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GENETIC DIVERSITY ANALYSIS IN SORGHUM (*Sorghum bicolor* L. Moench) BY USING RAPD MARKERS

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Abstract: *The experiment was conducted during the year 2014-2015 in College of Agricultural Biotechnology, Loni with a view to assess the genetic diversity in sorghum by RAPD analysis.*

A total of 6 sorghum varieties were evaluated using RAPD. DNA was extracted from leaves, quantified and subjected to PCR analysis by using 4 different primers. PCR products were evaluated by using 1.5% agarose gel electrophoresis. Data analysis was done using (NTSYS) software program. Results show the appreciable amount of genetic diversity among the sorghum varieties. Out of the 20 amplification products scored, 20 bands (100%) were found to be polymorphic. The pair wise similarity values shows that variety Phule Anuradha and Phule maulee are showing closest relationship with highest similarity value i.e., 70%. The lowest pair wise similarity values was showed by variety Phule Panchami and Phule Maulee. i.e.,20%.

Keywords: *Polymorphism, Amplicons, Fingerprinting, NTSYS.*

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal grain crop originated from West Africa about 5000-8000 years ago (De Candolle *et al.* 1884) and Indian sub-continent is the secondary origin. It is a staple food crop for millions of people in semi-arid tropics of Africa and Asia (Anglani *et al.* 1998) and it is fifth in acreage among the cereal crops in the world (Mehmood *et al.* 2008). Sorghum is derived from the Latin word 'Sorgo' which means rising above. Sorghum belongs to natural order Gramineae, family Poaceae, subfamily Panicoideae, tribe Andropogona and the sub tribe Sorghastrae (Hosmani and Chittarpur, 1997). Sorghum bicolor (L.) Moench includes annual sorghum with 10 pairs of chromosome (2n = 20, 1C = 735 c).

Sorghum is cultivated for a wide variety of purposes by people of different cultural heritage. Owing to the selection pressure imposed by such diverse environmental factors and cultivators, the sorghum crop has evolved into a vast array of forms (Morden *et al.* 1989). Sorghum is drought resistant, low input cereal grain cultivated throughout the world in most of the countries. It is used primarily as animal feed but in Africa and India as human feed (Agrama and Tunitra, 2003).

In India 37.70 lakh hectares area is under kharif sorghum with an annual production of 39.50 lakh tones and 1048 kg per hectare productivity. In rabi 47.76 lakh hectares area is under sorghum cultivation with annual production of 37.69 lakh tones and 789 kg per hector productivity. Sorghum is cultivated in Maharashtra, Karnataka, Madhya Pradesh, Andhra Pradesh, Gujarat and Rajasthan states. Maharashtra being the main sorghum producing state of the country and has total of 47.40 lakh hectores area under its cultivation with annual production of 37.13 lakh tones and 897 kg per hectare productivity. Out of these, 14.72 lakh hectares are grown in kharif with an annual production of 17.64 tones and 1198 kg per hectare productivity. In rabbi season, it is grown on 32.68 lakh hectare with annual production of 19.49 lakh tones and 597 kg per hectare productivity

Sorghum yield is limited by inadequate and/or erratic rainfall, poor soil fertility, pest and disease invasions and high temperatures. Drought is perhaps

the most important abiotic factor limiting crop productivity around the world and is of significance in semi-arid tropics, where rainfall is generally scanty and its distribution is erratic. One of the most effective ways to alleviate the problems of crop production in drought prone areas is the development of crops that withstand moisture stress.

Plant genetic resources play an important role in generating new crop varieties with the high yield potential and resistance to biotic and abiotic stresses. The germplasm of a particular crop collected from the local sources provides greater genetic variability and can furnish useful traits to broaden the genetic base of the crop species. Morphological, biochemical and molecular procedures are currently being employed in evaluating plant genetic resources. Until recently, most of the characterization and evaluation has been based on the recording of either qualitative or quantitative morphological characters. Biochemical markers have received more attention in recent years as the data reflect more truly the genetic variability because they are the direct products of genes (Perry McIntosh, 1991). Besides biochemical markers, DNA based markers provide powerful and reliable tools for discerning variation within crop germplasm and to study evolutionary relationships (Gepts, 1993). PCR based techniques have been used successfully in DNA fingerprinting of plant genomes and in genetic diversity studies. These techniques include RAPD (Randomly amplified polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), SSR (Simple Sequence Repeat) and AFLP (Amplified fragment Length Polymorphism). In RAPD technique short oligonucleotides of arbitrary sequences are used singly to support the amplification of the plant genomeand amplification products are separated by gel electrophoresis.

Analysis of genetic relationship in crop species is important component of crop improvement program as it serves to provide information about genetic diversity and is a platform for stratified sampling of breeding for diverse application including analysis of genetic variability, identifying diverse parental combinations to create segregating progenies which maximum genetic variability for further selection (Barret and Kidwell, 1998)

The distance between two clusters is measure of the degree of divergence. Genotypes can be grouped into different clusters by following Tocher's method (Rao, 1952). The wide range of genetic diversity in sorghum indicates the possibility of improving its productivity, to meet growing demand of food and feed.

Assessment of genetic variation based on morphology or pedigree has its limitations. Complex and quantitatively inherited traits are difficult to trace based solely on morphology. Therefore, DNA-based methods have been employed in studies of sorghum genetic diversity and in genetic improvement of the crop. Molecular marker are considered constant landmark in the genome. Genetic distance estimated determined by molecular marker help identify suitable germplasm for incorporation into plant breeding stock.

Material and Methods

A total six genotype of Sorghum (*Sorghum bicolor*) was collected from All India Coordinated Sorghum Improvement Project (AICSIP) at Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra. Seeds of these selected varieties were grown on germination paper and 8 to 10 days old seedlings were taken for DNA isolation and stored at -80°C.

DNA extraction: DNA extraction was done following the modified method of Saghai and Maroof using CTAB. Approximately two or three leaves were taken in an autoclaved mortar and crushed to fine paste using 2 ml CTAB buffer. The homogenate was incubated at 65°C for 45 minutes in block heater (Stuart Scientific). Material was centrifuged at 13000 rpm for 10 minutes. An equal volume of chloroform was added in supernatant, mixture vortexed and centrifuged for 10 minutes at 13000 rpm. The supernatant was transferred to another eppendorf tube and mixed with an equal volume of isopropanol. DNA was recovered as a pellet by centrifugation and washed with 70%

ethanol. The pellet was dried and stored in 50 µl of TE buffer and 1 µl/100 µl of RNase to digest RNA and to obtain pure DNA.

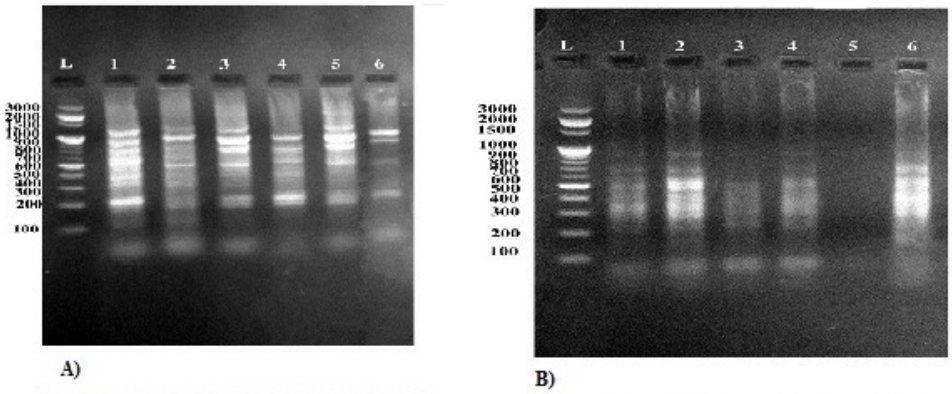
Agarose gel electrophoresis: The quality of DNA was checked by running it on 1.5% agarose gel and stained in Ethidium bromide solution. The stained gel was photographedand quality of the DNA was assessed using U.V transilluminator. The DNA was further quantified by using Nano-Drop machine at 260 and 280 nm and dilutions were made for further RAPD analysis.

DNA ampification: For polymerase chain reaction 10 oligonucleotide (decamer) primers were used. The PCR reaction mixture (15 µl) contained 20 ng of genomic DNA, 1.5 µl 10x reaction buffer, 1.5 µl 10x dNTPs, 0.7 µl Magnesium chloride (50 mM), 1µl of primer, 0.2 µl of taq polymerase and 9.1 µl nano pure water. Amplification was performed in programmable thermal cycler which was set for one cycle of 4.5 min., at 94°C, 1.30 min., at 37°C and 2 min., at 72°C. Then 40 cycles of 1 min at 94°C, 1.30 min at 37°C and 2 min at 72°C and hold at 4°C. The PCR product was loaded on 1.5% agarose gel in TAE buffer and stained with ethidium bromide. DNA fragments were then visualized by illumination with UV light (Kodak EDAS 290). In all cases DNA Ladder from Hi-Media was used as molecular marker.

Data analysis: Photographs from Ethidium bromide stained agarose gel were used to score RAPD data for analysis. The presence of a particular band was scored as 1 and absence as 0. Bands with same mobility were treated as identical fragments. The positions of PCR bands were compared with molecular weight standards. Data analysis was performed using the software NTSYS (Numerical Taxonomic System). After processing the Gel images, all pair wise similarity values were calculated with a Jaccard's similarity coefficient. The similarity matrix was converted into Dendrogram using UPGMA (i.e., unweighted pair group method with the arithmetic average) clustering algorithm.

Results and Discussion: Out of 7 primers of OPC, OPB, OPN, OPA series screened, 4 primers showed distinct and good banding pattern. The four primers amplified a total of 20 fragments out of all 20 were polymorphic. The number of amplified fragments per primer varied from 5 to 7 with a mean of 5.

The level of polymorphism for each primer differ and is summarized in (table 4.1). The percentage polymorphism over four primers used in the present study is 100%. This level of polymorphism observed was more as comparable to the reports of several RAPD studies by various workers. Tao *et al.,* (1994), Agrama and Tuinstra (2003), Akhre (2008), and Nkongola and Nsapato (2003) reported 55%, 58%, 54.44% and 52% polymorphism respectively. The average number of polymorphic bands amplified for each primer tested in this study is less as comparable to that reported in sorghum accession by Tao *et al.,* 1994 (117 bands), Menkri *et al.,* 1997 (4.2 bands) and Agrama and Tainstra, 2003 (39 bands). The results however are comparable to that reported by Ayana *et al.,* 2000a (7.35 bands) and ayan *et al.,* 2000b (9.00 bands), Thimmaraju *et al.,* (2000) obtained 12.84 average number of RAPD bands in sorghum per primer which are higher than present study.



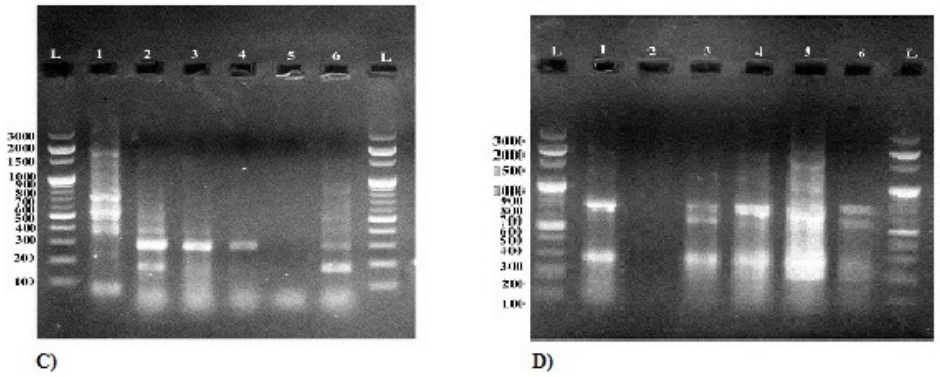


Fig. 1: RAPD pattern of sorghum varieties with different OPC primers: (a) OPC-01; (b) OPC -02; (c) OPB-01; (d) OPB-03. The DNA used as a template from 1, Phule Suchitra ; 2, Phule Anuradha ; 3, Phule Panchami; 4, Phule Vasudha ; 5, Phule Maulee; 6, Phule Revati

Table 1: List of RAPD primers and polymorphic amplicons generated

Primer	Sequence 5' to 3'	Score Bands	Polymorphic Bands	Polymorphic Rate %
OPC-01	5' TTCGAGCCAG 3'	7	7	100
OPC-02	5' GTGAGGCGTC 3'	4	4	100
OPb-01	5' GTTTCGCTCC 3'	4	4	100
OPB-03	5' CATCCCCCTG 3'	5	5	100
Total		20	20	100

Table 2: Binary similarity matrix based on Jaccard's similarity coefficient

	Phule Suchitra	Phule Anuradha	Phule Panchami	Phule Vasudha	Phule Maulee	Phule Revati
Phule Suchitra	1.00					
Phule Anuradha	0.31	1.00				
Phule Panchami	0.50	0.32	1.00			
Phule Vasudha	0.38	0.25	0.47	1.00		
Phule Maulee	0.50	**0.70**	**0.22**	0.40	1.00	
Phule Revati	0.24	0.25	0.29	0.27	0.24	1.00

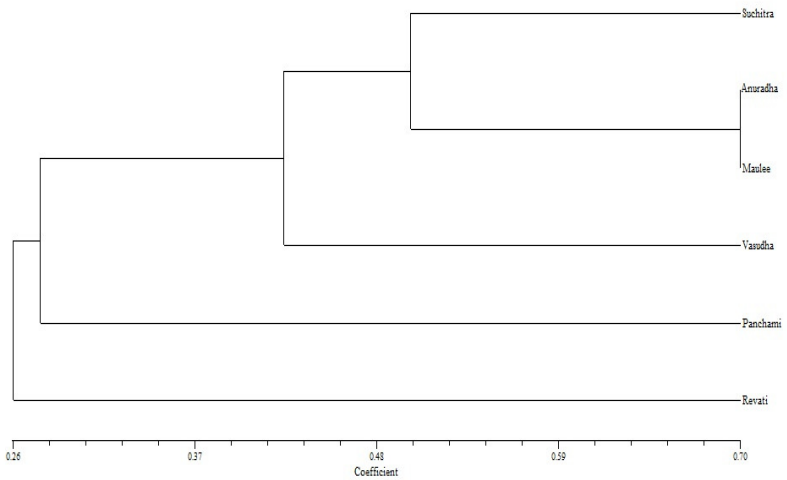


Fig. 2: UPGMA Dendogram with all tested primers showing the pair wise similarities values among the ten sorghum varieties: 1, Phule Suchitra; 2, Phule Anuradha; 3, Phule Panchami; 4, Phule Vasudha ; 5, Phule Maulee; 6, Phule Revati

Sorghum **(*Sorghum bicolar*** (L.) Moench) has worldwide economic importance among cereal crops. It is staple food crop in semi-arid tropics of Africa and Asia (Zindenga, 2000). In India it is mainly grown in Western region where geography and climate is suitable. Several sorghum varieties are grown with wide genetic diversity. This reflects in tremendous variation in yield. Therefore, still there is scope to isolate most divergent genotypes which would put in development of sorghum varieties with higher grain and fodder yield.

AICRP on Sorghum has developed several varieties and genotypes by its regular crop improvement programme. These lines needs to be studied at field for genetic variability and supplement to the results by analyzing at molecular level. With the view this investigation was undertaken during 2006 - 2009.

Diversity analysis in Sorghum by RAPD method

Genetic diversity still studied in many crops **on** the basis **of** morphological markers, however availability of special markers is lacking in many cultivars. The mapping of these is also tedious and time consuming (Akhare et al. **2008).** Further, biochemical marker like proteins and isoenzyme were used but it has less polymorphism and also influenced by environmental factors. This has shifted a focus to **DNA**-based molecular markers. Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next

Application of molecular marker developed during last few decades which overcome phenotype based. The technology like use of **RAPD** which has advantages its simplicity, rapidity, required small **DNA** and ability to generate more polymorphism (Cheng *et al.*1997). Thus it has been proved as powerful and useful tool for genetic analysis (Chapko, **1992)**

In contrast to morphological markers, which are based on visible traits and biochemical markers which are based on proteins produced by genes, molecular markers rely on DNA assay. DNA- based genetic markers are being increasingly utilized in cultivar development, quality control of seed production, measurement of genetic diversity for conservation management, varietals identification and intellectual property protection.

There are many different kinds of molecular markers including restriction fragment length polymorphism (RFLPs), random amplified polymorphism DNA (RAPDs), amplified fragment length polymorphism (AFLPs), microsatellite and single nucleotide polymorphisms (SNPs). PCR based method like RAPD's arc increasingly being used in the analysis of genetic diversity because of the relative ease with which PCR assay are carried out and also the prior knowledge about the genome is not known.

Genetic diversity is commonly measured by genetic distance or generic similarity both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). RAPD markers hold promise for the automation of the genome mapping extending the power of genetic analysis to organism which lacks an ample number of phenotypic markers to completely describe their genome. Random amplified polymorphic DNA markers (Williams *et al.,* 1990) have been successfully used for species identification in most of the plants due to technical simplicity and speed of RAPD technology (Gepts, 1993). The degree of polymorphism detected by different primers varied and thus there was considerable variation in the ability of individual primer to detect DNA polymorphism. Molecular markers can be used to better document for genetic diversity between possible parental material of breeding programme to accelerate the individual selection that combine favorable alleles and to establish the distinctness (Charcosset and Gallais, 2002).

Total genomic DNA was extracted and using PCR technique genetic diversity in 6 sorghum genotypes was studied using 7 random 10 mer primers. Out of which 3 didn't show clear amplification. Percent polymorphism shown by primers was 100 percent. It was found that, total 20

bands were generated by amplification out of which all 20 were polymorphic with an average of 100 per cent polymorphism. Ayana *et al.*, (2000a) reported maximum of 94 per cent polymorphism within the adopted zone (Ethiopia) sorghum cultivars. Similar reports of polymorphism were obtained by Arya *et al.* (2008) among Indian sorghum using AFLP were less.

The number of amplicons synthesized were ranged from 5 to 7 by 4 primers with an average of 5 amplicons per primer. Minimum seven polymorphic amplicon was found to be synthesized by OPC-02 and OPB-01 however, OPC-01, synthesized maximum 7 polymorphic amplicons. The average polymorphic amplicons were 5 percent per primer. The average number of polymorphic bands amplified for each primer recorded in the present study were comparatively higher than reported earlier in sorghum by Tao *et al.*, (1994) (0.117 bands), Menkir *et al.,* (1997) (4.2 bands) and Agrama and Tuinstra (2003) (3.9 band). The results however are comparable to that reported by Ayana *et al.,* (2000a) (7.35 bands) and Ayana *et al.,* (2000b) (9.00 bands), Kachapur *et al.,* (2009) (8.45 bands) while Thimmaraju *et al.*, (2000) obtained 12.84 bands per primer which are higher than that obtained in the present study.

To examine the genetic relationship among the 6 sorghum genotypes under study based on the RADP results, the data scored from 4 primer were compiled and analyzed using the Jaccard's similarity coefficient.The genetic similarity matrices based on the Jaccard's coefficient ranged from 0.22 to 0.70.

The genetic similarity matrix also revealed that Phule Anuradha and Phule Maulee were closely related with a genetic similarity coefficient of 70%.

To sum up, considerable diversity existed among the sorghum accessions. This study identified diverse genotypes i.e., Phule Panchami and Phule Maulee for use in hybridization program for sorghum improvement.

The dendrogram generated based on UPGMA method of cluster analysis related two major clusters A and B (Fig.1) Cluster A comprised of two sub clusters Cluster A_1 and A_2, where sub cluster A_1 is further divided into two branch in which Phule Vasudha is in separate cluster, while Phule Suchitra, Phule Anuradha and Plhule Maulee were in another cluster of branched sub cluster A1 and second sub cluster A_2 comprises only one Phule Panchami. The cluster B comprises only one variety Phule Revati.

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References:

Agrama, H.A. and Tuinstra, M.R. 2003. Phylogenetic diversity and relationship among sorghum accession using SSRs and RAPDs, *Afr. J. Biotechnol.,* 2(10): 334-340.

Ajmone Marson, P., Agidy, G., Monfredini, T., Disilvestro, S. and Matto, 1993. RAPD markers in Maize genetic analysis. *Myadica,* 38 (4): 259-264.

Akhare, A.A., Sakhare, S.B., Kulwal, P.L., Dhumale D.B., Kharkar, A. 2008. RAPD profile study in sorghum for identification of hybrids and their parents. *Interna. J. Integrative Biol.,* 3(1) : 18.

Anas, and Yoshida, T.2004 b. Sorghum diversity evaluated by simple sequence repeat (SSR) markers and phenotypic performance. *Plant Prod. Sci.,* 7(5): 301-308.

Anas, and Yoshida, T. 2004 a. Genetic diversity among Japanese cultivated sorghum assessed with simple sequence repeats markers. *Plant Prod. Sci.,* 7(2): 217-223.

Anglani, C. 1998. Sorghum for human food, a review*, Plant Foods Hum. Nutri.* 52: 85-89.

Appa-Rao, S., Prasad Rao, K.E., Mendesha, M.H. and V. Gopal Reddy 1996: Morphological diversity in sorghum germplasm from India. Gen. Res. *Crop Evol.,* 43: 559-567.

Arya, L., Sandhia, G.S., Singh, S.K., Rana, M.K. and Malik, S.S. 2006. Analysis of Indian Sorghum [*Sorghum bicolor* (l.) Moench] cultivars and lines using RAPD markers. *J. Plant Bichem. and Biotech.,* 15:97-101.

Asthana, O.P., Sharma, R.L., Hirve, C.D. and Asthana, N. 1995. Genetic variability, heritability and genetic advance in exotic sorghum. *Adv. Plant Sci.,* 8(2): 230-237.

Chang, H.C. 1997. Identification of dried rhizomes of *captis species* using random amplified polymorphic DNA. *Bot. Bull. Acad. Sin.,* 38:241-244.

Choudhari, L. and Arora, S. 2001. Genetic variability and character association in sorghum.*Agric.Sci.Digest.*, 21(4): 219-222.

Dahlberg, J.A., Zhang, X., Hart, G.E.and Mullet, J.E. 2002. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurements. *Crop Sci.*,42(1): 291-296.

Jaiswal, P., Ranade S.A., Nath P. and Sane P.V. 1998. Mitochondrial and total DNA RAPD patterns can distinguish restorers of CMS lines in sorghum. *The or. Appl. Genet.*,96(6\7): 791-796.

Jeya Prakash, S.P., Baji, K. R., Michael Gomez, Ganesa Murthy, K. and Chandra Babu, R. 2006. Genetic diversity analysis of sorghum (Sorghum bicolar L. Moench) accessions using RAPD markers. *Indian J. Crop. Sci.*,1(1-2): 109-112.

Kachapur, R.M., Salimath, P.M. and Reddy, B.V.S. 2009. Genetic Diversity within Sweet Sorghum (Sorghum bicolar (L.) Moench). Accessions as revealed by RAPD markers. J. Maharashtra agric. Univ., 34(1): 038-042.

*McDonald, M.B. 1995. Genetic purity: from protein electrophoresis to RAPDs. Proceedings of the fiftieth Annual Corn and Sorghum Industry Research Conference. 256-271.

Mahmood, Sajid, Bashir, A., Ahmad, A., Akram, Z., Jabeen, N. and Gulfraz, M. 2008. Molecular characterization of regional Sorghum bicolar varieties from Pakistan. Pak. *J. Bot.*, 40(5):2015-2021.

* Maiti, R.K. and Rodriguiez, J.M. 1996. Studies of some biochemical characters of 5 glossy and 5 non glossy sorghum related to drought resistance. Sorghum Newsletter, 29: 91-92

Menkir, A., Goldshrough, P., and Ejeta, ag. 1997. RAPD based assessment of Genetic diversity in cultivated races of sorghum. *Crop Sci.*, 37: 564-569.

*Nguyen-Duy-Can., Haryanto-TAD, Yoshida, T. 1998. Genetic variability and character association analysis in grain sorghum. J. Faculty Agric. Kyushu Univ.,43: 1-2,25-30.

Nkongolo, K.K. and Nsapato L.2003. Genetic diversity in *Sorghum bicolor* (L.) Moench accessions from different ecogeographical regions in Malawi assessed with RAPDs. Gen. Res.*Crop Evol.*,50(2): 149-156.

Paul,S., Wachira, F.N., Powell, W. and Waugh, R. 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (Cammellia sinensis (L.)Kuntze) revealed by AFLP markers. Theor. Appl. *Genet.*,94:255-263.

Rao, C.R. 1952. Advance statistical method in biometric research. John Wiley and Sons Ind., New York pp.293.

Sabharwal, P.S., Yadav, D.K., Grewal, R.P. 1995. Studies on variability for morphological traits of forage sorghum. *Genetica a slechten.* 31(3):171-173.

Sharma A.D., Prabhjot Kaur Gill and Singh P. 2002. DNA isolation from dry and fresh samples of polysaccharides rich plants. Plant *Mol. Biol. Reporter*, 20:415a-415f.

Saghai-Maroof, M.A. Soliman, K.M., Jorgensen R.A. and Allard R.W. (1984). Ribosomal spacer length polyrhism in barley; Mendelian inheritance, chromosome location and population dynamics.*Proc.Natt Acad Sci.,*USA 81:8014-8019.

Thimmaraju, R., Krishna, T.G., Kuruvinashetti, M.S., Ravikumar, R.L. and Shenoy V.V.2000. Genetic among sorghum genotypes assessed with RAPD markers. Karnataka *J. Agril.Sci.*, 13(3):564-569.

Veerabadhiran, P., Kennedy, VJF. 2001. Estimates of genetic variability in selected genotypes of sorghum. *Madras Agric. J.,* 88:4-6,308.

Zindegna, T. 2004. DNA-based methods in sorghum diversity studies and improvement. ISB News Reporter. March.