC for 1 min for a cycle. Annealing temperature was determined for each primer. Microsatellite amplification products were resolved by electrophoresis on 2% agarose gels run in tris-acetate buffer, pH 8 for 2 hours at 80 volt. The gel was stained with Ethidium bromide (10mg/ml) and scanned with the gel documentation system.

Band scoring and Microsatellites Data analysis

Bands generated were sized and then binary coded as 1 for the presence and 0 for the absence of an allele in each genotype, and scored in a binary data matrix. Where a PCR product was not obtained, data for specific locus and genotype were treated as missing data. Molecular data generated was analysed using NTSYS pc ver.2.20q (Rohlf, 2000). To visualize the relationship between the accessions a dendogram was constructed by clustering of the accessions based on the Dice similarity matrix using unweighted pair group method with UPGMA arithmetic algorithm in Sequential Agglomerative Hierarchical Nested method (SAHN) Module. Polymorphic information content (PIC) was calculated as described by (Botstein *et al.,* 1980) and modified by Anderson *et al.,* (1993) for self-pollinated species as follows. PIC =1-ΣP2ij, where relative frequency of the *j*th allele for the *i*th locus summed across all the alleles for the locus over all accessions. PIC values ranged from 0 (monomorphic) to 1 (very highly discriminative), with many alleles in equal frequencies. Mean number of alleles and effective number of alleles were computed using the Popgene software.

Results

Allelic diversity at Microsatellites loci

The 24 microsatellite markers used in the study generated 64 alleles. Some of the microsatellites amplified more than one band per accession indicating residual heterogeneity within the accessions. Null alleles were observed in some of the accession. Twenty two out of the 24 markers were polymorphic. Two microsatellite loci, Xtxp 321 and Xcup53 amplified rare alleles. The number of alleles revealed by each locus ranged from 1 to 5 with an average of 2.773 (Table 3.0). The total number of alleles generated from these markers varied from the source data which might be the result of differences in the accessions. A sample of DNA profile generated by one of the primers, Xtxp265 is shown in fig 1.0. The mean number of alleles (na) value of 2.773 was not close to mean effective number of alleles (ne) value of 2.340, which indicates the presence of rare alleles. The polymorphism information content (PIC) value for the microsatellite loci ranged from 0.081 to 0.776 with an average of 0.575 (Table 3.0).

Based on their individual PIC values, five of the primer pairs are moderately informative (0.25<PIC<0.5), while 17 primer pairs are highly informative (PIC>0.5). Even though the mean number of alleles per locus (2.772) detected in the 60 sorghum accessions was low, the average PIC value of 0.575 gives an indication that the microsatellite markers are informative. Table 5.0 shows the summary heterozygosity statistics across all the loci

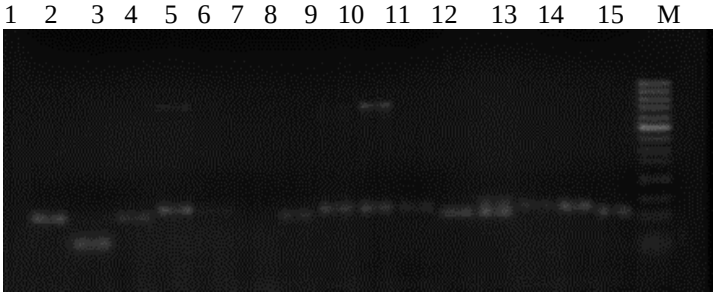


Fig 1.0 Sample of DNA profile generated by Xtxp265 M-100bp ladder; lanes 1-15 corresponding to the code of the 60 sorghum accessions in Table 1.

Microsatellites cluster analysis

Using the 64 shared alleles, cluster analysis resolved the 60 sorghum accessions into three major clusters (A, B and C) (Fig.1.0). The percent similarity between the sorghum accessions ranged from 56% to 89%. Accession pairs 28 and 30, and 48 and 49 in cluster A and 22 and 23 in cluster B were 89% similar (Fig. 3).

There was no accession at 100% similarity level indicating that there was no duplication among the accessions. Cluster A is the most homogenous; it consists of 22 sorghum accessions all from the same geographic origin, the Upper East Region (UER). Cluster B consists of 16 sorghum accessions comprising of accessions from two geographic origins, the Northern region (NR) and the sorghum introductions(SI) from Burkina Faso and Mali (SARI/MALI and SARI/BURKINA). Cluster C consists of 22 sorghum accessions, 20 from Nyakpala and the 2 from the Northern Region (NR). Clusters A and B share 60% similarity, with cluster C sharing 56% similarity with clusters A and B.

Discussion

Genetic Diversity

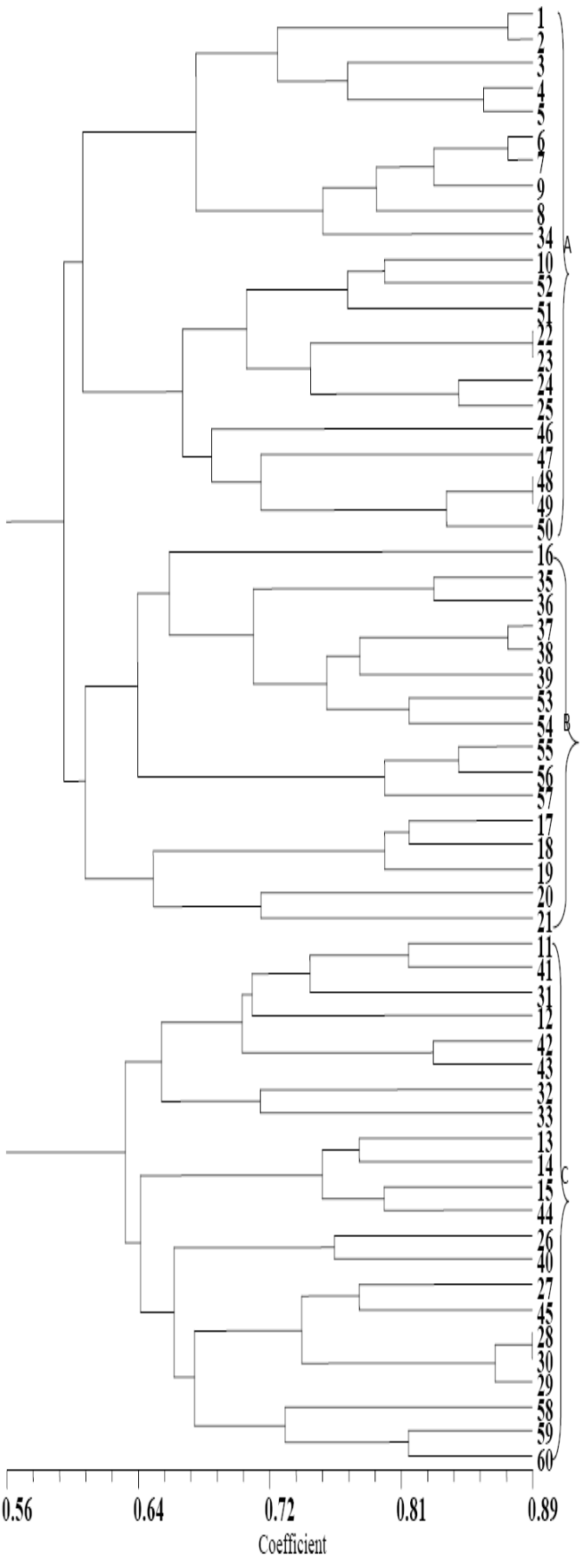
The study gives a medium range of genetic diversity among the 60 sorghum accessions assembled for the study compared with other genetic diversity studies in sorghum using similar microsatellites. The mean number of alleles per locus (2.772) was similar as recorded by Ali *et al.,* (2008) on 72 sorghum accessions (3.22) and Schloss *et al.,* (2002) on 25 sorghum accessions (3.4).

Kudajie (2005) and Egbadzor (2007) also observed (3.7 and 3.3) mean number of alleles per locus respectively. The mean number of alleles was however lower than reported by Agrama and Tuinstra (2003), Menz *et al.,* (2004) Pei *et al.,* (2010) (4.5, 5.9 and 4.96 respectively). The variations observed in the diversity indices of this study compared to those mentioned above; might be due to differences in sample size, origin and the background of the accessions.

The accessions for this study were assembled from Northern Ghana which is not geographically wide. Another factor that may influence the reported diversity in this study is the use of agarose gel in separating the PCR products. Agrama and Tuinstra (2003) noted in their study that Polyacrylamide gels gave greater resolving power than agarose gels. The lower resolution power of agarose gels could result in the detection of lower number of alleles per locus.

This may be particularly important for microsatellites containing di-nucleotide repeats whose amplification products are in the range of 130 to 200 base pairs (bp), as PCR products differing by two base pairs cannot be resolved by agarose gel (Agrama and Tuinstra, 2003).

Fig.2 Dendogram generated by UPGMA cluster analysis showing relationship among 60 sorghum Accessions based on Nei and Li similarity



The variability in the number of alleles per locus (2-5) may result from different locus specific mutation rates (Estoup *et al.*, 2002) and reflect differences in allelic diversity between microsatellites loci which affect estimating the genetic diversity. According to Nei (1973), this behaviour depends on both the number of alleles per locus and the respective allele frequency (McCouch *et al.*, 1997).

Besides locus specific mutation, the number of alleles and gene diversity can be affected by size homoplasy which occurs when different copies of a locus are identical in state, although they are not identical by descent (Estoup *et al.*, 2002). However, microsatellites are typically multiallelic markers (Matsuoka *et al*., 2002) with heterozygosity value much higher than other markers. Different authors have shown that microsatellites with three or more alleles per locus are more common than those with less than three alleles per locus in sorghum (Taramino *et al*., 1997; Kong *et al*., 2000) and in maize (Matsuoka *et al.*, 2002).

Even though the mean number of alleles per locus (2.773) detected in the 60 sorghum accessions was low, the average PIC value of 0.575 gives an indication that the microsatellites markers are informative. The PIC value represents the variation in locus specific genetic diversity for the accessions used in this study. The PIC values provide an estimation of the discriminating power of a marker by taking into account not only the number of alleles at a locus, but also the relative frequency of those alleles in the population under study (Smith *et al.*, 2000). The PIC value of 0.575 in this study was however, greater than (0.40) observed by Ali *et al.*, (2008) and lower than that of Agrama and Tuinstra (2003) who recorded 0.622.

Two microsatellite loci, amplified rare alleles. Rare alleles are defined as a frequency of <0.05 (Somers *et al.*, 2007; Casa *et al.*, 2005). These rare alleles could be of important interest as they are linked uniquely to some particular genotypes. Such alleles are important because they may be diagnostic for particular genotypes or for particular regions of the genome specific to a particular type of sorghum accessions (Agrama and Tuinstra, 2003). . The level of observed heterozygosity among the sixty was also very low 0 to 0.0333 as against the expected heterozygosity of 0.4263 to 0.7708; this indicates a high level heterozygous deficiency in the accessions. The predominantly autogamous breeding system of sorghum might contributes to explaining the patterns of and heterozygosity levels observed.

Clustering of the Sorghum accessions based on UPGMA

Sorghum accessions from the same geographical area tend to cluster together. The cluster analysis of the microsatellites data showed a narrow genetic background of the sorghum accession. Sorghum accessions from the UER all clustered together, this might be the result of the closeness of locations where these accessions were collected and the preference for similar sorghum genotypes in the region the accessions were collected from.

Exchange of seed between local farmers in the region and even between regions can also be the reason for the relatedness and clustering of the accessions together.

Sorghum introductions from Burkina Faso and Mali are expected to be separated from other accessions, but they clustered together with the accessions from the NR. Sorghum accessions collected at Nyakpala-SARI clustered together. This is expected since these accessions are collected in areas close to one another geographically.

Conclusion

Sorghum is one of the important staple cereals cultivated in Ghana; there is therefore the need to assess the dynamics of genetic diversity in this crop to draw up monitoring and conservation priorities. Assessing the genetic diversity with the

estimation of genetic relatedness in these accessions will also enhance the selection of these accessions for breeding programmes.

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Table 1.0 List of Sorghum accessions used in the genotyping

Code	Local Name	Location	Code	Local Name	Location
1	Belco- piilir	UER	31	2351	Nyakpala
2	Belcozia	UER	32	Stgb9218	Nyakpala
3	Nwaagured belco	UER	33	2663	Nyakpala
4	Belco manga	UER	34	Beko	UER
5	Bawku white	UER	35	Srgb11058	SARI/Burkina faso
6	Belco-wieg	UER	36	Kazinga	SARI/Burkina faso
7	Belco pielik 1	UER	37	2677	SARI/MALI
8	Beninga zebilla	UER	38	Stga11176	Nyakpala
9	Belco pielik	UER	39	Amanyiw	N/R
10	Mankaraga	UER	40	2668	Nyakpala
11	2443	NR	41	Stga113	Nyakpala
12	Weli	NR	42	Ssv 2006012	Nyakpala
13	Stga 11088	Nyakpala	43	Is6731	Nyakpala
14	2406	Nyakpala	44	2508	Nyakpala
15	2391	Nyakpala	45	Kadaga	Nyakpala
16	Yakpaji	NR	46	Naga	UER
17	Gong	NR	47	Kapelga	UER
18	Csm63e	SARI/Mali	48	Kuweigi	UER
19	Karazia	SARI/Mali	49	Kuweriga	UER
20	Toruk	NR	50	Dorado	UER
21	2443	Nyakpala	51	Nyanso	UER
22	Mankariga	UER	52	Isaxbergomanga	UER
23	Mankaraga	UER	53	38axksvii	SARI/Mali
24	Bunbawbo	UER	54	Kioedre	SARI/Burkina faso
25	Keriga gagbiri	UER	55	Extitak	SARI/Mali
26	IS30804 ca	Nyakpala	56	Toruk	SARI/Mali
27	Naa-yelinying	NR	57	Kiofdre	SARI/Burkina faso
28	Gpnx kazesheo	Nyakpala	58	NSV1	Nyakpala
29	Gpnx23767	Nyakpala	59	2361	Nyakpala
30	Gpnxcgm19/9-1-1	Nyakpala	60	2351	Nyakpala

Table 2: List of primers and their nucleotide sequences a used in the study

Code	Primers	Nucleotide Sequence of the Primers	Repeat Sequence
1	Xtxp 057	GGAACTTTTGACGGGTAGTGC CGATCGTGATGTCCCAATC	$(GT)_{21}$
2	Xgap 206	ATTCATCATCCTCATCCTCGTAGAA AAAAACCAACCCGACCCACTC	$(AC)_{13}/(AG)_{20}$
3	sbAGBO2	CTCTGATATGTCGTTGTGCT ATAGAGAGGATAGCTTATAGCTCA	$(AG)_{35}$
4	Xgab 84	CGCTCTCGGGATGAATGA TAACGGACCACTAACAAATGATT	$(AG)_{14}$
5	Xtxp015	CACAAACACTAGTGCCTTATC CATAGACACCTAGGCCATC	$(TC)_{16}$
6	Xtxp145	GTTCCTCCTGCCATTACT CTTCCGCACATCCAC	$(AG)_{22}$
7	Xcup 11	TACCGCCATGTCATCATCAG CGTATCGCAAGCTGTGTTTG	$(GCTA)_4$
8	Xtxp 021	GAGCTGCCATTTGGTCG ACCTCGTCCCACCTTTGTTG	$(AG)_{18}$
9	Xcup 53	GCAGGAGTATAGGCAGAGGC CGACATGACAAGCTCAAACG	$(TTTA)_5$
10	Xisep 0107	GCCGTAACAGAGAAGGATGG TTTCCGCTACCTCAAAAACC	$(TGG)_4$
11	Xtxp 321	GCCATGATAA TAACCCAAGCCTGAGCAT	$(GT)_4+(AT)_6+(CT)_2$
12	Xtxp 114	CGTCTTCTACCGCGTCCT CATAATCCCACTCAACAATCC	$(AGG)_8$
13	mSbCIR306	ATACTCTCGTACTCGGCTCA GCCACTCTTTACTTTTCTTCTG	$(AC)_{8.5}$
14	mbSCIR283	TCCCTTCTGAGCTTGTAAAT CAAGTCACTACCAAATGCAC	$(GT)_7$

Table 2: List of the primers and their nucleotide sequences used in the study (*Continued*)

			$(GAA)_{19}$
15	Xtxp265	GTCTACAGGCGTGCAAATAAAA TTACCATGGTGCACCCCTAAAAG	
16	gpsb089	ATCAGGTACAGCAGGTAGG ATGCATCATGGCTGGT	$(TG)_9$
17	Xcup 62	CGAGAAGATCGAGAGAACCC TGAAGACGACGACGACAGAC	$(GAA)_6$
18	Xtxp136	GCGAATAGCATCTTACAACA ACTGATCATTGGCAGGAC	$(GCA)_5$
19	gpsb148	CAACCACAAACCAAGAG ATAGAAATGGGGTGGAG	$(TC)_3+(CA)_5$
20	mSbCIR329	GCAGAACATCACTCAAAGAA TACCTAAGGCAGGGATTG	$(AC)_{8.5}$
21	mSbCIR240	GTTCTTGGCCCTACTGAAT TCACCTGTAACCCTGTCTTC	$(TG)_9$
22	Xtxp 012	GAGCTGCCATAGATTTGGTCG ACCTCGTCCCACCTTTGTT	$(AG)_{18}$
23	msbCIR248	GTTGGTCAGTGGTGGATAAA ACTCCCATGTGCTGAATCT	$(GT)_{7.5}$
24	Xtxp 278	GGGTTTCAACTCTAGCCTACCGAACTTCCT ATGCCTCATCATGGTTCGTTTTGCTT	$(TG)_9$

Table 4: Primers used in the analysis, number of alleles (na) and effective number of alleles (ne) for all the loci.

Code	Locus	na	Ne	PIC
1	Xtxp 057	2	1.8719	0.4994
2	Xgap 206	3	2.7922	0.6219
3	sbAGBO2	4	3.4310	0.7464
4	Xgab 84	5	4.2441	0.7761
5	Xtxp015	3	2.6473	0.5986
6	Xtxp145	3	2.8561	0.6636
7	Xcup 11	2	1.8952	0.4728
8	Xtxp 021	3	2.1637	0.5608
9	Xcup 53	3	1.7324	0.5139
10	Xisep 0107	3	2.9851	0.7119
11	Xtxp 321	3	2.3226	0.5831
12	Xtxp 114	2	1.8175	0.5445
13	mSbCIR306	2	1.9978	0.5797
14	mbSCIR283	3	2.2715	0.6508
15	Xtxp265	3	2.1225	0.6510
16	gpsb089	2	1.9956	0.4980
17	Xcup 62	2	1.9555	0.5900
18	Xtxp136	2	1.9782	0.5475
19	gpsb148	4	2.0466	0.0809
20	mSbCIR329	2	1.9606	0.6477
21	mSbCIR240	2	1.9953	0.5131
22	Xtxp 012	3	2.4064	0.5966
Means		2.773	2.340	0.575

Table 5.0 Summary of Heterozygosity statistics for all loci

Code	Locus	Obs.Het	Obs.Het
1	Xtxp 057	0.0084	0.4672
2	Xgap 206	0.0055	0.6470
3	sbAGBO2	0	0.7142
4	Xgab 84	0	0.7708
5	Xtxp015	0.0167	0.6267
6	Xtxp145	0.0056	0.6553
7	Xcup 11	0.0333	0.4762
8	Xtxp 021	0	0.5421
9	Xcup 53	0	0.4263
10	Xisep 0107	0	0.6706
11	Xtxp 321	0	0.5742
12	Xtxp 114	0	0.4535
13	mSbCIR306	0	0.5036
14	mbSCIR283	0	0.5641
15	Xtxp265	0.0055	0.5333
16	gpsb089	0	0.5031
17	Xcup 62	0.0167	0.4927
18	Xtxp136	0	0.4986
19	gpsb148	0.0209	0.4995
20	mSbCIR329	0.0084	0.4941
21	mSbCIR240	0.0084	0.5031
22	Xtxp 012	0	0.5894
Means		0.0058	0.5548