We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,500
Open access books available

136,000
International authors and editors

170M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Exploring Plant Genetic Variations with Morphometric and Molecular Markers

Bushra Sadia, Faisal Saeed Awan, Fozia Saleem, Javaria Altaf, Abdullah Bin Umar, Muhammad Nadeem, Samra Hameed, Farwa Ashraf and Mariam Nasir

Abstract

For centuries, crop improvement has served as the basis of food security of ever increasing human population. Though vast germplasm collections are available; their exploitation for crop improvement still depends upon efficient assessment of genetic diversity. Genetic variability is the key element in adaptation of plants to varying climates. While crops with narrow genetic diversity are vulnerable to stresses. The estimation of extent and pattern of genetic variability is a prerequisite for generating superior varieties. Genetic diversity analysis generates key information to dissect genetic variations in crop germplasm with the help of morphometrical, biochemical and molecular tools. Among these, DNA markers provide a reliable and detailed insight into the similarities and differences among crops. In this chapter, we discuss the applications of phenotypic and molecular markers to probe genetic divergence in crops and present case studies that describe the significance of these tools to characterize sorghum germplasm. Furthermore, we spotlight sorghum biodiversity exploration efforts worldwide and propose future directions.

Keywords: molecular markers, *Sorghum bicolor*, PCR, RAPDs, SSRs, SNPs, GWAS, association mapping, UPGMA, dendrogram

1. Introduction

The term "variability" refers to variation in one or more than one characters of living organisms. The cumulative influence of environment and the genetic factors brings about variations in a specific trait. Genetic variation refers to variation in sequences of genes between individuals in a population. Allelic variation is the building block of hereditary variation that is expressed in the form of different phenotypes. Processes like mutation, random mating and fertilization and gene duplication may introduce new genes and alleles thereby increasing genetic variation. Random mutations are the source of genetic variations. Mutations are either heritable or non-heritable; the beneficial heritable mutations exert a great influence on the genetic variations of living organisms. Likewise, gene flow is also a means of introducing new alleles to a population and thereby broadening the genetic diversity of living organisms. Genetic variability provides baseline for genetic diversity;

a broader term that reflects the degree/amount of variation existing within a population. Without genetic variability, populations fail to adapt to varying climatic conditions and are prone to extinction. Genetic variability is a source of natural selection, that is the key driver of evolution of living organisms.

Agriculture is directly influenced by environmental degradation and biodiversity loss leading to compromised quantity and quality of diverse and nutritious foods. Globally, people are relying on three major cereal crops wheat, maize and rice to fulfill their dietary needs and in turn are adopting similar dietary plans. Due to selective exploitation of few crops and large scale cultivation of genetically homogeneous cultivars, other wild and more nutritious crops are wiped out of global atlas. Not only we have compromised our health due to poor nutrition, the resilience of our food system is also at stake due to loss of crop diversity. Such lack of biodiversity was the root cause of Irish potato famine in the nineteenth-century. Presence of genetic diversity is the vital element of all variety development programs. Existence of genetic diversity in crop germplasm aides in the efficient selection of high yielding, better adapted crop plants with possible uses of direct introduction as a variety or one of the parents in crossing scheme of breeders for variety development programs. Since genetically diverse germplasm offers wider tolerance to biotic and abiotic stresses; such programs extensively involve exploring and exploiting diverse crop germplasm.

There is a continuous shift in the focus of agriculture from time to time. Agriculture in ancient times was focused on meeting subsistence food requirement. While, present day agriculture is focused to maximize yields for growing populations. That's why breeders are utilizing crop genetic resources for targeted and sustainable development of new high yielding and nutritious crop varieties in order to address malnutrition and balanced diet of human population. Under prevailing conditions of scarce water resources and escalating temperatures, development of climate resilient crop varieties is gaining momentum. Climate smart agriculture relies on cultivars with novel biotic/abiotic stress tolerance traits. However, depletion of natural variability persists in existing crop germplasm. Targeted breeding to improve specific traits and repeated use of few breeding parents has narrowed the genetic base of existing major crop varieties, raising serious concerns about genetic vulnerability of modern crops and making breeder's task even harder. In this context, new sources of desirable alleles are exploited from wild as well as closely related crop species and mutants. Hence, for ever changing breeding goals, it is imperative to conserve genetic diversity as germplasm resource. Crop genetic diversity is the core element of climate smart agro ecosystem to promise sustainable food availability and thereby to alleviate hunger and poverty.

A dire need exists to brought back underutilized and forgotten crops of every region to the canvas of agriculture for enhancing sustainable food production under anticipated harsh climates of the planet. A huge resource of alternative crops like sorghum, can replace the monoculture of three dominating cereal crops. Sorghum is a grass of multiple uses including food, feed, fiber, sugar, ethanol etc. Exploiting this and other nutritious and hardy crops is the best way to diversify present cropping system and enhance its resilience towards climate change. We need concerted collective efforts to increase awareness of farmers, policy makers and consumers towards benefits of diversification in agricultural systems.

2. Crop genetic diversity assessment methods

The assessment, extent and distribution of genetic divergence is the base line of preservation and exploitation of genetic variability within and between crop

species. Initially, morphometric, cytological and biochemical markers were frequently used to evaluate the extent of similarities and differences among crop germplasm. Genetic and molecular markers were developed in the genomics and post genomics era and now are the widely used method for crop genetic divergence estimation.

2.1 Morphological markers

Evaluation of phenotypic traits in glasshouse or field- grown plants has long been used for selection of diverse crop plants. Effective morphometric characterization involves field plantation of large number of plants following specific lay out design. The morphological traits are recorded at vegetative growth (germination percentage, number of leaves, nodes, leaf area index, leaf color, stem thickness etc.), reproductive growth (Days to flowering, days to maturity, flower color, morphology, brix value etc.) and maturity stage (Plant height, yield, dry biomass and grain weight etc.). Plants express physiological and morphological changes under biotic and a biotics stresses. Hence, phenotypic characterization is vital in the selection of tolerant plants under stress environment.

This approach is easy, simple, inexpensive and directly measurable. However, experienced staff is required for effective selection of promising plants. Such field-based evaluation is directly influenced by environmental factors. Moreover, labor and field requirements pose extra work. Morphological evaluations must be detailed involving all growth stages of plants. Presently high throughput phenomics approaches have refined the morphological data recording of large number of entries in the field with precision. The growth-stage dependent physio-morphological characterization provides a base line for breeders to develop diverse genotypes having stress tolerant attributes. Furthermore, good quality phenotypic data is the foundation of new genomics and molecular approaches to successfully dissect the molecular basis of complex quantitative traits such as yield, disease resistance etc. Morphological markers have limitation of delayed expression till the specific developmental stage of the plant. Moreover, genotype x environmental interactions render the morphological markers less reliable than other marker types.

2.2 Cytological markers

These markers are related to morphological variations in chromosome size, shape, number, length, arm ratio, volume, behavior in cell divisions and DNA content etc. These chromosomal features can be identified through microscopy and expressed by chromosome karyotype and bands. The G, Q, R and C banding patterns of chromosomes indicate regions of chromatin that are stained with the help of different fluorescent dyes, viz.; Quinacrine hydrochloride (Q bands) and Giemsa stain (G bands) [1]. The presence or absence of a chromosome band is associated with the specific traits. A thinnest chromosome band hosts over hundreds of genes. These are used to detect cytological mutations and track evolutionary chromosomal rearrangements. The fusion of chromosomal and molecular biology protocols in 1990 introduced fluorescence *in situ* hybridization (FISH) method. It is capable of physical mapping of nuclear content directly on the chromosomes and identifying protein content of a cell. A more advanced variant of *in situ* hybridization, "genomic *in situ* hybridization (GISH)" technique utilizes total genomic DNA of plant as a probe. Both GISH and FISH are powerful tools to characterize alien introgressions in crop species and dissect genetic makeup of natural and artificial hybrids [2]. However, cytological markers have limited use in genetic diversity estimation due to their small number and discrete detection.

2.3 Biochemical markers

Biochemical markers have been among the most widely used markers for assessing variations among and within crop species before the advent of molecular/DNA markers. The alternative forms of protein (isozymes) exhibit specific banding patterns on gel electrophoresis, owing to variations in charge- based protein mobility. Isozymes are the products of different alleles, their position can be mapped on to chromosomes and hence are used to map other genes. Protein/isozyme analysis is still among the simple, rapid and cheap methods and fits well in the projects where low level of genetic diversity estimation is desired. Though protein markers are more reliable than morphological markers, their expression is plant growth stage dependent and is readily influenced by the environment [3, 4].

2.4 Molecular markers

Molecular markers are based on DNA sequence polymorphism and bypass the limitations encountered in the use of morphological, cytological and biochemical markers. These have become the preferred method for evaluating crop genetic variations due to their simple inheritance, high reproducibility, widespread distribution in plant genome and being stable, highly polymorphic with minimum pleiotropic effects [5]. Molecular markers are not plant stage dependent and are least affected by environment. Large number of markers have been mapped on chromosomes of crop plants and livestock. Molecular markers show either dominant or co dominant inheritance mode. The codominant markers are preferred over dominant ones being more reliable and informative [6]. These have been extensively exploited for variety of applications like genetic fingerprinting, hybrid identification, functional genomics etc. In crop breeding, molecular markers help in early identification/selection of desired genotypes thereby shortening variety development time. These markers enhance breeders' capability of targeted breeding. The earlier version of hybridization- or PCR- based markers has now been upgraded to newer types based on sequencing or array platforms. Following are the groups of molecular markers based on principle techniques:

1. Nucleic acid hybridization- based markers: Restriction fragment length polymorphisms (RFLPs).

2. PCR- based markers: Randomly amplified polymorphic DNA (RAPDs), Amplified fragment length polymorphisms (AFLP), Microsatellites, or simple sequence repeats (SSRs), Randomly amplified microsatellite polymorphisms (RAMP), Sequence-related amplified polymorphism (SRAP), Inter simple sequence repeat (ISSR), Target region amplification polymorphism (TRAP)

3. PCR–RFLP markers: Cleaved amplified polymorphic sequences (CAPS)

4. Retrotransposons- based markers: Inter-retrotransposon amplified polymorphism (IRAP), Retrotransposon microsatellite amplification polymorphisms (REMAP), Retrotransposon-based insertion polymorphism (RBIP), Inter-primer binding site (iPBS).

5. Sequence-based markers: Single-nucleotide polymorphism (SNP)

6. Array-based platforms like Diversity Arrays Technique (DArT), restriction site-associated DNA (RAD), single feature polymorphism (SFP), etc. [7, 8]

7. Functional molecular markers (FMM): The term "Functional markers" was proposed by Andersen and Lübberstedt [9] for DNA markers that arise from sequence polymorphism among functional genes that are linked with variations in the desired phenotypic traits. Hence, these are more reliable and informative than all previous PCR- based markers.

Each marker system has its own benefits and disadvantages and variations exist on the basis of development cost, efficiency and reproducibility.

3. Need for genetic diversity assessment of sorghum germplasm

3.1 Sorghum origin

The word sorghum originated from "*Syricum*" in Latin, meaning "Grain of Syria" [10]. Sorghum (*Sorghum bicolor*) belongs to class *Liliopsida*, family *Graminea*, genus *Sorghum Moench* and has five groups named as: *Hetrosorghum*, *Chaetosorghum*, *Spitosorghum*, *Parasorghum* and *Eusorghum*. It is an ancient grain that has been cultivated for thousands of years. It originated mainly from Sudanese and Ethiopian grasslands more than 6000 years ago.

3.2 Global sorghum distribution and production

About 100 countries grow sorghum worldwide (**Figure 1**). USA is the top sorghum producer with five countries viz.; Nigeria, Ethiopia, Mexico, India and China follow in the order of production (**Figure 2**). The countries of Japan, Mexico, and Philippines are the major importers of North American sorghum, while China is the world's largest sorghum importer.

3.3 Sorghum in Pakistan

In Pakistan, sorghum is grown for fodder and forage of livestock. It is grown as kharif fodder in irrigated and rain fed areas of Punjab and Sindh provinces. Production of sorghum (*Sorghum bicolor*) in Pakistan is 1.45 million metric tons in 2020 (www.indexmundi.com). Sorghum is the second largest fodder crop after berseem (Pakistan Bureau of Statistics, 2016). Scarce record exists on use and adoption of

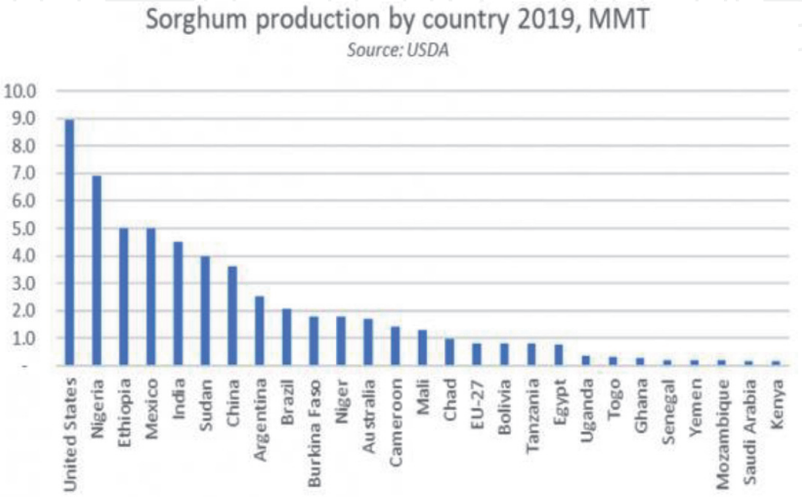

Figure 1.
Country-wise production of sorghum in the world.

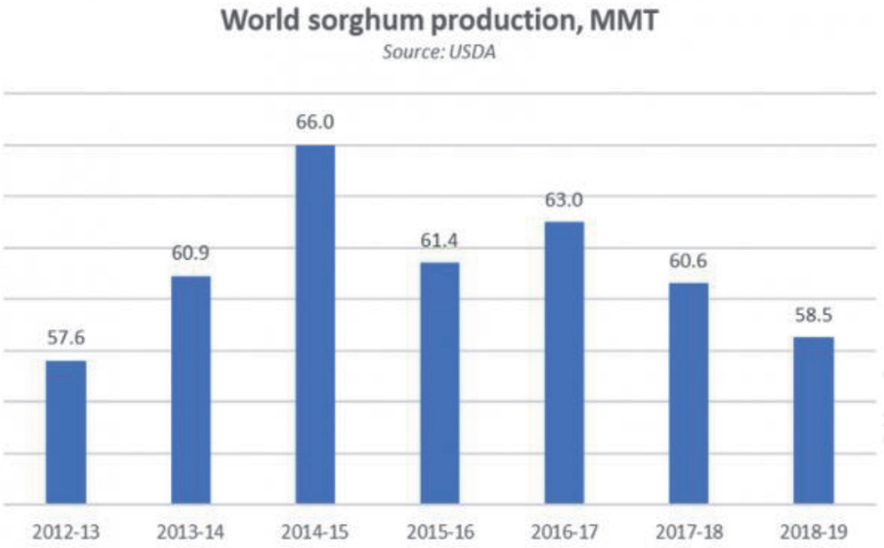

Figure 2.
Worldwide sorghum production statistics from 2012 to 2019.

grain and sweet sorghum types as silage, fodder and bioethanol source in Pakistan. Exploring diversity of different sorghum types is vital to develop better sorghums.

3.4 Multiple uses of sorghum

Sorghum is ranked as 5th most widely grown cereal crop of the world. It has C4 photosynthetic pathway which is useful for global food production. It is a staple food with significant nutritional qualities for about 500 million people around the globe. With growing world population, the demand for reliable food and feed sources has also escalated. In the context of possible limited water supplies and high temperatures, sorghum's role to feed the world will increase in importance owing to its higher adaptability. Sorghum has amazing range of multiple uses:

3.4.1 Sorghum grain as food

Sorghum grain is used for food and biofuels. Grain has an edible hull and retains the majority of its nutrients. It contains 86% total digestible nutrients, up to 15.6% protein and 3772 kcal/kg energy. Sorghum grain has higher levels of magnesium that help in higher absorption of calcium and thereby contribute to bone health. It is abundant in phenolic compounds and antioxidants that safeguard against age-onset degenerative diseases [11]. Sorghum grain is reported to reduce the risk of many important diseases like cancer, cardiac infarction and some neurological disorders [12]. The grain is consumed as whole or ground to nutritious flour for baking. Most importantly, sorghum food products are gluten free, have wide range of color, neutral flavor and low allergenicity.

3.4.2 Sorghum grain as feed

Sorghum grain is second to maize in consumption as feed in the USA. It is a significant component of animal feed in South America, Australia and China, and poultry feed in India. The low-tannin high digestible sorghum (HDS) varieties are quickly replacing corn in poultry feed.

3.4.3 Sorghum as feedstock for biofuels

Sorghum starch, sugar, and biomass are used as feedstocks for biofuel. High biomass sorghums developed by selective breeding are used as biofuel feedstock. Moreover, sweet sorghum has emerged as a promising contender of bioenergy. Its stalk, seeds and syrup are used for biomass and ethanol production [13].

3.4.4 Sorghum as fodder

For livestock feed, sorghum may be utilized in a number of ways like as green chop, grazed and made into hay or silage [14]. By adopting a combination of these systems, sorghum sufficiently meets the year round needs of stock farmers.

3.4.5 Sorghum as a climate smart crop

Worldwide climate change forecasts suggest incidences of low rainfall with variable distribution, flooding, extended droughts and elevated temperatures. Sorghum thrives exceptionally well under low water availability, heat, salinity and low inputs and thus is named as "the camel of crops". It is anticipated to perform high for food security of large number of masses with scant resources in arid zones of the world. According to climate predictions for 2050, sorghum will remain world's top crop to survive coming harsh weathers across the globe [15]. The crop is set to enjoy a relatively healthy future.

3.4.6 Sorghum as a diverse crop

Sorghum exhibits promising diversity in yield and quality traits as well as resilience to different environmental conditions in dry arid, semi-arid, temperate and tropical areas. In order to harness immense benefits of sorghum and for long term maintenance, there is a dire need to preserve this variability in the form of germplasm collections. Once this biodiversity in these collections is lost, it cannot be brought back. A crop with narrow genetic base cannot cope with drastic climatic stresses. Estimation of diversity among and within the species of any crop helps identify the germplasm with maximum variability that can be exploited in developing varieties of wide genetic background to withstand biotic and abiotic stresses.

4. Case studies on morphometric and molecular characterization of sorghum

We report morphological characterization of ten sweet sorghum genotypes from National Agriculture Research Center, Islamabad, Pakistan [16]. Data for Plant height (PH), Days of 50% flowering (DF), Brix value(BV), Number of leaves per plant (NL), Leaf length (LL), Leaf width (LW), Leaf area index (LAI), Stem girth (SG), Flag leaf width (FLW), Flag leaf length (FLL), Flag leaf area index (FLAI), Fresh weight (FW) and Dry weight (DW) were recorded. The means and standard error of means for each trait were calculated [17] and presented in **Table 1**.

Correlation for observed 14 morphological traits is presented in **Table 2**. Number of leaves per plant (NL) indicated positive strong correlation with BV, LL, LAI, DW and PH. Whereas, NL showed moderate to low correlation with DTF, FLL, FLW, FLAI and DTM. The morphological trait DW showed positive higher correlation with NL, BV, SG, LL, LW, LAI and FW. Significant ($p = 0.01$) strong positive correlation was obtained for Plant height (PH) with BV.

Variables	Range		Mean	Std. deviation
	Minimum	Maximum		
NL	8.55	11.89	10.00	1.17
DTF	58.33	77.56	71.45	6.02
BV	6.81	9.87	8.22	0.97
SG	1.60	5.67	3.71	1.12
LL	34.71	76.90	53.70	12.86
LW	3.33	7.23	4.97	1.15
LAI	130.71	518.36	277.51	126.90
FW	56.70	100.80	82.25	13.03
DW	32.55	52.85	41.90	6.19
FLL	21.84	33.75	27.66	4.03
FLW	2.36	3.36	2.71	0.35
FLAI	57.36	113.35	75.78	20.20
PH	158.71	230.02	191.40	22.50
DTM	106.33	124.78	117.45	5.84

Table 1.
Cumulative response of sorghum genotypes for fourteen phenotypic traits.

In PCA, three PCs were selected out of nine because their Eigen value is more than one. Selected PCs cover the character variability (**Tables 3** and **4**).

Bi-Plot (**Figure 3**) showed allocation of genotypes on the basis of performance. The characters which were far away from origin showed more variability.

Our group previously reported RAPD- based genetic diversity evaluation of sorghum germplasm of Pakistan [10]. We also performed molecular diversity analysis of twelve sweet sorghum genotypes with 17 RAPD primers viz.; GLA03, GLB10, GLC01, GLC 02, GLI06, GLL02, GLL05, GLL07, GLL09, GLL10, GLL12, GLL14, GLL15, GLL16, GLL17, GLL18 and GLL19 [18]. These markers yielded 77 fragments of different sizes and 6.41 bands per primer were produced on average (**Figure 4**). RAPD primers identified 83.33% polymorphism among sweet sorghum genotypes (**Figure 5**).

Genetic similarity was assessed among sorghum genotypes via Nei's similarity indices with popgen 1.32. The genotypes MN 2363 and Dobbs showed minimum similarity (76.92%). Whereas, Masaka and Dobbs exhibited the lowest similarity (44.87%) and hence the maximum divergence (**Table 5**).

The genetic relationship among sorghum genotypes was assessed by Popgen 1.32. All twelve sorghum genotypes were clustered in two groups with the help of Cluster analysis. Two genotypes (Malnal and Maska) were present in one group. While the rest of the genotypes constituted the second group. A close similarity was present among Masaka and Malnal that were clustered in Group A. Group B comprised of three genotypes, among these Dobbs and MN 2363 were clustered together and MN 2109 resided separately in this group. The genotypes Chedomba, Kamandri, Dura Huria and Juar were placed in Group C and IS12833, Juar 49 and Early Folger constituted Group D. The highest similarity was observed among Malnal and Masaka. On the other hand, the highest divergence was recorded between Malnal and Early Folger exhibited (**Figure 6**).

In a separate study, we explored genetic divergence of 24 sorghum genotypes with RAPD markers (OPL-7, OPL-8, OPA-13 and OPA-3) [19]. These markers produced

Variables	NL	DTF	BV	SG	LL	LW	LAI	FW	DW	FLL	FLW	FLAI	PH	DTM
NL	**1**	0.347	0.722	0.686	0.770	0.529	0.717	0.643	0.787	0.321	0.403	0.403	0.722	0.466
DTF	0.347	**1**	0.753	0.755	0.640	0.699	0.642	0.123	0.466	0.550	0.340	0.479	0.753	0.958
BV	0.722	0.753	**1**	0.883	0.903	0.937	0.957	0.404	0.739	0.572	0.543	0.614	1.000	0.764
SG	0.686	0.755	0.883	**1**	0.872	0.853	0.898	0.594	0.823	0.487	0.519	0.551	0.883	0.741
LL	0.770	0.640	0.903	0.872	**1**	0.810	0.947	0.488	0.799	0.504	0.367	0.482	0.903	0.662
LW	0.529	0.699	0.937	0.853	0.810	**1**	0.946	0.245	0.569	0.579	0.638	0.670	0.937	0.678
LAI	0.717	0.642	0.957	0.898	0.947	0.946	**1**	0.406	0.719	0.542	0.541	0.599	0.957	0.654
FW	0.643	0.123	0.404	0.594	0.488	0.245	0.406	**1**	0.888	0.004	0.109	0.064	0.404	0.137
DW	0.787	0.466	0.739	0.823	0.799	0.569	0.719	0.888	**1**	0.279	0.299	0.319	0.739	0.470
FLL	0.321	0.550	0.572	0.487	0.504	0.579	0.542	0.004	0.279	**1**	0.683	0.914	0.572	0.631
FLW	0.403	0.340	0.543	0.519	0.367	0.638	0.541	0.109	0.299	0.683	**1**	0.920	0.543	0.396
FLAI	0.403	0.479	0.614	0.551	0.482	0.670	0.599	0.064	0.319	0.914	0.920	**1**	0.614	0.552
PH	0.722	0.753	1.000	0.883	0.903	0.937	0.957	0.404	0.739	0.572	0.543	0.614	**1**	0.764
DTM	0.466	0.958	0.764	0.741	0.662	0.678	0.654	0.137	0.470	0.631	0.396	0.552	0.764	**1**

,: Significant at 5% and 1% probability level.*

Table 2.
Correlation matrix for different traits in sweet sorghum genotypes.

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigen value	9.292	2.103	1.154	0.604	0.381	0.281	0.114	0.054	0.017
Variability (%)	66.373	15.024	8.241	4.316	2.722	2.006	0.812	0.385	0.122
Cumulative %	66.373	81.397	89.638	93.953	96.675	98.681	99.493	99.878	100.000

Table 3.
Principle component analysis.

Variables	PC1	PC2	PC3
NL	0.750	0.394	−0.248
DTF	0.768	−0.206	0.528
BV	0.970	0.032	0.104
SG	0.939	0.170	0.066
LL	0.913	0.218	0.092
LW	0.911	−0.148	0.070
LAI	0.948	0.068	0.024
FW	0.469	0.747	−0.311
DW	0.781	0.556	−0.139
FLL	0.668	−0.577	−0.189
FLW	0.632	−0.476	−0.527
FLAI	0.712	−0.568	−0.397
PH	0.970	0.032	0.104
DTM	0.796	−0.229	0.433
Eigen value	9.292	2.103	1.154
Variability (%)	66.373	15.024	8.241
Cumulative %	66.373	81.397	89.638

Table 4.
PCA factor loadings for sorghum genotypes.

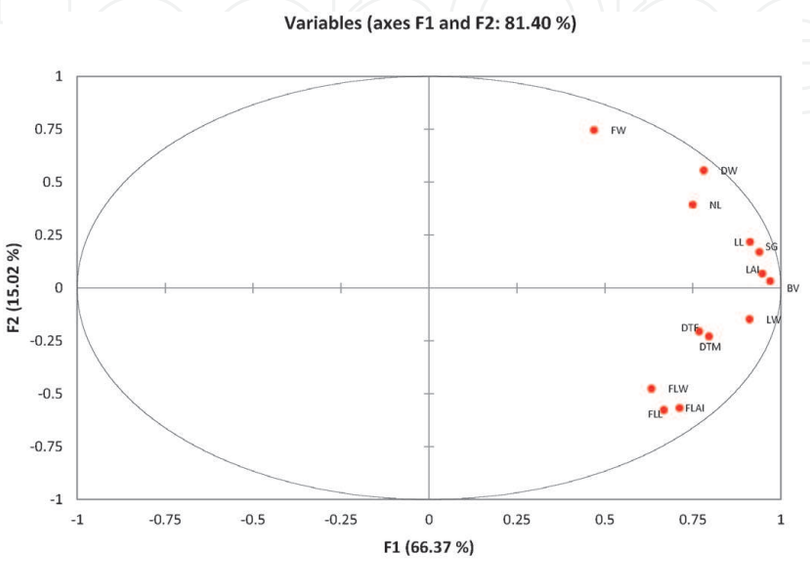

Figure 3.
PCA Biplot.

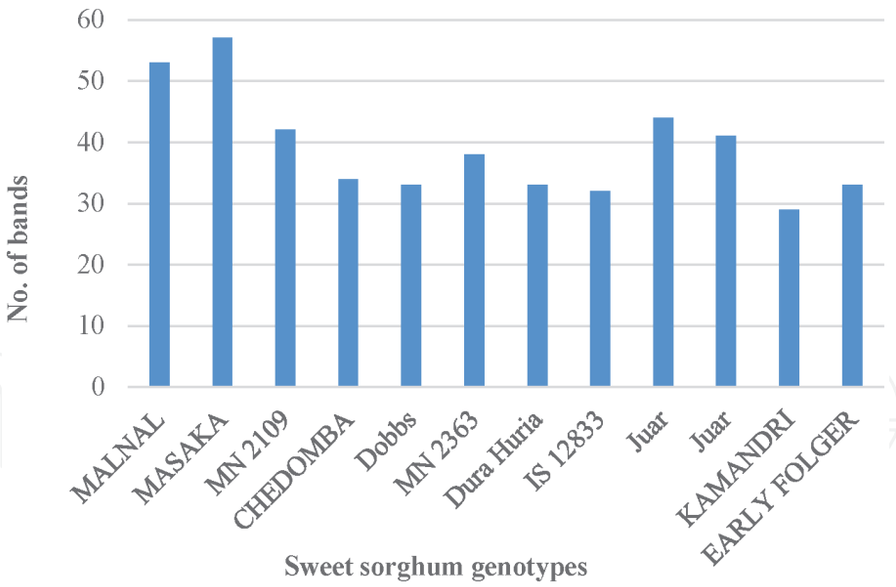

Figure 4.
Number of bands recorded per sorghum genotype.

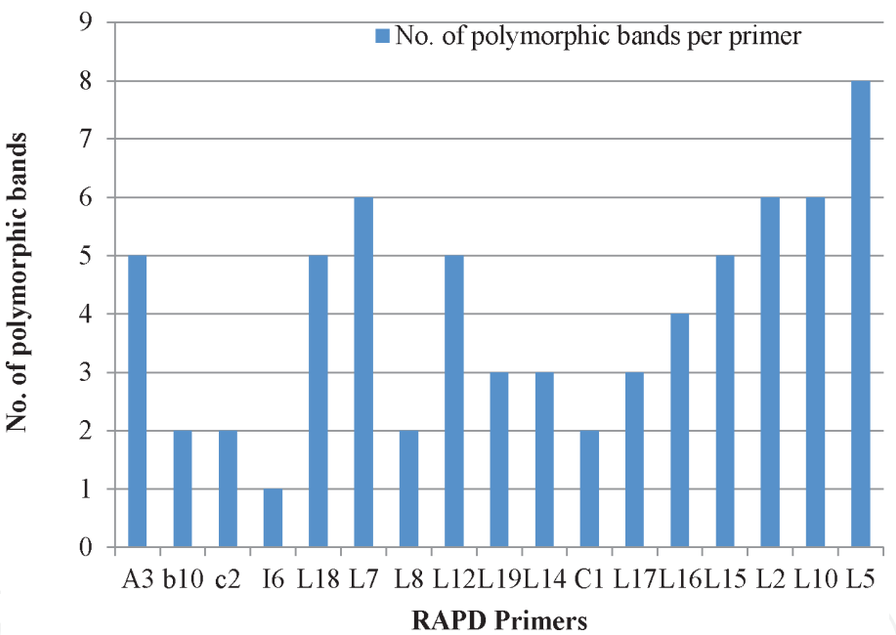

Figure 5.
Number of polymorphic bands per primer in sorghum genotypes.

74 bands of varying sizes/intensities. On average, each primer produced 18.5 bands. RAPD markers revealed 77.13% polymorphism among sorghum genotypes.

While, previous fingerprinting studies showed 58% [20] and 52% polymorphism [21] among various sorghum genotypes. The primer OPL7 produced the maximum number of fragments [22] whereas, the minimum number of fragments were generated by OPA3 (14) (**Figure 7**). The low level of similarity indicated high divergence among the sorghum germplasm under study.

More recently, we exploited sixteen SSR markers for DNA fingerprinting of fifty sorghum genotypes [8]. The molecular analysis indicated significant polymorphism among these genotypes.

The bands varied in size and intensity. The number of bands per primer per genotypes also varied. Some bands showed a high level of polymorphism indicating great variation among the sorghum germplasm (**Figure 8**). Marker diversity among

pop ID	1	2	3	4	5	6	7	8	9	10	11	12
1	****	0.6154	0.6410	0.5769	0.5769	0.5769	0.5000	0.5897	0.6795	0.5256	0.5256	0.6154
2	0.4855	****	0.5385	0.5513	**0.4487**	0.4744	0.5256	0.4872	0.5256	0.5769	0.4231	0.5641
3	0.4447	0.6190	****	0.7308	0.7564	0.7308	0.7308	0.6923	0.6538	0.7051	0.7051	0.5897
4	0.5500	0.5955	0.3137	****	0.6923	0.6410	0.6923	0.6282	0.6154	0.6667	0.7436	0.6795
5	0.5500	0.8014	0.2792	0.3677	****	0.**7692**	0.6154	0.6795	0.6154	0.5641	0.7436	0.6538
6	0.5500	0.7458	0.3137	0.4447	0.2624	****	0.6923	0.7564	0.6154	0.7179	0.6667	0.5513
7	0.6931	0.6431	0.3137	0.3677	0.4855	0.3677	****	0.7051	0.6154	0.6923	0.7436	0.5769
8	0.5281	0.7191	0.3677	0.4649	0.3864	0.2792	0.3494	****	0.7308	0.6538	0.7051	0.5641
9	0.3864	0.6431	0.4249	0.4855	0.4855	0.4855	0.4855	0.3137	****	0.5897	0.6154	0.6795
10	0.6431	0.5500	0.3494	0.4055	0.5725	0.3314	0.3677	0.4249	0.5281	****	0.6154	0.5256
11	0.6431	0.8602	0.3494	0.2963	0.2963	0.4055	0.2963	0.3494	0.4855	0.4855	****	0.5769
12	0.4855	0.5725	0.5281	0.3864	0.4249	0.5955	0.5500	0.5725	0.3864	0.6431	0.5500	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal). 1: Malnal, 2: Masaka, 3: MN 2109, 4: Chedomba, 5: Dobbs, 6: MN 2363, 7: Dura Huria, 8: IS 12833, 9: Juar 49, 10:Juar 48, 11: Kamandri, 12: Early Folger

***** are symbols just to separate above diagonal and below diagonal values*

Table 5.
Similarity matrix of 12 sweet sorghum genotypes.

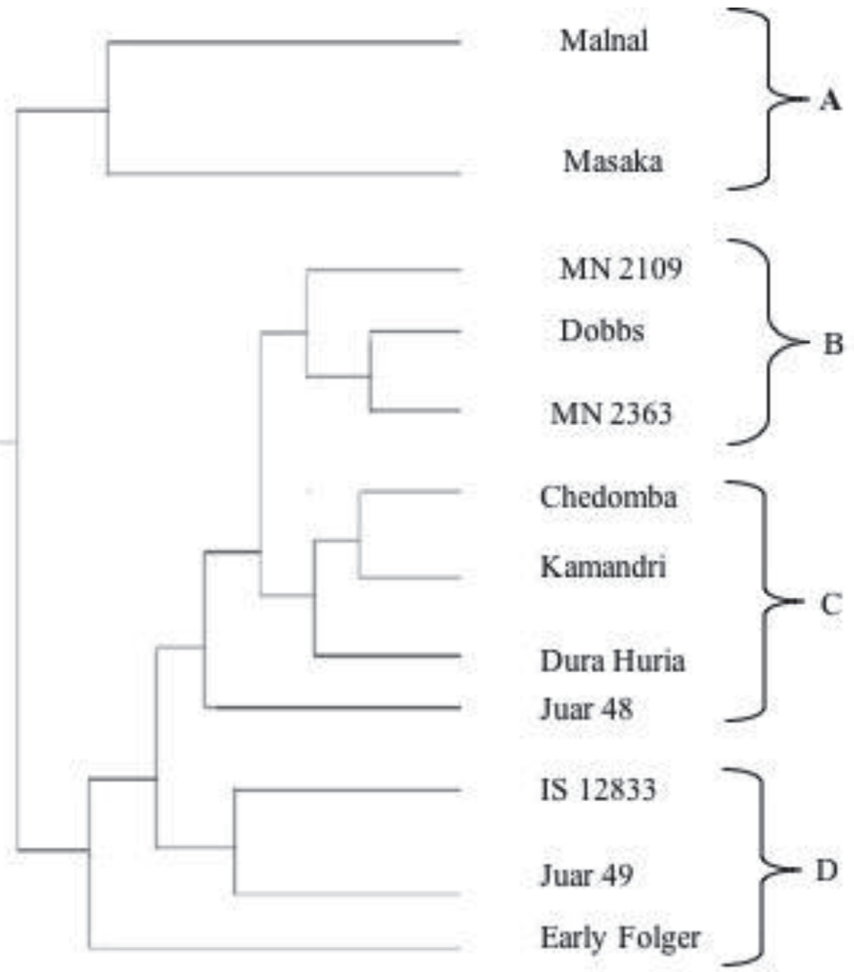

Figure 6.
Dendrogram of 12 sweet sorghum genotypes based on RAPD analysis.

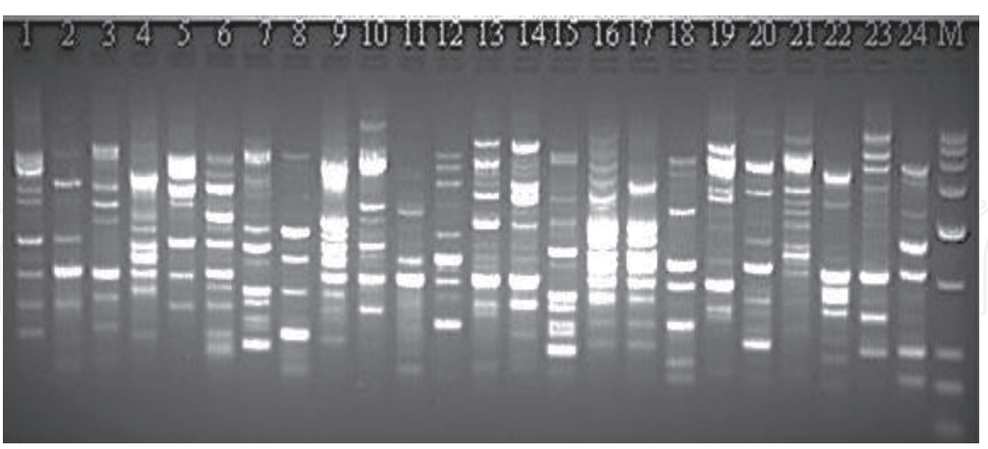

Figure 7.
PCR amplification of 24 sorghum genotypes with RAPD primer L-7. Lanes L: Ladder, 1–24: Sorghum genotypes.

fifty sorghum genotypes was studied using Powermarker software. The number of alleles per locus ranged from 2 to 3 with mean value of 2.875 alleles per locus. Genetic relationship among sorghum genotypes was evaluated by using popgen 1.32. All genotypes were grouped in two major clusters which were further divided into sub-groups. One small group consisted of eight genotypes (15, 39, 16, 35, 20, 22, 24, and 18) and the other large group contained remaining 42 sorghum genotypes. Maximum genetic distance was observed between 1st and 18th genotype.

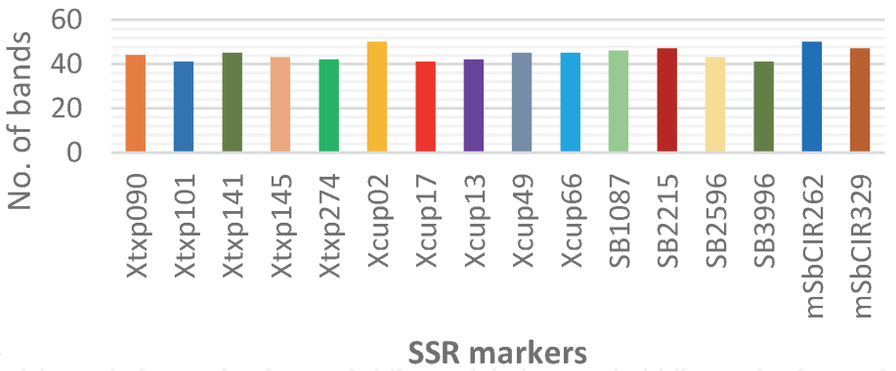

Figure 8.
No. of bands amplified by SSR primers in fifty sorghum genotypes.

This study revealed positive correlation among the allele number, gene diversity and PIC value. The ease of using these PCR-based markers for diversity evaluation, for allocating genotypes to heterotic groups, and for DNA fingerprinting proved advantageous for selecting biomass- related traits and for sorghum breeding programs.

5. Worldwide sorghum biodiversity exploration efforts

At present, extensive record is available on genetic diversity evaluation of sorghum using molecular markers. A review of global research on sorghum genetic diversity evaluation using morphological and molecular markers is presented in **Tables 6** and 7, respectively. Most of the studies analyzed vegetative and

Sr. #	Sorghum germplasm	Morphological traits	References
1	94 sorghum accessions	Area, Breadth, Circularity, Major axis length, Perimeter length and Rectangularity	Dahlberg et al. [22]
2	45 sorghum accessions including 34 landraces, 6 elite breeding lines and 5 improved cultivars	Ten qualitative (Plant color, Stalk juiciness, Leaf midrib color, Inflorescence exsertion, Panicle compactness and shape, Awns, Glume color, Grain covering, Grain color and Endosperm texture) and 16 quantitative (Days 50% flowering, Leaf number, Leaf length, Leaf width, Leaf area, Internode length, Leaf sheath length, Plant height, Panicle length, Panicle width, Number of primary branches Panicle head weight, Grain yield panicle, 1000-seed weight, Threshing percent and Grain size) traits	Geleta et al. [23]
3	40 sorghum landraces from Tanzania and 2 from Zambia	Five panicles average weight (g), Grain number/panicle, Height (cm), Hundred grain weight (g), Inflorescence length (cm), Inflorescence width (cm), Leaf length (cm), Number of leaves, Leaf width (cm), Leaf senescence, Main stem diameter (cm), Tillers diameter (cm), Number of tillers, Grain yield	Bucheyeki et al. [24]
4	320 sorghum accessions from more than 3500 germplasm collection	Days to heading (DTH), Days to flowering (DTF), Days to maturity (DTM), Culm diameter (CD), Grain weight per panicle (GWP), 100 grain weight (100GW), Culm length (CL), Number of tillers (NoT), Number of panicles (NoP), Panicle length (PL), Leaf	Shehzad et al. [25]

Sr. #	Sorghum germplasm	Morphological traits	References
		length (LL) and Leaf width (LW), Panicle shape (PS), Panicle type (PT), Coleoptile's color (CC), Quantity of lipid white powder on stem and leaves (LWP), Color of midrib (MC), Neck length of panicle (PNL), Awn presence (AP), Glume color (GC), Growth in early stage (GES), Endosperm type (ET), Aphid resistance (AR), Number of regenerated tillers (NRT), Regrowth (RG) and Resistance to insecticides (RI)	
5	124 sorghum from Burkina Faso	28 agro morphological traits (Vigor at emergence 5(Ve), Coleoptile color (Cc), Leaf anthocyanin pigmentation (Lap), Panicle compactness (Pc), Pedicellate spikelet length (Psl) and Persistence (Psp), Glume length (Gl) and opening (Go), Awn (Aw), Kernel shape (Ks), Kernel rotation (Kr), Glume color (Gc), Kernel color (Kc), Anthocyanin spots on kernels (Ask), Glume adherence (Ga), Seed coat or testa (Sc) and Kernel vitreousness (Kv), Plant height (Ph), Leaf number (Ln), Length (Ll) and Width (Lw) of the third leaf under the panicle, Number of effective tillers (Net), Panicle length (Pl), Panicle weight (Pw), Harvested seed weight (Hsw) and 1000-seed weight (1000-Sw)	Barro-Kondombo et al. [26]
6	156 sorghum accessions	Lowering time, Plant height, and panicle type/ inflorescence, Panicle type and glumes coverage, grain color	Sharma et al. [27]
7	25 sorghum genotypes	Seedling vigor, Number of leaves, Leaf area, Stay-green, Peduncle exertion, Panicle length and width, Plant height, Days to flowering and maturity, Grain yield, Biomass and Harvest index under Drought stress	Abraha et al. [28]
8	9 sorghum genotypes from Sudan	Days to flowering (DF), Days to maturity (DM), Plant height (PH) (cm), Panicle length (PL) (cm), Panicle exertion (cm), Head weight (HW) (g), Yield per panicle (YPP) (g), Thousand seed weight (TSW) (g), Biomass (BM) yield (ton/ha) and GY (kg/ha)	Sabiel et al. [29]
9	Recombinant inbred line of *Sorghum bicolor* made by crossing E-Tian, a sweet sorghum accession with Ji2731	Biomass and Biofuel traits	Mocoeur et al. [30]
10	Diallel set of 10 parents and their 90 crosses including reciprocals of sorghum	Days to flowering, Days to maturity, Plant height, Grain yield per plant, Panicle length, Number of tillers per plant, Panicle weight, Panicle exsertion, Thousand seed weight, Grain-filling period	Mohammed et al. [31]
11	40 accessions of sorghum from Tamil Nadu	Days to 50% flowering, Days to maturity, Plant height, Panicle length, Panicle width, Leaf length, Leaf breadth, Number of leaves per plant, Stem girth, Number of primary branches per panicle, Hundred-seed weight, Yield per plant, Panicle weight and Dry matter production	Sinha and Kumaravadivel [32]
12	267 sorghum genotypes from Ethiopia	Leaf rolling, Head compactness, Glume cover, Glume color, Leaf orientation, Midrib color,	Amelework et al. [33]

Sr. #	Sorghum germplasm	Morphological traits	References
		Panicle exsertion, Head shape, Grain color, Stay-green, Leaf color, Head orientation	
13	315 sorghum accessions	Plant height and Seed number	Jing Zhao et al. [34]
14	Two overlapping sets of RILs of sorghum	Grain yield, Flowering time, and Stay-Green traits	Sivakumar Sukumaran et al. [35]
15	100 sweet sorghum accessions	Bioenergy traits, Protein content and Ethanol yield	Da silva et al. [36]
16	Populations of sweet sorghum F4 families made by crosses between 11 tall sweet sorghum cultivars (used as males), and 3 short grain sorghums as females	Relationship between Sugar content and Plant height	Shukla et al. [37]
17	30 sorghum accessions	Days to 50% anthesis, Plant height, Flag leaf area, Brix percentage, Panicle length, Grain weight and Grain yield	Mumtaz et al. [38]
18	54 introgressed sorghum breeding lines	Drought stress imposed at pre-flowering and post-anthesis developmental stages, Panicle area, Width, Percent green leaf, Total above ground, Dry biomass and Dry panicle weight	Emendack et al. [39]
19	75 sorghum lines including 74 indigenous cultivars and 1 exotic cultivar	Glume color, Neck of panicle, Length of flower with pedicel, time of panicle emergence, color of dry anther, panicle length of branches, panicle shape and caryopsis color	Prajapati et al. [40]
20	196 sorghum accessions	Seedling vigor, Days to flowering, Days to maturity, Days to grain filling period, Plant height, Panicle exertion, Number of green leaf at physiological maturity, Panicle length, Panicle weight, Thousand seed weight, Panicle yield, Grain yield, Above ground dry matter, Harvest index	Derese et al. [41]
21	453 diverse photo-period sensitive sorghum lines	Moisture, Plant height	Fernandes et al. [42]
22	194 *Sorghum bicolor* and *S. bicolor sudanese* genotypes	Root system architecture	Parra-Londono et al. [4]
23	93 sweet grain sorghum accessions	Sweet grains in pasty stage	Sawadogo et al. [43]
24	329 accessions of sorghum	Seed morphology	Sakamoto et al. [44]
25	200 Sweet sorghum accessions from Serbia	Plant height, Plant biomass, Stem leaves, Panicle length and Yield of crude biomass	Bojović et al. [45]
26	98 accessions of South African sorghum	Genetic variability, Plant height, Panicle length, Width and exsertion, Rachis number, Panicle weight, Seed weight, Grain yield Per panicle	Mofokeng et al. [46]
27	12 *Sorghum bicolor* genotypes (5 sweet, 4 grain and 3 forage sorghums)	Green leaf area (cm2), Plant height (cm), Leaf number, Fresh biomass yield (t/ha), Cane yield (t/ha), Bagasse yield (t/ha), Brix degree and Juice yield (kl/ha)	Kanbar et al. [47]

Sr. #	Sorghum germplasm	Morphological traits	References
28	Seven groups of 44 parental lines of sorghum	Mid-season drought tolerance, Mid-season drought susceptibility, Stay green lines, Terminal drought tolerance, Saline-tolerance, Saline-susceptibility, High Fe–Zn lines	Pandian et al. [48]
29	Recombinant inbred line derived from a cross between an elite U.S. common parent RTx430 and 10 diverse founders	Inflorescence morphology	Olatoye et al. [49]
30	3 recombinant inbred line mapping populations of sweet sorghum	Stem lodging resistance, Mechanical stability analysis	Gomez et al. [50]
31	210 Ethiopian genotypes of grain sorghum	Days to flowering, Days to maturity, Plant height, Grain yield per plant, Panicle length, Number of tillers per plant, Panicle weight, Panicle exsertion, Thousand seed weight, Grain-filling period	Birhan et al. [51]
32	55 sorghum accessions comprising 11 Bicolor accessions, 15 Caudatum, 10 Durra, 9 Guinea and 10 Kafir	Inflorescence architecture	Li et al. [52]
33	21 diverse sorghum accessions	Transpiration efficiency, the ratio of plant carbon produced to water transpired and carbon isotope discrimination of leaf dry matter	Henderson et al. [53]

Table 6.
Studies on assessment of genetic variations using morphological markers in sorghum.

Sr. No	Sorghum germplasm	Molecular markers	Reference
1	25 accessions of sorghum	Microsatellites	Djè Y et al. [54]
2	415 sorghum accessions consisting of 391 landraces, 8 standard varieties and 16 introduced elite breeding lines	Allozymes and RAPD markers	Ayana, [55]
3	94 sorghum accessions	RAPDs	Dahlberg et al. [22]
4	100 accessions from a core collection of 293 sorghum	SSR markers	Folkertsma et al. [56]
5	45 sorghum accessions	SSRs and AFLP	Geleta et al. [23]
6	1 sorghum accession	SSRs	Wu et al. [57]
7	46 sorghum lines	AFLP and SSRs	Perumal et al. [58]
8	42 grain sorghum landraces	SSRs	Bucheyeki et al. [24]
9	40 sorghum genotypes	SSRs	Assar et al. [59]
10	320 sorghum accessions	SSR markers	Shehzad et al. [3]
11	124 sorghum landraces from Burkina Faso	Microsatellite markers	Barro-Kondombo et al. [26]
12	156 sorghum germplasm accessions	SSRs	Sharma et al. [27]

Sr. No	Sorghum germplasm	Molecular markers	Reference
13	Three populations of backcross-derived lines of sorghum	EST SSRs	Mohamed et al. [60]
14	160 plants of sorghum	SSR markers	Adugna et al. [61]
15	Recombinant Inbred Line of *sorghum bicolor* made by crossing E-Tian x Ji2731	PAV markers and SSRs	Mocoeur et al. [30]
16	Sorghum population derived from a cross between two sorghum landraces, Red Kafir and Takakibi	SSRs	Shehzad et al. [62]
17	Recombinant sorghum line (hugurtay x N-13 (resistance donor)	SSRs	Yohannes et al. [63]
18	Set of 1108 sorghum diverse collections	Microsatellite markers	Salih et al. [64]
19	22 sorghum accessions (landraces)	Microsatellites	Motlhaodi et al. [65]
20	267 genotypes from Ethiopia	SSRs	Amelework et al. [33]
21	Two overlapping sets of RILs of sorghum	SNPs	Sukumaran et al. [35]
22	A random collection of 44 genotypes of sorghum	SPAR - (ISSR, RAPD, DAMD)	Satish et al. [66]
23	315 sorghum accessions	SNP, SQNM	Zhao et al. [34]
24	100 sweet sorghum accessions	SNPs	Da silva et al. [36]
25	80 sorghum accessions	Microsatellites	Sifau et al. [67]
26	300 diverse accessions of sorghum	SNPs	Chopra et al. [68]
27	93 sweet grain sorghum accessions from Burkina Faso	Microsatellites	Sawadogo et al. [43]
28	194 *Sorghum bicolor* and *S. bicolor sudanese* genotypes	SNPs	Parra-Londono et al. [69]
29	41 sorghum accessions	22 SSRs	Danquah et al. [70]
	329 accessions of sorghum germplasm collection	SNPs analysis	Sakamoto et al. [44]
30	Seven groups of 44 parental lines of sorghum	ISSRs, RAPDs, DAMD	Pandian et al. [48]
31	46 accessions of *Sorghum bicolor*	RAPD	Ruiz-Chutan et al. [71]
32	214 sorghum accessions	SNPs	Afolayan et al. [72]
33	12 *Sorghum bicolor* genotypes (5 sweet, 4 grain and 3 forage sorghums)	RAPDs, ISSRs	Kanbar et al. [47]
34	150 accessions of *Broomcorn Sorghum*	SSRs	Zhu et al. [73]
35	20 sorghum accessions	SSRs	Joshi Akansha et al. [74]
36	10 *sorghum bicolor* genotypes collected from USA (Texas)	SSRs	Jessup et al. [75]
37	3 RIL mapping populations of sweet sorghum genotypes	SNPs	Gomez et al. [50]
38	Recombinant Inbred Line derived from a cross between an elite U.S. common parent RTx430 and 10 diverse founders	SNPs	Olatoye et al. [49]
39	21 diverse Sorghum accessions	SNPs	Henderson et al. [53]

Table 7.
Studies on assessment of genetic variations using molecular markers in sorghum.

morphological traits for characterizing sorghum followed by maturity characters. Plant height is the most researched trait in these studies. Majority of efforts related to DNA fingerprinting of sorghum employed SSRs, followed by SNP and RAPD markers.

6. Conclusions

Most of the modern cultivated crops exhibit narrow genetic base due to domestication, selection of few desired traits and repeated use of genetically similar varieties as breeding parents. Climate change poses a serious threat to agricultural communities with possible forecast of high temperature, water scarcity and altered pattern of showers round the globe. Climate variations and shift will be a key driver of crop production especially in arid and semi-arid rain fed areas of the world. Such effects will vary among crops depending upon their physiology and climate resilience traits of particular crop. Sorghum is a C4 grass cultivated in diverse regions of the world for variety of uses. It stands tall among other cereal crops owing to inherent biotic/abiotic stress tolerance and wider adaptability. It is among few climate smart crops with potential to withstand future harsh environmental conditions. Hence, development of high yielding sorghum varieties will contribute towards ensuring global food security. Breeders are exploiting high throughout phenotyping platforms as well as omics- assisted variability evaluation of sorghum germplasm to identify/select highly diverse types that will serve as a base line for breeding of broad genetic base sorghum varieties. So, dissecting phenotypic and molecular diversity of sorghum germplasm is strongly justified.

Conflict of interest

The authors declare no conflict of interest.

Author details

Bushra Sadia[1*], Faisal Saeed Awan[1], Fozia Saleem[1], Javaria Altaf[2], Abdullah Bin Umar[1], Muhammad Nadeem[1], Samra Hameed[1], Farwa Ashraf[1] and Mariam Nasir[1]

1 Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad (UAF), Pakistan

2 Department of Zoology, Government College University Faisalabad, Pakistan

*Address all correspondence to: bushra.sadia@uaf.edu.pk

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H. DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnology & Biotechnological Equipment. 2018 Mar 4; 32(2):261–85.

[2] Jiang J, Gill BS. Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research. Genome. 2006 Sep;49(9): 1057–68.

[3] Mateu-Andres I, De Paco L. Allozymic differentiation of the *Antirrhinum majus* and A. siculum species groups. Annals of botany. 2005 Feb 1;95(3):465–73.

[4] Hamouda M. Molecular analysis of genetic diversity in population of *Silybum marianum* (L.) Gaertn in Egypt. Journal of Genetic Engineering and Biotechnology. 2019 Dec 1;17(1):12.

[5] Platten JD, Cobb JN, Zantua RE. Criteria for evaluating molecular markers: comprehensive quality metrics to improve marker-assisted selection. PloS one. 2019 Jan 15;14(1):e0210529.

[6] Jiang GL. Molecular markers and marker-assisted breeding in plants. Plant breeding from laboratories to fields, Sven Bode Andersen. 2013 May 22:45–83. IntechOpen, DOI: 10.5772/ 52583

[7] RAK Rakshit S, Swapna M. DNA Markers in Diversity Analysis. InSorghum Molecular Breeding 2015 (pp. 23–46). Springer, New Delhi.

[8] Ashraf F. Genetic diversity analysis of exotic sorghum (*Sorghum bicolor* L. Moench) germplasm using single nucleotide polymorphisms. 2018. MPhil Dissertation. University of Agriculture, Faisalabad.

[9] Andersen JR, Lübberstedt T. Functional markers in plants. Trends in plant science. 2003 Nov 1;8(11):554–60.

[10] Iqbal A, Sadia B, Khan AI, Awan FS, Kainth RA, Sadaqat HA. Biodiversity in the sorghum (Sorghum bicolor L. Moench) germplasm of Pakistan. Genetics and Molecular Research. 2010; 9(2):756–64.

[11] Arias S, Bhatia SK. Sorghum. InMedical Applications for Biomaterials in Bolivia 2015 (pp. 33–39). Springer, Cham.

[12] Mrid RB, Bouargalne Y, El Omari R, Nhiri M. New insights into the therapeutic effects of phenolic acids from sorghum seeds. Journal of Reports in Pharmaceutical Sciences. 2019 Jan 1;8 (1):91.

[13] Dar RA, Dar EA, Kaur A, Phutela UG. Sweet sorghum-a promising alternative feedstock for biofuel production. Renewable and Sustainable Energy Reviews. 2018 Feb 1; 82:4070–90.Chris Duran, Nikki Appleby, David Edwards and Jacqueline Batley, " Molecular Genetic Markers: Discovery, Applications, Data Storage and Visualisation", Current Bioinformatics (2009) 4: 16. https://doi.org/10.2174/157489309787158198

[14] Janhi K, Matshaya Z, Chiduza C, Muzangwa L. Clipping Forage Sorghum Twice and Nitrogen Topdressing Offer an Option for Dual-Purpose Use for Cover Cropping and Fodder in Mixed Crop/Livestock Farming Systems. Agronomy. 2020 Jan;10(1):17.

[15] Nanaiah GK, Rakshit S. Genomic Designing for Climate Smart Sorghum. InGenomic Designing of Climate-Smart Cereal Crops 2020 (pp. 171–219). Springer, Cham.

[16] Nadeem M, Biodiversity evaluation of sweet sorghum (*Sorghum bicolor* L. Moench) for biomass potential. 2015. MPhil Dissertation. University of Agriculture, Faisalabad.

[17] Steel RG. Pinciples and procedures of statistics a biometrical approach. 1997.

[18] Hameed S. Assessment of genetic diversity of sweet sorghum (*Sorghum bicolor* L. Moench), an efficient biofuel crop. 2015. MPhil Dissertation. University of Agriculture, Faisalabad.

[19] Irshad B. Chemical and genetic diversity analyses of high biomass USDA sorghum (*Sorghum bicolor* M.) collections. 2017. MPhil Dissertation. University of Agriculture, Faisalabad.

[20] Agrama HA, Tuinstra MR. Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. African journal of biotechnology. 2003;2(10):334–40.

[21] Nkongolo KK, Nsapato L. Genetic diversity in Sorghum bicolor (L.) Moench accessions from different ecogeographical regions in Malawi assessed with RAPDs. Genetic Resources and Crop Evolution. 2003 Mar 1;50(2): 149–56.

[22] Dahlberg JA, Zhang X, Hart GE, Mullet JE. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurements. Crop Science. 2002 Jan;42(1):291–6.

[23] Geleta N, Labuschagne MT, Viljoen CD. Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. Biodiversity & Conservation. 2006 Sep 1;15(10):3251–65.

[24] Bucheyeki TL, Gwanama C, Mgonja M, Chisi M, Folkertsma R, Mutegi R. Genetic variability characterisation of Tanzania sorghum landraces based on simple sequence repeats (SSRs) molecular and morphological markers. African Crop Science Journal. 2009;17(2).

[25] Shehzad T, Okuizumi H, Kawase M, Okuno K. Development of SSR-based sorghum (Sorghum bicolor (L.) Moench) diversity research set of germplasm and its evaluation by morphological traits. Genetic Resources and Crop Evolution. 2009 Sep 1;56(6):809–27.

[26] Barro-Kondombo C, Sagnard F, Chantereau J, Deu M, Vom Brocke K, Durand P, Gozé E, Zongo JD. Genetic structure among sorghum landraces as revealed by morphological variation and microsatellite markers in three agroclimatic regions of Burkina Faso. Theoretical and Applied Genetics. 2010 May 1;120(8):1511–23.

[27] Sharma R, Deshpande SP, Senthilvel S, Rao VP, Rajaram V, Hash CT, Thakur RP. SSR allelic diversity in relation to morphological traits and resistance to grain mould in sorghum. Crop and Pasture Science. 2010 Mar 30;61(3):230–40.

[28] Abraha T, Githiri SM, Kasili R, Araia W, Nyende AB. Genetic variation among Sorghum (Sorghum bicolor L. Moench) landraces from Eritrea under post-flowering drought stress conditions. American Journal of Plant Sciences. 2015;6(09):1410.

[29] Sabiel SA, Noureldin I, Baloch SK, Baloch SU, Bashir W. Genetic variability and estimates of heritability in sorghum (Sorghum bicolor L.) genotypes grown in a semiarid zone of Sudan. Archives of Agronomy and Soil Science. 2016 Jan 2; 62(1):139–45.

[30] Mocoeur A, Zhang YM, Liu ZQ, Shen X, Zhang LM, Rasmussen SK, Jing HC. Stability and genetic control of morphological, biomass and biofuel traits under temperate maritime and continental

conditions in sweet sorghum (Sorghum bicolour). Theoretical and applied genetics. 2015 Sep 1;128(9):1685–701.

[31] Mohammed R, Are AK, Bhavanasi R, Munghate RS, Kavi Kishor PB, Sharma HC. Quantitative genetic analysis of agronomic and morphological traits in sorghum, Sorghum bicolor. Frontiers in plant science. 2015 Nov 3;6:945.

[32] Sinha S, Kumaravadivel N. Understanding genetic diversity of sorghum using quantitative traits. Scientifica. 2016 Jan 1;2016.

[33] Amelework B, Shimelis H, Tongoona P, Laing M, Mengistu F. Genetic diversity of lowland sorghum landraces assessed by morphological and microsatellite markers. Australian Journal of Crop Science. 2016 Mar;10(3):291.

[34] Zhao J, Mantilla Perez MB, Hu J, Salas Fernandez MG. Genome-wide association study for nine plant architecture traits in sorghum. The Plant Genome. 2016 Jul;9(2):1–4.

[35] Sukumaran S, Li X, Li X, Zhu C, Bai G, Perumal R, Tuinstra MR, Prasad PV, Mitchell SE, Tesso TT, Yu J. QTL Mapping for Grain Yield, Flowering Time, and Stay-Green Traits in Sorghum with Genotyping-by-Sequencing Markers. crop science. 2016 Jul;56(4):1429–42.

[36] Da Silva MJ, Pastina MM, de Souza VF, Schaffert RE, Carneiro PC, Noda RW, Carneiro JE, Damasceno CM, Parrella RA. Phenotypic and molecular characterization of sweet sorghum accessions for bioenergy production. PloS one. 2017 Aug 17;12(8):e0183504.

[37] Shukla S, Felderhoff TJ, Saballos A, Vermerris W. The relationship between plant height and sugar accumulation in the stems of sweet sorghum (Sorghum bicolor (L.) Moench). Field Crops Research. 2017 Mar 1;203:181–91.

[38] Mumtaz A, Hussain D, Saeed M, Arshad M, Yousaf MI. Estimation of genetic diversity in sorghum genotypes of Pakistan. Journal of the National Science Foundation of Sri Lanka. 2018 Sep 30;46(3).

[39] Emendack Y, Burke J, Sanchez J, Laza HE, Hayes C. Agro-morphological characterization of diverse sorghum lines for pre-and post-flowering drought tolerance. Australian Journal of Crop Science. 2018 Jan;12(1):135.

[40] Prajapati DK, Pahuja SK, Verma NK, Chaudhary S. Morphological characterization of sorghum [Sorghum bicolor (L.) Moench] germplasm for DUS traits. International Journal of Current Microbiology and Applied Sciences. 2018;7(2):2058–71.

[41] Derese SA, Shimelis H, Mwadzingeni L, Laing M. Agro-morphological characterisation and selection of sorghum landraces. Acta Agriculturae Scandinavica, Section B—Soil & Plant Science. 2018 Oct 3;68(7):585–95.

[42] Fernandes SB, Dias KO, Ferreira DF, Brown PJ. Efficiency of multi-trait, indirect, and trait-assisted genomic selection for improvement of biomass sorghum. Theoretical and applied genetics. 2018 Mar 1;131(3):747–55.

[43] Sawadogon N, Batieno TB, Kiebre Z, Ouedraogo MH, Zida WP, Nanema KR, Nebie B, Bationo-Kando P, Traore RE, Sawadogo M, Zongo JD. Assessment of genetic diversity of Burkina Faso sweet grain sorghum using microsatellite markers. African Journal of Biotechnology. 2018 Mar 21;17(12):389–95.

[44] Sakamoto L, Kajiya-Kanegae H, Noshita K, Takanashi H, Kobayashi M, Kudo T, Yano K, Tokunaga T, Tsutsumi N, Iwata H. Comparison of shape quantification methods for genomic

prediction, and genome-wide association study of sorghum seed morphology. PloS one. 2019 Nov 21;14(11):e0224695.

[45] Bojović R, Popović VM, Ikanović J, Živanović L, Rakaščan N, Popović S, Ugrenović V, Simić D. Morphological characterization of sweet sorghum genotypes across environments. JAPS: Journal of Animal & Plant Sciences. 2019 Jun 1;29(3).

[46] Mofokeng MA, Shimelis H, Laing M, Shargie N. Genetic variability, heritability and genetic gain for quantitative traits in South African sorghum genotypes. Australian Journal of Crop Science. 2019 Jan;13(1):1.

[47] Kanbar A, Shakeri E, Alhajturki D, Horn T, Emam Y, Tabatabaei SA, Nick P. Morphological and molecular characterization of sweet, grain and forage sorghum (Sorghum bicolor L.) genotypes grown under temperate climatic conditions. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology. 2020 Jan 2; 154(1):49–58.

[48] Pandian S, Satish L, Shilpha J, Ramesh M. Genetic Diversity Analysis Reveals Strong Population Structure in Sorghum Germplasm Collection. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2020 Mar;90(1):179–90.

[49] Olatoye MO, Marla SR, Hu Z, Bouchet S, Perumal R, Morris GP. Dissecting adaptive traits with nested association mapping: Genetic architecture of inflorescence morphology in sorghum. G3: Genes, Genomes, Genetics. 2020 May 1;10(5):1785–96.

[50] Gomez FE, Mullet JE, Muliana AH, Niklas KJ, Rooney WL. The genetic architecture of biomechanical traits in sorghum. Crop Science. 2020 Jan;60(1): 82–99.

[51] Birhan T, Bantte K, Paterson A, Getenet M, Gabizew A. Evaluation and Genetic Analysis of a Segregating Sorghum Population under Moisture Stress Conditions. Journal of Crop Science and Biotechnology. 2020 Jan;23 (1):29–38.

[52] Li M, Shao MR, Zeng D, Ju T, Kellogg EA, Topp CN. Comprehensive 3D phenotyping reveals continuous morphological variation across genetically diverse sorghum inflorescences. New Phytologist. 2020 Jun;226(6):1873–85.

[53] Henderson AN. The morphological, physiological, and genetic underpinnings of intraspecific salinity tolerance in Sorghum bicolor.

[54] Djè Y, Heuertz M, Lefebvre C, Vekemans X. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. Theoretical and Applied Genetics. 2000 Apr 1;100(6): 918–25.

[55] Ayana A. Genetic diversity in sorghum (Sorghum bicolor (L.) Moench) germplasm from Ethiopia and Eritrea (Doctoral dissertation, Addis Ababa University).

[56] Folkertsma RT, Rattunde HF, Chandra S, Raju GS, Hash CT. The pattern of genetic diversity of Guinea-race Sorghum bicolor (L.) Moench landraces as revealed with SSR markers. Theoretical and Applied Genetics. 2005 Aug 1;111(3):399–409.

[57] Wu YQ, Huang Y, Porter DR, Tauer CG, Hollaway L. Identification of a major quantitative trait locus conditioning resistance to greenbug biotype E in sorghum PI 550610 using simple sequence repeat markers. Journal of economic entomology. 2014 Sep 26; 100(5):1672–8.

[58] Perumal R, Krishnaramanujam R, Menz MA, Katilé S, Dahlberg J, Magill CW, Rooney WL. Genetic

diversity among sorghum races and working groups based on AFLPs and SSRs. Crop Science. 2007 Jul;47(4): 1375–83.

[59] Assar AH, Uptmoor R, Abdelmula AA, Wagner C, Salih M, Ali AM, Ordon F, Friedt W. Assessment of sorghum genetic resources for genetic diversity and drought tolerance using molecular markers and agro-morphological traits. University of Khartoum Journal of Agricultural Sciences. 2020 Feb 25;17(1).

[60] Mohamed A, Ali R, Elhassan O, Suliman E, Mugoya C, Masiga CW, Elhusien A, Hash CT. First products of DNA marker-assisted selection in sorghum released for cultivation by farmers in sub-saharan Africa. Journal of Plant Science & Molecular Breeding. 2014;3(3):1–0.

[61] Adugna A. Analysis of in situ diversity and population structure in Ethiopian cultivated Sorghum bicolor (L.) landraces using phenotypic traits and SSR markers. SpringerPlus. 2014 Dec 1;3(1):212.

[62] Shehzad T, Okuno K. QTL mapping for yield and yield-contributing traits in sorghum (Sorghum bicolor (L.) Moench) with genome-based SSR markers. Euphytica. 2015 May 1;203(1): 17–31.

[63] Yohannes T, Abraha T, Kiambi D, Folkertsma R, Hash CT, Ngugi K, Mutitu E, Abraha N, Weldetsion M, Mugoya C, Masiga CW. Marker-assisted introgression improves Striga resistance in an eritrean farmer-preferred sorghum variety. Field Crops Research. 2015 Mar 1;173:22–9.

[64] Salih SA, Herslman L, Labuschange MT, Mohammed AH. Assessment of genetic diversity of sorghum [sorghum bicolor (l.) Moench] germplasm in East and Central Africa. World Journal of Biotechnology. 2016;1 (03):113–20.

[65] Motlhaodi T, Geleta M, Chite S, Fatih M, Ortiz R, Bryngelsson T. Genetic diversity in sorghum [Sorghum bicolor (L.) Moench] germplasm from Southern Africa as revealed by microsatellite markers and agro-morphological traits. Genetic resources and crop evolution. 2017 Mar 1;64(3): 599–610.

[66] Satish L, Shilpha J, Pandian S, Rency AS, Rathinapriya P, Ceasar SA, Largia MJ, Kumar AA, Ramesh M. Analysis of genetic variation in sorghum (Sorghum bicolor (L.) Moench) genotypes with various agronomical traits using SPAR methods. Gene. 2016 Jan 15;576(1):581–5.

[67] Sifau MO, Oduoye OT, Oluwasanya OA, Aladele SE. Assessment of Genetic Variability in Sorghum Accessions (Sorghum bicolor L. Moench) at the National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria. Journal of Applied Sciences and Environmental Management. 2017;21(6):1143–7.

[68] Chopra R, Burow G, Burke JJ, Gladman N, Xin Z. Genome-wide association analysis of seedling traits in diverse Sorghum germplasm under thermal stress. BMC Plant Biology. 2017 Dec 1;17(1):12.

[69] Parra-Londono S, Kavka M, Samans B, Snowdon R, Wieckhorst S, Uptmoor R. Sorghum root-system classification in contrasting P environments reveals three main rooting types and root-architecture-related marker–trait associations. Annals of botany. 2018 Jan 23;121(2): 267–80.

[70] Danquah A, Galyuon IK, Otwe EP, Asante DK. Genetic diversity in some Ghanaian and Malian sorghum [Sorghum bicolor (L) Moench] accessions using SSR markers. African Journal of Biotechnology. 2019 Jul 31;18 (27):591–602.

[71] Ruiz-Chután JA, Salava J, Janovská D, Žiarovská J, Kalousová M, Fernández E. Assessment of genetic diversity in sorghum bicolor using RAPD markers. Genetika. 2019;51(3): 789–803.

[72] Afolayan G, Deshpande SP, Aladele SE, Kolawole AO, Angarawai I, Nwosu DJ, Michael C, Blay ET, Danquah EY. Genetic diversity assessment of sorghum (Sorghum bicolor (L.) Moench) accessions using single nucleotide polymorphism markers. Plant Genetic Resources. 2019 Oct;17(5):412–20.

[73] Zhu M, Chen J, Yuyama N, Luo L, Xiao X, Lv Y, Liu Y, Cai H. Genetic Diversity and Population Structure of Broomcorn Sorghum Investigated with Simple Sequence Repeat Markers. Tropical Plant Biology. 2020 Mar;13(1): 62–72.

[74] Joshi Akansha R, Kale Sonam S, Chavan Narendra R. Genetic diversity among elite sorghum (Sorghum bicolor L.) accessions genotyped with SSR markers to enhance use of global genetic resources. IJCS. 2020;8(2):1691–7.

[75] Jessup RW, Abed ZA, Najeep HF, Al-Azawi NM. Genetic analysis of sorghum cultivars from USA using SSR markers. Plant Archives. 2020;20(1): 1121–5.