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Phenotypic and biochemical screening of sorghum genotypes for growth and rice weevil resistance in Tanzania

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PHENOTYPIC AND BIOCHEMICAL SCREENING OF SORGHUM GENOTYPES FOR GROWTH AND RICE WEEVIL RESISTANCE IN TANZANIA

Emmanuel Thomas Mwenda

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The current study characterised sorghum genotypes based on their growth morphological variations; identified phenotypic and biochemical traits, and screened them for resistance to rice weevil (*Sitophilus oryzae*). A total of 117 sorghum genotypes were characterised using 10 qualitative and 10 quantitative growth morphological traits using sorghum descriptors. After preliminary yield screening, only 98 sorghum genotypes were used for the study. Data were subjected to one-way analysis of variance using GenStat version 15. Multivariate analysis and Pearson correlation was done using Mintab statistical software version 14. The study revealed significant levels of variability among sorghum genotypes ($p<0.001$) in terms of growth morphological traits, kernel phenotypic traits, biochemical and susceptibility to rice weevil. Positive and significant correlation was observed between grain yield and yield related parameters. Days to 50% flowering showed highly positive and significant association with plant height, number of leaves, and days to maturity. Genotypes IESH23022, IESV91104DL and IESV92172 were the best candidates for source of earliness and yield traits. Nine genotypes namely PATO, IESV92041SH, ATX623 x AIGD34533, UDO, Mbangala white, IESV74 DL, IESV92172, ICSA15 x R8602, and P9504A x ICSR172 recorded lower F1 progeny emergence, susceptibility index, median development period, kernel damage, and weight loss indicating that the genotypes were resistant to rice weevil. Kernel hardness indicated strong significant correlation with susceptibility parameters ($p<0.001$). Therefore, lines with adequate kernel strength are recommended for breeding against devastating storage pest, the rice weevil in Tanzania.

DECLARATION

I, Emmanuel Thomas Mwenda, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology (NM-AIST) that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Signed……………………....……   Date…………………………..…….
Emmanuel Thomas Mwenda

The above declaration is confirmed;

…………………………....…………………………   Date…………………
Dr. Ernest R. Mbega (Principal supervisor)

…………………………....…………………………   Date…………………
Dr. Justin H. Ringo (Co-supervisor)

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CERTIFICATION

This is to certify that, the dissertation titled "Phenotypic and Biochemical Screening of Sorghum Genotypes for Growth and Rice Weevil Resistance in Tanzania" submitted by Emmanuel Thomas Mwenda (M341/T.16) in partial fulfilment of the requirements for the award of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

…………………………....………………………   Date……………………

Dr. Ernest R. Mbega (Principal supervisor)

…………………………....………………………   Date……………………

Dr. Justin H. Ringo (Co-supervisor)

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Last but not least to our family including my lovely father Mr. Aidan Thomas Mwenda, and brothers Benedict, Mathew, Erick and Anthony for their enormous support and encouragements during the whole period of my study.

DEDICATION

With great respect, I dedicate this work to my father Mr. Aidan Thomas Mwenda, for laying foundation of my study, his support, prayers and tireless encouragement made me reach this high.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of variance
C	Centigrade
Cm	Centimetre
CV	Coefficient of variation
CREATES- FNS	Centre for Research, Agriculture Advancement, Teaching Excellence and Sustainability in Food and Nutrition Security
DNMRT	Duncan New Multiple Range Test
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
F1	First Filial generation
Fig	Figure
Fprob	Fixation probability
G	Gram
GenStat	General statistics
Ha	Hectare
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
ICRISAT	International Crops Research Institute for the Semi- Arid Tropics
Kg	Kilogram
LSD	Least Significant Difference

M	Metre
MDP	Median Development Period
MAS	Marker Assisted Selection
N	Nitrogen
NPGRC	Tanzania Plant Genetic Resources Centre
NM-AIST	Nelson Mandela African Institution of Science and Technology
P	Phosphorus
p	Probability
PCA	Principal component analysis
QTL	Quantitative Trait Loci
RCBD	Randomised complete block design
r.p.m	Revolution per minute
SI	Susceptibility Index
SNP	Single Nucleotide Polymorphism
SSRs	Simple Sequence Repeats
SUA	Sokoine University of Agriculture
TARI	Tanzania Agricultural Research Institute
TPRI	Tropical Pesticide Research Institute

CHAPTER ONE

INTRODUCTION

1.1 Background

Sorghum (*Sorghum bicolor* L. Moench) belongs to the Poaceae family which covers all grasses (OGTR, 2017). It is the world fifth important cereal crop next to wheat, maize, rice and barley (Weledesemayat *et al.,* 2016; Oyier *et al.,* 2016). Sorghum is important in semi-arid and arid areas where it out-performs many crops under harsh climatic conditions (Tsusaka *et al.,* 2015). It is cultivated in 40-45 million hectares (ha) with an estimated annual production of 55.7 million tons worldwide (Sarmiso, 2015). In Africa, sorghum is the second most important crop after maize with 22% of total area (Macauley, 2015). In Tanzania; sorghum occupies more than 700 000 ha with a production of about 500 000 MT (Schipmann-Schwarze *et al.,* 2014). The crop is used for food, feed and fodder and in the production of starch, fibre, dextrose syrup, biofuels and alcohol (Sinha and Kumaravadivel, 2016).

Sorghum has a wide genetic diversity in its physical structure and or chemical composition and therefore presenting benefits in breeding for various stresses (Bean, 2016). Understanding of morphological descriptors such as shape, colour, pigmentation, maturity, grain size, weight, yield and pest resistance is critical when studying the variability of sorghum genotypes (Prajapati *et al.,* 2018). These qualitative and quantitative traits provide descriptive evidence of the differences and similarities of sorghum genotypes (IBPGR/ICRISAT, 1993). Morphological evaluation of genetic materials employs morphological markers that can be visually analysed and provide estimates of the variability in plant resources (Ahmad *et al.,* 2017). Variation existing in kernel physical structure, nutritional composition and phytochemical composition is critical for selection of desired traits in sorghum breeding including insect pests (Gerrano, 2014).

According to OGTR (2017) farmers store about 60-80% of the sorghum grains produced. In storage 50% of grains loss is caused by biotic and abiotic factors (Nukenine, 2010). The abiotic factors include inappropriate temperature, humidity and rain while biotic factors includes insect pests, rodents and storage microbes (Kumar and Kalita, 2017). In Tanzania most farmers store harvested grains using unimproved traditional granaries which usually provide favourable conditions for development of storage insect pests (Rugumamu, 2003), of

which rice weevil (*Sitophilus oryzae* Linn.) is a most important grain feeding insect pest (Reddy *et al.,* 2002; Ladang *et al.,* 2008). This weevil is regarded as an economically important storage pest on sorghum and other cereals in tropical and sub-tropical regions with high humidity and temperature worldwide (Zunjare *et al.,* 2015). Rice weevil can cause approximately 83.5 percent infestation over a period of six months in susceptible sorghum grains in stores (Jagginavar, 2015). The weight loss in stored sorghum has been indicated to be caused by both larval and adult feeding, with a major damage being done by larvae eating inside the kernel (Bala and Vyas, 2016).

Rice weevils can induce up to 75% losses of sorghum seed stored under traditional storage system due to its ability to reproduce (Gerema *et al.,* 2017). For instance, in a period of 100 days a single rice weevil female can produce 24 adults. During this period, losses of grains due to weevils can range from 25 to 40 percent based on conditions and levels of susceptibility of stored sorghum (Pradeep *et al.,* 2015). Major losses due to rice weevil include reduction of seed weight and germination capacity, decreased nutritional quality and commercial values resulting from contaminants such as uric acid, insect body fragments, toxic substances and attack by storage fungi (Kiio *et al.,* 2012; Prasad *et al.,* 2015).

Different control strategies of rice weevil such as synthetic chemical insecticide, physical, cultural, biological and host plant resistance have been recommended worldwide (Mofokeng, 2016). However in Africa only few farmers can afford the use of commercial synthetic insecticides since they are associated with high price and in some locations they are less available (Gerema *et al.,* 2017). Moreover, application of synthetic chemical insecticides has been associated with inducing resistance to the insects as well as causing environmental and health problems (Talebi *et al.,* 2011). As a result, there is fear of their application especially in grains used for food (Gracen and Guthrie, 2015).

Of the control methods, crop resistance has been recommended to be the most important and sustainable approach against rice weevil (Chandrashekar and Satyanarayana, 2006). However there is limited information on the availability of resistant sorghum varieties especially in most African countries including Tanzania (Mofokeng, 2016). It is already known that both phenotypic characteristics and biochemical parameters of sorghum seed seem to be associated with resistance to rice weevil in sorghum grain (Pradeep *et al.,* 2015). There is variations among sorghum genotypes on seed size, pericarp thickness and pigmented testa which are associated with presence of condensed tannins (Valencia and Rooney, 2009).

According to Dasbak *et al.* (2009) the smaller and harder the sorghum grains are, the more resistant the grains to insect attack. In addition grain coat characteristics discourage oviposition, inhibits digestive enzyme and increase kernel hardness which enhance resistance to rice weevil (Gerema *et al.,* 2017).

Other factors associated with sorghum grain resistance to insect pests include grain nutrients and composition of compounds including amylaceous, protein, phenolic, flavonoids and other substances (Dobie, 1977; Torres *et al.,* 1996; Dykes and Rooney, 2006). For instance sorghum genotypes with a pigmented testa contain condensed tannins and black genotypes have higher level of 3-deoxyanthocyanidins (Waniska, 2000; Dykes *et al.,* 2014).

The current study intended to phenotype sorghum genotypes; and identify phenotypic and biochemical traits associated with resistance to the rice weevil. Resistant genotypes would be utilised in sorghum breeding programs for development of resistant sorghum varieties to rice weevils. This will contribute to improved sorghum breeding and reduced costs in weevil management to farmers.

1.2 Problem statement and justification

Rice weevil (*Sitophilus oryzae* Linn.) is a major devastating insect pests attacking sorghum grains during storage and causing significant losses to sorghum farmers (Gerema *et al.,* 2017). It has been reported that rice weevil can induce between 15 and 77 % sorghum grains losses when stored in unimproved traditional storages (Mofokeng, 2016). The damaged grain expresses decreased germination, nutritional values, reduced weight and market values (Gerema *et al.,* 2017). Large post-harvest losses and reduction in quality of sorghum grains caused by rice weevils is among the main impediments to achieving food security and better income among sorghum farmers in developing countries especially Tanzania.

Reports indicated that most of sorghum genotypes including improved released varieties, local cultivars and advanced breeding lines in Tanzania were not characterised based on their growth morphological variation and their susceptibility to rice weevils (Kiio *et al.,* 2012). Sorghum grain storage require use of synthetic insecticides which are expensive and few farmers can afford buying them (Stadlinger *et al.,* 2011). In addition, synthetic chemicals are associated with human health hazards such as poisoning, neurotoxicity, impaired development, endocrine disruption and cancer (Tago *et al.,* 2014). Improper application may kill beneficial organisms and creates genetic resistance to rice weevils (Talebi *et al.,* 2011).

Thus to reduce the risk of exposure to synthetic chemical application to farmer, there is urgent need to develop more sustainable methods of which resistance is highly recommended. In Tanzania, however, little has been done on the identification of resistant sorghum genotypes to rice weevil and the relationship between rice weevil resistance and the growth characteristics, kernel phenotypic traits and biochemical properties of sorghum genotypes has not been documented. This research therefore intends to phenotype sorghum genotypes and identify phenotypic and biochemical traits associated with resistance to the rice weevil.

1.3 Research objectives

1.3.1 Overall objective

To phenotype and screen sorghum genotypes for resistance to rice weevils and determine the association between resistance to phenotypic parameters and biochemical components of the seeds.

1.3.2 Specific objectives

(i)   To characterise sorghum genotypes based on their growth morphological variation.
(ii) To screen sorghum genotypes for resistance to rice weevils.
(iii) To identify phenotypic traits in sorghum grain associated with resistance to rice weevils.
(iv) To identify biochemical traits in sorghum grain associated with resistance to rice weevils.

1.4 Hypothesis

H_0: There is no relationship between phenotypic and biochemical characteristic of sorghum with resistance to rice weevil.

H_1: There is a relationship between phenotypic and biochemical characteristic of sorghum with resistance to rice weevil.

1.5 Significance of the study

This research will enable clients and users of this document to profile growth morphological variations, which are critical in germplasm collection, conservation and selection during hybridization. Understanding of phenotypic and biochemical variability in sorghum genotypes can enable selection of useful traits convening resistance to rice weevil. Host plant

resistance is more effective weevil management strategy compared to chemical control; the later develop genetic resistant to rice weevil, kills non-targeted organisms, and has many health hazards to human such as poisoning. Identification of weevil resistant genotypes can be selected as parental materials in sorghum breeding programs in order to develop new varieties and hybrids with adequate weevil resistance, in so this current research on screening sorghum resistant genotypes to rice weevil was eminent.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sorghum

2.1.1 Sorghum origin and distribution

Sorghum [*Sorghum bicolor* (L.) Moench] was originally domesticated about 7000 years ago in northern horn of Africa (Belay and Atsbha, 2016). Sorghum is mainly found in Africa, Asia, Australia, and some parts of America (Prasad and Staggenborg, 2009). The distribution is associated with several reasons including trade and human migration in Africa and Middle East. Currently, the main sorghum producing regions in the world include the Sub-Saharan Africa, North America, central India and northeast China. Large sorghum producing countries in the world are United States, Mexico, India, Nigeria, Niger and Sudan (Prasad and Staggenborg, 2014).

2.1.2 Sorghum genetics and classification

Sorghum is classified under the family of Poaceae, tribe Andopogoneae, subtribe Sorghinae and genus Sorghum (Prasad and Staggenbord, 2010). This crop is a short-day C4 plant (Mullet *et al.,* 2012), and its easy adaptable to hot and dry agro-ecologies making it a climate change responsive crop. Sorghum is self-pollinated by nature with an out crossing up to 6% depending on the genotype and growing conditions (Hariprasanna and Patil, 2015). Sorghum (2n = 2 × = 20) can be classified into two groups, the wild and the cultivated sorghums; the later has been classified into five major races; *bicolor, caudatum, durra, guinea* and *kafir* (Prasad and Staggenborg, 2009; Kumari, 2011; Gerema *et al.,* 2017).

2.1.3 Growth characteristics of sorghum

Growth in sorghum can be categorised into phases ranging from emergence to physiological maturity (White *et al.,* 2005). The first stage is known as vegetative growth; of which, vegetative structures such as tillers and leaves develops (Gerik *et al.,* 2003). However, as the number of leaves increase in sorghum delays maturity. The second stage involve the reproductive structures such as panicle initiation and development, flag leaf formation and booting (White *et al.,* 2005). The third stage include flowering characterised by yellow anthers, followed by seed formation processes such as milk, soft dough, hard dough and physiological maturity observed by appearance of black layer on seed attachment and seed formation (Gerik *et al.,* 2003; Cirino *et al.,* 2013). Morphologically, sorghum possess

qualitative and quantitative traits that contribute to growth variation (Shargie *et al.,* 2005; Sinha and Kumaravadivel, 2016). Growth characteristics can be evaluated through morphological, molecular and or biochemical procedures of genetic resources (Prajapati *et al.,* 2018). More research on growth characteristics including modelling experiment in sorghum is needed; as understanding of growth and phenology is necessary in variability studies.

2.1.4 Common methods used for characterizing sorghum genotypes

(i) Morphological characterisation

Morphological characterisation of genetic materials employs morphological markers that can be visually analysed and in plant resources it estimates the variability. According to Prajapati *et al.* (2018) morphological traits include colour, shape, pigmentation, texture, growth characteristics, maturity, yield and pest resistance. In conventional sorghum breeding, these traits can be identified through observational and visual selection (Ahmad *et al.,* 2017). Therefore characterization requires a collection of germplasm so as to identify materials suitable for hybridization and conservation of genetic resources (Prajapati *et al.,* 2018). The method is highly affected by the environment and agronomic practices. Despite its restraint, this method is very useful to sorghum breeders because it is easy and low cost. Therefore, morphological characterization is useful in evaluating sorghum genotypes based on growth descriptors to find the variability that can be exploited by breeders in resistant cultivar development.

(ii) Biochemical characterization

Bio chemicals are processes and substances found in organism made up by carbon and are important for normal processes for pursuing special engagement in activities such as respiration, digestion, growth and structural function. The compounds include protein, carbohydrate such as starches, sugars and cellulose, lipids and nucleic acid (Ogbaga *et al.,* 2016). In sorghum there are variations in chemical molecules including phenolic compound such as phenolic acids, tannin and flavonoids (Afify *et al.,* 2012; Chiremba *et al.,* 2015). Biochemical characterization of sorghum genotypes is required to profile important chemical related traits and ascertain its relationship with resistance to rice weevil. These chemicals could assist in breeding for weevil resistant cultivars.

(iii)   Molecular characterisation

Molecular based evaluation involves analysis of genetic materials at molecular level using DNA markers. According to Da Silva *et al.* (2017) molecular characterisation complements the phenotypic assessment. Genetic evaluation of sorghum genotypes may employ DNA based molecular markers such as Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP), Single Nucleotide Polymorphism (SNP), Random Amplified Polymorphic DNA (RAPIDs) or Restriction Fragment Length Polymorphism (RFLPs) (Ahmad *et al.,* 2017). Molecular markers are useful in genetic conservation and utilization of germplasm as it provide reliable data in analysing genetic diversity and divergence in a population (Mehmood *et al.,* 2015). Play role in mapping population; including identification of Quantitative Trait Loci (QTL) and for the marker assisted selection (MAS), enabling breeders in detection, labelling and tagging or tracking of genes and the overall genetic variation (Madhusudhana *et al.,* 2012). Unlike morphological evaluation, genetic characterization is not influenced by the environment and growth stage of the plant. The use of molecular marker (SSRs) is influenced by the simplicity, the extent of relatedness of samples, knowledge, objectives and high throughput. For instance, in sorghum simple sequence repeats appears to be very inexpensive and discriminative; have been effective in exposing crop diversity. The advantage of SNPs are codominance, high throughput analysis, and specificity (Liu *et al.,* 2010). According to Da Silva *et al.* (2017) the cost associated with this technique especially the processing time have been greatly reduced by range of available SNP genotyping platforms; therefore providing options of using SNP markers in breeding while exploiting techniques such as genotyping by sequencing that provides room for breeders utilising multiplexed sequencing and conducting genome wide scanning. Thus, molecular characterization is important to confirm traits identified through visual observation at DNA level. Utilization of this method could speed up sorghum breeding through exploitation of marker-assisted breeding.

2.1.5 Production constraints of sorghum

Sorghum production is constrained by several stresses from field to storage. Sorghum is highly affected by both abiotic and biotic factors (Pandey *et al.,* 2017). Abiotic factors include temperature and relative humidity (Kange *et al.,* 2014). Biotic factors include birds, parasitic weeds, pathogens and insects. The most prevalent insects in sorghum include aphids, green bug, cut worms, sorghum midge, and grain weevils; the later are regarded as

economic significance in postharvest or storages insect pest (Sarmiso, 2015). Therefore, there is a need of reducing postharvest losses in sorghum especially those related to pest attack such as rice weevil through sustainable approaches.

2.2 Rice weevil

2.2.1 Biology and the lifecycle of rice weevil

Grain weevils belongs to the family Curculionidae (Thompson, 1992), three important species known to cause damage in sorghum include *Sitophilus oryzae* (rice weevil), *Sitophilus zeamais* (maize weevil) and *Sitophilus granaries* (wheat weevil) (Campbell, 2002). They feed on whole grain and possess long snouts, with small white legless larvae grubs that develop inside sorghum kernel (Mason and Gibb, 2010). Apart from sorghum, these insects cause damage in many cereals including maize, wheat, and rice; they were also reported to feed on food staff such as cassava and nuts (Hill, 2002). Weevils are sometimes refers to internal feeders as they feed in the endosperm (Mason and Obermeyer, 2010) and or primary pest because their damage initiates secondary infestation from storage microbes (Nwilene *et al.,* 2013). Grain weevils have almost similar biology and life cycle, they differ in size, rice weevil is slightly smaller (2 mm in length) than maize weevil and granary weevil (Mason and McDonough, 2012). Rice weevils have been differentiated from others by having red or yellow oval shaped markings on their forewings (Mason, 2014). Grain weevils grows well in tropics where there is high temperature and humidity (Muzemu *et al.,* 2013; Kishor and Singh, 2017). They lay eggs in temperature ranging from 13 ^{o}C to 35 ^{o}C; however, the best temperature for oviposition is between 25 ^{o}C to 29 ^{o}C. Maize weevil and Rice weevil unlike granary weevil can fly from one area to another in storage areas (Throne and Cline, 1989; Plarre, 2010); and can infest sorghum in the fields and especially during late harvest. Ineffective local storage structures in many developing countries encourages weevil infestation (Arthur and Throne, 2002).

Females make a deep hole to about snout length in sorghum kernel, deposit eggs and covers using gelatinous materials to protect the egg (Mason and McDonough, 2012). Weevil can lay about 300 to 400 eggs, one per cavity. Larvae and pupae develop inside the grain. Larvae stage is the most destructive stage and feeding inside sorghum endosperm, while the adults feeds from outside the grain. The damage seems to vary based on temperature and relative humidity; total destruction may occur when the product is undisturbed. The maximum oviposition occurs one to two weeks after the adult emergence. The average developmental

period is around 25 days and 35 days at 25 ^{o}C and 27 ^{o}C respectively, however development period takes 94 days at 18.2 ^{o}C when there is a single larva in the kernel and to about 110 days when three larvae are oviposited (Mason, 2011). There are several stages of larval instars, each about five days, and a pupal period of five days inside the kernel. Adults emerge and may remain in the kernels up to five days (Jadhav, 2006). The adults may live for 7 months to 2 years (Young, 1977). Therefore, it is important to understand the life cycle of rice weevil and its interaction with sorghum grain in order to develop effective management strategies; including improvement of plant host resistance through breeding.

2.2.2 The effects of rice weevil in sorghum grain

Kernel damage caused by weevil infestation reduces sorghum grain quality through weight loss, nutritional loss, growth of microbes (Mason and McDonough, 2012). Damaged kernel have reduced thiamine/protein content (Venkatrao *et al.,* 1958). In addition, accumulation of insect urine increase chance of grain rancidity, poor seed germination and reduced market value of the crop (Mofokeng, 2016). There is urgent need of having sustainable rice weevil control mechanism to minimize weevil infestation and enhance food security in sorghum growing areas.

2.2.3 Control strategies of rice weevil

There are several control strategies of rice weevil; chemical control involve the use of insecticides such as fumigants and contact dust formulations such as Chlorpyrifos, phosphine and malathion (Satya *et al.,* 2016). Physical control include barriers and sealed structures (Divekar and Sharma, 2016). The cultural control involves sanitation of grain storage warehouses (Groot, 2004). Parasitoids such as *Theocola xelegans* has been used as biological control of rice weevils (Shadia and Aziz, 2011). While host plant resistance involve the use of resistant varieties to rice weevils (Ahmed and Yusuf, 2007). More research is needed to improve sorghum resistance to rice weevil. The present study therefore intends to identify sources of weevil resistance to be utilized in developing weevil resistant sorghum cultivars.

2.3 Kernel structure and the associated traits convening weevil resistance

Sorghum kernel possess some heritable traits conferring resistance to insect pests; comprised by physical aspects and biochemical molecules such as polyphenols and some resistance protein molecules (Adugna *et al.,* 2006; Morais *et al.,* 2012; Pradeep *et al.,* 2015; Sinha and Kumaravadivel, 2016). Kernel physical aspects include traits like pericarp thickness, testa,

seed size and kernel hardness or endosperm texture (Geleta and Labuschagne, 2005; Prajapati *et al.,* 2018). However, grain moisture content is none-heritable but has been associated with weevil resistance. According to Mofokeng *et al.* (2017) breeding for quality sorghums requires a clear understanding of heritability of the trait under consideration. The genetic diversity should be studied for selection of the best traits during crop development (Rakshit and Swapna, 2015). Therefore, there is a need of understanding better sorghum biology especially the kernel physical characteristics conferring resistance to rice weevil to come up with the best breeding strategies that can improve kernel resistance to insect pests; and alleviate crop losses resulted from weevil infestation.

Structure of sorghum kernel indicates three main parts including the outer layer known as pericarp, the germ (embryo) and the storage tissue referred to endosperm (Wall and Blessin, 1969; Waniska, 2000). The proportion of each layer differs based on the genotype and the environment where it was grown, for example there is larger proportion of embryo to endosperm when sorghum seed develops under stress condition (Haussmanna *et al.,* 2000). Da Silva (2003) and Chiremba (2012) reported the proportion of these materials based on hand-dissection kernel; of which 82% of the kernel comprised by endosperm; 10% the embryo and only 8% make up the pericarp. According to Waniska (2000) and Prasad *et al.* (2015) several factors influences general kernel appearance; these include colour of the pericarp and thickness, pigmented testa, and colour of the endosperm. The present study focused in critical analysis of kernel physical traits conferring resistance to rice weevil.

2.3.1 Sorghum kernel hardness

Kirleis and Cossby (1990), have described kernel hardness as textural characteristics of the endosperm. There is a variation among sorghum cultivars based on endosperm textural aspect; attributed by genetic and the environmental factors. For example Liu *et al.* (2013) reported a significant increase in sorghum kernel hardness as a result of decrease in irrigation levels. Zhao and Ambrose (2017) reported existing correlation between abrasive hardness and moisture content of the kernel. The hardness is mainly attributed by the structure of the cell wall and higher prolamin concentration especially α- and γ-kafirins; as prolamins in the endosperm acts as nitrogen repository (Holding, 2014), thus the hard sorghum kernel consists of high concentration of kafirins in the endosperm through various adjustment of protein size (Chiremba, 2012). Phenotypically kernel hardness can be recognised by observing the kernel endosperm and enumerate the corneous section against floury portion.

Different methods employed to determine kernel hardness in sorghum and other cereals. For instance, endosperm texture can used to reveal kernel hardness in sorghum. The method involve visual comparison of corneous portion with respect to floury in the endosperm (Gomez *et al.,* 1997); Fig. 1 A indicates the hard part of sorghum kernel described based on the proportion of corneous, 2B indicates an intermediate sorghum kernel (with almost equal proportion of corneous to that of floury or chalk), and 2C indicates soft sorghum kernel (floury overweigh corneous portion). Sorghum kernel hardness can also be determined by using Tangential Abrasive Dehulling Device (Reichert *et al.,* 1986), the dehulling equipment consists of grinding wheel running in proximity with a cup containing samples; when the wheel rotates seeds moves freely and dehulled in the surface of the abrasive (Gomez *et al.,* 1997). The Brabender Microhardness Tester has also been used to measure hardness, of which the degree of hardness is measured based on the farinograph (Anglani, 1998; Brabender, 2017). The other methods include resistance to grinding on the Stenvert Hardness Tester (Anglani, 1998); Percentage of floaters done by counting floating kernels in sodium nitrate (kernel density); and kernel water absorption capacity (Gomez *et al.,* 1997). It seems some variations exist among the mentioned laboratory procedures; Kirleis and Cossby (1990) assessed the efficient of six hardness measuring methods using 15 sorghum cultivars and concluded that particle size index method and the pearling index provided the best measure of kernel hardness. Hallgren and Murty (1983) reported a significant correlation between kernel density (percentage floaters) and corneous (endosperm texture) using 15-sorghum cultivars.

Kernel hardness is very important in determination of grain quality. The hardness has been useful in food processing, and plays greater role in resistance against pests including fungal infection and storage pest such as grain weevils. Several researchers documented that harder sorghum kernel is more resistant to insect attack compared to soft kernel. Implying that storage weevil attacks more floury or chalky endosperm than corneous endosperm. Studies documented the relationship between kernel hardness and rice weevil infestation in sorghum. For example, Russell (1962) reported fewer eggs and low oviposition rate in the harder sorghum varieties. Furthermore, Russell (1966) found a short adult life span in relation to kernel hardness; confirming the role of kernel hardness in resistance against rice weevil. Similar results have been reported by several researcher; Bamaiyi *et al.* (2007) found low susceptibility index among genotypes ICSV1079BF, BES, ICSV247, ICSV111, and ICSH89009NG measured by the lower F1 progeny emergence, small percentage of kernel damaged and low weight loss. The study also revealed kernel hardness as the main reason for

resistance to rice weevil. Prasad *et al.* (2015) reported a negative significant relationship between sorghum kernel hardness and weight loss; the study also found a positive significant association between median development period, hardness and 100 seed weight. This information is critical to sorghum breeders; because a better understanding of the contribution of hardness in kernel resistance to grain weevil; could enable breeders select the best parental materials with adequate strength during crop development. Varieties with harder grains will reduce the effect of this insect pest. This approach could help sorghum users to reduce application of synthetic chemical insecticides for storage purpose. Apart from insect resistance, the harder grains could increase the grain quality by offering better processing quality and protection against fungal infestation.
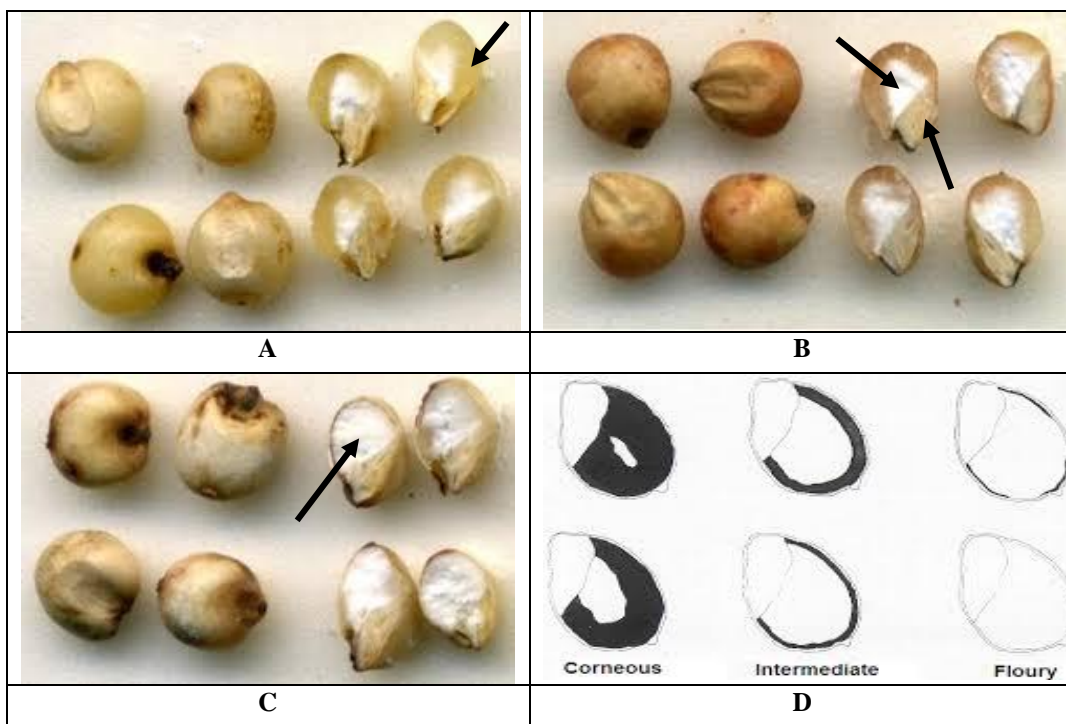
Figure 1: Illustration of endosperm texture describing sorghum kernel strength.

> Arrows pointing A) Corneous, B) Intermediate, and C) Floury and D) drawing indicating the proportion of endosperm texture. Source: Hikeezi, (2010).

2.3.2 Pericarp thickness and presence of testa

The pericarp refers to the outer layers of the sorghum kernel which is fused to the seed coat (USDA, 2013). With the help of electron microscope, Earp and Rooney (1982) researched the three sections of pericarp structure comprised by epicarp (outer layer), mesocarp and endocarp; and found thick walled rectangular cells and pigments in the epicarp section,

believed to influence kernel physical appearance. According to Earp *et al.* (2004), pericarp colour is genetically based controlled by R and Y genes resulting into red (R Y), yellow (rrY) and white (Ryy or rryy) sorghum colours. Pericarp thickness is genetically controlled by Z gene; if the gene is homozygous recessive the pericarp becomes thick; and when the gene is dominant pericarp becomes thin (Dykes and Rooney, 2006). Earp and Rooney, (1982) revealed differences on genotypes based on pericarp thickness and even within individual sorghum kernels; being thin in embryo and thick on crown. According to Earp and Rooney (1982) the endocarp represents the innermost layer of the pericarp containing cross tube cells. Earp *et al*. (2004) indicated that pericarp thickness varied among sorghum genotypes; because of the associated quantity of starch granules in the mesocarp, genotypes with thin pericarp have fewer starch granules than the thick ones. According to Bassey and Schmidt (1989) thin pericarps are strongly bound to the kernel.

In sorghum, the presence of testa is genetically controlled by gene B1 and B2 and its thickness ranged from 8 to 16 μm for US sorghum genotypes, 28 to 40 μm for the Malian genotypes and the range of 28 to 40 μm for the Sudanese genotypes (Earp and Rooney, 1982). It appears that pericarp colour is associated with biochemical concentration in sorghum; as black pericarp sorghum is associated with higher concentration of phenolic (Pfeiffer and Rooney, 2015). While, presence of purple or brown testa is associated with tannin content in sorghum (Cheng *et al.,* 2009). Most of these chemicals are concentrated in pericarp; and the higher concentration play crucial role in sorghum protection against insect pests mainly due to its antibiosis effect (Dykes and Rooney, 2007).

Russell (1962) reported deterrent effect of tannin in rice weevil oviposition when sorghum genotypes were mixed. Research conducted in maize crop found a negative correlation between pericarp thickness and maize weevil susceptibility (García-Laraa *et al.,* 2004). It was also reported that undamaged pericarp confer more resistance to rice weevil than damaged pericarp; as damage pericarp allow easy insect penetration Williams (1978). Also sorghums susceptible to weevils had thicker pericarp with clearly visible starch granules in the mesocarp; while the resistant ones had thinner pericarp (Williams, 1978). It appears that grain coat characteristics discourage oviposition, inhibit digestive enzymes and increase kernel hardness enhancing resistance to rice weevil (Dasbak *et al.,* 2009).

In addition, thick pericarp associated with kernel hardness makes dehulling by ponding more easier (Guindo *et al.,* 2016), hence dehulling is one criteria for industrial and traditional

acceptability of sorghum; making this parameter important in crop development (Guindo *et al.,* 2016). Generally, pericarp thickness and presence of testa in sorghum provides critical information to breeders in selection of parents with high tannin levels and the best strategy for hybridization. More research in this area is needed to confirm the role of pericarp thickness and testa to rice weevil resistance in an attempt of improving host plant resistance against weevil attack.

2.3.3 Kernel size

Sorghum kernel size have a significant role in the resistance to grain weevil. For instance, the large kernel size were reported to be preferred by weevils, due to large surface area, influencing insect oviposition compared to smaller grain size (Campbell, 2002). Kernel features such as size and moisture content determine the resources available for the larva. Studies indicate that female weevil laid more eggs in kernels size greater than 20 mg due to higher chance of larvae survival and large progeny size (Wongo, 1990). Stejskal and Kučerová (1996) reported that larger seed size had more than one egg compared to smaller kernel size. Also, female weevils may initiate the chewing of oviposition holes even in shrivelled kernels but were less likely to oviposit in them, it can be concluded that female weevils accept large kernels more quickly than small ones which affects oviposition (Campbell, 2002).

There are several studies confirming the role of kernel size on weevil infestation. Williams (1978) using a choice method found more oviposition on a sound kernels rather than broken kernels; however, under no choice method, the same number of eggs laid on both sound and halved kernels, indicating that large grain size were the sole reason for weevil preference. Similar results have been documented by Russell (1962) indicating that when sorghum genotypes were mixed, the insect oviposition preference and emergence was higher for the larger kernel size than smaller ones. Understanding of the relationship between kernel size and rice weevil preferences provides important hints in crop improvement program, telling that breeding should not consider only yield but also its susceptibility to insect pests.

2.4 Biochemical traits in sorghum grain related to rice weevil resistance

2.4.1 Nutrients composition

Sorghum is a source of nutrition molecules such as carbohydrate, protein, vitamins and minerals such as phosphorus (P), potassium (K), magnesium (Mg), iron (Fe) and zinc (Zn)

(Virupaksha and Sastry, 1968; Clark *et al.,* 1990; Pontieri *et al.,* 2014; Ajiboye *et al.,* 2014; Badigannavar *et al.,* 2016). According to Prasad *et al.,* (2015) sorghum grain nutrients may determine food suitability to storage insect pests. For instance, Keskin and Ozkaya (2013) found higher content of minerals in the wheat and flour samples infested by *Sitophilus granarius*, while the level of thiamine and riboflavin were found to be lower. However, there is an insufficient literature on the role of mineral elements in the weevil-sorghum interaction; therefore, more studies are needed to investigate the role of mineral elements in this antagonistic interaction. An understanding of the role of mineral elements in the interaction would help define the significance of these minerals in sorghum and enable to maximise the benefits from such minerals.

(i)   Starch concentration

Starch is a major chemical component of sorghum grain (Sang *et al.,* 2008), making up 69.5-83% of the endosperm (Wall and Blessin, 1969; Waniska *et al.,* 2004; Felix *et al.,* 2015). The starch granules of sorghum look like those of corn in size, range and shape, and their molecular structure shows linear chains of glucose linked by α-1,4 and α-1,6 glycosidic bonds forming two types of molecules namely amylopectin and amylose (Hernandez, 2012). About 70 – 80% of starch in sorghums is made up by amylopectin; with exception of waxy sorghums. The remaining 20 - 30% of starch in sorghum consist of amylose content. The proportions of amylopectin, amylase and glucan chains govern the structure of starch in sorghum (Mutisya *et al.,* 2013).

There is a positive correlation between starch depth and arrangement and the extent of resistance to damage by the rice weevil (Pendleton *et al.,* 2011). The higher the proportions of amylose the harder the grain, a trait controlled by a master gene which controls management of different biochemical events (Chandrashekar and Mazhar, 1999). Chippendale (1972) and Longstaff (1981) reported the effect of dietary carbohydrates in cereals and their role in feeding behaviour, consumption, and survival of rice weevil and found that, weevils survived well on diets with 72% (w/w) cereal starches and amylopectin, but not in diets having amylose, cellulose and mono/disaccharides; therefore concluded that there were significant contribution of amylopectin chains of cereal starches which, provide feeding stimulant and important nutrient to rice weevil in sorghum. Therefore, starch content and its chemistry seem to be an interesting parameter and can be used to describe susceptibility of sorghum genotypes to rice weevil. However, more research studies are

needed to find out if dietary starch containing amylose can be a source of resistance in sorghum grain, and assess its potential in sorghum improvement programs in developing countries to develop resistant sorghum cultivars to weevils.

(ii)   Protein content

Protein is the second major nutritional component in sorghum grain (Kulamarva *et al.,* 2012), and the content of protein varies between sorghum genotypes (Sastry *et al.,* 1986). The variation in grain composition may be due to climatic conditions, fertilizer application and soil types where sorghum is grown (Ebadi *et al.,* 2005). Protein content in sorghum genotypes ranges between 7.3 − 15.6% (Hulse *et al.*, 1980). In irrigation schemes, grain yield increases but protein content drops from 9.5% to 8.3% (Balko, 1975). Crop applied with nitrogen fertilizer sources boosted both protein and yield (Wall and Blessin, 1969; Salem, 2015). The total nitrogen (N content) on a dry basis of sorghum ranges from about 1-3% (Mosse *et al.,* 1988). Most of sorghum genotypes have deficient essential amino acids such as lysine, threonine, tryptophan and cysteine (Salunkhe *et al.,* 1977). Protein in sorghum is classified based on the solubility properties such as glutelin (44%), prolamin (26%), albumin and globulin (15%) (Ratnavathi and Patil, 2013). Prolamin subfamily include zeins and kafirin (Holding, 2014). Sorghum prolamins, termed kafirins, are categorized into subgroups a, b, and c (Kumar *et al.,* 2012). It appears that the biochemical basis has an implication on kernel hardness due to presence of prolamins (Holding, 2014), where the hard grains and vitreous part of the grain have c-prolamins which form the cement and a-prolamins forming bricks, the reason being that prolamins shapes the protein bodies through formation of disulphide bonds between proteins, thereby forming both physical and chemical (nutritional) barriers because of its resistance to digestion by grain weevil (Chandrashekar and Satyanarayana, 2006). The amount of prolamins in the endosperm and protein body and its distribution can be affected by the genetic and environmental conditions (Chandrashekar and Mazhar, 1999); for instance, sorghums grown under limited nitrogen are smaller in size and lack vitreous endosperm, because of smaller and less abundance of zein protein bodies, which fails the formation of glassy like structure, because certain ration of protein bodies, starch and viscous cytoplasm are needed (Holding, 2014). Also the amount of resistance to grain damage can be determined by kernel texture such as hardness (vitreous) and soft endosperm (opaque) (Wu *et al.,* 2010; Holding, 2014). This information implies that sorghum genotypes with less vitreous endosperm are more susceptible to grain weevils.

According to Mello and Silva-filho (2002), in crops like legumes, plant defence is associated with an array of storage protein in seeds with entomotoxic properties including α-amylase, proteinase inhibitors, lectins and also globulins. These protein fractions can also be found in sorghum grain and is associated with grain resistance to rice weevil (Boisen, 1983; Nwosu *et al.,* 2015). During interaction these molecules interfere with nutrients absorption and or inhibit digestive enzymes of insect especially when lectin makes contact with glycoprotein (Mello and Silva-filho, 2002). The α-Amylase Inhibitors function as digestive enzyme inhibitor and can be found in many plants including sorghum grain and are directed to interact with α-amylases from insects used for starch breakdown, as a result restrain rice weevil during interaction (Fürstenberg-hägg *et al.,* 2013).

Various studies investigated the relationship between protein concentration and number of adult's weevil emergence and grain damage parameters. For instance, Murthy and Ahmed (1978) investigated eight sorghum varieties against storage weevil and results indicated a positive correlation between number of adults emerged and protein content in different sorghum varieties; where genotype Y-75 had the lowest number of adults emergence and had lower protein content. Pradeep (2013) reported a positive correlation between sorghum protein content and the grain damage and population build-up of the rice weevil, where an increase of one milligram in protein content of the sorghum grain the grain damage increased by 0.85% and the population build-up of weevils increased to an extent of 0.50%. Nwosu *et al.* (2015) found that the susceptibility of maize to S. *zeamais* was increasing as protein level increases. However, Goftishu and Belete (2014) reported that the most important cause of resistance in sorghum against S. *zeamais* are lysine content in the grain, where the higher concentration of lysine in the genotype the higher resistant genotype it is. Thus, it is important to understand chemistry related to protein and associated resistant protein molecules in grain sorghum; due to its contribution in grain structure, grain strength and resistance to insect pest. Screening effort for reliable sources of protein and selection of appropriate breeding strategy to transfer this trait into farmers preferred varieties are needed.

2.4.2 Secondary metabolites in sorghum grain

Apart from nutritional chemical molecules sorghum grain is rich in phytochemicals, also known as secondary metabolites or anti-nutritional factors (Awika and Rooney, 2004). Phenolic compounds in sorghum have variety of genetically dependent levels including phenolic acids, condensed tannin and flavonoids (Dobie, 1977; Torres *et al.,* 1996; Dykes and

Rooney, 2006; Dykes and Rooney, 2007). Phenolic compounds in sorghum grain can also be divided into tannin and non-tannin polyphenols, where the tannin sorghums have proanthocyanidins as a component of their phenolic compounds but do not have tannic acid or hydrolysable tannins (Chandrashekar and Satyanarayana, 2006). These chemical compounds are the basis of antibiosis to storage pests such as grain weevil (Torres *et al.,* 1996; Kant *et al.,* 2015), and therefore associated with weevil resistance, and thus signifies its applicability in sorghum breeding for resistant weevil cultivars (Sharma *et al.,* 2005). To breed varieties with high phenolic compounds it needs to screen many genotypes to get reliable sources. Dykes *et al.* (2014) pointed out various techniques used to determine relative phenolic levels among sorghum genotypes including; colorimetric methods such as Folin–Ciocalteu, Prussian blue, ferric ammonium citrate, vanillin/HCl, and butanol–HCl; Other methods used to identify and quantify the specific phenolic compounds include High performance liquid chromatography (HPLC) coupled with photodiode array (PDA), fluorescent, and/or mass spectroscopy (MS) detectors. The role of phenolic compounds such as phenolic acid, condensed tannin and flavonoids in resistance against grain weevils is reviewed as under.

(i)   The role of phenolic acids in sorghum grain resistance against grain weevil

The phenolic acids of sorghum are present as benzoic or cinnamic acid derivatives (Awika and Rooney, 2004). Phenolic acids and their derivatives are everywhere in the plant kingdom; this implies that all sorghums contain phenolic acids (Dykes and Rooney, 2006) in various forms including soluble and bound forms, and plays greater role in cell wall structure (by assembling phenolic compounds, structural proteins, polysaccharides, and other cell wall materials) and defence. Phenolic amines and the soluble phenolic were known to lower insect attacks in grain; for example, phenolic amines are known to prevent glutamate dependent neuron receptors in insects; the compound is contained in the aleurone (Bergvinson, 2004).

The phenolic acids were reported to be in higher concentration in the pericarp and or cell walls of the endosperm, in addition the phenolic acid content in cereals were found to have an association with hardness of the grain which can be related to the mechanical contributions of phenolic dimers to the grain cell wall strength, it is also interesting to know that aleurone layer have phenolic acid amines containing toxic effects to insects (Pradeep, 2013). It was also reported that the presence of peroxidases and protein inhibitors build grain resistance against insects by catalysing the polymerization of phenolic acids in pericarp which limit

insect attack (Bergvinson, 2004). The presence of phenolic acid in sorghum is also associated with pigmentation of the grain (Lattanzio *et al.,* 2006).

Various studies indicated a negative correlation between level of phenolic acid and sorghum grain damage and population build up of the rice weevil, for example Pradeep (2013) concluded that an increase in phenol for one milligram decrease sorghum grain damage and population build up of weevil by 0.5%. Moreover, the study conducted to assess the role of phenolic acids on sorghum and maize hardness among eight cultivars of each of the cereals, revealed that the harder grains had more phenolic acids content than the soft grains; therefore one can deduce that the content of phenolic acids is a useful indicator of grain hardness and is useful when discriminating hard and soft sorghum cultivars (Chiremba *et al.,* 2012). Considering the importance of phenolic acids in developing grain strength and antibiosis effect against weevils, a better understanding on the best mechanism to increase its levels in susceptible sorghum cultivars is critical to elevate sorghum resistance to weevils.

**(ii)	The role of tannin in sorghum grain resistance against grain weevil**

Tannin is referred to complex phenolic polymers with aliphatic and phenolic hydroxyl groups and or carboxyl groups (Hagerman, 2002). There is a great variation of tannin content between sorghum genotypes; For instance, sorghum cultivars having pigmented testa contain condensed tannins or proanthocynidins (Waniska, 2000; Dykes and Rooney, 2006; Dykes *et al.,* 2014). Thus, there is a great relationship between grain colour of sorghum and the tannin content (Sedghi *et al.,* 2012). It is important to note that; the pericarp colour of sorghum is not the reliable indicator of presence of tannin (Rooney and Miller, 1981). The biosynthesis is controlled by Tan1 gene, which code for WD40 protein control for tannin biosynthesis in sorghum (Wu *et al.,* 2012). Tannin possesses a strong feeding deterrent to weevil and, therefore, considered as a defensive phytochemical (Bennett, 1994; War *et al.,* 2012). Various literatures documented the relationship existing between condensed tannin and sorghum grains resistance to rice weevil attack (Ramputh *et al.,* 1999; Hernandez, 2012).

For example, brown sorghums with high tannin levels have higher resistance to insect attack (Wongo, 1998). Ramputh *et al.* (1999) reported that the soluble phenolic content consisting primarily of proanthocyanidins can be an indicator of resistance to rice weevil in sorghum grain. Pradeep (2013) reported a negative correlation first with grain damage and second with population build-up of the rice weevil; where, a one milligram increase in tannin content

decrease kernel damage to the level of 0.90% and population build-up of rice weevil to the level of 0.69%. Understanding the implication of tannin in grain resistance to rice weevil is critical to breeders; including the methods of elevating its levels in susceptible genotypes to prevent weevil's damage.

(iii)   The role of Flavonoids in sorghum-weevil interaction

Dykes *et al*. (2009) reported that sorghums with red/purple secondary plant colour had the high levels of 3-deoxyanthocyanins, and highest concentration existing in black pericarp sorghums. Flavonoids plays a defensive role in plants by affecting the behaviour, development and growth of a number of insects (Lattanzio *et al.,* 2006). The main flavonoid derivatives in sorghum are the flavans containing double bond between C3 and C4 and hydroxylated at C3 are anthocyanidins (Waniska, 2000), mainly flavanols, isoflavones, flavanones, flavones, and anthocyanins (Dicko, 2005). Red pericarp sorghums with tan colour have been reported to have the highest levels of flavones, in addition Flavanones were also found in sorghum genotypes with a red pericarp; and secondary plant colour had no influence on the level of flavanones, these findings indicate that the level and composition of flavonoid were affected by sorghum genotype (Dykes *et al.,* 2009). Flavonoids are associated with grain defence against insect pests through toxicity and feeding deterrent (Lattanzio *et al.,* 2006). Therefore, this information could assist sorghum breeders to produce sorghum genotypes with required levels of flavonoids. There is a need to research more on the role of flavonoids in sorghum and concentration needed to bring deterrent effect to weevils. However, screening for genotypes with higher flavonoids concentration will provide breeders with reliable sources of this compound to be used in developing resistant cultivars to weevils.

2.5 Screening sorghum genotypes against rice weevil

Germplasm screening can reveal new sources of resistance to rice weevil, which can be used in breeding new resistant sorghum varieties to rice weevils (Hussain, 2015). First filial generation (F1) weevil progeny emergence has been a consistent parameter for discriminating genotypes into different susceptibility classes (Derera *et al.,* 2010). The Dobie method make use of F1 weevil progeny emergence and the median development period to calculate susceptibility index; the later differentiate sorghum genotypes into relative resistant such as resistant or susceptible based on magnitude of the index (Dobie, 1974). The present study employs Dobie susceptibility index to discriminate sorghum genotypes into different susceptibility classes.

2.6 Previous studies on screening sorghum genotypes against rice weevil

Source of resistance for rice weevil among diverse sorghum genotypes has been reported by a number of scientists. For example Russel (1966) screened four varieties against rice weevil and reported a short adult life of insect with increasing hardness of the grain. Reddy *et al.* (2002) evaluated 35 sorghum genotypes and reported greater levels of oviposition in both free choice tests and in no choice tests. While Bhanderi (2012) evaluated 12 genotypes of sorghum and noticed a negative relationship between population build up and tannin content of different genotypes. Reddy *et al.* (2002) found greater levels of antixenosis in terms of oviposition in genotypes "2077B, DJ 6514 and IS 11758" in a free-choice tests; and genotypes "2219B, M 148-138, P 721 and Nizamabad (M)" in a no-choice tests; and suggested the need to increase level of resistance in parental lines including A/B lines to be used in hybrid making so as protect sorghum from rice weevil. Bamaiyi *et al.* (2008) categorised five more sorghum genotypes such as BES, ICSV111, ICSV247, ICSV1079BF and ICSH89009NG as highly resistant genotypes to rice weevil due to lower F1 progeny emergence. Besides, Pradeep (2013) reported M 35-1, KMJ 1, AKJ 1, RSJ 1 and CSV 216R as resistant sorghum varieties to rice weevil using percentage grain damage. Goftishu and Belete (2014) categorised genotype "WB-77' as resistant sorghum variety to rice weevil, Prasad *et al.* (2015) categorised sorghum lines "EC 24, EC 22, PEC 8, PEC 7, EP 78, EP 57, AKR 354" as resistant genotypes to rice weevil and suggested the same to be used as sources of resistance in sorghum breeding program. While, Gerema *et al.* (2017) categorised "Lalo and Chemeda" as resistant sorghum varieties to rice weevil. Therefore, it is important to transfer the insect resistance genes in sorghum into male-sterile (CMS), maintainer lines, and restorer lines to allow materials to be used by public research institutions and private seed industry to develop grain weevil-resistant hybrids (Sharma *et al.,* 2005).

From the literature, the effect of rice weevil is well known. To tackle this problem chemical control measure has been widely used; though the method is associated with health, environmental problems and creation of weevil resistance. Despite many studies on sorghum genotype screening for sorghum resistance to rice weevil, none of them addressed genotypes from Tanzania, and less has been documented on the relationship between rice weevil resistance and the growth morphological characteristics, kernel phenotypic traits and biochemical properties of sorghum genotypes. This study has addressed this gap to enhance breeding process that will minimize crop loss during storages.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of the study

Sorghum genotypes were evaluated in a screen house at the Tanzania Agricultural Research Institute (TARI Ilonga centre), located in latitude 06^o42'S and longitude 37^o02'E on elevation of 506 meters above sea level. The region is characterised by bimodal type of rainfall. The monthly average amount of rainfall, minimum and maximum temperature during the growing season is as shown in Appendix 3. The identification of rice weevil was done at the Tropical Pesticide Research Institute (TPRI) laboratory in Arusha region. Susceptibility experiment and analysis of kernel phenotypic traits were conducted at TARI Ilonga crop protection laboratory. Biochemical analysis (protein and starch concentration) and identification of kernel phenotypic traits was carried at Sokoine University of Agriculture (SUA) and the Nelson Mandela African Institution of Science and Technology (NM-AIST).

3.2 Source of seed materials, insects and reagents

Sorghum genotypes were collected from Tanzania Agricultural Research Institute (TARI Ilonga centre), Tanzania National Gene Bank (NPGRC) and the International Crop Research Institute for the semi- Arid Tropics (ICRISAT) (The list of 117 sorghum genotypes used in this study is shown in Appendix 1). The genotypes comprised of local cultivars, commercial released varieties, advanced sorghum lines and hybrids submitted in the regional variety trial, participatory variety selection and National Performance Trials. Rice weevils were collected from sorghum granary at Tanzania Agricultural Research Institute (TARI Ilonga centre) in Kilosa Morogoro. The insects were collected in a previous season sorghum stock of variety Macia and Wahi. Chemical and reagents such as Hydrochloric acid, Selenium catalyst, boric acid indicator, Sodium Hydroxide, sulphuric acid, Ethanol, α – amylase enzyme, amyloglocosidase enzyme, D-glucose powder, sodium acetate, ρ-hydroxybenzoic acid and sodium azide mixture were purchased from SIGMA (Sigma Aldrich, USA) and Avanta Performance Material India Limited.

3.3 Experimental design, layout and data collection

A randomised complete block design (RCBD) was used in characterisation of sorghum genotypes based on their growth morphological variation. Similarly, RCBD was employed in suscptibility experiment using a no choice labororatory assay test method in discrimination of

sorghum genotypes based on their susceptiblity levels to rice weevil. Kernel phenotypic and bochemical traits was determine using laboratory procedures.

3.3.1 Morphological evaluation of sorghum genotypes

One hundred and seventeen (117) sorghum genotypes were planted in a screen house during the 2017/18 in cropping season. Treatments were arranged in a Completely Randomized Block Design with three replications. Four seeds per hill were sown in a 25 kg perforated plastic bags containing about 20 kg of sterile soil containing forest soil and sand at 3:1 ratio. A basal fertilizer application of 20 kg/ha (N/ha), and 20 kg/ha (P/ha) was applied during sowing. Thinning to two plants per hill was done two weeks after sowing. Hand weeding was done in the second week and fourth week after planting. Top-dressing with urea fertilizer at the rate of 45 kg/ha was done two times; irrigation and pesticide to control insects was applied as per requirements during the entire crop period. Selfing was done to maintain the genetic composition of genotypes by covering panicles with pollination bags. Qualitative and quantitative data were collected based on sorghum descriptors (IPGRI/ICRISAT, 1993) (Table 1).

Table 1: Descriptors used to characterise sorghum genotypes

Parameter	Description/ and code	Stage of
Awns	Present (1) and Absence (2)	Physiological
Hairiness	Hairiness (1), Middle (2) and Hairless (3)	
Panicle density at	Loose (1), Semi compact (2) and	Physiological
Panicle shape	Loose dropping primary branches (5)	Physiological
	Semi loose erect primary branches (6)	
	Semi loose dropping primary branches (7)	
	Semi compact elliptic (8), Compact elliptic (9)	
	Compact oval (10), Half broom corn (11)	
Glume covering	25% grain covering (1)	Physiological
	50% grain covering (3)	maturity of
	75% grain covering (5)	grain
	100% grain covering (7)	
Panicle exertion	Slightly exerted (<2cm but ligule of Hag leaf definitely below inflorescence base) (1)	Physiological maturity
	Exerted (2-10cm between ligule and inflorescence base) (2), Well exerted	
	(>10cm between ligule and inflorescence base) (3), Peduncle recurved (4)	
Glume colour	White (1), Brown (2),	Physiological
	Mahogany (greyed orange) (3),	
	Red (4), Purple (5), Black (6)	
Grain threshability	Easy (1), Medium (2), and Difficult (3)	Maturity
Grain shape	Elliptic (1) and Circular (2)	After threshing
Grain colour	White (1), Yellow (2), Red (3), Brown (4),	After threshing
	Buff (5) and Mixed (6)	
Plant height	Height of the plant taken from the base to the tip of the head	Physiological maturity
	<76 cm (very short),	
	76-150 cm (short),	
	151-225 cm (medium),tall	
	226-300 cm tall and very tall (>300 cm)	
Days to 50%	Days to when 50% of the plants entered flowering stage Very early (<56 days),Early (56-65 days), Medium (66-75 days)	Panicle
	Late (76-85 days), Very late (>85 days)	
Number of leaves	Counting leaves from first to the flag leaf.	Panicle
Panicle length	Recorded by measuring each panicle from the base to its tip.	Physiological maturity
Panicle width	Recorded at the widest part in natural position.	Physiological maturity
Panicle weight	Weight taken by measuring panicle after harvest	Physiological maturity
100 seeds weight	Measuring 100 seed weight after harvest	After harvest

3.3.2 Determination of qualitative phenotypic traits in sorghum grain

Testa layer was determined according to the procedure described by Gomez *et al.* (1997). Pericarp was removed using a scalpel and testa layer and the associated testa colour were visually observed and recorded in triplicates using 10 seeds per treatment.

Pericarp thickness was determined in triplicates of 10 seeds per tratment by scraping sorghum seeds with a scalpel and the pericarp were removed and observed using a magnifying glass, the pericarp's thickness recorded whether it was thick or thin. Thick pericarp comes off in thin flakes, while a thin one scrapes off in small fragments (Gomez *et al.,* 1997).

Endosperm texture was determined from seeds by holding tightly kernel in a piece of paper using forceps, and using the scalpel the kernel were cut longitudinally into two proportional halves. One half of the kernel were taken, pressed tightly and cut side up, onto a narrow piece of masking tape and sticky side up on the piece of paper. Using magnifying glass each sorghum kernel was observed and recorded the corneous score for each sorghum genotype. Texture was recorded in replicates and the average calculated using score: <25% (starchy/floury), 50% (intermediate) and >75% (corneous).

3.3.3 Determination of quantitative phenotypic traits in sorghum grain

The arithmetic mean diameters of sorghum kernel was taken in triplicates of 10 seeds per treatment as an average of the major diameter, minor diameter, and intermediate diameter of sorghum kernel using automatic calliper as per method described by Adinoyi *et al.* (2017).

Kernel hardness (firmness) was observed using Brookfield CT3 Texture analyser, using probe TA41 Cylinder 6 mm D, 35 mm L; with the recommended trigger value of 50 g for the Load cell of capacity of 50 kg, test speed was set at 10 mm/s, and deformation of 0.70 mm. The average of six samples (kernels) was taken as hardness.

3.3.4 Determination of nitrogen content

Total nitrogen and protein of sorghum genotypes was determined from grain through digestion, distillation and titration with hydrochloric acid as per Micro Kjeldahl Method (Bradstreet, 1953). Grain was grinded and sieved using 0.5mm sieve; a sample of 0.1 g of sorghum flour was placed into a digestion tube. 1g Selenium catalyst was mixed with the sample; 5 mL of sulphuric acid (96%) was added into the tube. The tube was heated slowly in the digestion apparatus until the digest is clear. The content was transferred into a 100 mL

volumetric flask where distilled water was added into a 100 mL graduated flask. Five millilitres of boric acid indicator solution were placed into the distillation apparatus. Ten millilitres of clear supernatant were then transferred into the apparatus where 10 mL of NaOH (46%) were added. Colour change was observed when distillation drops mixed with the boric acid indicator. One hundred and fifty millilitres of the distillate were titrated with sulphuric acids (0.0174N) where colour change from green to pink was observed, the titre volume was recorded. Finally, total nitrogen was determined using the following formula:

$$N \text{ (percentage)} = a \times N \times Mw \times 100 \times 100\% \, b \times c$$

Where; a = ml of sulphuric acid, N = Normality of sulphuric acid (0.0174), a = Titer volume, Mw = Molecular weight of Nitrogen (0.014), b = gram sample taken for analysis (0.1 g) and c = ml digest used for distillation (10 ml). Thus, the percentage crude protein = $6.25 \times$ % N.

3.3.5 Determination of starch concentration

Starch concentration was determined using (AOAC, 2002) official method 996.11 whereby, 100mg of finely ground sample was taken into 15 mL centrifuge tubes. Zero point two milliliter of 80% ethanol was added and vortexed. Three milliliter of 10% α – amylase enzyme in mM sodium acetate buffer was added. The mixture was incubated in a boiling water bath for 6 minutes with 2 minutes shaking intervals. The tubes placed in a water bath at 50 $^{\circ}$C and 0.1 mL of amyloglocosidase enzyme was added; the tubes was stirred using vortex and incubated for 30 minutes. The contents centrifuged for 10 minutes at 3000 rpm. A duplicate of 0.1 mL aliquot were placed into 15 mL test tube. Three milliliter of ρ-hydroxybenzoic acid and sodium azide mixture (1:1) was added and left to stand for 20 minutes at 20 $^{\circ}$C.

Five grams of D-glucose powder was taken into 100 mL volumetric flask, dissolved with sodium acetate buffer to make stock solution of 50 mg/mL. Serial dilution of 0 – 40 mg/mL prepared into 100ml volumetric flask. 0.1 mL diluted standard solution were taken into 15 mL test tube. Three milliliter ρ-hydroxybenzoic acid and sodium azide mixture (1:1) added and left to stand for 20 minutes at 20 $^{\circ}$C. Absorbencies of samples and standards were read at 510 nm using X-ma 3000 UV/Visible spectrophotometer.

3.3.6 Rearing of rice weevils

Multiplication of rice weevil was done to have adequate number of insect of the same generation. Parental weevils were cultured on a susceptible sorghum variety at the laboratory according to the method described by Dobie (1974) and Kasozi (2013). Forty kilograms of clean grain was kept in deep freezing at -20 ± 2 °C for disinfection for two weeks and left to acclimatize in the laboratory for one week, and placed in plastic jars of volume 3000 cm^3. About 300 unsexed weevils was introduced to infest the grain, the lids of plastic jars was perforated and gauze-wire mesh of pore size less than 1 mm were used to permit ventilation and prevent weevil from escape. Culture were kept at controlled temperature (24 ^{o}C – 27 ^{o}C) and relative humidity of (65±5%); an optimum condition for the ovipositor. After 14 days of oviposition, parental weevils were sieved out of the grain using mesh sieve. The grains were incubated again under the same condition, to allow eggs to hatch and newly F1 progenies to emerge. Newly emerged F1 rice weevil with aged 0-7 days were used in the susceptibility experiment.

3.3.7 Susceptibility experiment

Grain from each sorghum genotype was used for susceptibility experiment under no choice assay laboratory test method described by Dobie (1974). Grain were placed in refrigeration for two weeks for disinfection at -20 ± 2 °C and finally left to acclimatize in the laboratory for one week. The moisture content of the seeds was maintained at 12-13%. For each evaluated sorghum genotype, 25 g of sorghum grain was measured in three replicates and placed into a 250 cm^3 plastic jars, and infested with ten unsexed adult weevil (assumed equal ratio of male and female). In addition, 25 g seeds of each genotype was measured and subjected to the same condition without rice weevil to serve as control. Jars were covered with muslin cloth to allow ventilation and prevent insect escape. Jars was arranged in a Completely Randomized Block Design in the laboratory, randomization was achieved using excel computer program. This experiment was done at a controlled temperature (24 ^{o}C – 27 ^{o}C) and relative humidity (65±5%) and a 12:12 dark and light. Weevils were allowed to mate, feed and oviposit for 14 days. Then all parents were removed to ensure that the emerged insects are F1 generation. Data on number of parental weevils (alive and dead) and F1 weevil progenies were collected for 75 days until when no weevil emergence observed within three consecutive visit.

(i)   Susceptibility index

Susceptibility index (SI) was calculated using procedure described by Dobie (1974, 1977). The index was calculated using the number of F1 weevil progeny and the median development period, the later refer to the time from the middle of laying eggs (oviposition) to the fifty percent emergence of the F1 weevil progenies.

$$SI = \log e(\text{Number of F1 progeny emergence})/MDP \, X \, 100$$

Where $\log_e$ = natural logarithms, MDP = median development period.

Susceptibility index was used to discriminate sorghum genotypes based on their relative resistance using the following scale; 0-3 (Resistant), 4-7 (moderate resistant), 8-10 (susceptible) and >10 (highly susceptible).

(ii)   Loss in grain weight

The loss in grain weight loss was estimated through count and weigh method as described by Adams and Schulten (1978). The number and weight of damaged and undamaged seed was determined for each treatment. The following formula was used in the determination of weight loss;

$$\text{Weight loss (\%)} = \frac{(Wu \, x \, Nd) - (Wd \, xNd)}{Wu \, x(Nd + Nu)} x \, 100$$

Where; Wu = the weight of undamaged kernels, Nu = Number of undamaged kernels, Wd = Weight of damaged kernel, and Nd = Number of damaged kernel

3.4 Data analysis

The mean qualitative growth morphological traits and grains physical traits were converted into scores; and each genotype evaluated were taken as individuals and analaysed using excel program. Results on qualitative growth morphological traits were presented in bar charts..

Quantitative data on kernel phenotypic traits, biochemical traits, and susceptibility to rice weevil was subjected to one way analysis of variance using GenStat®v15, and differences on means were tested using Duncan multiple range test (DMRT). However, data on F1 rice weevil emergence undergone log transformation; while, percentage kernel damage and

percentage weight loss were subjected to square root transformation to attain normality. Descriptive statistics including mean, range and least significant difference were presented using tables.

Simple Pearson moment coefficients of correlation were computed between pairs of quantitative phenotypic, biochemical and weevil susceptibility traits using GenStat®v15 statistical software. Multivariate analyses including principal component (PCA) analysis was adopted to expose the percentage contribution of each trait and the pattern of variation using MINTAB statistical software version 14. The genotypes were finally assembled into clusters through an agglomerative hierarchical clustering procedure on the Euclidean Distance, while employing the Average Linkage Method.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.2 Morphological variation of sorghum genotypes used in the study

4.2.1 Qualitative traits

Results on presence of awns, hairiness, grain shape is shown in Fig. 2. Where 95.73% of sorghum genotypes were awn-less and only 4.27% possessed awns. Furthermore, 47.8% of sorghum genotypes were hairy, while the rest of materials were middle and hairless. The study classified grain shape into two categories "elliptic" and "circular"; of which 61.54% of genotypes had grains with elliptic shape, while 38.46% of genotypes had grains with circular shape in dorsal view.
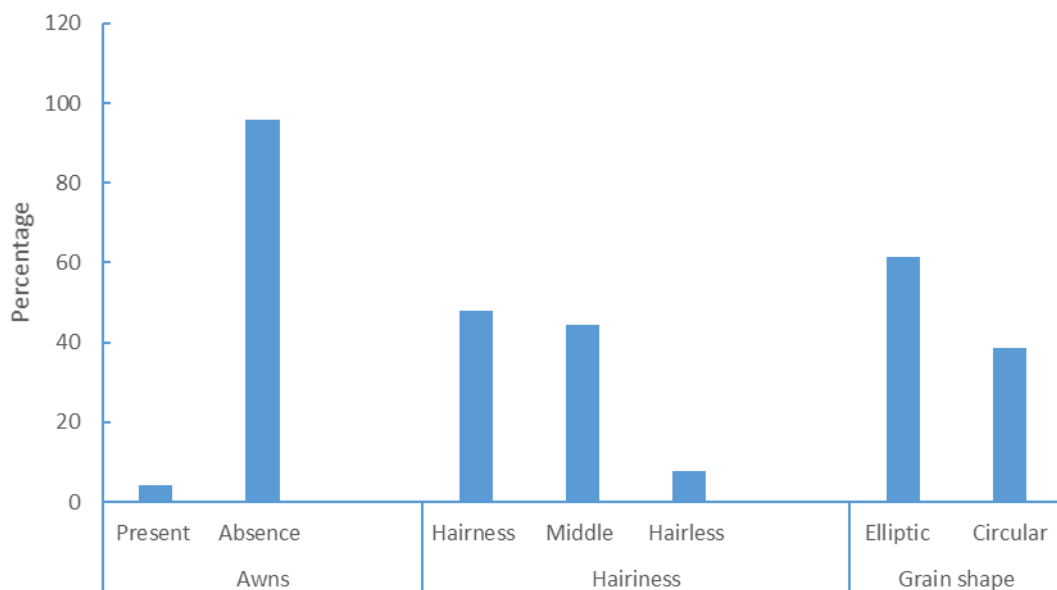
Figure 2: Qualitative growth variation of sorghum genotypes based on presence of awns, hairiness and grain shape.

Figure 3 below indicate results on growth morphological variability based on panicle shape, panicle density and threshability. Large portion (56.41%) of the evaluated genotypes exhibited semi compact elliptic while 26.50% had compact elliptic shapes. Other panicle shapes identified in the study were semi loose erect primary branches, compact oval, half

broom corn and loose dropping primary branches. In terms of panicle density; 55.56% of the evaluated sorghum genotypes displayed semi compact panicle density; 30.77% of them had compact panicle density; while 13.68% had loose panicles. Evaluation based on threshability revealed that 55.56% of sorghum genotypes were easily threshable; 38.46% were medium or partly threshable genotypes and 5.98% were difficult to thresh (more than fifty percent of sorghum seeds remains in the panicle).
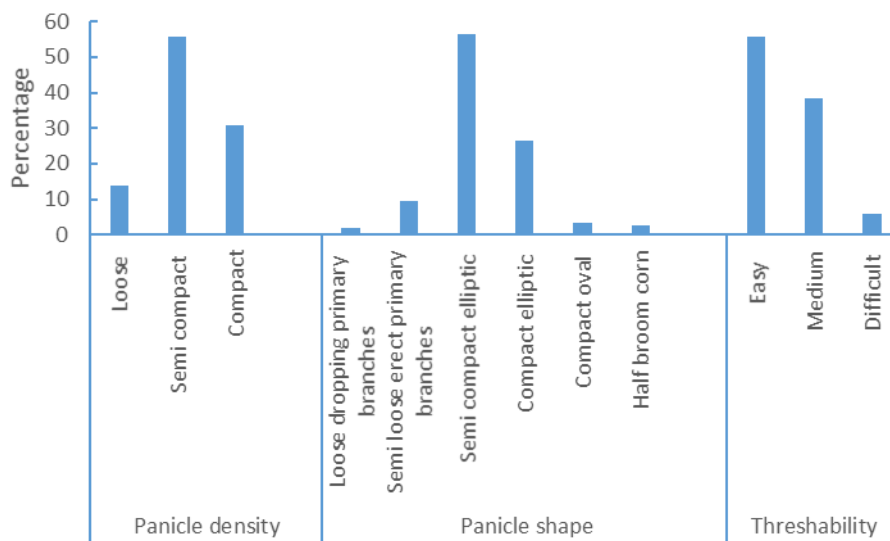
Figure 3: Qualitative growth variation of sorghum genotypes based on panicle density, panicle shape and threshability.

Sorghum genotypes were also assessed based on glume length and panicle exertion (Fig. 4); where, 51.28% of genotypes had fifty percent grain covered with glumes, 29.06% of genotypes had twenty-five percent grain covered with glumes, 17.09% of sorghum genotypes had grain covering of seventy five percent and only 2.56% of all genotypes had their grains covered by glumes hundred percent. Furthermore, variability was observed in terms of panicle exertion; where, 47.01% of evaluated sorghum genotypes were slightly exerted (<2cm but ligule of flag leaf definitely below inflorescence base), 23.08% were exerted (2-10cm between ligule and inflorescence base), 29.06% of genotypes were well exerted (>10cm between ligule and inflorescence base) and 0.85% of sorghum genotypes had peduncle recurved.
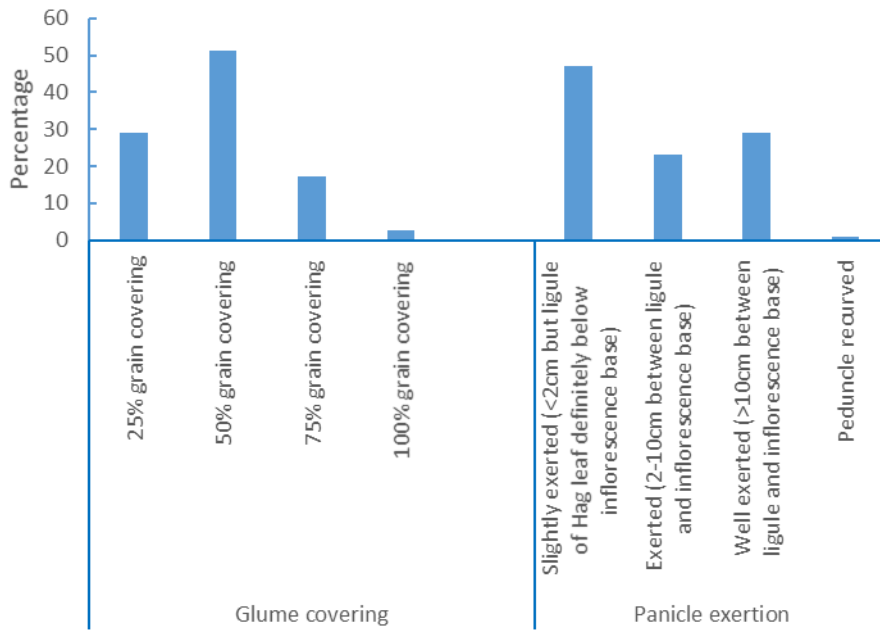
Figure 4: Qualitative growth variation of sorghum genotypes based on glume covering and panicle exertion.
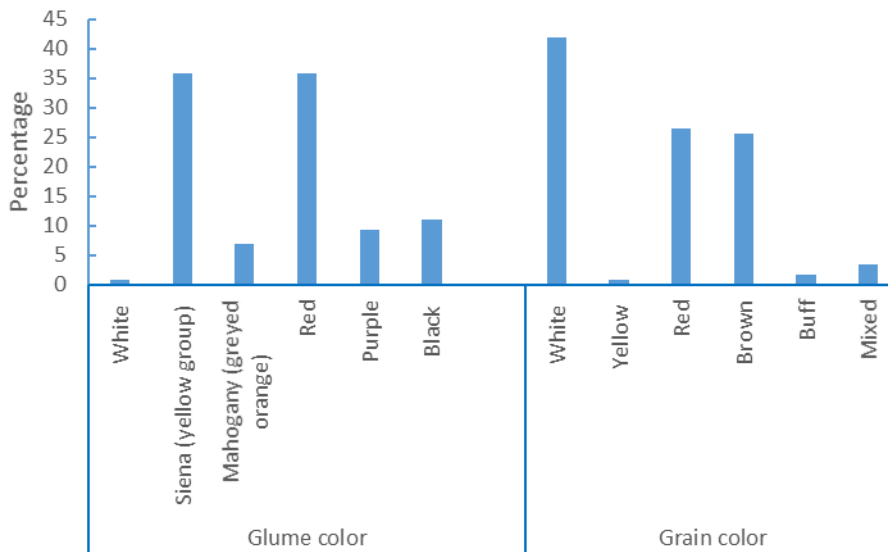
Figure 5: Qualitative growth variation of sorghum genotypes based on glume and grain colour.

Growth morphological variation observed in terms of glumes and grain colour is shown in Fig. 5. Results pointed out that 35.9% of these materials had glumes with sienna colour group, 35.9% of genotypes had glumes with red colour, and 11.11% had glumes with black colour; other glumes colour observed were purple, mahogany and white in small proportions. Additionally, variation of sorghum genotypes was assessed based on grain colour. The

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studied genotypes were categorised into six groups of which 41.88% had white coloured grains, 26.50% had red coloured grains, 25.64% had grain with brown colour and the rest of genotypes had buff, yellow and mixed colours grains. Pictorial presentation of the most recorded qualitative morphological variation among the studied sorghum genotypes is presented on Plate 1.

a.(Macia)	b. (IESH 22023)	c. (Mbangala white)
d. (IS 21881)	e. (Pato)	f. (IS 8193)
g. (IS 11167)	h. (R8602)	i. (TESO)

Plate 1: Pictorial presentation of morphological variability of sorghum in terms of grain colour, glume colour, panicle compactness, hairiness in glumes, glume covering and presence of awns for the evaluated genotypes.

From plate 1; (a) white grain, purple glumes, semi compact panicle, hairless, 50% covering, awns absent (b) Brown grain, red glumes, semi compact panicle, hairiness, 25% covering, awns absent (c) White grain, black glumes, semi loose panicle, hairiness, 50% covering, awns absent (d) Red grain, red glumes, compact panicle, hairless, 25% covering, awns absent (e) White grain, black glumes, semi loose panicle, hairiness, 50% covering, awns absent (f) Red grain, red glumes, semi compact panicle, hairless, 25% covering, absent (g) Brown grain, purple glumes, compact panicle, 50% covering, awns present (h) Red grain, black glumes, semi compact panicle, 50% covering, awns absent and (i) Brown grain, black glume, compact, hairiness, 25% covering, awns absent.

4.2.2 Quantitative traits

Table 2 shows descriptive statistics for the studied sorghum genotypes. A wide range of variability was observed among the studied traits. Result shows the mean values of days to 50% flowering; plant height, panicle length, panicle width, dry panicle weight, 100 seed mass, and grain yield were 66 days, 205.79 cm, 24 cm, 6 cm, 65.33 g, 4. 2 g, and 2.2 tons/ha respectively. The minimum and maximum days to 50 % flowering were (52 days, 92 days), plant height (99 cm, 396.5 cm), number of leaves (7, 18), panicle length (10.5 cm, 37 cm), panicle width (3 cm, 11.5 cm), panicle weight (16 g, 123 g), grain weight per panicle (7.5 g, 109 g), 100 seed mass (1.7 g, 7.5 g) and grain yield (0.33 tons/ha, 4.5 tons/ha).

Table 2: Descriptive statistics of the quantitative growth morphological traits in sorghum genotypes studied

	50%FD (days)	PH (cm)	NL	PL (cm)	PWd (cm)	DM (days)	DPWt (g)	GWt(g)	100 SWt (g)	GY (tons/ha)
Mean	66.74	205.79	11.75	24.07	6.02	103.72	65.33	49.66	4.17	2.21
SE Mean	0.38	3.66	0.10	0.26	0.06	0.33	1.11	1.04	0.05	0.05
Minimum	52.00	99.50	7.50	10.50	3.00	85.00	16.00	7.50	1.75	0.33
Maximum	92.00	396.50	18.00	37.00	11.50	120.00	123.00	109.00	7.50	4.84
Range	40.00	297.00	10.50	26.50	8.50	35.00	107.00	101.50	5.75	4.51
CV	10.78	3.50	5.40	6.50	9.20	6.01	7.00	8.60	9.80	8.60

Key: 50% FD= 50% Days of flowering, PH= Plant height, NL= Number of leaves, PL= Panicle length, PWd= Panicle width, DM= Days to maturity, DPWt= Dry panicle weight, GWt= Grain weight, 100SWt= 100 Seed weight, GY= Grain yield, SE Mean = Standard error of means, CV = Coefficient of variation.

4.2.3 Correlation between quantitative growth morphological traits

The correlation between sorghum genotypes were evaluated based on ten quantitative phenotypic traits to easy selection of appropriate traits during hybridization. Pearson correlation showed significant association between the studied traits (Table 3). For instance, Days to 50% flowering showed highly positive strong significant association with Plant height (r= 0.601, p=0.001), Number of leaves (r=0.800, p=0.001), Days to maturity (r=0.923, p=0.000). However, days to flowering showed weak negatively but high significant correlation with Panicle length (r= -0.352, p= 0.000), Grain weight (r=-0.171, p=0.001), 100

seed weight (r=-0.173, p=0.003), Grain yield (r=-0.171, p=0.001), and significant weak negatively association with dry panicle weight (r=-0.107, p=0.045).

Moreover, plant height indicated a highly positive significant correlation with number of leaves (r=0.577, p= 0.001), panicle width (r= 0.503, p= 0.001) and days to maturity (r=0.57, p=0.001). Hundred seed weight indicated highly positive significant association with panicle length, panicle width, dry panicle weight and grain weight, but highly negatively significant associated with days to 50% flowering. The grain yield showed highly positive significant association with dry panicle weight (r= 0.968, p= 0.001), and 100 seed weight (r= 0.536, p= 0.001); however, had a weak but high significant correlation with panicle length (r = 0.202, p = 0.001), panicle width (r= 0.267, p=0.001), and weak highly significant negative correlation with 50% days of flowering (r = -0.171, p = 0.001).

Table 3: Correlation coefficients between ten quantitative growth morphological traits for sorghum genotypes studied

	50% FD	PH	NL	PL	PWd	DM	DPWt	GWt	100 SWt
PH	0.601**								
NL	0.800**	0.577**							
PL	-0.352**	-0.064	-0.217**						
PW	0.282**	0.503**	0.221**	0.106*					
DM	0.923**	0.571**	0.767**	-0.283**	0.287**				
DPWt	-0.107*	0.092	-0.034	0.181**	0.276**	-0.013			
GWt	-0.171**	0.069	-0.081	0.202**	0.267**	-0.079	0.968**		
100 SWt	-0.173**	0.092	-0.074	0.205**	0.190**	-0.017	0.494**	0.536**	
GY	-0.171**	0.069	-0.081	0.202**	0.267**	-0.079	0.968**	1.000	0.536**

**P≤0.01; *P≤0.05

Key: 50% FD= 50% Days of flowering, PH= Plant height, NL= Number of leaves, PL= Panicle length, PWd= Panicle width, DM= Days to maturity, DPWt= Dry panicle weight, GWt= Grain weight, 100SWt= 100 Seed weight, GY= Grain yield.

4.2.4 Multivariate analysis

(i)   Principle component analysis for the quantitative traits

Three principal components PC1 to PC3 having eigenvalues of >1 were retained (Table 4). These components explained 80.9% of the variability observed among sorghum genotypes in terms of quantitative growth morphological traits. The first component contributed 33.6% of the total variation followed by PC2 (33.8%) and PC3 (10.3%). The first component was loaded on dry panicle weight (40.9% of the variation factor), grain weight (43.3% of the variation factor), 100 seed weight (31.4% of the variation factor), and grain yield 43.3% of the variation factor, and negative loaded with days to 50% flowering (34.4%). The second component were negatively loaded with factors such as 50% days of flowering (37.5%), plant height (40.4%), number of leaves (37.6%), panicle width (34.6%) and days to maturity (40%); and the third component were negatively correlated with panicle length, and plant height; but positively loaded with panicle width. The PCA indicated that dry panicle weight, grain weight per panicle and plant height largely contributed towards divergence.

Table 4: Principal component analysis (PCA) for the quantitative growth morphological traits of sorghum genotypes.

Variable	PC1	PC2	PC3
50% Days of flowering (50% DF)	-0.344	-0.375	0.116
Plant height (PH)	-0.154	-0.404	-0.322
Number of leaves (NL)	-0.288	-0.376	0.071
Panicle length (PL)	0.226	0.057	-0.712
Panicle width (PW)	0.033	-0.346	0.489
Days to maturity (DM)	0.288	-0.400	0.109
Dry panicle weight (DPWt)	0.409	-0.293	0.205
Grain weight (GWt)	0.433	-0.272	0.187
100 Seed weight (100SWt)	0.314	-0.193	-0.086
Grain yield (GY)	0.433	-0.272	-0.187
Eigenvalue	**3.6843**	**3.3788**	**1.0303**
% of total variance	**36.8**	**33.8**	**10.3**
Cumulative variance (%)	**33.6**	**70.6**	**80.9**

PC= Principle component analysis

The score plot (Fig. 6) placed genotypes across all quadrats based on first and second components. Genotypes were randomly spread despite of their types and origin. This pattern reveals significant variability among sorghum genotypes used in this study. Genotypes F6YQ212 (E108) and IESH 21023 (E11) were extreme from the origin. Names of genotypes for Codes (E1-E125) shown in the score plot is presented in Appendix 1.

Figure 6: Score plot showing the distribution of sorghum genotypes for the first two principal components based on quantitative growth morphological traits.

(ii) Cluster analysis of the quantitative growth morphological traits

Quantitative growth morphological traits were used to cluster genotypes based on Agglomerative hierarchical clustering done on the Euclidean Distance, utilizing Average Linkage method. These genotypes generated five main clusters at a 55.78% similarity level (Fig. 7). The number of genotypes ranges from one to sixty genotypes. Table 5 shows cluster means expounding the differences among clusters. Cluster I assembled one genotype IESH 22023 (E11) from ICRISAT; which is early maturing genotype with high yielding characteristics in terms of grain yield, 100 seed mass, grain weight per panicle, dry panicle weight and panicle length. Cluster II grouped 25 sorghum genotypes mixed of varieties, lines and hybrids with average grain yield, number of leaves, panicle width and grain weight irrespective of their origin. Cluster III assembled 10 genotypes with late flowering, late maturity, higher plant height and higher number of leaves; mostly local and advanced breeding lines regardless of their places of origin. Cluster IV grouped 60 genotypes including

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local cultivars, crosses, varieties, and breeding lines with average plant height, days to 50% flowering, days to maturity and grain yield without considering their origin. Cluster V assembled 21 sorghum genotypes without considering their place of origin; these genotypes had characteristics of short plant height and low yielding in terms of grain yield, 100 seed mass, grain weight, dry panicle weight, and panicle width.

Figure 7: Dendrogram of sorghum genotypes based on quantitative growth morphological traits.

Table 5: Cluster means of 117 sorghum genotypes based on quantitative growth morphological traits.

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
50%FD	61.000	71.840	76.700	64.117	63.714
PH	241.333	265.013	360.767	181.653	128.762
NL	10.667	12.653	14.567	11.172	11.056
PL	27.167	21.220	24.775	25.504	22.865
PWd	6.500	6.517	7.117	5.931	5.139
DM	98.000	108.507	110.700	101.783	100.516
DPWt	121.000	69.293	53.167	73.531	40.333
GWt	106.667	52.293	36.483	57.774	26.921
100SWt	5.667	4.369	3.692	4.415	3.417
GY	4.741	2.324	1.621	2.568	1.196

Key: 50% FD= 50% Days of flowering, PH= Plant height, NL= Number of leaves, PL= Panicle length, PWd= Panicle width, DM= Days to maturity, DPWt= Dry panicle weight, GWt= Grain weight, 100SWt= 100 Seed weight, and GY= Grain yield.

4.2.5 Identification of best sorghum genotypes for varietal development

Twenty five (25) best genotypes in terms of earliness and yield traits were presented in Fig. 8 (a). This pool comprises genotypes with the highest grain yield, early and medium maturity. These genotypes recorded 3.1 t/ha to 4.7 t/ha. The highest yield was recorded by genotypes IESH 22023 (4.7 t/ha), a cross of ATX623 × MACIA (4.1 t/ha) and ICSA 88006 X IESV92172DL (3.9 t/ha). Other genotypes include ICSA44 × IESV 91104DL (E37) (3.7 t/ha), IESV 91104DL (3.7 t/ha), IESV92172 (E32) (3.5 t/ha) and P9537A × MACIA (3.5 t/ha). Nevertheless, the commercial released varieties Naco Mtama 1 and Macia (check) yielded 3.3 t/ha each, and Pato (E3) yielded only 3.1 t/ha. Fifteen genotypes in this list were both high yielding and early panicle emergence (<56-65 days). For instance, genotypes Pato, Teso, IESV 92028 DL, and IESV 92038/2SH recorded less than 60 days to 50% flowering; Macia (check) recorded 65 days to panicle emergence (Fig. 8b).
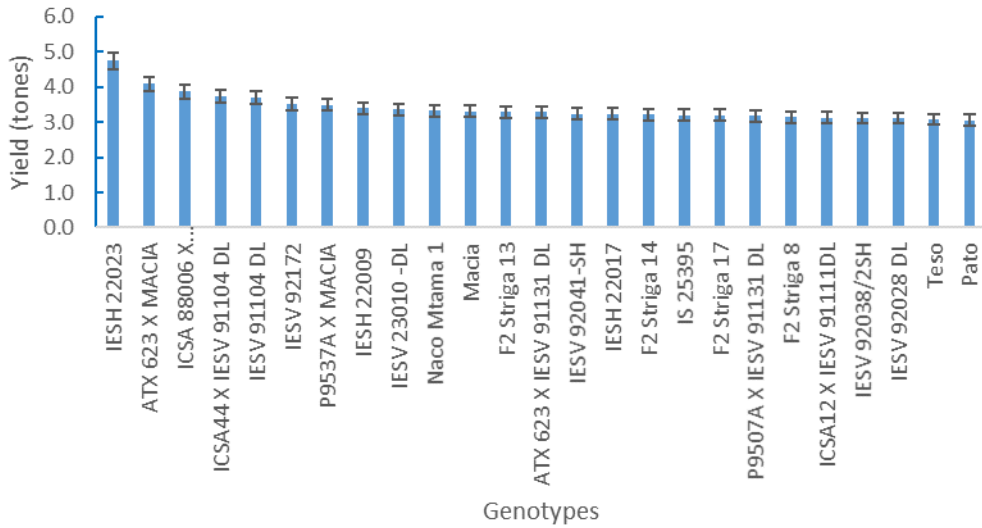
(a)
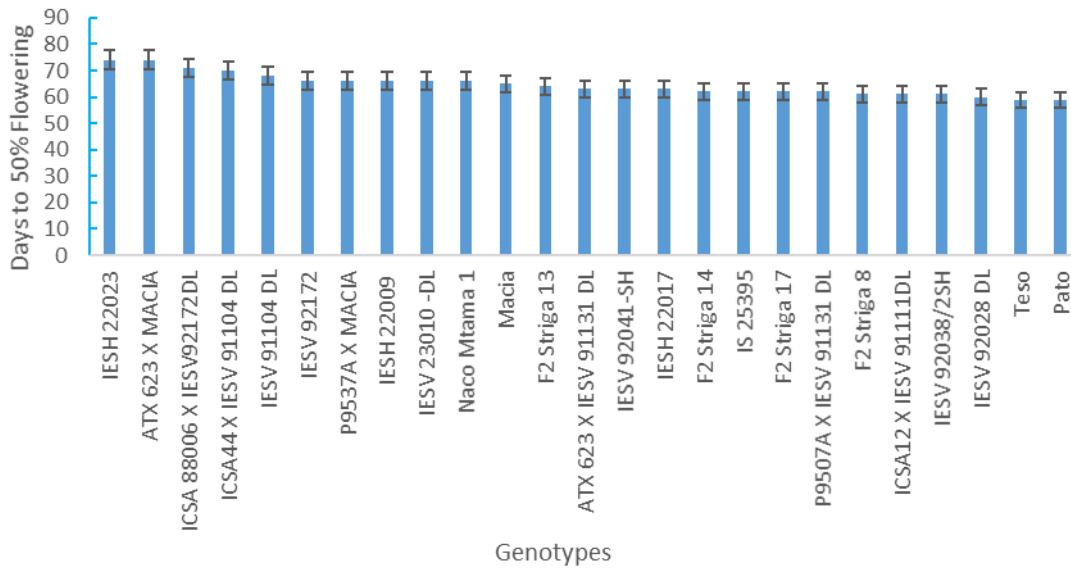
(b)

Figure 8: Promising sorghum genotypes identified based on (a) yield and (b) earliness traits

However, most of very early duration sorghum genotypes recorded very low yield of 0.4 t/ha to 1.4 t/ha therefore are not included in the list; these are Genotype F6YQ212, MAHUBE, FRAMIDA and ASARECA 15-3-1. The grain weight based on 100 seed ranged from 2-7 g. Genotypes IES11038 × A1GD 34553 recorded the highest weight (7.0 g), P9537A × MACIA (6.1g), ICSA44 × IESV 91104 DL (6.0 g). While, the least 100 seed weight were recorded in genotype F6YQ212 (2.0 g) and Mahube (2.1 g), these genotypes are early maturing genotypes. In terms of plant height; 11 genotypes recorded plant height of less than 3 m, the

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tallest genotype was IS 11167 with average height of (394.0 m). Results further pointed out that most of IS genotypes and local cultivars were the tallest genotypes, while the shortest genotypes were B35, ICS × 152 002-SB-11-1 and IS 8852 with an average height of (101.0 m, 103.7 m and 104m respectively).

Therefore, genotypes ATX 623 × MACIA (E55), ICSA 88006 × IESV92172DL (E34), ICSA44 × IESV 91104 DL (E37) and P9537A × MACIA (E44) are crosses that can be further advanced for release. Genotypes IESH 22023 (E11), IESV 91104 DL (E117), IESV 92172 (E32), IESV 23010DL (E12), MACIA, NACO Mtama 1 and PATO can be selected as best parental material in terms of yield and earliness in sorghum breeding programs.

4.3 Phenotypic traits in sorghum grain

4.3.1 Qualitative grain traits
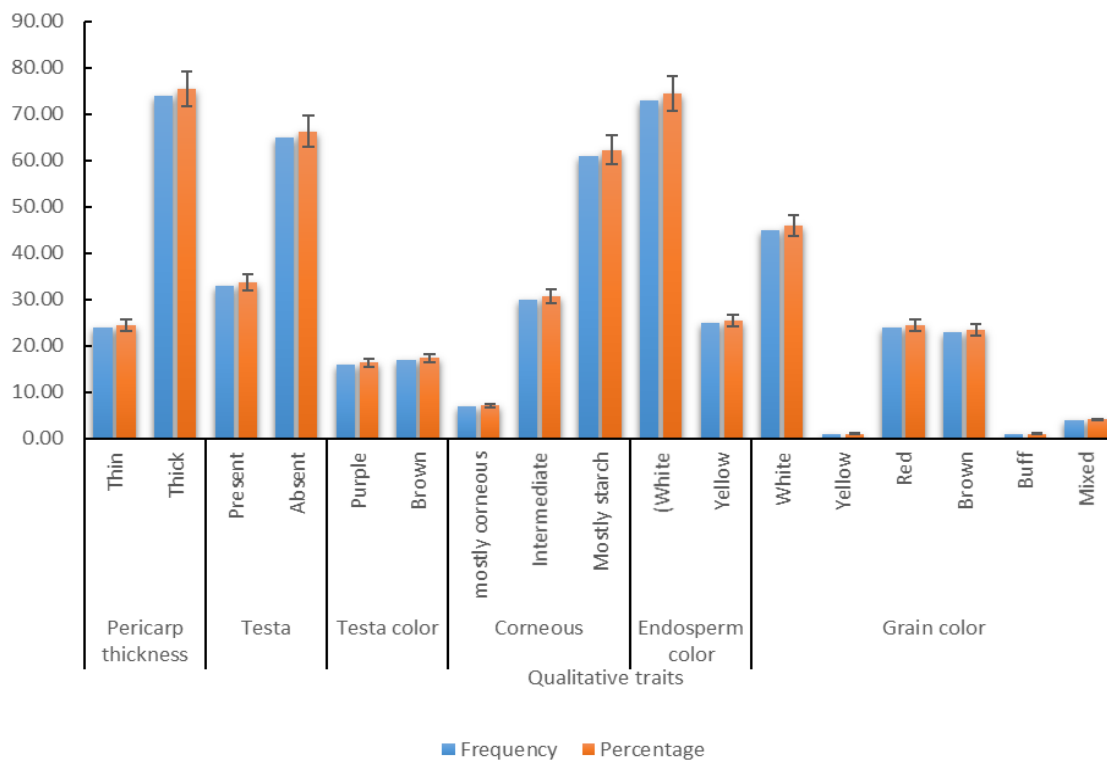
Figure 9: Frequencies and percentages of qualitative kernel phenotypic traits based on pericarp, presence of testa, corneous and grain colour of sorghum genotypes studied.

Most of the studied sorghum genotypes (75.51%) had thick pericarp (Fig. 9); while thin pericarp were observed in the rest of sorghum genotypes. Analysis of testa revealed that 33.67% had either purple or brown testa, while the rest of genotypes had no testa. In terms of

endosperm colour, 74.49% of the genotypes had white colour endosperm, while the rest were yellowish. Analysis of endosperm texture shows that only 7.14% of genotypes had mostly corneous endosperm texture, 30.61% had intermediate corneous indicating a relative balance between floury content and corneous; while the majority of genotypes were floury or complete starch. Considerable variation were also observed in terms of grain colour; where, 45.92% of the evaluated genotypes were white in colour, 24.49% were red, 23.47% of the genotypes were brown; the rest in small fraction were yellow, buff and mixed colours.

4.3.2 Analysis of variance (ANOVA)

Analysis of variance indicated a highly significant difference ($p < 0.001$) among the evaluated quantitative kernel phenotypic traits such as 100 seed weight, mean kernel diameter, and hardness of 98 sorghum genotypes; indicating greater genetic variability among these traits (Table 6).

(i)   Hundred seed weight (g)

A hundred seed weight ranged between 1.81 to 6.2 g. Genotypes F2Striga 5, P9537A x MACIA and IES11038 x A1GD 34553 recorded the highest hundred seed weight (6.20, 5.49 and 5.30 g), respectively; while genotype TZA 3983, CS x 152 001-SB-4-2, and ASARECA 15-3-1 had the least weight (1.81, 1.85, and 2.03 g), respectively (Table 6).

(ii)   Kernel hardness/strength

Kernel hardness varied between 14.94 newton to 110.33 newton; Genotypes PATO, IESV 92174DL, IESV 92028 DL, Mbangala white and F2Striga11 recorded the highest kernel hardness 110.33, 108.43, 103.90, 101.11 and 100.72 N, respectively; while genotypes IESV 92043DL, F2Striga15 and TZA3993 had the least kernel hardness (Table 6).

(iii)   Mean kernel diameter (mm)

The mean diameter ranged between 2.29 mm to 4.61 mm. Lines ICSx152 002-SB-13-2, F2Striga16 and IESH 22017 had the greater mean kernel diameter and genotypes IS 21055 had the lowest mean diameter. Other sorghum genotypes with highest average mean kernel diameter were, F2 Striga 16, IESH 22017, IS 15443, F2 Striga 18, N13 and WAHI recorded 4.54, 4.07, 3.82, 3.65, 3.62, and 3.60 mm, respectively (Table 6).

Table 6: Quantitative kernel phenotypic traits based on 100 seed mass, mean kernel diameter and hardness for 98 sorghum genotypes used in this study.

Genotype	Origin	Type	100swt (g)	MKD (mm)	Hardness (N)
NACO Mtama 1	Ilonga	Variety	4.3 A-F	3.07m-C	95.68H-K
HAKIKA	Ilonga	Variety	4.2 Zab	3.275 x-I	76.8wxy
PATO	Ilonga	Variety	4.258z-E	3.16 r-E	110.33P
WAHI	Ilonga	Variety	4.242 z-D	3.595 IJK	65.08 nop
TEGEMEO	Ilonga	Variety	4.108 yzA	3.535 F-K	78.25 w-z
TESO	ICRISAT	Line	3.85vwx	2.755 d-p	81.18 yzA
MACIA	Ilonga	Variety	3.583 r-u	3.27 w-I	99.2 K-N
IESV 92041-SH	ICRISAT	Line	3.533q-t	2.645 c-h	63.89 l-p
IESH 25002	ICRISAT	Line	3.858 vwx	3.025 j-A	72.27 s-v
IS 8193	ICRISAT	Line	3.483p-s	2.69c-k	63.7l-p
IESH 22023	ICRISAT	Line	4.983 NOP	3.485E-J	91.55E-H
IESV 23010 –DL	ICRISAT	Line	4.633 H-L	3.205 s-G	66.69 n-r
WAGITA	ICRISAT	Line	3.075 j-n	2.49 a-d	42.96 c
IS 25395	ICRISAT	Line	2.9 g-k	3.035 j-A	65.51 nop
ASARECA 14-1-1	ICRISAT	Line	2.083 c	2.775 d-q	67.25 o-r
IESV 92038/2SH	ICRISAT	Line	3.85vwx	2.92 f-w	70.38 r-u
IESV 92174 DL	ICRISAT	Line	2.033 bc	3.195 s-F	108.43P
PATO X WARD AKRA - H1/1/3/1-110-9	ICRISAT	Hybrid	3.692 r-w	2.625 b-h	64.33 m-p
ASARECA 15-2-1	ICRISAT	Line	3.667 r-v	2.805d-q	52.15efg
IS 15443	ICRISAT	Line	2.442de	3.82 KL	47.42 d
ASARECA 18-3-1	ICRISAT	Line	2.85 f-j	2.805 d-q	53.91fgh
IESV 24030 SH	ICRISAT	Line	3.633 r-v	2.705 c-l	96.12IJK
IESV 23007 DL	ICRISAT	Line	3.95wxy	3.19 s-F	90.07 D-G
KARI MTAMA 2	ICRISAT	Variety	3.633r-v	3.29y-I	64.45 m-p
R8602	ICRISAT	Line	2.258 cd	2.565 a-f	48.27 de
ASARECA 12-4-1	ICRISAT	Line	2.8 f-i	3.4B-J	59.45 jkl
IESV 92036 SH	ICRISAT	Line	4.583 G-K	3.55 G-K	81.35 yzA
ASARECA 13-1-1	ICRISAT	Line	2.083 bc	2.82 d-r	47.37 d
ASARECA 15-3-1	ICRISAT	Line	2.033abc	3.51 E-K	65.78n-q
ASARECA 24-4-1	ICRISAT	Line	2.85 f-j	2.775 d-q	48.3 de
IESV 92028 DL	ICRISAT	Line	4.35 A-G	2.92 f-w	103.9 O
IESV 92172	ICRISAT	Line	3.308 n-q	3.58 H-K	78.12w-z
P9507A X IESV 91131 DL	ICRISAT	Hybrid	5.117 PQ	2.76d-q	74.65 uvw
ICSA 88006 X IESV92172DL	ICRISAT	Hybrid	2.85 f-j	3.105o-D	81.96 zA
P9518A X IESV 92029 DL	ICRISAT	Hybrid	4.033 xyz	2.625 b-h	99.38K-N
P9507A X IESV 91131 DL	ICRISAT	Hybrid	4.8 K-O	2.89 e-u	74.22 t-w
ICSA44 X IESV 91104 DL	ICRISAT	Hybrid	5.3QR	3.31 z-J	51.23 d-g
IESA2 X PLOT *#142 SUDAN*	ICRISAT	Hybrid	3.117k-n	2.305 ab	57.25 hij
ICSA12 X IESV 91111DL	ICRISAT	Hybrid	3.7 r-w	3.245 v-H	87.26CDE
IES11038 X A1GD 34553	ICRISAT	Hybrid	6.2 S	2.75 d-o	87.64 CDE
ICSA 11040 X WAHI	ICRISAT	Hybrid	4.717 I-M	3.235 u-H	57.15 hij
P9504A X ICSR 172	ICRISAT	Hybrid	3.767 t-w	3.035 j-A	101.29 MNO
P9537A X MACIA	ICRISAT	Hybrid	5.492 R	3.29 y-I	86.34 BCD
ICSA75 X ICSR 38	ICRISAT	Hybrid	3.517 q-t	2.69 c-k	97.56 KLM
ICSA 232 X MACIA	ICRISAT	Hybrid	3.715 s-w	3.055l-B	88.34 DEF
ICSA 15 X R8602	ICRISAT	Hybrid	2.85 f-j	3.375 A-J	101.5 MNO
ATX623 X AIGD34533	ICRISAT	Hybrid	5.117 PQ	3.005 i-z	102.63 NO
ICSA 90001 X ICSR 172	ICRISAT	Hybrid	4.217 z-C	2.715d-m	57.09 hij
TZA 3993	Gene bank	Local	3.258m-p	2.54 a-e	33.85 b
IESH 22009	ICRISAT	Line	3.083 j-n	2.75d-o	95.5 H-K
ICSA 90001 X ICSR 160	ICRISAT	Hybrid	3.667 r-v	3.055 l-B	91.96 F-I

Table 6 continued

Genotype	Origin	Type	100swt (g)	MKD (mm)	Hardness (N)
ATX 623 X IESV 91131 DL	ICRISAT	Hybrid	4.217 z-C	2.785 d-q	82.28zAB
IESH 22017	ICRISAT	Line	4.492 E-I	4.07 L	76.47 vwx
ATX 623 X MACIA	ICRISAT	Hybrid	4.442 B-H	2.545a-e	54.49 f-i
IESV 91021DL/Flamida	ICRISAT	Line	3.483 p-s	3.31 z-J	52.43 efg
F2 Striga 4	ICRISAT	Line	4.483 D-I	2.74 d-n	95.08 H-K
F2 Striga 5	ICRISAT	Line	5.3QR	3.11 p-D	70.27 r-u
F2 Striga 6	ICRISAT	Line	3.2mn	2.82 d-r	92.84 G-J
F2 Striga 7	ICRISAT	Line	3.85 vwx	3.21 t-G	65.47 nop
F2 Striga 8	ICRISAT	Line	3.617 r-v	3.09 n-C	78.4 w-z
F2 Striga 11	ICRISAT	Line	3.033 i-m	2.77 d-q	100.72 L-O
F2 Striga 10	ICRISAT	Line	4.633 H-L	3.04 k-A	58.66 ijk
F2 Striga 12	ICRISAT	Line	4.45 C-H	2.835 d-r	96.71 JKL
F2 Striga 13	ICRISAT	Line	4.767 J-N	3.505 E-K	79.52 x-A
F2 Striga 14	ICRISAT	Line	5.033 OP	3.445 D-J	64.62m-p
F2 Striga 15	ICRISAT	Line	4.85 L-O	3.165 r-E	79.45 x-A
F2 Striga 16	ICRISAT	Line	4.217 z-C	4.54 M	80 x-A
F2 Striga 17	ICRISAT	Line	4.75 J-N	2.875 e-t	57.43 hij
F2 Striga 18	ICRISAT	Line	3.617 r-v	3.65 JK	83.57 ABC
ICS x 152 001-SB-2-2	ICRISAT	Line	2.7 fg	3.235 u-H	30.16 b
ICS x 152 001-SB-4-2	ICRISAT	Line	1.85 ab	2.36 abc	68.02 p-s
TZA 3943	Gene bank	Local	3.258m-p	2.295 ab	53.58 fgh
Udo	Ilonga	Local	2.667 fg	2.655 c-i	62.51 k-n
ICS x 152 001-SB-7-1	ICRISAT	Line	3.617 r-v	2.68 c-j	62.84k-o
Mbangala white	Ilonga	local	3.767 t-w	2.79d-q	101.11MNO
ICS x 152 001-SB-9-1	ICRISAT	Line	2.675 fg	3.07 m-C	51.11 d-g
TZA 3983	Gene bank	Local	1.808 a	2.96 h-z	54 fgh
ICS x 152 002-SB-4-1	ICRISAT	Line	3.617 r-v	3.115 q-D	47.62 d
ICS x 152 002-SB-8-1	ICRISAT	Line	3.517 q-t	2.585 a-g	59.49 jkl
ICS x 152 002-SB-8-2	ICRISAT	Line	3.85 vwx	2.855 e-s	63.85 l-p
ICS x 152 002-SB-10-1	ICRISAT	Line	3.8 u-x	2.655c-i	60.16 j-m
ICS x 152 002-SB-11-1	ICRISAT	Line	3.75 t-w	2.87 e-t	70.47 r-u
ICS x 152 002-SB-13-1	ICRISAT	Line	3.583 r-u	3.475 E-J	57.31 hij
ICS x 152 002-SB-13-2	ICRISAT	Line	3.8 u-x	4.605 M	65.19 nop
ICS x 152 003-SB-1-1	ICRISAT	Line	4.158 yzA	2.785 d-q	70 q-t
IS 8852	ICRISAT	Line	3.3n-q	2.925 g-x	55.61 g-j
IS 15107	ICRISAT	Line	3.187 lmn	2.68 c-j	76.97 wxy
AF28	ICRISAT	Line	2.95 h-l	2.645 c-h	57.1 hij
CR 35:5	ICRISAT	Line	3.717 s-w	3.005 i-z	54.78 f-i
GADAM	ICRISAT	Line	2.633 ef	2.625 b-h	47.08 d
IS 25395	ICRISAT	Line	3.133k-n	2.955 h-y	50.5def
FRAMIDA	ICRISAT	Line	2.767 fgh	3.42 C-IJ	71.78stu
SRN 39	ICRISAT	Line	4.533 F-IJ	2.91 f-v	41.91 c
N13	ICRISAT	Line	3.85 vwx	3.615 IJK	54.31 f-i
IESV 91104 DL	ICRISAT	Line	4.9M-P	2.605 a-h	62.58 k-o
IESV 92043 DL	ICRISAT	Line	3.75 t-w	2.68 c-j	14.94 a
IS 21881	ICRISAT	Line	3.45 o-r	3.21 t-G	34.09 b
IS 21055	ICRISAT	Line	3.217 mno	2.285 a	47.09d
		Mean	**3.02**	**3.713**	**70.02**
		LSD	**0.28**	**0.211**	**3.866**
		F prob	**<0.001**	**<0.001**	**<0.001**

Note: Means followed by the same letter a not significant different at ($p<0.05$).

- MKD = mean kernel diameter, 100Swt =100 seed weight; SE= Standard error of mean, SED = Standard of error of differences of means, LSD = Least significance difference of means (5% level).

4.4 Biochemical traits in sorghum grain and their correlation with phenotypic traits

4.4.1 Analysis of variance for protein and starch concentration of 98 sorghum genotypes used in this study

Analysis of variance shows highly significant difference (p<0.001) among 98 sorghum genotypes in terms of protein and starch concentration (Table 7).

(i)   Protein content

Protein ranges between 6.52 to 12.23%; of which genotypes Naco Mtama 1, IESV 24030SH, and ICSR75 × ICSR 38 recorded the highest protein content 12.23, 12.18 and 11.62%, respectively. Genotypes F2 Striga13, ICS ×152 001-SB-4-2 and ATX 623 ×MACIA had the lowest concentration 6.52, 6.55, and 6.55%, respectively.

(ii)   Starch concentration

The mean total starch concentration ranged between 21.88 to 79.05 g/100g. The higher starch concentration observed on genotypes ICSA 88006 × IESV92172DL, ICSA15 × R8602 and GADAM (79.05, 79.00, and 79.00 g/100g, respectively; while Tegemeo, ASARECA 18-3-1, and ICS × 152 002-SB-4-1 recorded the least starch concentration 21.88, 22.50 and 24.61 g/100g, respectively. Some genotypes recorded either lower or higher starch concentration due to high diversity of genotypes used in the present study.

Table 7: Protein content and starch concentration of 98 sorghum genotypes used in the study

Genotype	Origin	Type	Protein (%) (content	Starch(g/100g)
NACO Mtama 1	Ilonga	Variety	12.229M	47.17 A-G
HAKIKA	Ilonga	Variety	9.797 CDE	49.84 F-I
PATO	Ilonga	Variety	9.464 zA	33.41 e-h
WAHI	Ilonga	Variety	10.479 I	27.43 bc
TEGEMEO	Ilonga	Variety	7.347i-m	21.88 a
TESO	ICRISAT	Line	7.364 i-n	48.33C-H
MACIA	Ilonga	Variety	10.323 HI	37.81 j-q
IESV 92041-SH	ICRISAT	Line	7.382 i-n	44.93 v-C
IESH 25002	ICRISAT	Line	9.762 BCD	55.15 JK
IS 8193	ICRISAT	Line	10.777 J	59.76 LM
IESH 22023	ICRISAT	Line	11.582 L	35.21e-k
IESV 23010 -DL	ICRISAT	Line	8.344stu	34.7 e-j
WAGITA	ICRISAT	Line	9.832 C-F	49.13 D-I
IS 25395	ICRISAT	Line	8.082 r	52.37 IJ
ASARECA 14-1-1	ICRISAT	Line	7.049 d-h	42.93 s-z
IESV 92038/2SH	ICRISAT	Line	8.397 tuv	33.9 e-i
IESV 92174 DL	ICRISAT	Line	8.432uvw	41.62 q-v
PATO X WARD AKRA - H1/1/3/1-110-9	ICRISAT	Hybrid	9.832 C-F	35.62 f-l
ASARECA 15-2-1	ICRISAT	Line	10.199 GH	52.56 IJ
IS 15443	ICRISAT	Line	10.462 I	39.01 l-r
ASARECA 18-3-1	ICRISAT	Line	10.777 J	22.5a
IESV 24030 SH	ICRISAT	Line	12.177 M	45.67 w-D
IESV 23007 DL	ICRISAT	Line	7.399 j-n	48.17C-H
KARI MTAMA 2	ICRISAT	Variety	7.067 d-h	58.35 KL
R8602	ICRISAT	Line	6.601 ab	43.69 t-A
ASARECA 12-4-1	ICRISAT	Line	6.874 cde	40.45 n-u
IESV 92036 SH	ICRISAT	Line	8.082 r	32.01def
ASARECA 13-1-1	ICRISAT	Line	7.032 d-h	39.15l-s
ASARECA 15-3-1	ICRISAT	Line	11.039K	38.96l-r
ASARECA 24-4-1	ICRISAT	Line	10.549IJ	26.77 bc
IESV 92028 DL	ICRISAT	Line	11.214K	39.04 l-r
IESV 92172	ICRISAT	Line	10.532 IJ	41.33p-v
P9507A X IESV 91131 DL	ICRISAT	Hybrid	10.584 IJ	49.77E-I
ICSA 88006 X IESV92172DL	ICRISAT	Hybrid	10.077 FGH	79.05 QR
P9518A X IESV 92029 DL	ICRISAT	Hybrid	9.709 A-D	71.84 O
P9507A X IESV 91131 DL	ICRISAT	Hybrid	9.499zA	40.44 n-u
ICSA44 X IESV 91104 DL	ICRISAT	Hybrid	9.814 CDE	46.35 y-F
IESA2 X PLOT #142 SUDAN	ICRISAT	Hybrid	8.869 xy	34.61 e-j
ICSA12 X IESV 91111DL	ICRISAT	Hybrid	11.582 L	29.56 cd
IES11038 X A1GD 34553	ICRISAT	Hybrid	10.322 HI	34.38 e-j
ICSA 11040 X WAHI	ICRISAT	Hybrid	9.622 ABC	58.6 L
P9504A X ICSR 172	ICRISAT	Hybrid	8.502 uvw	49.56 E-I
P9537A X MACIA	ICRISAT	Hybrid	7.802 opq	42.31 r-x
ICSA75 X ICSR 38	ICRISAT	Hybrid	11.617 L	65.23 N
ICSA 232 X MACIA	ICRISAT	Hybrid	10.042 EFG	44 u-B
ICSA 15 X R8602	ICRISAT	Hybrid	7.399 j-n	79 QR
ATX623 X AIGD34533	ICRISAT	Hybrid	7.277 h-l	37.56 i-p
ICSA 90001 X ICSR 172	ICRISAT	Hybrid	8.677 wx	47.54 B-G
TZA 3993	Gene bank	Local	7.399 j-n	45.6 w-D
IESH 22009	ICRISAT	Line	6.734 abc	29.8 cd
ICSA 90001 X ICSR 160	ICRISAT	Hybrid	8.642 vwx	31.93 def
ATX 623 X IESV 91131 DL	ICRISAT	Hybrid	7.2 g-l	50.54GHI

Table 7 (Continue)

Genotype	Origin	Type	Protein (%) (content	Starch(g/100g)
IESH 22017	ICRISAT	Line	6.57 a	31.5 de
ATX 623 X MACIA	ICRISAT	Hybrid	6.55 a	46.03 x-E
IESV 91021DL/Framida	ICRISAT	Line	8.484 uvw	38 j-q
F2 Striga 4	ICRISAT	Line	7.592 mno	41.3 p-v
F2 Striga 5	ICRISAT	Line	10.182 GH	39.54 m-s
F2 Striga 6	ICRISAT	Line	9.972 D-G	32.78d-g
F2 Striga 7	ICRISAT	Line	10.094 GH	56.68 KL
F2 Striga 8	ICRISAT	Line	10.497 I	34.76 e-j
F2 Striga 11	ICRISAT	Line	6.892 c-f	49.8 F-I
F2 Striga 10	ICRISAT	Line	7.137 e-j	36.7 h-n
F2 Striga 12	ICRISAT	Line	7.784 op	38.88k-r
F2 Striga 13	ICRISAT	Line	6.515 a	42.18 r-w
F2 Striga 14	ICRISAT	Line	8.099 rs	64.56N
F2 Striga 15	ICRISAT	Line	8.537 uvw	70.4 O
F2 Striga 16	ICRISAT	Line	10.182GH	34.48e-j
F2 Striga 17	ICRISAT	Line	6.839bcd	42.72 r-y
F2 Striga 18	ICRISAT	Line	6.944 c-g	62.06MN
ICS x 152 001-SB-2-2	ICRISAT	Line	7.434 lmn	51.89 HIJ
ICS x 152 001-SB-4-2	ICRISAT	Line	6.55 a	46.08 x-F
TZA 3943	Gene bank	Local	7.784 op	40.83 o-u
Udo	Ilonga	Local	8.502 uvw	37.43 i-o
ICS x 152 001-SB-7-1	ICRISAT	Line	9.464 zA	46.53 z-F
Mbangala white	Ilonga	local	8.169 rst	63.23 MN
ICS x 152 001-SB-9-1	ICRISAT	Line	7.784 opq	44.75 v-C
TZA 3983	Gene bank	Local	8.169 rst	64.85N
ICS x 152 002-SB-4-1	ICRISAT	Line	6.944 c-g	24.61 ab
ICS x 152 002-SB-8-1	ICRISAT	Line	8.344 stu	78.25 QR
ICS x 152 002-SB-8-2	ICRISAT	Line	9.359z	33.85 e-i
ICS x 152 002-SB-10-1	ICRISAT	Line	7.154 f-k	62.52 MN
ICS x 152 002-SB-11-1	ICRISAT	Line	8.467 uvw	46.85 A-G
ICS x 152 002-SB-13-1	ICRISAT	Line	8.484 uvw	39.26 l-s
ICS x 152 002-SB-13-2	ICRISAT	Line	9.517 zAB	37.79 j-q
ICS x 152 003-SB-1-1	ICRISAT	Line	9.797 CDE	46.01 x-E
IS 8852	ICRISAT	Line	6.731 abc	72.79 OP
IS 15107	ICRISAT	Line	6.594 ab	40.18 n-t
AF28	ICRISAT	Line	7.627 no	51.28HI
CR 35:5	ICRISAT	Line	6.962 c-g	42.93 s-z
GADAM	ICRISAT	Line	7.119 e-i	79 R
IS 25395	ICRISAT	Line	7.417 k-n	44.77 v-C
FRAMIDA	ICRISAT	Line	10.497 I	62.54 MN
SRN 39	ICRISAT	Line	8.467 uvw	40.76o-u
N13	ICRISAT	Line	7.294 h-l	36.37 g-m
IESV 91104 DL	ICRISAT	Line	7.399 j-n	31.44 de
IESV 92043 DL	ICRISAT	Line	8.029 pr	59.81 LM
IS 21881	ICRISAT	Line	9.009 y	51.53 HI
IS 21055	ICRISAT	Line	8.047 r	75.61 PQ
		Mean	**8.656**	**45.95**
		LSD	**0.226**	**3.131**
		F prob	**<0.001**	**<0.001**

Means followed by the same letter in the same column are not significant different at (p<0.05) using DMRT.
MKD = mean kernel diameter, 100Swt =100 seed weight; SE= Standard error of mean, SED = Standard of error of differences of means, LSD = Least significance difference of means (5% level).

4.4.2 The correlation and multivariate analysis of the physiochemical traits evaluated among 98 sorghum genotypes

(i)   Correlation analysis

Results on the relationship between kernel physical traits and biochemical components for 98 sorghum genotypes used in the present study is shown in Table 8. Pearson correlation analysis indicated a weak but positive significant correlation between 100 seed weight and kernel hardness (r=0.250, p=0.013); while kernel hardness had a positive but weak significant correlation with protein concentration (r=0.225, p=0.026). Starch concentration had a weak negatively significant association with mean kernel diameter (r=-0.200, p=0.048). However, starch concentration showed a negative weak correlation with almost all the studied parameters.

Table 8: Pearson correlation based on kernel phenotypic traits and biochemical properties of 98-sorghum genotypes evaluated for these parameters

	Mean diameter	100 Seed weight	Kernel hardness	Protein
100 Seed weight	0.169			
Kernel hardness	0.143	0.250*		
Protein	0.140	0.132	0.225*	
Starch	-0.200*	-0.158	-0.064	-0.087

*significant at p<0.05

(ii)   Principal component analysis for the physiochemical traits

Table 9 shows analysed results on principal component analysis for the quantitative hysiochemical traits identified in 98 sorghum genotypes. The principle component analysis (PCA) grouped five traits into five components. Retention of PCs were based on proportion of variance criterion described by Hair *et al.* (1998). Four components can be retained based on adequate cumulative amount of variance explained (>80%). About 85.9% of the variances contained in the dataset were retained by the first four principal components. The first component explained 32.7% of the total variation. The high contributing factor loading is 100 seed weight, kernel hardness, mean kernel diameter (MKD), and protein content. The second principle component (PC2) accounted 20.1% of the total variation; mainly a function of starch concentration and kernel hardness with negative loadings. With similar scenario, in the third component (PC3) protein content have higher positive loading and 100Swt with the largest negative loading. The PC4 accounted 15.8% of the total variation with high negative loadings from starch

concentration and the mean kernel diameter. According to Hair *et al.* (1998) loading greater than ±0.40 were considered to be the best representing the corresponding PC axis. The score plot for the first two components is shown in Fig. 10. Genotypes ICSx152002-SB-4-1, IESH 22023, and ICSA75 x ICSR38 were the extremely genotypes because of great variability.

Table 9: Principle component analysis of quantitative kernel phenotypic and biochemical traits in 98 sorghum genotypes.

Variable	PC1	PC2	PC3	PC4	PC5
MKD	0.452	0.386	0.256	-0.761	0.042
100Swt	0.492	-0.051	-0.657	0.076	0.563
Hardness	0.484	-0.485	-0.205	-0.066	-0.697
Protein	0.425	-0.394	0.677	0.300	0.342
Starch	-0.373	-0.677	-0.051	-0.567	0.281
Eigenvalue	1.6346	1.0060	0.8639	0.7907	0.7048
% variance	32.7	20.1	17.3	15.8	14.1
Cumulative % variance	32.7	52.8	70.1	85.9	100

PC= principal component, MKD = mean kernel diameter, 100Swt =100 seed weight;

Figure 10: Score plot of first and second principle components explaining kernel phenotypic and biochemical variation among the evaluated sorghum genotypes.

(iii)   Cluster analysis of the physiochemical traits among 98 sorghum genotypes

Cluster analysis for the phenotypic kernel traits and biochemical parameters indicated a clear separation of the evaluated sorghum genotypes (Fig. 11). Four main clusters were observed namely; cluster I, II, III and IV formed at 59.68% similarity level. Table 9 indicates cluster means, explaining the differences among groups of the evaluated sorghum genotypes. Cluster I grouped twenty (20) sorghum genotypes formed based on lowest concentration of starch and small mean kernel diameter, and the highest hundred seed weight, kernel hardness and protein content. Cluster II grouped seven (7) genotypes consisting of hybrids, breeding lines and a local cultivar (Mbangala white) with the average protein content, the highest mean kernel diameter, and starch concentration. Cluster III grouped sixty seven (67) sorghum genotypes based on average mean kernel diameter, 100 seed weight, kernel hardness, protein content and starch concentration (irrespective of their origin). Cluster IV grouped four (4) sorghum genotypes originated from ICRISAT and Tanzania namely IESV92043DL, IS 21881, ICSx152001-SB-2-2 and TZA3993 these genotypes had the lowest mean kernel diameter, 100 seed weight, kernel hardness and protein content.

Figure 11: Dendrogram showing various clusters among 98 sorghum genotypes evaluated in terms of physiochemical properties.

Table 10: Cluster means of the phenotypic and biochemical traits for clusters 1-4

Clusters	MKD	100Swt	Kernel hardness	Protein	Starch
1	3.0173	4.0499	96.0791	9.2978	39.4972
2	3.0571	3.6405	92.0763	8.9215	70.1171
3	3.0278	3.6451	62.4233	8.4778	44.9705
4	2.9163	3.2896	28.2581	7.9678	52.2067

MKD = mean kernel diameter, 100Swt = 100 seed weight

4.5 Susceptibility of sorghum genotypes to rice weevil

4.5.1 Adult mortality, F1 Progeny emergence and Median development period.

(i)   Adult mortality

Results showed significant differences (p<0.001) among sorghum genotypes based on weevil adult mortality, F1 progeny emergence, and the median development period. The highest weevil mortality was observed in genotypes PATO (24.54%), IESV 92041-SH (18.46), ATX623 × AIGD34533 (18.2%) and IESV 92172 (16.86%); while the lowest mortality was recorded in genotypes F2 Striga 11 (1.15%), ICS ×152 003-SB-1-1 (1.19%), and ICS ×152 002-SB-13-2 (1.33%) (Table 11).

(ii)   F1 progeny emergence

A wide variation observed in terms of F1 progeny emergence. Progeny emergence ranged from 11 to 491 weevils. Genotypes with significant higher number of weevil emergence were ICS ×152 002-SB-8-2, ICS ×152 003-SB-1-1, ICS ×152 002-SB-10-1, IS 15443 and TZA 3993; recording 492, 478, 471, 469 and 467 insects respectively. However the least number of progeny emergence was observed in genotypes PATO, Mbangala white, ATX623 ×AIGD34533, IESV 92041-SH, and UDO; with weevil emergence of 11, 14, 15, 15 and 16 insects respectively (Table 11).

(iii)   Median development period

Median development period ranged from 28 days for the genotype IS 8193, IS 15107, IS 15443 and ICS x 152 002-SB-8-2; to 40 days for genotypes ATX623 x AIGD34533, IESV 92041-SH, UDO, IESV 92172, IESV 74 DL, ICSA 15 X R8602 and PATO (Table 11). It was observed that as the median development period increases, the F1 emergence decreases. Genotypes with higher F1 weevil emergence recorded short median development time and vice versa.

Table 11: The adult mortality, progeny emergence and median development period of rice weevil; observed in 98 sorghum genotypes evaluated for weevil susceptibility.

Genotype	% Insect mortality	F1 Progeny Emergence	Median Development Period
ICS ×152 002-SB-8-2	1.332a	491.67K	28a
IS 15443	1.889a-d	469.00JK	28a
IS 15107	1.381a	465.67JK	28a
IS 8193	2.525a-j	457.67IJK	28a
TZA 3993	1.884a-d	467.33JK	28.67ab
F2 Striga 5	1.978a-d	460.00IJK	28.67ab
F2 Striga 14	1.881a-d	455.00IJK	28.67ab
ICS ×152 002-SB-13-2	1.227a	446.00IJK	28.67ab
ICS x 152 003-SB-1-1	1.197	478.33JK	29abc
ICS x 152 002-SB-13-1	1.586ab	437.67IJK	28.67ab
IESV 92038/2SH	2.273a-g	429.00HIJ	28.67ab
IS 25395	2.248a-f	426.67HIJ	28.67ab
IESV 91021DL/Framida	1.656abc	441.33IJK	29abc
IS 8852	1.755abc	412.67GHI	28.67ab
ICS x 152 002-SB-10-1	1.702abc	470.67JK	29.33a-d
F2 Striga 18	2.468a-i	407.67GHI	28.67ab
TZA 3943	1.945a-d	467.00JK	29.33a-d
ASARECA 12-4-1	2.281a-g	428.33HIJ	29abc
F2 Striga 10	2.799a-l	326.67DE	28.67ab
P9537A X MACIA	2.19a-f	305.00DE	28.67ab
ICS x 152 002-SB-4-1	2.723a-l	386.00GH	30b-f
F2 Striga 17	3.063a-n	311.33DE	29abc
ICSA 11040 X WAHI	1.865a-d	289.33CD	28.67ab
ICS x 152 001-SB-2-2	2.415a-h	339.67EF	29.67b-e
IESH 22023	3.137a-o	311.00DE	29.33a-d
ICS x 152 001-SB-9-1	1.983a-d	446.00IJK	31.33f-i
IESV 92043 DL	2.314a-g	439.33IJK	31.33f-i
ICS x 152 002-SB-8-1	2.176a-f	439.67IJK	31.33f-i
TESO	2.563a-j	332.00DE	30b-f
KARI MTAMA 2	2.399a-h	452.33IJK	31.67g-j
FRAMIDA	3.022a-n	302.33DE	29.67b-e
TZA 3983	3.525a-q	258.33BC	29.33a-d
IESH 22017	2.668a-k	333.67E	31e-h
IS 21881	2.805a-l	376.33FG	31.67g-j
SRN 39	2.847a-m	213.33A	29abc
WAGITA	2.143a-e	247.00B	32.67i-l
ASARECA 15-2-1	1.742abc	210.67A	32.67i-l
ASARECA 24-4-1	2.416a-h	194.00Za	33j-m
ICSA44 X IESV 91104 DL	3.23a-p	144.67wx	31.33f-i
ASARECA 13-1-1	3.99b-s	116.67s-v	30.33c-g
IS 21055	4.039b-s	175.00yz	33j-m
ASARECA 15-3-1	2.568a-j	149.00xy	32.33h-k
IS 25395	4.819g-v	143.33vwx	33j-m
F2 Striga 16	3.495a-q	110.33r-u	31.67g-j
ASARECA 14-1-1	4.924h-v	126.67t-x	32.67i-j
IESA2 X PLOT *#142 SUDAN*	3.699a-r	92.33o-r	30.67d-g
IESV 23010 –DL	4.176c-t	154.33xy	34.67n-r
F2 Striga 15	3.205a-o	94.00p-s	31.67g-j
N13	4.726f-u	129.33u-x	34l-p
ASARECA 18-3-1	3.148a-o	120.33t-x	33.67k-o

Table 11 (Continue)

Genotype	% Insect mortality	F1 Progeny Emergence	Median Development Period
WAHI	2.838a-m	115.33stu	33.67k-o
PATO X WARD AKRA -	2.783a-l	129.67u-x	34.67n-r
ATX 623 X MACIA	2.92a-m	146.33wx	36.33s-v
TEGEMEO	3.714a-r	119.67t-w	35.67q-u
F2 Striga 7	4.635e-u	70.67k-o	32.33h-k
ICS x 152 001-SB-4-2	4.677e-u	90.33o-r	34.33m-q
IESV 24030 SH	4.17c-t	119.67t-w	37u-x
GADAM	4.647e-u	72.67l-o	33.33k-n
ICS x 152 001-SB-7-1	5.198k-v	101.67q-t	36.33s-v
ICSA 90001 X ICSR 172	5.636o-v	70.00j-n	33.67k-o
HAKIKA	4.398d-t	76.67m-p	35.67q-u
AF28	5.465n-v	66.33i-n	34.67n-r
IESV 23007 DL	4.377d-t	83.67n-q	36.67t-w
NACO Mtama 1	5.053j-v	67.00i-n	35o-s
P9507A X IESV 91131 DL	6.354s-x	57.33h-m	34.67n-r
IESV 91104 DL	4.808g-v	56.00g-l	34.67n-r
ICS x 152 002-SB-11-1	5.784q-w	59.33h-m	35.67q-u
IESH 25002	5.338m-v	58.33h-m	35.67q-u
F2 Striga 13	5.006i-v	66.67i-n	37.33vwx
ICSA12 X IESV 91111DL	4.663e-u	55.67g-l	36r-v
ATX 623 X IESV 91131 DL	5.724p-w	49.33d-i	35.67q-n
CR 35:5	6.006q-x	51.00e-j	37u-x
F2 Striga 4	6.13r-x	52.67e-k	37.33vwx
F2 Striga 12	6.625t-x	53.00f-k	38wxy
ICSA 88006 X IESV92172DL	5.216l-v	40.33c-g	35.67q-u
ICSA75 X ICSR 38	3.515a-q	43.33c-h	36.33s-v
F2 Striga 8	10.597ABC	35.00cd	34.33m-q
IES11038 X A1GD 34553	5.488n-v	40.00c-g	35.67q-u
IESV 92036 SH	8.991yzA	43.67c-h	37.33vwx
P9507A X IESV 91131 DL	9.274y-B	36.33cde	35.67q-u
ICSA 232 X MACIA	7.196v-y	38.33c-f	36.67t-w
F2 Striga 6	11.34BCD	32.67c	35.33p-t
P9518A X IESV 92029 DL	8.056w-z	38.67c-f	37u-x
IESV 92028 DL	9.563Zab	36.33cde	37u-x
IESH 22009	8.306xyz	33.67cd	36.33s-v
R8602	9.455Zab	32.33c	36.67t-w
ICSA 90001 X ICSR 160	7.039u-y	32.67c	37u-x
MACIA	9.795z-C	30.67c	36.67t-w
F2 Striga 11	1.15a	31.33bc	36.67t-w
P9504A X ICSR 172	14.352E	19.33a	39yzA
ICSA 15 X R8602	11.917CD	20.33ab	39.67Za
IESV 92172	16.86F	18.33a	40.33A
IESV 74 DL	14.612E	17.00a	40A
Mbangala white	11.99CD	14.33a	38.33xyz
Udo	13.399DE	16.33a	40.33A
ATX623 X AIGD34533	18.211F	14.67a	40.33A
IESV 92041-SH	18.467F	14.67a	40.33A
PATO	24.545G	11.00a	39.67Za
Mean	5.166	191.4	33.337
LSD (0.05)	1.9854	32.15	1.274
Fprob	<0.001	<0.001	<0.001

Means followed by the same letter in the same column are not significant different at (p<0.05) using NDMRT. Original or the back-transformed data are presented in this table. Though log and square root transformed data were used during the analysis.

4.5.2 Susceptibility index, Percentage kernel damage and weight loss.

Results on analysis of variance indicated a highly significant difference (p<0.001) for the susceptibility index, kernel damage and weight loss of 98 sorghum genotypes (Table 12).

(i)   Percentage kernel damaged

The percentage kernel damaged ranged from 5.6% to 73.3%. The highest kernel damage were observed in genotypes IS 15443 (73.3%), ICS x 152 002-SB-8-2 (51%), IESV 92043 DL (50%), while, the least percentage were recorded in genotype Mbangala white (5.7%), ICSA 15 x R8602 (5.7%), P9504A x ICSR 172 (6.7%), PATO (7.0%), IESV 92041-SH (7.0%) and IESV 92172 (7.0%). The percentage kernel damage directly related to weevil emergence (Table 12).

(ii)   Percentage weight loss

The percentage weight loss varied from 2.9% - 48.51%. The highest percentage weight loss was recorded in genotypes; IS 15443 (48.5%), ICS x 152 002-SB-8-2 (48.1%), IESV 92043 DL (42.9%), IS 15107 (40.2%). While the least percentage weight loss was recorded in genotypes IESV 92041-SH (2.9%), PATO (3.1%), Mbangala white (4.4%), IESV 74 DL (4.4%), ICSA 15 X R8602 (5.2%), P9504A X ICSR 172 (5.3%), and IESV 92172 (5.3%). Percentage weight loss directly related to the weevil emergence (Table 12); thus, genotypes recorded high weevil emergence had higher percentage weight loss.

(iii)   Susceptibility index (SI)

The susceptibility index (SI) varied between 2.6 to 9.6. Genotype PATO, IESV 92041-SH, ATX623 X AIGD34533, UDO, Mbangala white, IESV 74 DL, IESV 92172, ICSA 15 X R8602, and P9504A X ICSR 172 had the lowest index of susceptibility, while genotype ICS x 152 002-SB-8-2, IS 15443, IS 15107, IS 8193 recorded the highest susceptibility index. This implies that out of 98 sorghum genotypes evaluated only 9 can be categorised as resistant to rice weevil and the rest can be rated as moderate resistant, susceptible and highly susceptible genotypes as per Dobie (1974) scale of susceptibility (Table 12). Resistant sorghum genotypes to rice weevil comprised by the improved variety, local cultivar and advanced breeding lines. However, most of commercial release varieties were categorised as moderate resistant genotypes with exception of PATO. The SI were related to percentage kernel damage and percentage weight loss.

Table 12: The susceptibility index, percentage kernel damage and percentage weight loss of 98 sorghum genotypes evaluated for their susceptibility to rice weevil.

Genotype	Susceptibility index	%Weight loss	% Damaged kernel
ICS x 152 002-SB-8-2	9.613P	48.13JK	51.00O-P
IS 15443	9.538OP	48.51K	73.33P
IS 15107	9.527OP	40.22H-K	48.00MNO
IS 8193	9.501NOP	37.76GHI	41.33J-O
TZA 3993	9.313M-P	32.04C-H	36.33D-L
F2 Striga 5	9.286M-P	37.29F-I	39.33H-N
F2 Striga 14	9.273M-P	27.39y-E	30.00x-l
ICS x 152 002-SB-13-2	9.242M-P	35.77E-I	40.33l-O
ICS x 152 003-SB-1-1	9.239M-P	35.72E-I	39.33H-N
ICS x 152 002-SB-13-1	9.212MNO	38.54G-K	39.67H-O
IESV 92038/2SH	9.183MNO	38.36G-J	44.00K-O
IS 25395	9.17MNO	27.41y-E	44.00K-O
IESV 91021DL/Flamida	9.119LMN	35.10D-I	37.33G-M
IS 8852	9.118LMN	30.31B-G	36.67E-L
ICS x 152 002-SB-10-1	9.113LMN	33.29C-I	36.00C-L
F2 Striga 18	9.105LM	26.30y-D	27.67v-G
TZA 3943	9.102LM	28.28z-F	31.33y-J
ASARECA 12-4-1	9.074LM	31.21B-H	35.33B-L
F2 Striga 10	8.77KL	33.12C-H	36.33D-L
P9537A X MACIA	8.668JK	31.11B-H	31.67y-J
ICS x 152 002-SB-4-1	8.627JK	25.54x-C	28.33w-G
F2 Striga 17	8.594IJK	30.15B-G	32.33z-J
ICSA 11040 X WAHI	8.583IJK	33.86C-I	40.33H-O
ICS x 152 001-SB-2-2	8.532IJK	33.60C-I	39.00H-N
IESH 22023	8.5H-K	30.20A-G	33.67A-K
ICS x 152 001-SB-9-1	8.45H-K	25.41x-C	31.33y-J
IESV 92043 DL	8.433H-K	42.90IJK	50.33NO
ICS x 152 002-SB-8-1	8.432H-K	33.84C-H	38.00F-M
TESO	8.39G-K	34.66D-I	40.00H-O
KARI MTAMA 2	8.385G-K	30.54B-H	36.00C-L
FRAMIDA	8.37G-J	38.48G-J	40.67J-O
TZA 3983	8.225GHI	25.26x-C	30.00x-H
IESH 22017	8.134GH	39.32G-K	45.33L-O
IS 21881	8.133GH	33.19C-H	43.67K-O
SRN 39	8.031G	38.79G-K	46.00L-O
WAGITA	7.325F	19.66q-y	26.00s-B
ASARECA 15-2-1	7.114RF	22.84v-B	27.33u-F
ASARECA 24-4-1	6.925DE	20.44r-z	22.00p-x
ICSA44 X IESV 91104 DL	6.889CDE	22.03t-z	24.33q-z
ASARECA 13-1-1	6.821CDE	22.12u-A	23.33q-z
IS 21055	6.801CDE	20.41r-z	26.33t-C
ASARECA 15-3-1	6.719BCD	21.15s-z	23.33q-z
IS 25395	6.539ABC	25.11z-C	27.00u-E
F2 Striga 16	6.448ZzAB	23.18w-B	26.33t-C
ASARECA 14-1-1	6.433 zAB	11.64h-p	16.67i-q
IESA2 X PLOT *#142 SUDAN*	6.366 zAB	16.99p-w	17.67j-r
IESV 23010 -DL	6.31yzA	14.98m-t	21.00o-w
F2 Striga 15	6.228yzA	30.18B-G	33.33A-K
N13	6.21yzA	11.86h-p	18.00k-s
ASARECA 18-3-1	6.179x-A	21.38t-z	27.33u-F

Table 12 (continue)

Genotype	Susceptibility index	% Weight loss	% Damaged kernel
WAHI	6.124xyz	11.13g-p	23.00q-y
PATO X WARD AKRA - H1/1/3/1-110-9	6.091xyz	16.65o-w	26.67u-D
ATX 623 X MACIA	5.96wxy	16.59o-w	18.33k-t
TEGEMEO	5.82vwx	10.94g-p	23.33q-z
F2 Striga 7	5.711uvw	14.58l-t	15.00f-p
ICS x 152 001-SB-4-2	5.685uvw	20.59r-z	22.00p-x
IESV 24030 SH	5.618t-w	14.39l-s	20.00n-w
GADAM	5.584s-t	20.58r-z	24.33q-A
ICS x 152 001-SB-7-1	5.529r-v	14.12k-r	19.33m-v
ICSA 90001 X ICSR 172	5.438q-u	16.04n-v	18.33k-t
HAKIKA	5.282p-t	11.27g-p	27.33u-F
AF28	5.25o-s	15.44m-u	28.67w-G
IESV 23007 DL	5.239o-s	10.42f-n	13.67d-n
NACO Mtama 1	5.202n-r	10.31e-n	14.00d-o
P9507A X IESV 91131 DL	5.072m-q	8.04b-i	10.67b-i
IESV 91104 DL	5.034m-p	18.27q-x	25.33r-A
ICS x 152 002-SB-11-1	4.964l-p	13.23i-q	22.00p-x
IESH 25002	4.947k-p	8.01b-i	10.33a-f
F2 Striga 13	4.882j-o	14.91m-t	16.67i-q
ICSA12 X IESV 91111DL	4.844i-n	8.26b-j	16.67g-q
ATX 623 X IESV 91131 DL	4.737h-m	9.17d-l	11.33b-j
CR 35:5	4.614g-l	13.46j-q	18.00k-s
F2 Striga 4	4.591f-k	8.25b-j	10.33a-h
F2 Striga 12	4.539f-j	8.61d-j	12.33c-l
ICSA 88006 X IESV92172DL	4.5e-i	8.86d-j	14.67e-o
ICSA75 X ICSR 38	4.498e-i	11.91h-p	12.67c-m
F2 Striga 8	4.482e-i	8.34b-j	9.33a-f
IES11038 X A1GD 34553	4.477e-i	8.53d-j	10.33a-g
IESV 92036 SH	4.385d-h	8.52c-j	19.33l-u
P9507A X IESV 91131 DL	4.359d-h	10.61g-o	16.67i-q
ICSA 232 X MACIA	4.286d-g	8.85d-k	13.00d-m
F2 Striga 6	4.285d-g	7.50b-h	9.67a-f
P9518A X IESV 92029 DL	4.266d-g	9.99e-m	13.00d-m
IESV 92028 DL	4.213def	7.69b-h	23.67q-z
IESH 22009	4.2def	8.72d-k	11.33b-j
R8602	4.115de	7.94b-i	14.00d-o
ICSA 90001 X ICSR 160	4.087d	7.04b-h	9.00a-e
MACIA	4.054d	6.62b-g	9.33a-f
F2 Striga 11	4.041d	7.50b-h	11.67b-k
P9504A X ICSR 172	3.292c	5.28a-d	6.67ab
ICSA 15 X R8602	3.277c	5.24a-d	5.67a
IESV 92172	3.133bc	5.32a-d	7.00ab
IESV 74 DL	3.077bc	4.45abc	7.33abc
Mbangala white	3.002bc	4.35ab	5.67a
Udo	2.983bc	5.74a-f	9.67a-f
ATX623 X AIGD34533	2.89ab	5.76a-e	8.33a-d
IESV 92041-SH	2.872ab	2.90a	7.00ab
PATO	2.614a	3.14a	7.00ab
Mean	**6.402**	**20.20**	**24.94**
LSD (0.05)	**0.3239**	**6.510**	**7.431**
Fprob	**<0.001**	**<0.001**	**<0.001**

Means followed by the same letter in the same column are not significant different at (p<0.05) using NDMRT. Original or the back-transformed data are presented in this table. Though log and square root transformed data were used during the analysis.

4.5.3 Relationship between grain resistance to rice weevil with grain yield and days to 50% flowering

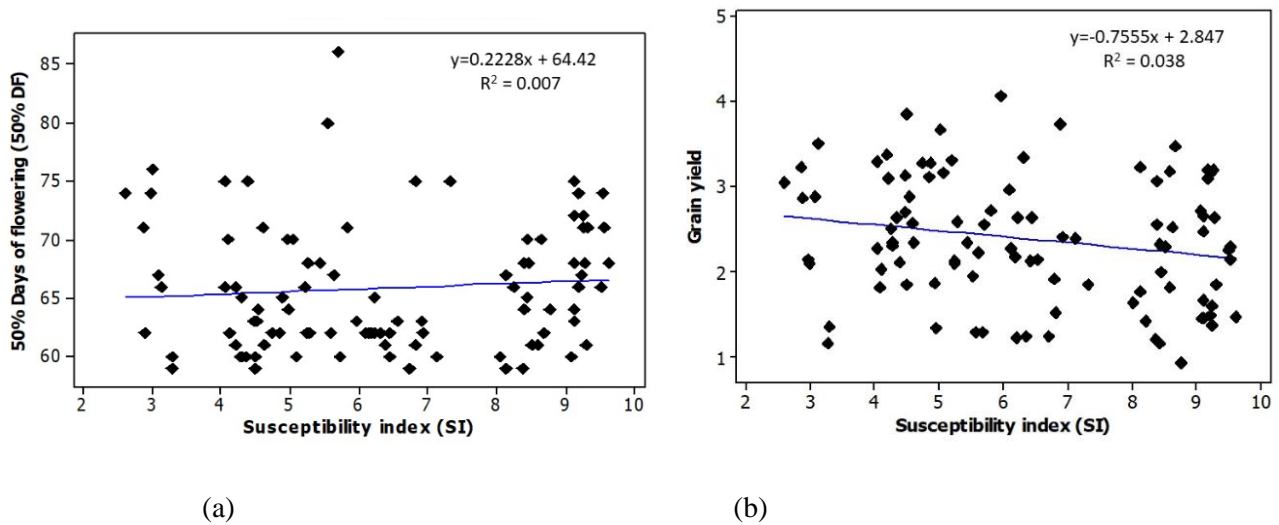(a)   (b)

Figure 12: Fitted line plot for the relation between Susceptibility index with other growth morphological traits (a) grain yield, and (b) days to 50% flowering.

Figure 12 indicate the relationship between weevil resistance with growth morphological traits. The correlation between susceptibility index and grain yield and days to flowering traits indicates insignificant relationship among these traits portrayed by parallel scattering of coordinates alongside the x −axis and small R squared values (less than 4% for yield) and (less than 1% for days to flowering).

4.5.4 Frequency distribution of sorghum genotypes into rice weevil susceptibility categories

Distribution of sorghum genotypes based on susceptibility index is shown in Fig. 14a. It was revealed that only 9% of entire set of 98 sorghum genotypes categorised as resistant genotypes to rice weevil, the rest were moderate resistant and susceptible genotypes. Frequency distribution of genotypes on susceptibility categories based on their origin is presented in Fig. 14b (N=11 and 87, for Tanzania and ICRISAT respectively). Where, 3 genotypes from Tanzania and 6 genotypes from ICRISAT portrayed adequate resistance to rice weevil and therefore categorised into resistant class.
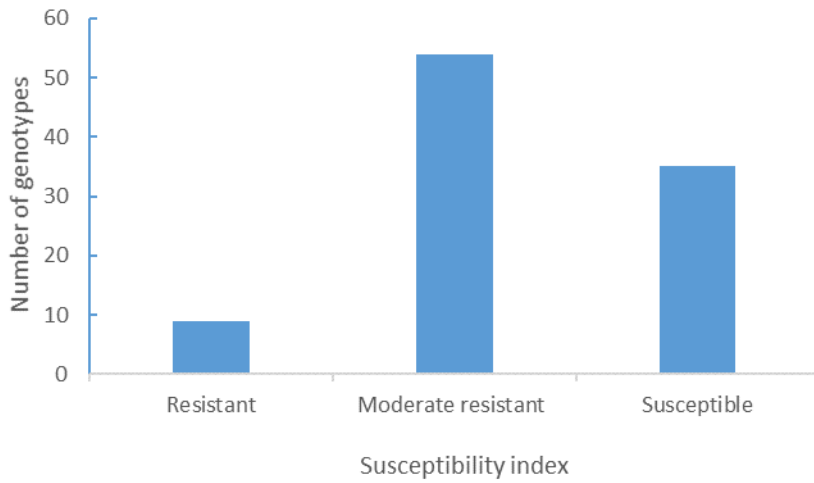
(a)
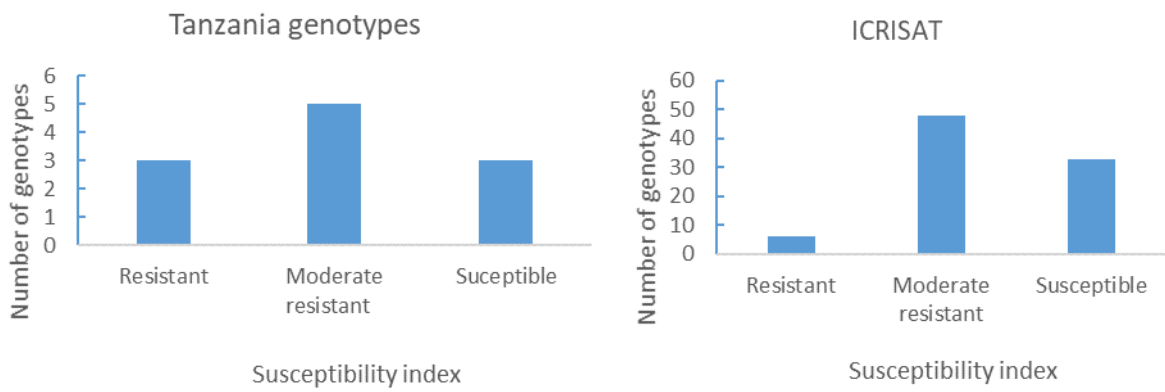
(b)

Figure 13: Genotypes distribution to rice weevil susceptibility categories a) overall frequency distribution of sorghum genotypes into susceptibility categories (N=98); b) frequency distribution of sorghum genotypes into susceptibility categories based on their origin.

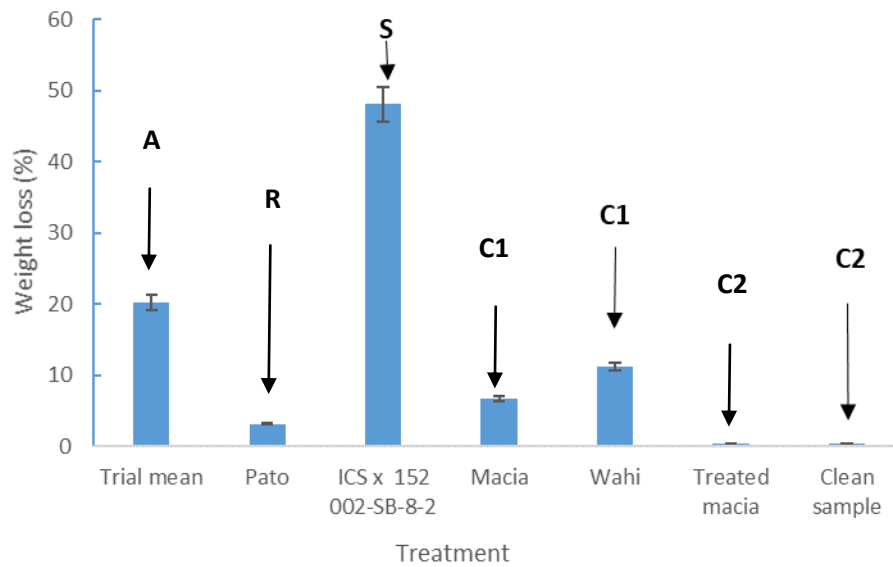**4.5.5 Comparison of various treatments on weevil susceptibility**

Figure 14: Comparison of different treatments on weevil susceptibility

Figure 14 shows the variation observed among different treatments based on trial mean, resistant and susceptible sorghum genotype and the insect free sample used as control in this study measured on percentage weight loss as a result of weevil infestation. Resistant genotype (Pato) recorded lower mean parentage weight loss compared to other treatments, the checks Macia and Wahi recorded slightly lower percentage weight loss than susceptible, checks and trial mean. Susceptible genotype recorded greater percentage weight loss than trial mean and other treatments. Clean sample (insect free sample) maintained in the same experimental site indicated a negligible weight loss

4.5.6 Pearson correlation coefficient between susceptibility index, kernel phenotypic traits and biochemical traits

Relationship between susceptibility factors, kernel phenotypic and biochemical traits of studied sorghum genotypes is shown in Table 13. Susceptibility index showed positive strong significant correlation with other genetic resistance variables like F1 progeny emergence, percentage weight loss, and percentage kernel damage. The median development period negatively associated with the Susceptibility index (r=-0.962, p<0.001) and F1 progeny emergence (r=-0.866, p<0.001). The results revealed that F1 progeny emergence, positively correlated with percentage weight loss (r=0.899, p<0.001) and percentage kernel damage (r=0.859, p<0.001). Kernel strength seems to have significance relationship with susceptibility parameters; kernel strength negatively correlated with Dobie Susceptibility Index (r=-0.582, p<0.001), F1 progeny emergence, damaged kernel, and weight loss. However, the kernel hardness had strong and highly positively significant correlation with median development period (r=-0.560, p<0.001).

Table 13: Correlation analysis between DSI, MDP, F1 progeny emergency, weight loss, kernel damage, kernel phenotypic traits, and biochemical traits of sorghum genotypes

	%MR	F1 PE	MDP	DSI	%DK	%WL	MKD	100Swt	KH	PT
%MR										
F1PE	0.853**									
MDP	-0.734**	-0.866**								
DSI	0.814**	0.950**	-0.962**							
%DK	0.728**	0.859**	-0.848**	0.890**						
%WL	0.757**	0.899**	-0.902**	0.932**	0.954**					
MKD	0.110ns	0.138ns	-0.145ns	0.151ns	0.181ns	0.155ns				
100Swt	-0.068ns	-0.043ns	0.020ns	-0.025ns	-0.039ns	-0.014ns	0.169ns			
KH	-0.458**	-0.506**	0.560**	-0.582**	-0.571**	-0.565**	0.143ns	0.250*		
PT	-0.196ns	-0.158ns	0.077ns	-0.121ns	-0.069ns	-0.110ns	0.141ns	0.132ns	0.225*	
Starch	-0.134ns	0.097ns	-0.074ns	0.073ns	0.057ns	0.097ns	-0.200*	-0.158ns	-0.064ns	-0.087ns

$**P \leq 0.01$; $*P \leq 0.05$; ns = not significant

Key: %MR = Mortality rate, F1PE = F1 Progeny emergence, MDP = Median development period, DSI= Dobie susceptibility index, %DK = Damaged kernel, %WL = Weight loss, MKD= Mean kernel diameter, 100Swt= 100 seed weight, KH=Kernel hardness, PT=Protein

4.6 Discussion

Understanding the growth morphological variation is critical in germplasm collection, conservation and breeding through selection of parental materials with best recombination. The present study showed growth morphological variability among 117 sorghum genotypes based on 20 qualitative and quantitative traits. The study further identified best genotypes in terms of earliness and yield traits. Variabilities observed in the studied sorghum genotypes for grain yield, 100-seed weight, panicle weight and plant height was similar to earlier reports (Desmae *et al.,* 2016). The variability in terms of grain yield can be exploited in development of high biomass cultivars for feed and fodder purposes. The multivariate statistical analysis is important in estimation of morphological variability among genotypes (Hailu *et al.,* 2006; Kumar *et al.,* 2012); in this study, the principle component analysis (PCA) showed that three axes explained a large portion (80.9%) of the total variation among the evaluated sorghum genotypes. Other studies on genetic variability reported the higher contribution of the first principle component in explaining total variability among variables (Dossou-Aminon *et al.,* 2015; Sinha and Kumaravadivel, 2016).

In breeding programs, it is important to understand the correlation among traits for proper selection of genotypes and traits needed (Alam *et al.,* 2001). In this study correlation, analysis indicated important associations between evaluated quantitative traits. For instance, days to 50% flowering indicated a positive correlation with Plant height, Number of leaves, panicle width and Days to maturity and were negatively highly significant correlated with Panicle length and 100 seed weight, but negatively correlated with Panicle length, grain weight, 100 seed weight, grain yield and dry panicle weight. Thus, traits that are positive correlated with earliness have to be highlighted in selection and developing of early maturing sorghum varieties and hybrids. Such varieties and hybrids will best suit ever changing climate associated with little and unreliable rainfall pattern experienced by majority of sorghum growing areas in Tanzania. Early maturing materials could be able to escape long drought periods. Pearson correlation also indicated that plant height had highly positive significant correlation with Number of leaves, Panicle width, and Days to maturity. Besides, the grain yield showed highly positive significant association with Panicle length, Panicle width, Dry panicle weight and 100 seed weight. The positive association among yield related traits suggested that these parameters are imperative and can be selected in developing high yielding sorghum cultivars in breeding programs. Importance of high yielding cultivars cannot be overlooked due to the fact that farmers are more concerned on yields for food and

selling surplus to full fill other requirements. Other studies Dossou-Aminon *et al.* (2015) and Sinha and Kumaravadivel (2016) reported similar findings.

Genotypes were clustered using quantitative growth morphological traits based on Agglomerative hierarchical clustering done on the Euclidean Distance, utilizing Average Linkage method. The twelve main clusters revealed genotypes with similar characteristics and therefore, simplified selection for parental lines for hybridization. Genotypes assembled into five clusters at 55.78% similarity level. Genotypes similar in terms of earliness and yield traits were clustered together regardless of their type and origin. In most cases, apart from high yielding, farmers selection on varieties is based on earliness and grain yield traits (Dossou-Aminon *et al.,* 2015). Maturity is also a key trait for adaptation of the plant to its environmental conditions. The need for earliness varieties and hybrids necessitated by the need for climate change adaptation and resilience due to unpredictable rainfall and long periods of dry spells. In addition, plant height is an important trait to sorghum growers; as the taller genotypes produces exceptional green and dry fodder, due to rationing, thicker stem, grain yield and higher juicy content; extremely needed during the dry seasons in the arid and semiarid regions where sorghum is highly cultivated. Studies indicate that Sorghum fodder contribute to about 45% of the total dry weight of animal feeds during rain seasons to about 60% during dry seasons. Therefore, plant height identifies the total biomass of the crop. Plant height is independent of stem structural composition such as cellulose, hemicelluloses and lignin content (Sadia *et al.,* 2018); thus, taller genotypes can be bred to contain higher cellulose and stalk sugars, and smaller amount of lignin which is ultimate for bioethanol production. On the other hand, the short genotypes could serve as potential parents for production of hybrids, especially for early maturity and short plants for sub-humid and dry low land areas.

The study analysed phenotypic and biochemical traits and revealed a wide variability among sorghum genotypes in these traits related to weevil resistance. High variations in terms of grain qualitative traits in sorghum was reported by other researchers; Earp and Rooney (1982) reported a variation in pericarp thickness in sorghum using electron microscope consisting of very thin (8 to 32 μm) to very thick (40 to 160 μm). Genotypes with testa layer indicates the possibility of having higher levels of tannin concentration compared to non-testa genotypes. Dykes and Rooney (2006) characterized sorghum into three different groups namely; Type I sorghums that lacking pigmented testa and have no tannin, Type II sorghums having pigmented testa with tannin and Type III sorghums having tannin in the testa and

pericarp of the kernel. Endosperm texture is related to kernel hardness which contribute to little insect especially rice weevil damage as compared to soft kernel.

Protein concentration ranges between 6.52 to 12.23% indicating the need for fortification to improve nutrition to sorghum growing and dependent communities. This finding corresponds with results from other studies. For instance, Afripro (2003) reported crude protein range of 6% to 16.6%. Dicko *et al.* (2006) reported protein content range from 7 - 15% using data from FAO and other studies. Mofokeng *et al.* (2018) reported protein range of 7.16 - 16.18% using 59 sorghum genotypes from South Africa. However, Mutwali *et al.* (2018) confirm the fact that protein contents varies due to environment and genotype. In addition, the starch concentration range of 21.88 to 79.05 g/100g was recorded in this study. Other studies reported a diverse starch concentration range; Dicko *et al.* (2006) reported starch concentration range of 60-75 g/100g; Gerrano *et al.* (2014) reported starch concentration range of 44.39% to 68.08% using 22 sorghum accessions mostly from Ethiopia and South Africa. However, it was suggested that starch concentration in sorghum is highly affected by genotype and environment (Boudries *et al.,* 2009). Kernel strength varied from 14.94 N to 110.33 N. This finding correspond with other researchers; for instance Subramanian and Jambunathan (1982) reported hardness range of 3 kg to 12 kg using forty-five sorghum genotypes.

To understand variability among phenotypic and biochemical traits multivariate analysis was performed. Four principal components explained 85.9% of the total variability among these traits. The first and second components accounted over fifty percent of the variation demonstrating existence of relationship among traits. Sinha and Kumaravadivel (2016) reported large contribution of the first two components using forty sorghum accessions. Similar findings has been reported by Gerrano *et al.* (2014) using 22 sorghum accessions. The distributed genotypes across all quadrants indicate a high genetic variability among them. The closer genotypes in the PC axes indicate the close genetic relationship, which can be explained by the shared traits. Genotypes ICSx152002-SB-4-1, IESH 22023, and ICSA75 x ICSR38 were the extremely genotypes indicating that some of these lines can be selected for hybridization of traits of interest to improve sorghum cultivars. However, cluster analysis assembled genotypes into four main clusters at similarity level of 59.68%. Dendrogram showed that genotypes from the same origin and or the same type; were not necessarily assembled within similar clusters based on their physiochemical properties.

Furthermore, the study analysed the relationship between kernel phenotypic traits and biochemical parameters. Results portrayed weak correlation between kernel hardness and protein content of the grain. This finding implies that as kernel weight increases, there is lower possibility of existence of a relationship with the increase in kernel hardness; likewise, the increase in kernel hardness has lower likelihood of existence of a relationship with the increase in protein content of the genotypes. The weak correlations observed in the present study paves a way for further research in this area in future to produce more evidence. Kumari and Chandrashekar (1994) found greater levels of protein content in corneous portion of the endosperm than floury endosperm in sorghum. According to Zunjare *et al.* (2015) the hard sorghum kernel is critical in resistance against fungal and insect attack due to presence of prolamins (War *et al.,* 2012). The higher variability among studied genotypes in terms of kernel phenotypic and biochemical traits is critical in selection of appropriate traits during cultivar development.

The findings in the present study revealed potential variability among sorghum genotypes based on their susceptibility to rice weevil. Considerable variation observed in terms of F1 progeny emergences, susceptibility index, median development period, weevil mortality, percentage kernel damage and percentage weight loss. The index of susceptibility ranged from 2.6 for genotype PATO to 9.6 for ICS x 152 002-SB-8-2. The nine genotypes with SI range of 1-3 namely; PATO, IESV92041SH, ATX623 x AIGD34533, UDO, Mbangala white, IESV74 DL, IESV92172, ICSA15 x R8602, and P9504A x ICSR172 are readily available source of breeding for resistance to destructive storage pests especially rice weevil. In this study, the insect free sample used as a control showed negligible weight loss unlike the infested samples confirming the effect of rice weevil. The commercial released varieties Macia and Wahi recorded higher weight loss compared to resistant genotypes, but less than trial mean indicating existence of potential weevil resistant materials in the studied genotypes. The mean separation clustered sorghum genotypes into three important clusters based on index of susceptibility ranging from resistant, moderate resistance and susceptible. The study revealed insignificant relationship between susceptibility index and growth parameters like days to panicle emergence and grain yield portrayed by parallel scattering of coordinates alongside the x –axis and small R-squared values in the fitted line. This finding implies that the improvement of the studied set of sorghum genotypes does not necessarily compromise with traits like grain yield and days to 50% flowering.

The highest F1 progeny emergence recorded in genotypes ICS x 152 002-SB-8-2, ICS x 152 003-SB-1-1, ICS x 152 002-SB-10-1, IS 15443 and TZA 3993 and less adult emergence were observed in the resistant sorghum genotypes PATO, Mbangala white, IESV 92041-SH, ATX623 x AIGD34533, and Udo. Torres *et al.* (1996), Goftishu and Belete (2014), and Bamaiyi *et al.* (2007) reported less number of adult emergent in resistant sorghum genotypes due to antibiosis effect. Rice weevil multiplication noticed to be fast in susceptible sorghum genotypes compared to resistant ones. For instance genotypes IS 8193, IS 15443, ICS x 152 002-SB-8-2, and IS 15107 took only 28 days for weevil to develop from egg to adult. While resistant sorghum genotypes such as PATO, IESV92041SH, ATX623 x AIGD34533, UDO, Mbangala white, IESV92174 DL, IESV92172, ICSA15 x R8602 and P9504A x ICSR172 recorded 40 days for the median development period. This implies that a short median development time enable more generations per year and great susceptibility of the sorghum genotype to rice weevil. In addition, as the median development period increases F1 progeny emergence decreases. The F1 progeny emergencies showed a positive highly significant difference with resistance parameters like damaged kernel and weight loss. Implying that sorghum genotypes with the highest F1 progeny emergence had higher percentage weight loss and kernel damage. This is because as the progeny emerge and feed on grain the kernel damage and weight loss in grams becomes higher. Bamaiyi *et al.* (2007) reported positive significant difference between progeny emergence and weight loss. The present results suggests that the extension of weevil development period is urged to tremendous decrease sorghum grain and seed losses in storages.

Several studies identified aspects liable for sorghum resistance to rice weevils; these include the phenotypic and biochemical composition of the grain. In this study, kernel strength showed negative significant correlation with the F1 progeny emergencies ($r=-0.506$, $p<0.001$), Susceptibility index ($r=-0.582$, $p<0.001$), kernel damage ($r=-0.571$, $p<0.001$), and weight loss ($r=-0.565$, $p<0.001$); but positively correlated with the median development time ($r=0.560$, $p<0.001$). This finding implies that kernel strength has an implication on sorghum susceptibility to rice weevil; meaning that increase in strength reduces chances of oviposition and thus weevil emergence. The insects took more days to develop from egg to adult, which reduces the number of weevil generations. According to Gerema *et al.* (2017) grain coat characteristics discourage oviposition, inhibits digestive enzyme and increase kernel hardness which enhance resistance to rice weevil. Pradeep (2013) reported the relationship between kernel hardness and levels of phenolic acids in grain coat related to the mechanical

contributions of phenolic dimers to the grain cell wall strength containing toxic effects to insects. Furthermore, Russell (1966), Bamaiyi *et al.* (2007) and Prasad *et al.* (2015) reported a short adult life span and negative significant relationship between sorghum kernel hardness and weight loss.

In this study, none of the susceptibility parameters correlated with the studied biochemical traits such as protein and carbohydrate. Insignificance weak correlation was found between susceptibility parameters with protein content (r=-0.12) and carbohydrate concentration (r=0.073). Weak and none significant correlation between SI with protein and carbohydrate has been reported by several authors; for instance, Dobie (1977) reported (r=-0.74) for protein content and (r=0.08) for carbohydrate. Further, Torres *et al.* (1996) using 29 sorghum genotypes reported (r=-0.18) for protein, (r=0.06) for the carbohydrate. However, Goftishu and Belete (2014) reported that the most important cause of resistance in sorghum against *Sitophilus zeamais* are lysine content in the grain, where the higher concentration of lysine in the genotype the higher resistant genotype it is. However, most of sorghum genotypes have deficient essential amino acids such as lysine, threonine, tryptophan and cysteine (Salunkhe *et al.,* 1977). From these findings, protein content and starch concentration alone could not predict well reasons for susceptibility. More research should be done using grains raised in multi-location for the wider prediction. In addition, the trend shown by resistant sorghum genotypes such as few weevil emergence and longer median development period suggest the need of confirmation of antibiosis effect as also reported in sorghum by Derera *et al.* (2001) mainly attributed by levels several biochemical. According to Carcia-Lara *et al.* (2007) higher levels of phenolic and peroxidases largely contributes to antibiosis in cereals.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study evaluated sorghum genotypes based on growth morphological variation, rice weevil susceptibility; and phenotypic and biochemical traits convening resistance to rice weevil. The studied genotypes revealed a wide variability based on qualitative and quantitative traits providing room for selection in sorghum breeding. Genotypes IESH 22023, IESV 91104 DL, IESV 92172, IESV 23010 DL, MACIA, NACO Mtama 1 and PATO could be used as parental materials in terms of yield and earliness. In addition, crosses of ATX 623 × MACIA, ICSA 88006 × IESV92172DL, ICSA44 × IESV 91104 DL and P9537A × MACIA can be further advanced for release.

The wide genetic variability was observed on kernel phenotypic and biochemical traits in terms of mean kernel diameter, 100 seed weight, kernel hardness, and protein and starch concentration. Sorghum lines F2Striga5, F2Striga14 and IESH 22017 are potential in breeding to improve yield and yield components. The lines PATO, IESV 92174 DL, IESV 92028 DL, and Mbangala white; represents potential sources of kernel strength. Genotype NACO Mtama 1, IESV 92174 DL, IESH 22023 and IESV 92028 DL could be potential parental materials to improve protein content in sorghum cultivars. These traits are the determinants of food quality and protection sorghum grain against pests. However, weak correlation observed among these traits indicating the need for further research; especially multi-location and or multi-season study to confirm potentiality of these genotypes in order to account the effect of genetic environmental interaction.

The study identified resistant sorghum genotypes to rice weevil namely PATO, IESV 92041-SH, ATX623 X AIGD34533, UDO, Mbangala white, IESV 74 DL, IESV 92172, ICSA 15 X R8602, and P9504A X ICSR 172. These genotypes recorded the least F1 progeny emergence, few days of median development time, low percentage of weight loss and less number of damaged kernels. Resistant genotypes include variety, local cultivars, crosses and advanced breeding lines. These materials can be confirmed through multi-location study and be included in crop improvement program as potential parental materials for rice weevil resistance in sorghum. Therefore, information drawn from this study contributes in the development of weevil management strategies in sorghum and other cereal crops.

5.2 Recommendations

Based on the conclusion the following recommendations are put forward:

(i)   Molecular characterization of the same set of sorghum genotypes to confirm the genetic variability at molecular level. This could aid early and accuracy selection and tracking of useful growth and weevil resistance related traits in sorghum breeding programs.

(ii)   Study on genetic potential and heritability of these traits is recommended for breeding precision. Both narrow and broad sense heritability of identified traits particularly earliness, yield and weevil resistance could be more informative to sorghum breeders in making appropriate decision regarding selection of an effective breeding line.

(iii)   There is a need to further research on growth morphological variation and grains susceptibility to rice weevil using a multi-location and or multi-season approach to confirm the variability and potentiality of these traits, while accounting the effect of genetic environmental interaction.

(iv)   Extensive research, involving wider genetic traits on physical and biochemical traits responsible for grain resistance to rice weevil is highly needed; particularly the thickness of pericarp, analysis of lysine, peroxidase, and secondary metabolites such as tannin and phenolic acids with significant antibiosis effect to insects pests.

(v)   This study recommends genotypes IESH 23022, IESV 91104DL, IESV 92172 IESV 23010 DL, MACIA, NACO Mtama 1 and PATO to be selected as source of earliness and yield traits in sorghum breeding programs. Genotype NACO Mtama 1, IESV 92174 DL, IESH 22023 and IESV 92028 DL to be included in improvement of protein content of sorghum cultivars. Genotype PATO, IESV92041SH, ATX623 x AIGD34533, UDO, Mbangala white, IESV 92174 DL, IESV92172, ICSA15 x R8602, and P9504A x ICSR172 with adequate resistance to be included in developing weevil resistant varieties. Lines with adequate kernel strength PATO, IESV 92174 DL, IESV 92028 DL and Mbangala white to be selected in breeding against devastating storage pest, the rice weevil in Tanzania.

(vi)   Introgression of weevil resistant traits in potential high yielding sorghum cultivars is of paramount important and could reduce application of synthetic insecticides during storage hence reduce health hazards and storage cost to farmers and end users.

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APPENDICES

Appendix 1: List of sorghum genotypes used in the study, their origin and type

Sn	Genotype	Code	Source	Type	Sn	Genotype	Code	source	Type
1	NACO Mtama 1	E1	Ilonga	Variety	59	F2 Striga 4	E59	ICRISAT	line
2	HAKIKA	E2	Ilonga	Variety	60	F2 Striga 5	E60	ICRISAT	line
3	PATO	E3	Ilonga	Variety	61	F2 Striga 6	E61	ICRISAT	line
4	WAHI	E4	Ilonga	Variety	62	F2 Striga 7	E62	ICRISAT	line
5	TEGEMEO	E5	Ilonga	Variety	63	F2 Striga 8	E63	ICRISAT	line
6	TESO	E6	ICRISAT	line	64	F2 Striga 9	E64	ICRISAT	line
7	MACIA	E7	Ilonga	Variety	65	F2 Striga 11	E65	ICRISAT	Line
8	IESV 92041-SH	E8	ICRISAT	line	66	F2 Striga 10	E66	ICRISAT	line
9	IESH 25002	E9	ICRISAT	line	67	F2 Striga 12	E67	ICRISAT	line
10	IS 8193	E10	ICRISAT	line	68	F2 Striga 13	E68	ICRISAT	line
11	IESH 22023	E11	ICRISAT	line	69	F2 Striga 14	E69	ICRISAT	line
12	IESV 23010 -DL	E12	ICRISAT	line	70	F2 Striga 15	E70	ICRISAT	line
13	WAGITA	E13	ICRISAT	line	71	F2 Striga 16	E71	ICRISAT	line
14	IS 25395	E14	ICRISAT	line	72	F2 Striga 17	E72	ICRISAT	line
15	ASARECA 14-1-1	E15	ICRISAT	line	73	F2 Striga 18	E73	ICRISAT	line
16	IESV 92038/2SH	E16	ICRISAT	line	74	F2 Striga 20	E75	ICRISAT	line
17	IESV 74 DL	E17	ICRISAT	line	75	F2 Striga 21	E76	ICRISAT	line
18	PATO X WARD AKRA - H1/1/3/1-110-9	E18	ICRISAT	hybrid	76	F2 Striga 22	E77	ICRISAT	line
19	ASARECA 15-2-1	E19	ICRISAT	line	77	ICS x 152 001-SB-	E79	ICRISAT	line
20	IS 15443	E20	ICRISAT	line	78	ICS x 152 001-	E81	ICRISAT	line
21	ASARECA 18-3-1	E21	ICRISAT	line	79	ICS x 152 001-SB-	E82	ICRISAT	line
22	IESV 24030 SH	E22	ICRISAT	line	80	TZA 3943	E83	Gene bank	Local cultivar
23	IESV 23007 DL	E23	ICRISAT	line	81	Udo	E84	Ilonga	Local cultivar
24	KARI MTAMA 2	E24	ICRISAT	variety	82	ICS x 152 001-	E85	ICRISAT	line
25	R8602	E25	ICRISAT	line	83	Mbangala white	E86	Ilonga	Local cultivar
26	ASARECA 12-4-1	E26	ICRISAT	line	84	ICS x 152 001-	E87	ICRISAT	line
27	IESV 92036 SH	E27	ICRISAT	line	85	ICS x 152 001-	E88	ICRISAT	line
28	ASARECA 13-1-1	E28	ICRISAT	line	86	TZA 3983	E89	Gene bank	Local cultivar
29	ASARECA 15-3-1	E29	ICRISAT	line	87	ICS x 152 002-	E90	ICRISAT	line
30	ASARECA 24-4-1	E30	ICRISAT	line	88	ICS x 152 002-	E91	ICRISAT	line
31	IESV 92028 DL	E31	ICRISAT	line	89	ICS x 152 002-	E92	ICRISAT	line
32	IESV 92172	E32	ICRISAT	line	90	ICS x 152 002-	E93	ICRISAT	line
33	IESV 91131 DL	E33	ICRISAT	hybrid	91	ICS x 152 002-	E94	ICRISAT	line
34	ICSA 88006 X IESV92172DL	E34	ICRISAT	hybrid	92	ICS x 152 002-	E95	ICRISAT	line
35	P9518A X IESV 92029 DL	E35	ICRISAT	hybrid	93	ICS x 152 002-	E96	ICRISAT	line
36	P9507A X IESV 91131 DL	E36	ICRISAT	hybrid	94	ICS x 152 003-	E97	ICRISAT	line
37	ICSA44 X IESV 91104 DL	E37	ICRISAT	hybrid	95	ICS x 152 003-	E98	ICRISAT	line
38	IESA2 X PLOT *#142 SUDAN*	E38	ICRISAT	hybrid	96	ICS x 152 003-	E99	ICRISAT	line
39	ICSA12 X IESV 91111DL	E39	ICRISAT	hybrid	97	IS 8884	E101	ICRISAT	line
40	IES11038 X A1GD 34553	E40	ICRISAT	hybrid	98	IS 8852	E103	ICRISAT	line
41	ICSA 11040 X WAHI	E41	ICRISAT	hybrid	99	IS 15107	E104	ICRISAT	line
42	P9504A X ICSR 172	E42	ICRISAT	hybrid	100	AF28	E105	ICRISAT	line
43	IESA2 X R8602	E43	ICRISAT	hybrid	101	IS 11167	E106	ICRISAT	line
44	P9537A X MACIA	E44	ICRISAT	hybrid	102	IS 11758	E107	ICRISAT	line
45	ICSA75 X ICSR 38	E45	ICRISAT	hybrid	103	F6YQ212	E108	ICRISAT	line
46	ICSA 232 X MACIA	E46	ICRISAT	hybrid	104	CR 35:5	E109	ICRISAT	line
47	ICSA 15 X R8602	E47	ICRISAT	hybrid	105	GADAM	E110	ICRISAT	line
48	ATX623 X AIGD34533	E48	ICRISAT	hybrid	106	MAHUBE	E111	ICRISAT	line
49	ICSA 90001 X ICSR 172	E49	ICRISAT	hybrid	107	IS 25395	E112	ICRISAT	line
50	TZA 3993	E50	Tanzania	Local	108	B35	E113	ICRISAT	line
51	IESH 22009	E51	ICRISAT	line	109	FRAMIDA	E114	ICRISAT	line
52	ICSA 90001 X ICSR 160	E52	ICRISAT	hybrid	110	SRN 39	E115	ICRISAT	line
53	ATX 623 X IESV 91131 DL	E53	ICRISAT	hybrid	111	N13	E116	ICRISAT	line
54	IESH 22017	E54	ICRISAT	line	112	IESV 91104 DL	E117	ICRISAT	line
55	ATX 623 X MACIA	E55	ICRISAT	hybrid	113	IESV 92043 DL	E118	ICRISAT	line
56	IESV 91021DL/Flamida	E56	Line	line	114	IESV 24029 SH	E119	ICRISAT	line
57	F2 Striga 2	E57	Line	line	115	IS 21881	E121	ICRISAT	line
58	IESV 92027DL/Flamida	E58	Line	line	116	IS 21185	E122	ICRISAT	line
				line	117	IS 21055	E125	ICRISAT	line

Appendix 2: Means of morphological quantitative traits for 117 sorghum genotypes evaluated for their growth variation in this study

Genotype	50%DF	PH	NrL	PaL	PaWd	Dmt	Dpwt	Gwt	100Swt	Gyld
NACO Mtama 1	66	192.83	11	25.33	5.42	104	86.00	74.83	5.00	3.33
HAKIKA	62	158.50	12	27.67	6.50	104	79.83	58.33	4.92	2.59
PATO	74	277.17	13	18.83	6.67	108	90.33	68.67	4.92	3.05
WAHI	62	132.50	11	24.75	5.42	98	71.83	51.50	4.58	2.29
TEGEMEO	71	202.17	13	22.17	5.75	108	73.33	61.17	4.67	2.72
TESO	64	195.83	11	29.67	6.17	101	85.83	69.00	4.50	3.07
MACIA	66	151.00	13	20.67	5.08	100	89.83	74.17	4.17	3.30
IESV 92041-SH	71	225.50	12	19.67	5.67	103	88.33	72.83	4.17	3.24
IESH 25002	70	160.17	12	29.00	5.75	103	59.67	42.25	4.42	1.88
IS 8193	66	215.33	11	22.42	6.42	102	62.17	50.83	4.17	2.26
IESH 22023	61	241.33	11	27.17	6.50	98	121	106.6	5.67	4.74
IESV 23010 -DL	62	193.83	10	24.83	6.58	100	97.50	75.50	5.17	3.36
WAGITA	75	335.50	13	22.67	7.42	108	57.17	41.67	3.75	1.85
IS 25395	66	209.00	12	24.83	5.83	104	87.83	72.00	3.50	3.20
ASARECA 14-1-1	60	135.33	9	21.00	5.00	96	66.00	48.17	2.67	2.14
IESV 92038/2SH	74	255.50	13	24.50	6.33	108	84.00	70.00	4.50	3.11
IESV 74 DL	67	139.33	10	21.00	4.67	106	87.00	65.00	2.67	2.89
PATO X WARD AKRA - H1/1/3/1-110 0	62	176.50	10	23.92	6.50	99	86.00	67.00	4.33	2.98
ASARECA 15-2-1	60	141.17	11	22.00	5.67	96	67.33	54.00	4.33	2.40
IS 15443	71	242.50	12	16.83	6.00	108	68.67	48.50	3.08	2.16
ASARECA 18-3-1	62	136.50	10	22.50	5.25	98	67.00	49.00	3.50	2.18
IESV 24030 SH	67	208.50	13	21.67	5.33	106	69.00	50.17	4.17	2.23
IESV 23007 DL	62	203.00	9	28.00	6.00	98	55.67	48.17	4.50	2.14
KARI MTAMA 2	68	190.67	12	21.50	6.42	108	75.50	57.50	4.17	2.56
R8602	62	141.17	12	27.67	5.75	97	57.17	45.83	2.92	2.04
ASARECA 12-4-1	60	132.17	10	23.58	5.67	96	70.67	61.50	3.50	2.73
IESV 92036 SH	75	256.33	14	27.17	6.58	116	67.00	47.83	5.17	2.13
ASARECA 13-1-1	61	135.00	10	20.00	4.67	96	55.17	34.33	2.67	1.53
ASARECA 15-3-1	59	136.50	10	21.17	5.67	97	41.17	28.00	2.67	1.24
ASARECA 24-4-1	62	135.83	10	22.17	5.83	97	73.50	54.17	3.50	2.41
IESV 92028 DL	66	245.33	13	25.25	5.17	108	90.00	70.00	5.00	3.11
IESV 92172	66	136.17	10	25.83	4.08	104	94.50	79.00	3.92	3.51
IESV 91131 DL	60	171.67	10	28.42	6.00	96	75.50	59.67	5.83	2.65
ICSA 88006 X IESV92172DL	63	170.67	12	34.67	5.33	100	102.3	87.00	3.50	3.87
P9518A X IESV 92029 DL	60	157.83	10	33.17	6.00	103	71.00	56.67	4.67	2.52
P9507A X IESV 91131 DL	60	182.67	11	29.33	7.67	104	86.83	71.33	5.50	3.17
ICSA44 X IESV 91104 DL	63	259.00	12	21.75	7.75	103	104.0	84.17	6.00	3.74
IESA2 X PLOT #142 SUDAN	61	134.67	10	25.92	5.50	97	36.17	28.00	3.83	1.24
ICSA12 X IESV 91111DL	62	185.50	11	30.50	6.25	98	86.00	70.33	4.50	3.13
IES11038 X A1GD 34553	63	196.67	11	28.67	6.75	99	68.17	61.00	7.00	2.71

Appendix 2 (continue)

Genotype	50%DF	PH	NrL	PaL	PaWd	Dymat	Dpawt	Gwt	100Swt	Gyld
ICSA 11040 X WAHI	61	195.83	11	30.33	5.67	98	54.00	41.17	5.33	1.83
P9504A X ICSR 172	59	183.33	10	28.75	5.50	98	49.50	30.50	4.33	1.36
IESA2 X R8602	62	136.33	10	28.50	5.08	97	22.17	12.00	2.67	0.53
P9537A X MACIA	62	206.50	12	27.83	5.58	102	92.50	78.50	6.08	3.49
ICSA75 X ICSR 38	60	190.67	11	24.00	5.58	102	62.83	41.83	4.08	1.86
ICSA 232 X MACIA	65	195.00	13	29.83	5.67	103	71.00	52.00	4.25	2.31
ICSA 15 X R8602	60	152.33	10	31.83	5.67	97	36.00	26.33	3.50	1.17
ATX623 X AIGD34533	62	213.00	10	30.83	6.17	102	74.83	64.50	5.83	2.87
ICSA 90001 X ICSR 172	68	169.67	13	31.00	5.67	108	74.17	53.00	4.83	2.36
TZA 3993	71	295.50	12	29.67	7.17	108	53.17	41.83	3.92	1.86
IESH 22009	61	181.67	11	30.50	5.83	98	100.8	76.17	3.67	3.39
ICSA 90001 X ICSR 160	70	209.50	12	28.83	5.92	108	53.17	40.83	4.33	1.81
ATX 623 X IESV 91131 DL	62	175.17	11	29.50	6.25	98	94.67	74.00	4.83	3.29
IESH 22017	59	218.33	11	23.83	6.50	102	87.33	72.83	5.08	3.24
ATX 623 X MACIA	63	175.33	12	27.17	6.00	101	119.1	91.83	5.08	4.08
IESV 91021DL/Flamida	64	204.17	11	25.75	6.42	101	71.67	59.83	4.17	2.66
F2 Striga 2	65	203.33	12	26.67	6.50	99	54.50	45.67	3.50	2.03
IESV 92027DL/Flamida	61	182.83	10	26.33	5.25	98	49.00	35.67	3.97	1.59
F2 Striga 4	71	255.83	12	23.67	6.17	106	70.67	57.83	5.17	2.57
F2 Striga 5	61	171.33	11	27.00	5.83	104	69.00	59.50	6.00	2.64
F2 Striga 6	60	174.17	10	23.00	6.33	97	63.17	52.83	4.00	2.35
F2 Striga 7	60	207.83	12	29.83	6.00	99	70.50	57.67	4.50	2.56
F2 Striga 8	59	210.67	9	25.50	6.67	99	81.67	70.50	4.33	3.13
F2 Striga 9	67	268.33	14	25.33	5.17	105	29.67	18.17	3.08	0.81
F2 Striga 11	75	186.17	13	22.25	6.00	112	78.33	51.50	3.67	2.29
F2 Striga 10	64	152.67	10	21.00	4.92	100	35.67	21.00	5.17	0.93
F2 Striga 12	64	164.33	14	25.33	6.67	103	77.17	65.00	5.00	2.89
F2 Striga 13	65	265.00	11	24.17	6.42	104	87.00	74.00	5.33	3.29
F2 Striga 14	68	262.83	13	20.50	6.50	106	93.67	72.17	5.67	3.21
F2 Striga 15	62	256.00	10	25.67	6.33	104	74.17	59.67	5.50	2.65
F2 Striga 16	62	191.00	11	24.50	6.67	100	75.33	59.50	4.83	2.64
F2 Striga 17	61	232.67	12	23.83	7.00	102	86.33	71.83	5.50	3.19
F2 Striga 18	63	144.00	11	26.33	5.33	103	52.83	37.50	4.33	1.67
F2 Striga 20	75	239.83	13	22.17	5.42	111	67.50	48.33	4.83	2.15
F2 Striga 21	64	248.33	11	18.83	6.17	104	58.67	39.67	5.00	1.76
F2 Striga 22	62	121.83	10	22.67	4.42	99	22.33	15.17	2.08	0.67
ICS x 152 001-SB-2-2	83	304.50	16	21.00	8.00	117	67.50	51.83	3.50	2.30
ICS x 152 001-SB-4-1	83	170.50	15	16.33	7.83	118	68.83	50.50	2.67	2.24
ICS x 152 001-SB-4-2	86	275.17	15	27.33	6.42	120	46.50	29.00	2.50	1.29
TZA 3943	75	141.83	13	25.17	7.50	111	46.83	33.00	3.92	1.47
Udo	74	263.00	10	23.58	10.33	110	74.50	48.33	3.33	2.15

Appendix 2 (continue)

Genotype	50%DF	PH	NrL	PaL	PaWd	Dymat	Dpawt	Gwt	100Swt	Gyld
ICS x 152 001-SB-7-1	80	363.67	13	20.33	6.58	116	58.50	43.83	4.33	1.95
Mbangala white	76	379.50	15	24.92	9.42	112	62.50	47.17	4.33	2.10
ICS x 152 001-SB-8-2	85	369.33	17	25.33	6.50	116	35.33	20.00	3.17	0.89
ICS x 152 001-SB-9-1	68	145.67	13	19.00	5.08	108	58.50	45.17	3.25	2.01
TZA 3983	66	360.83	14	21.75	6.33	103	50.83	32.00	2.42	1.42
ICS x 152 002-SB-4-1	70	159.17	13	20.17	5.92	108	74.00	57.00	4.33	2.53
ICS x 152 002-SB-8-1	70	111.50	12	20.33	4.33	108	50.17	26.17	4.33	1.16
ICS x 152 002-SB-8-2	68	144.00	13	21.00	4.42	104	49.33	33.17	4.50	1.47
ICS x 152 002-SB-10-1	68	222.83	12	26.00	6.25	104	44.50	33.00	4.50	1.47
ICS x 152 002-SB-11-1	64	103.67	10	21.42	5.17	103	43.33	30.17	4.50	1.34
ICS x 152 002-SB-13-1	67	116.17	11	18.17	6.00	106	44.17	33.67	4.17	1.50
ICS x 152 002-SB-13-2	71	115.83	14	18.83	5.83	107	43.17	31.00	4.50	1.38
ICS x 152 003-SB-1-1	72	362.17	13	33.17	7.50	108	57.83	36.33	4.92	1.61
ICS x 152 003-SB-1-2	80	189.33	12	20.67	5.17	117	43.00	30.00	3.50	1.33
ICS x 152 003-SB-3-1	62	112.00	12	21.50	5.25	99	31.00	17.83	2.00	0.79
IS 8884	88	289.00	16	11.00	6.00	116	48.33	31.67	3.17	1.41
IS 8852	72	265.17	13	14.92	5.58	108	70.50	55.67	4.00	2.47
IS 15107	74	266.67	12	27.00	8.83	108	81.17	51.83	3.83	2.30
AF28	68	335.33	13	32.33	7.25	100	61.50	47.33	3.50	2.10
IS 11167	92	394.00	18	14.08	5.92	120	57.17	33.17	3.33	1.47
IS 11758	78	363.33	16	31.50	4.75	116	37.00	20.17	3.33	0.90
F6YQ212	52	117.83	8	23.33	5.00	85	31.50	15.67	2.00	0.70
CR 35:5	61	154.83	10	22.17	6.17	96	62.67	52.83	4.33	2.35
GADAM	62	135.50	11	18.50	4.75	98	42.00	29.33	3.17	1.30
MAHUBE	59	104.50	11	26.83	4.42	96	20.17	9.67	2.08	0.43
IS 25395	63	206.67	11	23.83	5.50	98	57.00	48.50	3.67	2.16
B35	66	101.00	12	21.00	3.17	103	28.17	12.33	3.50	0.55
FRAMIDA	59	185.50	10	27.67	5.42	96	50.67	27.33	3.33	1.21
SRN 39	60	164.50	10	17.08	5.08	98	48.67	37.00	5.17	1.64
N13	65	284.67	11	13.25	6.33	103	35.83	27.83	4.50	1.24
IESV 91104 DL	70	275.67	14	21.25	6.50	108	95.17	82.83	5.50	3.68
IESV 92043 DL	65	229.00	11	21.33	5.58	104	68.50	52.50	4.50	2.33
IESV 24029 SH	69	166.50	13	25.00	5.83	108	67.67	41.50	4.67	1.84
IS 21881	67	266.50	12	13.33	5.58	104	55.17	40.00	4.00	1.78
IS 21185	86	278.50	17	12.17	5.92	116	50.67	35.00	3.22	1.56
IS 21055	75	344.00	14	21.67	9.50	108	53.83	43.17	3.83	1.92
Mean	66.74	205.79	11.7	24.07	6.02	103	65.33	49.66	4.17	2.21
SE mean	0.384	3.66	0.1	0.26	0.06	0.33	1.11	1.04	0.05	0.05
CV	10.78	3.5	5.4	6.5	9.2	6.01	7.0	8.6	9.8	8.6

Appendix 3: Weather data for the growing season
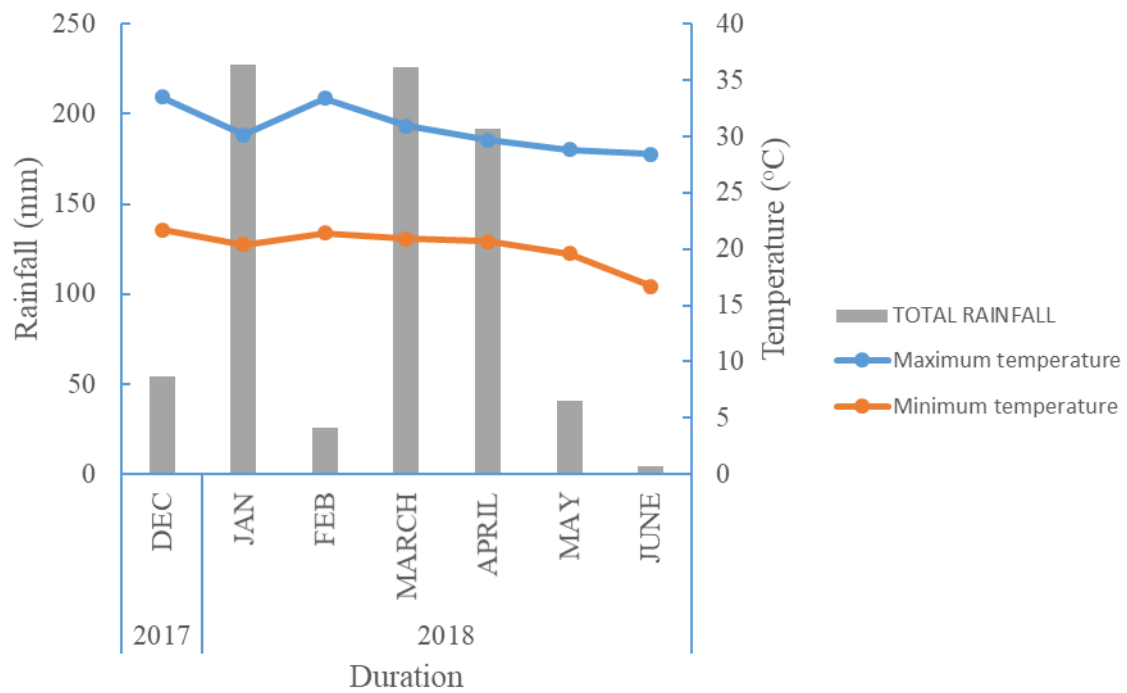
Source: Tanzania Meteorological Agency (TMA), Ilonga Agrometeorological Station.

Appendix 4: Conceptual framework

Figure shows the interaction between rice weevil, phenotypic and biochemical traits. Several mechanisms can be drawn; first is weevil attack strategies and second sorghum grain defensive strategies using either phenotypic and or biochemical traits. Breeders can use these traits as the source of resistance to rice weevils. The resulting physical and chemical related traits is enhanced by the environment and growth characteristics including morphological variability.

Phenotypic traits Seed size, pericarp thickness, kernel hardness		**Resistant genotypes** Low Susceptibility index
Variation in qualitative & quantitative traits	**Sorghum genotypes**	Grain resistance to rice weevil
Biochemical traits Starch, protein, phenolic compounds		**Susceptible genotype** High Susceptibility index

Output 1: Research paper

Physiochemical Properties and Identification of Elite Genotypes for Improved Sorghum Breeding in Tanzania

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Variability in physiochemical properties in sorghum is critical in cultivar development for optimum grain quality and crop resistance against fungal and insect pests. These traits are not well studied. The objective of this study was to characterize sorghum genotypes based on kernel phenotypic and biochemical traits and identify promising genotypes for better utilization of these traits in sorghum breeding. 98 sorghum genotypes comprised by the released varieties, breeding lines, hybrids and local cultivars were studied using qualitative and quantitative parameters. 75.51% of these

genotypes have thick pericarp, 33.67% have testa layer, and 7.0% showed mostly-corneous endosperm texture. Results revealed a wide variability among studied genotypes in terms of phenotypic and biochemical properties (p<0.001). A cross IES11038 X A1GD 34553 recorded the highest 100 seed weight (6.2g). Pato and IESV 92174DL were the hardest genotypes with 110.33 and 108.4N respectively. Protein content ranged from 6.52 to 12.23%, of which Naco Mtama 1 and IESV 24030SH were the promising genotypes. Genotypes ICSA 88006 x IESV92172DL, ICSA15 x R8602 and GADAM recorded the highest starch concentration (79 g/100g). The identified elite genotypes could enable selection and hybridization of useful traits.

Physiochemical Properties and Identification of Elite Genotypes for Improved Sorghum Breeding in Tanzania

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

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IESV 24030SH were the promising genotypes. Genotypes ICSA 88006 x IESV92172DL, ICSA15 x R8602 and GADAM recorded the highest starch concentration (79 g/100g). The identified elite genotypes could enable selection and hybridization of useful traits.

1. INTRODUCTION

Sorghum is the main source of calories and protein to most people in Africa and Asia [1], widely grown in semi-arid areas. The crop is known to withstand harsh environmental condition including drought [2]. Sorghum have a wide genetic diversity in its physical structure and or chemical composition and therefore presenting benefits in hybridization [3]. Variation in structure, nutritional composition and phytochemical composition is critical for selection of desired traits in sorghum breeding [4]. The inheritable qualitative traits in sorghum kernel consist of pericarp color, pericarp thickness, presence of testa, testa color, and endosperm texture; while quantitative traits include grain size and weight [5,6]. Literature indicated that starch is the largest portion of sorghum grain weight made up by amylose and amylopectin molecules held by hydrogen bonds [7]. Amylopectin is made up by large branched polymer unlike the amylose structure. Sorghum starch contain 70-80% amylopectin and 20-30% of amylose; mainly for feed and industrial use [8]. Moreover, Protein concentration in sorghum grain usually varied based on the genotype, water, temperature, and soil fertility status of the soil. According to [9] drought condition is known to increase protein concentration while reducing starch content. Sorghum genotypes with higher yield is known to have smaller concentration of protein; while the application of nitrogenous fertilizer increases protein concentration particularly prolamin, kafirns and glutelins in the sorghum endosperm [10]. In addition, the germ portion comprised by albumin and globulins with highest concentration of lysine [11]. The physical appearance of sorghum kernel structure largely guided by its associated biochemical traits including the phenolic compounds. According to [12] Phenolic compounds consist of benzene ring and hydroxyl group. Plants materials contains phenolic compounds, which reflects the taste, color and appearance. Sorghums has a wide variability of phenolic acids. In addition, [12] screened a number of sorghum genotypes and found high phenolic content in high tannin sorghums. Further [13] concluded the health benefits derived from phenolic such as low digestibility,

reduction of diseases like cardiovascular, anti-carcinogenic and lowering of cholesterol; This is due to antioxidant capacity of phenolic compounds as lowers amount of free radicals in the body. Some sorghum cultivars comprised by tannins or proanthocynidins which is genetically based controlled by genes B1,B2 in the testa [14]. [15] characterized sorghum as Type I (sorghums without condensed tannins), Type II sorghums are genotypes with extractable tannins using 1% acidified methanol and not the pure methanol and Type III sorghums have tannin that can be extracted using both one percent acidified methanol and the pure methanol. Sorghum tannins bind protein and makes it unavailable in the digestion through ionic, hydrogen, hydrophobic and covalent bonding [16]. These compounds were also reported to protect plants against insects [17]. For this case breeders must screen large pool of germplasm to identify genotypes with higher levels of phenolic [18].

Several studies attempted to characterize sorghum genotypes based on physical and biochemical composition. For instance, [19] assessed the phytochemical properties of forty five sorghum genotypes based on weight, protein and sugar content; [4] documented a wide variability in terms of nutritional and stalk sugar content in sorghum. [20] screened four improved sorghum varieties and observed considerable variability in terms of biochemical composition including mineral concentration, crude protein, starch, fat and even ash content. The current study therefore contributes to the general understanding of kernel traits related to phenotypic and biochemical properties for effective utilization of these traits. In Tanzania, many sorghum genotypes were not previously evaluated and their phenotypic and biochemical potential is not understood and or documented; therefore, it is important to characterize a broad range of sorghum genotypes. The study intended to characterize sorghum genotypes based on kernel phenotypic traits and biochemical composition to establish potential of these traits in cultivar development. The study also identified promising sorghum genotypes to be used as parental materials during hybridization.

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2. MATERIALS AND METHODS

2.1 Site and Source of Materials

Ninety eight (98) sorghum genotypes collected from TARI Ilonga center, Tanzania National Plant Genetic Resource Centre (NPGRC) and International Crops Research Institute for the Semi-arid Tropics (ICRISAT); comprised by commercial varieties, hybrids, local cultivars and breeding lines (Table 1). The known agronomic properties of these materials include high yielding, midge resistance, striga resistance, anthracnose, stay green and earliness. Materials were raised at Tanzania Agricultural Research Institute Ilonga in Kilosa, Morogoro Tanzania; located at latitude 06°42'S, longitude 37°02'E and altitude of 506 meters above sea level with a bimodal type of rainfall. Materials were planted in the cropping season 2017/18. All agronomic management including supplementary irrigation, weeding, fertilizer application and insect control were applied as per recommendation. Harvested grains were cleaned and sorted for analysis of phenotypic and biochemical traits at the Nelson Mandela African Institution of science and Technology and food processing laboratory of the Sokoine University of Agriculture.

2.2 Determination of Qualitative Kernel Traits

Ten sound kernel selected randomly for each physical analysis according to procedure described by [21]. Pericarp thickness was determined by scratching sorghum kernel using scalpel and observe the pericarp thickness using a magnifying glass. The presence of testa layer and the associated color was recorded after removal of pericarp. Endosperm texture; was determined by cutting each kernel into half and observe the proportion of corneous material with the aid of magnifying glass; materials were characterized into starch, intermediate and pearly based on the score. Grain color was determined through visual examination using color chart and codes as per sorghum descriptors guide [22].

2.3 Determination of Quantitative Kernel Physical Traits

100 sound sorghum kernels were manually counted and weight measured in replicates using analytical balance TPA 500. Kernel hardness (firmness) was observed using Brookfield CT3 Texture analyzer, using probe TA41 Cylinder 6 mm D, 35 mm L; with the recommended trigger value of 50 g and Load Cell of capacity of 50 kg, test speed was set at 10 mm/s, and deformation of 0.70 mm. The average of six samples (kernels) per test was taken as hardness. Furthermore, the arithmetic mean diameters was taken as average of the major diameter, minor diameter, and intermediate diameter of sorghum kernel using automatic caliper [23].

2.4 Determination of Nitrogen Content

Total nitrogen and protein of sorghum genotypes was determined from grain through digestion, distillation and titration with hydrochloric acid as per Micro Kjeldahl Method [24]. Grain was grinded and sieved using 0.5mm sieve; 0.1 g was placed into a digestion tube. 1g Selenium catalyst mixture weighed and mixed with the sample; followed by addition of 5 ml of sulphuric acid (96%) into the tube. The tubes was heated slowly in the digestion apparatus until the digest is clear. The content was transferred to a 100 ml volumetric flask where distilled water was added into a 100 ml graduated flask. 5ml of boric acid indicator solution were placed into the distillation apparatus. 10ml of clear supernatant were then transferred into the apparatus where 10 ml of NaOH (46%) were added. Color change were observed when distillation drops mixed with the boric acid indicator. 150 ml of the distillate were titrated with sulphuric acids (0.0174N) where color change from green to pink was observed, the titer volume was recorded. Finally, total nitrogen was determined using the following formula:

$$N \text{ (percentage)} = \frac{a \times N \times Mw \times 100 \times 100\%}{b \times c}$$

Where, a = ml of sulphuric acid, N = Normality of sulphuric acid (0.0174), a = Titer volume, Mw = Molecular weight of Nitrogen (0.014), b = gram sample taken for analysis (0.1 g) and c = ml digest used for distillation (10 ml). Thus, the percentage crude protein = $6.25 \times$ % N.

2.5 Determination of Starch Content

Starch concentration was determined using [25] official method 996.11 whereby, 100mg of finely ground sample were taken into 15ml centrifuge tubes. 0.2 ml 80% ethanol was added and vortexed. 3 ml of 10% α – amylase enzyme in mM sodium acetate buffer were added and incubated in a boiling water bath for 6 minutes with 2 minutes shaking intervals. The tubes placed in a water bath at 50 °C and 0.1ml of amyloglocosidase enzyme was added; the tubes

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was stirred using vortex and incubated for 30 minutes. The contents were then centrifuged for 10 minutes at 3000 rpm. A duplicate of 0.1ml aliquot was placed into 15 ml test tube. 3.0 ml of p-hydroxybenzoic acid and sodium azide mixture (1:1) and left to stand for 20 minutes at 20 °C.

5.0 g of D-glucose powder was taken into 100 ml volumetric flask, dissolved with sodium acetate buffer to make stock solution of 50 mg/ml. Serial dilution of 0 – 40 mg/ml prepared into 100 ml volumetric flask. 0.1 ml of diluted standard solution were taken into 15 ml test tube. 3.0 ml p-hydroxybenzoic acid and sodium azide mixture (1:1) and left to stand for 20 minutes at 20 °C. Absorbencies of samples and standards was read at 510 nm using X-ma 3000 UV/Visible spectrophotometer.

2.6 Data Analysis

Qualitative data including pericarp thickness, testa presence, corneous, and endosperm color was analyzed using excel program; where frequencies and percentage presented in bar chart. Data on mean kernel diameter, 100seed weight, kernel hardness, protein and starch concentration were subjected into analysis of variance (ANOVA) using GenStat version 15 software and means were compared using Duncan new multiple range test. Pearson correlation employed to determine the association between quantitative traits. MINTAB version 14 software were used in multivariate analysis such as principal component and cluster analysis.

3. RESULTS AND DISCUSSION

3.1 Qualitative Traits

Most of sorghum genotypes studied (75.51%) had thick pericarp (Fig. 1), while the rest possessed thin pericarp. Other researchers; [26] reported a variation in pericarp thickness in sorghum using electron microscope consisting of very thin (8 to 32 μm) to very thick (40 to 160 μm).

Only 33.67% of sorghum genotypes had either purple or brown testa, while the rest of genotypes had no testa. Genotypes with testa indicates the possibility of having higher levels of tannin concentration compared to non-testa genotypes. [15] characterized sorghum into three different groups namely; Type I sorghums that lacking pigmented testa and have no tannin, Type II sorghums having pigmented testa with tannin

and Type III sorghums having tannin in the testa and pericarp of the kernel. (74.49%) of the evaluated sorghum genotypes had white color endosperm, while the rest were yellowish. While, 7.14% of all genotypes had mostly corneous endosperm texture; 30.61% had intermediate corneous indicating a relative balance between floury content and corneous; while the majority of genotypes were floury or complete starch. Endosperm texture is related to kernel hardness; such that mostly corneous endosperm referring to hard kernel and floury endosperm referring to soft kernel [27]. Great variation were also observed in terms of grain color;where,45.92% of the evaluated genotypes were white in color, 24.49% were red, 23.47% of the genotypes were brown; the rest in small fraction were yellow, buff and mixed colors. However, qualitative traits in sorghum play bigger role in processing and flour quality; for instance, genotypes producing grains of uniform sizes is most preferred in milling than non-uniform because smaller kernels normally taken out with bran.

3.2 Analysis of Variance (ANOVA)

Analysis of variance indicated a highly significant difference ($p < 0.001$) among evaluated genotypes, showing greater genetic variability among traits under consideration (Table 1). Kernel mean diameter ranged between 2.29mm to 4.61mm. Lines ICSx152002-SB-13-2, F2Striga16 and IESH 22017 had the greater mean kernel diameter and genotypes IS 21055 had the lowest mean kernel diameter. 100 seed weight ranged between 1.81to 6.2 g. Genotypes F2Striga5, P9537A x MACIA and IES11038 x A1GD 34553 recorded the highest hundred seed weight; while genotype TZA 3983 had the least weight. Kernel hardness varied between 14.94 newton to 110.33 newton; Genotypes PATO, IESV 92174DL, and IESV 92028 DL recorded the highest kernel hardness, while genotypes IESV 92043DL, F2Striga15 and TZA3993 had the least kernel hardness. [19] reported hardness range of 3 kg to 12 kg using forty-five sorghum genotypes.

Protein concentration ranges between 6.52 to 12.23%; where genotype Naco Mtama 1 and IESV 24030SH recorded the highest concentration and genotypes F2 Striga 13 and ICS x 152 001-SB-4-2 had the lowest concentration. This finding corresponds with results from other studies. For instance, [28] reported crude protein range of 6% to 16.6%. [14] reported protein content range from 7 -15% using data from FAO and other studies. [29]

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Reported protein range of 7.16- 16.18% using 59 sorghum genotypes from South Africa. However, [30] confirm the fact that protein contents varies due to environment and genotype.

The mean total starch concentration ranged between 21.88 to 79.05 g/100 g. The higher concentration observed on genotypes ICSA 88006 X IESV92172DL, ICSA15 x R8602 and GADAM; while Tegemeo and ASARECA 18-3-1 recorded the least concentration. Some genotypes recorded either lower or higher starch concentration due to high diversity of genotypes used in the present study. [14] reported starch concentration range of 60-75 g/100 g; [4] reported starch concentration range of 44.39% to 68.08% using 22 sorghum accessions mostly from Ethiopia and South Africa. However, it was suggested that starch concentration in sorghum is highly affected by genotype and environment [31]. According to [32] sorghum starch is resistant impairing digestion making it useful to people with obesity and diabetic. The higher variability among studied genotypes in terms of kernel phenotypic and biochemical traits is critical in selection of appropriate traits during cultivar development.

3.3 Correlation between Quantitative Traits

Pearson correlation analysis indicated a weak positive significant correlation between 100 seed weight and kernel hardness (r=0.250, p=0.013) (Table 2); while kernel hardness had a positive but weak significant correlation with protein concentration (r=0.225, p=0.026). Starch concentration had a weak negatively significant association with mean kernel diameter (r=-0.200, p=0.048). However, starch concentration showed a negative weak correlation with all studied parameters. This finding implies that as kernel weight increases, there is lower possibility of existence of a relationship with the increase in kernel hardness; likewise, the increase in kernel hardness has lower likelihood of existence of a relationship with the increase in protein content of the genotypes. The weak correlations observed in the present study necessitates the need for further research to confirm these findings. However, [33] found greater levels of protein content in corneous portion of the endosperm than floury endosperm in sorghum. The hard sorghum kernel is critical in resistance against fungal and insect attack such as *Sitophilus oryzae* [34]; due to presence of prolamins [17]. Hardness is also a good determinant of grain quality relating to cooking qualities such as stiffness and the milling qualities [33].

3.4 Principal Component Analysis

Principle component analysis (PCA) grouped five traits into five components. Retention of PCs were based on proportion of variance criterion described by [35]. Four components can be retained based on adequate cumulative amount of variance explained (>80%). About 85.9% of the variances contained in the dataset were retained by the first four principal components. The first component explained 32.7% of the total variation. The high contributing factor loading are 100 seed weight, kernel hardness, mean kernel diameter (MKD), and protein content (Table 3). The second principle component (PC2) accounted 20.1% of the total variation; mainly a function of starch concentration and kernel hardness with negative loadings. With similar logic, in the third component (PC3) protein content have higher positive loading and 100Swt with the largest negative loading. PC4 accounted 15.8% of the total variation with high negative loadings from starch concentration and the mean kernel diameter. According to [35] loading greater than ±0.40 were considered to best represent the corresponding PC axis. The first and second components accounted over fifty percent of the variation demonstrating existence of relationship among traits. [36] reported large contribution of the first two components using forty sorghum accessions. Similar findings has been reported by [4] using 22 sorghum accessions.

Further, the score plot for the first two components (Fig. 2) indicate existence of genetic variation among sorghum genotypes in terms of studied physiochemical traits. The scattered genotypes across all quadrants indicate a high genetic variability among them. Genotypes from different origin and or type were scattered. The closer genotypes in the PC axes indicate the close genetic relationship, which can be explained by the shared traits. Genotypes ICSx152002-SB-4-1, IESH 22023, and ICSA75 x ICSR38 were the extremely genotypes; therefore some of these lines can be selected for hybridization of traits of interest to improve sorghum cultivars.

3.5 Cluster Analysis

Cluster analysis for the phenotypic kernel traits and biochemical parameters indicated a clear

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separation of the evaluated sorghum genotypes (Fig. 3). Four main clusters was observed namely; cluster I, II, III and IV formed at 59.68% similarity level. (Table 4) indicates cluster means, explaining the differences among groups of the evaluated genotypes. Cluster I grouped twenty (20) sorghum genotypes formed based on the lowest concentration of starch and small mean kernel diameter, and highest hundred seed weight, kernel hardness and protein content.

Cluster II grouped seven (7) genotypes consisting of hybrids, breeding lines and a local cultivar (Mbangala white) with the average

protein content, highest mean kernel diameter, and starch concentration. Cluster III grouped sixty seven (67) sorghum genotypes based on average mean kernel diameter, 100 seed weight, kernel hardness, protein content and starch concentration. Cluster IV grouped four (4) sorghum genotypes originated from ICRISAT and Tanzania namely IESV92043DL, IS 21881, ICSx152001-SB-2-2 and TZA3993 these genotypes had the lowest mean kernel diameter, 100 seed weight, kernel hardness and protein content. Dendrogram shows that genotypes from the same origin and or the same type; were not necessarily assembled within similar clusters.
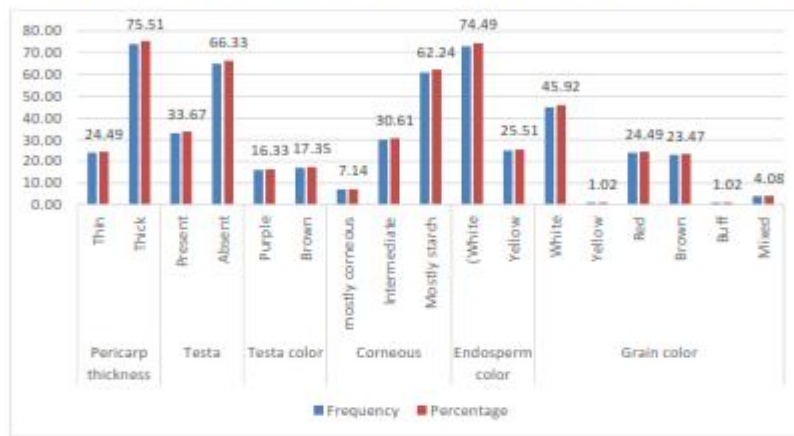
Fig. 1. Frequencies and percentages among qualitative kernel traits

Table 1. Simple statistics

Variable	MKD	100Swt	Hardness	Protein	Starch
Mean	3.02	3.713	70.02	8.656	45.95
SE	0.1	0.075	1.377	0.08	1.116
Minimum	4.6050	1.8083	14.94	6.515	21.88
Maximum	2.2850	6.2000	110.33	12.229	79.05
CV	4.7	2.9	2.8	1.3	3.4
F prob	<0.001	<0.001	<0.001	<0.001	<0.001

MKD = mean kernel diameter, 100Swt =100 seed weight;
SE= Standard error of mean, CV= coefficient of variation,

Table 2. Pearson correlation among the studied traits in terms of phenotypic and biochemical properties

	Mean diameter	100 seed weight	Kernel hardness	Protein
100Seed weight	0.169			
Kernel hardness	0.143	0.250*		
Protein	0.140	0.132	0.225*	
Starch	-0.200*	-0.158	-0.064	-0.087

*significant at p<0.05

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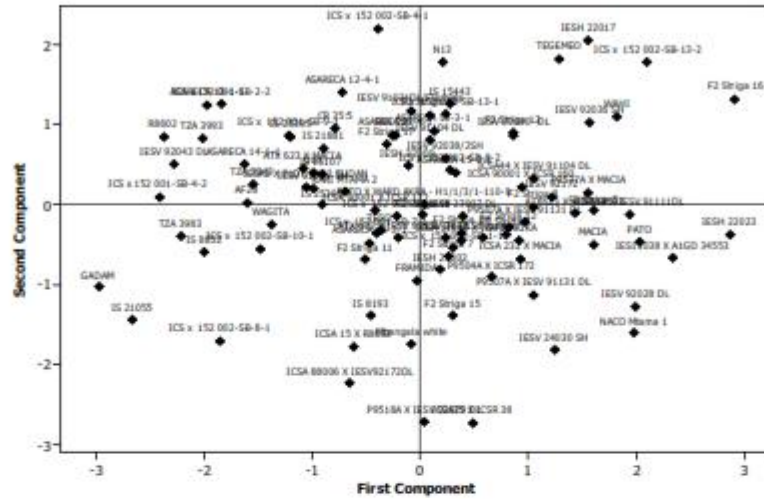
Fig. 2. Score plot of first and second principle components explaining kernel phenotypic and biochemical variation among the evaluated sorghum genotypes
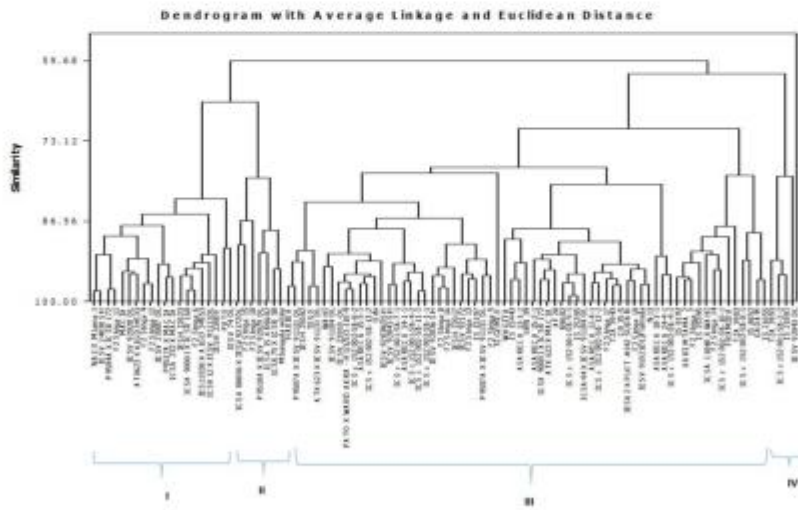
Fig. 3. Dendrogram showing various clusters among 98 sorghum genotypes evaluated in terms of physiochemical properties

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Table 3. Principle component analysis of quantitative physiochemical traits in 98 sorghum genotypes

Variable	PC1	PC2	PC3	PC4	PC5
MKD	0.452	0.386	0.256	-0.761	0.042
100Swt	0.492	-0.051	-0.657	0.076	0.563
Hardness	0.484	-0.485	-0.205	-0.066	-0.697
Protein	0.425	-0.394	0.677	0.300	0.342
Starch	-0.373	-0.051	-0.051	-0.567	0.281
Eigenvalue	1.6346	1.0060	0.8639	0.7907	0.7048
% variance	32.7	20.1	17.3	15.8	14.1
Cumulative % variance	32.7	52.8	70.1	85.9	100

PC= principal component, MKD = mean kernel diameter, 100Swt =100 seed weight;

Table 4. Cluster means of the phenotypic and biochemical traits in the evaluated sorghum genotypes

Clusters	MKD	100Swt	Kernel hardness	Protein	Starch
1	3.0173	4.0499	96.0791	9.2978	39.4972
2	3.0571	3.6405	92.0763	8.9215	70.1171
3	3.0278	3.6451	62.4233	8.4778	44.9705
4	2.9163	3.2896	28.2581	7.9678	52.2067

MKD = mean kernel diameter, 100Swt =100 seed weight;

3.6 Identification of Elite Genotypes for Breeding

Few sorghum genotypes performed better In terms of 100 seed weight; these include genotype IES11038 X A1GD 34553 (6.20 g), P9537A X MACIA (5.49 g), F2Striga5 (5.30 g), ICSA44 X IESV 91104 DL (5.30 g), ATX623 X AIGD34533 (5.12 g), P9507A X IESV 91131 DL (5.12 g) and F2 Striga 14 (5.03 g). Lines F2Striga5 and F2Striga14 can be recommended for crop improvement in terms of yield. However, genotypes with highest average mean kernel diameter were ICS x 152 002-SB-13-2, F2 Striga 16, IESH 22017, IS 15443, F2 Striga 18, N13 and WAHI recorded 4.61, 4.54, 4.07, 3.82, 3.65, 3.62, and 3.60 mm respectively.

Lines with the highest Kernel hardness include PATO, IESV 74 DL; IESV 92028 DL, Mbangala white and F2 Striga 11 which recorded 110.33, 108.43, 103.90, 101.11 and 100.72 N. The highest protein content were recorded in genotype NACO Mtama 1 (12.23), IESV 92174 DL (12.18), IESH 22023 (11.58), IESV 92028 DL (11.21) and ASARECA 15-3-1 (11.04). These genotypes can be potential source of hardness and protein content in breeding programs. Hence, hardness and protein correlated with corneous portion in the endosperm; the later play significant role in resistance against pests including storage weevils. Improvement of these traits in commercial released varieties could be

necessary for sustainable management of storage insects. Nevertheless, more research is needed; a multi-location study is recommended to confirm potentiality of these genotypes.

4. CONCLUSION

The present study revealed a wide variability for the qualitative and quantitative parameters studied. Analysis of variance for the mean diameter, 100 seed weight, kernel hardness, protein and starch concentration showed a high significance difference (p<0.001). Crosses performed better in terms of yield possibly due to heterosis. The best genotypes in terms of 100 seed weight were IES11038 x A1GD 34553 and P9537A x MACIA. However, lines F2Striga5 and F2Striga14 can be recommended to improve yield component. Promising genotypes in terms of mean kernel diameter were ICS x 152 002-SB-13-2, F2Striga16 and IESH 22017. Lines with the upper most kernel hardness include PATO, IESV 74 DL; IESV 92028 DL, and Mbangala white; representing potential sources of kernel hardness. Genotype NACO Mtama 1, IESV 92174 DL, IESH 22023 and IESV 92028 DL could be potential parental materials to improve protein content in sorghum cultivars. However, weak correlation among these traits indicate the need for multi-location or multi-season study to confirm potentiality of these genotypes while accounting the effect of genetic environmental interaction. The studied materials were clustered

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into four main clusters at 59.68% similarity level; genotypes clustered together indicates the possibility of easy selection during hybridization. Physiochemical traits are useful in determination of food quality, processing and kernel protection against pests in sorghum. Variability identified in the present study could aid selection of useful traits for breeding precision.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Table 5. Analysis of variance for the quantitative traits

Genotype	Origin	Type	100swt	MKD	hardness	Protein	Starch
NACO Mtama 1	Ilonga	Variety	4.3 A-F	3.07m-C	95.68H-K	12.229M	47.17 A-G
HAKIKA	Ilonga	Variety	4.2 Zab	3.275 x-I	76.8wxy	9.797 CDE	49.84 F-I
PATO	Ilonga	Variety	4.258z-E	3.16 r-E	110.33P	9.464 zA	33.41 e-h
WAHI	Ilonga	Variety	4.242 z-D	3.595 IJK	65.08 nop	10.479I	27.43 bc
TEGEMEO	Ilonga	Variety	4.108 yzA	3.535 F-K	78.25 w-z	7.347i-m	21.88 a
TESO	ICRISAT	Line	3.85vwx	2.755 d-p	81.18 yzA	7.364 i-n	48.33C-H
MACIA	Ilonga	Variety	3.583 r-u	3.27 w-I	99.2 K-N	10.323 HI	37.81 j-q
IESV 92041-SH	ICRISAT	Line	3.533q-t	2.645 c-h	63.89 l-p	7.382 i-n	44.93 v-C
IESH 25002	ICRISAT	Line	3.858 vwx	3.025 j-A	72.27 s-v	9.762 BCD	55.15 JK
IS 8193	ICRISAT	Line	3.483p-s	2.69c-k	63.7l-p	10.777 J	59.76 LM
IESH 22023	ICRISAT	Line	4.983 NOP	3.485E-J	91.55E-H	11.582 L	35.21e-k
IESV 23010 -DL	ICRISAT	Line	4.633 H-L	3.205 s-G	66.69 n-r	8.344stu	34.7 e-j
WAGITA	ICRISAT	Line	3.075 j-n	2.49 a-d	42.96 c	9.832 C-F	49.13 D-I
IS 25395	ICRISAT	Line	2.9 g-k	3.035 j-A	65.51 nop	8.082 r	52.37 IJ
ASARECA 14-1-1	ICRISAT	Line	2.083 c	2.775 d-q	67.25 o-r	7.049 d-h	42.93 s-z
IESV 92038/2SH	ICRISAT	Line	3.85vwx	2.92 f-w	70.38 rstu	8.397 tuv	33.9 e-i
IESV 92174 DL	ICRISAT	Line	2.033 bc	3.195 s-F	108.43P	8.432uvw	41.62 q-v
PATO X WARD AKRA - H1/1/3/1-110-9	ICRISAT	Hybrid	3.692 r-w	2.625 b-h	64.33 m-p	9.832 C-F	35.62 f-l
ASARECA 15-2-1	ICRISAT	Line	3.667 r-v	2.805d-q	52.15efg	10.199 GH	52.56 IJ
IS 15443	ICRISAT	Line	2.442de	3.82 KL	47.42 d	10.462 I	39.01 l-r
ASARECA 18-3-1	ICRISAT	Line	2.85 f-j	2.805 d-q	53.91fgh	10.777 J	22.5a
IESV 24030 SH	ICRISAT	Line	3.633 r-v	2.705 c-l	96.12IJK	12.177 M	45.67 w-D
IESV 23007 DL	ICRISAT	Line	3.95wxy	3.19 s-F	90.07 D-G	7.399 j-n	48.17C-H
KARI MTAMA 2	ICRISAT	Variety	3.633r-v	3.29y-I	64.45 m-p	7.067 d-h	58.35 KL
R8602	ICRISAT	Line	2.258 cd	2.565 a-f	48.27 de	6.601 ab	43.69 t-A
ASARECA 12-4-1	ICRISAT	Line	2.8 f-i	3.4B-J	59.45 jkl	6.874 cde	40.45 n-u
IESV 92036 SH	ICRISAT	Line	4.583 G-K	3.55 G-K	81.35 yzA	8.082 r	32.01def
ASARECA 13-1-1	ICRISAT	Line	2.083 bc	2.82 d-r	47.37 d	7.032 d-h	39.15l-s
ASARECA 15-3-1	ICRISAT	Line	2.033abc	3.51 E-K	65.78n-q	11.039K	38.96l-r
ASARECA 24-4-1	ICRISAT	Line	2.85 f-j	2.775 d-q	48.3 de	10.549IJ	26.77 bc

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Genotype	Origin	Type	100swt	MKD	hardness	Protein	Starch
IESV 92028 DL	ICRISAT	Line	4.35 A-G	2.92 f-w	103.9 O	11.214K	39.04 I-r
IESV 92172	ICRISAT	Line	3.308 n-q	3.58 H-K	78.12w-z	10.532 IJ	41.33p-v
IESV 91131 DL	ICRISAT	Hybrid	5.117 PQ	2.76d-q	74.65 uvw	10.584 IJ	49.77E-I
ICSA 88006 X IESV92172DL	ICRISAT	Hybrid	2.85 f-j	3.105o-D	81.96 zA	10.077 FGH	79.05 QR
P9518A X IESV 92029 DL	ICRISAT	Hybrid	4.033 xyz	2.625 b-h	99.38K-N	9.709 A-D	71.84 O
P9507A X IESV 91131 DL	ICRISAT	Hybrid	4.8 K-O	2.89 e-u	74.22 t-w	9.499zA	40.44 n-u
ICSA44 X IESV 91104 DL	ICRISAT	Hybrid	5.3QR	3.31 z-J	51.23 d-g	9.814 CDE	46.35 y-F
IESA2 X PLOT #142 SUDAN	ICRISAT	Hybrid	3.117k-n	2.305 ab	57.25 hij	8.869 xy	34.61 e-j
ICSA12 X IESV 91111DL	ICRISAT	Hybrid	3.7 r-w	3.245 v-H	87.26CDE	11.582 L	29.56 cd
IES11038 X A1GD 34553	ICRISAT	Hybrid	6.2 S	2.75 d-o	87.64 CDE	10.322 HI	34.38 e-j
ICSA 11040 X WAHI	ICRISAT	Hybrid	4.717 I-M	3.235 u-H	57.15 hij	9.622 ABC	58.6 L
P9504A X ICSR 172	ICRISAT	Hybrid	3.767 t-w	3.035 j-A	101.29 MNO	8.502 uvw	49.56 E-I
P9537A X MACIA	ICRISAT	Hybrid	5.492 R	3.29 y-I	86.34 BCD	7.802 opq	42.31 r-x
ICSA75 X ICSR 38	ICRISAT	Hybrid	3.517 q-t	2.69 c-k	97.56 KLM	11.617 L	65.23 N
ICSA 232 X MACIA	ICRISAT	Hybrid	3.715 s-w	3.055I-B	88.34 DEF	10.042 EFG	44 u-B
ICSA 15 X R8602	ICRISAT	Hybrid	2.85 f-j	3.375 A-J	101.5 MNO	7.399 j-n	79 QR
ATX623 X AIGD34533	ICRISAT	Hybrid	5.117 PQ	3.005 i-z	102.63 NO	7.277 h-l	37.56 i-p
ICSA 90001 X ICSR 172	ICRISAT	Hybrid	4.217 z-C	2.715d-m	57.09 hij	8.677 wx	47.54 B-G
TZA 3993	Gene bank	Local	3.258m-p	2.54 a-e	33.85 b	7.399 j-n	45.6 w-D
IESH 22009	ICRISAT	Line	3.083 j-n	2.75d-o	95.5 H-K	6.734 abc	29.8 cd
ICSA 90001 X ICSR 160	ICRISAT	Hybrid	3.667 r-v	3.055 I-B	91.96 F-I	8.642 vwx	31.93 def
ATX 623 X IESV 91131 DL	ICRISAT	Hybrid	4.217 z-C	2.785 d-q	82.28zAB	7.2 g-l	50.54GHI
IESH 22017	ICRISAT	Line	4.492 E-I	4.07 L	76.47 vwx	6.57 a	31.5 de
ATX 623 X MACIA	ICRISAT	Hybrid	4.442 B-H	2.545a-e	54.49 f-i	6.55 a	46.03 x-E
IESV 91021DL/Flamida	ICRISAT	Line	3.483 p-s	3.31 z-J	52.43 efg	8.484 uvw	38 j-q
F2 Striga 4	ICRISAT	Line	4.483 D-I	2.74 d-n	95.08 H-K	7.592 mno	41.3 p-v
F2 Striga 5	ICRISAT	Line	5.3QR	3.11 p-D	70.27 r-u	10.182 GH	39.54 m-s
F2 Striga 6	ICRISAT	Line	3.2mn	2.82 d-r	92.84 G-J	9.972 D-G	32.78d-g
F2 Striga 7	ICRISAT	Line	3.85 vwx	3.21 t-G	65.47 nop	10.094 GH	56.68 KL
F2 Striga 8	ICRISAT	Line	3.617 r-v	3.09 n-C	78.4 w-z	10.497 I	34.76 e-j
F2 Striga 11	ICRISAT	Line	3.033 i-m	2.77 d-q	100.72 L-O	6.892 c-f	49.8 F-I
F2 Striga 10	ICRISAT	Line	4.633 H-L	3.04 k-A	58.66 ijk	7.137 e-j	36.7 h-n
F2 Striga 12	ICRISAT	Line	4.45 C-H	2.835 d-r	96.71 JKL	7.784 op	38.88k-r
F2 Striga 13	ICRISAT	Line	4.767 J-N	3.505 E-K	79.52 x-A	6.515 a	42.18 r-w

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Genotype	Origin	Type	100swt	MKD	hardness	Protein	Starch
F2 Striga 14	ICRISAT	Line	5.033 OP	3.445 D-J	64.62m-p	8.099 rs	64.56N
F2 Striga 15	ICRISAT	Line	4.85 L-O	3.165 r-E	79.45 x-A	8.537 uvw	70.4 O
F2 Striga 16	ICRISAT	Line	4.217 z-C	4.54 M	80 x-A	10.182GH	34.48e-j
F2 Striga 17	ICRISAT	Line	4.75 J-N	2.875 e-t	57.43 hij	6.839bcd	42.72 r-y
F2 Striga 18	ICRISAT	Line	3.617 r-v	3.65 JK	83.57 ABC	6.944 c-g	62.06MN
ICS x 152 001-SB-2-2	ICRISAT	Line	2.7 fg	3.235 u-H	30.16 b	7.434 lmn	51.89 HIJ
ICS x 152 001-SB-4-2	ICRISAT	Line	1.85 ab	2.36 abc	68.02 p-s	6.55 a	46.08 x-F
TZA 3943	Gene bank	Local	3.258m-p	2.295 ab	53.58 fgh	7.784 op	40.83 o-u
Udo	Ilonga	Local	2.667 fg	2.655 c-i	62.51 k-n	8.502 uvw	37.43 i-o
ICS x 152 001-SB-7-1	ICRISAT	Line	3.617 r-v	2.68 c-j	62.84k-o	9.464 zA	46.53 z-F
Mbangala white	Ilonga	local	3.767 t-w	2.79d-q	101.11MNO	8.169 rst	63.23 MN
ICS x 152 001-SB-9-1	ICRISAT	Line	2.675 fg	3.07 m-C	51.11 d-g	7.784 opq	44.75 v-C
TZA 3983	Gene bank	Local	1.808 a	2.96 h-z	54 fgh	8.169 rst	64.85N
ICS x 152 002-SB-4-1	ICRISAT	Line	3.617 r-v	3.115 q-D	47.62 d	6.944 c-g	24.61 ab
ICS x 152 002-SB-8-1	ICRISAT	Line	3.517 q-t	2.585 a-g	59.49 jkl	8.344 stu	78.25 QR
ICS x 152 002-SB-8-2	ICRISAT	Line	3.85 vwx	2.855 e-s	63.85 l-p	9.359z	33.85 e-i
ICS x 152 002-SB-10-1	ICRISAT	Line	3.8 u-x	2.655c-i	60.16 j-m	7.154 f-k	62.52 MN
ICS x 152 002-SB-11-1	ICRISAT	Line	3.75 t-w	2.87 e-t	70.47 r-u	8.467 uvw	46.85 A-G
ICS x 152 002-SB-13-1	ICRISAT	Line	3.583 r-u	3.475 E-J	57.31 hij	8.484 uvw	39.26 l-s
ICS x 152 002-SB-13-2	ICRISAT	Line	3.8 u-x	4.605 M	65.19 nop	9.517 zAB	37.79 j-q
ICS x 152 003-SB-1-1	ICRISAT	Line	4.158 yzA	2.785 d-q	70 q-t	9.797 CDE	46.01 x-E
IS 8852	ICRISAT	Line	3.3n-q	2.925 g-x	55.61 g-j	6.731 abc	72.79 OP
IS 15107	ICRISAT	Line	3.187 lmn	2.68 c-j	76.97 wxy	6.594 ab	40.18 n-t
AF28	ICRISAT	Line	2.95 h-l	2.645 c-h	57.1 hij	7.627 no	51.28HI
CR 35:5	ICRISAT	Line	3.717 s-w	3.005 i-z	54.78 f-i	6.962 c-g	42.93 s-z
GADAM	ICRISAT	Line	2.633 ef	2.625 b-h	47.08 d	7.119 e-i	79 R
IS 25395	ICRISAT	Line	3.133k-n	2.955 h-y	50.5def	7.417 k-n	44.77 v-C
FRAMIDA	ICRISAT	Line	2.767 fgh	3.42 C-IJ	71.78stu	10.497 I	62.54 MN
SRN 39	ICRISAT	Line	4.533 F-IJ	2.91 f-v	41.91 c	8.467 uvw	40.76o-u
N13	ICRISAT	Line	3.85 vwx	3.615 IJK	54.31 f-i	7.294 h-l	36.37 g-m
IESV 91104 DL	ICRISAT	Line	4.9M-P	2.605 a-h	62.58 k-o	7.399 j-n	31.44 de
IESV 92043 DL	ICRISAT	Line	3.75 t-w	2.68 c-j	14.94 a	8.029 pr	59.81 LM
IS 21881	ICRISAT	Line	3.45 o-r	3.21 t-G	34.09 b	9.009 y	51.53 HI
IS 21055	ICRISAT	Line	3.217 mno	2.285 a	47.09d	8.047 r	75.61 PQ

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Genotype	Origin	Type	100swt	MKD	hardness	Protein	Starch
		Mean	3.02	3.713	70.02	8.656	45.95
		SE	0.1	0.075	1.377	0.08	1.116
		SED	0.14	0.106	1.948	0.114	1.578
		LSD	0.28	0.211	3.866	0.226	3.131
		CV	4.7	2.9	2.8	1.3	3.4
		F prob	<0.001	<0.001	<0.001	<0.001	<0.001

MKD = mean kernel diameter, 100Swt =100 seed weight;
SE= Standard error of mean, SED = Standard of error of differences of means, LSD = Least significance difference of means (5% level), CV= coefficient of variation

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Output 2: Poster Presentation