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Original scientific paper

ASSESSMENT OF GENETIC DIVERSITY IN *Sorghum bicolor* USING RAPD MARKERS

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Sorghum is one of the most important cereal crop and ranks fifth among cereals after wheat, rice, maize and barley for economic importance. Because the demand of food is increasing, sorghum will increase in importance as a source of food, feed, fibre, and fuel; specially in the European continent where sorghum is little cultivated, mainly due to the lack of sorghum varieties well adapted to the soil and climate conditions such as photoperiod, cold and drought; for this reason, the genetic diversity analysis, through molecular characterization, is an important requirement to begin a plant breeding program. The analysis was performed in 46 sorghum genotypes obtained from the Czech Plant Gene Bank, Crop Research Institute, Prague. Genetic variability values were estimated, through the genetic distance using Dice's coefficient, and dendrogram constructed using DARwing software. Four out of fifteen of the primers evaluated were completely polymorphic (100%), A hundred and twenty-six scorable bands were identified and 89% of them were polymorphic, the bands ranged from 200 to 2000 bp. The dendrogram grouped the accession into six

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clusters. The results indicate the existence of high genetic distance values up to 0.8776 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 were originated most of the wild and cultivated species known nowadays. 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 Plant Gene Bank for future plant breeding program.

Keywords: sorghum, genetic variability, molecular marker, RAPD, genetic distance, Dice´s coefficient, dendrogram

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) 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, fibre, dextrose syrup, biofuels, alcohol and other products (JEYA *et al.*, 2006). Sorghum as a crop with C4 photosynthesis is well adapted to hot, semi-arid tropical environments with 400-600 mm rainfall; as a result, it is a very stable source of nutrition (DOLCIOTTI *et al.*, 1998; SMITH *et al.*, 2000). Sorghum is able to grow at an altitude ranging from sea level to 1,000 m above sea level due to its ability to adapt to different climatic conditions. 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).

Sorghum is an ancient crop (MANN *et al.*, 1983) indicated that the origin and early domestication of sorghum took place in north-eastern 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; DAHLBERG, 1996; DAHLBERG *et al.*, 2011). HARLAN and STEMLER (1976) confirmed that "all of the races belong to the same biological species and are fully fertile when hybridized." For the cereal sorghums, they have identified four wild races and five cultivated races. 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) based on MURDOCK (1959) and HARLAN (1995) describe how the classification of *S. bicolor* in races as follows: (1) bicolor, the primitive type, (2) guinea, (3) kafir, (4) caudatum, and (5) durra. 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 in plant breeding programs (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 to the no requirement for DNA probes, or for any sequence information for the design of specific primers (WILLIAMS *et al*., 1990). RAPD markers

offer many advantages such as higher frequency of polymorphism, rapidity (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 and also is still used in various plant polymorphism studying, including inter and intraspecific variability or species identification (SUBUDHI and HUANG, 1999; ŠTEFÚNOVÁ and BEŽO, 2003; MILELLA *et al*., 2011; PAVLOVIČ *et al.*, 2012; HUO *et al.*, 2013; BOJOVIČ *et al.*, 2013). Beside RAPD technique, such as independency from genomic data knowledge possess microsatellite and retrotransposon length polymorphism based techniques (ŽIAROVSKÁ *et al*., 2013a; ŽIAROVSKÁ *et al*., 2013b; SENKOVÁ *et al*., 2013).

Sorghum bicolor has a relative small genome size when comparing it to other crops that is about 760 Mb with 2n=20 chromosomes. It is the first plant of African origin whose genome was sequenced. Actually, representative genome: of Sorghum bicolor is accessed in NCBI under four Genome Assembly and Annotation reports and all ten Sorghum chromosomes are characterized in reference sequence databases. Identification of genome-wide patterns was reported by ZHENG *et al*. (2011) and gene annotations and transcriptome atlas of this crop was reported by MCCORMIC *et al*. (2018). A wide range of different molecular markers were applied in the whole genome analysis of sorghum up today. Five races of Sorghum bicolor were subjected to DArT markers analysis by MACE *et al*. (2008), microsatellite markers represented by SSR and ISSR were reported for different sorghum accessions (EL HUSSEIN *et al*., 2014; TOO *et al*., 2018) and AFLP markers were applied by GERRANO *et al*. (2014). RAPD markers were applied previously in the screening of population variability of 33 populations of the Moroccan landraces (MEDRAOUI *et al*., 2007); 37 accessions including released varieties, hybrids and their parental lines were analyzed by ARYA *et al*. (2006), 32 accessions by JEYA *et al*. (2006) and 40 accessions of sorghum with different technological use by SINHA *et al*. (2014). This shows that RAPDs are still a very usable marker to reveal a genetic diversity of this crop.

Due to the impact of sorghum as one of the most important cereal crops around the world, it is possible to find a wide number of studies related to determination of genetic diversity of this species (AKRAM *et al*., 2011; BAHAA *et al*., 2013; DAHLBERG, 2011; LEKGARI and DWEIKAT, 2014; MADHUSUDHANA *et al*., 2012; MBEYAGALA *et al*., 2012; MOFOKENG *et al*., 2012; RITTER *et al*., 2007). In this sense, the present study is aimed to assess the genetic diversity of sorghum genotypes from the Czech Plant Gene Bank, Crop Research Institute in Prague, through Random Amplified Polymorphic DNA molecular markers (RAPD).

MATERIALS AND METHODS

Biological material

Total number of 46 accessions of *Sorghum bicolor* were selected and obtained from the sorghum genetic resources collection of the Czech Plant Gene Bank, Crop Research Institute (CRI). Those samples were donated from Vavilov Research Institute of Plant Industry, Saint Petersburg, Russia (RUS), USDA ARS, Plant Genetic Resources Conservation Unit (USA), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Republic of Moldova (MOL). The complete list of genotypes is stated in

Table ***1***. All the samples were sown on germination trays and placed inside of greenhouse. The temperature 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 fresh young leaves and was collected 30 days after sowing.

Table 1.Characteristics of analysed Sorghum bicolor genotypes.

No.	Register number	Name of genotype	Donor entity	Collection site	Type of genotype
1	PI246594	IS 3098	GRIN	Russia	L
2	01Z1800012		Unknown	Hungary	E
3	Ruzrok 2B		CRI	Czech Republic	E
4		JantarRannij 161	RUS	Russia	L
5	Selection from PI326289	GaolianVoskovidnyjnízky	RUS	Russia	E
6	Selection from PI326289	GaolianVoskovidnyjvysoky	RUS	Russia	E
7	PI314743	IS 14015	GRIN	Russia	E
8	PI326293	Efremovskoe 2	RUS	Russia	L
9	PI619662	IS 30470	GRIN	Russia	L
10	PI619663	IS 30471	GRIN	Russia	L
11	PI619665	IS 30473	GRIN	Russia	L
12	PI619666	IS 30474	GRIN	Russia	L
13	PI619667	IS 30475	GRIN	Russia	L
14	PI619668	IS 30476	GRIN	Russia	L
15	PI619670	IS 30478	GRIN	Russia	L
16	PI619677	IS 30485	GRIN	Russia	L
17	PI246594	IS 3098	GRIN	Russia	L
18	PI262567	Stavropolskoye 98	RUS	Russia	L
19	PI267123	K-460	GRIN	Russia	L
20	PI284975	Barnard Red	GRIN	Argentina	L
21	PI326292	Belozernoe Utr-110	RUS	Russia	L
22	PI326294	PenzenskojeRanneje 97	RUS	Russia	E
23	PI326295	DzugaraMestnaja	RUS	Russia	L
24	PI392391	2256	GRIN	Russia	L
25	PI495001	Hazine-4	GRIN	Russia	L
26	PI495010	YantarZernogradskij	RUS	Russia	L
27	PI539066	AJC544	GRIN	Russia	E
28	PI 76035	Sor 9	GRIN	Germany	E
29	PI177551 03SD		GRIN	Turkey	E
30	PI174380 03SD		GRIN	Turkey	L
31	PI170800 01SD		GRIN	Turkey	L
32	PI246595	Saccharatum	GRIN	Russia	E
33	PI408814 02SD		GRIN	China	L
34	PI922267 03SD		GRIN	China	L
35	IS18758		ICRISAT	Ethiopia	L
36	IS18813	Wild	ICRISAT	Egypt	L
37	IS2262	Landrace	ICRISAT	Sudan	L
38	IS12804	Landrace	ICRISAT	Turkey	L
39	IS32050	Landrace	ICRISAT	Yemen	L
40	IS20727	Landrace	ICRISAT	USA	L
41	IS20351	Landrace	ICRISAT	Nigeria	L
42	IS20709	Landrace	ICRISAT	USA	L
43	IS23988	Landrace	ICRISAT	South Africa	L
44	IS2367	Landrace	ICRISAT	Nigeria	L
45		PRO-BIO (Moldova 1)	MOL	Moldova	L
46		PRO-BIO (Moldova 2)	MOL	Moldova	L
47	01Z2300022	*Setaria italica*		France	

E means those materials with early flowering / maturity. L means those materials with late flowering / maturity.

DNA extraction

Genomic DNA was isolated by DNeasy Plant Mini Kit (QIAGEN) following the manufacturer´s instruction. The extracted DNA was separated using agarose electrophoresis gel (1%) and it was visualized under UV light and photographed.

PCR amplification of DNA

Conditions for DNA amplification were standardized for all 10-mers used as random primers (Operon Technologies). PCR (Polymerase Chain Reaction) reactions were performed in a 25 µl reaction mixture containing 1X *Taq* DNA polymerase buffer with 2 mM $MgCI_2$, 0.1 mM each dNTP, 20 pmol primer, one unit AmpONE *Taq* DNA polymerase (GENEALL BIOTECHNOLOGY), 25 to 30 ng template DNA and sterile distilled water (

Table ***2***).

Table 2. Concentrations and volumes of PCR reagents in master mix for one reaction

Components	Concentration	Volume
Distilled water	-	19.2 µl
10X PCR buffer	10x	2.5 µl
$MgCl_2$	20mM	1.5µl
dNTPs	25mM	0.1µl
Primer	100µM	0.5µl
Taq DNA polymerase	5U/µl	0.2µl
Template DNA	25-30 ng/µl	1 µl
Total		25µl

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 3. List of RAPD primers 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

PCR products electrophoretic separation and data analysis

Amplified products were mixed with loading dye (GENEALL BIOTECHNOLOGY) and loaded into the agarose gel. Electrophoretic separation was performed with 10 µl of amplified products on 1.5% agarose gel (CAMBREX) in 1x TAE 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 (Thermo Fisher 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.

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 x100% where n is the number of polymorphic bands within the group of accessions and N is the total number of bands analyzed. The binary matrix was exported, as unicode text file (.txt), and then was used to calculate a genetic distance matrix using Dice's coefficient (DICE, 1945). 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 dendrogram was also showed as radial scheme for easily creation of sub-clusters. This scheme was also created with DARwin 6.0.012 software.

RESULTS AND DISCUSSION

A total of 15 decamer primers (Table 3) that showed clear bands were selected through screening of 20 RAPD total primers in 47 sorghum genotypes. For those selected primers, all of them provided at least three polymorphic bands (Table 4). The amplification products ranging from 250 to 2,000 bp that yielded only sharp and strong bands were scored to build a binary matrix. 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 2,599 bp. A smaller range, from 200 to 700 bp, was reported by MOKOFENG *et al.,* (2012).

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% (Table 4). This value is similar to those obtained by MEHMOOD *et al*., (2008) with 78.94%while the value of polymorphic bands obtained by AKRAM *et al*., (2011) was higher with a value of 99.5%. Similar RAPD polymorphism was obtained by JEYA *et al*., (2006) with the 97.4% of polymorphic bands, while TAO *et al.* (1993), AGRAMA and TUINSTRA (2003), NKONGOLO and NSAPATO (2003) AMRAPALI *et al.* (2008) BAHAA *et al.,* (2013), reported 55.47%, 58.68%, 52%, 54.44% and 58.72% polymorphic bands, respectively.

The mean number of polymorphic bands amplified per primer was 7.4 in this study and it is higher as compared with values reported by TAO *et al.* (1993) and MENKIR *et al.* (1997) who ranged 1.17 and 4.2 polymorphic band per primer as was also described by AMRAPALI *et al.,* (2008). Nevertheless, the result obtained in this study is comparable to the values reported by AYANA *et al.,* (2000a), THIMMARAJU *et al.,* (2000) AYANA *et al.,* (2000b), AGARAMA and TUINSTRA (2003), AMRAPALI *et al.,*(2008), who obtained 7.35, 12.84, 9.0, 6.6 and 8.16 bands per primer, respectively.

Table 4. 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 amplification (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**

The highest 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 4). The largest number of polymorphic bands per primer was produced by OPB 2 (13 polymorphic bands) while the lowest number of polymorphic bands was observed by primer OPB 16 (3 polymorphic bands).

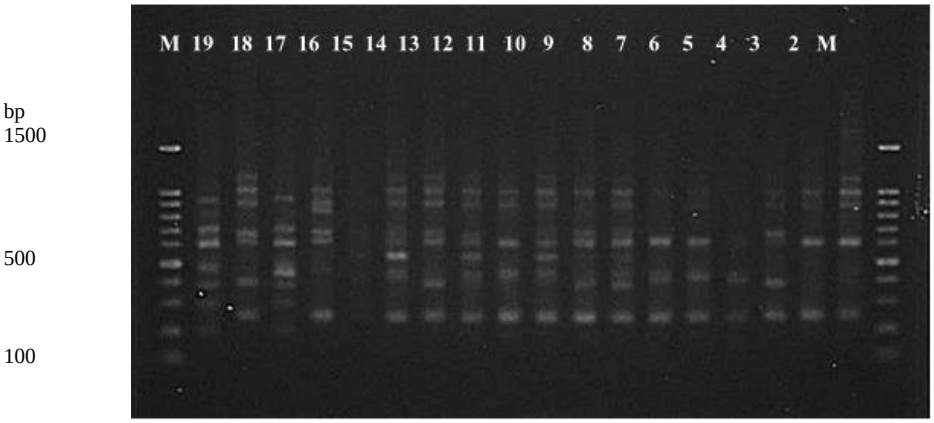

Figure 1. RAPD profiles generated by OPB 02 primer. The number corresponding to the samples is at the top of each lane. M is the 100bp molecular weight marker 100 bp.

The highest number of monomorphic bands was observed by primer OPB 12 producing 3 bands that means 25% of the total of produced bands for this primer; while the lowest number of monomorphic bands was 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 a specific banding pattern for genotype number 18, as possible to see in Figure 1.

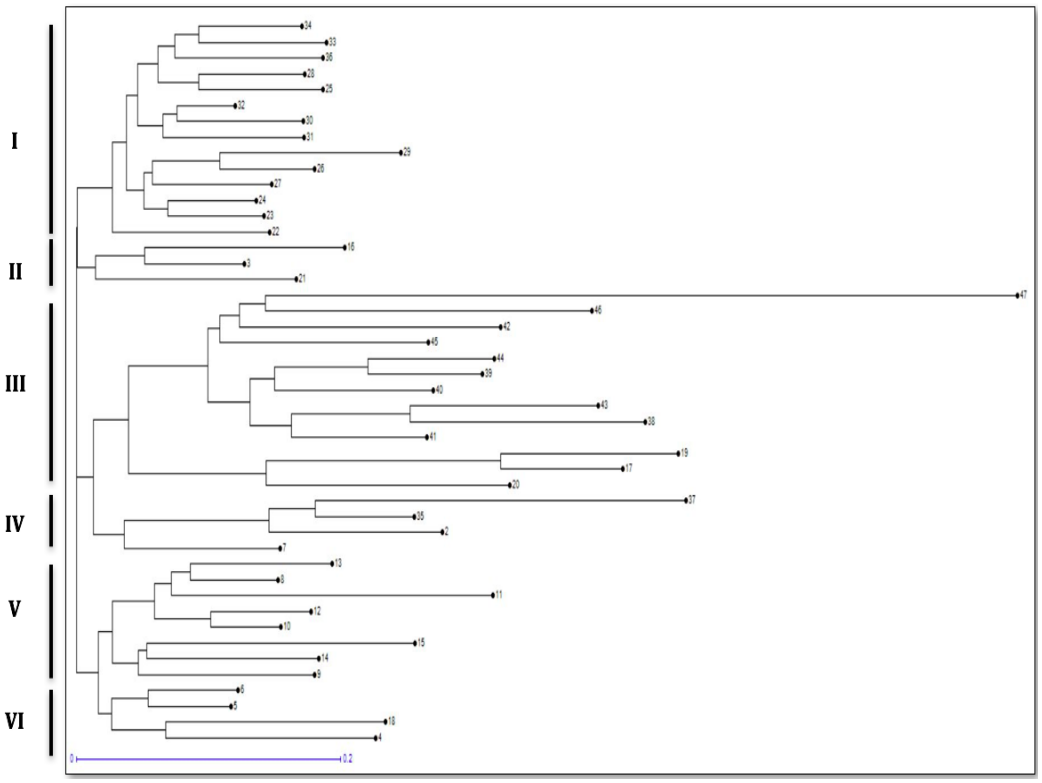

Figure 2. Neighbour joining dendrogram based on Dice's coefficient showing relationship among sorghum accessions. The dendrogram was constructed based on genetic distance matrix generated with gained RAPD marker.

The genetic distance among the all sorghum genotypes were found in the range of 0.13 to 0.8776. The highest genetic distance value among the sorghum accessions (0.8776) was found between the accession No. 37 and accession No. 16. Through the neighbour joining dendrogram the genotypes were classified into six clusters (**Error! Reference source not found.**). Cluster I consist of 14 genotypes from Russia, Germany, Egypt, Turkey, and China as the collection sites. It is also possible to identify early and late genotypes. Cluster II included three genotypes from Russia, as collecting site, and early and late flowering genotypes. Cluster III included 12 sorghum genotypes and the only one *Setaria* accession. All those genotypes were from Russia,

Argentina, Turkey, Yemen, United States, Nigeria, South Africa, Nigeria and Moldova. Cluster IV included 4 sorghum genotypes from Russia, Ethiopia and Sudan as the collecting sites. Cluster V included 8 sorghum genotypes belonging to Russia as the collecting site. Finally, cluster VI contained 4 sorghum genotypes all of them belonging to Russia.

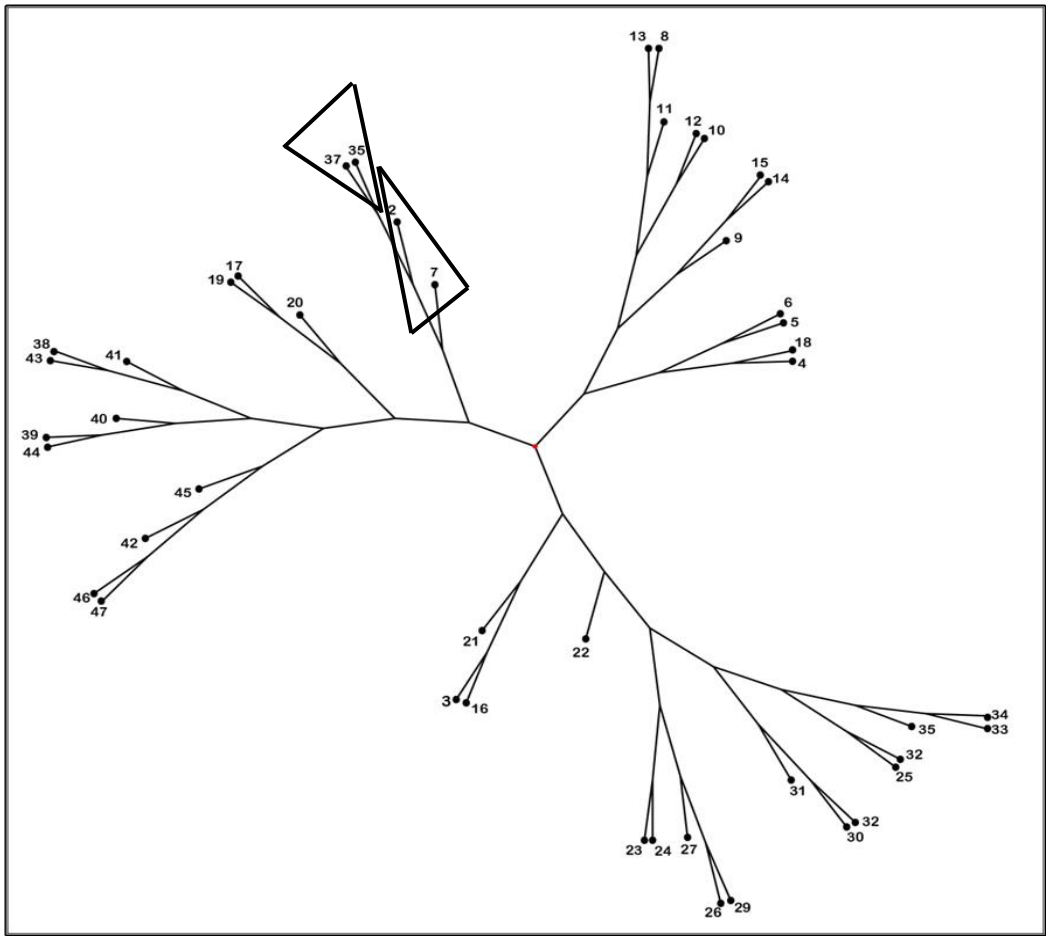

Figure 3. Neighbour joining radial scheme showing sub-cluster division among sorghum accessions and the numbers correspond to table 1. Radial representation constructed based on RAPD markers gained. All edge lengths were established to one to exhibit a better radial scheme structure.

Taking in consideration that cluster I ranged the lowest values of genetic distance, it is possible to determine that this is the most homogeneous cluster, in terms of genetic similarity, but this condition is not related with the collecting site, due to the genotypes included into this cluster were from Europe, Africa and Asia. Through the radial representation (Figure 3) it was possible to identify into the first sub-cluster, named IA, the genotypes No. 25, 28, 33, 34 and 36 with collecting site in Russia, Germany, China (accessions 33 and 34) and Egypt respectively.

Even if the accessions had different collecting sites, located far away among them, it is possible to explain the low genetic distance.

According to MURDOCK (1959) and HARLAN (1995) *S. bicolor* is divided into 5 races, originated in Sudan and Ethiopia, and many of the current *S. bicolor* caused by hybridization of these 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 migration of people across 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 describe that *S. bicolor* is not only widely distributed in Africa due to it is also apparently ancient in Asia, coastwise from India to Indonesia and China. Is not clear at all how *S. bicolor* migrated out of Africa, but KIMBERT *et al* (2012) suggests that people were the responsible 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 BC. This may have been one avenue on which sorghum moved off the coast of Africa and into India.

The relationship among the accessions grouped in sub-cluster IB (Figure 3) is also explained through the process of evolution and domestication of the sorghum previously described. Genotypes from Turkey were grouped together with accession from Russia, so there is a high probability that these genotypes were developed from one of the five ancient races located in African continent and then spread out from origin centre. Because of different environmental conditions between the collecting sites, it is possible to explain the existence of late flowering genotypes for accessions from Turkey and early flowering genotype from Russia, due to the phenotype is highly affected by the environment; even if the genetic distance is lower, as in this case, the phenotype will express different agronomic traits. The sub-cluster IC showed similar conditions than as sub-cluster IB with three genotypes from Russia and two accessions from Turkey. The sub-cluster ID is represented only by accession No. 22 from Russia and an early genotype. Although this accession was not included into any other sub-cluster, it is valid to locate into main cluster I because only three Russian genotypes, from cluster I, are classified as early flowering genotype and accession 22 is one of them.

Two genotypes from Russia, with late flowering phenotype, and the only accession from the Czech Republic, with early flowering phenotype, are located into the second main cluster. The genotypes from cluster II are geographically more related than genotypes from cluster I. In this case, the relations between the genetic distance and the geographic distance are not in contrast, due to the lower genetic distance value, the lower the geographic distance among the accessions. About the difference between early and late flowering genotypes, it could be due to the difference of latitudes because the closer to North latitude, the harder climatic conditions for most crops as sorghum.

About cluster III, thirteen genotypes from America, Africa, Asia and Europe were grouped into this cluster. Attending the idea described by MURDOCK (1959) and HARLAN (1995) 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 on the spreading of genetic material and despite the genotypes have been collected from far away sites, there is a remaining of genetic material. It is possible to recognize a genetic distance relatively closer, in comparison with geographical distance. 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, crosspollination 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 found in sub-cluster IV B and probably these materials were developed from the same ancient race, as described by KIMBERT *et al.* (2012), due to the low genetic distance values in comparison 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 A and probably these two accessions belong to the same ancient race.

CONCLUSIONS

RAPD markers were proved here to be suitable tool to identify a high level of polymorphism among sorghum genotypes through the visualization of high percentage of polymorphic bands, making evident the existence of genetic diversity. Taking in consideration the data obtained from the evaluation done by RAPD markers, and after the statistical analysis, the accessions were classified in six main groups by the genetic distance values obtained. This way of grouping evidences one more time the large genetic diversity of *Sorghum bicolor*. 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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OCENA GENETIČKE RAZNOVRSNOSTI KOD *Sorghum bicolor* POMOĆU RAPD MARKERA

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Izvod

Sirak je jedna od najvažnijih žitarica i zauzima peto mesto među žitaricama posle pšenice, pirinča, kukuruza i ječma po ekonomskom značaju. Budući da je potražnja za hranom sve veća, sirak će dobiti na značaju kao izvor hrane, hrane za životinje, vlakana i goriva; posebno na evropskom kontinentu gde se sirak malo gaji, uglavnom zbog nedostatka sorti sirka koje su dobro prilagođene zemljištu i klimatskim uslovima kao što su fotoperiod, hladnoća i suša; iz tog razloga, analiza genetske raznolikosti, molekularnom karakterizacijom, je važan zahtev za pokretanje programa oplemenjivanja biljaka. Analiza je izvršena na 46 genotipova sirka dobijenih iz Češke banke biljnih gena, Prag. Vriednosti genetičke varijabilnosti procenjene su kroz genetsku udaljenost pomoću Dice-ovog koeficijenta i dendrograma konstruisanog pomoću DARving softvera. Četiri od petnaest ocenjenih prajmera bili su potpuno polimorfni (100%). Identifikovano je 126 uporednih traka, a 89% je bilo polimorfno, a trake su bile od 200 do 2000 bp. Dendrogram je grupisao uzorke u šest klastera. Rezultati ukazuju na postojanje visokih vrednosti genetske udaljenosti do 0,8776 među procenjenim uzorcima, čak i ako su oni prikupljeni u istoj zemlji ili, naprotiv, na nižu genetsku raznolikost među uzorcima prikupljenim u različitim zemljama. To bi moglo biti posledica postojanja pet drevnih rasa sirka iz kojih su poreklom do sada bile poznate divlje i kultivisane vrste. Uglavnom, migracije ljudi iz oblasti porekla sirka, smeštenog u Etiopiji i Sudanu, objašnjavaju širenje genetskog materijala van Afrike. Informacije dobijene ovim istraživanjem trebale bi biti korisne za bolje razumevanje genetske raznolikosti od germplazme sirka čuvanog u Češkoj banci biljnih gena za budući program oplemenjivanja biljaka.

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