Open Journal of Ecology, 2014, 4, 371-393
Published Online May 2014 in SciRes. http://www.scirp.org/journal/oje
http://dx.doi.org/10.4236/oje.2014.47034

Scientific Research

Assessment of Genetic Variability of 142 Sweet Sorghum Germplasm of Diverse Origin with Molecular and Morphological Markers

A. Lekgari, I. Dweikat

Department of Agronomy and Horticulture, University of Nebraska, Lincoln, USA
Email: Idweikat2@unl.edu

Received 3 January 2014; revised 3 February 2014; accepted 10 February 2014

Abstract

Sorghum bicolor **(L.) Moech is the fifth most important crop in the world. Recently, its agronomics and genetics have drawn interest among scientists. Sweet sorghum, a variety of sorghum, may potentially become a bioenergy source because of the high sugar content in its juicy stems. Exploring the diversity of sweet sorghum around the world is important to the development and improvement of the crop as an energy source. In exploring the diversity of sweet sorghum, three types of markers (simple sequence repeats [SSR], sequence-related amplified polymorphisms [SRAP], and morphological markers) are used on 142 sweet sorghum accessions from around the world. The accessions show a high significance ($P < 0.05$) for all the morphological traits measured. The morphological markers cluster the accessions into five groups based primarily on plant height (PH), anthesis data (AD), and moisture content (ML), with the principal component analysis (PCA) showing these traits to explain 92.5% of the total variation. The furthest accessions were PI571103 from Sudan, and N99 from the United States. The Nei's genetic standard distances ranged from 0.024 to 1.135 and 0.078 to 0.866 for SSR and SRAP, respectively. As expected, accessions of the same origin or breeding history had the lowest genetic distance (e.g. Mokula and Marupantse, both from Botswana; NSL83777 and NSL83779 from Cameroon). Neighbor joining clusters the sweet sorghum accessions into five major groups using SSR and four major groups using SRAP, based on their origin, or breeding history. The three marker types complement each other, and the presence of accessions of different origins across clusters indicate similar genetics, and evidence of germplasm movement between countries.**

Keywords

SRAPs, SSRs, Energy Crops, Genetic Distance

How to cite this paper: Lekgari, A. and Dweikat, I. (2014) Assessment of Genetic Variability of 142 Sweet Sorghum Germplasm of Diverse Origin with Molecular and Morphological Markers. *Open Journal of Ecology*, **4**, 371-393.
http://dx.doi.org/10.4236/oje.2014.47034

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one crop species that can survive harsh climatic conditions of arid environments [1]. *Sorghum bicolor* contains both cultivated and wild races and possess a significant amount of genetic diversity for traits of agronomic importance [2]. Commonly, it is used as a source of grain food, syrup fuel, and feed for livestock. Sweet sorghum, a variety of sorghum with high sucrose accumulation in their stems, has recently received a lot of attention as a source of biofuel [3]. Sweet sorghums are selected to accumulate high levels of sucrose in the parenchyma of the juicy stems [4] [5]. The stems are desired for food grade syrup, fresh chewing, and alcohol production in areas like Brazil [5], India, and Africa [6]. In the United States, sweet sorghum has been researched for biofuel for over 30 years [7]. The primary research regarding sweet sorghum, its development, and breeding began in the late 1970s [5] because of the high oil costs and the need for alternative energy sources. Under favorable conditions, a sweet sorghum crop is capable of producing up to 13.2 metric tons per hectare of total sugars, which is equivalent to 7682 liters of ethanol per hectare [5]. Sweet sorghum has a compelling advantage for cellulosic biofuel production over seed-based ethanol production, and its adaptation to marginal lands makes the per unit value of biomass production economical [8].

Sweet sorghum gene pool creation has not received much attention, primarily because it is not considered to be among the important crops in the US, and the pedigree information is scarce and incomplete. Most sweet sorghums released in the US are developed by public breeding programs in the 1900s and are mainly open pollinated [9]. The crop improvement is done mainly on the sweet sorghums' syrup, sugar concentration, and biomass, with lines primarily selected for improved disease resistance [5]. Genetic diversity or knowledge of patterns of diversity of genetic resources is of great importance (Warburton *et al.*, 2001) and is a key component in crop improvement and plant breeding.

Murray *et al*. [5] stated that the majority of the sweet sorghum cultivars released in the United States have a narrow genetic base that can be traced to six African landraces. There are currently no criteria (molecular or morphological markers) to differentiate sweet sorghums from grain sorghums [5], and most of the accessions lack the proper information to help distinguish them. Therefore, when requesting sweet sorghum germplasm, one is limited to a few characters that are common in sweet sorghum, such as tall leafy plants (high biomass), and the brix degree where available, which is also subjective as there is no definite value for distinguishing grain sorghums from sweet ones. The Meridian, Mississippi Station attempted to curate what may be the world sweet sorghum collection, and when it closed, materials were transferred to the USDA sorghum collection in Griffin, Georgia [10]. Thus, many diversity studies have concentrated on cultivars/lines that are common and known, leaving the vast majority of the collection (genetic sources) unexploited. In this study, we tried to incorporate the commonly used lines together with some of the uncommon lines, and accessions from other sorghum collections.

The use of morphological traits in plants as markers for determining the genetic relationship dates back many years. Mendel followed visible phenotypic traits in progeny of sexual crosses, and the use of morphological markers has continued to the present day [11]. Phenotypic variables include continuous variables such as height, maturity, and yield, as well as discrete variables such as grain color, texture, and insect and disease resistance [12]. Franco *et al*. [12] stated that the truth underlying homogeneous groups or sub-populations of genotypes and their shape and structure is unknown. This is due to the fact that the association between the traits affects the shape of the groups and their structure is dependent on the composition of the group.

However, clustering methods attempt to recover the true shape and structure of the sub-population. When using both the morphological and molecular marker data, two types of hierarchical classification are carried out independently. The morphological marker data first utilizes the computation of standard distances (e.g. Euclidean distances) and clustering strategies such as UPGMA or neighbor joining are applied. On the other hand, when applying the molecular marker data, genetic similarities or dissimilarities using each band fragment as an attribute (0 for absence and 1 for presence) are determined, then a clustering strategy applied [12]. This enables genotypes to be clustered into as homogenous groups as possible. Phenotypic and genetic diversity are important in genetic conservation, evaluation, and utilization of genetic resources, and the study of breeding germplasm for determining uniqueness and genetic constitution for the purpose of breeder's property rights [12]. The morphological markers are highly influenced by environmental conditions. Therefore, there is a need to supplement or complement their clustering with molecular marker data.

Polymerase chain reaction (PCR) is widely used in genomic DNA analysis, and one of its main applications has been in the development of DNA-based markers for map construction, breeding taxonomy, evolution, and gene

cloning [11] [14]. Molecular markers are basically nucleotide sequence corresponding to a physical position in the genome, and their polymorphisms between accessions allow the pattern of inheritance to be easily traced [11]. The availability of molecular markers to assess diversity is a quicker way to help breeders select suitable lines/genotypes for crossing. The use of molecular markers as a tool to assess relatedness in and between cultivated and wild sorghum have been successfully used [1] [15]-[19]. PCR-based markers are widely used in fingerprinting crops because of their high level of polymorphisms [20] and their ease of detection [21]. Several PCR-based markers vary in their complexity, reliability, and information generating capacity.

Simple sequence repeats (SSR), also known as microsatellites, are based on tandem repeats of one to six core nucleotide elements. These codominant markers are dispersed throughout the genome, and have multiple alleles that often have conserved loci between related species [11] [22]. Powell *et al*. [23] stated that SSRs are able to discriminate among closely related individuals and have advantage over other markers in their ability to trace pedigrees in plants. Therefore, SSRs have been used in a variety of genetic studies such as diversity analysis, quantitative trait locus mapping, gene tagging, and cultivar identification.

Several studies involving either SSR markers alone or in combination with other marker types have been conducted on sorghum varieties [5] [24]-[27]. Polymerase chain reaction made possible the development of many other marker methods. Schulman [11] indicated that some marker methods detect specific, cloned, and sequenced targets in the genome, while others use conserved or general primers that amplify from many anonymous sites throughout the genome.

Sequence-related amplified polymorphism (SRAP) markers are based on two primer amplification, which preferentially amplifies open reading frames (ORFs) or coding regions that result in a number of dominant and codominant markers [14] [28]-[30]. Li and Quiros [14] and Zhao *et al*. [30] explained that the forward primer amplifies the exon regions while reverse primer amplifies the intron and promoter regions. They also stated that the polymorphisms resulted from the variation in length of these exons, introns, promoters, and spacers among both individuals and species. Sequence-related amplified polymorphism markers are more reproducible, stable, and less complex [30] [31], in addition to being more powerful in revealing the genetic diversity among closely related individuals than other marker types [32]. Sequence-related amplified polymorphism markers have also been used in a wide range of plant species such asalfalfa [28], *Brassica* [14], buffalo grass ([28], 2004b), cotton [33], *Cucubita* [34], tree peony [31] and wheat [35] [36]. Ferriol *et al*. [34] also reported that the information obtained from SRAP markers agreed with the morphological variations and evolutionary history of morphotypes more than that found with AFLP.

Several diversity studies of sorghum and/or its wild relatives [1] [15] [16] [26] are limited to either grain sorghum or to germplasm from or within an individual country. In this era, germplasm sharing is an important factor in breeding as breeders try to develop modern cultivars with improved agronomic performance. The use of molecular markers has proven to be an effective tool in assessing the genetic relatedness of different species [1]. Regarding this, many types of markers have been used in sorghum. These studies have revealed both a wide and narrow genetic variation between agroecological zones. Folkertsma *et al*. [37] indicated that there is a wide variability within accessions in the semi-arid regions of Africa. However, the south Asian accessions have narrower diversity compared to those in Africa. Therefore, it is important to establish the genetic similarity among some of the world germplasm collection of sweet sorghum, especially since its potential as an agro-industrial crop continues to draw more attention. Therefore the objectives of this study are to:

Examine the genetic variability within sweet sorghum germplasm from different regions of the world for traits associated with biofuel production.

Classify/group the sweet sorghum germplasm based on SSRs, SRAPs, and several morphological data.

2. Materials and Methods

2.1. Plant Material

One hundred and forty-two 142 sweet sorghum accessions were used in this study (**Appendix 1**). These accessions were obtained from the USDA-ARS, University of Nebraska-Lincoln, NE; National Center for Genetic Resources Preservation (NCGRP), Fort Collins, CO; National Plant Germplasm System, Griffin, GA; Texas Agricultural System Station, College station, TX; University of Kentucky, KY; and the Department of Agricultural Research (Ministry of Agriculture), Botswana. These 142 sweet sorghum accessions consisted of landraces, released improved cultivars, and breeding lines. The available pedigree information were obtained from GRIN

website (http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl), ICRISAT website (http://www.icrisat.org), and other resource publications [5] [37], or accompanied in the seed information.

2.2. Agronomic Traits

The 142 sorghum lines and two check cultivars (M81-E and sugar drip) were planted under rain-fed conditions at Mead, NE during the 2009 growing season. The experiment was laid in an incomplete block design with 12 incomplete blocks of 12 entries each (12 × 12 alpha lattice) and two replications. Single row plots measuring five meters long with between row spacing of 0.75 m were over sown at the rate of 160,000 seeds per hectare. The seeding rate was assumed to compensate for situation where there might be low seed viability, and the final population density was on average of 140,000 plants per hectare.

Four agronomic traits were measured and included anthesis date measured as the duration in days from planting to 50% of the plants within a plot were 1) shedding pollen; 2) plant height measured as the distance from the base of the plant to the tip of the panicle; 3) dry matter yield in $Mg \cdot ha^{-1}$ when plants had reached their physiological maturity; and 4) moisture content as the percentage difference between wet and dry biomass weight. Dry matter yield was calculated from a sample taken at harvest as follows: DM = (Dry weight of total 0.50 m row)/(plot area in m^2) then calculated as $Mg \cdot ha^{-1}$. Plants were weighed immediately after cutting the 0.5 m samples, bagged, and placed into an oven at 120˚C - 160˚C for ten days to completely dry the samples. Samples were reweighed to obtain the dry weight.

2.3. DNA Extraction and Marker Analysis

Genomic DNA of each accession was extracted from fresh leaf tissues from plants planted in the greenhouse using cetyltrimethyl ammonium bromide (CTAB) protocol [39]. The ground tissue was incubated in an extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1 mM 1,10-phenathroline and 0.15% 2-mercaptoethanol) at 65˚C for one hour; then equal volume of chloroform:isoamyl alcohol (24:1) was added to the tissue mixture. After centrifugation at 3000 rpm, the supernatant was transferred to a new clean tube and DNA was precipitated with equal volume of cold isopropanol. DNA was air died at room temperature for one hour and then re-suspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) with 20 ng RNase and incubated at 37˚C overnight. Equal volume of 24:1 chloroform:isoamyl alcohol was added to the DNA-RNase mix and centrifuged at 3000 for five minutes, and the resulting supernatant was transferred to new tube. Two volumes of cold absolute ethanol and 5 µl of 8 M ammonium acetate were added to the supernatant in order to precipitate the DNA. After centrifugation, DNA pellets were air dried at room temperature, and later re-suspended with 200 to 400 µl TE buffer, depending on the size of the pellet. DNA concentration was determined using a spectrophotometer (TKO 100 Fluorometer, Hoefer Scientific Instruments, San Francisco, California).

A collection of 82 oligonucleotide primer pairs that included 33 sorghum SSRs ([40]; Lubbock, TX [unpublished]) and 49 SRAP combinations [14] [41] were synthesized, and marker assays were conducted following the procedure of Kuleung *et al*. [42]. A 25 µl total/reaction was used, which consisted of 75 ng genomic DNA, 100 ng primer pair, 125 µMdNTP, 50 mM KCl and 10 mM Tris-HCl, 2 mM $MgCl_2$, and one unit Taq polymerase. The amplification procedure consisted of one cycle at 94˚C for three minutes, followed by 35 cycles of one minute at 94˚C, one minute at 55˚C to 58˚C for SSRs depending on the primer pair, and 47˚C for SRAPs, one minute at 72˚C, and final extension step at 72˚C for five minutes. The reaction was then cooled to a resting temperature of 4˚C and resolved by electrophoresis in 12% non-denatured polyacrylamide gels (37:1 of acrylamide: bis-acrylamide). The gels were stained in 1 µg/ml ethidium bromide for 10 minutes, distained in deionized water for 15 minutes, then photographed using the Gel Doc2000 (Bio-Rad, Hercules, Califorlia).

2.4. Data Analysis

Analysis of variance was performed on agronomic data using PROC MIXED, where incomplete blocks were treated as random effects. In the next step, principal component analysis using a correlation matrix from least square means (LSMEAN) was done using PROC PRINCOMP to determine the traits that account for most variation between lines. A simple Pearson correlation was done on the means of the four agronomic traits measured. Due to the large difference in the unit of each trait, agronomic data were standardized using the standard deviation of mean by PROC STANDARD. Afterwards, we used PLOC CLUSTER using “Average Linkage Cluster

Analysis" based on Euclidean distance [43] for the clustering. Average Linkage algorithms were used for cluster analysis and then dendrogram was constructed using PROC TREE [44].

Two genetic distance and clustering methods were used for the marker data to determine how the sorghum accessions grouped, using a band scoring of "1" to indicate the presence of an allele and "0" when absent. Polymorphism information content (PIC) values were calculated as per formula developed by Anderson *et al*. [45], which assumes homologous alleles. Polymorphic information content is calculated as:

$$PIC = 1 - \Sigma P_{ij}^{2}$$

where P_{ij} is the frequency of j^{th} allele of i^{th} locus, summed across all the alleles for the locus over all lines. A marker with a PIC value of more than 0.5 is considered highly informative, between 0.25 and 0.5 is considered informative, and less than 0.25 is considered slightly informative [46]. The genetic diversity was estimated by similarity index calculation from band sharing data of each pair of DNA fingerprints. Genetic similarity (GS) between cultivars *i* and *j* was calculated using all loci for both the SSR and SRAP markers according to Nei and Li's formula for estimating coefficient of similarity [47] [48] based on shared allele frequency. The formula is as follows:

$$S = 2n_{ab} / \left(n_a + n_b \right)$$

where *S* is the similarity coefficient, n_{ab} is the number of bands common to A and B cultivars, n_a and n_b are number of bands in A and B cultivar, respectively. A similarity matrix was used to construct a similar dendogram by cluster analysis using the neighbor joining method to determine how sorghum accessions were related. The genetic distances were calculated based on Nei's [49] standard genetic distance, as follows:

$$Ds = -\ln\left(Jxy / \sqrt{JxJy} \right)$$

where $Jx = \Sigma\Sigma\, X_{ij}^{2}/\mathrm{r}$, $Jy = \Sigma\Sigma\, Y_{ij}^{2}/\mathrm{r}$, and $Jxy = \Sigma\Sigma\, X_{ij}Y_{ij}/\mathrm{r}$ with X_{ij} and Y_{ij} being the frequencies of allele *i* at *j* locus of populations *X* and *Y*, respectively [50]. Population genetics software (Version 1.2.30) was used for genetic distance calculation (http://bioinformatics.org/~tryphon/populations). The dendogram construction from the POPULATIONS program used the TreeView program (version 1.6.6) (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html; [51]).

3. Results and Discussion

3.1. Agronomic Traits

Harvest was done at the same time for all lines. However, due to the wide range of maturity existing among them, some lines were past the physiological maturity stage at the time of harvest. The analysis of variance showed a highly significant differences ($P < 0.01$) for all the traits measured among sorghum accessions (**Table 1**). The anthesis data showed a wide range of maturity among the lines (70 to 147 days to anthesis) (**Appendix 2**). Plant height ranged from 76.0 cm to 423.8 cm, moisture content ranged from 45.4% to 80.6%, and dry matter yield weight ranged from 3.81 Mg·ha^{-1} for PI 276804 to 59.19 Mg·ha^{-1} for N99 (**Appendix 2**).

Table 1. Mean squares of anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) measured at Mead in 2009 season.

Source	DF†	Anthesis	PH	ML	DM
Rep	1	4.53	1961.99	122.45***	9.87
Block (Rep)	22	9.82	1171.73**	19.95**	99.98**
Line	138	282.67***	9346.66***	66.21***	186.29***
Residual	112	7.18	665.85	9.54	47.92
Mean		**92.4**	**273.9**	**64.3**	**24.06**
CV (0.05)		**2.91**	**9.42**	**4.83**	**28.77**
Range		**69.5 - 147.2**	**76.0 - 423.8**	**45.4 - 80.6**	**3.81 - 59.19**

†The degree of freedom for lines was less than expected because of some missing data; **, *** indicate significance at probability values of 0.05 and 0.01 respectively.

PI 276804 is an Ethiopian landrace with moderate tillering and medium height (ICRISAT website), while N99 is an F_7 selection from a cross between a Fremont forage sorghum and Theis sweet sorghum [38]. Theis is a high biomass producer that may contribute to N99 high yield. There were highly significant correlations between anthesis date and plant height ($r = 0.53^{***}$), anthesis date and dry matter yield ($r = 0.57^{***}$), and plant height with moisture content and plant height with dry matter yield ($r = 0.285^{***}$ and 0.712^{***} respectively) (see **Table 2**).

Cluster analysis of agronomic traits grouped the lines into five main groups (**Figure 1**, **Appendix 3**). Although the agronomic traits did not distinctly group lines according to their geographic origin/area, materials from the same area tended to cluster together within each group, indicating that their origin played a role in the selection or development of germplasm used (**Figure 1**). Apart from the germplasm origin, the lines tended to group together according to plant height and percent moisture content. For example, Group 1 consisted of materials that were 248.0 cm tall (176.3 - 288.3 cm) on average Group 2 averaged 328.5 cm (287.3 - 390.5 cm); Group 3 averaged 399.5 cm (379.2 - 423.8 cm); Group 4 averaged 170.7 cm (104.3 - 200.8 cm) and Group 5 was 123.8 cm tall (76.0 - 281.8 cm) on average.

Data obtained from the principal component analysis in which anthesis date, plant height, and moisture loss accounted for the 79.1% of the variation (*i.e.* principal component 1 and 2) supported the above reasoning (**Table 3**). Principal component 3 was mainly associated with anthesis date, together with the first two principal components, which accounted for 92.5% of the variation. The two furthest genetic distances exist between accession PI 571103 (a landrace from Sudan) and N99 with distance of 7.818. On the other hand, the closest accessions were PI 569520 (a breeding line from Sudan) and ICSR90017 (a restorer line from ICRISAT), with a genetic distance coefficient of 0.189.

3.2. Molecular Marker Data

From the 33 SSR marker pairs screened, 29 produced 84 polymorphic alleles with a mean of 2.90. This was lower

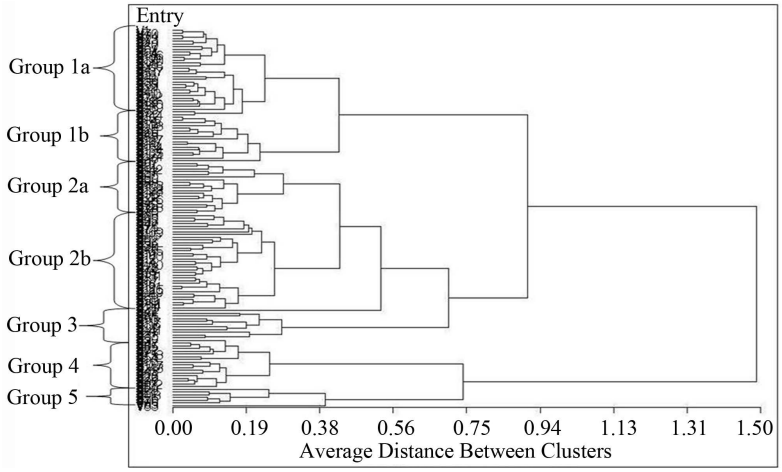

Figure 1. The dendrogram using average distances of 142 accessions based on anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) measured at Mead in 2009. Five major groups at threshold distance of 0.40.

Table 2. Correlation coefficients of anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) measured at Mead in 2009 season (n = 142).

	AD	PH	ML	DM
AD	1			
PH	0.530^{***}	1		
ML	0.054^{ns}	0.285^{**}	1	
DM	0.570^{***}	0.712^{***}	-0.005^{ns}	1

***Significance at probability value of 0.01 respectively; ^{ns}Non-significance at $P < 0.05$.

Table 3. The eigenvalues and principal components for anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) measured at Mead in 2009 showing the proportion explaining variation.

	Eigenvalue	Diff	Proportion	Cumulative
1	2.105	1.046	0.526	0.526
2	1.059	0.525	0.265	0.791
3	0.534	0.233	0.134	0.925
4	0.301		0.075	1
	Prin1	**Prin2**	**Prin3**	**Prin4**
Anthesis	0.540	−0.135	0.828	0.069
Height	0.602	0.172	−0.305	−0.717
Moist. Loss	0.131	0.938	0.041	0.317
DM Yield	0.573	−0.268	−0.468	0.616

than the 3.22 that Ali *et al*. [26] observed, or the 3.4 that Schloss *et al*. [40] observed. The polymorphic information content of SSR markers ranged from 0.22 to 0.75 with a mean value of 0.52 (**Table 4**). These values were higher than those of 0.40 and 0.44 observed by Ali *et al*. [26] and Folkertsma *et al*. [37], respectively. The differences may be attributed to the number of bands scored and the type of SSR markers used. Botstein *et al*. [46] suggested that markers with PIC > 0.5 be considered highly informative. Thus, we could conclude that the mean PIC value of 0.52 indicated that the markers used were highly informative. By using 72 US sorghums in their experiment, Ali *et al*. [26] reported PIC value range of 0.03 to 0.87.

For each pairwise similarity estimate, a dendrogram was constructed using Nei's standard genetic distance [49]. The accessions were grouped mainly according to their origin or breeding history (**Figure 2**). Nei's standard genetic distance ranged from 0.024 to 1.135, with Marupantse and Mokula having the smallest genetic distance, while PI 154844 and NSL 55404 had the largest genetic distance, followed by NSL 55429 and NSL 87920, and PI 602982 and PI 571103 with a value of 1.099. Marupantse and Mokula are both from Botswana. Marupantse is an advanced/improved cultivar while Mokula is of unknown parentage. However, the two do not belong to the same sorghum race (kafir vs. durra-caudatum). PI 154844 is a landrace from Uganda and NSL 55404 is from India. However, both belong to the Durra race. NSL 53429 is a landrace from India, while NSL 87920 is from Cameroon; PI 602982 is a line developed in Mali with pedigree (SPV 35/E35-1)/CS 3541, and PI 571103 is a landrace from Sudan.

By using Nei's standard genetic distances [49] through neighbor joining (**Figure 2**), cluster analysis grouped the accessions into five major groups. Group 1 consisted of germplasm mainly from East Africa (Sudan, Kenya, and Ethiopia), while Group 2 consisted of germplasm from different regions. Group 3 is occupied by Nebraska lines (released and breeding lines) and some Ethiopian germplasm. The largest group was Group 4, which consisted of 47 accessions, and occupied mainly by germplasm from Botswana and the US (particularly Nebraska). The SC accessions from Botswana might have been part of the late 1960s USDA sorghum conversion program, thus creating a link between Botswana sorghums and the US sweet sorghums, e.g. 65D, which is an introduction to Botswana from the United States, with unknown parentage. The last group was the smallest (nine accessions) and also had germplasm from different regions.

Within each major group, accessions from the same country/region grouped together to form smaller clusters. This is in agreement with Wang *et al*. [52] and Murray *et al*. [5], who observed that both sweet and grain sorghums germplasm corresponded well with the geographic locations where the accessions originated. Since most of the accessions used were landraces with unknown parentage, it can only be assumed that accessions with the same origin may be highly related. However, those with known parentage such as the Nebraska breeding lines, the ones with similar pedigree, tend to cluster together. For example, the lines that have wheatland in their parentage (05C09882 [5] tan, 05C09881 [4] ppbmr, and 05C09892 [6] ppbmrsw, etc.) were closer to wheatland, while lines like 05C09889 [1] vtallsw grouped with N99. Ali *et al*. [26] reported that Dale, N108, Theis, Cowley, and Norkan clustered in the same major group but different subgroups. In this study, they were also in the same major group.

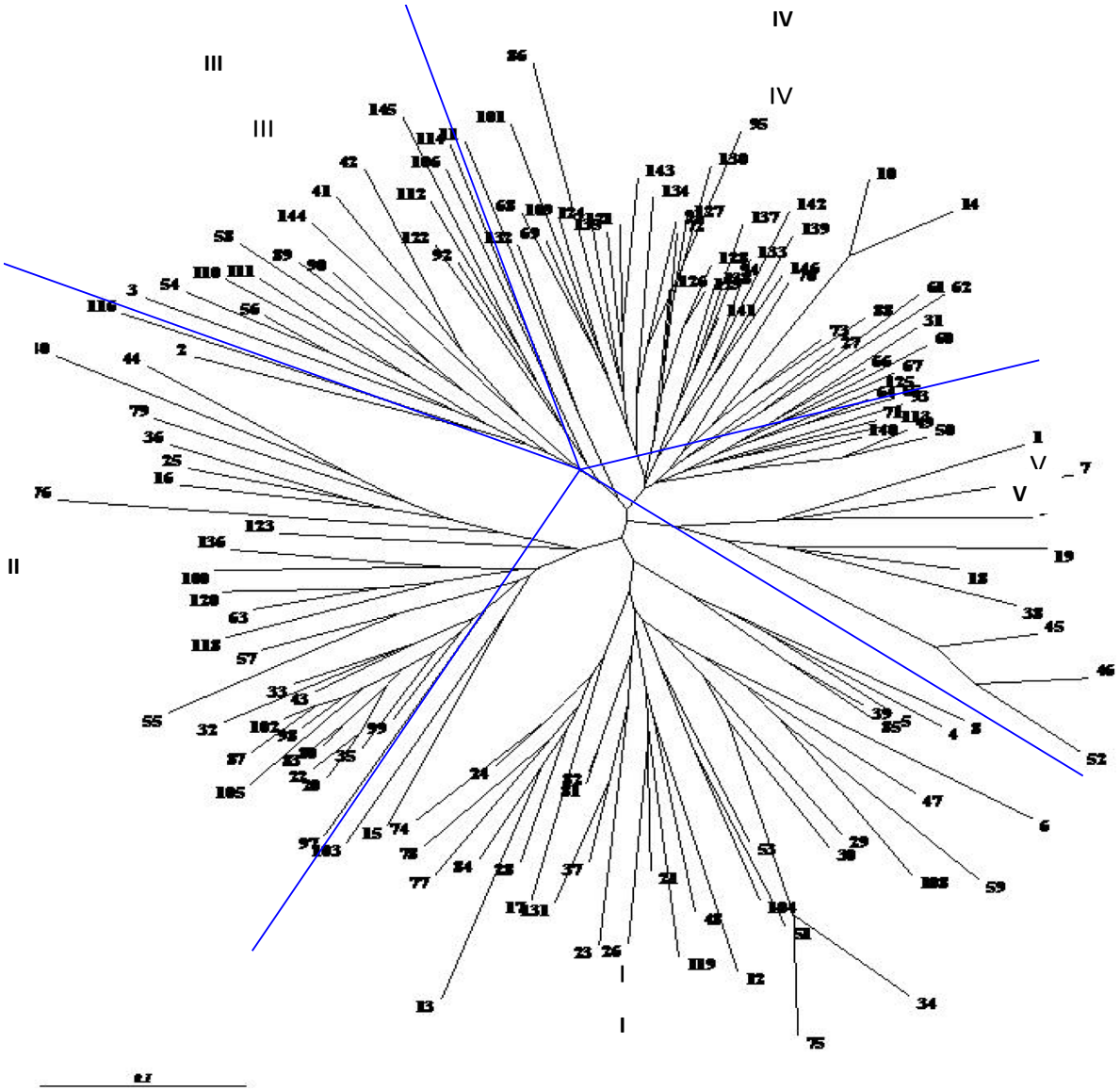

Figure 2. Dendrogram constructed by neighbor joining analysis using Nei's (1972) genetic standard distances of 142 sorghum accessions based on SSRs data.

Table 4. Polymorphic information content (PIC) of markers used to analyze 142 sorghum accessions.

Marker Type	Markers Screened	Polymorphic Markers	Number of Bands	PIC		
				Min	Max	Mean
SRAP	49	40	109	0.145	0.939	0.557
SSR	33	29	84	0.221	0.75	0.519

From the 49 SRAP marker pairs screened, 40 polymorphic pairs produced 109 alleles, with a mean of 2.73 alleles. This value was lower than that of SSR markers. However, the PIC for SRAP was higher than that of the SSR because SRAP markers had lower allele frequency, and it ranged from 0.15 to 0.94 with a mean of 0.56 (**Table 4**). The pairwise similarity estimate using Nei's standard genetic distance ranged from 0.078 (NSL 83777 and NSL 83779) to 0.866 (PI 286245 and Orange). Both NSL 83777 and NSL 83779 are sorghum landraces from Cameroon, PI 286245 is from Sudan, and orange has no clear origin, as there are various versions of orange from

different origins. The SRAP markers also grouped the accessions according to their origin or breeding history, although the groups were different from the SSR groups. The differences between markers in clustering the accessions may be due to differences in genomic regions amplified by each marker type.

Cluster analysis based on neighbor joining using Nei's standard distances produced four major groups (**Figure 3**). Group 1 was occupied by accessions from East and West Africa and consisted of 59 accessions. Group 2 was the smallest with 9 accessions mainly from East Africa. Group 3 consisted of germplasm from both Botswana and North and South America. Unlike the SSRs grouping, the Nebraska lines featured in this group were very few and were mainly the released ones. Finally, Group 4 consisted mainly of the Nebraska breeding lines with some ICRISAT and India accessions. The SRAP markers seem to have separated the accessions well based on their breeding and origin compared to SSRs. Budak *et al*. [32] reported that SRAP markers are suitable in showing true variation within and among buffalo grass cultivars. Zhao *et al*. [30] also observed SRAPs clustering seemed to agree with morphological classification, although that was not the case with this study.

This study focused more and was limited by the number of morphological traits measured as well as the number of field experiments conducted. When looking at other types of molecular markers, Ritter *et al*. [1] observed that clusters developed based on agronomic data could not approximate groupings produced by molecular markers. When looking at within group clustering, one could observe smaller subgroups aligned to each country or breeding program .The main difference between the marker clusters could be due to the differences in marker type. Simple sequence repeats amplified randomly in the genome, whereas the SRAP amplified from the open reading frames or promoters of genes. Therefore, based on the study, SRAP was more informative in grouping accessions based on their breeding history.

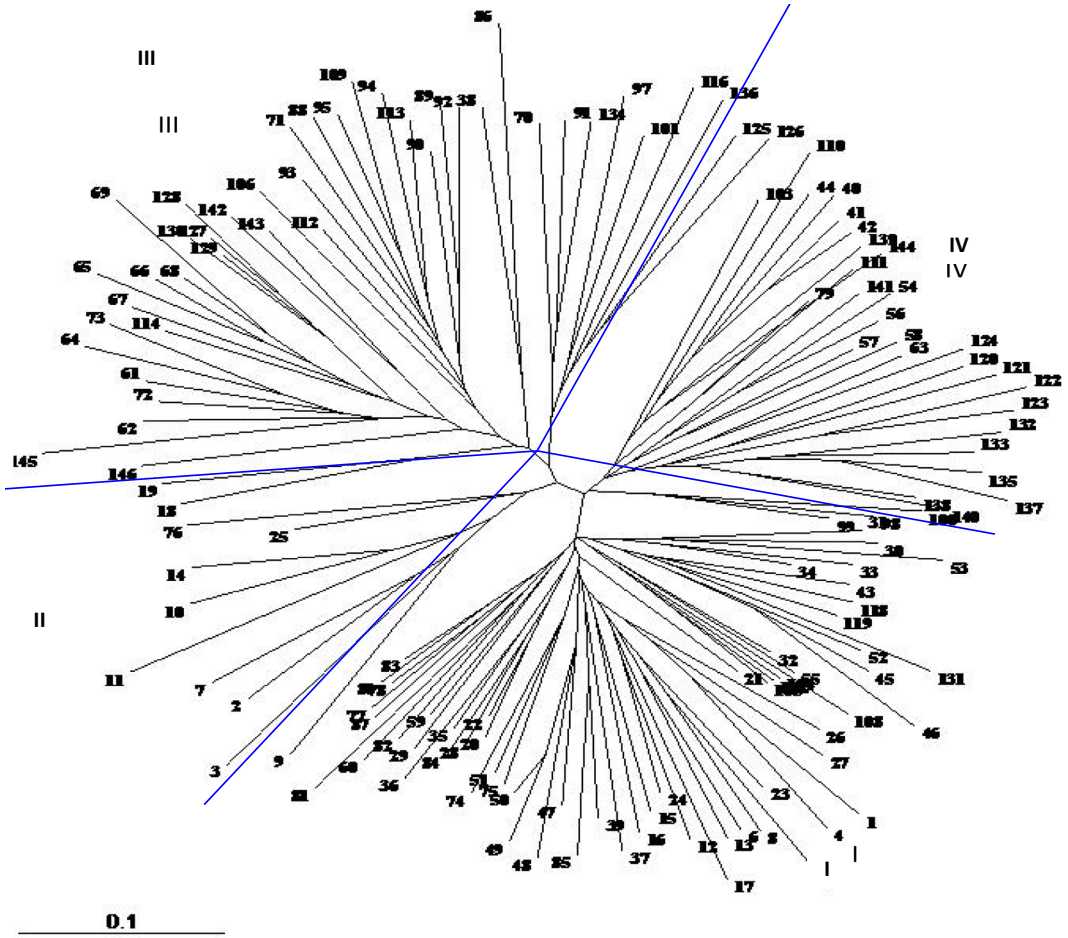

Figure 3. Dendrogram constructed by neighbor joining based on Nei's (1972) standard distances of 142 sorghum accessions based on SRAP data.

Comparing the three clustering procedures, it has been shown that each data type has its own strength but all seem to reflect the breeding history of the germplasms. The morphological data grouped accessions were based primarily on plant height, which is one of the characters that breeders base their selection on. Therefore, what the molecular markers (particularly SRAP) showed was what potential genes were selected for in these accessions. Wang *et al*. [52] reported that most of agronomic traits are affected by different levels of population structure and may therefore contribute to the differences observed between clustering conducted with different marker data.

4. Conclusions

The agronomic and molecular marker data produced distinct cluster groups for the sorghum accessions evaluated. Although the groups were not identical, they complemented each other. The agronomic data clusters provided clues to which important characters separated individual accessions, while SSR clusters further narrowed the groups based on their origin. The SRAP markers then even refined the groups as they showed the breeding pattern/history of the accessions. Perumal *et al*. [25] made similar observations, indicating that a more comprehensive and composite index based on pedigree, morphological, biochemical, and molecular data is expected to improve accuracy of grouping individuals.

This study also showed that there was a considerable amount of germplasm movement across different regions of the world, and there is still a large genetic diversity even within some regions. For example, some of the lines that were far from each other came from the same region. Previous reports have also indicated that the diversity of sorghum is limited in certain regions compared to others. Therefore, sorghum improvement will benefit from this wide range of diversity, and germplasm exchange will be the key to the success of improving sweet sorghum cultivars as a source for biofuel. This study has also strengthened the point that the use of molecular markers is essential and beneficial to plant breeders. The molecular markers are used to compliment the agronomic data when pedigree information is limited or unavailable.

References

[1] Ritter K.B., McIntyre, C.L. Godwin, I.D. Jordan, D.R. and Chapman, S.C. (2007) An Assessment of the Genetic Relationship between Sweet and Grain Sorghums, within *Sorghum bicolor* ssp. *bicolor* (L.) Moench, Using AFLP Markers. *Euphytica*, **157**, 161-176. http://dx.doi.org/10.1007/s10681-007-9408-4

[2] Hart, G., Schertz, K., Peng, Y. and Syed, N. (2001) Genetic Mapping of *Sorghum bicolor* (L.) Moench QTLs That Control Variation in Tillering and Other Morphological Character. *Theoretical and Applied Genetics*, **103**, 1232-1242. http://dx.doi.org/10.1007/s001220100582

[3] Ferraris, R. (1981) Early Assessment of Sweet Sorghum as an Agro-Industrial Crop. I. Varietal Evaluation. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **21**, 75-82. http://dx.doi.org/10.1071/EA9810075

[4] Vietor, D.M. and Miller, F.R. (1990) Assimilation, Partitioning, and Nonstructural Carbohydrate in Sweet Compared with Grain Sorghum. *Crop Science*, **30**, 1109-1115. http://dx.doi.org/10.2135/cropsci1990.0011183X003000050030x

[5] Murray, S.C., Rooney, W.L., Hamblin, M.T., Mitchell, S.E. and Kresovich, S. (2009) Sweet Sorghum Genetic Diversity and Association Mapping for Brix and Height. *Plant Genome*, **2**, 48-62. http://dx.doi.org/10.3835/plantgenome2008.10.0011

[6] Vaidyanathan, S., Rao, K.E.P., Mengesha, M.H. and Jambunathan, R. (1987) Total Sugar Content in Sorghum Stalks and Grains of Selected Cultivars from the World Germplasm Collection. *Journal of the Science of Food and Agriculture*, **39**, 289-295. http://dx.doi.org/10.1002/jsfa.2740390403

[7] Lipinsky, E.S., Kresovich, S., McClure, T.A. and Lawhon, W.T. (1977) Fuels from Sugar Crops. First Quarterly Report, Battelle Columbus Labs, Columbus.

[8] Paterson, A.H. (2008) Genomics of Sorghum: A Review. *International Journal of Plant Genomics*, **2008**, Article ID: 362451, 6 p. http://dx.doi.org/10.1155/2008/362451

[9] Swanson, A. and Laude, H. (1934) Varieties of Sorghum in Kansas. *Kansas Bulletin*, **266**, 2-50.

[10] Freeman, K.C. (1979) Germplasm Release of Sweet Sorghum Lines with Resistance to Downy Mildew, Leaf Anthracnose and Rust with Adequate Combining Ability to Produce Progeny with Agronomic Characters Acceptable for Commercial Sirup and/or Sugar Production. Mississippi Agricultural and Forestry Experiment Station Research Report, Vol. 4, No. 2.

[11] Schulman, A. (2007) Molecular Markers to Assess Genetic Diversity. *Euphytica*, **158**, 313-321. http://dx.doi.org/10.1007/s10681-006-9282-5

[12] Franco, J., Crossa, J., Ribaout, J.M., Betran, J., Warburton, M.L. and Khairallah, M. (2001) A Method for Combining

Molecular Markers and Phenotype Attributes for Classifying Plant Genotypes. *Theoretical and Applied Genetics*, **103**, 944-952. http://dx.doi.org/10.1007/s001220100641

[13] Ramakrishnan, A.P., Meyer, S.E., Walters, J., Stevens, M.R., Coleman, C.E. and Fairbanks, D.J. (2004) Correlation between Molecular Markers and Adaptively Significant Genetic Variation in *Bromus tectorum* (Poaceae), an Inbreedingannual Grass. *American Journal of Botany*, **91**, 797-803. http://dx.doi.org/10.3732/ajb.91.6.797

[14] Li, G. and Quiros, C.F. (2001) Sequence-Related Amplified Polymorphism (SRAP), a New Marker System Based on a Simple PCR Reaction: Its Application to Mapping and Gene Tagging in Brassica. *Theoretical and Applied Genetics*, **103**, 455-461. http://dx.doi.org/10.1007/s001220100570

[15] Tao, Y., Manners, J., Ludlow, M. and Henzell, R. (1993) DNA Polymorphisms in Grain Sorghum (*Sorghum bicolor* (L.) Moench). *Theoretical and Applied Genetics*, **86**, 679-688. http://dx.doi.org/10.1007/BF00222656

[16] Ahnert, D., Lee, M., Austin, D., Livini, C., Woodman, W., Openshaw, S., Smith, J., Porter, K. and Dalon, G. (1996) Genetic Diversity among Elite Sorghum Inbred Lines Assessed with DNA Markers and Pedigree Information. *Crop Science*, **36**, 1385-1392. http://dx.doi.org/10.2135/cropsci1996.0011183X003600050049x

[17] Uptmoor, R., Wenzel, W., Friedt, W., Donaldson, G., Ayisi, K. and Ordon, F. (2003) Comparative Analysis on the Genetic Relatedness of *Sorghum bicolor* Accessions from Southern Africa by RAPDs, AFLPs and SSRs. *Theoretical and Applied Genetics*, **106**, 1316-1325.

[18] Anas and Yoshida, T. (2004) Genetic Diversity among Japanese Cultivated Sorghum Assessed with Simple Sequence Repeats Markers. *Plant Production Science*, **7**, 217-223.

[19] Menz, M.A., Klein, R.R., Unruh, N.C., Rooney, W.L., Klein, P.E. and Mullet, J.E. (2004) Genetic Diversity of Public Inbreds of Sorghum Determined by Mapped AFLP and SSR Markers. *Crop Science*, **44**, 1236-1244. http://dx.doi.org/10.2135/cropsci2004.1236

[20] Warburton, M.L., Reif, J.C., Frisch, M., Bohn, M., Bedoya, C., Xia, X.C., Crossa, J., Franco, J., Hoisington, D., Pixley, K., Taba, S. and Melchinger, A.E. (2008) Genetic Diversity in CIMMYT Nontemperate Maize Germplasm: Landraces, Open Pollinated Varieties, and Inbred Lines. *Crop Science*, **48**, 617-624. http://dx.doi.org/10.2135/cropsci2007.02.0103

[21] Sharon, D., Cregan, P.B., Mhameed, S., Kusharska, M., Hillel, J., Lahav, E. and Lavi, U. (1997) An Integrated Genetic Linkage Map of Avocado. *Theoretical and Applied Genetics*, **95**, 911-921. http://dx.doi.org/10.1007/s001220050642

[22] Brown, S.M., Hopkins, M.S., Mitchell, S.E., Senior, M.L., Wang, T.Y., Duncan, R.R., Gonzalez-Candelas, F. and Kresovich, S. (1996) Multiple Methods for the Identification of Polymorphic Simple Sequence Repeats (SSRs) in Sorghum [*Sorghum bicolor* (L.) Moench]. *Theoretical and Applied Genetics*, **93**, 190-198. http://dx.doi.org/10.1007/BF00225745

[23] Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996) The Comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) Markers for Germplasm Analysis. *Molecular Breeding*, **3**, 225-238. http://dx.doi.org/10.1007/BF00564200

[24] Casa, A.M., Mitchell, S.E., Hamblin, M.T., Sun, H., Bowers J.E., Paterson, A.H., Aquadro, C.F. and Kresovich, S. (2005) Diversity and Selection in Sorhum: Simultaneous Analyses Using Simple Sequence Repeats. *Theoretical and Applied Genetics*, **111**, 23-30. http://dx.doi.org/10.1007/s00122-005-1952-5

[25] Perumal, R., Krishnaramanujan, R., Menz, M.A., Katilé, S., Dahlberg, J., Magill, C.W. and Rooney, W.L. (2007) Genetic Diversity among Sorghum Races and Working Groups Based on AFLPs and SSRs. *Crop Science*, **47**, 1375-1383. http://dx.doi.org/10.2135/cropsci2006.08.0532

[26] Ali, M.L., Rajewski, J.F., Baenziger, P.S., Gill, K.S., Eskridge, K.M. and Dweikat, I. (2008) Assessment of Genetic Diversity and Relationship among a Collection of US Sweet Sorghum Germplasm by SSR Markers. *Molecular Breeding*, **21**, 497-509. http://dx.doi.org/10.1007/s11032-007-9149-z

[27] Klein, R.R., Mullet, J.E., Jordan, D.R., Miller, F.R., Rooney, W.L., Menz, M.A., Franks, C.D. and Klein, P.E. (2008) The Effect of Tropical Sorghum Conversion and Inbred Development on Genome Diversity as Revealed by High-Resolution Genotyping. *Crop Science*, **48**, 512-526.

[28] Budak, H., Shearman, R.C., Parmaksiz, I., Gaussoin, R.E., Riordan, T.P. and Dweikat, I. (2004) Molecular Characterization of Buffalograss Germplasm Using Sequence-Related Amplified Polymorphism Markers. *Theoretical and Applied Genetics*, **108**, 328-334. http://dx.doi.org/10.1007/s00122-003-1428-4

[29] Ariss, J.J. and Vandemark, G.J. (2007) Assessment of Genetic Diversity among Non-Dormant and Semi-Dormant Alfalfa Populations Using Sequence-Related Amplified Polymorphism. *Crop Science*, **47**, 2274-2284. http://dx.doi.org/10.2135/cropsci2006.12.0782

[30] Zhao, W., Fang, R., Pan, Y., Yang, Y., Chung, J.W., Chung, I.M. and Park, Y.J. (2009) Analysis of Genetic Relationships of Mulberry (*Morus* L.) Germplasm Using Sequence-Related Amplified Polymorphism (SRAP) Markers. *African Journal of Biotechnology*, **8**, 2604-2610.

[31] Han, X., Wang, L., Liu, Z.A., Jan, D.R. and Shu, Q. (2008) Characterization of Sequence-Related Amplified Polymorphism Markers Analysis of Tree Peony Bud Sports. *Scientia Horticulturae*, **115**, 261-267. http://dx.doi.org/10.1016/j.scienta.2007.09.003

[32] Budak, H., Shearman, R.C., Parmaksiz, I. and Dweikat, I. (2004) Comparative Analysis of Seeded and Vegetative Biotype Buffalograss Based on Phylogenetic Relationship Using ISSRs, SSRs, RAPDs, and SRAPs. *Theoretical and Applied Genetics*, **109**, 280-288. http://dx.doi.org/10.1007/s00122-004-1630-z

[33] Lin, Z.X., Zhang, X.L. and Nie, Y.C. (2004) Evaluation of Application of a New Molecular Marker SRAP on Analysis of F2 Segregation Population and Genetic Diversity in Cotton. *Acta Genetica Sinica*, **31**, 622-626.

[34] Ferriol, M., Pico, B. and Nuze, F. (2003) Genetic Diversity of a Germplasm Collection of *Cucurbita pepo* Using SRAP and AFLP Markers. *Theoretical and Applied Genetics*, **107**, 271-282. http://dx.doi.org/10.1007/s00122-003-1242-z

[35] Fufa, H., Baenziger, P.S., Beecher, B.S., Dweikat, I., Graybosch, R.A. and Eskridge, K.M. (2005) Comparison of Phenotypic and Molecular Marker-Based Classifications of Hard Red Winter Wheat Cultivars. *Euphytica*, **145**, 133-146. http://dx.doi.org/10.1007/s10681-005-0626-3

[36] Zaefizadeh, M. and Goliev, R. (2009) Diversity and Relationships among Durum Wheat Landraces (Subconvars) by SRAP and Phenotypic Marker Polymorphism. *Research Journal of Biological Sciences*, **4**, 960-966.

[37] Folkertsma R., Frederick, H., Rattunde, W., Chandra, S., Raju, G. and Hash, C. (2005) The Pattern of Genetic Diversity of Guinea-Race *Sorghum bicolor* (L.) Moench Landraces as Revealed with SSR Markers. *Theoretical and Applied Genetics*, **111**, 399-409. http://dx.doi.org/10.1007/s00122-005-1949-0

[38] Gorz, H.J., Haskins, F.A. and Johnson, B.E. (1990) Registration of 15 Germplasm Lines of Grain Sorghum and Sweet Sorghum. *Crop Science*, **30**, 762-763. http://dx.doi.org/10.2135/cropsci1990.0011183X003000030089x

[39] Dweikat, I. (2005) A Diploid, Interspecific, Fertile Hybrid from Cultivated Sorghum, *Sorghum bicolor*, and the Common Johnsongrass Weed *Sorghum halepense*. *Molecular Breeding*, **16**, 93-101. http://dx.doi.org/10.1007/s11032-005-5021-1

[40] Schloss, S.J., Mitchell, S.E., White, G.M., Kukatla, R., Bowers, J.E., Paterson, A.H. and Kresovich, S. (2002) Characterization of RFLP Probe Sequences for Gene Discovery and SSR Development in *Sorghum bicolor* (L.) Moench. *Theoretical and Applied Genetics*, **105**, 912-920. http://dx.doi.org/10.1007/s00122-002-0991-4

[41] Riaz, A., Li, G., Quresh, Z., Swati, M.S. and Quiros, C.F. (2001) Genetic Diversity of Oilseed *Brassica napus* Inbred Lines Based on Sequence-Related Amplified Polymorphism and Its Relation to Hybrid Performance. *Plant Breeding*, **120**, 411-415. http://dx.doi.org/10.1046/j.1439-0523.2001.00636.x

[42] Kuleung, C., Baenziger, P. S. and Dweikat, I. (2004) Transferability of SSR Markers among Wheat, Rye and Triticale. *Theoretical and Applied Genetics*, **108**, 1147-1150. http://dx.doi.org/10.1007/s00122-003-1532-5

[43] Flury, B., and Riedwyl, H. (1986) Standard Distance in Univariate and Multivariate Analysis. *The American Statistician*, **40**, 249-251.

[44] SAS Institute Inc. (2008) User's Guide, Version 9.2. SAS Institute Inc., Cary.

[45] Anderson, J.A., Churchill, G.A., Autrique, J.E., Sorells, M.E. and Tanksley, S.D. (1993) Optimizing Parental Selection for Genetic Linkage Maps. *Genome*, **36**, 181-186. http://dx.doi.org/10.1139/g93-024

[46] Botstein, D., White, R.L. Skolnick, M. and Davis, R.W. (1990) Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphism. *The American Journal of Human Genetics*, **32**, 314-331.

[47] Nei, M. and Li, W.H. (1979) Mathematical Model for Studying Genetic Variation in Terms of Restriction Endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 5269-5273. http://dx.doi.org/10.1073/pnas.76.10.5269

[48] Weising, K., Nyborn, H., Wolff, K. and Kahl, G. (2005) DNA Fingerprinting in Plants: Principles, Methods, and Applications. 2nd Edition, CRC Press, New York. http://dx.doi.org/10.1201/9781420040043

[49] Nei, M. (1972) Genetic Distance between Populations. *The American Naturalist*, **106**, 283-292. http://dx.doi.org/10.1086/282771

[50] Takezaki, N. and Nei, M. (1996) Genetic Distances and Reconstruction of Phylogenetic Tree from Microsatellite DNA. *Genetics*, **144**, 389-399.

[51] Page, R.D.M. (1996) TreeView: An Application to Display Phylogenetic Trees on Personal Computers. *Computer Applications in the Biosciences*, **12**, 357-358.

[52] Wang, M.L., Zhu, C., Barkley, N.A., Chen, Z., Erpelding, J.E., Murray, S.C., Tuinstra, M.R., Tesso, T., Paterson, G.A. and Yu, J. (2009) Genetic Diversity and Population Structure Analysis of Accessions in the US Historic Sweet Sorghum Collection. *Theoretical and Applied Genetics*, **120**, 13-23. http://dx.doi.org/10.1007/s00122-009-1155-6

Appendix 1

List of germplasm accessions used in diversity study, their origin, year of registration and parentage.

Entry	Name	Designation/Accession No.	Registration Year	Place of Origin	Parentage/Pedigree
1		PI 217892	1954	Sudan	
2		PI 246698	1958	India	
3		PI 276804	1961	Ethiopia	
4		PI 286245	1963	India	Sudan collection
5		PI 287611	1963	Zimbabwe	
6		PI 329336	1968	Ethiopia	
7	Durra	PI 329761	1968	Ethiopia	
8		PI 562943	1992	Sudan	Landrace
9		PI 569009	1993	Sudan	Wild collection
10		PI 569154	1993	Sudan	Landrace
11		PI 569283	1993	Sudan	Landrace
12		PI 569295	1993	Sudan	Landrace
13		PI 569520	1993	Sudan	Cross 45/6
14	PN 4135	PI 569590	1993	Sudan	PN 4135 (Breeding line)
15	PN 4288	PI 569597	1993	Sudan	PN 4288 (Breeding line)
16	PN 5043	PI 569644	1993	Sudan	PN 5043 (Breeding line)
17	PN 6058	PI 569670	1993	Sudan	PN 6058 (Breeding line)
18	Waramsara	PI 570717	1993	Sudan	Landrace
19	Mesera	PI 570718	1993	Sudan	Landrace
20	Sinidyl	PI 570731	1993	Sudan	Landrace
21	Thok brown	PI 570747	1993	Sudan	Landrace
22	Ani-el-gaong	PI 570753	1993	Sudan	Landrace
23	SBI 100	PI 570759	1993	Sudan	
24	UT 69	PI 570761	1993	Sudan	
25	Maluk	PI 570775	1993	Sudan	Landrace
26	Wad akar 9	PI 570877	1993	Sudan	Landrace
27	Feterita	PI 570957	1993	Sudan	Landrace
28	Kawanda L53	PI 571067	1993	Sudan	Landrace
29	Kawanda L31	PI 571068	1993	Sudan	Landrace
30	Msambiji	PI 571073	1993	Sudan	Landrace
31	Zerazera	PI 571120	1993	Sudan	Landrace
32	Kalili	PI 571126	1993	Sudan	Landrace
33	Karinaka	PI 571176	1993	Sudan	Landrace
34	A 154	PI 571276	1993	Sudan	Landrace

Continued

35	A 211	PI 571284	1993	Sudan	Landrace
36		PI 571370	1993	Sudan	Landrace
37	Wad bashir 3	PI 586791	1967	Sudan	Landrace
38		NSL 50393 (PI 651101)	1968	Indiana	Landrace
39		NSL 54316	1967	Uganda	Breeding line
40		NSL 55404	1967	India	Landrace
41		NSL 55429	1967	India	
42		NSL 55431	1967	India	
43	EC 21415	NSL 55645	1967	Uganda	
44	Hundijowar	NSL 76942	1970	India	Landrace
45		NSL 82099	1972	Cameroon	Landrace
46		NSL 83601	1973	Cameroon	Landrace
47		NSL 83611	1973	Cameroon	Landrace
48		NSL 83656	1973	Cameroon	Landrace
49		NSL 83777	1973	Cameroon	Landrace
50		NSL 83779	1973	Cameroon	Landrace
51		NSL 83984	1973	Cameroon	Breeding line
52		NSL 87920	1974	Cameroon	Landrace
53		NSL 92446	1976	Ethiopia	Landrace
54		NSL 92465 (Orange-red)	1976	Ethiopia	Landrace
55		NSL 103374	1979	Cameroon	Landrace
56		NSL 92465 (White)	1976	Ethiopia	Landrace
57		NSL 92465 (Red)	1976	Ethiopia	Landrace
58	Green leaf	NSL 4028	1955	Texas	Leoti-Sudan 2/Leoti-Sudan 4
59	Roma		1993	South Africa	Sudan grass type variety grown in Texas
60	Theis	CSR 216	1978	Mississippi	(Wiley/C.P. Special)/(MN1054/ White African)/MN660
61	Dale	NSL 74333	1973	Mississippi	Tracy/MN960 (PI 152857)
62	Cowley	NSL 189405	1985	Texas	Mer.64-7/Mer.64-6 (F2 selection)
63	05CO9810 (4) F3		2005 - nusery	Nebraska	
64	Mall			Botswana	Sweet sorghum collection
65	SC - 154			Botswana	Sweet sorghum collection
66	PMC - 18	PI 510906	1980	Botswana	Landrace
67	PMC - 5	PI 510893	1980	Botswana	Landrace
68	SC - 163			Botswana	
69	SC - 15			Botswana	
70	SC - 161			Botswana	

Continued

71	SC - 157			Botswana	
72	PSA - 160	PI 511004	1980	Botswana	Landrace
73	PMK - 80	PI 510942	1980	Botswana	Landrace
74	IPWA 1	IS 19674	1975	Zimbabwe	Landrace
75	A 157	IS 9890	1974	Sudan	Landrace
76		IS 22636	1980	Cameroon	Landrace
77	Ikumba	IS 20962	1979	Kenya	Landrace
78	Evsitu (short)	IS 21005	1979	Kenya	Landrace
79		IS 21991	1979	India	Landrace
80	Andiwo ma rabour	IS 21229	1979	Kenya	Landrace
81	Ochuti ma rabour	IS 21235	1979	Kenya	Landrace
82	Sabina	IS 20984	1979	Kenya	Landrace
83	Andiwo	IS 21100	1979	Kenya	Landrace
84	Andiwo ma rabour	IS 21260	1979	Kenya	Landrace
85	Hegari 6645-27-1-4-2	IS 131	1974	Ohio, USA	Hegari 6645-27-1-4-2
86		IS 20888	1979	Angola	Breeding line
87	Olusi	IS 20963	1979	Kenya	Landrace
88	Sabina	IS 20974	1979	Kenya	Landrace
89	N98 short	PI 535783	1990	Nebraska	(Waconia//AN39/ N4692-Rio)/Fremont
90	N98 tall	PI 535783	1990	Nebraska	(Waconia//AN39/N4692-Rio)/ Fremont
91	N99	PI 535784	1990	Nebraska	Fremont/Theis
92	N100	PI 535785	1990	Nebraska	Waconia/Wray
93	N108	PI 535793	1990	Nebraska	Inbred derived from Saccharum sorgo
94	Wheatland	CIso 918	1936	Oklahoma	Milo/Kafir
95	Norkan	NSL 4002	1942	Kansas	Atlas/Early Sumac
97	ICSR56	IS 84, IS 517		ICRISAT	Restorer line
98	ICSR160	IS 84, IS 517		ICRISAT	Restorer line
99	ICSR196	IS 84, IS 517		ICRISAT	Restorer line
100	ICSR90017	IS 1055		ICRISAT	F1 MS/Jowar BP53 (MS/IS 1055)-Restorer line
101	ICSRP3034			ICRISAT	Restorer line
102	ICSV700	IS 3443		ICRISAT	Restorer line
103	S35	PI 602982	1980	Nigeria	(SPV 35/E35-1)/CS 3541
104	E36-1			Ethiopia	
105	NTJ2				
106	Seredo			Kenya	
108	Grassl	PI 154844 01 SD	1946	Uganda	Introduced as “Lwera”
109		PI 175919 01 SD	1949	Maryland	Turkey
110	Suki	PI 217768 02 SD	1954	Sudan	

Continued

111	Chinese Amber	PI 22913 04 SD	1908	China	
112	Chinese Amber	PI 248298 01 SD	1958	India	
113	Mf.G.F.:383	PI 257294 02 SD	1959	Argentina	
114	Mf.G.F.:581	PI 257295 03 SD	1959	Argentina	
116	Perennial sweet Sudan	PI 562717 01 SD	1992	Texas	
118	Ajax Sweet	PI 571103 01 SD	1993	Sudan	
119		PI 591038 01 SD	1995	Nigeria	
120		05C09880-1(2)	2006 - nursery	Nebraska	(ms7//Tx430)/mix of Dale, Wray & Sugar Drip
121		05C09881msTAN	2006 - nursery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
122		05C09889-1-3 tall tan	2006 - nursery	Nebraska	(122 ms3/Wheatland-bmr12)/Dale
123		05C09890(1) PP bmr	2006 - nursery	Nebraska	(122 ms3/Wheatland-bmr12)/Dale
124		05C09892 (3)-2 tanmedbmr	2006 - nursery	Nebraska	(128 ms3/Wheatland-bmr6)/Wray
125		05C09654(3) sw	2006 - nursery	Nebraska	(ms3/Wheatland-bmr6)/C297
126	65D			Botswana	Unknown introduction from USA
127	Kanye standard	PI 540519		Botswana	Landrace
128	Marupantse	PI 540516		Botswana	Landrace
129	Mokula			Botswana	Landrace
130	Segaolane	PI 540518		Botswana	Landrace
131	Sureno	PI 561472		Hunduras	[(SC423/CS3541)E35-1]-2
132		05C09882 (1) tanbmr	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
133		05C09882(3) tanbmr	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
134		05C09889(1) vtallsw	2005 - nusery	Nebraska	(122 ms3/Wheatland-bmr12)/N99
135		05C09892(3)-4 tallbmr	2005 - nusery	Nebraska	(122 ms3/Wheatland-bmr12)/N99
136		05C09880(3)tan	2005 - nusery	Nebraska	(ms7//Tx430)/mix of Dale, Wray & Sugar Drip
137		05C09881(4)ppbmr	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
138		05C09882(5) tan	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
139		05C09882(8) tanbmr	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
140		05C09882(9) tanOP	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/mix of Dale, Wray, Sugar Drip
141		05C09892(6) ppbmrsw	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/Wray
142		05C09892(3) tanbmr	2005 - nusery	Nebraska	(128 ms3/Wheatland-bmr6)/Wray
143		05C09891(2) bmr	2005 - nusery	Nebraska	(122 ms3/Wheatland-bmr12)/N98
144	SN372 - Chinese Amber			Texas	
145	Orange				
146	Blackstrap			Kansas	

Appendix 2

Least square means of anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) for the 142 accessions evaluated at Mead in 2009.

Accession Number/Name	AD (days)	PH (cm)	ML (%)	DM (Mg·ha^{-1})
PI 217892	81.9	278.3	67.8	18.21
PI 246698	92.5	288.3	66.8	29.23
PI 276804	76.7	161.2	67.4	3.81
PI 286245	98.5	262.7	60.1	22.67
PI 287611	116.6	380.1	60.2	41.86
PI 329336	121.5	338.0	67.5	38.51
PI 329761	101.3	247.2	67.6	24.56
PI 562943	96.8	336.1	57.4	49.32
PI 569009	93.8	356.2	57.0	52.40
PI 569154	85.7	248.5	66.3	8.59
PI 569283	84.5	175.1	53.7	6.42
PI 569295	88.7	307.3	74.5	17.55
PI 569520	84.3	287.3	73.6	16.89
PI 569590	90.9	318.4	70.7	23.26
PI 569597	87.7	226.6	53.3	19.82
PI 569644	86.2	290.7	64.3	17.85
PI 569670	84.4	303.1	78.6	17.33
PI 570717	102.4	262.8	58.0	27.90
PI 570718	84.3	207.6	62.6	20.00
PI 570731	87.9	287.6	64.8	31.93
PI 570747	112.9	318.4	61.9	41.42
PI 570753	83.0	193.0	60.6	15.48
PI 570759	89.8	76.0	61.4	7.63
PI 570761	120.0	423.8	56.9	52.28
PI 570775	90.0	235.8	63.8	21.05
PI 570877	75.2	204.3	56.8	6.88
PI 570957	138.1	389.1	63.7	36.69
PI 571067	81.0	313.3	76.0	17.68
PI 571068	83.2	189.2	68.3	11.50
PI 571073	110.7	350.3	60.8	24.27
PI 571120	76.9	317.3	71.5	18.53
PI 571126	93.6	335.6	67.9	33.00
PI 571176	122.8	393.1	65.8	20.81
PI 571276	78.5	281.1	69.8	26.06

Continued

PI 571284	98.8	299.4	70.4	15.05
PI 571370		356.0	70.2	28.57
PI 586791	111.1	375.8	70.0	42.54
NSL 50393	95.4	337.3	62.6	21.67
NSL 54316	82.0	250.8	62.8	22.97
NSL 55404	95.0	348.3	76.0	28.52
NSL 55429	91.0	253.9	62.6	22.79
NSL 55431	100.2	323.5	52.2	33.20
NSL 55645	77.5	176.8	71.8	10.00
NSL 76942	147.2	303.7	67.6	25.92
NSL 82099	97.5	242.2	68.8	25.36
NSL 83601	93.8	328.8	65.2	27.70
NSL 83611	87.5	159.4	61.0	11.89
NSL 83656		311.0	71.9	31.91
NSL 83777	108.3	323.6	70.7	32.14
NSL 83779	99.6	301.7	62.9	30.84
NSL 83984	91.0	92.4	49.0	12.37
NSL 87920	91.4	317.5	56.2	32.78
NSL 92446	105.1	390.5	56.4	42.63
NSL 92465	97.2	176.3	59.8	23.73
NSL 103374	.	281.0	71.1	32.68
NSL 92465	88.0	104.3	58.9	14.79
NSL 92465	98.3	281.8	61.8	32.45
Green leaf	84.4	259.5	73.9	12.95
Roma	108.8	316.6	61.0	34.74
Theis	89.0	200.8	62.8	30.50
Dale	116.8	375.1	66.5	37.25
Wray	95.8	279.5	68.9	34.31
05CO9810 (4) F3	98.8	180.6	66.2	19.68
Mall	83.0	270.1	67.5	21.14
SC-154	88.8	235.4	66.4	23.84
PMC-18	99.2	375.3	65.6	38.62
PMC-5	80.2	290.6	72.3	15.17
SC-163	90.5	224.3	67.9	12.13
SC-151	84.5	209.0	64.8	17.16
SC-161	79.8	280.1	69.6	18.96
SC-157	121.0	346.3	60.0	39.90

Continued

PSA-160	75.5	260.0	67.2	21.19
PMK-80	80.8	279.5	69.0	26.53
IS 19674	85.0	316.9	69.1	25.59
IS 9890	109.3	319.9	56.1	31.22
IS 22636	75.7	97.5	50.5	9.00
IS 20962	75.5	146.6	55.6	7.75
IS 21005	83.2	345.6	70.4	19.12
IS 21991	89.3	352.3	55.6	31.89
IS 21229	93.4	351.9	63.3	32.39
IS 21235	99.4	408.3	73.2	34.63
IS 20984	85.8	151.3	61.5	10.09
IS 21100	84.4	129.7	57.9	7.30
IS 21260	88.0	229.2	57.0	21.28
IS 131	89.5	105.3	61.6	5.89
IS 20888	78.3	256.0	69.5	16.90
IS 20963	86.1	188.2	62.6	12.86
IS 20974	91.6	341.1	56.1	33.81
N98 short	88.8	267.2	57.7	33.94
N98 tall	116.0	395.7	68.8	25.92
N99	120.7	419.3	68.4	59.19
N100		212.5	75.8	11.30
N108	116.9	330.6	70.3	37.73
N104	83.1	188.9	71.2	20.44
N110	86.8	161.7	64.5	16.09
ICSR56	79.9	320.9	55.3	29.66
ICSR160	104.5	219.5	61.6	8.45
ICSR196	69.5	265.2	67.9	13.01
ICSR90017	80.8	247.6	68.5	14.79
ICSRP3034	85.9	309.1	71.6	15.20
ICSV700	99.1	304.5	64.6	28.11
S35	79.8	186.0	64.1	13.74
E36-1	93.3	346.7	60.7	38.93
NTJ2	86.5	218.6	68.8	19.66
Seredo	95.3	223.3	63.7	28.88
PI 154844	73.0	264.9	62.2	20.71
PI 175919	83.3	174.8	59.1	11.13
PI 217768	75.3	226.5	61.0	22.42

Continued

PI 22913	112.9	325.9	79.6	16.46
PI 248298	87.0	248.5	65.7	16.51
PI 257294	83.8	251.1	68.1	20.38
PI 257295	87.1	338.2	59.0	19.61
PI 562717	80.4	217.2	55.1	16.30
PI 571103	97.5	307.0	64.5	30.24
PI 591038	122.8	311.4	61.6	23.13
05C09880-1 (2)	101.9	388.7	59.6	30.21
05C09881	93.8	379.2	60.0	29.20
05C09889-1-3	76.2	84.1	48.1	6.25
05C09890(1)	85.6	277.0	75.4	20.92
05C09892 (3)-2	84.8	274.3	71.5	18.28
05C09654 (3)	86.2	300.9	66.7	28.78
65D	81.8	245.1	65.9	21.50
Kanye standard	96.1	337.7	51.2	30.32
Marupantse	97.6	220.0	66.4	23.76
Mokula	83.9	297.6	67.9	25.32
Segaolane	84.1	337.6	67.5	20.18
Sureno	73.6	229.7	45.4	19.29
05C09882 (1)	76.8	350.9	68.7	25.69
05C09882 (3)	91.5	290.1	80.6	19.00
05C09889 (1)	98.8	322.8	69.7	31.61
05C09892 (3)-4	84.5	325.1	77.5	24.24
05C09880 (3)	102.1	351.5	63.4	46.57
05C09881 (4)		234.5	71.7	18.89
05C09882 (5)	94.8	225.8	67.1	23.58
05C09882 (8)	95.2	323.7	62.7	23.09
05C09882 (9)	90.0	272.1	66.9	24.58
05C09892 (6)	101.5	269.2	60.6	26.69
05C09892 (3)	87.2	160.8	49.2	16.93
05C09891 (2)	71.8	248.1	62.9	13.66
SN372	80.5	257.1	63.8	24.70
Orange	100.8	407.3	63.9	53.80
Blackstrap	121.0	390.5	67.5	44.95
Mean	92.4	275.5	64.6	24.20
CV	2.91	9.42	4.83	28.77

Appendix 3

The cluster groups based on average distances of anthesis date (AD), plant height (PH), moisture content (ML) and total biomass (DM) planted at Mead in 2009.

Group 1					
Entry	**Label**	**Origin**	**Entry**	**Label**	**Origin**
V1	PI 217892	Sudan	V73	PMK-80	Botswana
V2	PI 246698	India	V84	IS 21260	Kenya
V4	PI 286245	India	V86	IS 20888	Angola
V7	PI 329761	Ethiopia	V89	N98 short	Nebraska
V10	PI 569154	Sudan	V97	ICSR160	ICRISAT
V15	PI 569597	Sudan	V98	ICSR196	ICRISAT
V18	PI 570717	Sudan	V99	ICSR90017	ICRISAT
V19	PI 570718	Sudan	V104	NTJ2	
V20	PI 570731	Sudan	V105	Seredo	Kenya
V25	PI 570775	Sudan	V106	PI 154844	Uganda
V26	PI 570877	Sudan	V108	PI 217768	Sudan
V34	PI 571276	Sudan	V110	PI 248298	India
V39	NSL 54316	Uganda	V111	PI 257294	Argentina
V41	NSL 55429	India	V113	PI 562717	Texas
V45	NSL 82099	Cameroon	V119	05C09890(1)	Nebraska
V57	NSL 92465	Ethiopia	V120	05C09892 (3) - 2	Nebraska
V58	Green leaf	Texas	V122	65D	Botswana
V62	Wray	Texas	V124	Marupantse	Botswana
V64	Mall	Botswana	V127	Sureno	Hunduras
V65	SC-154	Botswana	V134	05C09882(5)	Nebraska
V68	SC-163	Botswana	V136	05C09882(9)	Nebraska
V69	SC-151	Botswana	V137	05C09892(6)	Nebraska
V70	SC-161	Botswana	V139	05C09891(2)	Nebraska
V72	PSA-160	Botswana	V140	SN372	Texas
Group 2					
Entry	**Label**	**Origin**	**Entry**	**Label**	**Origin**
V5	PI 287611	Zimbabwe	V71	SC-157	Botswana
V6	PI 329336	Ethiopia	V74	IS 19674	Zimbabwe
V8	PI 562943	Sudan	V75	IS 9890	Sudan
V9	PI 569009	Sudan	V78	IS 21005	Kenya
V12	PI 569295	Sudan	V79	IS 21991	India
V13	PI 569520	Sudan	V80	IS 21229	Kenya
V14	PI 569590	Sudan	V88	IS 20974	Kenya

Continued

V16	PI 569644	Sudan	V93	N108	Nebraska
V17	PI 569670	Sudan	V96	ICSR56	ICRISAT
V21	PI 570747	Sudan	V100	ICSRP3034	ICRISAT
V28	PI 571067	Sudan	V101	ICSV700	ICRISAT
V30	PI 571073	Sudan	V103	E36-1	Ethiopia
V31	PI 571120	Sudan	V109	PI 22913	China
V32	PI 571126	Sudan	V112	PI 257295	Argentina
V35	PI 571284	Sudan	V114	PI 571103	Sudan
V37	PI 586791	Sudan	V115	PI 591038	Nigeria
V38	NSL 50393	Indiana	V121	05C09654 (3)	Nebraska
V40	NSL 55404	India	V123	Kanye standard	Botswana
V42	NSL 55431	India	V125	Mokula	Botswana
V44	NSL 76942	India	V126	Segaolane	Botswana
V46	NSL 83601	Cameroon	V128	05C09882 (1)	Nebraska
V49	NSL 83777	Cameroon	V129	05C09882 (3)	Nebraska
V50	NSL 83779	Cameroon	V130	05C09889 (1)	Nebraska
V52	NSL 87920	Cameroon	V131	05C09892 (3)-4	Nebraska
V59	Ror 9	South Africa	V132	05C09880 (3)	Nebraska
V61	Dale	Mississippi	V135	05C09882 (8)	Nebraska
V66	PMC-18	Botswana	V142	Blackstrap	Kansas
V67	PMC-5	Botswana			
Group 4			**Group 3**		
Entry	**Label**	**Origin**	**Entry**	**Label**	**Origin**
V3	PI 276804	Ethiopia	V24	PI 570761	Sudan
V47	NSL 83611	Cameroon	V81	IS 21235	Kenya
V95	N110	Kansas	V53	NSL 92446	Ethiopia
V82	IS 20984	Kenya	V117	05C09881	Nebraska
V77	IS 20962	Kenya	V116	05C09880-1 (2)	Nebraska
V138	05C09892(3)	Nebraska	V91	N99	Nebraska
V54	NSL 92465	Ethiopia	V141	Orange	
V63	05CO9810 (4) F3	Nebraska	V27	PI 570957	Sudan
V11	PI 569283	Sudan	V33	PI 571176	Sudan
V107	PI 175919	Maryland	V90	N98 tall	Nebraska
V143	Sugar Drip				
V43	NSL 55645	Uganda		Group 5	
V94	N104	Oklahoma	Entry	Label	Origin

Continued

V22	PI 570753	Sudan	V23	PI 570759	Sudan
V29	PI 571068	Sudan	V51	NSL 83984	Cameroon
V87	IS 20963	Kenya	V118	05C09889-1-3	Nebraska
V102	S35	Nigeria	V56	NSL 92465	Ethiopia
V60	Theis	Mississippi	V76	IS 22636	Cameroon
			V85	IS 131	Ohio
			V83	IS 21100	Kenya