African Journal of Biotechnology Vol. 12(1), pp. 1-7, 2 January, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2909
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Genetic variability of tissue cultured *Sorghum bicolor* (L) Moench as revealed by morphological traits and simple sequence repeats (SSR) markers

E. G. O. Omondi[1]*, M. N. Makobe[1], L. G. Matasyoh[2] and C. A. Onyango[3]

[1]Department of Botany, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 6000, Nairobi, Kenya.
[2]Department of Biological Science, Chepkoilel University College, Nairobi, Kenya.
[3]Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.

Accepted 17 August, 2012

To evaluate their performance for seedling traits at seedling stage (under hydroponics), plant water relations under water stress and ultimately grain yield, and to estimate the genetic variability of the regenerates, the parent plants of local sorghum cultivars in Kenya using simple sequence repeats (SSR) markers were analyzed. The research involved Seredo, El Gardam and Mtama 1 sorghum cultivars. The TC (Tissue culture) regenerates were produced through somatic embryogenesis in Linsmaier and Skoog (LS) media augmented with an osmoticum, NaCl (100 mM) in attempt to improve the parent plant for water stress tolerance. The genetic variability was investigated using five SSR markers and seven morphological traits associated with water stress tolerance. The amplified DNA fragments were screened by gel electrophoresis using 2% agarose. Cluster and principal component analysis (PCA) were done using NTSYS-pc version 2.2. The SSR detected 11 alleles with an average of 2.2 alleles per locus. The number of alleles per locus ranged from two to three and the allelic polymorphism information content (PIC) value ranged from 0.344 for *Xcup14* to 0.730 for *Xgap72* with an average of 0.559. These results show that the cultivars differed for morphological and SSR markers. The average genetic similarity based on the morphological traits (0.922 with a range of 0.845 to 0.989) was higher than SSR markers (0.625 with a range of 0.306 to 0.944). The results also show that the classification of these sorghum cultivars may be useful to the breeders to plan crosses for preferred traits. The results also show that the sorghum microsatellites can be used to distinguish the cultivars and to estimate genetic diversity due to somaclonal variation. Overall, this study shows that tissue culture technique might not add value in producing somaclonal variation in sorghum.

Key words: Cultivars, tissue culture regenerates, water stress tolerance, simple sequence repeats.

INTRODUCTION

Plant cell and tissue culture methods are now considered generate somaclonal variation. Tissue culture techniques to be simple and easy to use for plant propagation and to are used to produce minimal changes in plant variety where other changes are not desired. Cytodifferentiation and transformation of embryonic callus for wheat has been reported by Ebiamado et al. (2000). Evaluation of tissue culture derived somaclones of sorghum has resulted in variants with increased or decreased levels of tannins (Cai et al., 1995). Sorghum somaclonal variants resistant to head smut (*Sphaselothe careliana*) have also been established (Wang et al., 1997).

A comprehensive analysis of genetic variation is essential for genetic conservation strategies that may include

*Corresponding author. E-mail: eomondi2008@gmail.com. Tel: 254 733 229 682.

Abbreviations: UPGMA, Unweighted pair group method of arithmetic average; **TC,** Tissue culture.

breeding programmes in future. This approach has also enabled unique collection of favorable alleles (Swaminathan, 1997; Camlin, 2003). Traditional methods of cultivar identification frequently are based on the evaluation of sets of morphological traits. Though these methods are cost-effective, they have limitations such as insufficient variation among cultivars (especially if the cultivars to be compared share a closely related pedigree), subjectivity in the analysis, influence of the environment and management practice, and expression of some characters only in certain developmental stages. These necessitate alternative means of cultivar identification, including allozyme analyses, cytogenetics, analysis of secondary metabolites, and DNA profiling (Camlin, 2001).

Genetic finger printing is one of the DNA based techniques with various applications in crop biology including assessment of taxonomy and phylogeny, diversity analysis, hybridity testing, gene mapping, molecular breeding and somaclonal variations (Mehmood et al., 2008). Molecular markers are considered constant landmarks in the genome. Markers are identifiable DNA sequences found at specific locations of the genome for example, simple sequence repeats (SSRs), and transmitted by the standard laws of inheritance from one generation to the next. Due to its global socio-economic importance, there has been substantial interest in characterizing the levels of genetic diversity present within sorghum using both phenotypic and molecular markers especially SSRs (Grenier et al., 2001; Ghebru et al., 2002; Uptmoor et al., 2003; Menz et al., 2004). SSR markers are a preference for studying genetic diversity because they are abundant in plant and animal genomes (Roder et al., 1995), have high levels of polymorphism (Schug et al., 1998) and are adaptable to automation (Mitchell et al., 1997). Numerous SSR markers have been developed and mapped for sorghum (Taramino et al., 1997; Schloss et al., 2002).

In this study, the three local cultivars of sorghum were collected from Katumani research station, Kenya. The three cultivars Mtama 1 (creamish in colour, no testa and no tannin), Seredo (brown with testa and high tannin) and El Gardam (chalky white with testa and no tannin) are a representative of all sorghum cultivars with respect to tannin levels. The objectives of this research were: (i) to evaluate their performance for seedling traits at seedling stage (under hydroponics), plant water relations under water stress and ultimately grain yield, (ii) to estimate the genetic variability of the regenerates and the parent plants using SSR markers. The ultimate goal of the study was to establish the possible use of tissue culture to develop somaclonal variant for sorghum resistant to water stress.

MATERIALS AND METHODS

In vitro selection of the sorghum cultivars

For salt screening, 0, 50 100 and 200 mM (selected based on salinity ranges for soils in Kenya where wild sorghum grow) NaCl was added to the culture media before autoclaving. Calli from growth chamber weighing 0.5 g were transferred to sterilized bottles containing 10 ml of the culture media. Each treatment had 50 replicates per cultivar. The treatments were maintained in the sealed bottles for one week in a growth chamber with continuous light at 26°C. Triphenyl tetrazolium chloride (TTC) viability test was done to determine the optimum NaCl concentration for the treatment of sorghum calli to induce drought tolerance without killing calli. For viability test, 100 mM NaCl was selected as the optimum concentration to which calli was subjected for the selection of drought tolerant from non-tolerant ones. The tolerant ones were further cultured for 100 replicates in media containing 100 mM NaCl for one week then transferred to culture media containing no NaCl to enable further multiplication of the treated calli. After four sub-cultures, seven days embryonic culture were visually selected and inoculated in Linsmaier and Skoog (LS) regeneration media. Regenerates, after rooting and shooting were transferred to the green house and potted in plastic bags containing 500 g vermiculite for acclimatization. They were watered twice a week and after two weeks they were transferred to the field conditions at Jomo Kenyatta University of Agriculture and Technology (JKUAT) experimental plots (Plate 1).

Field experiments

On the basis of rainfall, Kenya is divided into seven agro-climatic zones. Agriculturally, agro-climatic zones 1 to 3 are categorized as having high potential while agro-climatic zones 4 to 7 where Juja falls are categorized as having marginal or low potential. It is for this reason that field experiments were done at JKUAT Horticulture farm, agronomic division. The sorghum cultivars were planted in the first week of July, 2008 and first week of January, 2009 in triplicate randomized complete block design (RCBD). During sowing, the seeds were manually drilled into 10-row plots of 3.4 m length, spaced 0.3 m apart. The plots were weeded as frequently as needed. The agronomic traits evaluated included total chlorophyll content measured in intact leaves in the field using a portable chlorophyll meter (SPAD-502, Manitola camera), plant height (cm) measured in from ground level to the base of the spike length, peduncle length (cm) from last node to the base of spikelet maturity, extrude of spike from flag leaf (cm) from base of the flag leaf to base of spike, spike length (cm) from base to tip of spike, flag leaf area (cm^2), 1000 grain weight (g), grain yield ($kgha^{-1}$) and water potential (ψ).

For seedling root and shoot lengths (cm), hydroponics experiments were carried out at JKUAT Horticulture Lab. Hydroponics involved growing plants with their roots submerged in the aerated nutrient solution. The plants were suspended from a floating Styrofoam board with the roots hanging in the aerated Hoagland nutrient solution which was kept in circulation by an air pump (Resun® Air-2000) to provide even nutrient and air supply for the plant roots.

DNA extraction

Total genomic DNA was extracted from leaf tissue per each variety. Young leaves from two weeks- old plants were cut as tissue samples for DNA extraction. DNA was isolated from these genotypes as described by Murray and Thompson (1980). The DNA was resuspended in 100 µl of Tris-EDTA (TE) and stored in the fridge (-20°C) awaiting quantification.

DNA quantity and quality checking

This involved ethidium bromide fluorescence and spectrophotometric determination (Sambrook and Russell, 2001). In ethidium bro-

Plate 1. (a) Callus initiation, (b) organogenesis, (c) advanced regeneration.

bromide fluorescence, a portion of 5 µl of DNA from each sample was mixed with 5 µl of 1X gel loading dye III (0.01% bromophenol blue (w/v), EDTA pH 8.0 (0.5 M) and 50% glycerol (v/v) and run in 2% agarose gels containing ethidium bromide (0.5 µg/ml) buffered in 1X TBE [(1 M Tris – HCl pH 7.5), 1 M boric acid and 0.5 M EDTA (pH 8.0)] in a horizontal electrophoresis apparatus. DNA was also loaded and the gels run at 80 V for 1.30 h. Gels were viewed under an ultraviolet (UV) transilluminator Bio Doc-It™ System and photographed using its UVP printer (Mitsubishi). DNA concentration of the sorghum samples was estimated by comparing DNA band size and ethidium bromide staining intensity of the test 1 kb molecular weight marker.

In spectrophotometric determination, a Bio photometer (Eppendorf) was used to determine DNA concentration of sorghum samples. A 1:50 dilution of DNA in dSDW was made and the absorbance (optical density) measured at 260 nm. DNA concentration was calculated as follows:

DNA conc. of sample (µl/ml) = A_{260} × dilution factor × 50

Where, A_{260} is the absorbance of DNA sample at 260 nm. DNA purity was estimated from the optical density (OD) ratios for the DNA samples at 260 and 280 nm when compared to the OD_{260} / OD_{280} for pure DNA preparations, which is equal to 1.8.

Polymerase chain reaction using SSR primers

After screening of the 10 pairs of SSR markers, five were found to amplify scorable and reproducible banding profiles.

The polymerase chain reaction (PCR) amplifications were performed with a Gene-Amp PCR system 9700 (Applied Biosystems) in a 20 µl final volume containing 1 µl (10 ng) of genomic DNA, 1.5 µl (10X) PCR buffer with $MgCl_2$ (Gene script), 1.5 mM dNTP, 0.25 µl *Taq* DNA polymerase 0.5 µM of each primer and 14.75 µl of sterile water. The microtubes were placed in a thermocycler (a Gene-Amp PCR system 9700 (Applied Biosystems)) and the thermocycling reactions done in the following scheme: denaturation at 94°C for 5 min, 40 cycles for 30 s at 94°C; 1 min between 46 and 52°C; 45 s at 72°C; and a final elongation for 4 min at 72°C to reduce the probability of false scoring of stutter bands as alleles (Table 1).

Data collection and diversity analysis

Marker polymorphism

To measure the informativeness of the SSR markers, the polymerphism information content (PIC) for each was calculated according to the formula: where, k is the total number of alleles detected for a locus of a marker and P the I frequency of the ith allele in the set of seven varieties investigated.

Genetic similarity estimation and cluster analysis

Each SSR band was scored as present (1) and absent (0) for the different varieties. Genetic similarity (GS) between two varieties i and j was estimated by the formula: gsij = 2Nij/(Ni+Nj), where Nij is the number of bands present in varieties i and j, Ni (resp. Nj) is the number of bands present in variety I (resp. j). Based on the genetic similarity matrix, unweighted pair group method of arithmetic average (UPGMA) that is, cluster were selected on the basis of their known genetic analysis were used to assess pattern of diversity among the sorghum cultivars. All calculations were performed

Table 1. Microsatellite marker name used in this study.

SSR locus	Repeat motif	Forward primer:5'-3'	Reverse primer:3'-5'
Xcup02	(GCA)6	GACGCAGCTTTGCTCCTATC	GTCCAACCAACCCACGTATC
Xcup11	$(GCTA)_4$	TACCGCCATGTCATCATCAG	CGTATCGCAAGCTGTGTTTG
Xcup14	$(AG)_{10}$	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG
Xgap072	$(AG)_{16}$	TGCCACCACTCTGGAAAAGGCTA	CTGAGGACTGCCCCAAATGTAGG
Xgap084	$(AG)_{14}$	CGCTCTCGGGATGAATGA	TAACGGACCACTAACAAATGATT

Source: Taramino et al. (1997).

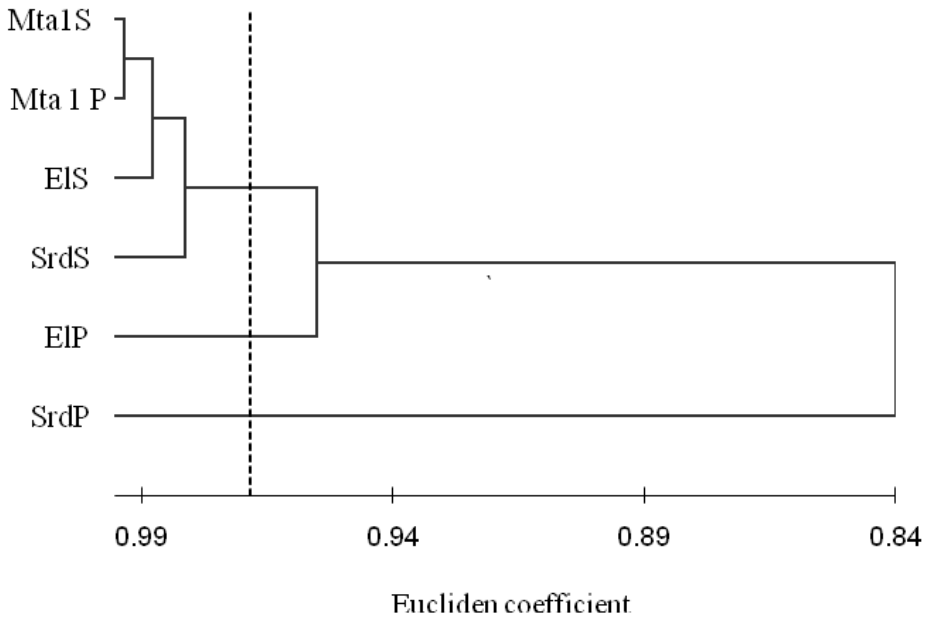

Figure 1. A dendrogram of six sorghum cultivars based on seven morphological traits.

using the NTSYS-pc version 2.1 software.

Statistical analysis

The average data recorded for seedling traits and physiological characters during 2008 and 2009 were subjected to the analysis of variance according to Steel et al. (1997). The data was submitted to PCA, using the XLSTAT 2008 statistical package. Cluster analyses were carried out on the principal components using the neighbor joining method (Nei, 1973) or hierarchic ascendant analysis and Euclidian average distance.

The presence or absence of each single fragment amplified by the microsatellite primers were coded by 1 or 0, respectively and scored for a binary data matrix. GS were calculated for each pair of cultivar using the Dice similarity. Genetic diversity is referred to as the PIC (Anderson et al., 1993).

RESULTS

Morphological characters analyses

Distance estimates based on the seven morpho-physiological traits ranged from 0.845 to 0.989 with an average of 0.922 (Figure 1). The cluster analysis based on the morpho-physiological data assigned the cultivars into two groups. The first cluster include seredo parent while the second cluster was divided into two sub-groups, the first included El Gardam parent while the second included Seredo regenerate, El Gardam Regenerate, Mtama 1 Regenerate and Mtama 1 Parent.

Microsatellite polymorphism

Five microsatellite markers for five loci were used to evaluate the genetic diversity of the five sorghum cultivars. A total of 11 alleles were detected among the sorghum regenerates and their parents. The number of alleles per locus ranged from two for *Xcup02, Xcup14* and *Xgap72* to three for *Xgap84* with an average number of 2.2 allele per locus (Table 2). The PIC values ranged from 0.344 for the *Xcup14* to 0.73 for *Xgap72* with an average value of 0.559 (Table 2).

Table 2. Polymorphism detected by 5 SSR markers in the parents and the regenerates of the 3 Sorghum cultivars.

Marker name	Total no. of alleles	Allele size range	Abundant allele (%)	[a]PIC values
Xcup02	2	192-204	42.30	0.510
Xcup14	2	211-225	25.10	0.344
Xgap84	3	183-217	24.80	0.726
Xcup11	2	165-172	23.60	0.485
Xgap72	2	183-205	25.00	0.730
Mean	2.2		28.16	0.559

[a] PIC=1-$\sum(pi^2)$ where, Pi is the frequency of the i^{th} allele detected.

Table 3. Similarity matrix among the sorghum cultivars.

Sorghum cultivar	Seredo P	Seredo R	El Gar. P	El Gar. R	Mta.1 P
Seredo P	-				
Seredo R	0.944	-			
El Gar. P	0.167	0.222	-		
El Gar. R	0.278	0.222	0.889	-	
Mta.1 P	0.389	0.333	0.556	0.667	-
Mta.1 R	0.444	0.389	0.611	0.722	0.944

Genetic relationships among the six sorghum cultivars

The dice similarity among the six sorghum cultivars ranged from 0.167 to 0.944 similarity coefficient with an average of 0.675. This indicated that there is a fair amount of similarity among the sorghum cultivars within each cultivar (regenerates and parents). About 30% of the pair-wise comparisons among the sorghum cultivars showed similarity greater than 0.675 while about 70% showing genetic similarity lower than 0.675. There was strong similarity between the sorghum cultivars Seredo regenerates (Seredo R) and Seredo parent (Seredo P) (cluster A) and also between Mtama 1 regenerates (Mta.1 R) and Mtama 1 parent (Mta.1 P) (cluster B) (Figure 1). Lowest similarities were shown between El Gardam parents (El Gar. P) and Seredo parent (Seredo P) (Table 3).

Phylogenetic analysis

UPGMA analysis clustered the six sorghum cultivars into two main clusters namely cluster A and B (Figure 2). The simple matching coefficient ranged between 0.306-0.944. Cluster A had two cultivars (Seredo regenerants and Seredo parent). The rest of the cultivars, Mtama 1 and El Gardam, clustered as B.

DISCUSSION

Microsatellite markers have been used to show genetic variations in many important crops including *Oryza sativa* L. (Bligh et al., 1999), Sorghum bicolor (Smith et al., 2000), cassava cultivars (Moyib et al., 2007) sweet potato (Karuri et al., 2009). This study identifies five oligonucleotide primers which can prove useful to analyze amount of polymorphism available in the sorghum cultivars and their regenerates.

The dice similarity indices and the consensus tree were developed on the basis of the scorable banding patterns of the three sorghum cultivars with their regenerates using the five SSR primers as shown in Table 1 and Figure 2. The similarity indices showed that the parents and regenerates of two cultivars, Mtama 1 and Seredo parents, were the closest with the similarity index of 0.994. This may be an indication of low percentage of somaclonal variation that occurred at callus formation level. This level of polymorphism for sorghum is in the range 3.0 per locus reported by Rolf et al. (2005), using sorghum varieties from East Africa and the great lake region. This lower genetic variation could be attributed to the fact that the variation between the parent and the regenerates could be as a consequence of simple chimerism during callus maintenance during regeneration (Frank et al., 2000). Furthermore, the detected genetic diversity for the 3 sorghum varieties and their regenerates is also lower than that (0.645) reported by Smith et al. (2000), using elite sorghum inbred lines assessed with simple sequence repeats.

The genetic similarities among the cultivars revealed by their clustering into distinct groups suggest the presence of different sources of relationships among the sorghum cultivars. The UPGMA dendrogram analysis (Figure 2) based on the 5 SSR markers (Table 2) showed two main clusters, the first included El Gardam and Mtama 1 cultivars while the second included Seredo cultivar.

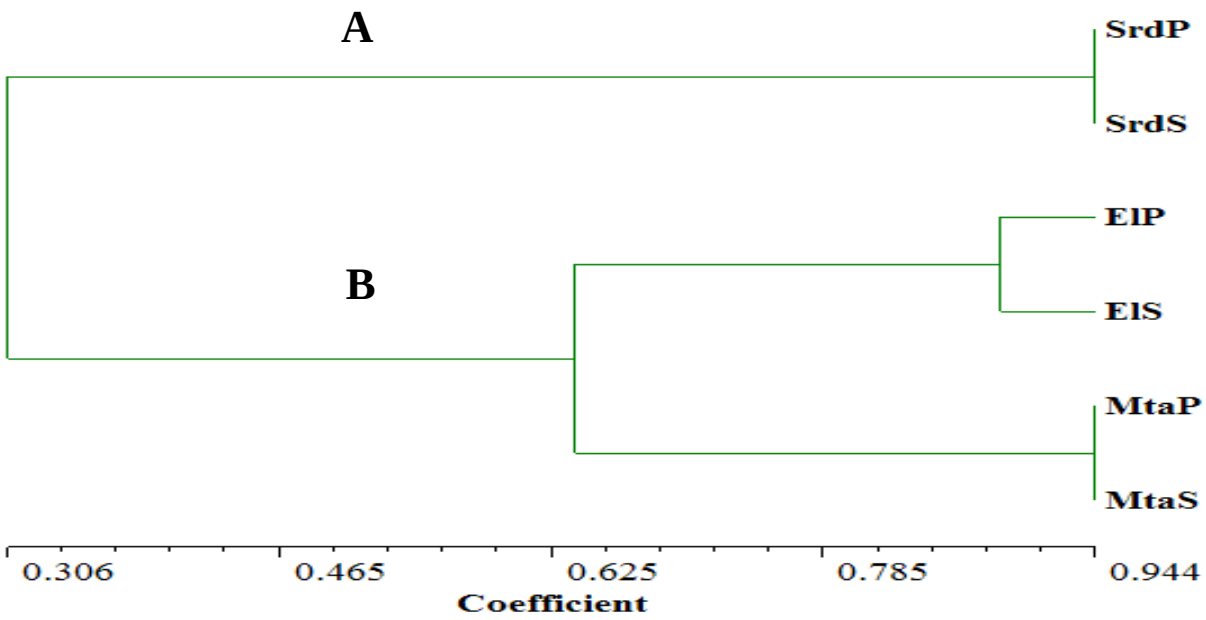

Figure 2. UPGMA dendrogram of 6 sorghum cultivars based on genetic similarity matrix calculated from SSR markers. SrdP, Seredo parent; SrdS, Seredo regenerant; ElP, El Gardam parent; ElS, El Gardam regenerant; MtaP, Mtama 1 parent; MtaS, Mtama 1 regenerant.

The results of the genetic diversity study provide estimates on the level of genetic variation among the cultivars that can be used in germplasm management and sorghum improvement. In this study, morphological data analysis was coupled with molecular analyses (SSR markers) to investigate the genetic relationships among the three sorghum cultivars and their regenerates.

The range of genetic distance based on the morphological traits was on average lower than SSR markers which might be a reflection of the environmental influence on the performance of the materials. Therefore, the DNA markers and morpho-physiological traits will not necessarily gain closely matching results and Mertnez et al. (2005), believed that the correspondence between different methods might be improved by analyzing more morphological characters and DNA markers. Two reasons for low correlations between DNA markers and morphological as well as protein data have been suggested by Semagn (2002). These are: (i) DNA markers cover a larger proportion of the genome, including coding and non-coding regions, than the morphological markers and (ii) DNA markers are less subjected to artificial selection compared to the morphological markers.

Success of these primers in establishing polymorphism in sorghum is confirmed by other studies by Rolf et al. (2005) that showed PICs of the primers to be in the same ranges as those obtained in this study. These findings clearly demonstrate the reliability of SSR in analyzing genomic diversity. Thus, it is possible to establish a collection of highly polymorphic SSRs for genetic diversity studies cultivar identification and plant variety protection in sorghum. The genetic diversity levels observed in this study would be useful indicators if such an approach is planned for the sorghum genome.

Conclusion

In summary, our data shows significant variation among the parent sorghum plants and their tissue culture regenerates. The data can be used in selecting diverse parent in breeding programme and in maintaining genetic variation in germplasm. This study also shows that tissue culture may not add much practical value in introducing somaclonal variation.

ACKNOWLEDGEMENT

The author wish to express a profound gratitude to Research, Production and Extension (RPE), (JKUAT) for funding and Institute for Biotechnology Research (IBR), Botany and Horticulture Departments (JKUAT) for providing physical facilities.

REFERENCES

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993). Optimizing parental selection for genetic linkage maps. Genome 36:181-186.

Bligh HFJ, Blackhall NW, Edwards KY (1999). Using amplified length polymorphism to identify cultivars of brown and white milled rice. Crop Sci. 3a(6):1715-1721.

Cai T, Ejeta G, Butter LG (1995). Screening for grain and phosyphenol variants from high-tannin sorghum somaclones. Theo. Applic. Gen. 90: 211-320.

Camlin MS (2001). Possible future roles for molecular techniques in the identification and registration of new plant cultivars. Acta. Hortic. 546:289–296.

Camlin MS (2003). Plant cultivar identification and registration-the role for molecular techniques. Acta. Hortic. 625:37–47.

Ebiamado AB, Alena G, Olesen A, Sven BD (2000). Quality of initial callus explants is is profound importance in encouraging proliferation of Type II cultures. J. Exp. Bot. 51:187-196.

Ghebru B, Schmidt RJ, Bennetzen JL (2002). Genetic diversity of Eritrean sorghum landraces assessed with simple sequence repeat (SSR) markers. Theor. Appl. Genet. 105:229-236.

Grenier C, Bramel-Cox PJ, Hamon CP (2001). Core Collection of Sorghum: I.Stratification Based on Eco-Geographical Data. Crop Sci. 41:234-240.

Karuri HW, Ateka EM, Amata R, Nyende AB, Muigai AWT (2009). Characterization of Kenyan sweet potato genotypes for SPVD resistance and high dry matter content using simple sequence repeat markers. Afr. J. Biotechnol. 8(10):2169-2175.

Mehmood S, Bashir A, Amad A, Akram Z (2008). Molecular characterization of regional *Sorghum bicolor* varieties from Pakistan. Pak. J. Bot. 40:2015-2021

Menz MA, Klein RR, Unruh NC, Rooney WL, Klein PE, Mullet JE (2004). Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. Crop Sci. 44:1236-1244.

Mertnez L, Cavagnaro P, Masuelli R (2005). Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. Elect. J. Biotechnol. 6:37-45.

Mitchell SE, Kresovich S, Jester CA, Javier Hernandez C, Szewc-McFadden AK (1997). Application of multiplex PCR and fluorescence-based, semi automated allele sizing technology for genotyping plant genetic resources. Crop Sci. 37:617–624

Moyib OK, Odunola OA, Dixon AGO (2007). SSR markers reveal genetic variation between improved cassava cultivars and landraces within a collection of Nigerian cassava germplasm. Afr. J. Biotechnol. 6(23):2666-2674.

Murray MG, Thompson WF (1980). Rapid isolation of high molecular weight plant DNA, Nucleic Acids Res. 8: 4321–4325.

Nei M (1973). Analysis of gene diversity in subdivided populations. Proc. Natl Acad. Sci. USA 70:3321–3323.

Roder MS, Plaschke J, Konig SU, Borner A, Sorrells ME,Tanksley SD, Ganal MW (1995). Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Genet. Gen. 246:327–333

Rolf TF, Frederick H, Chandra WR, Raju GS, Hash CT (2005). The pattern of genetic diversity of Guinea-race Sorghum bicolor (L.) Moench landraces as revealed with SSR markers. Theoret. Appl. Genet. 111:399–409.

Sambrook J, Russell DW (2001). Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schloss SJ, Mitchell SE, White GM, Kukatla R, Bowers JE, Paterson AH, Kresovich S (2002). Characterization of RFLP clone sequences for gene discovery and SSR development in Sorghum bicolor (L). Moench. Theor. Appl. Genet. 105:912–920.

Schug MD, Hutter CM, Wetterstrand KA, Gaudette MS, Mackay TFC, Aquadro CF (1998). The mutation rates of di-, tri- and tetra-nucleotide repeats in Drosophila melanogaster. Mol. Biol. Evol. 15:1751–1760.

Semagn K (2002). Genetic relationships among ten endod types as revealed by a combination of morphological, RAPD and AFLP markers. Hereditas 137:149-156.

Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee M, Porter K (2000). Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Sci. 40:226–232.

Swaminathan MS (1997). Implementing the benefits-sharing provisionsof the convention on biological diversity, challenges and opportunities. Plant Genet. Res. Newslett. 112:19-27.

Taramino G, Tarchini R, Ferrario S, Lee PME (1997). Characterisation and mapping of simple sequence repeats (SSR) in *Sorghum bicolor*. Theoret. Appl. Genet. 95:66-72.

Uptmoor R, Wenzel W, Friedt W, Donaldson G, Ayisi K, Ordon F (2003). Comparative analysis on the genetic relatedness of Sorghum bicolor accessions from Southern Africa by RAPDs, AFLPs and SSRs. Theor. Appl. Genet. 106:1316-1325

Wang C, Wang L, Bai Z, Wang F, Zheng L (1997). Improvement of a sorghum R line through tissue culture. Plant. Physiol. 38:98-118.