Australian Journal of Crop Science AJCS

AJCS 11(02):142-148 (2017) ISSN:1835-2707
doi: 10.21475/ajcs.17.11.02.p127

Breeding strategies to improve sorghum quality

Alina M. Mofokeng[1], Hussein Shimelis[2], Mark Laing[2]

[1]Agricultural Research Council, Grain Crops Institute, Private Bag X 1251, Potchefstroom, 2520, Republic of South Africa
[2]African Centre for Crop Improvement, School of Agriculture, Earth and Environmental Sciences, Private Bag X 01, Scottsville, 3209, Republic of South Africa

***Corresponding author: MofokengA@arc.agric.za**

Abstract

Sorghum is an important grain crop grown for human consumption worldwide. The crop is classified into various races with the wild and cultivated sorghums included. The cultivated sorghums have a great phenotypic diversity which breeders can exploit for further improvement. The exploitation of the genetic diversity requires effective characterization of the genetic pool. The genotypes can be characterized using morphological, molecular markers, and analysis of nutritional quality traits. Characterization is performed with the purpose of sourcing new genes for crop improvement to combat hunger and malnutrition in arid and semi-arid regions of the world. Attempts were made to improve sorghum protein quality through mutation and genetic engineering but information on the use of classical breeding methods to improve sorghum protein quality and the combining ability of parental lines for improved protein quality is still limited. The effects of gene action on sorghum for yield and protein quality are essential for researchers and plant breeders for their breeding programmes aiming to develop hybrids and for further genetic improvement. Moreover, outcomes of nutritional quality studies are of paramount importance to scientists in various research disciplines. This article reviews the characterisation, combining ability and efforts for improving sorghum protein quality.

Keywords: Characterization, combining ability, protein quality, amino acids, sorghum.

Introduction

Sorghum is one of the world's major cereal crops and a dietary staple for more than 500 million people in sub-Saharan Africa and Asia. In developed countries, it is primarily used as animal feed, but is becoming more popular in food products due to a rise in demand for specialty grains, especially those that are gluten-free (Taylor et al., 2006). It ranks fifth after maize, rice, wheat, and barley and second after maize in Sub-Saharan Africa. Sorghum can be used for human consumption, livestock feed and fodder, ethanol production and other industrial purposes. Numerous studies have demonstrated that sorghum is a very diverse crop, with cultivated sorghums exhibiting great phenotypic variability. Many plant breeders exploit this genetic diversity for crop improvement. The exploitation of this variability first requires effective characterization or analysis of genetic diversity. The characterisation of the sorghum genotypes involves the use of phenotypic, nutritional quality and molecular marker techniques. Of late, plant breeders resort to use phenotypic and molecular marker technology for assessing existence of genetic variation due to their advantageous nature over biochemical markers. On the other hand, sorghum genotypes can be characterised in terms of nutritional quality. Evaluation of genetic diversity among the nutritional quality traits is important for sourcing genes of novelty for crop improvement.

Traditionally, the research focus was based on biotic and abiotic factors such as diseases, insect pests, and environmental stresses such as drought, heat, toxicity, as well as processing and marketing products. Nutritional aspects such as vitamins, mineral elements such as iron, and zinc received little attention, but efforts are being made to improve them (Sleper and Poehlman, 2006) in various crops including sorghum. Among other nutritional needs, proteins are essential to people and animal nutrition. They contribute to tissue building which is highly dependent on amino acids, also referred to as building blocks of life. The amino acids are supplied by proteins from plants and animals. These proteins are composed of numerous amino acids, and only eight are regarded essential. These essential amino acids include leucine, phenylalanine, threonine, tryptophan, isoleucine, lysine, methionine and valine (Ferreira et al., 2006), which must be supplied in a human diet. However, sorghum has inferior levels of amino acids such as lysine, hence it is important to determine the genetic variation present in the available sorghum genetic pool. The main aim of the study is to review efforts made thus far in sorghum nutritional quality with special emphasis on protein and amino acids and their mode of gene action.

Sorghum genetics and classification

Sorghum is a diploid (2n=2x=20) crop classified into two groups, the wild and the cultivated sorghums (Ayana et al., 2002). The wild sorghums include *Sorghum halepense*, *Sorghum propinquum*, *Sorghum bicolor*, subspecies *drummondii* and *Sorghum bicolor* subspecies *verticilliflorum*. The cultivated sorghum has been classified

into five major races, *bicolor*, *caudatum*, *durra*, *guinea* and *kafir*, and 10 intermediate races based on panicle morphology. According to Harlan and De Wet (1972) the *bicolor* race is characterised by loose panicle with grains covered by large closed glumes, and is mostly distributed in Asia and Africa. The *caudatum* race is characterised by asymmetric grain, flattened on the ventral surface and convex on the dorsal. The panicle morphological structure varies with shape. This race is mainly found in Central and East Africa. The *durra* sorghums have very compact panicle with curved penducle, and the tiny glumes are attached to globular grain. This race is mostly grown in East Africa, the Middle East and India. The *guinea* sorghums are tall with loose panicle, spikelets with open glumes enclosing an elliptical grain and photoperiod sensitive. These sorghums are found in West Africa. The *kafir* varieties are small sorghums with relatively compact and cylindrical panicle consisting of symmetrical grain flattened on the ventral surface and convex on the dorsal. This race is grown mostly in eastern and southern Africa (Mann et al., 1983). The morphological intermediate races have been reported in Africa. Due to the vast diversity in cultivated sorghum, breeders developed interest to exploit this diversity, and develop improved varieties in their programmes using various breeding methods and technologies. To develop cultivars it is important to characterise the germplasm available to identify potential candidate genotypes for further improvement.

Characterization and analysis of genetic diversity in sorghum

Accurate estimates of genetic diversity among and within crop plant species are becoming increasingly important for crop improvement. This is because they can assist to reduce population bottlenecks, threats of genetic losses and also for determination of variation of landraces at various levels such as country, regional and local levels. This estimation and evaluation of diversity levels are a prerequisite for utilization in cultivar improvement. The germplasm can be characterized morphologically and genotypically using phenotypic descriptors, biochemical and molecular markers. The characterisation of the genotypes gives descriptive information of the traits and aids in understanding the similarities and differences among genotypes.

Morphological characterization

Morphological or phenotypic descriptors are used to distinguish one accession from the other. Most of the characterisation and evaluation has been based on the recording of either or both qualitative and quantitative morphological characters. Geleta and Labuschagne (2005) characterised sorghum germplasm using qualitative traits that include leaf midrib colour, grain colour, glume colour, endosperm texture, pericarp colour, leaf trichomes, awns, testa color, pericarp thickness, panicle compactness. Abdi et al. (2002) reported patterns of variation in sorghum for qualitative traits in Ethiopia. On the other hand, the quantitative traits are also useful for determination of genetic diversity among genotypes. The quantitative traits include plant height, days to maturity, leaf area, leaf width, leaf length, number of leaves, panicle length, grain yield per plant, grain size, 1000 grain weight, grain number per panicle, panicle width, number of primary branches per panicle, and panicle weight (Punitha et al., 2010). Gambin and Borras (2011) reported great variation in grain filling patterns in sorghum. Rao et al. (1996) observed great morphological and agronomical diversity in sorghum germplasm in India. Ganesamurthy et al. (2010) observed great phenotypic variability among sorghum landraces collected in Tamil Nadu. Morphological characterisation is useful for design of future breeding programmes, identify duplicates, study pattern of genetic variation and establish the relationship between agronomic traits for direct and indirect selection.

Although the agronomical and morphological characterization provides useful information to breeders, the challenge is that they may be easily influenced by the environmental factors. This process/procedure may take a long time and the plants must be assessed during a fixed vegetative phase of the crop. Of late, breeders resort to the use of molecular marker systems because they are not subjected to environmental influences, have fixed plant developmental stages and have potential to give results within a short time.

Characterisation using molecular markers as new technologies

Traditionally, breeders used to classify sorghum gene pool based on morphological characters. Recently, biotechnology has provided ways of improving crops with special reference to sorghum. This is through the use of various techniques such as molecular markers, gene identification and cloning, *in vitro* protocols for efficient plant regeneration, genetic engineering and gene transfer technology to introduce desirable traits into sorghum genomes and genomics, and germplasm databases (O'Kennedy et al., 2006). The genetic improvement has been made easily accessible through the use of easily assayable molecular DNA genetics of DNA markers that enable accurate identification of genotype without the confounding effect of environment, thereby increasing heritability. Marker assisted selection (MAS) in sorghum reduces the length of time required for introgression of characters unlike with the use of the pedigree breeding method. Also the selection of progenies based on genetic values derived from molecular marker data substantially increases the rate of genetic gain, particularly if the number of cycles of evaluation or generations can be reduced (Meuwissen et al., 2001).

In contrast to morphological or biochemical marker techniques, molecular markers are discrete, co-dominant or dominant, and free from epistatic interaction. The DNA-based methods are independent of environmental factors and give rise to a high number of polymorphisms. Various molecular markers that have been used in sorghum breeding including randomised amplified polymorphic DNA (RAPD) (Prakash et al., 2006), amplified fragment length polymorphism (AFLP) (Wu et al., 2006), and simple sequence repeat (SSR) (Manzelli et al., 2006), single nucleotide polymorphism (snp) (Zeng et al., 2011), microarrays (Buchanan et al., 2005), and diversity array technology (DArT) (Mace et al., 2009). The latter technique has been reported recently in several studies. But in Africa, there are limited studies conducted using DArTs. These markers were mainly used for genetic diversity, cultivar identification, gene mapping and discovery, and gene pyramiding. For instance, The β-, γ- and δ-kafirin genes were sequenced from 35 sorghum genotypes to investigate the allelic diversity of seed storage proteins (Laidlaw et al., 2010). Marker-assisted selection (MAS) is becoming an increasingly useful tool to breeders, and it is our goal to add them to the toolbox by providing a genetic analysis of

sorghum protein digestibility. Winn et al. (2009) mapped QTLS governing protein digestibility in sorghum. The results uncovered that two major QTLs on chromosome 1 are associated with protein digestibility—one QTL (locus 1 from the high digestibility/ high lysine content parent) unfavorably affects digestibility and one QTL (locus 2 from the HD parent) only 20 cM away favorably affects digestibility. A contrast analysis between genotypic groups at these two loci shows that a higher level of protein digestibility may be obtained when this linkage in repulsion is broken and favorable alleles are allowed to recombine.

Molecular markers are useful for fingerprinting of collections for identification and germplasm management in plant breeding. They are also useful in a wide range of applications including genetic mapping and genome analysis (Li et al., 2000), gene and quantitative trait locus analysis (Blair and McCough, 1997) and in marker assisted breeding (Weising et al., 1998). For genotyping, SSRs were used in genetic diversity studies among elite sorghum inbred lines (Menz et al., 2004), among germplasm collections from different geographic locations (Muraya et al., 2011), and in the assessment of population genetic structure and relatedness within or among landraces (Folkertsma et al., 2005). On the other hand, Smith *et al.* (2010) determined genetic diversity of sorghum hybrids used widely in the US. Furthermore, Wu and Huang (2006) used SSR for mapping sorghum genome in comparison with the existing genetic linkage maps. In addition, SSRs can be used in conjunction with other molecular techniques (Geleta et al., 2006).

Omics application in sorghum

Proteomics is essential as a worldwide tool for transitional and studies of expression of genes. Information on plant proteomics lags far behind than the animal. With regards to sorghum protein analysis, polyacrylamide gels and mass spectrometric were used for accumulation of protein profiles (Ndimba and Ngara, 2012). In other studies, sorghum protein was conjugated with dextran or galactomannan under controlled conditions (60 °C, 79% relative humidity), or cross-linked with transglutaminase (TGase) to improve the functional properties (Babiker and Kato, 1998).

On the other hand, genomics deepens understanding about gene networks for cereal development and agronomy through molecular maps, genomic and EST sequences QTLs gene pathways. It further accelerates application of biotechnology in cereals by providing target genes. Also, it simplifies and advances breeding techniques through marker assisted selection and application of mutagenic agents. The increasing availability of tools such as transcriptomics, metabolomics, and proteomics has helped to facilitate the discovery of previously cryptic connections between genotype and phenotype. Transcriptomics has been used in studies that include drought and heat (Johnson et al., 2014). The results show evidence for both cross-talk and specificity in the sorghum response to combined heat and drought stress. The first transcriptome data was generated by d*e novo* transcriptome assembly for sorghum variety Taejin by next-generation sequencing in Korea (Yeonhwa et al., 2016).

Metabolomics is an emerging method to improve our understanding of how genetic diversity affects phenotypic variation in plants. Recent studies have demonstrated that genotype has a major influence on biochemical variation in several types of plant tissues, however, the association between metabolic variation and variation in morphological and physiological traits is largely unknown. The use of metabolomics to explain relationships between two or more morpho-physiological traits was explored and showed chlorogenic and shikimic acid to be associated with photosynthesis, early plant growth and final biomass measures in sorghum. Taken together, Turner et al. (2016) demonstrated the integration of metabolomics with morpho-physiological datasets to elucidate links between plant metabolism, growth, and architecture. In recent studies, metabolomics has been used in such applications as characterization of biochemical variation within species (Kusano et al., 2015), discovery of potential metabolic engineering targets (Tsogtbaatar et al., 2015) examination of plant responses to the whole environment (Steinfath et al., 2010; Heuberger et al., 2014) as well as responses to individual biotic (Scandiani et al., 2015) and abiotic (Ganie et al., 2015; Sanchez-Martin et al., 2015) stressors. However, research work on protein quality using transcriptomics and metabolics is still limited.

Characterization of nutritional quality: protein and amino acids

Protein is one of the most important nutritional attributes of sorghum quality. It is located in the endosperm, germ, and pericarp with about 80, 30 and 16% respectively (Taylor and Schussler, 1986). The average protein content varies from 7.3-15.6% (Hulse et al., 1980). The major protein fractions in sorghum are kafirins or prolamins accounting for 80% of the total grain protein (Hamaker et al., 1995), and glutelins. These fractions are located primarily within the protein bodies and protein matrix of the endosperm (Wong et al., 2009).

The prolamins are characterised by their low contents of essential amino acids particularly lysine, which accounts for only 0.2% of the total amino acids in sorghum kafirin, less than 2% in the endosperm and less than 3% in the whole grain, all values are g/100 g and based on values cited in Serna-Saldivar and Rooney (1995). The accumulation of the fractions and the protein amounts were previously increased significantly by nitrogen fertilization (Warsi and Wright, 1973). The germ is rich in albumins, and globulins, while the endosperm contains kafirins and glutenins. The albumins, globulins, and glutenins fractions are rich in lysine and other essential amino acids. Cultivars exhibiting improved protein quality usually contain more of these with a corresponding lower proportion of kafirins. These cultivars were selected and bred to contain a larger germ-to-endosperm ratio to yield more albumins, globulins, and glutenins (Mohan and Axtell, 1975). The poor nutritional quality of the kafirins is compounded by the fact that they are difficult to digest and their digestibility decreases on cooking (Duodu et al., 2003).

Song et al. (2004) reported the presence of ten genes in α-kafirins at a single locus. The genes were characterised using macro-tandem repeats. Izquierdo and Godwin (2005) characterized methionine σ-kafirin gene that is expressed in low levels and further comprised of 1% of total seed storage protein. Freitas et al. (1994) characterised and cloned cysteine-rich γ-kafirin which are more abundant than the β-kafirin. The γ-kafirin contains 9-12% of the total kafirin fraction and 19-21% of opaque endosperm, whereas the σ-kafirin contains 1% total seed storage protein. Laidlaw et al. (2010) reported characterization of alleles for presence of genetic diversity among the seed storage proteins in sorghum cultivars. The sorghum cultivars were sequenced and characterised for grain protein quality where the β-kafirin showed large variation in grain quality, and cysteine rich γ-kafirin and σ-kafirin comprised less variation.

Gerrano et al. (2011) assessed biodiversity among Ethiopian and South African sorghum genotypes for protein, starch, and other nutritional quality traits. Assessment of biodiversity of mineral elements is also important for further breeding in nutritional quality improvement (Shegro et al., 2012). The success of crop breeding programmes depends on the availability of genetic variation in the genetic pool available. The genetic variation further enhances genetic gain and selection, and improves chances of heritability of traits in monogenic or multi-genic form. Studies and outcomes of nutritional diversity assessment are important for crop breeders, curators, millers, dietetics, and nutritionists for handling and conservation of the genetic material.

Efforts for balance of diet and protein quality improvement in sorghum

Use of protein supplements

Different approaches have been employed with the purpose of improving quality of protein in sorghum for the society to have a balanced diet. In many regions of the world sorghum is grown as a staple food and is important for people suffering from celiac disease. The challenge with this crop is that its protein is indigestible once cooked and has low levels of lysine as compared to other cereals (Hulse et al., 1980). There are reports of malnutrition and diseases related to malnutrition (Kwashiorkor) (FAO, 2010). However, some studies showed that low amounts of sorghum lysine in human diets can be balanced with the use of supplements. The reports so far, indicated that sorghum can be consumed together with other protein rich leguminous crops to serve as protein supplements for human beings and ruminants.

However, sorghum is mainly grown by resource poor farmers in various arid and semi-arid regions of the world as a staple food crop. The reason that sorghum can survive, unlike other cereal crops such as maize is mostly preferred by many rural households and communities for food. The use of protein supplements can approximately work for large-scale farmers mainly situated in developing regions or countries. Emphasis must be put on improving the strategies employed by scientists in order to solve the challenge of malnutrition. Attempts to improve nutrition were made in various and across disciplines in the agricultural and other life science sectors.

Mutation

Researchers attempted to improve sorghum nutritional quality based on the identification of high lysine mutants (Nelson et al., 1965). Two mutants were identified in sorghum, the *hl* gene in an Ethiopian line (Singh and Axtell, 1973) and the P721 opaque gene which was induced with the chemical mutagen diethyl sulphate (Axtell et al., 1979). Studies show that the lines contain ''low prolamin'' in which the proportion of kafirin is reduced by 50% with compensatory increases in other more lysine-rich proteins and free amino acids. The lysine content was enhanced by 40-60%. The challenge of protein improvement is the association with deleterious effects on seed weight and yield. Oria et al. (2000) reported the identification of a novel line with high protein digestibility from a cross involving the high lysine P721 opaque mutant. Sorghum lines from the African Centre for Crop Improvement, and breeding lines from other sources were mutagenised with gamma irradiation and cyclotron to improve agronomic and nutritional traits (Brauteseth, 2009). The author used the FOSS near-infrared spectroscopy (NIR) for analysis of nutritional quality traits such as protein, and starch among others. Significant differences and deviations were observed among the populations.

Genetic engineering/Biofortification

Sorghum was genetically transformed with the use of *Agrobacterium* (Zhao et al., 2000) to improve nutritional quality. Because sorghum is low in lysine, a high-lysine gene *HT12* was inserted into the sorghum gene using *Agrobacterium* vector together with a herbicide resistant gene *bar*. Ultimately, increased levels (40-60%) of lysine were observed in hemizygous sorghum grains (Zhao et al., 2003). This implicates that an improved lysine transgenic variety can benefit communities by eradicating malnutrition. Furthermore, Africa Biofortified Sorghum (ABS) project developed improved sorghum lines through the process of genetic engineering techniques (Africa Biofortified Sorghum Project, 2009). da Silva et al. (2011) transformed sorghum lines to suppress synthesis of various kafirin sub-classes (alpha, gamma and delta-kafirins) or backcrossed into transgenic lines with improved protein quality. The transgenic lines had high protein digestibility, improved amino acid score, and protein digestibility corrected amino acid score in contrast to untransformed sorghum lines. According to Vendemiatti et al. (2008) a commercial sorghum line, Massa 03, and nine ICRISAT high lysine enriched sorghum lines from India, were evaluated for storage protein content and amino acid composition. The protein content and amino acid composition showed variation among the lines evaluated. These lines served as potential source of food due to balanced amino acid profile.

Gene action in sorghum for yield, protein and amino acid composition

The combining ability of cultivars can be classified into general combining ability (GCA) which are mainly due to additive gene action and specific combining ability (SCA) due to non-additive gene action (Falconer, 1989). GCA and SCA effects and variances are effective genetic parameters of direct utility to decide the next phase of the breeding programme (Dabholkar, 1992). Combining ability helps in selection of parents for construction of synthetics, selection of suitable F_1's for a multiple crossing or composite breeding programme and the possibility of employing an appropriate selection technique like modified mass selection, recurrent selection and reciprocal selection (Dabholkar, 1992). It also contributes to heritability (Simmonds and Smartt, 1999) and is very important in hybrid breeding programmes. Kenga et al. (2004) used combining ability primarily to identify parental lines for possible combinations to make hybrids. Studies have shown the importance of GCA and SCA for grain yield in sorghum (Tadesse et al., 2008; Makanda et al., 2011). Hicks et al. (2002) reported good combining ability effects in sorghum feed quality including protein among other traits and seed weight. A significant GCA effect for grain yield, protein, and lysine was reported for restorer lines and for male sterile testers only for yield and protein; the SCA was insignificant (Collins and Pickett, 1972). Monyo et al. (1988) reported significant GCA effect on protein content and lysine and SCA effects on grain yield. Significant differences in GCA for grain yield, protein, and lysine were reported among sorghum genotypes and restorer lines (Collins and Pickett, 1972). The hybrids showed high heterotic effects for yield as well as protein. Furthermore, Khandelwal et al. (2004) reported effects of general and specific combining ability in

yield and protein quality among the sorghum genotypes tested and also affected by environment. Patel and Patel (2010) reported combining ability effects on sorghum fodder yield and quality traits. The ratio of general combining ability to specific combining ability variance indicated the non-additive gene action. The combining ability studies allow breeders to choose the good combiners and discard the poor combiners.

Genotype x environment interaction in sorghum for nutritional quality

Genotype x environment interaction refers to the variation in performance of genotypes in various environments. Genotype x environment interaction occurs when various genotypes respond in a different way to environmental changes. It can be cross-over or non-cross over interaction. However, breeders prefer the genotypes that show stable performance in favourable and non-favourable environments for cultivar recommendation. Genotypes that show cross-over interaction slows down selections in breeding programmes. Although sorghum thrives well in various environments where other cereals fail, breeders are interested in cultivars that show best stable performance in a variety of environments in terms of yield (Annicchiarico, 2002). Many studies focus on developing hybrids with high yield and stable performance across environments, as this is one of essential approaches in combating hunger and malnutrition (Kenga et al., 2004). The protein content and the amino acid composition vary due to genotype and environmental conditions during growth and development. The environmental conditions may include temperature, soil fertility, and water availability among others. According to Hulse et al. (1980) there are several reports that cultivars possessing high protein content in one location may have different grain protein content when planted in another or in different seasons. Genotype x environment interaction plays a major role in the composition and content of proteins produced in sorghum cultivars. Cultivars that have good protein quality in various environments may be essential in developing and under developed countries where human diets consist mainly of cereals. Hence, it is essential to determine the stability of sorghum hybrids with improved protein content in various environments to guide future breeding activities.

Various methods are employed for analysis of interaction of genotype x environment. The methods include linear regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Freeman and Perkins, 1971), and principal component analysis (Hill and Goodchild, 1981), non-parametric analyses (Hill, 1975; Lin et al., 1986; Crossa, 1990), additive main effects and multiplicative interaction (AMMI) (Alanís et al., 2010), and genotype and genotype by environment (GGE) biplot analysis (Yan et al., 2007). The AMMI method can separate the genotype, environment, and the genotype by environment for breeding purposes as well as the structural variation from noise. Of late, the GGE biplot is used in evaluation of hybrids for g x e interaction (Ma et al., 2004) even where the AMMI method is limited (Yan et al., 2007). The GGE biplot analysis can be used for both genotype and the environment evaluations. It is also effective for analysis of mega-environments to show genotypes adapted for specific environments (Yan et al., 2007).

Conclusion

Knowledge of different morphological and molecular markers, biodiversity methods of analysis as well as g x e, methods is essential for cultivar and hybrid improvement and their release to smallholder and large-scale farmers. The effects of gene action on sorghum for yield and protein quality are essential for researchers and plant breeders for their breeding programmes aiming to develop hybrids and for further genetic improvement. Information of nutritional diversity assessment is also important for plant breeders, curators, millers, dieticians, and nutritionists for handling and conservation of the sorghum genetic material.

Acknowledgement

Agricultural Research Council and Alliance for a Green Revolution in Africa are sincerely thanked for funding the first author.

References

Abdi A, Bekele E, Asfaw Z, Teshome Z (2002) Patterns of morphological variation of sorghum [*Sorghum bicolor* (L.) Moench] landraces in qualitative characters in north shewa and south welo, Ethiopia. Hereditas. 137:161-172.

Africa Biofortified Sorghum (2009) Africa biofortified sorghum project. DOI: http:/biosorghum.org/.

Alanís HW, Quintero VP, García FZ, García NM, Vázquez AJG, Cavazos GA, Gracia MAG, Hernández SM, Salinas LA (2010) Finlay and wilkinson model vs. ammi model in the analysis of genotype-environment interactions in sorghum. Rev Fitotec Mex. 33:117-123.

Annicchiarico P (2002) Genotype x environment interactions: challenges and opportunities for plant breeding and cultivar recommendations. FAO Plant Production and Protection Paper 174. FAO, Rome.

Axtell JD, Van Scoyoc SW, Christensen PJ, Ejeta G (1979). Current status of protein quality improvement in grain sorghum. p. 357 Seed Protein Improvement in Cereals and Grain Legumes. IAEA, Vienna.

Ayana A, Beckele E, Bryngelsson T (2002) Genetic variation in wild sorghum (*Sorghum bicolor*) Ssp. *Verticilliflorum* (L.) Moench) germplasm from ethiopia assessed by random amplified polymorphic DNA (RAPD). Genet Resour Crop Evol. 45:499-510.

Blair MW, McCough SR (1997) Microsatellites and sequence tagged site markers diagnostic for the bacterial blight resistance gene, xa-5. Theor App Genet. 95:174-184.

Buchanan CD, Lim S, Salzman RA, Kagiampakis I, Morishige DT, Weers BD, Klein RR, Pratt LH, Cordonnier-Pratt MM, Klein PE, Mullet JE (2005) *Sorghum bicolor*'s transcriptome response to dehydration, high salinity and aba. Plant Mol Biol. 58:699-720.

Collins FC, Pickett RC (1972) Combining ability for yield, protein, and lysine in an incomplete diallel of *Sorghum bicolor* (L.) Moench. Crop Sci. 12:5-6.

Crossa J (1990) Statistical anlyses of multilocation trials. Adv Agron. 44:55-85.

Dabholkar AR (1992) Elements of biometrical genetics. Concept Publishing Company, New Delhi, India.

Da Silva LS, Jung R, Zhao ZY, Glassman K, Taylor J, Taylor JRN (2011) Effect of suppressing the synthesis of different kafirin sub-classes on grain endosperm texture, protein body structure, and protein nutritional quality in improved sorghum lines. J Cer Sci. 54:160-167.

Duodu KG, Taylor JRN, Belton PS, Hamaker BR (2003) Factors affecting sorghum protein digestibility. J Cer Sci. 38:117-31.

Eberhart SA, Russell WA (1966) Stability parameters for comparing varieties. Crop Sci. 6:36-40.

Falconer DS (1989) Introduction to quantitative genetics. 3rd ed. Longman Scientific and Technical CO., Essex, England.

FAO (1995) Sorghum and millets in human nutrition. http://www.fao.org/docrep/T0818E/T0818E04.htm.

FAO (2010) The state of food insecurity in the world. Addressing food insecurity in protracted crises. Food and Agriculture Organization of the United Nations, Rome.

Ferreira RR, Meinhardt LW, Azevedo RA (2006) Lysine and threonine biosynthesis in sorghum seeds: Characterization of aspartate kinase and homoserine dehydrogenase isoenzymes. Ann Appl Biol. 149:77-86.

Finlay KW, and Wilkinson GN (1963) The analysis of adaptation in a plant breeding programme. Austr J Biol Sci. 14:747-754.

Folkertsma RT, Rattunde FH, Chandra S, Soma Raju W, Hash CT (2005) The pattern of genetic diversity of Guinea-race *Sorghum bicolor* (L.) Moench landraces as revealed with SSR markers. Theor Appl Genet. 111:399-409.

Freeman GH, Perkins JM (1971) Environmental and genotype-environmental components of variability.VIII. Relations between genotypes grown in diferent environments and measures of these environments. Heredity. 27:15-23.

Freitas FA, Yunes JA, Silva MJ, Arruda P, Leite A (1994). Structural characterisation and promoter activity analysis of the *gamma* kafirin gene from sorghum. Mol Genet Genom. 245:177-186.

Gambín BL, Borrás L (2011) Genotypic diversity in sorghum inbred lines for grain-filling patterns and other related agronomic traits. Crop Past Sci. 62:1026-1036.

Ganesamurthy K, Punitha D, Elangovan M (2010) Genetic diversity among the land races of sorghum collected in Tamil Nadu. Electr. J Plant Breed. 1:1375-1379.

Ganie A H, Ahmad A, Pandey R, Aref IM, Yousuf PY, and Ahmad S (2015). Metabolite profiling of low-P tolerant and low-P sensitive maize genotypes under phosphorus starvation and restoration conditions. PLoS One. 10(6): e0129520

Geleta N, Labuschagne MT (2005) Qualitative traits variation in sorghum (*Sorghum bicolor* (L.) Moench) germplasm from eastern highlands of Ethiopia. Biodivers Conserv. 14:3055-3064

Geleta N, Labuschagne MT, Viljoen CD (2006) Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. Biodivers Conserv. 15:3251-3265.

Gerano AS (2011) Biodiversity in plant, grain and nutritional characteristics of sorghum [*Sorghum bicolor* (L.) Moench] accession from Ethiopia and South Africa Department of Plant Sciences (Plant Breeding). University of the Free State, Bloemfontein, South Africa.

Hamaker BR, Mohammed AA, Habben JE, Huang CP, Larkins BA (1995) Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents that the conventional method. Cer Chem. 72:583-588.

Harlan JR, de Wet JMJ (1972) A simplified classification of cultivated sorghum. Crop Sci. 12:127-176.

Heuberger A L, Robison FM, Lyons SMA, Broeckling CD, and Prenni J.E (2014). Evaluating plant immunity using mass spectrometry-based metabolomics workflows. Front Plant Sci. 5:291.

Hill J (1975) Genotype-environment interactions-a challenege for plant breeding. J Agric Sci. 85:477-493.

Hulse JH, Laing EM, Pearson OE (1980) Sorghum and the millets: their composition and nutritive value. Academic Press, London.

Irzuierdo L, Godwin ID (2005) Molecular charcterisation of a novel methionine-rich delta-kafirin seed storage gene in sorghum (*Sorghum bicolor* L.). Cereal Chem. 82:706-710.

Kenga R, Alabi SO, Gupta SC (2004) Combining ability studies in tropical sorghum [*Sorghum bicolor* L. (Moench)]. Field Crop Res. 88:251-260.

Khandelwal V, Sharma V, Choudhary L (2004) Heterosis and combining ability for grain yield and other traits in sorghum [*Sorghum bicolor* (L.) Moench]. Indian J. Genet Plant Breed. 64:235-236.

Kusano M, Yang Z, Okazaki Y, Nakabayashi R, Fukushima A, and Saito K (2015). Using metabolomic approaches to explore chemical diversity in rice. Mol Plant. 8:58-67.

Laidlaw HKC, Mace ES, Williams SB, Sakrewski K, Mudge AM, Prentis PJ, Jordan DR, Godwin ID (2010) Allelic variation of the β-, γ- and σ- kafirin genes in diverse *Sorghum* genotypes. Theor Appl Genet. 121:1227-237.

Li YC, Fahima T, Peng JH, Roder MS, Kirzhner VM, Beiles A, Nevo E (2000) Edaphic microsatellite DNA divergence in wild emmer wheat, *Triticum dicocoides*, at a microsite: Tagigha, Israel. Theor Appl Genet. 101:1029-1038.

Lin CS, Binns MR, Lefkovitch LP (1986) Stability analysis: Where do we stand? Crop Sci. 26:894-900.

Mace ES, Jean-Francois R, Bouchet S, Klein PE, Klein RR, Kilian A, Wenzel P, Xia L, Halloran K, Jordan DR (2009) A consensus genetic map of sorghum that integrates multiple component maps and high-throughput diversity array technology (DArT) markers. BMC Plant Biol. 9:13.

Makanda I, Tongoona P, Derera J, Sibiya J, and Fato P (2011) Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low- and mid-altitude environments. Field Crops Res. 116:75-85.

Mann JA, Kimber CT, Miller FR (1983) The origin and early cultivation of sorghums in Africa. Texas Agricultural Experiment Station. Bullet 1454., Texas A&M University. College Station, TX.

Manzelli M, Pileri L., Lacerenza N, Benedettelli S, Vecchio V (2006) Genetic diversity assessment in Somali sorghum (*Sorghum bicolor* (L.) Moench) accessions using microsatellite markers. Biodivers Conserv. 16:1715-1730.

Menz M, Klein R, Unruh N, Rooney W, Klein P, Mullet J (2004) Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. Crop Sci. 44:1236-1244.

Meuwissen THE, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genet. 157:1819-1820.

Mohan DP, Axtell JD (1975). Diethyl sulfate induced high lysine mutant in sorghum. Proceedings of 9th Biennial Grain Sorghum Research and Utilization Conference., Lubbock, TX, USA.

Monyo ES, Ejeta G, Nyquist WE, Axtell JD (1988) Combining ability of high lysine sorghum lines derived from P-721 opaque. Crop Sci. 28:70-75.

Muraya MM, de Villiers S, Parzies HK, Mutegi E, Sagnard F, Kanyenji BM, Kiambi D, Geiger HH (2011) Genetic structure and diversity of wild sorghum populations (*Sorghum* spp.) from different eco-geographical regions of Kenya. Theor Appl Genet. 123:571-583.

O'Kennedy MM, Grootboom A, Shewry PR (2006) Harnessing sorghum and millet biotechnology for food and health. J Cer Sci. 44:224-235.

Oria MP, Hamaker BR, Axtel JD, Huang CP (2000) A highly digestible sorghum mutant cultivar exhibits a unique folded structure of endosperm protein bodies. Proceedings of the National Academy of Sciences, USA p. 5065-5070.

Patel KV, Patel AD (2010) Combining ability analysis for green fodder yield and quality traits attributes in sorghum (*Sorghum bicolor* (L.) Moench). Adv Res J Crop Improv. 1:187-193.

Prakash SPJ, Biji KR, Gomez SM, Murthy KG, Babu RC (2006) Genetic diversity analysis of sorghum (*Sorghum bicolor* L. Moench) accessions using rapd markers. Indian J Crop Sci. 1:109-112.

Punitha D, Ganesamurthy K, Rajarathinam S (2010) Metroglyph analysis of morphological variations in sorghum germplasm collections. Electr J Plant Breed. 1:536-541.

Rao SA, Rao KEP, Mengesha MH, Reddy VG (1996) Morphological diversity in sorghum from India. Genet Resour Crop Evol. 43:559-567.

Scandiani MM, Luque AG, Razori MV, Casalini LC, Aoki T, and O'donnell K (2015). Metabolic profiles of soybean roots during early stages of Fusarium tucumaniae infection. J Exp Bot. 66:391-402.

Serna-Saldivar S, and Rooney L (1995) Structure and chemistry of sorghum and millets. In: Dendy, D. A. V. (ed.) Sorghum and millets, chemistry and technology. American Association of Cereal Chemists, St Paul, MN..

Shegro A, Shargie NG, van Biljon A, and Labuschagne MT (2012) Diversity of starch, protein, and mineral composition of sorghum landrace accessions from Ethiopia. J Crop Sci Biotech. 15:275-280.

Sanchez-Martin J, Heald, Kingston-Smith A, Winters A, Rubiales D, and Sanz M (2015) A metabolomic study in oats (*Avena sativa*) highlights a drought tolerance mechanism based upon salicylate signalling pathways and the modulation of carbon, antioxidant and photo-oxidative metabolism. Plant Cell Environ. 38:1434–1452

Singh R, and Axtell JD (1973) High lysine mutant gene (h1) that improves protein quality and biological value of grain sorghum. Crop Sci. 3:535.

Sleper DA, Poehlman JM (2006) Breeding field crops. 5th ed. Blackwell Publishing, Iowa.

Smith S, Primomo V, Monk R, Nelson B, Jones E, Porter K (2010) Genetic diversity of widely used U.S. sorghum hybrids 1980-2008. Crop Sci. 50:1664-1673.

Song RT, Segal G, and Messing J. (2004). Expression of the sorghum 10-member kafirin genes cluster in maize endosperm. Nucleic Acid Res. 32:8.

Steinfath M, Strehmel N, Peters R, Schauer N, Groth D, and Hummel J (2010). Discovering plant metabolic biomarkers for phenotype prediction using an untargeted approach. Plant Biotechnol J. 8:900-911.

Tadesse T, Tesso T, and Ejeta G (2008) Combining ability of introduced sorghum parental lines for major morpho-agronomic traits. SAT eJournal 6:1-7.

Taylor JRN, Schussler L (1986) The protein composition of the different anatomical parts of sorghum grain. J Cer Sci. 4:361-365.

Taylor J R N, Schober T J, and Bean SR (2006) Novel food and non-food uses for sorghum and millets. J Cer Sci. 44:252-271.

Tsogtbaatar E, Cocuron J, Sonera MC, Alonso AP (2015) Metabolite fingerprinting of pennycress (*Thlaspi arvense* L.) embryos to assess active pathways during oil synthesis. J Exp Bot. 66:4267-4277.

Turner MF, Heuberg AL, Kirkwood JS, Collins CC, Wolfrum EJ, Broekling CD, Prenni JE, and Jahn CE (2016) Non-targeted metabolomics in diverse sorghum breeding lines indicates primary and secondary metabolite profiles are associated with plant biomass accumulation and photosynthesis. Front Plant Sci. 7:953

Vendemiatti A, Ferreira RR, Gomes LH, Medici LO, and Azevedo RA (2008) Nutritional quality of sorghum seeds: storage proteins and amino acids. Food Biotech. 22:377-397.

Warsi AS, Wright BC (1973) Effects of rates and methods of nitrogen application on the quality of sorghum grain. Indian J Agric Sci. 43:722-726.

Weising K, Winter P, Huttel B, and Kahl G (1998) Microsatelite markers for molecular breeding. J Crop Produc. 1:113-143.

Wong JH, Lau T, Cai N, Singh J, Pederson JF, Vensel WH, Hurkman WJ, Wilson JD, Lemaux PG, Buchanan BB (2009) Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. J Cer Sci. 49:73-82.

Wu Y, Huang Y (2006) An SSR genetic map of *Sorghum bicolor* (L.) Moench and its comparison to a published genetic map. Genome. 50:84-89.

Yan W, Kang MS, Ma B, Woods S, Cornelius PL (2007) GGE Biplot vs AMMI analysis of genotype-by-environment data. Crop Sci. 47:643-653.

Zeng LY, Guo SX, He B, Sun LJ, Peng Y, Dong SS, Liu TF, Jiang S, Ramachandran S, Liu CM, Jing HC (2011) Genome-wide patterns of genetic variation in sweet and grain sorghum (*Sorghum bicolor*). Genom Biol. 12.