



Sorghum (*Sorghum bicolor* (L.) Moench) grain nutritional quality traits with breeding and genetics perspectives: A review

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is a staple food grain in many semi-arid and tropical areas of the world, especially in sub-Saharan Africa and some parts of Asia. In a breeding program for sorghum quality traits, the breeder needs to understand the nutritional and grain quality traits of the crop and the target environments. Therefore, the objective of the article is to review the major nutritional and antinutritional factors of sorghum grain concerning seed protein, starch, and phenolic compounds in line with their breeding and genetics perspectives. The sorghum grain major nutritional quality traits such as content and digestibility of protein and starch, nutritional and antinutritional factors regarding phenolic compounds with their corresponding genetics and breeding perspectives were discussed in this article. Moreover, genotypes and mutants, which have superior grain nutritional quality traits and QTLs and genes that govern quality traits such as lysin, amylose/amylopectin, and tannin content and also inheritance pattern were discussed.

Keywords: food sorghum, genetics, grain nutrition, starch, protein, phenolic compounds

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important staple food crop in the semi-arid tropics especially in parts of Africa and Asia. Unique features like tolerance; to drought, waterlogging, saline-alkaline, infertile soil and high temperature leads the crop for globally cultivated cereal. Sorghum is grown as one of the major food cereals and more than one-third of the produced sorghum grain is used directly for human food and is consumed mostly in the form of porridges (thick or thin) and flat breads (Awika and Rooney, 2004) [1].

In Africa and Asia, sorghum grain is used for human food as porridge, leavened and unleavened bread, boiled whole grain, roasting, popping, steam-cooked products, snacks, and for making beer (Awika and Rooney 2004) [1]. In these part of the world, low-income peoples do not have access to foods which are rich in nutrient and sorghum grain is characterized by its high starch, micronutrients, and crude fiber but low in fat (Mudge *et al.*, 2016) [2]. Therefore, there should be a research intervention to avail dietary energy, macro and micronutrient requirements of the peoples into the product which they can access easily.

Grain quality of the end product is another important objective in plant breeding, which decides the suitability and consumer preference of end product. The shape of sorghum grain are spherical with different sizes and colors. The color ranged from black, red, purple, brown, yellow to white. The basic anatomical components are pericarp (outer layer), germ (embryo), and endosperm (storage tissue). The distribution of these three parts vary based on varieties and environment, with an average of 8%, 82%, and 10% for pericarp,

endosperm, and germ, respectively. As the proportion of pericarp and germ becomes less the in-vitro protein digestibility will be increased resulted in increase quality (Duodu *et al.*, 2002) [3]. The color of the sorghum grain, size, and endosperm texture is major characteristics to be considered in breeding for improved food quality.

In promoting the health and nutrition of human beings sorghum grain plays important role. Since sorghum is a gluten-free cereal it is considered safe for people suffering from celiac disease. In developed country, the demand for gluten-free foods is growing especially for sorghum. Besides, the slow release carbohydrate property of sorghum increases satiety and has a positive roll in controlling diabetes. It is a source of energy, protein, starch, vitamins, minerals, and nutraceuticals such as antioxidant phenolics and cholesterol-lowering waxes (Taylor *et al.*, 2006) [4]. Moreover, it has very great potential as health-promoting food because it is rich in bioactive phenolic compounds. There is a growing tendency to use sorghum in pasta and noodle formulations, which are among the most widely consumed products in the world. Sorghum grain is also utilized for livestock feed, fuel, construction materials and an increasing number of industrial products. This diversity of uses increases the complexity of breeding for improved quality.

Grain and nutritional quality vary among different types of sorghum and their cultivated environments. Genetic improvement of sorghum quality traits can help sorghum to adapt to varying demands for end-use products. There is a substantial variation among sorghum genotypes for levels of proteins, lysine, lipids, carbohydrates, fiber, calcium, phosphorus, iron, thiamine, and niacin. Nutritional aspects

such as vitamins, mineral elements like iron, and zinc received little attention, but efforts are being made to improve them in various crops including sorghum. Among other nutritional needs, proteins are essential to people and animal nutrition. The amino acids are supplied by proteins from plants and animals and considered as building blocks of life. Concerning protein content sorghum foods has inferior nutritional quality compared to major cereals which have low nutritional value and digestibility of seed storage protein with low content of essential amino acids, lysine and threonine (Rhodes *et al.*, 2017^[5]; Mudge *et al.*, 2016)^[2].

The other important nutritional component of sorghum is starch which provides energy to humans and livestock and accounts for ~70% of dry grain weight in sorghum and other cereals. There are two types of starch in cereal grains, amylose and amylopectin which play a significant role in grain structure and quality. Sorghum with pigmented testa can develop condensed tannins. Some of these tannins retard the hydrolysis of food by reducing the digestibility of proteins and starch, thus increasing dietary fiber levels (Awika *et al.*, 2005)^[6].

The exploitation of genetic diversity requires effective characterization of the genetic pool. The genotypes can be characterized using morphological, molecular markers, and analysis of nutritional quality traits. However, in the present market economy, product quality has become increasingly important. Breeding for improved quality, consideration is given to the physical and chemical characteristics of the product harvested that affect its nutritional value, processing and utilization. Thus, the objective of this article is to review the major nutritional and antinutritional factors of sorghum concerning seed protein, starch, and phenolic compounds in line with their breeding and genetics perspectives.

Sorghum grain nutrition

The proportions of tannins, starch and proteins, are the most prominent factor that affect the nutritional quality and end use value of sorghum grains. Sorghum nutritional contents such as starch, vitamin, fat and minerals are comparable with fine cereals including rice and wheat however, the protein quality is slightly inferior (Kimani *et al.*, 2020)^[7]. As protein is essential part of the dietary pattern of people and animal nutrition several research have been done to improve the protein quality of sorghum grain. Grain quality traits such as digestibility and nutritional value depend heavily upon the content of the cereal proteins, which are primarily attributed to their amino acid composition (Rhodes *et al.*, 2017)^[5].

The main components of sorghum are the polysaccharides (starch and nonstarch), followed by proteins and lipids. In a sorghum association panel with 390 accessions, the protein content ranged from 8.1% to 18.8%, fat content ranged from 1.0% to 4.3%, and the starch content ranged from 61.7% to 71.1% (Rhodes *et al.*, 2017)^[5]. Apart from starch, other carbohydrates present are simple sugars, cellulose, and hemicelluloses. Sucrose being the major constituent (0.85%) is followed by glucose (0.09%), fructose (0.09%), maltose, and stachyose. Sorghum bran is low in protein and ash and rich in fiber components. Germ fraction is rich in ash, protein, and oil but very poor in starch. Over 68% of the total mineral

matter and 75% of the oil of the whole kernel are located in the germ fraction. Its contribution to the kernel protein is only 15%. Sorghum germ is also rich in B-complex vitamins. Endosperm, the largest part of the kernel, is relatively poor in mineral matter, ash, and oil content. It is, however, a major contributor to the kernel's protein (80%), starch (94%), and B-complex vitamins (50%-75%). The other important biochemical parameters are dietary fiber, phytic acid content, total phenols, polyphenols, flavan-4-ols, antioxidant activity, b-glucan content, in vitro protein digestibility, in vitro starch digestibility, minerals, and vitamins. Sorghum grains also contain resistant starch along with dietary fiber (Ratnavathi, 2019)^[8].

Sorghum grain protein

Grain sorghum is low in protein content. Lysine, and threonine are the limiting amino acids in the proteins of grain Sorghum (Mudge *et al.*, 2016)^[2]. Protein fractionation studies have shown that prolamine and glutelin are the principal protein fractions. An increase in protein content of grain sorghum leads to an increase in prolamin fraction and decreases the nutritional quality. Environmental factors such as location, chemical fertilizers, plant population and chemical treatments influence the protein content and amino acid pattern.

Protein is one of the most important nutritional attributes of sorghum quality. The proteins are composed of numerous amino acids, and only eight are regarded as essential. These essential amino acids include leucine, phenylalanine, threonine, tryptophan, isoleucine, lysine, methionine and valine, which must be supplied in a human diet. Grains of most cereal species, like wheat, maize and sorghum, which represent the world's largest providers of food and consequently important economical commodities, contain inadequate levels of some essential amino acids, particularly lysine, threonine, tryptophan and methionine (Mudge *et al.*, 2016)^[2]. Lysine content was reported to vary from 71 to 212 mg per gram of nitrogen and the corresponding chemical score varied from 21 to 62. The methionine and tryptophan content on average are 87 and 63 mg per gram of nitrogen. These deficiencies arise from the amino acid composition of the grain storage proteins, called kafirins, which account for up to 80% of the total grain proteins. These deficiencies are also exacerbated by cooking, which reduces sorghum protein digestibility. Similarly, binding of tannins to proteins can also reduce digestibility in high tannin lines (Taylor *et al.*, 2006)^[4]. As in other cereals, lysine is the first limiting amino acid in sorghum.

Protein is located in the endosperm, germ, and pericarp with about 80, 30 and 16% respectively (Duodu *et al.*, 2002)^[3]. The average protein content varies from 7.3-15.6%. The major protein fractions in sorghum are kafirins or prolamins accounting for 80% of the total grain protein, and glutelins. These fractions are located primarily within the protein bodies and protein matrix of the endosperm (Wong *et al.*, 2009)^[9].

The prolamins are characterized 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. Significant variability in accumulation of the fractions and the protein amounts were observed upon genotypic variation and nitrogen fertilization (Ahmed *et al.*, 2014) ^[10]. 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. 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) ^[11].

Sorghum grain starch

Starch is the predominant storage carbohydrate in plants and the most important source of carbohydrate in the human diet where it occupies an important source of energy. In sorghum grain, starch is the major proximate component (61.7% to 71.1%) and the major energy supplier. Starch granules consist of 20-30% amylose (linear polysaccharide) and 70-80% amylopectin (branched polysaccharide) of starch with different properties and they are not suitable for the same applications (Rhodes *et al.*, 2017) ^[5].

The proportion of amylose and amylopectin affects the rheological properties, such as gelatinization, retrogradation, gelling, and digestibility of starch. Starches exist in a highly organized manner in which amylose and amylopectin molecules are held together by hydrogen bonds and are arranged radially in spherical granules. It is composed of linear chains of glucose joined by α -1,4-glycosidic bonds called amylose and branched chains of glucose joined by α -1,4- and α -1,6-glycosidic bonds called amylopectin. Amylose is a linear chain of glucose averaging 1500 units. Hydrated amylose forms a helix that can interact with iodide to form a blue or purple color. Amylopectin is a much larger, branched polymer composed of about 3000 chains averaging 15-20 units. Starch granules are generally polygonal, but some are misshapen or contain dents because of compressive effects from the protein bodies in the corneous (vitreous) endosperm (Ratnavathi, 2019) ^[8]. The size of starch granules in sorghum are generally \approx 10-16 μ m (but has a range of 2-25 μ m). Both waxy and regular starches contain free sugars up to 1.2%. Normal sorghum starch has \approx 24%-33% amylose (Beta and Corke, 2001) ^[12]. Starch from waxy varieties contains very low amylose. Waxy sorghum starch comprises almost exclusively amylopectin. In both red and white sorghum cultivars, the amylose chains are reported to have an average degree of polymerization (DP) of \approx 1330-1390. Normal (nonwaxy) sorghum contains average amylose chain lengths of 260-270 on a weight basis (Hanashiro and Takeda, 1998) ^[13].

Sorghum grain starch and protein digestibility

Sorghum grain has been reported to have the lowest raw starch digestibility due to restrictions in accessibility to starch caused by endosperm proteins. The digestibility of the starch, dependent on hydrolysis by pancreatic enzymes, determines

the available energy content of cereal grain. The chemical nature of the starch, particularly the amylose and amylopectin content, is yet another factor that affects its digestibility. The starch digestibility was reported to be higher in low-amylose, i.e., waxy, sorghum than in normal sorghum (Gerrano *et al.*, 2014) ^[14].

The presence of tannins in the grain contributes to the poor digestibility of starch in some varieties of sorghum. Tannins isolated from sorghum grain were shown to inhibit the enzyme X-amylose, and they also bind to grain starches to varying degrees. The low starch digestibility has also been attributed to a high content of dietary fiber. Reduction in sorghum digestibility also observed in cooked sorghum and it has been estimated that the digestibility decreases by around 24–31%. It is indicated that starch digestibility in cooked sorghum flour has been attributed to the formation of disulphide bonds during cooking, which leads to toughening at the surface and interior of protein bodies (Elkonin *et al.* 2013) ^[15]. Sorghum with pigmented testa can develop condensed tannins. Some of these tannins retard the hydrolysis of food by reducing the digestibility of proteins and starch, thus increasing dietary fiber levels. The presence of condensed tannins can therefore be considered as an antinutritional factor for monogastric animals (Awika *et al.*, 2005) ^[6].

A nutritional constraint to the use of sorghum is the poor protein digestibility when wet cooked. A protein with high digestibility is potentially of better nutritional value than one of low digestibility because it would provide more amino acids for absorption on proteolysis. In mixed diets containing marginal or low protein contents and where the percentage of sorghum is high, increased protein digestibility would provide much needed protein to the consumer (Duodu *et al.*, 2003) ^[11]. As per Duodu *et al.*, (2003) ^[11] factors that contribute to the poor protein digestibility of sorghum may be divided into two broad categories: exogenous and endogenous factors. Exogenous factors arise out of the interaction of sorghum proteins with non-protein components like polyphenols, non-starch polysaccharides, starch, phytates and lipids. Whereas endogenous factors resulted from changes within the sorghum proteins themselves without interaction with non-protein components.

The first extraneous factors that affect sorghum protein digestibility is the organizational structure of the sorghum grain. Sorghum protein digestibility depends on the form in which the grain is provided and a study indicated the variability upon different levels (Duodu *et al.*, 2002) ^[2]. In vitro protein digestibility assays have been conducted on either whole grain, decorticated grain, endosperm and or some undefined commercial grain fraction which resulted differing proportions of pericarp, endosperm and germ and also different types of protein. A general picture which emerges is that in vitro protein digestibility of sorghum is improved as the proportion of pericarp and germ material becomes less (Duodu *et al.*, 2002) ^[2].

In high-tannin sorghum varieties, the formation of indigestible protein–tannin complexes is a major limiting factor to protein utilization. Sorghum tannins are known to inhibit enzymes such as amylases. However, it has been suggested that the antinutritional effect of sorghum tannins lies in their ability to

form less digestible complexes with dietary protein and not by inhibition of digestive enzymes (Butler *et al.*, 1984^[16]; Wong *et al.*, 2009)^[9].

The phytate molecule is highly charged with six phosphate groups and so is an excellent chelator, forming insoluble complexes with mineral cations and proteins (Hung, 2016)^[17]. This leads to reduced bioavailability of trace minerals and reduced protein digestibility.

In sorghum and maize endosperm, starch granules and protein bodies are in very close association with each other. The largely polygonal, tightly packed starch granules are surrounded with numerous, largely spherical protein bodies embedded in a protein matrix (Duodu *et al.*, 2002)^[3]. The implication of such a close association between starch and protein may be that the starch, especially when gelatinised after cooking, could reduce the accessibility of proteolytic enzymes to the protein bodies and therefore reduce protein digestibility.

One of the endogenous factors that affect protein digestibility is protein crosslinking during processing, the physical and chemical conditions proteins encountered can result in changes ranging from subtle changes in the hydration of the protein to thermal destruction (pyrolysis) with potential formation of mutagens. The other one is racemization and isopeptide formation. The process whereby L-amino acids are converted to the D form is known as racemization. Racemization of amino acids is believed to be a prelude to the formation of isopeptide bonds in proteins (Friedman *et al.*, 1981)^[18]. These isopeptide crosslinks may decrease the digestibility and bioavailability of proteins. Disulphide crosslinking affects protein digestibility since it has a direct correlation with kafirin solubility during cooking. Kafirin and zein hydrophobicity also reduce protein digestibility and both are known to be hydrophobic proteins. It has also been suggested that the kafirins are less soluble (therefore more hydrophobic) than the zeins (Wong *et al.*, 2009)^[9]. Enzymes function in an aqueous environment. Therefore, if the kafirins are indeed more hydrophobic, they may be generally less accessible to enzymes and hence less digestible than the zeins. In certain sorghum varieties, the presence of condensed polyphenols or tannins in the grains is another factor that adversely affects protein digestibility and amino acid availability. A decrease in the protein digestibility of sorghum on cooking was attributed to the reduced solubility of prolamin and its reduced digestibility by pepsin (Elkonin *et al.*, 2013)^[15]. Tannins bind sorghum kafirin (prolamine rich protein) resulting in reduced protein levels, and consequently lower and slower starch digestibility of tannin sorghum (Butler *et al.*, 1984)^[16].

The prolamin storage proteins found in sorghum endosperm are called kafirins. Four subclasses, α -, β -, δ - and γ -kafirin, have been described based on their protein characteristics. The kafirins are stored in discrete protein bodies, which form a tight matrix with the starch granules in mature endosperm and contribute to grain hardness, digestibility and processing quality of sorghum (Wong *et al.*, 2009)^[9]. The β - and γ -kafirins are highly cysteine-rich proteins and appear to form both intra- and inter molecular disulfide bonds. This in turn leads to a higher degree of protease resistance in these seed

storage proteins. As these kafirins are on the periphery of the protein body in the mature grain they contribute to sorghum's lower protein digestibility relative to other cereals (Oria *et al.*, 2000)^[19].

Phenolic compounds

Phenolic compounds found in all sorghums with the highest content compared to other cereals (Hung, 2016)^[17]. However, the amount of the compound present in any particular genotype is influenced by the genotype and its growing environment. Sorghum phenols protect plants against insects and diseases and act as antioxidant to play a role like antifungal, antibacterial, and antiviral agents. Phenolic compounds can affect the color, appearance, and nutritional quality of grain and products and it can be divided into three categories: tannins, flavonoids, and phenolic acids. It has advantages and disadvantages for nutritional quality and health. The phenolics mostly responsible for astringent taste of the product but nowadays it acts as antioxidants which prevent cancer development in human body (Awika and Rooney, 2004)^[1]. Phenolic compounds are a heterogeneous group of compounds extensively studied for their polyphenol composition of food, and on polyphenol bioavailability, metabolism and biological effects (Hung, 2016)^[17].

The specific profile and proportion of phenolic compounds in sorghum depends on the pericarp color and presence of a pigmented testa or red and brown sorghum grains (Chhikara *et al.*, 2019)^[20]. On the other hand, several researchers indicated the intensity of the pericarp color does not correlate with the content of condensed tannins, and one of the erroneous ideas about sorghum is that all red and brown varieties contain condensed tannins. There were numerous reports that this cereal cannot be used as an ingredient in human or animal diet. However, even sorghum high in tannins are already used to prepare many foods and beverages, from beer to popped sorghum. Sorghum offers the opportunity to produce foods with various natural colors, high levels of dietary fibers and antioxidants (Llopart *et al.*, 2013)^[21].

Tannins are the secondary metabolites found in sorghum, barley, millet and many plant species but absent in major cereals. According to their type, content and distribution of the individual oligomers and polymers tannins in sorghum classified into three types. There are no significant levels of tannins (Type I), tannins that are extractable only in acidified methanol (Type II), and tannins that are extractable in methanol and acidified methanol (Type III) (Hahn and Rooney, 1984)^[22].

Sorghum grain contains approximately 10% protein and therefore in theory, high-tannin cultivars would contain more than enough tannin (2–4%) to bind all the seed protein (Butler *et al.*, 1984)^[16]. Lower protein yields were obtained for high-tannin compared with low-tannin (condensed tannin free) sorghum on subjecting both grains to the Landry–Moureaux protein fractionation procedure (Wong *et al.*, 2009)^[9]. This was due to interactions between tannin and the albumin, globulin and prolamin proteins, rendering most of the proteins insoluble. Furthermore, electrophoresis indicated that proteins extracted from high-tannin sorghum were bound to tannins. Generally, the characteristics of proteins that bind strongly to

sorghum tannin are that they are relatively large, have a loose, open structure and are rich in proline (Butler *et al.*, 1984)^[16].

Tannins are structurally very diverse, and derived their name from their ability to tan leather, which involves the tannins binding to collagen protein. Tannins are also known for their interaction with salivary proteins which causes the feeling of astringency commonly experienced when consuming wine, tea, fruit skin, etc. Tannins in the grain reduce the availability of minerals, proteins, and starch. Although the anti-nutritional effects, there are several merits for stress resistance of the crop and also oligomers of tannin in foods produced antioxidants as health promoting food. Sorghum genotypes with high tannin mostly have a brown color and it makes the crop resistant to bird attack and fungal infection. Sorghum grains are a major source of anthocyanins which is used as food coloring agent and antioxidant. Tannins are reported to have vascular protective and anti-inflammatory, anticancer, and chemoprotective property (Larsson *et al.*, 2005)^[23].

The other major phenolic compound in sorghum is flavonoids. Flavonoids are widely distributed secondary plant metabolites which is a class of phenolic compounds that share a basic C6-C3-C6 structure, consisting of two aromatic rings joined by a three carbon heterocyclic ring. More than 5000 flavonoids occur in nature differing in substitution patterns on the benzene rings and the heterocyclic ring. Common classes of flavonoids based on heterocyclic ring structure included anthocyanins, flavanols, flavones, isoflavones, flavanones, flavonols, and the polymeric proanthocyanidins. Flavonoids play an integral role in plant growth, reproduction and function. High concentration helps to protect against disease, pests, and UV damage and also produced inducibly by plants during stress (Nicholson and Hammerschmidt, 1992)^[24].

Flavonoids found in sorghum are very diverse and commonly not found in other cereal grains. The high levels of compounds like 3-deoxyanthocyanins, pro (3-deoxy) anthocyanidins, flavones, and flavanones in certain sorghum varieties are especially of interest both from a commercial and health perspective. The high levels of flavanones in lemon yellow sorghum varieties, and proanthocyanidins in type III sorghum could provide an economical way to obtain these well-known bioactive compounds for nutritional applications. The epidemiological evidence that indicate sorghum consumption may be more chemoprotective indicating that 3-deoxyanthocyanins are more cytotoxic to cancer cells (Larsson *et al.*, 2005)^[23]. Sorghum phenolic extracts are more powerful antioxidants than other grains, or fruits and vegetables, that can significantly contribute to chronic disease prevention. The antioxidant activity in various sorghum varieties is strongly correlated with their phenol and flavonoid content. From tested genotypes of sorghum, tannin sorghums have the strongest antioxidant capacity; this is attributed to the generally higher free radical scavenging power of tannins relative to simple flavonoids (Awika *et al.*, 2005)^[6]; Awika and Rooney, 2004)^[1]. These flavonoids in sorghum are controlled by a set of well documented genes and its possible to breed sorghum for desired level of flavonoids for food/health applications or other purposes.

Phenolic acids exhibit high antioxidant activity in vitro and thus are beneficial for human health. These acids are

derivatives of benzoic and cinnamic acids and found in sorghum grain as well as other cereals (Nicholson and Hammerschmidt, 1992)^[24]. Hydroxybenzoic acids and hydroxycinnamic acids are the two classes of these acids. Free phenolic acids are found in the outer layers of the kernel (pericarp, testa, and aleurone), whereas the bound phenolic acids are associated with the cell walls. Sorghum phenolic compounds are mostly concentrated in the bran and found in bound forms. As per Hung (2016)^[17], there are several abundant bound phenolic acid in sorghum including syringic, protocatechuic, caffeic, p-coumaric, sinapic and ferulic in which the last one found to be the most abundant. Gallic acid is found only in bound form, whereas cinnamic acid is found only in free form and salicylic acid found in sorghum (Hung, 2016)^[17].

Breeding and genetics perspective of sorghum for nutritional quality traits

The first challenge for a breeder is to determine which traits are most important and which trait is most responsive to give a breeding solution. For a better understanding of the traits and to plan our breeding strategy first we have to characterize the genotypes and get descriptive information about the traits similarities and differences among genotypes. Morphological, nutritional and genotypic characterization of genotypes could be possible using phenotypic descriptors, biochemical and molecular markers. Accurate estimation of genotypic and varietal differences does have paramount importance for crop improvement efforts and helps the breeder to determine variation and decrease genetic loss. This estimation and evaluation of diversity levels are a prerequisite for utilization in cultivar improvement.

Even though the agronomical, morphological and nutritional characterization provides useful information to breeders, the challenges are that they may be easily influenced by environmental factors, with long growing duration and the plants must be assessed during a fixed growing phase of the crop. Therefore, breeders tend to use molecular marker systems because to avoid those problems associated with environmental variation, time, developmental stages, and increase effectiveness and find reputable data. Understanding the natural variation and genetic architecture of grain quality traits in sorghum is a first step towards improvement of the nutritional quality through conventional and molecular breeding.

Sorghum, considered as often cross-pollinated crop and it is suitable to exploit breeding procedures applicable for both self and cross-pollinated crops. This means that a sorghum breeder can develop a homozygous line as a variety or can exploit heterosis through hybridization. The general distinction of breeding techniques characterized by three main steps namely; induction of variation by creation of crosses or by variation inducing treatments, selection of desired traits in new varieties and propagation and multiplication of breeding lines. At each step different techniques can be applied. It can generally distinguish between plant breeding techniques which have an impact at plant/population level, cell/tissue level and DNA level.

General combining ability (GCA) expressed as additive gene

action and specific combining ability (SCA) due to non-additive gene action is important genetic parameter to decide the next phase of breeding program. The combining ability studies allow breeders to choose the good combiners and discard the poor combiners. 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 hybrids showed high heterotic effects for yield as well as protein (Collins and Pickett, 1972)^[25].

The nutritional value of sorghum becoming increasingly important in current plant breeding programs. The nutritional quality and end use value of sorghum grains are primarily influenced by the proportions of tannins, starch and proteins, but the genetic basis of these grain quality traits remains complex. Research conducted on the grain composition about protein, fat and starch all having a strong correlation across the years. From this protein was the most consistent at 73–82% correlation between the three years. Similarly, protein had the highest narrow-sense heritability ($H^2 = 0.90$), followed by fat ($H^2 = 0.85$) and starch ($H^2 = 0.80$). Pearson's correlations between protein, fat, and starch depicts there was a strong negative correlation between starch and both protein and fat, and a strong positive correlation between protein and fat (Rhodes *et al.*, 2017)^[5].

Genetic variability was observed in sorghum for protein digestibility. The inheritance of protein digestibility in sorghum showed that in vitro protein digestibility inherited as additive trait and F1 hybrids had intermediate level of digestibility (Hicks *et al.*, 2002)^[26]. In contrast Winn *et al.*, (2009)^[27] and Elkonin *et al.*, (2013)^[15] indicated that two different loci involved in genetic control of high digestibility traits in sorghum.

The major limiting factor in sorghum protein content improvement is lysine content. Several researchers have been done a lot of efforts to improve sorghum nutritional quality based on the identification of high lysine mutants (Nelson *et al.*, 1965)^[28]. In sorghum two mutants were identified, the *hl* gene in an Ethiopian line (Singh and Axtell, 1973)^[29] and the P721 opaque gene which was induced with the chemical mutagen diethyl sulphate (Axtell *et al.*, 1979)^[30]. Lines containing these gene contain more lysine-rich proteins enhance up to 50% and free amino acids with compensatory low prolamins which results reduced kafirin by about 50%. This improvement of protein quality is usually associated with a negative effect on seed weight and yield. Many research were carried out to develop line with high protein digestibility from a cross involving the high lysine P721 opaque mutant and other sources through mutation using gamma irradiation and cyclotron (Oria *et al.*, 2000)^[19].

Efforts has been done to transform sorghum genetically with the use of *Agrobacterium* and through the process of genetic engineering techniques for improvement of nutritional quality. Yohannes *et al.* (1999)^[31] made the first attempt to improve sorghum grain quality through genetic engineering and resulted mutated gene *dhdps-rl* which lead to increased lysine accumulation. Sorghum is low in essential amino acid, lysine, and increasing this limiting amino acid is a good strategy to improve sorghum grain quality. 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)^[32]. Sorghum transformation also conducted to suppress synthesis of various kafirins (α , δ and γ kafirins) to get sorghum transgenic lines with high protein digestibility, improved amino acid score, and protein digestibility corrected amino acid score. Methods of genome editing, in particular, editing the nucleotide sequences of the α - or γ -kafirin genes, opens wider prospects for improving the digestibility of kafirins and increasing the nutritional value of sorghum grain. This implicates that an improved lysine transgenic variety can benefit communities by eradicating malnutrition.

Significant genetic variation of starch digestibility in sorghum hybrids had been shown, although digestibility level of the F1 had not been compared with the digestibility of their parental lines. In vitro starch digestibility content in the F1 hybrids corresponds to the level of maternal parent where parental lines significantly differed one from another by this trait. Assuming that genetic input of both parents in endosperm development in F1 hybrids is equal a study suggest possible involvement of any cytoplasmic factor(s) in genetic control of starch digestibility. The research also indicated that the higher the total starch the lower the digestible starch and vice versa. It also testify that total starch content is not enough for characterization of entry nutritive value, and a more correct conclusion could be obtained by analysis of the digestible starch level (Elkonin *et al.* 2013)^[15].

Starch of non-waxy sorghum has approximately 75% and 25% amylopectin and amylose, respectively while waxy starch contains approximately 100% amylopectin (Rooney and Pflugfelder, 1986)^[33]. The amylose content of sorghum plants depends on the genotypes and the growing environment. A sufficient amount of variability was observed for amylose content between sorghum genotypes which depicts a possible improvement through breeding intervention (Gerrano *et al.*, 2014)^[14]; Beta and Corke, 2001)^[12]. Grains with low amylose contents (1-2%) are termed “waxy”, and are associated with homogenous recessive genes (*wxwxwx*). Studies have shown that starch is more readily digested in waxy sorghum than in non-waxy type of sorghum (Rooney and Pflugfelder, 1986)^[33]. Non-waxy grains are those with normal levels of amylose (23-28%). Accessions grouped as heterowaxy contain lower amylose content than non-waxy grains, which contains 24 to 30% amylose. Heterowaxy grains have lower amylose contents than normal grains (20%) but display many of the physical attributes of nonwaxy grains (McDonough *et al.*, 1998)^[34]. Alkali spreading phenotype is one of a good indicator for starch quality. Griebel *et al.* (2019)^[35] showed that *SSIIa* and *SBEIIb* are key genes controlling the alkali spreading phenotype in mutant populations of sorghum. On the other hand these genes have contrasting effects on starch GT, amylose content, and paste viscosity profiles (Griebel *et al.*, 2019)^[35]. Therefore, further study should be conducted to evaluate the effect of alkali spreading phenotype on starch quality and the contribution of identified genes.

Tannins are the secondary metabolites found in sorghum which act as anti-nutritional compound and on the other hand as defensive mechanism for the plant from harmful

pathogens. There are different type of tannin in sorghum as mentioned in above however, sorghum grain contains approximately 10% protein and therefore in theory, high-tannin cultivars would contain more than enough tannin to bind all the seed protein (Butler *et al.*, 1984)^[16]. The genetics of tannin accumulation in sorghum is well documented. Generally sorghums without the pigmented testa do not contain tannins; the presence of the pigmented testa is controlled by *B1_B2_* genes (Rooney *et al.*, 2000)^[36]. Dominance at both loci is required for a pigmented testa to be present; for example, *B1_b2b2* or *b1b1B2_* will not have the pigmented testa, and thus will not contain any tannins. Therefore, marker assisted breeding techniques could be usefull to reject these dominant gene through selection process and produce good quality sorghum with low level of tannin. Research conducted on antinutritional factors of wild and cultivated sorghum accessions revealed there is a high level of variation and some of Sudanese wild genotypes contain less amount of antinutrients than those of released sorghum cultivars with regard to tannin, total polyphenol, and phytic acid content (Ahmed *et al.*, 2014)^[10]. Therefore, efforts must be devoted to identify valuable alleles which have been left behind in the wild ancestors of crop plants and to reintroduce them into cultivated crops to make the breeding program more effective.

Conclusion

Understanding major quality traits of sorghum will lead to prioritization of the breeders objective to conduct targeted breeding that result a product which reduce hunger and malnutrition. Several authors indicated that sufficient amount of variability were observed in sorghum accessions and their wild relatives on grain nutritional quality traits such as seed protein, starch, tannin, flavonoids, starch and protein digestibility which will certainly allow selection for specific applications to produce improved varieties. Grain sorghum is low in protein content especially in essential amino acids such as lysine, and threonine. Apart from these protein digestibility is critical problem that needs to be adressed. In sorghum grain, starch is the major component and energy supplier and amylose/amylopectin ratio is an important characteristic of starch that governs much of starch functionality. Sorghum grain starch from waxy varieties contains very low amylose which resulted enhanced starch digestibility. Generally, several candidate genes and QTLs for lysin, amylose/amylopectin ratio, tannin and other phenolic compounds have been identified and thus further study should be conducted to verify and utilize the genes effectively in the breeding program.

Conflict of interest

The author declares that there is no conflict of interest.

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