Variability in some sorghum accessions assessed with SSR markers

by

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Abstract

Variability in forty-nine accessions of sorghum [Sorghum bicolor (L.) Moech] assembled at Savannah Agricultural Research Institute of Ghana was assessed using ten microsatellite markers. DNA of plants from the various accessions was extracted and amplified using ten microsatellite primers. The amplified products were ran on 3% agarose gel at 45 volts for two hours, visualized under ultraviolet radiation and photographed. Alleles identified in the form of bands were scored '+' for presence and '0' for absence. Eight of the ten primers discriminated among the sorghum accessions with two to five alleles each. An average of 3.3 alleles was scored per primer pair. Allele information for each accession from the ten primers was recorded in a Microsoft excel sheet and analysed with NTSYSpc. A dendrogram was drawn using Sequential Agglomerative Hierarchical Nested method (SAHN). Similarity within the sorghum accessions ranged from 63 to 100%.

Keywords: Sorghum, SSR, molecular

Introduction

The primary resource of plant breeding programs is the genetic variability available within germplasm closely related to the crop of interest. However, the success of crop improvement programs is highly reliant on the power and efficiency with which this genetic variability can be manipulated (Crouch and Ortiz, 2004). Genetic markers are molecules that could be used to trace a desired gene(s) and they offer plant breeders the potential of making genetic progress more precisely and more rapidly than through phenotypic selection (Ovesna and Leisova, 2002).

Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers are highly effective for germplasm evaluation and certain molecular breeding approaches. Both can be used without prior

knowledge of the genome to rapidly and efficiently screen the genome (Crouch and Tenkouano, 1999). However, RAPD analysis suffers from problems of repeatability in many systems, especially when transferring between populations or laboratories as is frequently necessary with marker assisted selection programs. Conversely, AFLP analysis has significant practical limitations for routine screening of large breeding populations in marker assisted selection systems. Markers based on known genomic sequence information, e.g. SSR, EST, SNP are expensive and time consuming to develop as they require extensive cloning and sequencing efforts (Paterson et al., 1991; Sharma et al, 2002). However, markers based on genomic sequence information are extremely effective and appropriate tools for molecular breeding as they are based on simple protocols yet readily provide reliable high quality data (Crouch and Ortiz, 2004).

Microsatellites (SSR) consist of tandemly repeated units, each between one and 10 base-pairs in length, such as (TG)n or (AAT)n (Bruford and Wayne, 1993). They are widely dispersed through eukaryotic genomes and are often highly polymorphic. These markers are one of the molecular tools of choice for biodiversity studies because of their high information content (Morin and Woodruff, 1996).

SSR markers have been found to be very efficient in sorghum diversity studies by Agrama and Tuinstra (2003). They (Agrama and Tuinstra, 2003) observed that SSR markers revealed more diversity in sorghum compared with Restriction Fragment Length Polymorphism (RFLPs). Kudadjie (2004) also observed diversity between and within sorghum accessions characterized with SSR makers.

Sorghum, a crop of the family Poaceae (Purseglove, 1998) originated from Ethiopia and Eritrea area (Zohary and Hopf, 2000; Lupien, 1990; Zidenga, 2004, Gbebru et al., 2002). Sorghum is an important food crop and is ranked the fifth most important cereal crop worldwide in terms of consumption after wheat, rice, maize and barley (Agrama and Tuinstra, 2003, Lupien, 1990). Sorghum is also used for the production of syrup and livestock feed (Rainford, 2005). Sorghum stems are used for a range of purposes, including: the construction of walls, fences and thatches, and production of brooms, mats, baskets, fish-traps and sun shades (Oelke, 1971). It is also important source of fuel in some parts of the world.

The objective of the research was to characterize forty-nine accessions of sorghum assembled from Savanna Agriculture Research Institute of Ghana using ten SSR markers developed by ICRISAT.

Materials and Methods

There were forty-nine accessions of sorghum assembled from Savannah Agricultural Research Institute (SARI) for the studies. The collection and local names of the sorghum accessions are presented in Table 1. Plant materials used for the genotyping assay were grown in Sinnai's garden at Crop Science Department, University of Ghana. One accession was sown per hill in a nursery box filled with sterilized soil. Seedlings were harvested when they were four days old for DNA extraction.

DNA Extraction

Total DNA was extracted from each of the sorghum accessions using DNeasy Mini Kit. Extraction was done exclusively on ice using liquid nitrogen in the Biotechnology Laboratory of the Crop Science Department, University of Ghana.

Grinding of plant material was done in a porcelain mortar with a pestle. Plant tissue was kept refrigerated by the addition of liquid nitrogen. To each sample (100 mg), 400 µl of buffer AP1 and RNase A stock solution (100 mg/ml) was added. The mixture was vortexed vigorously for 30 seconds. The mixture was then incubated for 10min at 65ºc after which 130 µl of buffer AP2 was added. It was mixed and incubated for 5 min. on ice. The lysate was centrifuged for 5 min. at 13000 rpm. The lysate was applied to the QIA Shredder Mini Spin Column (lilac) placed in a 2 ml collection tube and centrifuged for 3 min at 13000 rpm. The flow-through fraction was transferred to a new tube without disturbing the cell-debris pellet.

Buffer AP3/E (1.5 volume) was added to the cleared lysate and mixed. 650 µl of the mixture was applied to the DNeasy Mini Spin Column sitting in a 2 ml collection tube. This was centrifuged for 1min. at 8000 rpm. The flow-through was discarded. The centrifugation was done again and the flow-through discarded as before. DNeasy Mini Spin Column was placed in a new 2 ml collection tube. 500 µl buffer AW was added to the DNeasy Mini Spin Column and centrifuged for 1min. at 8000 rpm. Flow-through was discarded. 500 µl of buffer AW was added to the DNeasy Mini Spin Column and centrifuged for 3 min. at 13000 rpm to dry the membrane. The flow-through together with the collection tube was discarded. DNeasy Mini Spin Column was transferred to a 2 ml microcentrifuge tube.

100 µl of buffer AW was applied directly onto the DNeasy membrane. Incubation was done for 5 min at room temperature (25ºc) and then centrifuged for 1 min at 8000 rpm to elute. Buffer AE was pipette again onto the DNeasy membrane. Incubation followed again for 5 min at room temperature and then centrifugation was done for 1 min at 8000 rpm for second elution. The extracted DNA samples were run on 1 % agarose in 1 × TAE buffer (40 mM Tris – acetate and 1 mM EDTA, pH 8.0). The DNA samples were diluted to final concentration of 10 ng/µl and stored at -20 ºc for Polymerase Chain Reaction (PCR).

Table 1: Accession number, collection I.D. and local names of the sorghum accessions.

ACCESSION NUMBER	COLLECTION I.D.	LOCAL NAME/AREA OF COLLECTION
1	WA 202	KASHEGU
2	WA105	MURAPAW
3	WA 113	SAMSORG 40
4	WA 215	CHARI KPONG
5	WA 206	SAMSORG 3
6	LAWRA202	NYONSO
7	LAWRA201	SARSORG E 14
8	LAWRA 104	BUNGAGO
9	LAWRA 108	KARIGA 1
10	LAWRA 203	DORADO
11	LAWRA 102	AYUFUFUL
12	WA 203	KAZEA MANGA
13	WA 206	CHERI
14	WA 206	SANSORG 41
15	WA 205	SARSORG M 15
16	WA 103	YAKPAAJI
17	SABOBA 206	KAZEA
18	SABOBA 220	KAZEA SHEO
19	SABOBA 107	SARSORG E 1-2
20	SABOBA 205	KAPAALA
21	SABOBA 212	MANKARANGA

22	GBD 216	KAZEA TAM
23	GBD 105	KAZEA GYERI
24	GBD213	SARSORG E 5-2
25	SABOBA215	KAPELI TAM
26	SABOBA 214	NWARAGU (RED BELKO)
27	BIMBILA109	CHARI -BILE
28	BIMBILA 107	POGKUORI LATUORI
29	BIMBILA 210	BELKO PIELIK 2
30	BIMBILA 103	BERKO MANGA
31	BIMBILA 112	SARSORG E 3
32	AGIRI ABANIA 32	MANKARAG TAM
33	KAS-NAN 203	SAMSORG 3
34	WUBUGU WHITE 209	GONO
35	BONGO 106	GLOBAL 2000
36	LAWRA 111	BERKO
37	BULSA 211	KERIGA 2
38	BULSA 120	SARSORG MB-2
39	BULSA 227	BELKO
40	BULSA 104	RED BELKO
41	BULSA 119	KYERE
42	BULSA 115	BELKO PIELIK 1
43	BWD 226	ZELK
44	BWD 230	
45	BWD 104	
46	BWD 221	
47	BWD 112	
48	BWD	

49	KAS-NAN 203(1)	SAMSORG 3

DNA Amplification

Ten SSR primer pairs listed in Table 2 were used in the genotyping assay. PCR reactions were performed in a Therma Hybaid thermocycler in a final volume of 25 µl containing 10 ng of sorghum genomic DNA, 5 pmol of primer and a PCR bead. A PCR bead contains 1.5 units of Taq polymerase, 10 mM Tris-HCl, (pH 9.0), 1.5 mM MgCl2, 200 uM of each dNTP and stabilizers, including BSA.

PCR cycling conditions were: 2 min. initial denaturation at 95 ºc, 30 cycles of (1 min at 95 ºc denaturation, 1 min at 60 ºc (Tm), 1 min elongation at 72 ºc) followed by a final elongation at 72 ºc for 1 min for a cycle. Amplification products were resolved by gel electrophoresis in 3% agarose gels run in TAE buffer, pH 8 for 4 h at 45 volt. The gel was stained with Ethidium bromide (10mg/ml) and the DNA fragments (bands) were visualized using trans-illumination device and photographed using a Polaroid camera.

Analysis of SSR data

A band was scored as present (+) or absent (0) for each of the genotypes. Cluster analysis was based on similarity matrices obtained with the unweighted pair-group method using the arithmetic average (UPGMA). The resulting dendrogram from Sequential Agglomerative Hierarchical Nested method (SAHN) showing the phylogenic relationships is shown in Figure 1. The data analysis was performed with the software package NTSYS-pc version 2.11.

Table 2 Oligonucleotide Primers used in DNA Amplification

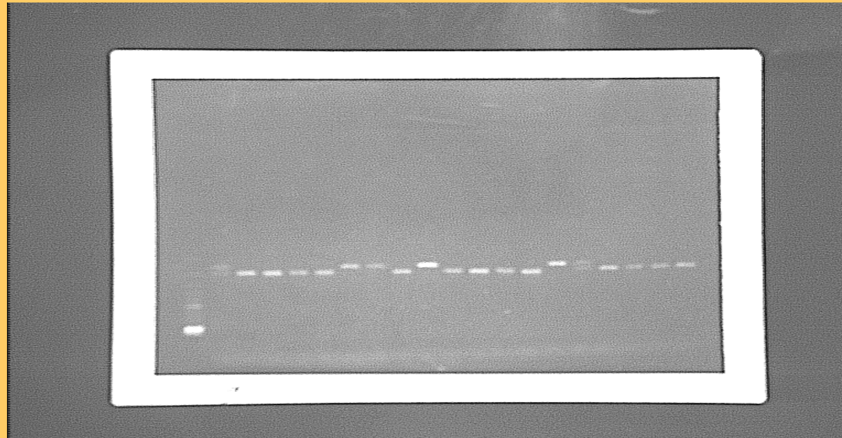
Lab No primer	Sequence of Forward primer	Sequence of Reverse
P1	ATGGCGTCTGTCCAGGT	CAGTTGCTAATCTTTGACCG
P2	GTTAACGACCAATCACCC	TAGAGGTGTCACTGATGAGC
P3	GCAGCTCAGGGACAAATAC	CTGCTTCAGGTAAGGATCG
P4	ACAGGGCTTTAGGGAAATCG	CCATCACCGTCGGCATCT
P5	CGCTGAACAACGAAAGGAATAAGTC	GAAAAATCTCCGTCAATCCCAAAATAA
P6	GCTTTCGGCGAGCATCTTACAA	GCGGTTGGATTCGCCATG
P7	ATAGAGAGGATAGCTTATAGCTCA	CTCTGATATGTCGTTGTGCT
P8	AAACATCATACCGGAGCTCATCAATG	TCCTGTTTGACAAGCGCTTATA
P9	CTTTGAAACCCTGAACTCAT	AGCACTGCTTGACACTCC
P10	GGAAGAAAGGAATGACTTGA	AGCTCTCAGCCTTTCACAAT

Results and discussion

Clustering using SSR markers results

The number of alleles scored by each of the ten microsatellite markers used is listed in Table 3. With the exception of SbAGF06 and Sb1-10 all the remaining 8 microsatellite loci were polymorphic. The fragments produced by the primers were within the range of 100 and 200 bp in size. Number of alleles scored ranged from 1 to 5. A sample photograph of electrophoresed PCR products is shown in Plate 1. The total number of alleles scored by the ten primers was 33 giving an average of 3.3. The dendrogram generated from the allele scoring is shown in Figure 1.

Plate 1: Sample photograph of electrophoresed PCR products



Showing polymorphic

A dendrogram generated from the results of the analysis is shown in Figure 1. An average of 3.3 alleles per locus was detected using the 10 primers. In their study to determine the degree of genetic diversity among 22 sorghum accessions using 28 SSR primers, Agrama and Tuinstra (2003) detected an average of 4.5 alleles per locus. Kudadjie (2006) observed an average of 3.7 alleles per locus in similar studies using 14 primers. The fewer alleles per locus observed in this studies may be due to the fewer number of primers (10) used or relatively less diversity in the sorghum accessions. The level of polymorphism observed nevertheless was high (94%) in this study.

The percentage similarity between the sorghum accessions ranges from 63% to 100% (Figure 1). This result is similar to 66% to 100% obtained by Kudadjie (2006) in diversity studies in sorghum using SSR markers. Kudadjie (2006) observed 4 clusters at 70% similarity coefficient. In the present studies, 4 clusters were observed at 72% level of similarity coefficient.

Accession 6 (Nyonso) an outlier is linked with the rest of the collection at 63% level of similarity. Similar to accessions 7, 8, and 9, accession 6 was collected from Lawra. However, accession 6 is not related to these accessions more than to other accessions in the collection. Accession 8 (Bungago) and 10 (Dorado) are not genetically different according to this data. Morphological differences observed in Bungago and Dorado as the basis of sampling them as different accessions may be due to the environmental differences.

Table 3: SSR markers and the number of alleles the produced

SSR marker	Lab no.	No. of alleles
SbAGF08	1	4
SbAGF06	2	1
SvPEPCAA	3	5
Sb1-10	4	1
Sb4-121	5	3
SbAGB02	7	2
SB1-1	8	5
Sb6-57	9	4
SbAGE03	10	4

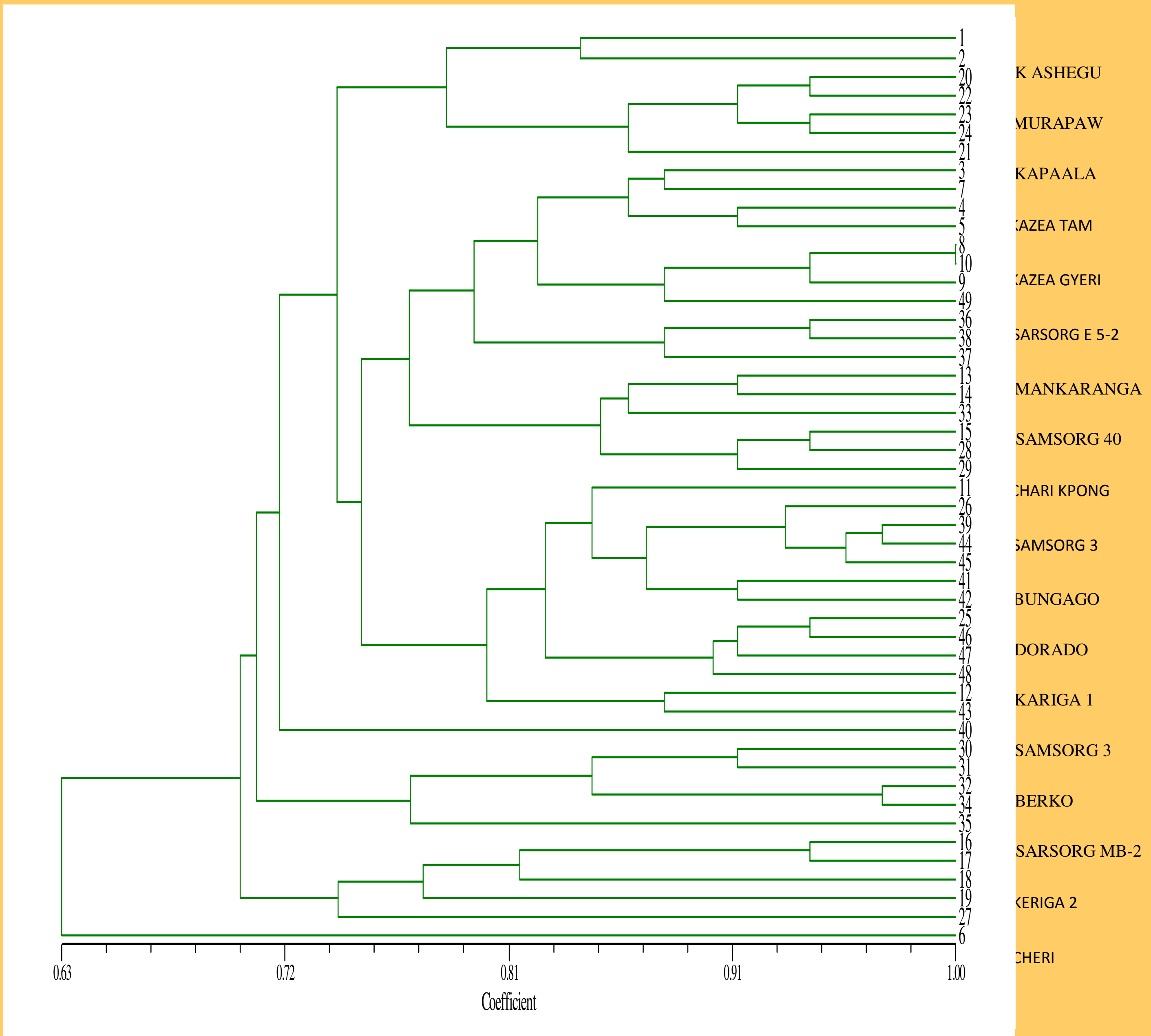


Fig 1: Tree plot of sorghum accessions by UPGMA cluster analysis based on qualitative and quantitative data with SAHN method

Note: The local names of the following accession are not known; 44, 45, 46, 50,51,52,53, 54. 56.

At 74% Jaccard similarity coefficient, the dendrogram has four clusters. At the bottom of the tree is cluster one with one accession, Nyonso, which has been discussed above. Cluster two is made up of 5 accessions; accession 27 (Chari-Bile), 19 (Sarsorg E 1-2), 18 (Kazea Sheo), 17 (Kazea) and 16 (Yakpaji). Cluster three also has 5 accessions including: accession 35 (Global 2000), 34 (Gono), 32 (Mankarang Tam), 31 (Sarsorg E 3) and 30 (Berko Manga). Red Belko alone made cluster number 4. The fifth cluster, which is the largest, had 37 accessions.

Cluster 2 had accession numbers following each other; 16, 17 18 and 19. This is probably due to the closeness of the collection points. Accession 17, 18 and 19 were collected from Saboba. The names of accessions 17 and 18 (Kazea and Kazea Sheo) suggests a close relationship between them observed by the local growers. Sorghum can cross-pollinate, leading to this reduced variability among accessions planted close to each other. Cluster 3 also had members collected from close geographic locations. These accessions have either intermediate or mostly starchy endosperm.

At an 81% level of similarity, 7 clusters were formed by cluster 5. The first cluster has accession 12 and 43 (Kazea Manga and Zelk respectively).

The 2nd to the 6th group had members ranging from 5 to 11. The 7th cluster has two members; accession 1 and 2 which separated at 83% level of similarity.

Acknowledgement

The author wish to express a profound gratitude to the following: Profs, E.Y. Danquah and S.K. Offei, Dr. I.D.K. Atokple, Messrs. Ben and E. Addo. Each of the listed personalities played very important roles in carrying out the experiment.

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Reviewer´s Comments

1. Comment by Theresa Fulton
Director of Outreach & Training, Institute for Genomic Diversity
Cornell University , 130C Biotechnology Building
Ithaca, NY 14853 USA

Attached please find the paper you asked me to review with some minor internal edits, as well as a document with my comments, both to you and the reviewer. This is a very nice article; I have suggested a couple of additions that would be helpful. In the interest of full disclosure

To the editor:

This is a very well-written paper, and recommended for submission with a few edits and additions. Most notably, it would be good to have a little more discussion of the results or at least some concluding remarks, as well as a bit of information on why this analysis was done.

To the author:

This paper is well written and very clear.

Some minor grammatical edits are included within the document (using the Track Changes function).

Please include information about why you wanted to study the diversity of these particular 49 accessions. Was there any reason to believe they were variable, or related? Can you suggest any reason for why they turned out to be so highly variable?

If the DNA extraction protocol was the DNeasy Mini protocol with the kit, you do not need to give all the details of the extraction procedure. Indeed, you could probably eliminate everything from “grinding of plant….” And keep only “The extracted DNA samples were run on 1% agarose….”

I don’t think mentioning the cassava example is helpful, so I deleted it (and the reference was not included in the reference list anyways).

Please add some conclusions – why is this information interesting? What will you do with it? At minimum, something like “This data suggests that there has been a lot of genetic exchange between farmers’ accessions that are located geographically near each other, and yet the collection still maintains a good level of diversity”. Is it possible 8 and 10 are the same? More discussion is needed.

2. Comment by Rosane Collevatti

Universidade Católica de Brasília, Brasília, Brazil

Review of the manuscript -Variability in forty-nine accessions of sorghum [Sorghum bicolor (L.) Moech]

The authors aimed to characterize 49 accessions of sorghum assembled from Savanna Agriculture Research Institute

of Ghana using ten microsatellite loci developed by ICRISAT.

The manuscript has some important flaws that must be reviewed before publication.

Introduction should be rewritten. The second paragraph should be omitted. Characteristics and differences of those molecular markers and definition of microsatellite markers are already recognized and well established. More emphasis should be given on the importance of the characterization of the accessions.

Material and Methods – detailed description of DNA extraction is not necessary as the protocol is described in the manual (cite the kit manufactory).

Statistical analysis should be reviewed. The method of band score and similarity estimation is not appropriate for microsatellites. The authors should use appropriate genetic distance estimation for microsatellites. If they are interested in the pattern of similarity among accessions, the authors may use the Neighbor-joining method of cluster. UPGMA has many problems including short branch attraction and with heterogeneity in evolutionary rate among branches. Additionally, it would be useful to characterize the microsatellite markers, showing the number of alleles and heterozigosities.

Results and Discussion should be rewritten following modifications on Material and Methods.

Comment of Author

I have seen the area Dr. Rosane (I hope I'm correct) is comming from. As a beginner, I chose a simple method for my analysis. The fact that one method is better does not make the other bad. My dendogram is enough to tell a farmer that this accession is closer to the other than another one.
Thank you.

An Additional Comment by Theresa Fulton

Hello Ken and Nagib,
I would respectfully disagree with the comment about UPGMA analysis - however, there are certainly many variable opinions.

I am attaching a recent paper (Assessment of the Genetic Diversity in Argentine Rice Cultivars with SSR Markers by L. E. Giarrocco, M. A. Marassi, and G. L. Salerno - CROP SCIENCE, VOL. 47, MARCH–APRIL, 2007)

where it is used for rice. I think you could add a sentence or a comment to the effect of "While there are many possible analyses, in this case we used the UPGMA, as did (and cite this other work). If you do a search on "ssr upgma analysis" you will find many other examples too. If you are concerned though you could easily do another type of analysis and mention if it confirmed the same results you found here.