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Assessment of genetic diversity in *Sorghum bicolor* using RAPD marker

M.Sc. Thesis

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DECLARATION

I, José Alejandro Ruiz Chután, hereby declare that this thesis, submitted in partial fulfilment of requirements for the master degree in Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, is wholly my own work written exclusively with the use of the quoted sources.

In Prague, 2016

José Alejandro Ruiz Chután

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ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereal crops and ranks fifth among cereals after wheat, rice, maize and barley for economic importance. Because the demand of food is increasing and also the competition for global water resources is becoming more intense in the twenty-first century, sorghum will increase in importance as a source of food, feed, fiber, and fuel; specially in the European continent where sorghum is little cultivated, mainly due to the lack of sorghum varieties well adapted to the agronomic conditions such photoperiod, cold and drought tolerance; for this reason the genetic diversity analysis, through molecular characterization, is an important requirement to begin a plant breeding program. In this sense, the present study is aimed to assess the genetic diversity of sorghum accession through Random Amplified Polymorphic DNA molecular marker (RAPD). The analysis was performed in 45 sorghum accessions obtained from the Gen Bank of the Crop Research Insitute, Prague. Genetic variability values were estimated, through the dissimilarity index using Dice's coefficient, and dendogram constructed using DARwing software. Four out of fifteen of the primers evaluated were completly polimorphic (100%), 126 scorable bands were identified and 89% of them were polymorphic, the bands ranged from 200 to 2000 bp. The dendogram grouped the accession into six clusters. The results indicate the existance of high genetic diversity with dissimilarity values up to 0.2809 among the evaluated accessions, even if the accessions were collected in the same country, or by the contrary, lower genetic diversity among accessions collected in different countries. It may be due to the existence of five ancient races of sorghum, from which most of the wild and cultivated species known nowadays were originated. Mainly, the migration of people from the origin centre of sorghum, located in Ethiopia and Sudan, explain the spread of the genetic material out of Africa. The information generated by this study should be useful for a better understanding of the genetic diversity from the sorghum germplasm stored in the Czech gene bank for future plant breeding program.

Key words: *Sorghum bicolor*, genetic diversity, DNA, RAPD

RESUMEN

El sorgo (*Sorgum bicolor* L. Moench) es uno de los cereales más importantes en el mundo y, dada su importancia económica, se ubica en el quinto lugar después del trigo, arroz, maíz y cebada; debido al incremento en la demanda de alimentos aunado a una competencia global por los recursos hídricos cada vez más intensa en el siglo veintiuno, el sorgo incrementará su importancia como una fuente de alimentación Humana y animal, de fibra y combustible; especialmente en el continente europeo, donde el sorgo es poco cultivado, principalmente por la falta de variedades que estén adaptadas a las condiciones agronómicas propias de la región como el fotoperíodo, resistencia a bajas temperaturas y sequía. Por esta razón el estudio de la diversidad genética, a través de la caracterización molecular, es un requisito importante para empezar un programa de fitomejoramiento. En este sentido, el presente estudio tuvo como objetivo la evalución de la diversidad genética de accesiones de sorgo, a través del marcador molecular denominado Amplificación Aleatoria de ADN Polimórfico (RAPD por sus siglas en inglés). El análisis fue realizado en 45 accesiones de sorgo obtenidas del Banco de Genes del Instituto de Investigacion de Produccion de Cultivos, Praga. La elaboración del dendograma y la estimación de los valores de variabilidad genética, calculados a través del índice de dismilitud utilizando el coeficiente de Dice, fueron realizados con el programa estadístico DARwin. Cuatro de los quince cebadores evaluados fueron completamente polimórficos (100%), 126 bandas fueron identificadas, de las cuales el 89% fueron polimórficas, dichas bandas oscilaron desde 200 hasta 2000 pares de bases. El dendograma agrupó las accesiones en seis conglomerados. Los resultados indican la existencia de una alta diversidad genética con valores de disimilitud de hasta 0.2809 entre las accesiones evaluadas, incluso si las accesiones fueron colectadas en el mismo país, o por el contrario, la diversidad genética fue más baja en accesiones colectadas en países diferentes. Esto puede deberse a la existencia de 5 razas antiguas de sorgo, de las cuales la mayoría de especies silvestres y cultivadas, conocidas hoy en dia fueron originadas. La migración de personas, desde el centro de origen del sorgo, ubicado en Etiopía y Sudán explica la dispersión del material genético fuera de África. La información generada a través de este estudio debería ser útil para una mejor compresión de la diversidad genética del germoplasma de sorgo, almacenado en el banco de genes checo para un futuro programa de fitomejoramiento.

Palabras clave: *Sorghum bicolor*, diversidad genética, DNA, RAPD.

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LIST OF USED ABBREVIATIONS

AFLP	=	Amplified Fragment Length Polymorphism
ARC	=	Agricultural Research Corporation
bp	=	base pair
CAAS	=	China Academy of Agricultural Sciences
CIRAD	=	Centre De Coopération Internationale En Recherche Agronomique Pour Le Développement
CRI	=	Crop Research Institute
DNA	=	Deoxyribonucleic Acid
dNTP	=	Deoxynucleotide
DPI	=	Department of Primary Industries
EMBRAPA	=	Empresa Brasileira de Pesquisa Agropecuária
IBC	=	Institute of Biodiversity Conservation
ICRISAT	=	International Crops Research Institute for the Semi-Arid Tropics
IER	=	Institut d'Economie Rurale
ISSR	=	Inter Simple Sequence Repeat
NBPGR	=	National Bureau of Plant Genetic Resources
NCGRB	=	Center for Genome Research and Biocomputing
NGBK	=	National Genebank of Kenya
NPGRC	=	National Plant Genetic Resources Centre
PCR	=	Polymerase Chain Reaction
PGRCU	=	Plant Genetic Resources Conservation Unit
PGRU	=	Plant Genetic Resources Unit
RAPD	=	Random Amplified Polymorphic DNA
RFLP	=	Restriction Fragment Length Polymorph
RNA	=	Ribonucleic Acid
SNP	=	Single Nucleotid Polymorphis
SSR	=	Short Sequence Repeat
USA	=	United State of America
USDA	=	United State Department of Agriculture
VIR	=	Vavilov Research Institute for Plant industry

1 INTRODUCTION

Sorghum is ranked the fifth most produced food crop in the world, and is a dietary staple for over 500 million people in over 30 countries (Haussmann *et al*, 2002). It has gained importance as a fodder (green/dry) and feed crop in the last decade. Besides being an important food, it provides raw material for the production of starch, fiber, dextrose syrup, biofuels, alcohol and other products (Jeya *et al.,* 2006). Sorghum is well adapted to hot, semi-arid tropical environments with 400-600mm rainfall; as a result, it is a very stable source of nutrition (Smith, 2008). Due to its ability to adapt to different climatic conditions, sorghum is able to grow at an altitude ranging from sea level to 1,000 m above sea level. It is also found in temperate regions and at altitudes of up to 2300 m.a.s.l. in the tropics (Mamoudou *et al*., 2006).

In plant breeding programs, assessment of genetic diversity is useful for determining the uniqueness and distinctness of a phenotype, genetic constitution of genotypes and selection of parents for hybridization (Bretting and Widrelechner, 1996). DNA markers have been used to assess genetic diversity in different crop species (Cooke, 1995). Molecular markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant (Winter and Kahl, 1995). The attraction for RAPDs is due that there is no requirement for DNA probes, or for any sequence information for the design of specific primers, as is needed with SSRs. (Williams *et al.,* 1990). RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity (Fahima *et al*. 1999 by Jeya *et al*, 2006), technical simplicity, requirement of a few nanograms of DNA, no requirement of prior information of the DNA sequence and feasibility of automation (Subudhi and Huang, 1999).

Due the impact of sorghum (*Sorghum bicolor*) as one of the most import cereals around the world, is possible to find a wide number of studies related to determination of genetic diversity of this specie (Agrama and Tuinstra, 2003; Akram *et al*., 2011; Amparapali *et al*., 2008; Ayana *et al*., 2000a; Ayana *et al*., 2000b; Bahaa *et al*., 2013; Dahlberg, 2011; Dje *et al*., 2000; Hart *et al*., 2001; Horn *et al*., 2001; Jeya *et al*, 2006; Lekgari and Dweikat, 2014; Madhusudhana et al., 2012; Mbeyagala *et al*., 2012; Mehmood *et al*., 2008; Menkir *et al*., 1997; Mofokeng *et al*., 2012; Ritter *et al*., 2007; Smith *et al*., 2000; Tao *et al*., 1993; Tawanda, 2004).

2 LITERATURE REVIEW

2.1 SORGHUM (*Sorghum bicolor*)

Sorghum (*Sorghum bicolor* L. Moench) ranks fifth among cereals after wheat, rice, maize, and barley (Iqbal *et al*, 2010; Mofokeng *et al*, 2012), for economic importance with an annual production of 60 million tons in the world. It is an important food, feed, forage and provides raw material for producing of starch, fibre, dextrose syrup, biofuels, alcohol and other products (Iqbal, 2010).

Sorghum (*Sorghum bicolor* (L.) Moench) is one crop species that can survive harsh climatic conditions of arid environments (Ritter *et al*, 2007). *Sorghum bicolor* contains both cultivated and wild races and possess a significant amount of genetic diversity for traits of agronomic importance (Hart *et al,* 2001).

Sweet sorghum is a saccharinae C4 crop which is often considered to be one of the most drought resistant agricultural crops as it reduces evapotranspiration. Thus, the crop has a potential of conserving water and the capability of remaining dormant under conditions of drought and high temperatures (Prashant, 2007).

Like other sorghum types, sweet sorghum originated in East Africa and spread to other parts of African, Southern Asia, Europe, Australia and the U.S. (Tawanda, 2004). Although a native to the tropics, sweet sorghum is well adapted to temperate climates (Prashant, 2007).

Sorghum is well adapted to hot, semi-arid tropical environments with 400-600mm rainfall; as a result, it is a very stable source of nutrition (Smith, 2008). Due to its ability to adapt to different climatic conditions, sorghum is able to grow at an altitude ranging from sea level to 1,000 m above sea level. It is also found in temperate regions and at altitudes of up to 2300 m.a.s.l. in the tropics (Mamoudou *et al*., 2006).

Its value in hot, arid or semi-arid areas is due to its ability to withstand dry conditions (Tawanda, 2004). The rainfall requirements for sorghum vary within the range of 350-700 mm per growing season depending on the length of the growing cycle,

short growing cycle is 90 days and a long growing cycle is more than 130 days. In many semi-arid areas where sorghum is mainly grown for food, the annual rainfall ranges from 300-750 mm. Sorghum is deep rooted and grows on soil pH ranging from pH 5 to 8.5. It can withstand temperatures above 38 ^{0}C, but the best yields are realized at the temperature range of 24-27^{0}C. The crop is adapted to a wide range of soils, especially the heavy soils commonly found in the tropics (Mamoudou *et al*., 2006).

2.1.1 BOTANICAL CLASSIFICATION OF SORGHUM (*Sorghum bicolor*)

Several authors have discussed the systematics, origin, and evolution of sorghum (de Wet and Harlan, 1971; de Wet and Huckabay, 1967; Harlan, 1975; by Dahlberg *et al*, 2011). provides an excellent overview of the present day classification using an integrated classification system to describe the variation found within cultivated sorghums (Dahlberg *et al*, 2011).

Sorghum is classified under the genus Sorghum, De Wet (1978 by Dahlberg *et al,* 2011) recognized *S. bicolor*, representing all annual cultivated, wild and weedy sorghums along with two rhizomatous taxa, *S. halepense* and *S. propinquum*. *Sorghum bicolor* was further broken down into three subspecies: *S. bicolor* subsp. *bicolor*, *S. bicolor* subsp. *drummondii*, and *S. bicolor* subsp. *verticilliorum*. Cultivated sorghums are classified as *S. bicolor* sub sp. *bicolor* and are represented by agronomic types such as grain sorghum, sweet sorghum, sudangrass and broomcorn. Additionally, there are at least two weedy sorghums wide-spread in temperate zone (Dahlberg *et al,* 2011).

2.1.2 BOTANICAL DESCRIPTION OF SORGHUM (*Sorghum bicolor*)

Annual grass up to 5 m tall, with one to many tillers, originating from the base or stem nodes; roots concentrated in the top 90 cm of the soil but sometimes extending to twice that depth, spreading laterally up to 1.5 m; stem (culm) solid, usually erect. Leaves alternate, simple; leaf sheath 15–35 cm long, often with a waxy bloom, with band of short white hairs at base near attachment, reddish in dye cultivars, auricled; ligule short, 2 mm long, ciliate on upper free edge; blade lanceolate to linear-

lanceolate, 30–135 cm × 1.5–13 cm, initially erect, later curving, margins flat or wavy. Inflorescence a terminal panicle up to 60 cm long; rachis short or long, with primary, secondary and sometimes tertiary branches, with spikelets in pairs and in groups of three at the ends of branches. Spikelet sessile and bisexual or pedicelled and male or sterile, with 2 florets; sessile spikelet 3–10 mm long, with glumes approximately equal in length, lower glume 6–18-veined, usually with a coarse keel-like vein on each side, upper glume usually narrower and more pointed, with central keel for part of its length, lower floret consisting of a lemma only, upper floret bisexual, with lemma cleft at apex, with or without kneed and twisted awn, palea, when present, small and thin, lodicules 2, stamens 3; ovary superior, 1-celled with 2 long styles ending in feathery stigmas; pedicelled spikelet persistent or deciduous, smaller and narrower than sessile spikelet, often consisting of only two glumes, sometimes with lower floret consisting of lemma only and upper floret with lemma, 2 lodicules and 3 stamens. Fruit a caryopsis (grain), usually partially covered by glumes, 4–8 mm in diameter, rounded and bluntly pointed (Balole and Legwaila, 2006).

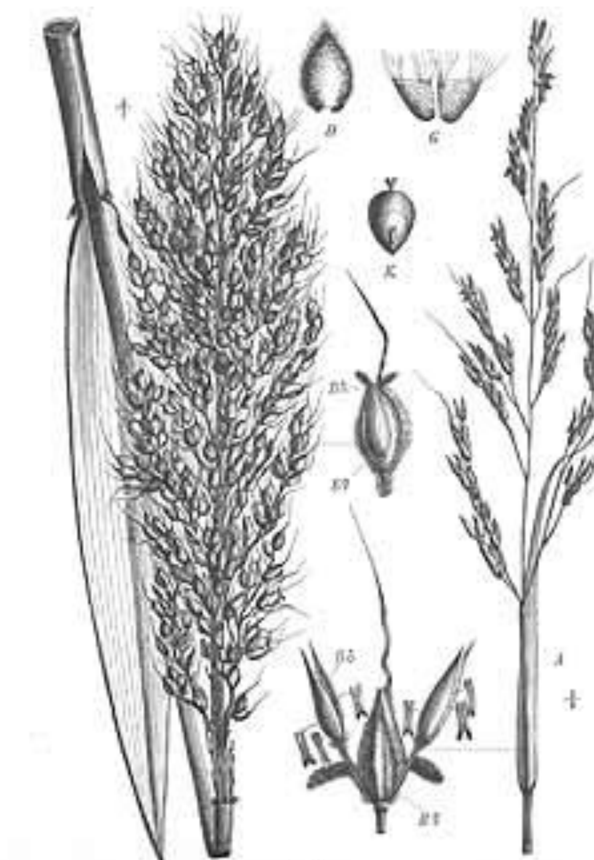

Figure 1. ***Sorghum bicolor*** (Popescu and Condei, 2014)

2.1.3 ORIGIN OF SORGHUM

Sorghum is an ancient crop (Mann *et al* 1983 by Dahlberg *et al.,* 2011) indicated that the origin and early domestication of sorghum took place in northeastern Africa north of the Equator and east of 10°E latitude, approximately 5,000 years ago. However, carbonized seeds of sorghum with consistent radiocarbon dates of 8,000 years BP have been excavated at an early Holocene archaeological site at Nabta Playa near the Egyptian Sudanese border (Wendorf et *al.*, 1992; Wasylikowa, 1996 by Dahlberg *et al.,* 2011).

These sorghums are 3,000 years older and 10-15° latitude further north than had been previously reported and suggests an early interest in sorghum by hunter and gathers and early agriculturalists. These early domestication events followed major trading and migratory paths of early Africans and Asians. As these early domesticated sorghum spread throughout Africa and Asia, plants were selected and dispersed throughout a broad range of environments and utilization giving rise to a widely adapted genetic base that has been further exploited throughout the agricultural process to create the current crop known as cultivated sorghum (Dahlberg *et al.,* 2011).

2.1.4 EVOLUTION AND DOMESTICATION OF THE SPECIES

Harlan (1995) based on experiments and on the work of Snowden (1936 by Kimbert *et al.,* 2012) confirmed that "all of the races belong to the same biological species and are fully fertile when hybridized." For the cereal sorghums, they identify four wild races and five cultivated races (Harlan and Stemler 1976 by Kimbert *et al.,* 2012). The four wild races of *Sorghum bicolor* are arundinaceum, virgatum, aethiopicum, and verticilliflorum. They are now placed in *S. bicolor* subspecies *verticilliflorum*, formerly subspecies *arundinaceum*. Using de Wet and Rao's interpretation, Doggett (1988) puts the four weedy races under *S. bicolor* subspecies *verticilliflorum*. (Kimbert *et al.,* 2012).

Kimber *et al.,* (2012) cited Murdock (1959) and Harlam (1995) and based on the information developmented by them, describes how the classification of *S. bicolor* in races as follow: (1) bicolor, the primitive type, (2) guinea, (3) kafir, (4) caudatum,

and (5) durra. Intermediates that are caused by hybridization of races exhibit characters of both parents. All will also breed with wild species with which they are sympatric. These cultivated races are placed in *S. bicolor* subspecies *bicolor*. Comparison of the distribution of the wild and cultivated races indicates a narrower concentrated band of the cultivated races in the east–west line across and north–south line on the eastern part of the continent than for the wild races. Such a distribution is support for the dispersal of sorghum taxa by migrating peoples across the Sahel-Sudan grasslands and southward from the Nile Valley region along the Great Rift.

According to Snowden (1936 by Kimbert *et al.,* 2012) wild race aethiopicum gave rise to races durra and bicolor, arundinaceum to guinea, and verticilliflorum to kafir. de Wet and Huckabay (1967 by Kimbert *et al.,* 2012) had much the same understanding except they proposed that durras came out of kafirs. Doggett (1965 by Kimbert *et al.,* 2012) suggested that the diversity seen in the wild forms might reflect human manipulation and intervention associated with the selection of domesticated types.

S. bicolor is distributed widely but is nowhere dominant among the African regions with cultivated sorghums. Kimbert et al., 2012 based on Wet and Price (1976) describe that bicolor, is not only widely distributed in Africa but is also apparently ancient in Asia, coastwise from India to Indonesia and China. *S. bicolor* migrated out of Africa is unknown, as are the people who were responsible for its diffusion. Cultivated sorghum may have reached China from Indochina by way of the Mekong River or other river valleys (Kimbert *et al.,* 2012). Another way to explain the migration of *S. bicolor*, out of Africa, is through the semitic speakers from Africa carried their culture to India before 3000 B.C. This may have been one avenue on which sorghum moved off the coast of Africa and into India (Hawkes, 1973 by Kimbert *et al.,* 2012).

Today, we see the four wild races distributed throughout the African continent, based on their biological traits. The wild race arundinaceum is distributed largely in wet and humid parts of forested central and west Africa along stream banks and in clearings, which are not suitable environments for the cultivated races. The Nile Valley proper is a region of seasonal flooding, with wild grasses such as *S. virgatum* among the first

colonizers after the waters recede. It also is found in disturbed riparian habitats in the Sudan. Based on the biology of this wild species, Harlan's group rejects it as the sou

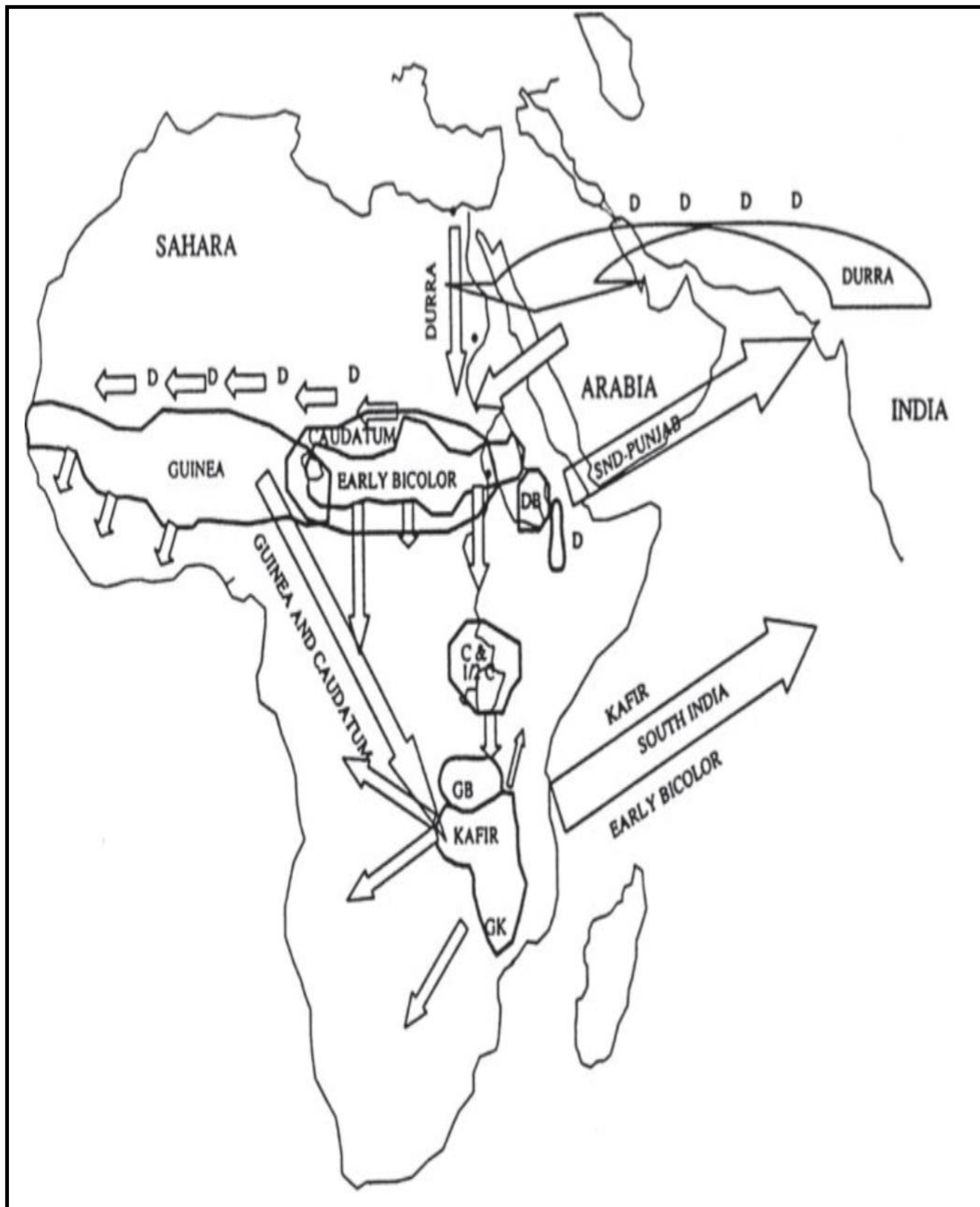

Figure 2. Areas of origin and development for the domesticated races of bicolor (Kimber *et al.,* 2012).

of the cultivated types. Race aethiopicum is found in the Kassala region of the Sudan, more sparsely to the west along the fringes of the Sahara, and in Ethiopia. Based on the ecology of the wild races, this leaves *S. verticilliflorum*, widely distributed throughout

the sorghum-growing areas, including the savanna zone of eastern and southern Africa (although not well represented in Nigeria), and morphologically appropriate as the parent race for *S. bicolor* (Harlan 1992 by Kimber *et al*., 2012). This position is a change from the position 20 years previously when Harlan and de Wet considered aethiopicum/verticilliflorum as a complex that was the primitive primogeniture of the cultivated races.

2.1.5 GENETIC DIVERSITY

Diversity is the variation in living organisms within a given ecosystem (Yang *et al*., 2006). Genetic variation is recognized as one of the three fundamental levels of biodiversity, the other two being ecological diversity and species diversity. Genetic diversity is the variety of genes found among the individuals within a species. It is the raw material available to plant breeders (Huttner *et al*., 2004; Arif, 2010). 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 (Mehmood, Bashir et al., 2008; Mondini, 2009). An assessment of the genetic diversity within species is a prerequisite for future sustainable breeding efforts. The degree of success in the genetic improvement depends on the amount of genetic diversity existing among cultivated accessions as well as their wild relatives (Ojuederie *et al.,* 2014).

Characterization of sorghum germplasm is an important aspect in plant breeding programs to find new sources of genetic variation. Exploitation of diversity at the genotypic level requires an efficient system such as molecular marker technology. Genetic diversity assessment is one key step in any plant breeding programme and knowledge of the genetic relationships among different accessions is essential for developing appropriate strategies for breeding and germplasm management (Madhusudhana *et al.,* 2012). The variability in sorghum germplas is an invaluable input for sustaining and improving sorghum productivity (Mbeyagala *et al.,* 2012). RAPD marker have been used to study genetic diversity in sorghum germplasm (Agrama and Tuinstra, 2003).

2.1.6 CENTERS OF DIVERSITY OF SORGHUM AROUND THE WORLD

The efforts for to continue collecting new and additional landraces from isolated farmers' fields, and various collections have been assembled world-wide that represents much of the genetic diversity that can be found in sorghum. Major collections from the site of origin of sorghum exist for Ethiopia and Sudan, while other collections representing the major races and working groups of sorghum have been compiled in areas such as Mali, South Africa, India, and China. Internationally, two collections have worked to bring most all of these various collections together; the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) located in Andhra Pradesh, India and the National Center for Genetic Resources Preservation, located in Fort Collins, Colorado, USA (Kimbert *et al.,* 2012).

Table 1. Major sorghum collection from various countries around the world (GCDT, 2007).

Country	Institute	No. of accessions	% of total holdings (194,250 acc.)
USA	USDA-ARS-PGRCU	43,104	22.2
Global	ICRISAT	36,774	18.9
India	NBPGR	18,853	9.7
China	CAAS	18,250	9.4
Ethiopia	IBC	9,772	5
Brazil	EMBRAPA	8,017	4.1
Russia	VIR	7,335	3.8
Zimbabwe	NPGRC	7,009	3.6
Austrialia	DPI	5,403	2.8
Sudan	PGRU-ARC	4,191	2.2
Mali	IER	2,975	1.5
France	CIRAD	2,690	1.4
Kenya	NGBK	1,320	0.7
Zambia	NPGRC	1,005	0.5
South Africa	NPGRC	428	0.5
Malawi	NPGRC	401	0.2
Nigeria	NCGRB	159	0.2
Serbia	Inst. field and vegetable crops	152	0.1
Global	ILRI	52	0.1
	Total 19 institutions	**167, 890**	**86**

2.1.7 SORGHUM PRODUCTION

The world sorghum production reached 63,811 thousand metric tons in 2014, the main producers being the USA, Mexico, Nigeria, India, Argentina, Ethiopia, Sudan and China. The world consumption of sorghum reached 63,148 thousand metric(USDA, 2007; by Popescu and Condei, 2014). The record yield for grain sorghum was 20.1t ha^{-1} (Dahlberg, *et al,* 2011). Sweet sorghum tend to be extremely high yielding as well. Nebraska,USA has averaged 23t ha^{-1} (United States Department of Agriculture National Agricultural Statistics Service, 2011). However, sorghum is not a miracle crop. For maximum yield it too requires inputs such as moisture and nutrients. Yield losses in grain sorghum are attributed to unfavourable physiochemical environments, accounting for approximately 79 % of the yield reduction, that prevents it from obtaining record yields (Ali *et al.*, 2013). The abiotic stress tolerance of sorghum, especially the enhanced tolerance to drought and adaptation to marginal lands has been well documented (Berenji, 1993).

Under the same stressed environment the adaptation and yield stability of sorghum is more enhanced than that of maize. The present as well as the future of European eld crop production should not neglect this comparative advantage sorghum possesses. Europe should exploit sorghum as an alternative for more efficient and stable grain and biomass production in regions where water is limiting and heat stress is a problem for maize (Berenji *et al,* 2004 by Dahlberg *et al.,* 2011). With the expected increase of temperatures and decrease of precipitation as the result of global climate change and their effect on production of high input crops such as maize, the wide adaptability of sorghum could help alleviate crop losses in areas affected by abiotic stresses (Dahlberg *et al.,* 2011).

2.1.8 PESTS AND DISEASES

Very few biotic stresses occur on sorghum in Europe, which could be considered favourable for its utilization in this part of the world. Europe does not face the same pest problems as countries such as the US, which have greenbug (*Schizaphi graminum*) problems and Australia, which has to deal with midge (*Contarinia sorghicola*); both of

which can have serious economic impacts on the production of this crop. Until recently maize in Europe has been ranked among the crops with very few biotic stress problems; however, during the last decade the Western Corn Rootworm (*Diabrotica virgifera virgifera*), previously unknown in Europe, has become a considerable pest problem for maize producers. It was detected in July 1992 in the locality of Surčin (Serbia) near the Belgrade international airport. The origin of this introduction remains unknown (Čamprag, 1995 by Dahlberg *et al.*, 2011).

The pest started to spread at a rather rapid pace in the Danube Basin. In addition, several identications outside of the Danube basin have now been reported. In 2002, it was found for the rest time in France near airports close to Paris. This pest is likely to survive and develop wherever maize is grown in Europe. Insecticide use is quite limited, although growers can manage the rootworm problem very successfully through crop rotation. As sorghum is not among the hosts for Western Corn Rootworm, grain sorghum is an excellent replacement for corn in infected areas. Furthermore, sorghum could successfully replace corn in feed rations in the diets of many of the animals that currently depend on corn in areas where Western Corn Rootworm is an issue. The newly developed transgenic corn may change this situation, but in the light of Europe's current position on transgenic plants, transgenic corn for Western Corn Rootworm control will certainly not be a solution in the short or medium term in Europe (Dahlberg *et al.,* 2011).

Assessing sorghum [*Sorghum bicolor* (L) Moench] germplasm for new traits: food, fuels and unique uses. The disease situation with sorghum could also be considered favourable in Europe (Berenji, 2000 by Dahlberg, Berenji, Sikora, Latković, 2011). Diseases like head smut (*Sporisorium reilianum*) or downy mildew (*Peronosclerospora sorghi*), which can cause significant losses to sorghum growers in other parts of the world, are almost unknown in Europe. Anthracnose (*Colletotrichum graminicola*), fusarium (*Fusarium spp*.) and maize dwarf mosaic virus (have been observed in Europe but without significant economic losses. As large-scale production of sorghum in Europe develops, pest and disease problems could also develop, but in the meantime european sorghum farmers could benefit from the lack of these pests and diseases (Dahlberg *et al.,* 2011

2.1.9 OTHER IMPORTANT USES

***Cover Crop*:** Sorghum is used as a drought tolerant, summer annual rotational cover crop either alone or seeded in a warm season cover crop mixture. There are multiple cultivars of sorghum available for use as a cover crop. All sorghum species have the potential to smother weeds, suppress nematode species, and penetrate compacted subsoil. Sorghum cover crops can also be used as livestock forage in a cropping system (Dial, 2012)

***Soil Compaction*:** Sorghums have extensive root systems that can penetrate up to 8 feet into the soil and extend more than 3 feet away from the stem (Shoemaker and Bransby, 2010). These aggressive root systems alleviate subsoil compaction. To encourage more significant root growth, sorghum stalks should be cut at least once during the growing season when they reach 3–4 feet tall (Clark, 2007 by Dial, 2012).

Weed Management: Sorghums are quick growing grasses that have the potential to shade out and/or smother weed populations when planted at a high density. In addition, root exudates of sorghum have been shown to reduce the growth of weeds such as velvet leaf, thorn apple, redroot pigweed, crabgrass, yellow foxtail and barnyardgrass (Stapleton et al., 2010).

One of the most studied root exudates of sorghum is the compound sorgoleone. Sorgoleone is produced exclusively by sorghum species and suppresses the growth of many plant species, but it is most active on small seeded species. Sorgoleone activity in the soil is similar to the activity of a pre-plant incorporated herbicide. Detectable levels of sorgoleone have been measured up to seven weeks after incorporation (Dayan, et al., 2010).

Pest Management: Sorghum-Sudangrass hybrids have been reported to inhibit some species of nematodes in subsequent crops. The suppressive activity of the hybrids is due to their production of natural nematicidal compounds, their poor host status, general stimulation of microbial antagonists, and the release of toxic products during decomposition. For maximum suppression of soilborne diseases, cut or chopped Sudangrass must be immediately well incorporated (Clark, 2007 by Dial, 2012).

Forage: Sorghum and sorghum-Sudangrass hybrids are all very palatable and are highly valued as forage crops. They can be used as silage, hay, green chop, or in pastures. Sorghum and Sudangrass plants contain a compound called dhurrin, which can break down to release prussic acid (hydrogen cyanide, HCN) (Undersander, 2003 by Dial, 2012).

Biofuel Production: Sorghum cultivars are studied intensively as potential biofuel sources due to their high biomass yield and sugar production. The sugars sorghums produce give it an economical advantage over starch based crops for biofuel use. Other desirable characteristics of sorghum that make it an attractive biofuel crop for use on marginal lands include its wide range of adaptation, drought resistance, and salinity tolerance (Shoemaker and Bransby, 2010 by Dial 2012).

2.2 MOLECULAR MARKERS

In the last decade, the use of DNA markers for the study of crop genetic diversity has become routine, and has revolutionized biology (Spooner *et al*., 2005). Many promising new alternative molecular marker techniques have been developed in plant genetics, largely due to rapid growth in genomic research initiating a trend away from random DNA markers towards gene targeted functional markers (Poczail, Vargal, Laos *et al*., 2013). The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics (Semagn, Bjørnstad, Ndjiondjop, 2006; Mbeyagala, Kiambi, Okori, Edema, 2012).

DNA markers are defined as a fragment of DNA revealing mutations/variations, which can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool. DNA markers can be also defined as 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 (Schulman, 2007).

Such fragments are associated with a certain location within the genome and may be detected by means of certain molecular technology. Simply speaking, DNA marker is a small region of DNA sequence showing polymorphism (base deletion, insertion and

substitution) between different individuals (Jian, G. 2013; Semagn *et al.*, 2006). They are used to flag the position of a particular gene or the inheritance of a particular character (Datta *et al*., 2011). The availability of molecular markers to assess diversity is a quicker way to help breeders select suitable lines/genotypes for crossing (Schulman, 2007).

There are two basic methods to detect the polymorphism: Southern blotting, a nuclear acid hybridization technique (Southern 1975), and PCR, a polymerase chain reaction technique (Mullis, 1990). Using PCR and/or molecular hybridization followed by electrophoresis, the variation in DNA samples or polymorphism for a specific region of DNA sequence can be identified based on the product features, such as band size and mobility (Patwardhan *et al*, 2014). DNA markers are also called molecular markers in many cases and play a major role in molecular breeding (Jian, G. 2013)

Depending on application and species involved, ideal DNA markers should meet the following criteria:

• High level of polymorphism • Even distribution across the whole genome (not clustered in certain regions) • Co-dominance in expression (so that heterozygotes can be distinguished from homozygotes) • Clear distinct allelic features (so that the different alleles can be easily identified) • Single copy and no pleiotropic effect • Low cost to use (or cost-efficient marker development and genotyping) • Easy assay/detection and automation • High availability (un-restricted use) and suitability to be duplicated/multiplexed (so that the data can be accumulated and shared between laboratories) • Genome-specific in nature (especially with polyploids) (Jian, 2013). Molecular markers are phenotypically neutral (Datta *et al*., 2011).

Since first used DNA restriction fragment length polymorphism (RFLP) in human linkage mapping (Botstein, 1980 by Schulman, 2007), substantial progress has been made in development and improvement of molecular techniques that help to easily find markers of interest on a largescale, resulting in extensive and successful uses of DNA markers in human genetics, animal genetics and breeding, plant genetics and breeding, and germplasm characterization and management (Jing, 2013).

Among the techniques that have been extensively used and are particularly promising for application to plant breeding, are the restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeat (SSR), and single nucleotide polymorphism (SNP) (Jian, 2013). The use of molecular markers as a tool to assess relatedness in and between cultivated and wild sorghum have been successfully used (Schulman, 2007). In sorghum and other crop plants, efforts to estimate genetic variation have been greatly enhanced with the advent of molecular techniques. Seed storage proteins (Shechter, De Wet, 1975 by Ayana, *et al*., 2000), isozymes, restriction fragment length polymorphism (RFLP) (Aldrich and Doebley, 1992) and random amplified polymorphic DNA (RAPD) (Menkir *et al*., 1997) have been used to estimate genetic variation in sorghum. These markers differ in the level at which they detect genetic variation (and hence extent of genome coverage), extent of polymorphism, degree of environmental stability, number of loci, molecular basis of the polymorphism, practicality, and amenability to statistical estimation of population genetics parameters (Ayana *et al.,* 2000).

Table 2. Comparison of the five most widely used DNA markers in plants (Semagn *et al.*, 2006).

Feature	RFLP	Microsatellite	RAPD	AFLP	ISSR
Genomic abundace	High	Medium	Very high	Very high	Medium
Part of genome surveyed	Low copies coding regions	Whole genome	Whole genome	Whole genome	Whole genome
Amount of DNA required	High	Low	Low	Medium	Low
Type of Polymorphism	Singles base changes, insertion, deletion	Changes in length of repeats	Singles base changes, insertion, deletion	Singles base changes, insertion, deletion	Singles base changes, insertion, deletion
Inheritance	Codominant	Codominant	Dominant	Dominant	Dominant
Detection of alleles	Yes	Yes	No	No	No
Ease to use	Labour intensive	Easy	Easy	Difficul inicially	Easy
Automation	Low	High	Medium	Medium	Medium
Reproducibility	High	High	Intermediate	High	Medium to high
Types of probes/primers	Low copy genomic DNA or cDNA clones	Specific repeat DNA sequence	Usually 10 bp random nucleotide	Specific sequence	Specific repeat DNA sequence
Cloning and/or sequencing	Yes	Yes	No	No	No
Utility for genetic mapping	Species specific	Species specific	Cross specific	Cross specific	Cross specific

2.2.1 RANDOM AMPLIFIED POLYMORPHISC DNA, RAPD

Estimation of genetic variations are increasingly being based upon information at the DNA level by various molecular techniques such as Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), RFLP, SSR and microsatellite. Among them, RAPD, markers generated by Polymerase Chain Reaction (PCR) is widely used since 1990’s to assess intra specific genetic variation at nuclear level (Welsh and McClellan, 1990).

RAPD is a PCR based technique for identifying genetic variation. It involves use of single arbitrary primer in a PCR reaction, resulting in amplification of many discrete DNA. RAPD technology provides a quick and efficient screen for DNA sequence based polymorphism at a very large number of loci. The major advantage of RAPD includes that, it does not require pre-sequencing of DNA. Genetic variation and divergence within and between the taxa of interest are assessed by the presence or absence of each product, which is dictated by changes in the DNA sequence at each locus (Liu and Cordes, 2004).

Random amplified polymorphic DNA (RAPD) is one of the promising techniques for characterizing the cultivars/ hybrids because of its simplicity and rapidity (Amrapali *et al.,* 2008). The PCR based RAPD's profiling have quickly gained the widespread acceptance and application because it has provided a tool for genetic analysis in biological systems (Welsh and McClelland, 1990). The information obtained through germplasm characterisation using RAPD is extensively used for the identification of germplasm, screening of duplicates, assessing genetic diversity and monitoring the genetic stability of conserved germplasm (Amrapali *et al*, 2008).

The vast range of potential primers that can be used, give the technique great diagnostic power (Datta *et al,* 2011). Reproducible RAPD bands can be found by careful selection of primers, optimization of PCR condition for target species and replication to ensure that only reproducible bands are scored. RAPD analysis has been extensively used for various purposes which include identification and classification of

accessions, identification of breeds, and genetic diversity analysis. The advent of Randomly Amplified Polymorphic DNA (RAPD) (Liu and Cordes, 2004).

RAPD polymorphisms can occur due to base substitutions at the primer binding sites or to indels in the regions between the sites. The potential power is relatively high for detection of polymorphism; typically, 5 –20 bands can be produced using a given primer pair, and multiple sets of random primers can be used to scan the entire genome for differential RAPD bands (Liu and Cordes, 2004).

RAPD markers are inherited as Mendelian markers in a dominant fashion and scored as present/absent. A RAPD band is produced by homozygotes as well as heterozygotes, and though band intensity may differ, variations in PCR efficiency makes scoring of band intensities difficult. As a result, distinguishing homozygous dominant from heterozygous individuals is not generally possible. In addition, it is difficult to determine whether bands represent different loci or alternative alleles of a single locus, so that the number of loci under study can be erroneously assessed. This is especially true if the RAPD is caused by deletion or insertion within the locus rather than at the primer binding sites (Liu, Cordes, 2004). RAPD proved to be reliable, rapid and practical technique of revealing relationship among sorghum varieties (Akram *et al*, 2011).

2.2.1.1 Advantages of RAPD

Main advantages of the RAPD technology include (i) suitability for work on anonymous genomes, (ii) applicability to problems where only limited quantities of DNA are available, (iii) efficiency and low expense (Liu and Cordes, 2004). Non involvement with radioactive assays (Kiss *et al,* 1993 by Jonah *et al,* 2011).

High number of fragments are formed. It is a simple technique. Arbitrary primers used for this technique can be easily purchased and there is no need for initial genetic or genomic information and the unit cost per assay is low. RAPDs have the advantage that they can be obtained at a reasonable cost and will generally amplify a range of fragments of most DNA and show polymorphisms. Certain primers will produce unrelated patterns between unrelated species and identical ones for very closely

related species. Presumably, primer sites are randomly distributed along the target genome and flank both conserved and highly variable regions. Wide variation in band intensity can be shown to be reproducible between experiments, which could be the result of multiple copies of the amplified regions in the template or the efficiency with which particular regions are amplified. The polymorphic bands obtained from RAPDs can also be cloned for further analysis (Liu and Cordes, 2004).

3 OBJECTIVES

1. The main objective of this study is to assess the genetic diversity of *Sorghum bicolor* of 45 accessions from the Gene Bank of the Crop Research Institute, Prague.

Specific objectives

1. To evaluate the polymorphism for 45 accessions of *Sorghum bicolor* using RAPD molecular marker.

2. To analyze the genetic relationship between 45 accessions of *Sorghum bicolor* through the use of statistical methods.

4 HYPOTHESIS

1. The Random amplified polymorphism RAPD is an poweful and effective techinique to identify the genetic diversity in many species as *Sorghum bicolor* as mencioneted by Ayana *et al* (2000*);* Horn *et al* (2007); Amparali (2008) and Mehmood (2008).

2. The Random amplified polymorphism RAPD is not the most appropriate techinique to identify the genetic diversity in *Sorghum bicolor* as mencioned by Agrama and Tuinstra (2003); Bahaa *et al*., (2013) and Mofokeng *et al.,* (2012).

5 MATERIALS AND METHODS

5.1 PLANT MATERIAL

As first stage, 15 samples was collected, directly from the field for the optimization of DNA extraction protocol and also for screening all the available primers and to identify, wich of them, was useful for detecting polymorphic bands.

Total number of 45 seed accessions of *Sorghum bicolor.* Selected accessions were obtained from the Czech Gene Bank of the Crop Research Institute (CRI) Prague, Czech Republic. Those samples were donated from Russia Federation (RUS), Germplasm Resources Information Network (GRIN), International Crops Research Insitute for the Semi-Arid Tropics (ICRISAT) and Republic of Moldova (MOL). The complete list of accessions is grouped in Table 3.

All the samples were sown on germination trays and placed inside of greenhouse. The temperatura inside of greenhouse was between 24°C - 26°C with 16 hours of photoperiod. The germination period was around 10 days for all the genotypes. The biological material collected consisted of adult fresh young leaves and was collected 30 day after sowing.

Leaves samples were cut and individually packed in small bags. From each genotype 10 leaves were collected. The samples were transported to the laboratory of the Division of Crop and Plant Health in the Crop Research Institute. The samples were storaged in a refrigerator at -20 °C.

Table 3. List of accessions used. Early genotype (E), Late genotype (L).

No.	Field Number	Register number	Name of genotype	Donor entity	Collection site	Type of Genotype
2	1	Z18-0012 1A		Unknow	Hungary	E
3	2	Ruzrok 2B		CRI	Czech Republic	E
4	13		Jantar Rannij 161	RUS	Russia	L
5	18a	PI 326289	Gaolian Voskovidnyj nízky	RUS	Russia	E
6	18b	PI 326289	Gaolian Voskovidnyj vysoky	RUS	Russia	E
7	17	PI 314743	IS 14015	GRIN	Russia	E
8	19	PI 326293	Efremovskoe 2	RUS	Russia	L
9	20	PI 619662	IS 30470	GRIN	Russia	L
10	21	PI 619663	IS 30471	GRIN	Russia	L
11	22	PI 619665	IS 30473	GRIN	Russia	L
12	23	PI 619666	IS 30474	GRIN	Russia	L
13	24	PI 619667	IS 30475	GRIN	Russia	L
14	25	PI 619668	IS 30476	GRIN	Russia	L
15	26	PI 619670	IS 30478	GRIN	Russia	L
16	27	PI 619677	IS 30485	GRIN	Russia	L
17	29	PI 246594	IS 3098	GRIN	Russia	L
18	34	PI 262567	Stavropolskoye 98	RUS	Russia	L
19	36	PI 267123	K-460	GRIN	Russia	L
20	40	PI 284975	Barnard Red	GRIN	Argentina	L
21	45	PI 326292	Belozernoe Utr-110	RUS	Russia	L
22	46	PI 326294	Penzenskoje Ranneje 97	RUS	Russia	E
23	47	PI 326295	Dzugara Mestnaja	RUS	Russia	L
24	48	PI 392391	2256	GRIN	Russia	L
25	49	PI 495001	Hazine-4	GRIN	Russia	L
26	52	PI 495010	Yantar Zernogradskij	RUS	Russia	L
27	53	PI 539066	AJC544	GRIN	Russia	E
28	57	PI 576035	Sor 9	GRIN	Germany	E
29	105	PI 177551 03SD		GRIN	Turkey	E
30	114	PI 174380 03SD		GRIN	Turkey	L
31	118	PI 170800 01SD		GRIN	Turkey	L
32	150	PI 246595	Saccharatum	GRIN	Russia	E
33	168	PI 408814 02SD		GRIN	China	L
34	183	PI 922267 03SD		GRIN	China	L
35	188	IS 18758		ICRISAT	Ethiopia	L
36	194	IS 18813	Wild	ICRISAT	Egypt	L

Table 3. List of accessions used. Early genotype (E), Late genotype (L).

37	195	IS 2262	Landrace	ICRISAT	Sudan	L
38	196	IS 12804	Landrace	ICRISAT	Turkey	L
39	201	IS 32050	Landrace	ICRISAT	Yemen	L
40	276	IS 20727	Landrace	ICRISAT	USA	L
41	313	IS 20351	Landrace	ICRISAT	Nigeria	L
42	315	IS 20709	Landrace	ICRISAT	USA	L
43	316	IS 23988	Landrace	ICRISAT	South Africa	L
44	318	IS 2367	Landrace	ICRISAT	Nigeria	L
45	319		PRO-BIO (Moldavsko 1)	MOL	Moldova	L
46	320		PRO-BIO (Moldavsko 2)	MOL	Moldova	L
47	Setaria	01Z2300022	*Setaria italica*		France	

5.2 DNA EXTRACTION

The DNA isolation was made using the DNeasy Plant Mini Kit (QUIAGEN). This method was chosen because it is not necessary high concentration of DNA for RAPDs. The first step of the method was homogenizing the samples; the leaves were put into a mortar and frozen with liquid nitrogen. The the samples were disrupted using mortar and pestle until to pulverize the tissue. One hundred miligrams of the powder was transfered into 2 mL sterilized microtubes; 400 µl of buffer AP1 and 4 µl RNase A was added, put in vortex and incubated for 10 minutes at 65 °C. After, 130 µl buffer P3 was added, mixed and incubated for 5 minutes on ice. Then the samples were centrifuged for 5 minutes at 12,000 rpm. The lysate was pipetted into a QIAshredder spin colum and placed in a 2 mL collection tube. The samples were centrifuged for 2 minutes at 14,000 rpm. The flow-through was transfered into a new tube, without disturbing the pellet located at the bottom of the tube, 375 µl buffer AW1 was added and mixed by pipetting.

Then 650 µl of the mixture was transfered into a DNeasy Mini spin column and placed in a 2 mL collection tube. The samples were centrifuged for 1 minute at 8,000 rpm. The flow through was discared. The spin column was placed in a new 2mL collection tube collection and 500 µl buffer AW2 was added and centrifuged for 1 minute at 6,000 rpm. The flow-through was discared. Another 500 µl AW2 buffer was added and the samples were centrifuged for 1 minute at 10,000 rpm. The spin column was transfered to a new 1,5 mL tube, 100 µl buffer AE was added, incubated for 5

minutes at room temperature and centrifuged for 1 minute at 6000 rpm. Finally, the genomic DNA was separated using agarose electrophoresis gel (1%) it was visualized under UV light and photographed.

5.3 PCR-AMPLICATION OF DNA AND FRAGMENTS VISUALIZATION

Condition for DNA amplification were standardised for all 10-mers used as random primers (Operon Technologies) (Table 6). PCR (Polymerase Chain Reaction) reactions were performed using a 25 µl reaction mixture containing 10X *Taq* DNA polymerase buffer with $MgCl_2$, 20 mM $MgCI_2$, 10 mM dNTPs, 30 pg primer, one unit AmpoONE *Taq* DNA polymerase (GENANLL BIOTECHNOLOGY), 25 to 30 ng template DNA and sterile distilled water.

Table 4. PCR composition.

Substance	Concentration	Volumen
Destilled water	-	19.02 µl
10X Buffer	10x	2.5 µl
$MgCl_2$	25mM	1.5 µl
dNTP	10mM	0.1 µl
Primer	10mM	0.5 µl
Taq* DNA *polymerase	5U/ µl	0.2 µl
DNA	25-30 ng/ µl	1 µl
Total		25 µl

For DNA amplification, the DNA thermal cycler (PTC-200 of MJ Research) was programmed as follows: one cycle of 2 minutes at 94°C followed by 40 cycles at 94°C for 20 seconds, 36°C for 1 minute, ramp + 0.2°C/s, 72°C for 1 minute, and final extension 72°C for 7 minutes (Table 5).

Table 5. PCR profile.

Step	Temperature	Time	Number of cycles
Initial denaturation	94°C	2 minutes	1
Denaturation	94°C	20 seconds	
Annealing	36°C	1 minute	40
Extension	72°C	2 minutes	
Final extension	72°C	7 minutes	1
Soak	10°C	Indefinite	1

Table 6. List of RAPD primer used.

No	Primer	Sequence
1	**OPB 01**	5´ GTTTCGCTCC 3´
2	**OPB 02**	5´ TGATCCCTGG 3´
3	**OPB 04**	5´GGACTGGAGT 3´
4	**OPB 05**	5´ TGCGCCCTTC 3´
5	**OPB 06**	5´TGCTCTGCCC 3
6	**OPB 08**	5´GTCCACACGG 3´
7	**OPB 10**	5´CTGCTGGGAC 3´
8	**OPB 11**	5´GTAGACCCGT 3´
9	**OPB 12**	5´CCTTGACGCA 3´
10	**OPB 13**	5´TTCCCCCGCT 3´
11	**OPB 14**	5´TCCGCTCTGG 3´
12	**OPB 15**	5´GGAGGGTGTT 3
13	**OPB 16**	5´TTTGCCCGGA 3´
14	**OPB 17**	5´AGGGAACGAG 3´
15	**OPB 18**	5´CCACAGCAGT 3

Amplified products were mixed with loading dye (GENALL BIOTECHNOLOGY) and loaded onto the agarose gel. Electrophoretic separation was performed with 10 µl of amplified products on 1,5% agarose gel (CAMBREX) in 1x TBE buffer. The size (bp) of most intensely amplified band for each RAPD marker was determined based on its migration relative to molecular weight size 100 bp DNA ladder (Cleaver Scientific). Gels were run for about 1.5 - 2 h at 4 $V.cm^{-1}$. Gels were stained with ethidium bromide (Promega), and visualized with a UV transilluminator and photographed (Figure 3).

Figure 3. RAPD profile generated by OPB 02 primer. The number corresponding to the samples is at the top of each lane. M is the 100bp molecular weight marker 100 bp.

5.4 DATA ANALYSIS

RAPD fragments were scored for the presence (1) or absence (0) of bands in the gel profile. Only strong and clear bands were used to construct a binary matrix in Microsoft® Excel. A determination of the proportion of polymorphic loci (P) was calculated with the formula: $P = n/N \times 100\%$ where n is the number of polymorphic bands within the group of accessions and N is the total number of bands that we analysed. The binary matrix was exported, as unicode text file (.txt), and then was used to calculate a dissimilarity matrix using Dice's coefficient (Dice 1945) using following formula: $d_{ij} = (b+c)/2a + (b+c)$, where a represents number of

variables where $x_i$is present and xj is is present; b stands for variables where x_i is present and x_j in absent; and finally c is the number of variables where x_i is absent and x_j is present. The d_{ij} means the dissimilarity between units i and$j$.

Data were analyzed using DARwin 6.0.012 software (Perrier and Jacquemoud-Collet, 2006), and then a final Neighbour joining (NJ) dendrogram (Saitou and Nei 1987) was constructed by means of the UnWeighted Neighbor-Joining method. The final dendogram was also showed as radial scheme for easily creation of sub-clusters. This scheme was also created with DARwin 6.0.012 software.

A distance matrix was constructed by tree distance tool using DARwing 6.0.012 software. This matrix was used as dissimilarity matrix and was useful for determination of dissimilartiy distance among the sorghum accesions. On this study, the term distance does not mean distance in Euclidean space; DARwing 6.0.012 software use this term as a subjective measure of dissimiliarty.

Finally, the dissimilarity index data, from distance matrix, was used to calculate the mean number and standard deviation per each cluster. This calculations were done by Microsoft® Excel.

6 RESULTS

6.1 RAPD PROFILE AND ANALYSIS

A total of 15 decamer primers (Table 6) that showed clear bands were selected trough screening of 20 RAPD total primers in 15 sorghum accessions. These 15 primers were then used to analyze the genetic diversity of 45 sorghum accessions and 1 setaria accession. For those selected primers, all of them provided at least three polymorphic bands (Table 7). The lenght of fragments ranged from about 100 to 2500 base pair (bp), but the results suggest that fragments out of range between 200 and 2000 were rarely reproducible. The amplication products ranging from 250 to 2,000 bp that yielded only sharp and strong were scored to build a binary matrix.

A total of 126 bands were identified with mean number of 8.4 scorable bands per primer and 111 of the total scorable bands, were identified as polymorphic bands, that means 89%; while only 15 bands from the total scorable bands were identified as monomorphic bands, that means 11%. Absolute polymorphism (100%) was observed with primers OPB 02, OPB 06, OPB 15 and OPB 16; while the lowest level of polymorphism (66,6%) was obtained with primer OPB 14 (Table 6). The highest number of polymorphic bands per primer was produced by OPB 2 (13 polymorphic bands) as it is possible to see it in Figure 3; while the lowest number of polymorphic bands was observed by primer OPB 16 (3 polymorphic bands).

The higher number of monomorphic bands were observed by primer OPB 12 producing 3 bands that means 25% of the total of produced bands for this primer; while the lower number of monomorphic bands were observed by primers OPB 02, OPB 06, OPB 15 and OPB 16 producing no monomorphic bands.

Any primer was able to provide a specific banding pattern for each accession, nevertheless, primer OPB 02 and OPB 08 generated an specific banding pattern for accesion number 18, as possible to see on Figures 3 and 4 respectively.

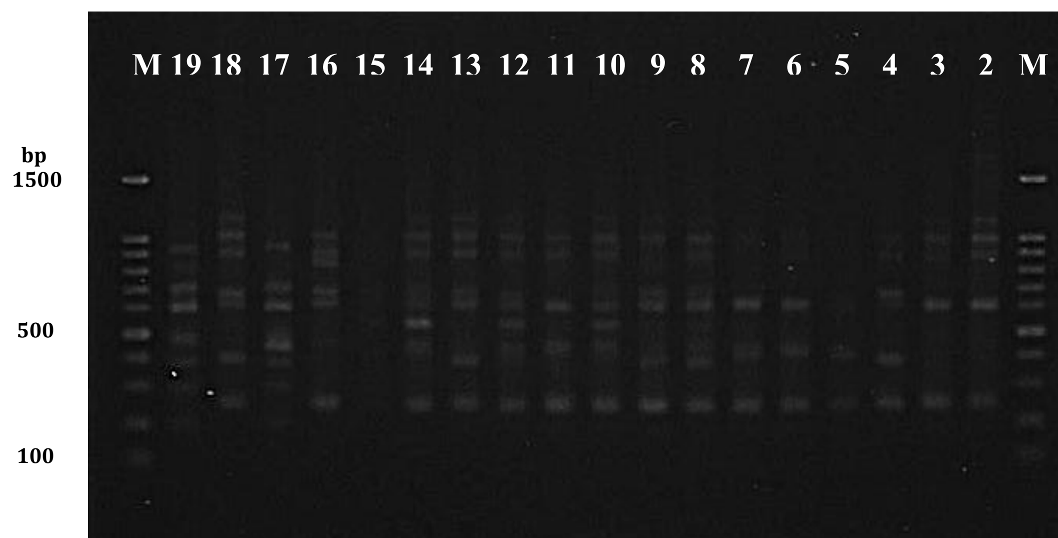

Figure 4. RAPD profile generated by OPB 08 primer. The number corresponding to the samples is at the top of each lane. M is the molecular weight marker.

Table 7. Primers used and schematic description of monomorphic and polymorphic bands obtained with RAPD marker.

Primer	Monomorphic bands	Polymorphic bands	Total scorable bands	% Polymorphic bands per primer	Range of amplificacation (bp)
OPB 01	1	7	8	87.5	350 – 1000
OPB 02	0	13	13	100	250 – 2000
OPB 04	1	11	12	91,6	200 – 2000
OPB 05	1	7	8	87.5	350 – 950
OPB 06	0	8	8	100	200 – 900
OPB 08	1	10	11	90.9	200 – 1200
OPB 10	1	6	7	85.7	400 – 1300
OPB 11	2	6	8	75	300 – 1200
OPB 12	3	9	12	75	200 - 1200
OPB 13	1	5	6	83.3	500 – 1400
OPB 14	2	4	6	66,6	300 - 900
OPB 15	0	7	7	100	300 – 1200
OPB 16	0	3	3	100	700 – 900
OPB 17	1	5	6	83.3	400 – 1300
OPB 18	1	10	11	90.9	200 – 1700
Total	**15**	**111**	**126**	**-**	**-**
Mean	**1**	**7.4**	**8.4**	**89**	**-**
Range	**0 - 3**	**3 - 13**	**3 - 13**	**66.6 - 100**	**200 - 2000**

6.2 GENETIC DIVERSITY AND CLUSTER GROUPING BASED ON THE RAPD GENOTYPING PROFILE

A dendrogram based on Neighbour joining analysis of the RAPD data was constructed in order to be able to infer the phylogenetic relationships among 45 sorghum accessions and 1 setaria accession. The accessions were divided in clusters (Figure 5) and then subdivided in smaller cluster through the radial scheme based on Neighbour joining analysis (Figure 6). The radial scheme was constructed in order to better understanding of phylogenetic relationship among the accessions.

The mean number values for the dissimilarity index per cluster ranged from 0.0909 to 0.1790 (Table 8). The standard deviation values for the dissimilarity index ranged from 0.0222 to 0.1212. The dissimilarity index values per accessions ranged from 0.06260 for accession number 5, to 0.566482 for accession number 47 corresponding to setaria genus (Table 9). The dissimilarity index values among all accessions can be observed through the distance matrix based on RAPD data (table 10).

Table 8. Mean number, standard deviation of dissimilarity index grouped by clusters, number of sub-clusters and level of homogeneity.

Cluster	sub-clusters	Dissimilarity index		Homogeneity
Cluster	sub-clusters	Mean	Standard deviation	Homogeneity
I	4	0.0909	0.0222	1
II	0	0.1251	0.0331	2
III	3	0.179	0.1212	6
IV	2	0.1536	0.0767	5
V	2	0.1264	0.0615	4
VI	2	0.1143	0.0488	3

According to the dendogram, all accesions were divided into six clusters. Cluster I consist of 14 accessions (Table 9) from Russia, Germany, Egypt, Turkey, and China as the collection sites. It is also possible to identify early and late genotypes. The dissimilarity index values ranged from 0.046925 for accession number 32, to 0.134011 for accession number 29. The mean number for dissimilarity index was 0.0909 and standard deviation was 0.0222. This cluster was divided into four sub-clusters for better understanding about the origin and type of genotype of the accessions grouped into this maincluster.

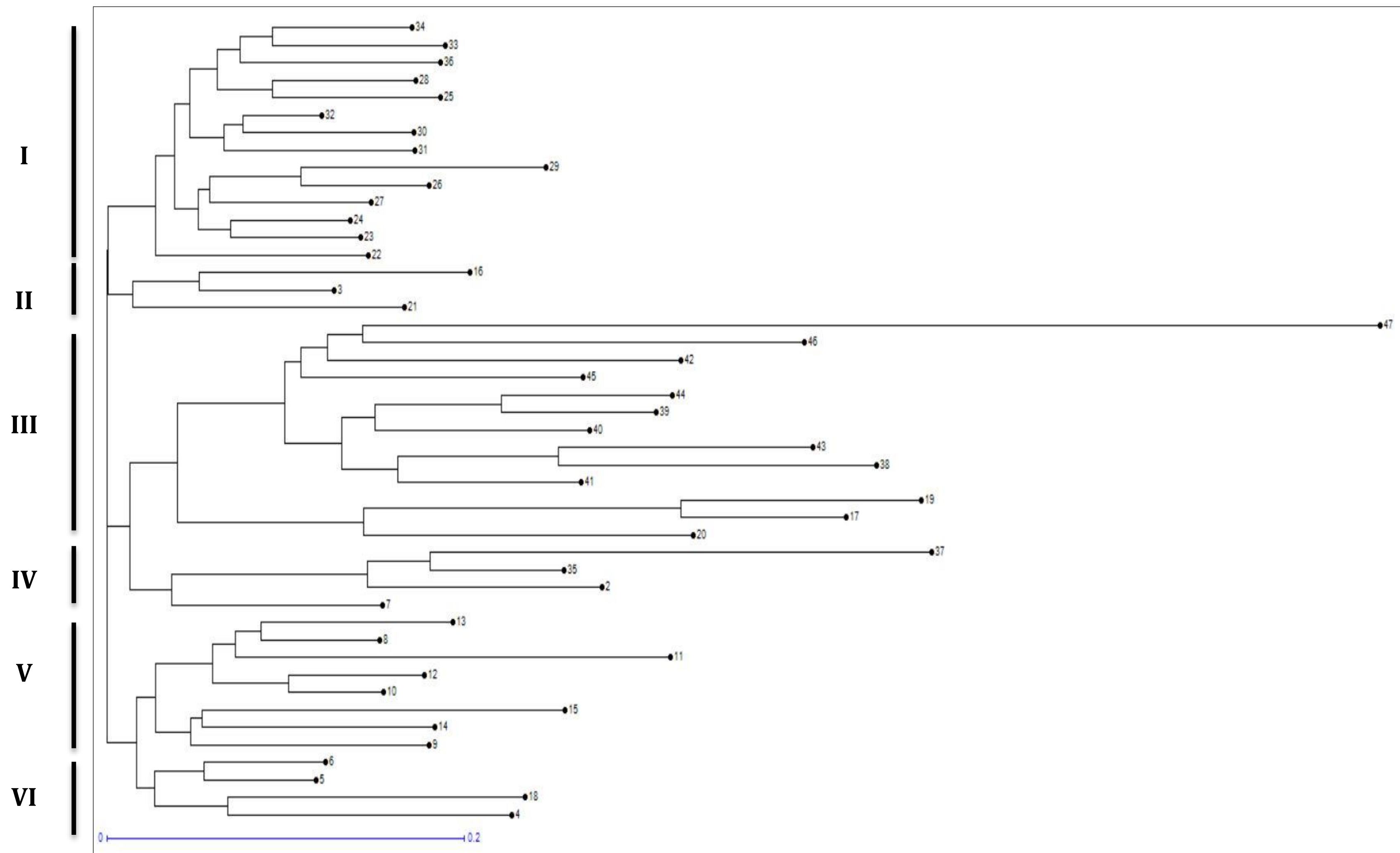

Figure 5. Neighbou joining dendogram showing relationship among sorghum accessions. Dendogram constructed based on gained RAPD marker

Table 9. Sorghum accessions grouped by cluster number.

Number of accesion	Cluster	Dissimilarity index value	Origin	Genotype
22	I	0.1203	**Russia**	E
23	I	0.0729	**Russia**	L
24	I	0.0671	**Russia**	L
25	I	0.0908	**Russia**	**L**
26	I	0.0759	**Russia**	L
27	I	0.0888	**Russia**	E
28	I	0.0844	**Germany**	E
29	I	0.134	**Turkey**	E
30	I	0.0933	**Turkey**	L
31	I	0.1077	**Turkey**	L
32	I	0.0469	**Russia**	E
33	I	0.0978	**China**	L
34	I	0.0775	**China**	L
36	I	0.1153	**Egypt**	L
3	II	0.0783	**Czech Republic**	E
16	II	0.149	**Russia**	L
21	II	0.1481	**Russia**	L
17	III	0.0927	**Russia**	L
19	III	0.135	**Russia**	L
20	III	0.1879	**Argentina**	L
38	III	0.1763	**Turkey**	L
39	III	0.0873	**Yemen**	L
40	III	0.1223	**USA**	L
41	III	0.1076	**Nigeria**	L
42	III	0.2053	**USA**	L
43	III	0.1444	**South Africa**	L
44	III	0.0958	**Nigeria**	L
45	III	0.1552	**Moldova**	L
46	III	0.2517	**Moldova**	L
47	III	0.5665	**France**	--
2	IV	0.1311	**Hungary**	E
7	IV	0.127	**Russia**	E
35	IV	0.0754	**Ethiopia**	L
37	IV	0.2809	**Sudan**	L
8	V	0.0732	**Russia**	L
9	V	0.1358	**Russia**	L
10	V	0.0526	**Russia**	L
11	V	0.2388	**Russia**	L
12	V	0.0771	**Russia**	L

Table 9. Sorghum accessions grouped by cluster number**.**

13	V	0.1007	**Russia**	L
14	V	0.1319	**Russia**	L
15	V	0.2014	**Russia**	L
4	VI	0.1687	**Russia**	L
5	VI	0.0626	**Russia**	E
6	VI	0.0687	**Russia**	E
18	VI	0.1572	**Russia**	L

Cluster II (figure 5) was conformed to tree accessions from Russia, as collecting site, and early and late genotypes. The dissimilarity index values ranged from 0.078276 for accession number 3, to 0.148996 for accession number 16. The mean value for dissimilarity index was 0.1251 and standard deviation 0.0331.

Cluter III was conformed to 12 sorghum accessions and the only one setaria accession. All those accessions were from Russia, Argentina, Turkey, Yemen, United States, Nigeria, South Africa, Nigeria and Moldova. The dissimilarity index value for setaria accessions was 0.566482. For sorghum accesions, the dissimilarity index values ranged from 0.107611 for accession number 41, to 0.135016 for accession number 19. The mean number for dissimilarity index was 0.1790 and standard deviation was 0.1212. This cluster was divided into tree sub-clusters for better understanding about the origin and the type of genotype of the accessions.

Cluster IV was conformed to 4 sorghum accessions from Russia, Ethiopia and Sudan as the collecting sites. The dissimilarity index values ranged from 0.126968 for accession number 7, to 0.280881 for accession number 37. The mean value for dissimilarity index was 0.1536 and standard deviation was 0.0767. This cluster was divided into two sub-clusters for better understanding of phylogenetic relationship.

Cluster V was conformed to 8 sorghum accessions belonging to Russia as the collecting site. The dissimilarity index value ranged from 0.0732 for accession number 8, to 0.2388 for accession 11. The mean number for dissimilarity index was 0.1264 and standard deviation was 0.0615. This cluster was also divided as cluster IV.

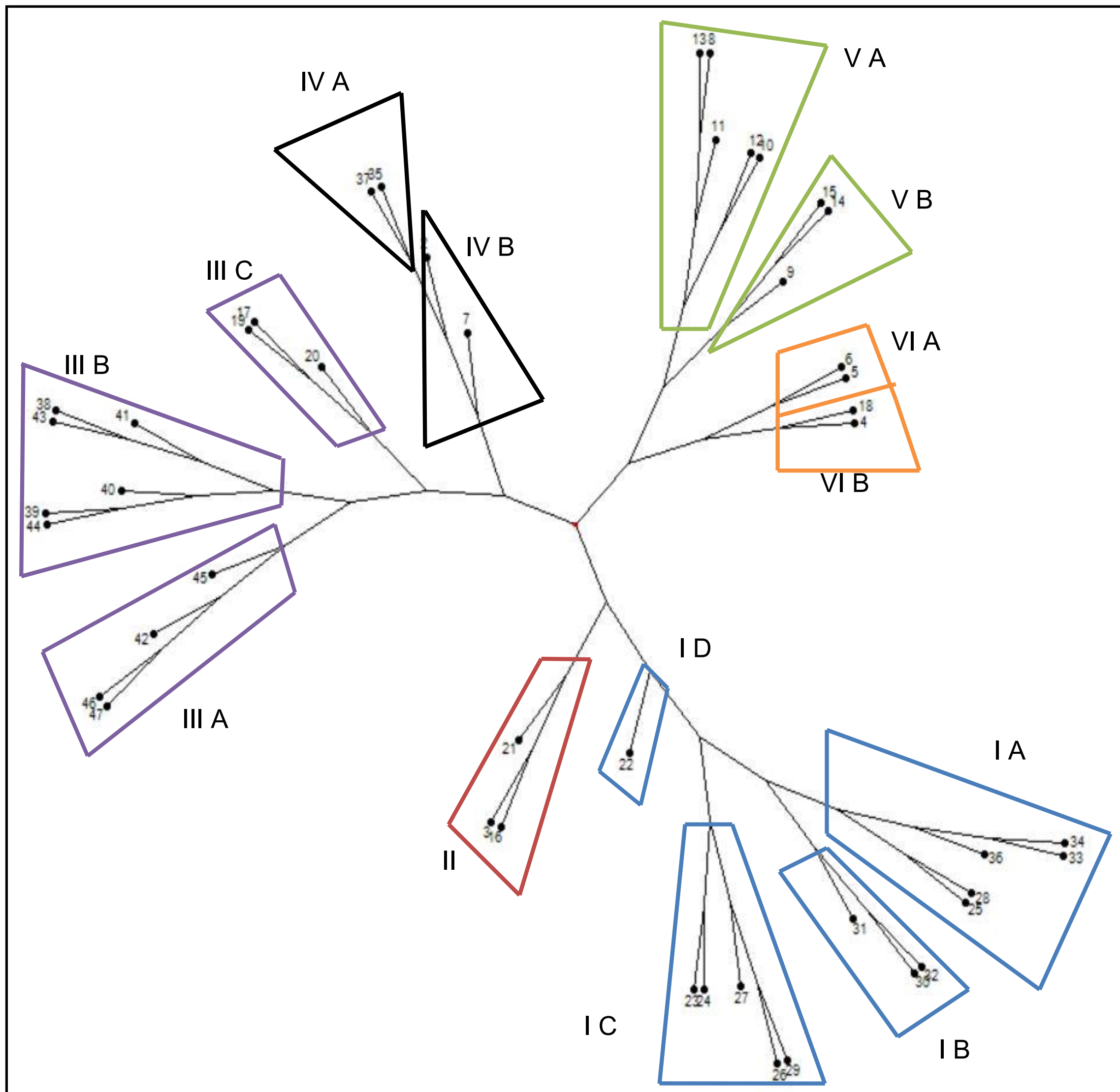

Figure 6. Neighbour joining radial scheme showing sub-cluster division among sorghum accessions. Radial representation constructed based on RAPD markers gained. All edge lengths were established to one to exhibit a better radial scheme structure.

Finally, cluster VI was conformed to 4 sorghum accessions all of them belonging to Russia. The dissimilarity index value ranged from 0.0626 for accession number 5, to 0.1687 for accesion 11. The mean value for dissimilarity index was 0.1143 and standard deviation was 0.0488. This cluster was also divided into two sub-clusters for better understanding of the relationship among the accessions.

The distance matrix among the all sorghum accessions was found in the range of 0.13 to 0.89, with the mean of 0.456. The lowest value among sorghum accessions ranged 0.13 (table 10), and it was found between the accession No.10 and accession No. 12, while the lowest value between setaria accesion and sorghum accessions ranged (0.7533) and was found between the accession No. 47 (setaria) and sorghum accession No. 46.

The highest genetic distance value among the sorghum accessions (0.8776)was found between the accession No. 37 and accession No. 16, while the highest value between setaria accesion and sorghum accessions (0.9126) was found between the accession No. 47 (setaria) and sorghum accession No. 36.

Table 10. Genetic distance matrix among sorghum accessions base on RAPD data. The rest of data can be observed in appendices.

No. accessions	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	0														
3	0.42	0													
4	0.516	0.355	0												
5	0.412	0.251	0.3	0											
6	0.418	0.257	0.306	0.131	0										
7	0.365	0.309	0.405	0.301	0.307	0									
8	0.434	0.273	0.353	0.249	0.255	0.323	0								
9	0.462	0.301	0.381	0.277	0.283	0.351	0.298	0							
10	0.441	0.28	0.36	0.256	0.262	0.33	0.2	0.306	0						
11	0.579	0.418	0.498	0.394	0.4	0.468	0.332	0.444	0.346	0					
12	0.466	0.305	0.385	0.281	0.287	0.355	0.225	0.33	0.13	0.37	0				
13	0.461	0.3	0.38	0.276	0.282	0.35	0.174	0.326	0.228	0.359	0.252	0			
14	0.462	0.301	0.381	0.277	0.283	0.351	0.298	0.271	0.306	0.444	0.33	0.326	0		
15	0.531	0.371	0.451	0.346	0.353	0.42	0.368	0.341	0.375	0.513	0.4	0.395	0.333	0	
16	0.491	0.227	0.426	0.322	0.328	0.38	0.344	0.372	0.351	0.489	0.376	0.371	0.372	0.441	0

7 DISCUSSION

Due the impact of sorghum (*Sorghum bicolor*) as one of the most import cereals around the world, it is possible to find a wide number of studies related to determination of genetic diversity of this species (Agrama and Tuinstra, 2003; Akram *et al*., 2011; Amparapali *et al*., 2008; Ayana *et al*., 2000a; Ayana *et al*., 2000b; Bahaa *et al*., 2013; Dahlberg, 2011; Dje *et al*., 2000; Hart *et al*., 2001; Horn *et al*., 2001; Jeya *et al*, 2006; Lekgari and Dweikat, 2014; Madhusudhana et al., 2012; Mbeyagala *et al*., 2012; Mehmood *et al*., 2008; Menkir *et al*., 1997; Mofokeng *et al*., 2012; Ritter *et al*., 2007; Smith *et al*., 2000; Tao *et al*., 1993; Tawanda, 2004). Nevertheless, there is not any study to determine the intra-specific relationship among the sorghum accessions and inter-generic relationship among *Sorghum* and *Setaria* genus, stored in the Gene Bank of the Crop Research Institute, Prague.

7.1 RAPD MARKERS

In RAPD analysis 126 bands were observed, of wich the 89% were polymorphic bands. This value is similar to those obtained by Mehmood *et al*., (2008) with 78.94%. The value of polymophic bands obtained by Akram *et al*., (2011) was considerably higher, due the value ranged 100%, similar value was obtained by Jeya *et al*., (2006) 97.4% of polymophic bands; while Tao *et al.* (1993), Agrama and Tuinstra (2003), Nkongola and Nsapato (2003) Amrapali *et al.* (2008) Bahaa *et al.* (2013), reported 55.47%, 58.68%, 52%, 54.44% and 58.72% polymorphism, respectively.

The mean number of polymophic bands amplified per primer, used in this study, was 7.4 and it is higher as compared with values reported by Tao *et al.* (1993), Menkir (1997) who ranged 1.17 and 4.2 polymophic band per primer as was also described by Amrapali *et al.,* (2008). Neverthless, the result obtained in this study is comparable to the values reported by Ayana *et al.,* (2000), Thimmaraju *et al.,* (2000) Ayana *et al.,* (2000b), Agarama and Truinstra (2003), Amrapali *et al.,* (2008), who obtained 7.35, 12.84, 9.0, 6.6 and 8.16 bands per primer, respectively.

The 15 primers used in this study produced fragments ranged from 200 to 2000 bp. The range obtained is comparable with values reported by Ayana et al., (2000), Agrama and Tuinstra (2003) with a range from 300 to 2400 bp and 225 to 2600 bp respectively. Similar range was obtained by Amrapali *et al.,* (2008) who reported a range from 330 to 2599 bp. A smaller range, from 200 to 700 bp, was reported by Mokofeng *et al.,* (2012).

7.2 GENETIC DIVERSITY AND CLUSTER GROUPING ANALYSIS BASED ON THE RAPD GENOTYPING PROFILES

The whole set of accessions were divided into six main clusters, as possible to see on Neighbour joining dendogram (Figure 5). This grouping was made through the location of the accessions around the NJ dendogram and also taking in consideration the dissimilarity index (Table 9). After this grouping, the relationship between the accessions and their collecting site, was not completely clear. Similiar situation was observed with the relationship between the grouping of the accessions and the early or late genotypes. For better understanding, a second grouping was made through the NJ tree, represented as radial scheme.

Taking in consideration that cluster I ranged the lowest values of dissimilarity index, expressed as standard deviation, it is possible to determine that this is the most homogeneus cluster, in terms of genetic distance, but this condition is not related with the collecting site, due the the accessions included into this cluster were from Europe, Africa and Asia. Through the radial representation it was possible to identify the first sub-cluster named IA. Accessions No. 25, 28, 33, 34 and 36 with collecting site in Russia, Germany, China (accessions 33 and 34) and Egypt repectively, were grouped into this smaller cluster. Even if the accessions had different collecting sites, located far away among them, it is possible to explain the low genetic distance.

According to Kimbert *et al* (2012) cited to Murdock (1959) and Harlam (1995) explains that *S. bicolor* is divided into 5 races, originated in Sudan and Ethiopia, and many of the current *S. bicolor* caused by hibridization of this main races. As second important point, Kimbert *et al* (2012) also describes that the current distribution of sorghum was influenced by the dispersion caused by by migration of people across the

the Sahel-Sudan grasslands and southward from the Nile Valley region along the Great Rift. To explain the relationship among accessions from different continents, Kimbert *et al* (2012) through the previous studies from Wet and Price (1976) describe that *S. bicolor* is not only widely distributed in Africa due it is also apparently ancient in Asia, costwise from India to Indonesia and China. Is not clear at all how *S. bicolor* migrated out of Africa but Kimbert *et al* (2012) suggest that people were the responsable for its diffusion.

In more detailed way, Hawkes (1973) suggests that cultivated sorghum may have reached China from Indochina by way of the Mekong River or other river valleys. Another way to explain the migration of *S. bicolor*, out of Africa, is through the semitic speakers from Africa carried their culture to India before 3000 B.C. This may have been one avenue on which sorghum moved off the coast of Africa and into India (Hawkes, 1973 by Kimbert *et al.,* 2012).

The relationship among the accessions grouped in sub-cluster IB is also explained through the process of evolution and domestication of the sorghum previously described. Accessions from Turkey were grouped together with accession from Russia, so there is a high probability that this accessions were developed from one of the five ancient races located in African continent and then spread out from origin center. Becasuse of different enviromental conditions, between the collecting sites, is possible to explain the existence of late genotypes for accessions from Turkey and early genotype from Russia, due the phenotype is highly affected by the environment; even if the genetic distance is lower, as this case, the phenotype will express different agronomic traits. The sub-cluster IC showed similar conditions than as sub-cluster IB with three accession from Russia and two accessions from Turkey. The sub-cluster ID is represented only by accession No. 22 from Russia and early genotype. Although this accession was not included into any other sub-cluster is valid to located into main cluster I because only three Russian accessionsi, from cluster I, are classified as early genotype and accession 22 is one of them.

Finally, after the evolution and domestication analysis of sorghum, it is possible to accept the cluster I as the most homogeneus, in term of genetic distance, as showed the

standard deviation calculated from dissimilarity index, although the accessions were collected from three different continents. The low value of standard deviation means that the value of dissimilarity, from the fourteen accesions of cluster I, is closer to the mean value calculated for the cluster I, in comparisson with the rest of accessions grouped in other clusters. For this cluster, the mean and standard deviation showed the lowest values from the six main clusters, but it is important to explain that this is not an specific condition to categorize one cluster, as more homogeneus or less homogeneus, in terms of dissimilarity. In this case, coincidentally, both values were the lowest, but in case of cluster VI, the standard deviation is able to categorize this cluster as the third most homogeneous, but in consideration of mean value, the same cluster is categorized on the fourth place. The standard deviation was used to categorize the clusters (Table 8).

Two accessions from Russia, with late fenotype, and the only accession from the Czech Republic, with early fenotype, are located into the second main cluster, who is also the second one in order of homogeneity (table 8). The accessions from cluster II are geographically more related than accessions from cluster I. In this case, the relation between the genetic distance and the geographic distance are not contrary, due the lower the dissimiliraty the lower the geographic distance among the accessions. About the difference between early and late genotypes, it could be due the difference of latitudes because closer to north latitude harder climatic conditions for agricultural activities for the most of species as sorghum.

The highest standard deviation value was ranged in cluster III, it means that this set of accessions had the highest dissimilarity values. Thirteen accessions from America, Africa Asia and Europe were grouped into this cluster. Attending the idea described by Murdock (1959) and Harlam (1995) cited by Kimbert *et al* (2012) that is possible to speculate about the idea of more than one ancient race as source of genetic material through cross pollination process; this could be the main reason for explaining the wide range of genetic distance among the accessions into this cluster. About the geographic origin of the accessions, it is valid to argue that migration of people has a high influence about the spread of genetic material and despite the accessions have been collected from

far away sites, there is a remaining of genetic material and it is possible to recognize a genetic distance relatively closer, in comparison with geographical distance.

The only setaria accession was included into this main cluster. The sub-cluster III A grouped the two available accesions from Moldava, one accession from the USA and the only setaria accession. The genetic distance value among the accessions from Moldova ranged a low value of 0.09; while the genetic distance value among setaria and sorghum accession no. 45 ranged 0.4113; this high value was expected, due the banding pattern for setaria accession, after the electrophoresis, it was highly different with the most of 15 evaluated primers, in comparison with the banding pattern from sorghum accessions; this condition can be also explained through the difference of morphological traits. Although the high dissimilarity value of setaria contributed to increase the value of standard deviation, the rest of accessions also showed high dissimilarity values, for this reason this main cluster was categorized as the most heterogeneous. In sub-cluster III B it was also found a wide range of genetic distance and geographical collecting sites located far away among them, like Turkey and the USA. As it is described by Agrama and Tuinstra (2003) based on Doggett (1988) multiple origins for domesticated sorghums, cross-pollination between selected races, and outcrossing between domestic cultivars and highly variable wild species all are considered to be factors contributing to the extensive genetic diversity observed in this sub-cluster.

Cluster IV grouped accessions with small differences of genetic distance values and also of geographical collecting site. Accessions No. 2 and No. 7 were founded into sub-cluster IV A and probably these materials were developed by the same ancient race, as described by Kimbert (2012), due the low genetic distance values in comparisson with values from cluster III. Similar condition was found with the accessions No. 35 and No. 37 with collecting site in Ethiopia and Sudan grouped into sub-cluster IV B and probably this two accessions belongs to the same ancient race.

The remaining accessions were grouped into cluster V and VI. All those accessions were collected in Russia and for cluster V all the evaluated materials were late genotype. For cluster V, taking in consideration the late or early fenotype, the accessions were divided in sub-cluster A and B respectively. Although the collecting

site is the same, it is possible to identify the effect of the human domestication of sorghum because due the genetic distance is short enough to keep the samples into the same main cluster, then the accessios were divided in more detailed way due it was possible to find differences at DNA level through the visualization of polymophic bands.

8 CONCLUSIONS

1. The sorghum accessions, evaluated by RAPD marker, showed high level of polymorphism through the visualization of high porcentaje of polymophic bands, making evident the existance of genetic diversity among the accessions and confirming the first hypothesis.

2. Taking in consideration the data obtained from the evaluation done by RAPD marker, and after the statical analysis, the accession were classified in six main groups by the dissimilarty index values obtained. This way of grouping evidence one more time the large genetic diversity of sorghum bicolor.

3. Through the analysis of collecting site, type of genotype and genetic distance data, is possible to infer in the high impact of the human activities over *S. bicolor* genetic evolution and dispersion around the world.

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10 APPENDICES

Figure A 1. ***Sorghum bicolor*** **collection. Crop Research Institute.**

Figure A 2. Germination of sorghum seed accessios used for DNA extraction.

Table A 1. Standard deviation of dissimilarity index grouped by sub-clusters and level of homogeneity.

Cluster	Sub-Cluster	Dissimilarity index Standard deviation	Homogeneity/Cluster
I	A	0.0129	1
	B	0.8164	3
	C	0.0255	2
	D	0.1203	4
II		0.0331	1
III	A	0.0331	2
	B	0.0304	1
	C	0.0389	3
IV	A	0.1027	2
	B	0.0021	1
V	A	0.0669	2
	B	0.0318	1
VI	A	0.003	1
	B	0.0057	2

Table A 2. Genetic distance matrix among sorghum accessions base don RAPD data.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
2	0																																													
3	0.42	0																																												
4	0.52	0.36	0																																											
5	0.41	0.25	0.3	0																																										
6	0.42	0.26	0.31	0.13	0																																									
7	0.36	0.31	0.41	0.3	0.31	0																																								
8	0.43	0.27	0.35	0.25	0.25	0.32	0																																							
9	0.46	0.3	0.38	0.28	0.28	0.35	0.3	0																																						
10	0.44	0.28	0.36	0.26	0.26	0.33	0.2	0.31	0																																					
11	0.58	0.42	0.5	0.39	0.4	0.47	0.33	0.44	0.35	0																																				
12	0.47	0.3	0.38	0.28	0.29	0.35	0.22	0.33	0.13	0.37	0																																			
13	0.46	0.3	0.38	0.28	0.28	0.35	0.17	0.33	0.23	0.36	0.25	0																																		
14	0.46	0.3	0.38	0.28	0.28	0.35	0.3	0.27	0.31	0.44	0.33	0.33	0																																	
15	0.53	0.37	0.45	0.35	0.35	0.42	0.37	0.34	0.38	0.51	0.4	0.4	0.33	0																																
16	0.49	0.23	0.43	0.32	0.33	0.38	0.34	0.37	0.35	0.49	0.38	0.37	0.37	0.44	0																															
17	0.65	0.55	0.64	0.54	0.55	0.54	0.56	0.59	0.57	0.71	0.59	0.59	0.59	0.66	0.62	0																														
18	0.5	0.34	0.33	0.29	0.29	0.39	0.34	0.37	0.35	0.49	0.37	0.37	0.37	0.44	0.41	0.63	0																													
19	0.69	0.59	0.69	0.58	0.59	0.58	0.6	0.63	0.61	0.75	0.64	0.63	0.63	0.7	0.66	0.23	0.68	0																												
20	0.57	0.47	0.57	0.46	0.47	0.46	0.48	0.51	0.49	0.63	0.52	0.51	0.51	0.58	0.54	0.46	0.55	0.5	0																											
21	0.44	0.31	0.41	0.3	0.31	0.33	0.33	0.35	0.33	0.47	0.36	0.35	0.35	0.42	0.38	0.57	0.4	0.61	0.49	0																										
22	0.42	0.29	0.38	0.28	0.29	0.31	0.3	0.33	0.31	0.45	0.33	0.33	0.33	0.4	0.36	0.55	0.37	0.59	0.47	0.27	0																									
23	0.43	0.3	0.4	0.29	0.3	0.32	0.32	0.34	0.32	0.46	0.35	0.34	0.34	0.41	0.37	0.56	0.39	0.6	0.48	0.29	0.26	0																								
24	0.43	0.3	0.39	0.29	0.29	0.32	0.31	0.34	0.32	0.46	0.34	0.34	0.34	0.41	0.37	0.56	0.38	0.6	0.48	0.28	0.25	0.14	0																							
25	0.46	0.33	0.42	0.32	0.33	0.35	0.34	0.37	0.35	0.49	0.37	0.37	0.37	0.44	0.4	0.59	0.41	0.63	0.51	0.31	0.28	0.26	0.26	0																						
26	0.46	0.33	0.42	0.32	0.32	0.35	0.34	0.37	0.35	0.49	0.37	0.37	0.37	0.44	0.4	0.59	0.41	0.63	0.51	0.31	0.28	0.23	0.22	0.29	0																					
27	0.43	0.3	0.39	0.29	0.29	0.32	0.31	0.34	0.32	0.46	0.34	0.34	0.34	0.41	0.37	0.56	0.38	0.6	0.48	0.28	0.25	0.18	0.18	0.26	0.22	0																				
28	0.45	0.32	0.42	0.31	0.32	0.34	0.34	0.36	0.34	0.48	0.37	0.36	0.36	0.43	0.39	0.58	0.41	0.62	0.5	0.31	0.28	0.26	0.25	0.18	0.28	0.25	0																			
29	0.51	0.38	0.48	0.38	0.38	0.4	0.4	0.43	0.41	0.54	0.43	0.43	0.43	0.5	0.46	0.64	0.47	0.69	0.56	0.37	0.34	0.28	0.28	0.34	0.21	0.28	0.34	0																		
30	0.45	0.32	0.41	0.31	0.32	0.34	0.33	0.36	0.34	0.48	0.36	0.36	0.36	0.43	0.39	0.58	0.4	0.62	0.5	0.3	0.27	0.25	0.25	0.26	0.27	0.25	0.25	0.33	0																	
31	0.45	0.32	0.42	0.31	0.32	0.34	0.34	0.36	0.34	0.48	0.37	0.36	0.36	0.43	0.39	0.58	0.41	0.62	0.5	0.31	0.28	0.26	0.25	0.26	0.28	0.25	0.26	0.34	0.21	0																
32	0.4	0.27	0.37	0.26	0.27	0.29	0.28	0.31	0.29	0.43	0.32	0.31	0.31	0.38	0.34	0.53	0.36	0.57	0.45	0.25	0.22	0.21	0.2	0.21	0.23	0.2	0.21	0.29	0.14	0.16	0															
33	0.45	0.32	0.42	0.31	0.32	0.34	0.34	0.36	0.34	0.48	0.37	0.36	0.36	0.43	0.39	0.58	0.41	0.62	0.5	0.31	0.28	0.26	0.25	0.25	0.28	0.25	0.25	0.34	0.25	0.26	0.21	0														
34	0.43	0.3	0.4	0.29	0.3	0.32	0.32	0.34	0.32	0.46	0.35	0.34	0.34	0.41	0.37	0.56	0.39	0.6	0.48	0.29	0.26	0.24	0.23	0.23	0.26	0.23	0.23	0.32	0.23	0.24	0.19	0.18	0													
35	0.24	0.4	0.5	0.39	0.4	0.34	0.41	0.44	0.42	0.56	0.45	0.44	0.44	0.51	0.47	0.63	0.48	0.67	0.55	0.42	0.4	0.41	0.41	0.44	0.44	0.41	0.43	0.49	0.43	0.43	0.38	0.43	0.41	0												
36	0.46	0.33	0.42	0.32	0.32	0.34	0.34	0.37	0.35	0.48	0.37	0.37	0.37	0.44	0.4	0.58	0.41	0.63	0.51	0.31	0.28	0.26	0.25	0.26	0.28	0.25	0.25	0.34	0.26	0.26	0.21	0.23	0.21	0.44	0											
37	0.45	0.61	0.7	0.6	0.6	0.55	0.62	0.65	0.63	0.76	0.65	0.65	0.65	0.72	0.68	0.84	0.69	0.88	0.76	0.63	0.6	0.62	0.61	0.64	0.64	0.61	0.64	0.7	0.63	0.64	0.59	0.64	0.62	0.36	0.64	0										
38	0.65	0.55	0.65	0.55	0.55	0.54	0.57	0.6	0.57	0.71	0.6	0.59	0.6	0.66	0.62	0.73	0.64	0.77	0.65	0.58	0.55	0.57	0.56	0.59	0.59	0.56	0.59	0.65	0.58	0.59	0.53	0.59	0.57	0.63	0.59	0.84	0									
39	0.54	0.44	0.53	0.43	0.44	0.43	0.45	0.48	0.46	0.6	0.48	0.48	0.48	0.55	0.51	0.61	0.52	0.65	0.53	0.46	0.44	0.45	0.45	0.48	0.47	0.44	0.47	0.53	0.47	0.47	0.42	0.47	0.45	0.52	0.47	0.72	0.48	0								
40	0.51	0.4	0.5	0.4	0.4	0.39	0.42	0.45	0.42	0.56	0.45	0.44	0.45	0.52	0.47	0.58	0.49	0.62	0.5	0.43	0.4	0.42	0.41	0.44	0.44	0.41	0.44	0.5	0.43	0.44	0.39	0.44	0.42	0.48	0.44	0.69	0.44	0.28	0							
41	0.5	0.4	0.5	0.39	0.4	0.39	0.41	0.44	0.42	0.56	0.45	0.44	0.44	0.51	0.47	0.57	0.48	0.62	0.49	0.42	0.4	0.41	0.41	0.44	0.44	0.41	0.43	0.49	0.43	0.43	0.38	0.43	0.41	0.48	0.44	0.69	0.37	0.32	0.29	0						
42	0.56	0.46	0.56	0.46	0.46	0.45	0.48	0.51	0.48	0.62	0.51	0.5	0.51	0.58	0.53	0.64	0.55	0.68	0.56	0.49	0.46	0.48	0.47	0.5	0.5	0.47	0.5	0.56	0.49	0.5	0.45	0.5	0.48	0.54	0.5	0.75	0.57	0.46	0.42	0.42	0					

Table A 2. Genetic distance matrix among sorghum accessions base don RAPD data.

43	0.62	0.52	0.62	0.51	0.52	0.51	0.53	0.56	0.54	0.68	0.57	0.56	0.56	0.63	0.59	0.69	0.61	0.74	0.62	0.54	0.52	0.53	0.53	0.56	0.56	0.53	0.55	0.62	0.55	0.55	0.5	0.55	0.53	0.6	0.56	0.81	0.32	0.45	0.41	0.34	0.54	0				
44	0.55	0.45	0.54	0.44	0.44	0.44	0.46	0.49	0.47	0.61	0.49	0.49	0.49	0.56	0.52	0.62	0.53	0.66	0.54	0.47	0.44	0.46	0.45	0.49	0.48	0.45	0.48	0.54	0.47	0.48	0.43	0.48	0.46	0.53	0.48	0.73	0.49	0.18	0.29	0.33	0.47	0.45	0			
45	0.5	0.39	0.49	0.39	0.39	0.39	0.41	0.44	0.42	0.55	0.44	0.44	0.44	0.51	0.47	0.57	0.48	0.61	0.49	0.42	0.39	0.41	0.4	0.43	0.43	0.4	0.43	0.49	0.42	0.43	0.38	0.43	0.41	0.48	0.43	0.68	0.5	0.39	0.36	0.35	0.38	0.47	0.4	0		
46	0.62	0.52	0.62	0.51	0.52	0.51	0.54	0.57	0.54	0.68	0.57	0.56	0.56	0.63	0.59	0.7	0.61	0.74	0.62	0.55	0.52	0.54	0.53	0.56	0.56	0.53	0.56	0.62	0.55	0.56	0.5	0.56	0.54	0.6	0.56	0.81	0.63	0.52	0.48	0.48	0.47	0.6	0.53	0.44	0	
47	0.94	0.84	0.93	0.83	0.84	0.83	0.85	0.88	0.86	0.87	0.88	0.88	0.88	0.95	0.91	0.9	0.92	1.05	0.93	0.86	0.84	0.85	0.85	0.88	0.87	0.84	0.87	0.93	0.87	0.87	0.82	0.87	0.85	0.92	0.87	0.84	0.95	0.83	0.8	0.79	0.78	0.92	0.84	0.75	0.82	0