Sudan University of Science and Technology

College of Graduate Studies

Assessment of Genetic Diversity of some Sweet Sorghum Genotypes (*Sorghum bicolor* L.) using Agro-Morphological and Molecular Markers (SSR)

تقويم التباين الوراثى لبعض الطرز الوراثية لمحصول الذرة الحلوة بإستخدام الصفات المظهرية ووالواسمات الجزيئية (التسلسل المتكرر البسيط)

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الآيـة

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قـالتعـالـى:

وَفِي الْأَرْضِ قِطَعٌ مُّتَجَاوِرَاتٌ وَجَنَّاتٌ مِّنْ أَعْنَابٍ وَزَرْعٌ وَنَخِيلٌ صِنْوَانٌ وَغَيْرُ صِنْوَانٍ يُسْقَىٰ بِمَاءٍ وَاحِدٍ وَنُفَضِّلُ بَعْضَهَا عَلَىٰ بَعْضٍ فِي الْأُكُلِ إِنَّ فِي ذَٰلِكَ لَآيَاتٍ لِّقَوْمٍ يَعْقِلُونَ (4)

الرعد - الآية 4

Dedication

I dedicate my humble and modest work:

To whom surrounded me with emotions and cares

(My Parent) and also to my beloved brothers and sisters especially

(Siddig, his wife Hawaa and their Sons),

Also dedication extended to those are always awesome

My colleagues in B.Sc, M.Sc and in Environmental and Natural Resources and

Desertification Research Institute (ENRDRI)

Especially Amir Alhag, Eltayeb Abass and Dr. Atif Abuali.

To all of you.

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Initially, all thanks and praise due to ALLAH, who provide me the power and patience to complete this work. The prophet Mohamed (PUBH) said a man doesn't thanks human; does not thanks ALLAH. So I am thankful and grateful to everyone help me to finish this work, thanks my Supervisor Dr. Bahaeldeen Babiker for uncountable support and assistance and valuable advices, much thanks to Dr. Maria Elnasikh the head dean of sweet sorghum project, my thanks extended to Amal Ibrahim for her unlimited efforts, Sarah Suleiman, Elsadig Eltayeb and Ali Ismail. I am really appreciate your much contribution, I will never forget my teachers in department of crop production, faculty of Agricultural studies, Sudan university of science and technology to you, I sending a lot of greetings and respects, finally special thanks to Prof. Mahmoud F. from dept. of agronomy, University of Khartoum, also my great acknowledges to Dr. Mohamed Ismail and Mohamed Adam from university of Bakht AlRuda for their huge field experiment contributions Thanks to everyone I forgot.

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ABSTRACT

Genetic diversity plays important role for successful genetic improvement. In this study morphological and molecular data were used to evaluate twenty Sudanese sweet sorghum (*Sorghum bicolor* L. Moench) genotypes. Two field and laboratory experiments were conducted to assess the genetic diversity between these genotypes. The field experiment for morphological data was conducted in the summer season of 2014 at demonstration farm, Faculty of Agriculture and Natural Resources, University of Bakhat Al– Ruda, Elduiem, Sudan. A randomized complete block design with four replications was used and four morphological characters were measured included plant height (cm), stem diameter (cm), leaf area (cm^2) and leaf area index. Phenotypic (PCV) and genotypic (GCV) coefficients of variation and phenotypic correlation between morphological traits were estimated. For the laboratory experiment, 14 SSRs markers were used to estimate the genetic diversity based on molecular level in the sweet sorghum genotypes. Genetic analysis was performed using principal coordinates analysis, dissimilarity between genotypes ranged from 0.13 to 1.14, the Dendrogram was constructed depending on Percentage Disagreement Value using Weighted Pair Group Method with Arithmetic Mean (UPGMA) clustering three clusters were grouped. The twenty sweet sorghum genotypes revealed several patterns of variation, although some of them share same eco-geographic location. The results indicated that 13 primers (93%) were polymorphic, generated 29 alleles 26(90%) of this alleles were polymorphic. The significance which reveled from the twenty sweet sorghum genotypes based on morphological and SSR markers data could be of great value in any sweet sorghum breeding program.

المستخلص

يلعب التنوع الوراثي دورا هاما في إنجاح التحسين الوراثي. في هذه الدراسة تم إستحدام البيانات المظهرية والجزيئية لتقييم عشرون طرازا من الذرة الحلوة السودانية. تم إجراء تجربتين حقلية ومعملية لتقويم التباين الوراثي بين هذه الطرز. تم إجراء التجربة الحقلية لغرض أخذ البيانات المظهرية وذلك للموسم الصيفي للعام 2014 بالمزرعة التوضيحية لكلية الزراعة والموارد الطبيعية، جامعة بخت الرضا، الدويم، السودان. تم إستحدام تصميم القطاعات الكاملة العشوائية بأربعة مكررات وتم أخذ أربعة قياسات تشمل (طول النبات و قطر الساق) بالسم ومساحة الورقة بالسم المربع و مؤشر سطح الورقة. تم تقدير معامل التباين الوراثي والمظهري و الارتباط المظهري بين الصفات المظهرية. أظهرت النتائج أن هنالك فروقات معنوية لكل الصفات المدروسة عدا طول النبات. سجلت أعلى نسبة تباين وراثي ومظهري لمؤشر سطح الورقة، ووجد أن هنال إرتباط معنوي عالي وموجب بين قطر الساق ومساحة سطح الورقة. تم إجراء التجربة المعملية بوحدة الأحياء الجزيئية، معهد أبحاث البيئة والموارد الطبيعية والتصحر، المركز القومي للبحوث، تم إستخدام 14 بادئ من الواسم الجزيئي التسلسل البسيط المتكرر لتقدير التباين الوراثي بناءاً على المستوى الجزيئي لطرز الذرة الحلوة. تم إجراء التحليل الوراثي بإستخدام النسق التحليلي الإحداثي، تراوح عدم التشابه الوراثي بين الطرز ما بين 0.13 الى 1.14 ، تشكلت شجرة القرابة الوراثية اعتماداعلى نسب عدم التوافق بتطبيق متوسطات المجموعات الزوجية غيرالمزانة مكونة ثلاث مجموعات. أظهرت النتائج أن 13 بادئاً (93%) منها بأنها متعددة الأشكال، منتجة 29من الاليلات 26 (90%)من هذه الأليلات متعددة الأشكال. المعنوية التي تم الحصول عليها من العشرين طرازاً وراثيا من الذرة الحلوة بناءا علي البيانات المظهرية وبيانات الواسم الجزيئي التسلسل البسيط المتكررقد يكون لها قيمة كبيرة في اي برنامج لتربية ال الذرة الحلوة.

CHAPTER ONE

INTRODUCTION

Sorghum (*Sorghum bicolor* L.Moench) is a cereal crop belongs to Family (Gramineae),it gets significant importance as a multipurpose crop, yielding grains for food, and fodder from its leaves and stems. Generally Sorghum is regarded as the most important crop for semi-arid regions, it grown globally on total area approximately about 47 million hectares in about 105 countries of Africa, Asia, Americas and Oceania. The USA, India, Mexico, Nigeria, Sudan and Ethiopia are regarded as the main producer. Farmers in small-scales in drier areas regard as majority of producers over the world especially in Africa and Asia (FAO, 2013), moreover, the crop is ranked as the second grain-based bio-fuel crop after Maize (Dahlberg *et al.*, 2011).Sweet sorghum belong to same species of grain sorghum, has highest photosynthetic efficiency, stem possess high concentration of fermentable sugar and capable of producing high biomass and sugar yield, so the crop achieved more interest in Europe and USA as the most ideal bio-fuel crop (Shiringani *et al.*, 2010 and Cai*et al.*, 2013), besides that the crop have another features such as low water and fertilizers requirement, and relative short growth cycle (4 months) and it capable to well adapted to harsh environmental conditions, this make the crop as the promising raw material for bio-ethanol production (Wu *et al.*, 2008). Ankolib, is common name of Sweet sorghum in Sudan, grown mainly for fresh chewing, but in some cases the peeled stalks are dried and used instead of sugar when sugar cane not available (Sirelkatim, 2003).

Genetic diversity or knowledge of patterns of diversity of genetic resources is of great importance and is a key component in crop improvement and plant breeding (Warburton*et al.*, 2002). Genetic biodiversity on sorghum varieties are genetically diverse based on morphological traits, genetic polymorphism and differences in isoenzymes (Deu *et al.*, 1994 and Tao *et al.*, 1993). The

chromosome number of Sorghum 2n = 20, but the difference in sweet sorghum from grain sorghum genetically on the genes which controlling plant height and ratio of juice and sugar in stalk (Paterson *et al*., 2003).(Mamoudou *et al*., 2005) reported that the difference between grain sorghum and sweet sorghum in genes that controlling height of plant, ratio of juice and sugar content of stalk. Genetic biodiversity it can be detected by using morphological markers and DNA molecular markers, There were various PCR systems (Random Amplified Polymorphism (RAPD), Simple sequence repeat (SSR), and amplified fragment length polymorphism (AFLP)… etc.) contrasting in complexity and reliability, due to their high values for polymorphic information content (PIC), and shanon diversity index (Geleta *et al*., 2006). Molecular marker are basic nucleotide sequence corresponded to physical position among genome, and their polymorphism between genotypes tracing inheritance easily (Schulman, 2007). Breeders use this tool because of fabulous features it regard as fast and quick way to assessing diversity and to select superior genotype lines, furthermore discover the relatedness between cultivated and wild plant (Ritter *et al*., 2007 and Menz *et al*., 2004). DNA molecular markers have major applications which useful include evolution, gene cloning, taxonomy and plant breeding. SSR (microsatellite) are based on tandem of one to six core nucleotide elements. These co-dominant markers are dispersed throughout the genome and have a multiple alleles that often have conserved loci between related species (Schulman, 2007). Powell *et al*., (1996) stated that SSRs can be used for genetic studies such as variability analysis, quantitative trait locus mapping and gene tagging. However, the objective of this study was to assess genetic diversity among different Sudanese sweet sorghum genotypes by using Agro- morphological traits and molecular markers (SSR).

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of Sorghum

Sorghum was believed to be originated and domesticated in northeastern of Africa (Sudan and Ethiopia) north of the Equator and east of 10˚E latitude since more than 5000 years ago (Mann *et al*., 1983), moreover archaeological location at Napta 10-15˚ latitude north near the Sudanese- Egyptian borders there sorghum seed fossil agree with radiocarbon dates of 8,000 years BP (Dahlberg and Wasylikowa, 1996). Sorghum is regard as a native wild plant of Africa, and (Ziggers, 2006) stated that sorghum was cultivated since 700 BC in Assyria. Sudan and Ethiopia have wide spread of genus sorghum varieties during second century AD or even earlier, sorghum were spread from Ethiopia through local tribes into Eastern Africa, then the crop was carried by Bantu tribes into western and Southern regions of continent, later its expansion took place (FAO, 1995).

The main features of Sorghum and cereals domestication and their development resulted due to remaining of grain on spike at mature consequence of genetic mutations (Kimber, 2000). The absence of abscission layer in spike and spikelet this is consider the main difference between wild and cultivated sorghum (Kambal, 2015).

Africa has significant variation in sorghum species for both cultivated and wild species (Dogget, 1970, de Wet and Harlan 1971). Dogget, (1965) suggested that the sorghum was domesticated in Ethiopia before 5000 year from *Sorghum aundiinaceaum*result of disruptive selection and from Ethiopia spread to rest of Africa with migration and trade exchanging. Harlan and Stemler,(1976) stated that the sorghum was originated from wild parent in Sudan area between Chad lake and Ethiopia highlands. Whereas (Evelyn,

1951) by virtue of great variation of cultivated and wild sorghum in Kurdufan in which sorghum was originated. But (Snowden, 1936) suggested it is not necessary determine only one center of origin, so there are many central origin of sorghum i.e. cultivated sorghum (bicolor and Dura) domesticated from wild species (*aethiopicum*) while Guienia from *aundiinaceaum*, Kafir originated from *verticillforum*. This hypothesis was supported by de Wet and Hukabay, (1967) they suggested that the sorghum of western Africa which domesticated from wild species (*S. aundiinaceaumvaraundiinaceaum*) whereas Eastern Africa sorghum (*S. aundiinaceaum va r aethiopicum*) and the sorghum of East and West Africa from *(S. aundiinaceaum var verticillforum*), later de Wet *et al*., (1976) suggested that sorghum was domesticated from *S. verticillforum* because this species has wide spread over Africa. Generally according to (Purseglove, 1972) the majority of evidence indicated that the northern east zones of Africa 10 south to 25 East longitudes this area has high significant degree of variation to both wild and cultivated sorghum.

2.2 Botanical Classification of Sorghum

Although Sorghum is difficult to be classified because of its wide diversity but, many authors such as (de Wet and Harlan,1971, de Wet and Huckabay, (1967); Harlan, (1975); Snowden (1936) and Dahlberg (2000) were contribute much overview to present sorghum classification; they were used integrated system to describe the variation between cultivated sorghum. Sorghum was classified under family Poaceae (Graminae). Dogget, (1970) reported that (Linnaeus) in 1753 included sorghum under genus (Holcus), but Moench,(1794) suggested a solitary Genus and named it (Sorghum)in the past sorghum was gave several scientific names i.e. *Sorghum vulgarepers*, *Holcus sorghum* (L.), but *Sorghum bicolor* (L.) this is most common now days. Later sorghum was classified under genus *Sorghum* (Clayton and Renvoize, 1986). de Wet (1978) recognizing as sorghum bicolor which includes all annual cultivated, while wild and weedy are represent in *S. propinquum* and *S. halepense*. Furthermore *S. bicolor* was divided into three subspecies *S.*

bicolor subsp. *bicolor, S. bicolor* subsp. *drummondii*, and *S. bicolor* subsp. *verticilliflorum*. Berenji and Dahlberg, (2004) stated that the agronomic types such as grain sorghum, sweet sorghum, Sudan grass and broomcorn are classified under subspecies bicolor, furthermore there are wide spread of another sorghum species in temperate zones i.e. Johnsongrass and spontaneous sorghum (shattercane),Johnsongrass (*S. halepense*), generally there are close relation between sorghum species both cultivated and wild.Harlan and de Wet, (1972) suggested simple classification which depends on shape of inflorence, structure of spikelet and shape of the grains. According to this classification, sorghum was divided into 5 main races (dura, caudatum, kafir, Gueinia and bicolor), this classification was preferred by plant breeders because it is simple, and easy to be applied in field.

2.3 Sudanese Sorghum Cultivars

It was believed that sorghum originated and domesticated in Sudan and in which there are wide spread and diversity of sorghum traditional and improved cultivars. Some farmers favor own local and adapted cultivars to adverse conditions i.e. drought and *Striga* tolerant. The desirable traditional cultivars include ArfaGadamak, Kurakulu, Ariana in Gadarif, and Geshish and Hemeisi in Butana,Wadakkar, Hegeiri in Blue Nile state , Wadfahal in Elgezeira, Kulum in south Kurdufan, Zonari and Nagadha in north Kurdufan, Breberi in Darfour , Debeikri , Abu70 in River Nile state. Kambal, (2015) stated that the released sorghum cultivars such as WadAhmed due to their high productivity in grain and forages and cultivar Tabat for seed quality although, low striga-tolerant and low forage productivity, and Butana, Arfaagadamak for early mature.

2.4 History of sweet sorghum

The local name of sweet sorghum in Sudan called “Ankolib” which is grown in minor areas for Human consumption (Kambal, 2015). Sweet sorghum was

developed as Bio-fuel initially in Brazil to produce traditional Alcoholics by rural communities using traditional equipment (Schafert, 1992).

2.5 Sweet Sorghum Adaptation

Sweet sorghum can grow in wide range of soils from light texture (sand) to heavy clay soils and pH (5-8.5). Moreover sweet sorghum has wide adaptability to adverse conditions i.e. drought, water logging, relative to salinity, alkalinity and poor aeration. It exactly requires 500-1000 water (rain fall or irrigation) per season to produce 500- 1000 kg ha^{-1} fresh weight of total above ground biomass Smith and Frederiksen, 2000; Kangama and Rumei, 2005). The crop can grow in temperature ranges between 15 to $37^{O}C$ and the optimum is 32-$34^{O}C$ (Ustimenko-Bakumovsky, 1983). Sweet sorghum is adaptable to harsh condition in semi-arid and regard as the most efficient crop in dry land by converting atmospheric carbon dioxide into sugar. It can be grown in wide range of climatic conditions and regard as ideal warm humid and semi-arid areas.

Sweet sorghum needs about 315kg of water compared to requirement of corn is 370 kg to produce 1 kg dry mater, therefore, it is more efficient water use, the annual evapo-transpiration rate for sorghum 580 mm per annum, compared to 760 mm in corn (Chapman and Carter, 1976). Sweet sorghum is C4 plant so it characterized by efficient radiation utilization especially in tallest cultivars because it permit better light interception. With respect to grain production sweet sorghum has grain production similar to grain sorghum both of them can produce about (3-7 t ha^{-1}), moreover it produce from cane about 54 - 69 t ha^{-1}(Almodares *et al*., 2008).

2.6 Uses of sweet Sorghum

Sweet sorghum plant is characterized by yielding high biomass and sugar among the family Poaceae, which belongs to the same species *Sorghum bicolor* L. Moench, this genus include grain and forage sorghum. It regard as ideal multipurpose crop because simultaneous grain production from head as

food and feed, green foliage regard as good source as fodder and the stem juice is used as a raw material industrial products such as sugar, syrup, ethanol, starch, mono sodium glutamate, acids and vitamins (Athar, 2012). According to Kumar, *et al*., (2008**)** sweet sorghum can't substitute's sugarcane by all mean but can supplement the deficit in ethanol production during shortage periods. Furthermore Sweet sorghum has highest production of dry matter among sugar crops (sugarcane and sugar beet).

2.7 Improvement of sweet sorghum

2.7.1 **in the world**

Many studies were conducted over the world resulted in a new cultivars of sweet sorghum, in USA the research on sweet sorghum focused on increasing biomass, sugar content and quality regardless the seed production. In India the grain regarded as human food. The Sudanese cultivar Ankolib which characterized by thick glumes which covered the seeds, while the American cultivar M81E has small, brown grain, while the Indian cultivar ICSV25280 has large, white seeds, so it desirable to human consumption (Kambal, 2015).

2.7.2 **In Sudan**

Many of sweet sorghum were introduced from ICRISAT India some of it were evaluated in Shambat and Sugar research station in Kenana to the M81E cultivars which characterized by high productivity of bio-mass, sugar and Ethanol but late mature, while under rain-fed area in ELdamazein the cultivar ICSV25280 from ICRISAT gives better result in grain quality and sugar content (Kambal, 2015). Ankolib is the local name of sweet sorghum in Sudan, many studies were took place by Sudanese researchers Ahmed,(2003) and Alkhalifa, (2009) concentrated their studies on Ankolib races and this races exhibited large variations among races in morphological characters, cane and sugar production. They added sweet sorghum can be improved for sugar production through selection, in addition developing of dual-purpose sorghum require improvement of grain quality, because to brown colour,

colored testa covered by thick glumes that make it difficult to be threshed, thereforethese grainsare not edible for humans, on the other side there is local sweet sorghum genotype which called Abu-nafain which cultivated for grain production in Elgazeira Aba region areas, White Nile State, can also be used as dual purposes crop, this genotype was evaluated in Demonstration Farm, Faculty of Agriculture, University of Khartoum in Shambat and resulted of low forage productivity, and relative low sugar in juice, but fortunately it can be improved by pure line selection or/and to be crossed with sweet sorghum cultivars to produce a new cultivars have a desirable characters (Kambal, 2015).

2.8 Genotypic performance

Local Genotypes or varieties of sorghum have a significant role in crop production and improvement, sweet sorghum genotypes vary widely in their adaptation to different climatic and soil conditions(Lakkana*et al.,* 2009), and the number of leaves of potential Ethanol production variety are ranged between 5 to 25 among genetic resources (Chavan *et al.*, 2009, Ratnavathi *et al.*, 2010 and Davila-Gomez *et al.*, 2012),while sudewad, (1976) reported that it was ranged between 6 to 12 in some genotypes, with respect to plant height Meli, (1989) resulted the plant height varied from1to 3.5 meters among 10 sweet sorghum genotypes and stem diameter about 1.47 to 2.29 cm and the leaf area have significant difference in all plant stage, With respect the yield of stalk the average yield of commercial cultivars stalks ranged from 35-48 ton ha^{-1}, while extractable percentage of juice 45.5 to 50.5 and $Brix^{o}$ content ranges between 14.8 -26.2%.

2.9 Genetic Diversity

Diversity defined as any variation in living organisms within given eco-system (Yang *et al.*, 2006).Genetic diversity is the variety of genes which found among individuals through species, so it consider as raw material for plant breeders which contribute much for their activities (Peter *et al.*, 2004).

Plant genetic diversity comprises from modern cultivars, traditional genotypes, and wild species which have continuous utilization in plant breeders programs, so it consider a crucial resources for human life. Crop genetic diversity provides the biological and natural basis of our ability to grow food requirement now days, as well as to meet the challenges such as over increasing populations and climatic changes. thus the crop genetic diversity is essential element of sustainable crop production and the success of any breeding program or genetic conservation are depend heavily on genetic diversity identification among crops gene pools(Siddiqui and Naz, 2009).

2.10Assessment of genetic diversity methods

Information about genetic diversity and genetic relatedness among germplasm is basic element in plant breeding (Siddiqui and Naz, 2009).Accurate assessing genetic diversity may be used in difference applications including analysis of genetic variability in cultivars, identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection, and introgressing desirable genes from diverse germplasm into the available genetic base.

Genetic diversity Study is a process by which variation among individualsor groups of individuals or populations is analyzed bya specific method or a combination of methods. Researchers used diverse data sets to analyze genetic diversity in crops. They include pedigree data,passport data, morphological data and agronomic performance data (Mohammadi and Prasanna, 2003).

2.11 Morphological characteristics

Morphological measurement is considered as conventional tools of selection up to now. Using physical characteristics to discrimination between plants i.e maturity cycle, growth habit, leaf shape, hairiness, nature of corolla and panicle/pod/fruit size (Van der Maesen, 1990). Siddiqui and Naz, (2009)

stated that morphological traits can be adaptive prevailing environmental condition. While in phenotypic diversity studies are often influenced by the environment (Grenier *et al*., 2001).

2.12 Molecular markers

Many types of markers have been used to analyze genetic populations (Siddiqui and Naz, 2009). Include morphological markers, protein markers, ribozymes, mitochondrial-DNA and Molecular Markers. Any type of these marker to be applied in assessing diversity among accessions will depend on the crop species, technical expertise, lab infrastructures and cost, suitability for the specific study and the desired results (Chandra *et al*., 2001). Molecular markers plays a significant role in plant breeding development, because it considers powerful tools in applications of this science. By incorporation between molecular markers with genes that governing characters this can accelerating breeding program, furthermore provide dependable information of phylogeny among species. Since more than three decades Botstein *et al*., (1980) was reported first DNA molecular marker (RFLP). There are appreciable achievements was reported in discipline to deploying plant breeding regularly (Liu *et al*., 2012). Through this technique plant breeder can get a valuable information, origin clarification and relatedness among genotypes that can be considered for breeding purposes – generally, species and varieties of a given genus – this facilitate the design of crosses and later selection. Furthermore, the work of plant breeders is not finish by completing the crosses, the real work starts from this stage. The application of this technique has some important properties in the breeding program, so it can accelerate and facilitate the complicated process of selection.

2.13 Simple Sequence repeats (SSR) or Microsatellites marker

Microsatellites are also known as simple sequence repeats (SSR), this term coined first by Litt and Luty, (1989) Consisting of 1 to 6 base pairs of

simple repeated motifs in both coding or/ and non-coding regions. It has to be inherited as Mendelian factors as co-dominant markers. Furthermore, it has high distribution and abundance among genome and high polymorphism rates. All this make microsatellite common molecular markers in plant breeding (Morgante*et al.,* 2002).

2.14 Morphological and SSR markers for sorghum diversity assessment.

Assessment based on morphological traits it regard traditional process (Torkpo*et al*., 2006). Studies showed that phenotypic variance among and within landrace populations are related and close to geographical areas between the areas of origin, Therefore, an entire collection phenotypic diversity can be classified via eco-geographical information(Grenier *et al*., 20 01).

Sorghum improvement depends upon the using of genetic variability in genotypes originally maintained by farmers in traditional agricultural practices (Grenier *et al*., 2001). In the past, studies have been devoted to evaluating patterns of sorghum genetic variation based on morphology or pedigree. However, it is difficult to tracing based through morphology because there are complex quantitatively inherited traits. For this reason, DNA-based markers should be utilized simultaneously with morphological markers in studies of sorghum genetic diversity and in crop genetic improvement (Tawanda, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

The plant materials used in this study consisted from 20 sweet sorghum genotypes which collected from different regions of Sudan, figure 3.1 (8 from Eastern Sudan, 10 from central Sudan, 2 from western Sudan) as shown in Table (3.1).

3.2 Description of experiments of the study

Two field and laboratory experiments were used in this study, the field experiment was carried out in summer season (rainy season) of 2014 at demonstration farm, faculty of Agricultural and natural resource studies, University of Bakhat Al– Ruda, Elduiem, Sudan (long 32 20E, lat 13 39N and 385above sea level). In the period from 2014 to April 2015. Molecular experiment was conducted at Molecular Biology Unit laboratory, Environmental and Natural Resource and Desertification Institute (ENRDRI), National Center for Research, Ministry of Higher Education and Scientific Research, Khartoum, Sudan.

3.3 Field experiment

3.3.1 Cultural practices and design of the experiment

The randomized complete block design (RCBD) was used in this experiment. The field was well prepared by using chisel plough, disc harrow, leveled and ridged 75 cm apart and later was divided into plots (5× 3.75 m^2). 5 seeds of each genotype were planted in holes, spaced 20 cm between holes. Irrigation immediately done to ensure good germination and establishment, later the seedlings were thinned to 2 plants per hole, moreover the Nitrogen

fertilizer were applied with dose 80 kg/ fedan (urea 46%N) and the chemical pesticide (Furdan) was applied to control plants from stem borer insect.

Table 3.1: Sweet Sorghum genotypes used in the study

No.	Name	Area of collection
1	W.N.M-1	Eldueim, White Nile State
2	Eldueim-4	Eldueim, White Nile State
3	Elhoush	Elhoush, Algazeira state
4	Elswki-2	Elswki, Sinnar State
5	Elshwak-1	Elshwak, Elgadarif State
6	Alfao	Alfao, Elgadarif State
7	W.N.M-2	Eldueim, White Nile State
8	Elhawata-1	Elhawata, Elgadarif State
9	Elhawata	Elhawata, Elgadarif State
10	W.N.M-3	Eldueim, White Nile State
11	Elobied	Elobied, North Kurdufan State
12	Kosti	Kosti, White Nile State
13	Shawak-2	Elshwak, Elgadarif State
14	Elswaki -1	Elswaki, Sinnar State
15	Elgadarif	Gadarif, Gadarif State
16	Eldueim-1	Eldueim, White Nile State
17	Eldueim-10	Eldueim, White Nile State
18	Southern Gadarif	Gadarif, Gadarif State
19	Sinnar	Sinnar, Sinnar State
20	Elobied-1	Elobied, North Kurdufan State

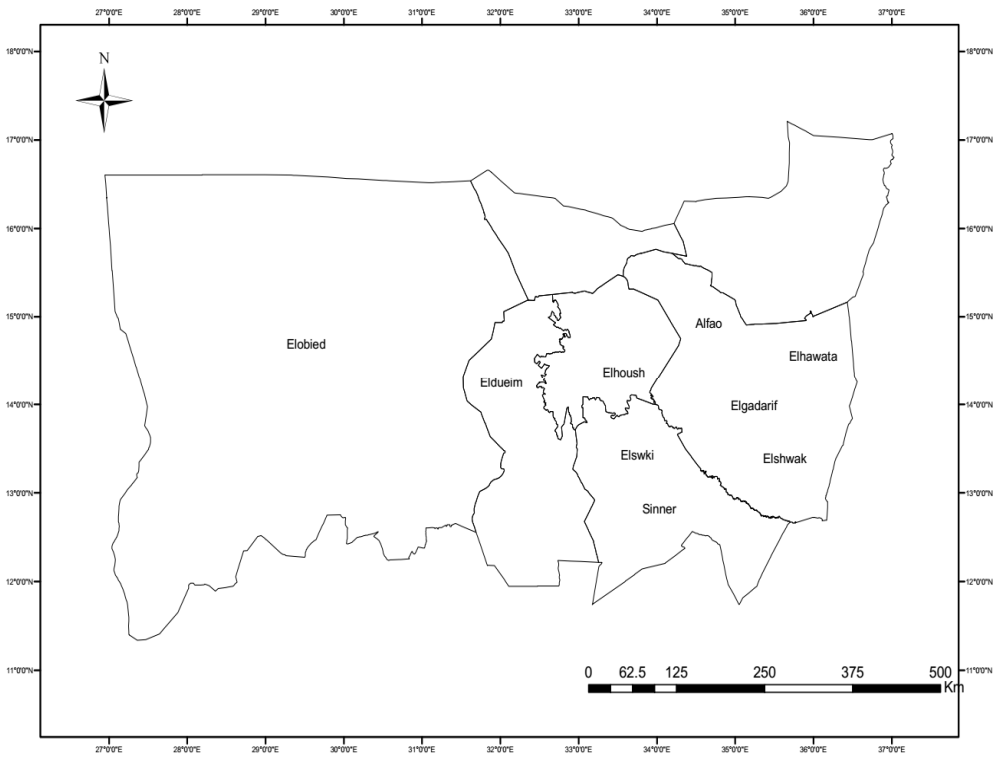

Figure 3.1 Map demonstrate areas from which the Seeds of Sweet Sorghum genotypes were collected

3.4 Data collection of phenotypic traits

The following traits were taken from five randomly selected sweet sorghum plants in the plot and later the average of the 5 plants was used.

3.4.1 Plant height PH (cm)

It was measured in cm, from the soil surface to tip the panicle of each genotype.

3.5.2Stem diameter (mm)

Measured as the thickness average of the stem at the middle of the fourth internodes from the plant base using digital vernia. Which expressed in mm?

3.5.3 Leaf area (cm^2)

Sticker *et al.*, (1961) was described formula to calculating the Leaf area (LA) = Maximum length×Maximum width

3.5.4 Leaf area index (LAI)

The leaf area index was measured by using the following formula of Kemp, (1960) who stated that:

$$LAI = \frac{\text{Total leaf area per plant x number of plants per unit area}}{\text{Ground area occupied by plants}}$$

3.6 Statistical analysis:

The analysis of variance (ANOVA) for Randomized Complete Blocks Design (RCBD), with four replications was carried out on the collected data which analyzed by using Genestat version 4 software package, while correlation acquired by using SPSS.

3.7 Genotyping

3.7.1 Genomic DNA

For extraction Genomic DNA Leaves of 2-week-old seedlings were collected

using the Cetyltrimethyl ammonium bromide (CTAB) method as described by Zhang and Stewart, (2000) with some modification. Sweet sorghum leaves were frozen in liquid nitrogen and grinded to a fine powder with a pestle and mortar. Fine powder transferred to 15 ml tube, in 5 ml of preheated (65 °C) 2X CTAB buffer (Cetyltrimethyl ammonium bromide, containing 100 m MTrisHCl (pH 8.0), 20mM Ethylene diamine tetra acetic acid (EDTA) pH 8.0, 1.4 M Sodium Chloride (NaCl) and 1% 2-Mercaptoethanol. The mixture was incubated at 65°C for at least one hour, and then mixed with equal volume of chloroform-isoamyl alcohol (24:1). Tubes were inverted gently 10-15 times and then centrifuged at 10,000 rpm for 10 minutes; the chloroform isoamylalchohol treatment was repeated when needed. The supernatant was transferred to a new tube. To precipitate the DNA, 2/3 isopropanol and 7.5 M ammonium acetate was added and the contents were incubated for 30 min on ice. Then centrifuged for 10 minutes at 5000 rpm, then Adding 1-2 mL of 70% EtOH and centrifuging for 1 minute at low speed. The DNA pellet then re-suspended in 100 µL of TE buffer or ddH20.

3.7.2 Agarose gel electrophoresis

DNA quality was checked in 1% agarose gel electrophoresis prepared in 0.5X TBE buffer and Ethidium Bromide (10 ng /100 ml) was added to the gel to stain the DNA bands. Before loading the DNA in the gel, 2 µl of bromophenol blue dye was added, 5µL of the reaction was loaded on gel. DNA ladder (1 kb) was also loaded on one side of the gel. Samples were runned in electrophoresis for approximately 30 minutes. After electrophoresis,the products were viewed under ultraviolet transilluminator and photographed using Saratoga, CA. 95070, USA Gel Documentation System. Working DNA samples (containing 50ng/µL) were stored at 4°C. For genotyping also gel electrophoresis used to analyze PCR product of SSR Markers 2% gel was used.

3.7.3. Sources of microsatellites markers

Microsatellites primer pairs were distinctly obtained from the following link

for sorghum http://archive.gramene.org/db/markers/marker_view, for Maize obtained from genetic and genomic database (http://www.maizegdb.org).

3.7.4 PCR protocols

PCR-amplifications were performed in a 20 µL reaction mix containing 1 µL 10× PCR buffer, 0.2 µLdNTPs (5 mm each), 0.1 µL 25 mM MgCl2, 0.1 µLTaq DNA polymerase, and 1µL (50ng) genomic DNA. Microsatellites were amplified by standard PCR procedures described by Zhang *et al.*, (2000). PCR amplification was carried out using thermo cycler with the following cycling profile 1 cycle of 3min at 95ºC, 30 cycles of 45s at 95ºC, 1min at 55ºC, 1min 45s at72ºCfollowed by 1cycle of 10 min at 72ºC, with a final extension step of 1minute at4ºC. Following thermocycler was used to carry out amplification (Fig. 3.2).

Molecular Data analysis

Each DNA fragment obtained by both marker types was scored as present (1) or absent (0), each of which was treated as an independent character. Similarity between the genotypes was analyzed on the basis of the scores. Data were then used to create a matrix to analyze genetic relationship using "STATISTICA 6". A dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the markers data for all sorghum genotypes following the Unweighted Pair Group Method Analysis (UPGMA) as described by Sokal and Michener, (1958).

The genetic diversity was estimated by similarity indices calculated from band sharing data of each pair of DNA fingerprints.

3.4.2 Coefficient of variation (C.V)

Coefficient of variation (c.v) for each trait was determined according to the following formula:

$$\text{CV\%} = \frac{\sqrt{\text{Mean square of error}}}{\text{Grand mean}} \times 100\%$$

3.4.3 Phenotypic and genotypic coefficient of variation (%):

They were according to formula suggested by Burton and Devane. (1953) as follows:

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\sigma^2 Ph}}{\text{Grand mean}} \times 100$$

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\sigma^2 g}}{\text{Grand mean}} \times 100\%$$

Where $\sigma^2 Ph$ is phenotypic variance

And $\sigma^2 g$ = genotypic variance

Table 3.2 Simple Sequence repeat markers used in this study.

No.	SSR locus & (Chromosome Location)	Forward (F) & Reverse (R) Primer Sequences (5' to 3')	No. of bp
1	Xgap 256(1)	F = AAT TTG CTT TTT GGT CCG TTT	21
		R = TAG GAA AGA CAG TAC TAG AGG TCA	24
2	Xtxp 003(2)	F = AGC AGG CGT TTA TGG AAG	18
		R = ATC CTC ATA CTG CAG GAC C	19
3	Xgap 236(3)	F = GCC AAG AGA AAC ACA AAC AA	20
		R = AGC AAT GTA TTT AGG CAA CAC A	22
4	Xtxp 012(4)	F = AGA TCT GGC GGC AAC G	16
		R = AGT CAC CCA TCG ATC ATC	18
5	Xtxp 030(5)	F = AAA AAG GAC GCG CAG CTG	18
		R = CTG GTC TCC ACC ATC CGT AG	20
6	Xtxp 176(6)	F = TGG CGG ACA TCC TAT T	16
		R = GGA GAG CCC GTC ACT T	16
7	Xtxp 040(7)	F = CAG CAA CTT GCA CTT GTC	18
		R = GGG AGC AAT TTG GCA CTA G	19
8	Xtxp 47(8)	F = CAA TGG CTT GCA CAT GTC CTA	21
		R = GGT GCG AGC TAG TTA AGT GGG	21
9	Xtxp 10(9)	F = ATA CTA TCA AGA GGG GAG C	19
		R = AGT ACT AGC CAC ACG TCA C	19
10	Xtxp 141(10)	F = TGT ATG GCC TAG CTT ATC T	19
		R = CAACAA GCC AAC CTA AA	17
11	p-umc1858	F = GTTGTTCTCCTTGCTGACCAGTT	23
		R = ATCAGCAAATTAAAGCAAAGGCAG	24
12	p-umc1327	F = AGGGTTTTGCTCTTGGAATCTCTC	24
		R = GAGGAAGGAGGAGGTCGTATCGT	23
13	p-umc1201	F = ACCCTAGAGGGGTCCACTGC	20
		R = GGAACGATCCTTACAAGTGAGAGAA	25
14	p-umc1111	F = CTACAGCCTTCTTTTTGGTGGAGA	24
		R = TGACTCTTAGCCGTTTCCATTTTC	24

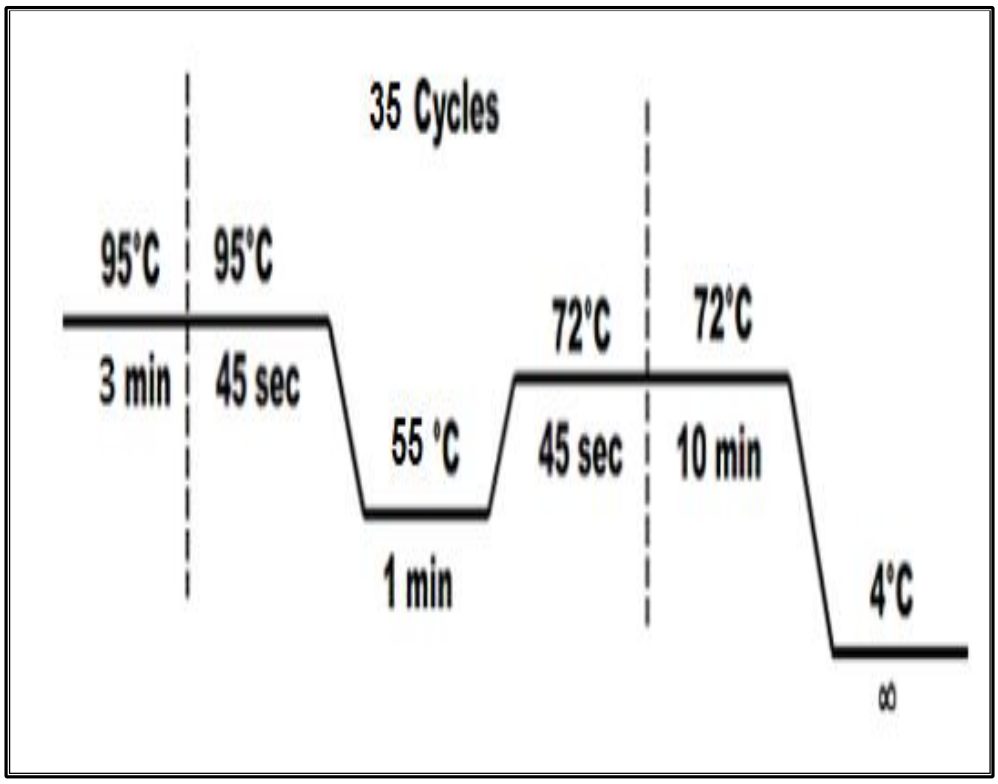

Fig 3.2 Thermo cycling profile for the amplification of PCR

3.4.1 Analysis of variance

Table3.3 The analysis of variance of randomized complete block design with four replications used in this study

Source of variation	Degree of freedom	Mean square
Replication	(r-1) = 3	M3
Treatment	(t-1) =19	M 2
Error	(r-1)(t-1) = 57	M 1
Total	Rt-1 = 79	

M1 ,M2=error and genotype mean squares

CHAPTER FOUR

RESULTS

4.1 Phenotypic Variability:

The results of the study reveal that there was highly significant in stem diameter and leaf area index, while plant height and leaf area resulted no significant differences.

4.1.1 Plant height (cm)

The analysis of variance (ANOVA) showed that there are no significant differences at ($P \leq 0.05$) among genotypes with respect to plants height (Table 4.1). The mean of highest value (245.9 cm) it was obtained from genotype 18 (South Gadarif), while genotype 3 (Elhoash) recorded lowest value (193.9 cm). The overall mean 215.34 cm and coefficient of variation (CV %) was 11.34 % (Table 4.1).

4.1.2 Stem Diameter (mm)

There was a highly significant difference of this trait due to analysis of variance at ($P \leq 0.01$) (Table 4.1) which demonstrate the stem diameter values (highest value recorded by genotype 6(Alfao) it was 2.26 cm, while lowest value which recorded by genotype 9 (Elhawata-1) 1.63 cm , The overall mean 1.86 cm and coefficient of variation (CV %) was 11.8 % (Table 4.1).

4.1.3 Leaf area (cm^2)

The result showed that there is no significant difference of leaf area among genotypes. Genotype 6(Alfao) resulted 708.9 cm this is regard highest value, while genotype 2(Eldueim-4) resulted lowest value (436.7cm). The overall mean was 529 cm and the coefficient of variation (CV%) was 18.2% (Table 4.1).

4.1.4 Leaf area index

It was showed very high significant differences among genotypes at ($P \leq 0.01$). Genotype 6(Alfao), gives highest value (11.8), while genotype 2(Eldueim-4) was gave the lowest (5.65), the overall mean of this parameter was (40.54) and the coefficient of variation (CV %) was 21.5% (Table 4.1).

4.1.5Estimates of phenotypic (o2ph) and genotypic (o2g) variance among sweet sorghum genotype

Among all values of phenotypic and genotypic variance it were ranged between (4021 LA of phenotypic) to (0.03 SD of genotypic).The mean of phenotypic variance is highly greater (701) than genotypic variance (270), with respect to phenotypic variance leaf area per plant (LA) resulted highest value (4021) while stem diameter (SD) resulted the lowest (0.04), whereas the Genotypic variance (2g) revealed values ranged between 1452 (LA) to 0.03 (SD).

4.1.6 Estimation of phenotypic (σ^2Ph) and genotypic (σ^2g) coefficient of variation traits.

The result indicated that phenotypic coefficient of variation (σ^2Ph) is highest (2.80) than genotypic coefficient of variation (σ^2g)2.46. Generally coefficient of variation for both ranged between -0.03 (Leaf area per plant for σ^2Ph) to 12.36 (Leaf area index for σ^2g). σ^2g ranged between 0.07 to 12.36 (LA and LAI respectively), whereas σ^2Phvaried from -0.03 to 8.54 (LA and LAI respectively). Table 4.2

4.1.7Phenotypiccorrelations between different traits

It was exhibited positive low significant correlation between leaf area index and plant height, low negative low correlation between leaf area (LA) and plant height (PH), while there is highly significant positive correlations between LA with LAI and LA with SD. Generally correlation ranged between -0.0339(LA with PH) to 0.8905 (LAI with LA). Table 4.3

Table4.1.Means of some growth traits of twenty genotypes ofsweet sorghum.

No.	Plant height(cm)	Stem diameter	Leaf Area	Leaf Area Index
1	208.55	1.80	539.65	7.40
2	205.60	1.68	436.70	5.65
3	193.88	1.65	533.70	7.55
4	195.60	1.68	505.93	7.03
5	219.90	2.00	522.38	8.48
6	219.25	**2.28**	**708.88**	**11.80**
7	222.85	1.80	626.63	8.85
8	213.25	1.80	592.65	8.58
9	208.40	1.63	475.30	6.43
10	220.95	1.75	542.83	7.53
11	218.80	2.03	547.03	8.15
12	213.35	2.03	572.20	9.45
13	**234.95**	2.10	652.23	10.50
14	**234.15**	1.88	533.53	7.65
15	214.75	2.18	642.70	9.95
16	231.20	1.73	577.78	8.13
17	206.75	1.8	574.83	7.45
18	245.85	2.0	531.03	8.73
19	202.25	1.63	525.40	6.33
20	196.60	1.68	518.03	6.00
Grand Mean	215.34	1.86	557.97	8.08
C.V	11.34	12.05	18.17	21.48
SE+-	17.26	0.16	71.69	1.23
LSD(0.05)	34.57	0.32	143.54	2.46

Table 4.2 Estimates of phenotypic (σ^2ph) and genotypic (σ^2g) coefficient of variation in sweet sorghum for growth and yield traits.

Traits	GCV	PCV
Plant height(cm)	0.45	0.01
Stem diameter(cm)	1.61	2.15
Leaf area per plant(cm)	0.07	-0.03
Leaf area index	12.36	8.54

Table 4.3 Phenotypic correlation between different traits

Traits	PH	SD	LA
SD	0.0684		
LA	-0.033	0.4263*	
LAI	0.1869	0.5779**	0.8905**

, significant according to R value*

Table 4.4 Mean squares of plant height (PH), Stem diameter (cm), Leaf area index (LAI) and leaf area (LA).

Traits	Replication DF =3	Genotype DF =19	Error DF=57
Plant height(cm)	4865.66**	779.21NS	595.84
Stem diameter (cm)	0.13 *	0.15**	0.06
Leaf area per plant(cm)	22306.70 NS	16084.40*	10277.10
Leaf area index	4.49 NS	9.54**	3.02

*** Highly significant at p=0.05 and p=0.01 respectively,*NS non- significant*

4.2 Molecular Marker Data

Genetic relationship and similarity among sweet sorghum genotypes

A total of 14 SSRs primers pairs screened revealed (84.6%) polymorphic band across 20 sweet sorghum genotypes, primer p-umc1201 revealed highest (100% polymorphic) while primer p-umc1858 and X1resultedlowest (55%) polymorphic. indicating significant homogeneity of sweet sorghum; A total of 29 putative alleles with different fragment sizes were found.

The average number of alleles per SSR marker was 2.07, ranging from 1 allele (a total of 9 SSR loci including X2, X3, X4, X5, X6,X8, X9 and X10) to 6 alleles (p-umc1201) (Table 4.2). 29 alleles were detected, 26 (90%) of alleles are polymorphic. The microsatellite markers obtained a great amount of variation in sampled genome, although the sweet sorghum genotypes showed relatively low polymorphism resulted high level genetic similarity. Regarding the pair-wise combination the genetic dissimilarity ranged between 0.13 between genotypes (5 with 8 and 3 with 16) those genotype regard most closely genotypes to 1.14 between genotypes 4 and 6 (highest genetic distance)

4.2.1. Matrix of genetic distances

The results based on percentage disagreements indicated that the highest genetic distance (1.14) between genotype 4(Elswki) with genotype 6(Alfao), while lowest genetic distance (0.13) obtained among genotypes 8(Elhawata-1) with 5(Elshwak-1) and 3(Elhoush) with 16(Eldueim-1).

4.2.2. Dendrogram Tree

A dendrogram based on similarity values resulted from SSRs the UPGMA was used to constructing cluster analysis (figure 4.1), for the 20 genotypes with 14 microsatellite primers the Dendrogram was constructed, based on similarity, the 20 sweet sorghum genotypes was showed several pattern of variation. At the base of Dendrogram we can find single genotype it was genotype 4(Elswki), then there is two independent sisters first genotypes

2(Eldueim-4) with 6(Alfao) and second genotypes 9(Elhawata) with 20(obied-1)) from the top of the Dendrogram (figure 4.1) there were three clusters and sub-clusters, clusters A have 2 sub cluster A1(possess genotypes 1(W.N.M-1) , 5(Elshwak-1), 8(Elhawata-1) and 13(Elshwak-2) and A2 contains(genotypes 3(Elhoush), 15(Gadarif), 16(1), and 18(South Gadarif). cluster B includes (11(Obied-2), 12(Kosti), 14(Elswki-1), and 19(Sinnar)), while cluster C possess (genotypes 9(Elhawata) 17(Eldueim-10), and 20(Obied-1).

4.5 Molecular characteristics of 14 SSR primers used for genotyping

Primer name	Total of band	Polymorphic	Percentage
X1	2	11	55%
X2	1	19	95%
X3	1	18	90%
X4	1	19	95%
X5	1	14	70%
X6	1	17	85%
X7	2	16	80%
X8	1	20	100%
X9	1	17	85%
X10	1	19	95%
p-umc1858	4	11	55%
p-umc1327	5	15	75%
p-umc1201	6	20	100%
p-umc1111	2	19	95%
Mean	2.07	16.8	84%

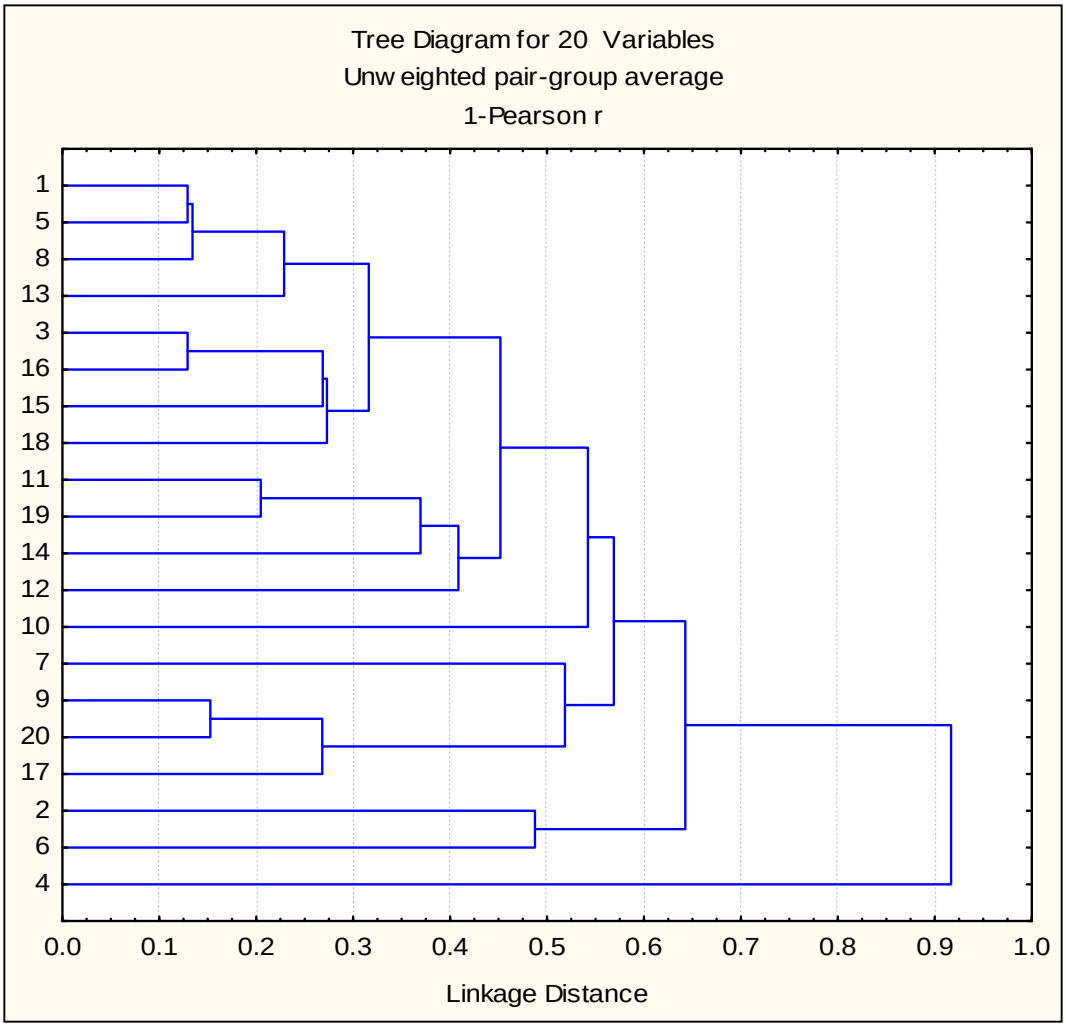

Fig 4.1 UPGMA tree showing genetic relationships of 20 Sudanese sweet sorghum genotypes as revealed by 14 SSR markers, the genotypes names from (1 to 20) as mentioned in Table 2

Table 4.6 Matrix of genetic distances based on percentage disagreements, the genotypes names from (1 to 20) as mentioned in table 2.1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.00																			
2	0.38	0.00																		
3	0.19	0.49	0.00																	
4	0.92	0.90	0.80	0.00																
5	0.13	0.41	0.21	0.91	0.00															
6	0.85	0.49	0.87	1.14	0.67	0.00														
7	0.63	0.54	0.49	0.96	0.59	0.52	0.00													
8	0.14	0.24	0.19	0.84	0.13	0.70	0.49	0.00												
9	0.63	0.69	0.49	0.96	0.59	0.52	0.46	0.49	0.00											
10	0.32	0.64	0.44	0.73	0.40	1.01	0.80	0.47	0.80	0.00										
11	0.48	0.61	0.55	1.02	0.34	0.76	0.68	0.34	0.68	0.67	0.00									
12	0.48	0.75	0.55	1.02	0.48	0.76	0.68	0.48	0.54	0.52	0.41	0.00								
13	0.28	0.38	0.33	0.92	0.27	0.70	0.49	0.14	0.49	0.47	0.21	0.34	0.00							
14	0.52	0.43	0.63	0.97	0.41	0.49	0.69	0.38	0.54	0.64	0.33	0.47	0.24	0.00						
15	0.38	0.57	0.21	0.83	0.27	0.78	0.54	0.24	0.54	0.64	0.33	0.33	0.24	0.43	0.00					
16	0.34	0.61	0.13	0.88	0.34	0.76	0.39	0.34	0.39	0.67	0.69	0.69	0.48	0.75	0.33	0.00				
17	0.56	0.67	0.47	0.84	0.55	0.70	0.49	0.42	0.20	0.78	0.62	0.62	0.42	0.52	0.52	0.34	0.00			
18	0.41	0.55	0.21	0.77	0.41	0.96	0.59	0.27	0.44	0.71	0.62	0.62	0.41	0.55	0.27	0.34	0.41	0.00		
19	0.41	0.69	0.34	0.98	0.28	0.81	0.73	0.41	0.59	0.56	0.20	0.34	0.27	0.41	0.27	0.48	0.55	0.55	0.00	
20	0.63	0.83	0.64	1.04	0.59	0.52	0.61	0.63	0.15	0.80	0.68	0.39	0.63	0.54	0.54	0.54	0.34	0.59	0.59	0.00

CHAPTER FIVE

DISCUSSION

5.1. Phenotypic and genotypic variability

The amount of variation which existing via populations plays a significant role of any successful improvement through selection. From the results which revealed from this study the phenotypic variation is highly greater than genotypic, although the GCV is higher in PH, LA and LAI. While PCV is higher in SD. The characteristic of 20 genotypes under this study varied considerably in some growth components which include (stem diameter and leaf area index, this differences may return to genetic makeup of genotypes or due to environmental conditions, and this finding agree with (Lakkana *et al*., 2009) who reported that the sweet sorghum genotypes vary widely in their adaptation to different climatic and soil conditions.

5.1.1. Phenotypic traits

The analysis of variance showed that the genotypes are not differing significantly on plant height and leaf area per plant, while leaf area index resulted highly significant and stem diameter resulted significant. The highest plant height was (245.9 cm) which obtained from genotype 18 (South Gadarif), while genotype 3 (Elhoash) recorded lowest value (193.9 cm). Plant height mostly affected by environmental conditions. However, our results similar to finding of Reddy *et al*., (2007) height ranging from 130-240 cm and less than reports of Ali *et al*., (2008) and Rao*et al.,* (2013), reported that sweet sorghum varieties had a height ranging from and 104 to 374, 280-332 cm respectively. The height of the plant also may be influenced by genotypic performance some genotypes being genetically taller with more number of internodes than others .Reddy et *al*., (2007) stated that there is linear correlation between the length, brix and cane

yield. 1.47 genotype 9 (Elhwata-1) to 2.29 genotype 6 (Alfao) and this similar to result that obtained by Meli, (1989) who reported that stem diameter(SD) of some sweet sorghum genotype ranged between 1.47 to 2.29, and less than finding of Bucheyeki *et al*, (2008) reported SD ranged between 1.59 to 2.69 cm. With respect to Leaf area index, genotype 6 (Alfao) resulted highest (11.8 cm) this very higher than finding of Kaplan and Kara, (2014) who resulted LAI ranged between 3.54 to 5.14.In this study, the phenotypic variances are greater than thegenotypic variances for all characters under study. Also all phenotypic coefficient are greater than genotypic coefficient for all characters. The genotypic coefficient of variation indicates the genetic variability present in various characters. Similar findings were Reported by Swarp *et al*., (1970); Phull *et al.,* (1972); Kumar andSingh (1986); Mohammed (2004) and Idris (2006).

5.1.2 Phenotypic correlation between different traits

Within measured morphological parameters, LAI and LA a highly significant positive correlations this finding is similar to that which revealed by Tesso *et al*., (2011) which stated that Phenological characters and plant height were significantly correlated with each other and with all leaf traits. There was positive correlation among leaf traits, between LA with LAI and LA with SD, it was exhibited positive low significant correlation between leaf area index and plant height this results are similar to which stated by Bucheyeki *et al.,* (2008) low negative low correlation between leaf area (LA) and plant height (PH) but this not agree with results which stated by Chikkarugi and Balikai (2011) they resulted that there are a significant and positive correlation between plant height with leaf area.

5.2 Molecular data

Simple sequence repeat marker are heavily being used in crops to discriminate among genotypes, cultivars and used as tools in selection based- markers (Dje *et*

al., 2000). Contrasts in genetic diversity revealed by previous studies using SSR can be referred to sample size, origin and background. For the genetic diversity, the primer sets yielded 29 alleles of which 26 were polymorphic across the 20 genotypes and fragment size were amplified by 14 SSR markers. 29 alleles were detected with average 2.07 alleles per primers, 26 of alleles produced (90%) polymorphic, similar result obtained by Ji *et al.*,(2011) 90% , our finding less than (Pei *et al.*, 2010) resulted polymorphic (100%), and more than Elhussein *et al.*,(2014) they were resulted 71.4% polymorphic .The number of polymorphic alleles detected per primer pair ranged from 1 to 6 alleles , with an average of 2.07 alleles per primer pair in our study was slightly different from study which shown by (Pei *et al.*, 2010) SSRs generated 2-6 alleles per locus with average 2.76 per locus, but lowest than that obtained by Agrama and Tuinstra,(2003) and Menz*et al.*, (2004) (4.5 and 5.9 alleles respectively). The results showed that the highest genetic distance between genotype 4(Elswki) with genotype 6(Alfao), it revealed genetic distance 1.14) while lowest (0.13) genetic distance between genotypes 8(Elhawata-1) with 5(Elshwak-1) and 3(Elhoush) with 16(Elduim-1). Although the genotypes 4(Elswki) and 6(Alfao) were located at the close geographic area but resulted the highest genetic distance, while the genotypes (Elhawata-1with Elshwak-1) and (Elhoush with and Elduim-1). Resulted closely genetic relatedness this either due to closely geographic or /and due to seeds transmission by farmers. this similar result that revealed by Lekgari and Dweikat (2014) that genetic distance ranged from 0.024 to 1.13 and more than that obtained by Abu Assar *et al.*, (2005), Elhussein *et al.*,(2014), (Pei *et al.*,2010) and (Geleta *et al.*, 2006) 0.0 to 0.91, 0.15 to 0.78, 0.042 to 0.89 and (0.152–0.762) respectively. for the 20 genotypes with 14 microsatellite primers the Dendrogram was constructed, based on similarity, all genotypes showed several pattern of variation, At the base of Dendrogram we can find single genotype (genotype 4(Elswki), then there are two independent sisters, first

sister genotypes(Elduim-4and Elfao), second sister genotypes ((Elhawata) with (Obied-1))from the top of the Dendrogram (figure 4.1) there were three clusters and sub-clusters, clusters A have 2 sub cluster A1(possess genotypes (W.N.M-1) , 5(Elshwak-1), (Elhawata-1) and (Elshwak-2), and A2 contains(genotypes (Elhoush), (Gadarif), (Elduim-1), and (South Gadarif). cluster B includes ((Obied-2), (Kosti), (Elswki-1), and (Sinnar)), while cluster C possess (genotypes (Elhawata), 17(Elduim-10), and (obied-1).Three clusters groups were Revealed among all genotype this is more than results which revealed by (Pecina-Quintero *et al.*, 2012) two clusters.It was clear that the clustering either referred to genotypes which have close geographic locations, i.e. sub- cluster A2 possess genotype from same geographic area (Elshwak-1) and 13(Elshwak-2), also sub-cluster A2 possess genotype from same geographic area (Gadarif), and (South Gadarif), while cluster B includes (Obied-2 and Kosti), (Elswki-1 and Sinnar), there is geographic relation between Elobied and Kosti (figure 3.1), and also Elswki and Sinnar. With respect cluster C it aggregate genotypes from different geographic sites i.e. From western, central and eastern (figure 3.1).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The combination of using morphological and molecular markers consider optimum tool in plant breeding. SSR molecular markers, provide possibly fast, accurate fingerprinting. It concluded that, highly significant different between sweet sorghum genotypes used in this study. However, this genetic diversity between the genotypes can be used in sweet sorghum breeding programs in future. Moreover, it is very important for sweet sorghum germplasm conservation, these genotypes could be identified obviously using selected SSR markers. The highest genetic distant between genotypes can be used by breeders for further evaluation

6.2 Recommendations

1. Future research in this aspect is required for collection of more genotypes which represent all Sudanese regions.
2. More Markers needed specially marker assisted selection
3. High throughput sequencing and genotyping by sequencing requested for future research.

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