EVALUATION AND GENOME WIDE ASSOCIATION MAPPING OF ETHIOPIAN SORGHUM LANDRACES (*Sorghum bicolor* (L.) Moench) UNDER MOISTURE STRESSCONDITIONS AT MIESSO, EASTERN ETHIOPIA

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EVALUATION AND GENOME WIDE ASSOCIATION MAPPING OF ETHIOPIAN SORGHUM LANDRACES (*Sorghum bicolor* (L.) Moench) UNDER MOISTURE STRESSCONDITIONS AT MIESSO, EASTERN ETHIOPIA

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A Thesis

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In Partial Fulfillment of the Requirements for the Degree of Master of Science in Plant Breeding

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DEDICATION

To my mother Genet Mulugeta and my father Tefera Shibiru

STATEMENT OF THE AUTHOR

First, I, Gezahegn Tefera, declare that this thesis is my work and that all source of material used for this thesis have been acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Plant Breeding at Jimma University, and it can be deposited at the University Library to be made available to borrowers under the rules of the library. I declare that this thesis is not submitted to any other institutions anywhere for the award of academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

Gezahegn was born on 24 May 1990 at Sululta woreda, special zone of Oromia regional state. He attended his elementary school from 1996 to2003 at Gorfo elementary school and then joined Chanco Aba Gada secondary school from 2004 to 2007. He then joined the Jimma University, Ambo Colleague of Agriculture and now Ambo University, 2008 and graduated with Bachelor of Science in crop production in 2010.

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Every step in my life including completion of this study is by the will of my Almighty God.

Nothing shall be impossible for God!

LIST OF ACRONYMS

Acronym	Meaning
AM	Association Mapping
BLINK	Bayesian Information and LD Interatively Nested Keyway
BLUP	Best linear unbiased prediction
CSA	Central Statistical Agency
DNA	Deoxyribonucleic Amino Acid
EBI	Ethiopia Biodiversity Institute
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agriculture Organization
GAM	Genetic Advance as a Percent of Mean
GBS	Genotype by Sequence
GCV	Genotypic Coefficient of Variation
GWAS	Genome Wide Association Study
IBPRG	International Plant Genetic Resource Institute
ICRISAT	International Crop Research Institute in Semi-Arid Tropics
LD	Linkage Disequilibrium
m.a.s.l	Meter Above Sea Level
MAF	Minor Allele Frequency
MAT	Marker Trait Association
PCV	Phenotypic Coefficient of Variation
QTL	Quantitative Trait Loci
SNNP	Southern Nations, Nationalities and Peoples
SNP	Single Nucleotide Polymorphism

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ABSTRACT

Drought is the primary cause of crop yield loss among abiotic factorsaround the world. It is a major problem in Ethiopia, leading to food shortages and is a challenge for small-holder farmers to produce enough sorghum grain when rainfall is low and erratic. Improvement of the crop for drought tolerance related traits requires studying the genetics of the traits.Therefore, the objectives of this study were to assessthe genetic variability among sorghum genotypes for drought tolerance related traits, and identify genomic regions associated with drought related traits. A total of 945 sorghum genotypes collected from different geographic regions were evaluated in an alpha-latticedesign with two replications in 2018/19 main cropping season at Miesso (Eastern Ethiopia). Analysis of variance showed that there was highly significant difference ($p<0.01$) among the genotypes for all the traits.Genotypic coefficient of variation (GCV) ranged from 4.27% to 52.96%, and phenotypic coefficient of variation ranged from 5.53% to 31.53%. Heritability ranged from 54.75% to 88.9% and genetic advance as a percent of mean (GAM) ranged from 6.78% to 102.87%. Among the traits with high GCV and heritability estimates, panicle length and width, leaf area and number oftillers per plant were in conjunction with higher values of GAM. This indicates that, these traits are controlled by additive genetic factors and less environmental influence. A total of 692 genotypes (73.62%) assigned to either one of the 5 sub-populationswith the admixture coefficient value >60%, while the remaining 248 genotypes (26.38%) were categorized as admixtures. A total of 91 significant ($p \leq 5.21E\text{-}5$) marker-trait associations were detected for 17 traits, explaining 6.32% to 36.82% of the phenotypic variations and 11 markers, out of 91 were found to be associated with more than one trait. The results of this study showed the existence of genetic variability in the studied genotypes and indicates the presence of opportunity to select a number of promising parental lines with desirable traits related to drought tolerance. The identified genomic regions cloudbe transferred to high yielding but drought susceptible varieties through marker-assisted breeding after a proper validation.

Keywords: Sorghum, GWAS, Drought, SNPs

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a self-pollinating, diploid(2n = 2x = 20) species belonging to the Poaceae family. It is a monocotyledon plant of tropical origin.Cultivated sorghum probably originated innortheastern Africa (Ethiopia, Sudan), where the greatest diversity of both wild and cultivated species occurs (Harlan and De Wet, 1972; Vavilov, 1951).As a C4 species,and sorghum has greater transpirationefficiency and hence survives and grows better than most other cereal crops under water-stress conditions (Doggett, 1988; Rooney, 2004).

Sorghum is the fifth most important food (grain) or feed (grainand biomass)cropin the worldafter wheat, maize, rice and barley(FAOSTAT, 2017), andthe second most important cereal crop (after maize) in Sub Saharan Africa(Zidenga, 2004).The crop is mainly grown in tropical and subtropical areas that are marginal and stress prone. Over 60 percent of the total area devoted to sorghum in the world is in Africa, where the area under sorghum production is about 23.14 million ha and total production and average yield being 23.35 million metric tons and 1.01 tons/ha, respectively (FAO, 2015). Ethiopia is the third largest sorghum producer in Africa next to Nigeria and Sudan (Chala *etal*., 2019) where the crop is one of the major food cereals.

In Ethiopia, sorghum is contributing 16.4% of the total annual cereal grain production (CSA, 2017). In Ethiopia, the area covered with sorghum is 1.9 million ha with a total grain production of 4.8 million tons (CSA, 2017). It is the dominant crop in the dry lowlands which accounts for 66% of the total cultivated areas of the country and the national average productivity of sorghum in Ethiopia is 2.7 tons/ha (CSA, 2018).

The area and production of sorghum in Ethiopia has steadily increased over the years. However, the national average productivity is 2.7 tons/ha (CSA, 2018) which is far below the genetic potential of the crop and compared to countries like USA (4.3 tons/ha), Argentina (4.9 tons/ha), and China (3.2 tons/ha) (FAOSTAT, 2014).

Various biotic and abiotic factors contribute to the low productivity of sorghum. Among the abiotic factors, drought is the major cause for low productivity of the crop (Asfaw, 2007). Worldwide, the annual yield loss due to drought is estimated to be around 10billion US dollar (Mutava, 2009). In Ethiopia it is a major problem leading to food shortages and

challenging small-holder farmers in Ethiopia to produce enough sorghum grain when rainfall is low and erratic. The effect of drought on crop yield is dependent on the stage of plant development.Assefa et al.(2010) has reported that water stress occurring during the vegetative stage alone could reduce yield by > 36% and > 55% at the reproductive stage.In Ethiopia, complete yield loss due to drought was recorded in some parts of the country, such as Mehoni area (EIAR, 2014). In2015/16 cropping season drought inflicted a total crop failure in the major lowland sorghum growing areas of the country(https://www.expogr.com/ethiopia/foodexpo/detail_news.php?newsid=4461&pageid=2).

Plants including sorghum resist drought stress by either of drought escape, drought avoidance or drought tolerance mechanisms.Drought tolerance in sorghum is a complex quantitative trait controlled by many genes coding for various traits contributing towards tolerance (Blum, 1979). Development of molecular markers and their use in Quantitative Trait Loci (QTL) analysis has become a powerful tool for studying the inheritance of complex traits and helps for improving drought tolerance in crops (Suji *etal.*, 2011). In crop plants, there are two approaches for dissecting genomic regions influencing expression of quantitative traits. The most common approach of QTL mapping is to identify QTLs in a bi-parental crosses (Shehzad*etal.*, 2009). However, biparental mapping is constrained by a lack of allelic diversity, which limits the characterization of genetic architecture, and a lack of recombination events, which limits the resolution of mapping (Myles *etal.*, 2009).

More recently, genome-wide association studies (GWAS) provide opportunities to capturemore allelic diversity existing in natural populations and recombination events. With its power in overcoming the major limitations of bi-parental mapping populations, it is becoming a more common approach in trait identification (Brachi *etal.*, 2011) particularly with recent advances in high throughput DNA sequencing technologies. In sorghum, association mapping has been employed to identify association between genomic regions and different phenotypic traits, including plant height, panicle exsertion, awns, days to flowering, culm length, number oftillers and panicle length(Shehzad *et al.*, 2009; Bhosale *et al.*, 2012, Girma *et al.*, 2019). GWAS analysis for drought tolerance traits including days to flowering, chlorophyll content, and tiller number was also conducted in a sorghum mini core collection (Morris*et al.*, 2015).

The development of drought tolerant genotypes is, however, based on the exploitation of genetic variability of the genotypes with the traits of interest.Ethiopia being first rank among countries that have contributed germplasm collections to the initial world collections of sorghum at ICRISAT (Rao *et al.*, 1989), it is a rich source of sorghum landraces. Landraces or farmers varieties have been found to have higher variability and stability (adaptation over time) in marginal environments, encompassing a population of genes and alleles that are adaptable to natural and human selection pressures (Ceccarelli and Grando, 2002). The existence of the different sorghum landrace accessions, which can respond to the recurrent moisture stress, is expected to provide an opportunity in screening and identifying best drought tolerance accessions with relatively stable yield.

In Ethiopia, many attempts have been made to address the drought problem in sorghum. Bekelle(2008) reported the presence of genetic diversity among Ethiopian sorghum germplasm accessions collected from the drought prone areas. Ayana et al.(2001) evaluated 415 accessions based on morphological traits and showed the presence of significant variation among Ethiopian sorghum accessions.

There are around 11,353 sorghum accessionscollected and conservedby the Ethiopian Biodiversity Inistitute(EBI, 2016).However, only a small portion of the germplasm resources have been used in breeding programs for enhancing sorghum production and productivity. One of the limitations for the utilization of these vital resources is lack of sufficient genetic information on the accessions (either for patterns of diversity or for specific drought tolerance mechanisms).Therefore, understanding the extent and pattern of genetic variability for key traits have paramount importance for sorghum breeding under water limited conditions. Drought tolerance in sorghum is a function of various physiological and morphological traitscontributing towards tolerance (Younis *etal.*, 2000).Evaluation of root characterized sorghum genotypes under target environments provides an opportunity to identify promising parental which combines desirable drought tolerance traits.However, very limited workshave been done to evaluate Ethiopian sorghum germplasm for drought tolerance.

Identification of desirable genotypes using morphological traits is less efficient and ineffective as morphological markers are strongly influenced by environmental factors for their expression and may not reflect the true genetic constitution of a genotype(Eagles *etal.*, 2001; Luzuriaga *et al.*, 2006). Moreover, morphological markers used for phenotypic traits are limited in number(Collard *et al.*, 2005) as compared to molecular markers, which are highly abundantin genomes, not dependent on stage of growth or part of the plant and they are phenotype and environment independent(Winter and Kahl, 1995).

Identification of genomic regions and loci underlying traits of interest in crops were primarily based on evaluation of genetic populations derived from bi-parental crosses. However, this approach has yielded limited genomic resolution and restricted allelic diversity as only allelic segregates between and among the parents of the particular recombinant progenies can be assayed. Genome wide association study (GWAS) is an alternative approach that does not require development of biparental crosses and several generations of progeny (Rafalski**,** 2010). GWAS has become a routinely used method to investigate the genetic mechanisms underlying genetic variation. However, identification of genomic regions associated with drought tolerance in the Ethiopian sorghum germplasm using this approach is limited. Hence, this study was conducted with the following objectives**:**

- To evaluate the performance of sorghum landraces for drought tolerance and identifying promising genotypes.
- To identify chromosomal regions associated with drought tolerance and other important traits.

2. LITERATURE REVIEW

2.1. Origin and domestication

The domestication of sorghum has its origins in Ethiopia and surrounding countries, commencing around 4000–3000 BC(Dillon*et al*., 2007). In early 200 AD sorghum made its way into Eastern Africa from Ethiopia via the local tribes(Ng'uni*et al*., 2011).Some archaeological evidence also indicates that cereal domestication practice was introduced from Ethiopia to Egypt about 3000 BC (Doggett, 1965).There are also suggestions that cultivated sorghum was domesticated by selections from a wild progenitor, subspecies *verticilliflorum*, about 5000-7000 years ago (Purseglove, 1972).

2.2. Taxonomicclassifications and races

Sorghum was first described by Linnaeus in 1753 under the name of *Holcus*. Later on in 1794,Moench distinguishedthe genus sorghum from the *Holcus* and gave it the binomial of *Sorghum bicolor*(Kumar, 2016). The current formal taxonomic concept of the sorghum genus and species agrees with the one established byMoench. *Sorghum* has 25 species, grouped into five subgenera or sections: *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum*, and *Stiposorghum*(Garber, 1950). Section Eusorghum includes cultivated sorghum S. bicolor (L.) Moench (2n=20) and its subspecies *Drummondii* and *Arundinaceum*, and the wild species *S. almum* Parodi, *S. propinquum* (Kunth) Hitch and *S. halepense* (L.) Pers. Cultivated sorghum has five basic races: Bicolor (B), Guinea (G), Caudatum (C), Kafir (K), and Durra (D), and 10 intermediate/hybrid races. All 15 races of cultivated sorghums are defined on the basis of spikelet, seed, and panicle morphology (Harlan and DeWet, 1972).

The cultivated *S.bicolor* subsp. *Bicolor*, race durra sorghums developed primarily in Ethiopia and the Horn of Africa, is adapted to the drier parts of Northern Africa and Asia (Morris *et al*., 2013). Race kafir types originated in the Southern Africaand adapted to the high rainfall regions of Eastern and Southern Africa (Kimber *et al*., 2013).Race Guinea sorghums are grown mainly in West and Eastern Africa.Races bicolor types are widely distributed in Africa and Asia, which is originated in the East Africa from the variety aethiopicum though the high genetic diversity found in Asia which was arose after its introduction to the region (Kimber *et al*, 2013).Race caudatum is dominantly in parts of Sudan, Chad, Nigeria and most of Uganda (Bitima, 2016).

2.3. Ecology and botany of sorghum

Sorghum is evolved in semi arid tropical Africa and widely adapted to regions lying between 40^0 N and 40^0 S of the equator (Doggett, 1988). It is a warm weather plant and its growth and maturity can be retarded when temperatures drop below 15^0 C.It can withstand maximum temperature of up to 37^0 C; however, it grows best at an optimum temperature of about 27^0 C (Wilson and Myers, 1954). Sorghum is a short-day plant that grows on a wide range of soil conditions, from heavy clay soils to light sand, with pH ranging from 5.0 to 8.5 (Smith andFrederickson, 2000). Sorghum is tolerant of arid and saline growing conditions, and reaches maturity in 90 to 180 days. It requires an annual rainfall of 400 to 800 mm, which should be well distributed over the cropping season (Ng'uni *et al*., 2011).

Sorghum is an annual and predominantly self-pollinated cereal with the degree of spontaneous cross pollination, in some cases, reaching up to 30% depending on panicle types (Dje *et al*., 2004). It is a vigorous, coarse, erect canelike grass of height ranging from 0.5 to 6 m tall, depending on variety and growing conditions (Purseglove, 1972). The sorghum plants have an extensive root system that can penetrate 1.5 to 2.5 meters into the soil and extend 1.5 meter away from the stem (Shoemaker and Bransby, 2010). It may produce two or more tillers. The stalk is solid. The center of the stem can be dry or juicy, insipid or sweet to taste. A dry stalked variety has leaves with a white or yellow midrib, while a juicy stalked variety has a dull green midrib because of the presence of the juice instead of air spaces in the pithy tissues. The number of leaves on the plant varies between 7 and 24 depending on the variety. The flowers open during the night or early morning. Those at the top of the panicle open first and it takes approximately 4 - 7 days for the entire panicle to flower (Acquaah, 2007).

2.4. Sorghum genetic variability

Genetic variability is the occurrence of differences among individuals due to differences in their genetic composition and/or the environment in which they are raised. Sorghum is a crop species with a wealth of genetic variability, which may have originated from the sympatric coevolution and intercrossing of the cultivated and wild species in Africa (Tesso *et al*., 2008). The diversity of new sorghum types, varieties and races created through the movement of people, disruptive selection, geographic isolation and recombination of these types in

different environments would have been large (Dillon *et al*., 2007). The high level of genetic variability in sorghum could also be related to the rate of outcrossing in the species, which can reach up to 30% depending on the head type. However, the predominantly self-fertilizing nature of the crop could help to fix and maintain novel genetic variations in the population (Rooney, 2004).

Being an indigenous crop with tremendous amount of variability (Asfaw, 2007), Ethiopia serves as the global reservoir for sources of favorable genes of various crops to which it is the Vavilonian center of origin and diversity including sorghum (Vavilov, 1951) ranking first among countries that have contributed sorghum collections at ICRISAT (Rao*et al*., 1989). All the races, except Kafir, and the corresponding intermediate races are naturally found in Ethiopia(Teshome *etal*., 1997,Stemler *etal*., 1977).Information on the nature and magnitude of genetic variability present in a crop species is thus important for developing effective crop improvement program.

The amount of the total genotypic and phenotypic variability that exists in a crop germplasm dictates the initiation of crop improvement programs, of the total variability present in a population the genetic component is the most important to the breeder as it could be transmitted to the progeny.In addition, proper management of this type of variability can produce permanent gain in the performance of the crop concerned (Welsh, 1991). Phenotypic variability is the observable traits of variation present in a population and it is a combined effect of genotypic value and environmental deviation. Genotypic variations, on the other hand, is the component of variation which is due to the genetic differences among individuals within a population and is the main concern of plant breeding(Allard, 1996).

In sorghum, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) above 20% for panicle length, productive tillers, grain yieldandplant height (Abraha *et al*., 2015). Tesso et al.(2011) has reported on phenology, plant height, a range of leaf traits and yield components to determine the extent of morphological variability among the Ethiopian durras and indicated that significant variation for all traits measured, which could be respond to selection pressure for sorghum improvement.Tirifesa(2009) reported higher genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV)

forhead length and 100 seed weight; andmodrate GCV and PCV fornumber of leaves, plant heightand days to flowering.

2.5. Heritability and genetic advance in sorghum

Heritability is the proportion of observed variability, which is due to heredity, the remainder being due to environmental causes (Allard, 1960). Heritability provides the degree of transmissibility of a character and indicates the effectiveness of selection. Further, estimates of heritability have to be considered in conjugation with genetic advance to find the expected genetic gain in next generation (Shukla *et al*., 2006). Genetic advance is the improvement over the base population that can potentially be made from selection for a character. It is a function of the heritability of the trait, the amount of phenotypic variation and the selection differential that is used by breeders (Singh *et al*., 2018)

High heritability coupled with high genetic advance was observed for traits such as plant height, hundred grain weight andpanicle width tested at Miesso (Mulualem*etal*., 2018).Warkad et al.(2011) found significant geneticvariability among sorghum genotypes for most of the agronomic traits studied. High heritability for number of leaves per plant (0.89), plant height (0.88),hundred grain weight (0.86) and panicle length (0.90) was reported by Tirifesa(2009).

2.6. Importance and constraints to sorghum production

Sorghum is the fifth most important cereal crop in the world, after wheat, maize, rice and barley grown in arid and semi-arid parts of the world (FAO, 2016).In Africa, sorghum is still largely a subsistence food crop(Taylor, 2003).In Ethiopia, sorghum is the third primary staple food crop after tef and maize (CSA, 2015).The highest proportion (74%) of the grain producedis consumedat the household level, with the remainder being used for sale and seed purposes (CSA, 2014).Sorghum grain is preferred next to tef, a small cereal grain crop, for the preparation of the staple leavened bread (*injera*). The grain is also used for the preparation of local beverages (*Tella* and *Areke*). In addition, the stover is used as animal feed (green chop, hay, silage, and pasture), fuel wood and construction (fencing and roofing material) purposes.Sorghum grows in a wide range of agro ecologies most importantly in the moisture stressed parts where other crops can least survive and food insecurity is rampant(Asfaw, 2007).

The productivity of sorghum in Ethiopiais very low as compared to research site (3-4 tons/ha) and farmers fields in major sorghum growing regions of the country (Geremew*et al*., 2004).Sorghum production and productivity in the country are constrained by several biotic and abiotic factors. Among the biotic factors are Striga, diseases (grain mold, anthracnose, rust and smut), insect (stalk borer, midge, and shoot fly) and Quellea birds (Wortmann et al., 2006). Important abiotic constraints include low soil fertility (nutrient deficiency) and drought(EIAR, 2014).Sorghum production constraints vary from region to region within Ethiopia.Drought and Striga in north and north eastern parts, quelea birds in the Rift Valley and Southwest lowlands (Wortmann*etal*., 2006), soil infertility and drought were seen as a major constraints in the eastern parts of the country (Shiferaw *etal*., 2015).However, drought and strigaare the most important problems across regions.Among abiotic factors droughtis a major constraint in sorghum production worldwide and is considered as the most important cause of yield reduction in crop plants.

In Ethiopia,over 80% of sorghum is produced under severe drought to moderate drought stress conditions.Complete yield loss was observed in some parts of the country (EIAR, 2014).The Ethiopian government is persuing a strategy of improving agricultural productivity primarily through agricultural intensification including seeds of improved crop varieties.

2.7. Drought and effect of drought stress on sorghum

From an agricultural point of view, drought is the availability of inadequate water including precipitation and soil water storage capacity, in quantity and distribution during the life cycle of a crop plant, which inhibits the expression of full genetic potential of the plant (Mitra, 2001).It is the major cause of poor crop performance and low yield, and sometimes it causes total crop failure. Drought is also unpredictable in its timing of occurrence, duration and intensity.In thetropics, the probability of drought is highest at the start and end of the growing season. Short duration drought stress mostly reduces grain yield while prolonged drought stress leads to complete death of plant. Drought stress occurs at different stages of growth and adversely affects and yield parameters which lead to reduction in yield. The extents of yield loss caused by drought stress vary with sorghum genotypes and their stage of growth (Reddy*et al*., 2007).

Drought response in sorghum has been classified into two distinct stages, pre-flowering(panicle differentiation to flowering) and post-flowering(flowering to grain development) (Sanchez et al., 2002). Pre-flowering drought tolerance responses of sorghum includes reductions in panicle size, seed number, grain yield, seed set, plant height,leaf rolling, irregular leaf erectness, delayed flowering and flower abortion. Post-flowering drought tolerance encompasses stalk logding, reduced seed size, susceptibility to charcoal rot, reduced biomass, loss of chlorophyll, degradation of photosynthesis, reduced seed weight, reduced grain number, reduced hundred seed weight and premature leaf and stalk senescence (Burke*et al*., 2010).Post-anthesis drought stress is considered more detrimental to grain yield regardless of the stress severity because photosynthesis per unit leaf area is decreased leading up to 70 % yield loss (Abraha*et al*., 2015).

2.8. Sorghum drought resistance mechanisms

Drought tolerance can be defined as a plant's ability to maintain physiological functions when little or no water is available to the plant(Mitra, 2001).Plants respond and adapt to and survive under drought stress by the induction of various morphological, physiological and biochemical responses. However, a plant may exhibit more than one of these strategies to cope with drought stress.There are evidences that sorghum is drought tolerant than other cereal crops. Sorghum had a greater ability to extract water from deeper soil layers compared to maize (Farre, 2006). Sorghum avoids effects of moisture stress at critical stages by delaying or hastening development. Early in the vegetative stage, delays its growth; when recovered it has the ability to compensate yield by producing tillers. If water stress occurs late in the growth stage, hastens its growth and quickly passes to the next developmental stage (Yared *et al.*, 2014).

2.8.1. Drought escape

Drought escape is the ability of plants to avoid drought by completing their life cycles before the onset of a dry period to sustain some reproduction (Manavalan *et al*., 2017). Early matured sorghum genotypes have less evapotranspiration when compared to late maturity genotype because of smaller leaf area which can help limit further water loss. Some sorghum cultivars also escape drought through remobilization of stem reserves (Seetharama *et al.*, 1982).Sorghum has a developmental plasticity, which delay or postpone their development

during stress and resume their development with the start of rain.Due to deep and extensive root formation, sorghum can escape drought (Tari *et al.*, 2013).

2.8.2. Drought avoidance

The drought avoidance mechanism avoids a low water status in tissues during water stress by maintaining cell turgor and cell volume. This is achieved either through aggressive water uptake by an extensive root system, leaf rolling, through reduction of water loss from stomatal transpiration and other non-stomatal pathways such as cuticular transpiration (Ludlow and Muchow, 1990). Most sorghum genotypes have a thick waxy cuticle that limits water loss during periods of water deficit, which reduce water loss from leave. The resistant sorghum lines showed more leaf-rolling than the susceptible lines in water stress condition, reducing the effective area of the uppermost leaves by about 75% (Matthews *et al.*, 1990).

2.8.3. Drought tolerance

Drought tolerance is a mechanism through which sorghum maintains metabolism even at a lower water potential.This mechanism involves physiological traits including osmotic adjustment, antioxidant capacity and genetic components such as pre-flowering drought tolerance and post anthesis drought tolerance (Subudhi*et al.,* 2002).The genetic components are expressed depending on the growth stage of the sorghum plant and are controlled by different genetic elements.Pre-flowering response in sorghum occurs when the plants are under significant moisture stress prior to anthesis and post flowering drought response in sorghum is expressed when moisture stress occurs during the grain filling stage(Rosenow and Clark, 1995).

2.8.4. Stay-green or delayed senescence

Stay-green refers to a drought tolerance mechanism that enables the sorghum plants to tolerate premature senescence under drought stress that occurs during grain filling. The stay-green trait results in greater functional photosynthetic leaf area during grain filling and even after physiological maturity. It is an important component of post-flowering drought response in sorghum (Harris *et al.*, 2007). Sorghum genotypes with the stay-green trait continue to fill their grain normally under drought and exhibit resistance to stalk lodging,charcoal rotand higher levelsof stemcarbohydrates (Borrell*et al.*, 2000).

2.9. History of sorghum breeding and achievements in Ethiopia

Sorghum research in Ethiopia started in 1953 at the then Jimma Agricultural Technical School, which is now called Jimma University and in 1957 the program moved to the then Alemaya College of Agriculture now called Haramaya University. In 1972 the initiation of Ethiopian Sorghum Improvement Project (ESIP) with the fund from International Development Research Centre (IDRC) can be considered to be the start of formal research on the crop in the country (Chemeda, 2018).In Ethiopia hybrid development research was inceptedin the mid-1970s using introduced inbred lines, with an objective of developing sorghum hybrids for the lowland and moisture stress ecological zones. In 1982 Institute of Agricultural Research (IAR), now the Ethiopian Institute of Agriculture Research (EIAR) was established and sorghum breeding started advancing (EIAR, 2014).Since the establishment of the program morethan 50 improved sorghum varieties have been released (http://www.eiar.gov.et/marc/index.php/anrl-research/crop-research).Amongwhich 23 sorghumvarieties and 4 hybrids were released for the dry lowland environment (http://www.eiar.gov.et/marc/index.php/anrl-research/crop-research).Currently, drought tolerant and high yielding varieties Melkam, Dekeba, Meko, and Teshale are popular varieties in the dry lowlands.

2.10. Breeding of sorghum for drought tolerance

In breeding for drought tolerance, a pure line selection method has been used in many national and regional sorghum research programmes (Acquaah, 2007). However, pedigree and bulk selection methods are commonly used in most international and national breeding institutions. Pedigree selection in segregating populations derived from planned crosses is the dominant breeding strategy to develop pure line varieties and hybrid parents in sorghum. However, if the transfer of few traits related todrought tolerance to a high yielding genotype is the aim, backcrossing is the appropriate methodology(Mitra, 2001).

Breeding for drought tolerance is complicated because of a negative correlation between some stress adaptive traits and crop yield. Zehui(1996) observed that the use of yield components as the unique indicators for drought tolerance is not sufficient. Drought tolerance is a complex trait whose expression depends on action and interaction of different morphological, physiological and biochemical characteristics (Mitra, 2001).Therefore, understanding of the

physiological mechanisms and genetic control of drought in crops is important as a base for improving the production and productivity of crops.

Selection and breeding for varieties that perform very well under drought conditions is a key factor to improve the production and productivity of sorghum. Selection based on plant developmental traits such as plant phenology (days to flowering and maturity), stay-green, leaf area, tillering, panicle size and peduncle exsertions are conducive for drought tolerance in sorghum genotypes (Ali*et al*., 2011).

2.11. Phenotypic traits associated drought resistance

Drought tolerancein sorghum is a complex trait and adaptation of a plant to drought is the result of overall expression of quantitative traits. Adaptive traits are effective only for certain aspects of drought tolerance and over a limited range of drought stress, no single trait can be used to improve the productivity of crop in a moisture deficitsenvironment(Vasant, 2012).For better understanding of plant responses to moisture deficitsand link this understanding with breeding of improved cultivars, drought-tolerance traits may be divided into phenological and plant-type traits (Farroq*et al*., 2009).

2.11.1. Days to 50% flowering

Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape, and is the most important trait for avoiding late season drought. Drought stress delays flowering in crops, which is due to low plant water status and longer delay in flowering is related to drought susceptibility (Kumar and Kujur, 2003). Flowering time tends to be associated with yield but in a rather unpredictable manner.Early flowering may be advantageous if it enables a cultivar to escape drought during the reproductive stages whereas late flowering may be beneficial in the cases where drought stress occurs early in the season.

2.11.2. Chlorophyll content

Chlorophyll content is an indicator of photosynthetic capacity of plant tissue. Drought stressleads to a significant changes in the ratio of chlorophyll ‘a’ and ‘b’ and carotenoids (Farooq *etal*., 2009). Chlorophyll content is positively associated with photosynthetic rate (Zhang *et al*., 2009). Significant relationships between chlorophyll content and yield and yield

componentsfacilitate selection of high yielding genotypes. In different studies, determination of chlorophyll content is used as a screening tool for selection of drought tolerant genotypes (Malala, 2010; Borrell *etal*., 2000b). In relation to this, Farshadfar et al. (2013) has reported drought tolerant lines of wheat have higher chlorophyll content under drought stress condition. Therefore, chlorophyll content measurement can be used as selection criteria for breeding programs.

2.11.3. Number of leaves, leaf area and tiller number

Plants generally limit the number and area of leaves in response to drought stress just to cut down the water budget at the cost of yield loss (Schuppler*et al*., 1998). Narrow leaves decrease the total leaf area per plant. Leaf area adjustment has been suggested as one of the most powerful means of avoiding stress. Blum(1979) has shown that early sorghum genotypes not only escape drought but also avoid it because of reduced transpiration demand as a result of small leaf area. Small leaf area is an advantage for conserving water loss due to transpiration, one of the drought tolerance mechanisms (Adugna and Tirfessa, 2014).Tillering ability is commonly associated with sorghum in regions with limited rainfall. Tillering is generally recognized as one of the most plastic traits affecting biomass accumulation and ultimately grain yield. Hammeret al.(1996) reported significant yield advantage of high-tilleringtypes in high-yielding seasons when water was plentiful, whereas suchtypes incurred a significant disadvantage in lower yielding under water-limited circumstances.

2.11.4. Grain yield and hundred seed weight

Yield is the principal selection index used commonly under drought stress condition. In addition, correlation analysis between grain yield and drought tolerance indices can be a good criterion for screening the best genotypes(Farshadfar*etal*., 2012). Grain yield and hundred seed weight showed significant difference between genotypes in stress and non-stressenvironments.Grain yield had the highest decrease percent of traits under drought stress condition that it was due to reduction in biological yield and number of seeds under drought stress. In sorghum genotypes,hundred seed weight reduced by drought stress due to decrease in the assimilation rate and lower photo assimilate translocation to physiological sinks and shortening the grain-filling period (Malala, 2010).

2.11.5. Stem diameter and plant height

Many sorghum traits such as stem diameter and sugar concentration are correlated with each other either positively or negatively. Zou et al. (2011) had reported significant positive correlation between stem diameter and sugar concentration (r=0.23),suggesting thicker stems have more sugar concentration. Sugar concentration in plant tissues constitutes an important signal, and sugar responsive genes have a role in the response of plants to drought stress (Koch, 1996; Smeekens 1998).Sugar responsive genes participate in the control of resource distribution among tissues and organs (Koch, 1996). Sorghum accumulates high content of sugar in its stem leading to strong drought tolerance (Njokweni, 2015). Water regime also affects stem reserves and grain yield. According to Duncan et al.(1981), the non-senescent or stay-green lines had significantly larger stem diameter and maintained a higher sugar concentration in the basal part of the stem. The stem diameter is greatly reduced under drought stress. This causes suppression of the main stem and lateral branch growth, resulting in reduced stem dry weight, which leads to final yield reduction(Sutro and Tirtoutom, 1989).

Plant height is a trait, which varies with genotype, soil fertility and moisture deficit. sorghum genotypes that exhibit greater plant height have overall larger plant size, intercept more light and use water faster by transpiration, that reduce water content of the plant, higher leaf death scores and more spikelet sterility (Kato *et al*., 2007). Less reduction in plant height in drought stress conditions at anthesis stage may be an important adaptive mechanism for environments characterized as drought tolerant.

2.11.6. Leaf angle

The radiative load on the individual leaf is maximized when solar radiation is received perpendicular to the leaf, especially around solar noon. Any deviation fromthis will reduce the load. Leaf inclination angle, the angle at which a leaf emerges with respect to the stem, is a feature of plant architecture that influences how a plant canopy intercepts solar radiation (Truong *eta1*., 2015).Erect leaf is a distinct morphological feature common in the cereals and often considered in breeding as a favorable component of canopy architecture. Erect leaves allow better distribution of irradiance into the canopy instead of just illuminating the top leaves. In this sense, erect leaves are generally at a better leaf-water status than lax leaves when subjected to droughtstress (Ludlow and Bjorkman, 1984). This is most

probably the reason why erect leaf lines yielded better than lax leaf lines of wheat under conditions of moisture stress.Edmeadesetal. (1999) concluded that erect leaves offer some adaptive advantage to maize under drought stress.

2.12. QTLs discovery in sorghum for drought tolerance

Many important traits for drought tolerance like yield, stem diameter, leaf number, leaf area and length, flowering time and chlorophyll content are controlled by many genes and are known as quantitative traits. Detection of quantitative trait loci (QTLs) controlling those traits, and then to utilize them for crop improvement to water deficits aids in our understanding of the genetics of drought tolerance and helps in developmentof drought tolerant varieties.QTLs are region within the genome that contains genes associated with a particular quantitative trait. QTL mapping is the process of constructing linkage maps and conducting Quantitative trait loci (QTL) analysis, which allows assessing the locations,numbers,magnitude of phenotypic effects and pattern of gene action (Ashraf M., 2010).

In sorghum, the best characterized form of drought tolerance is the stay-green under drought stress which is considered as an important trait for sustaining yield under stress during grain filling period (Borrell and Hammer, 2000; Sanchez *et al*., 2002). Using F7 RILs derived from the cross of B35 X Tx7000, Xu et al.(2000) reported four stay green QTLs located on the three linkage groups along with three QTLs for chlorophyll content.Several stay green QTLs co-localized with grain yield, flowering time and plant height have been reported (Sabadin *et al*., 2012).QTLs linked to days to 50% flowering, plant height, panicle weight, grain weight, grain weight per panicle, panicle harvest index and thousand grain weight were identified in 160 sorghum genotypes under well watered conditions (Endre and Bantte, 2016). Besufekad and Bantte (2013) reported a total of four significant SSR loci associated with days to 50% flowering, panicle exsertion and grain weight per panicle using 151 sorghum accessions and 39 SSR markers under drought stress conditions at two environments.

Recently, the idea of utilization of association mapping in crop plants, including sorghum is gaining moreattention than conventional or classical linkage mapping, which is based on biparental mapping populations. Unlike specific bi-parental populationsin which certain trait

differences exist, most of the assembled association mapping panels can be used to study a host of traits so that questions from different angles can be studied, including basic biology, plant architecture, development, agronomic performance, adaptive characteristics, and nutritional value (Atwell *et al.*, 2010; Flint-Garcia *et al.*, 2005).

2.13. Association mapping study tool

Association mapping (AM) is usually defined as the detection of non random associations between molecular markers on the one hand and phenotypic traits on the other hand in populations of genotypes without a simple genetic structure (Zhu *et al.,* 2008). Advances in molecular marker technology and statistical methods have made association mapping accessible and affordable. The main purpose of AM is to dissect complex traits and identify QTLs. QTLs detected using AM (also called signals, peaks or hits) are usually represented using Manhattan plots which show the association of markers with the trait along a chromosome. They-axis indicates – log10 (P value) for the association plotted against the SNPs along each chromosome on the x-axis, so the map positions of all markers used must be known (Verdepradoe*t al.*, 2018).

Association mapping can be classified into two broad categories: (1) candidate gene (CG) analysis and (2) Genome wide association studies (GWAS).While CG analysis is ahypothesis-driven approach based on prior studiesabout genes involved in the trait of interest, GWAS isa more comprehensive approach which does notrequire any initial information about the genetic controlof a trait of interest (Zhu*et al.*, 2008).

2.14. Genome wide association studies (GWAS)

The sorghum genome is 750 Mb (Yonemaru *et al.*, 2009), larger than that of rice (430 Mb)(Kurata *etal.*, 2002) but about 3-fold smaller than that of maize (2400 Mb)(Bennetzen *etal.*, 2005). Its small genome makes sorghum an attractive model for studyingthe functional genomics of C_4 grasses(Kumar *et al.*, 2011). Release of the sorghum genome sequence (Paterson*et al.*, 2009) greatly facilitated research in association mapping.Genome-wide association ofSNP variation will accelerate molecular breeding by expanding the diversity of germplasm accessible to crop improvement programs and increasing the resolution of GWAS. It has become a routinely used method to investigate the genetic mechanisms underlying natural phenotypic variation.

Genome-wide association studies (GWAS) have been used to successfully discover significant marker-trait associationsin cereal crops including maize, rice, barley and sorghum(Morris*et al*., 2013).Different authors reported about Genome-wide association mapping study on sorghum which revealed significant marker-trait association, i.e., days to flowering, culm length, number oftillers, number of panicles and panicle length (Shehzad *et al.*, 2009; Bhosale *et al.*, 2012), Plant Height, Panicle Exsertion, Awns, Panicle Compactness and Shape, Smut Resistance and Pericarp Color (Girma *et al*., 2019), Panicle erectness, Plant height and Flowering time (Morris *etal*., 2019).

2.15. Linkage disequilibrium (LD)

Linkage disequilibrium (LD) refers to a historically reduced level of the recombination of specific alleles at different loci controlling particular genetic variations in a population(Abdurakhmonov and Abdukarimov, 2008). D' and r^2 is the most commonly used measures of LD. Considering the objective, the most appropriate LD quantification measureneeded for association mapping is r^2 (the square of the correlation coefficient betweenthe two loci) which is also an indicativeof marker-trait correlations (Gubta *et al*., 2005). The value r^2 varies from 0 to 1, and it will be equal to 1 when only twohaplotypes (combination of alleles) are present. The r^2value of equal to 0.1 or above considered the significant threshold for the roughestimates of LD to reveal association between pairs of loci (Zuh *et al*., 2008).

LD can be calculated using available haplotyping algorithms (Oraguzie*etal*., 2007). Some computer software packages measuring LD such as Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) and PowerMarker have similar graphical display features. The large red blocks of haplotypes along the diagonal of the triangle plot indicate the high level of LD between the loci in the blocks, meaning that there has been a limited or no recombination since LD block formations.LD blocks are very useful in association mapping when sizes are calculated, which suggest the needs for the minimum number of markers toefficiently cover the genome-widehaplotype blocks in association mapping(Abdurakhmonov and Abdukarimov, 2008).

3. MATERIALS AND METHODS

3.1. Description of the experimental site

The experiment was conducted at Miesso (substation of Melkessa Agricultural Research Center), which is located in the eastern escarpment of the central rift valley of Ethiopia during the main cropping season of 2018/19. Miesso is located 302 km east of Addis Ababa at an altitude of 1425 m.a.s.1 on geographical coordinates of $9^0$13' N latitude and 40^0 45'E longitudes. The maximum and minimum temperatures are 26.5^0C and 16^0C, respectively. The average annual rain fall of the area is between 635 and 945 mm. The study area is predominantly categorized as hot and warm sub-moist plain agro-ecological zone. The soil texture is mainly silty clay loam with slightly alkaline pH, ranging from 7.8 to 8.3 (Lemma, 2008).

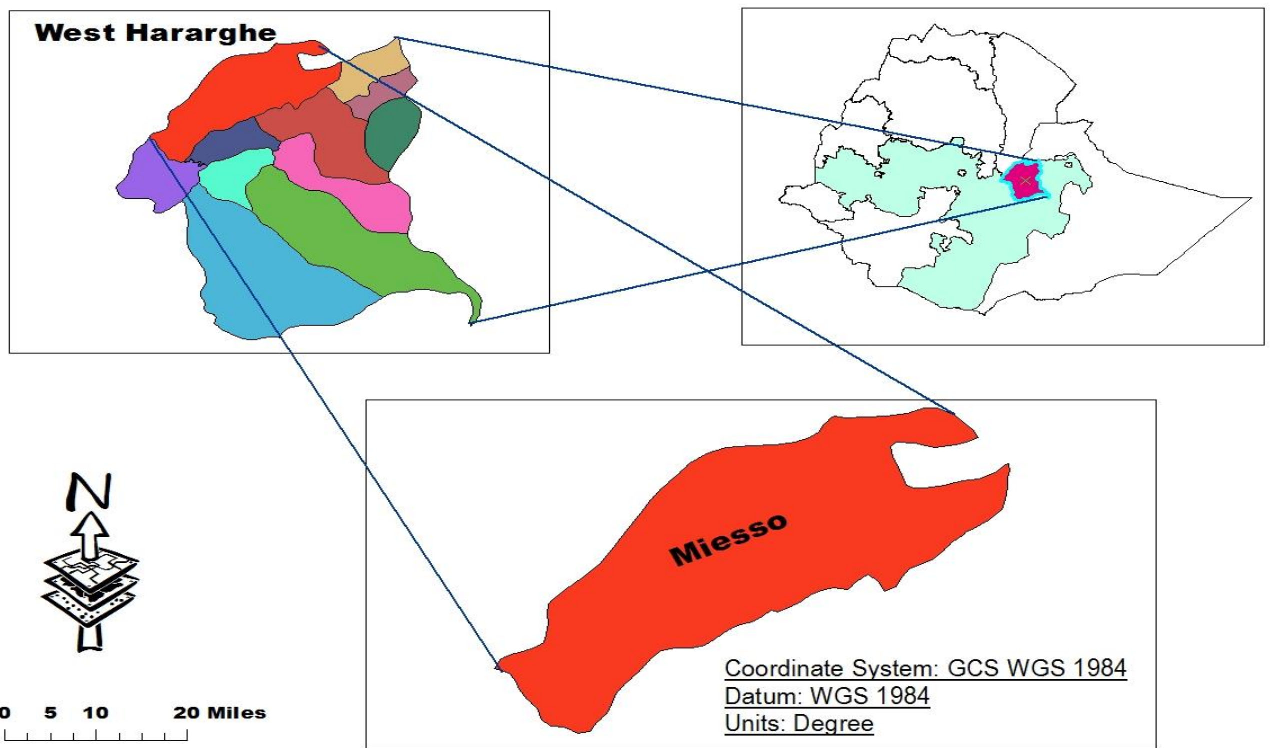

Figure 1. Map of the study area showing Ethiopia, Western Hereghe and Miesso (study site)

The meteorological data during the crop growth period from May to November was collected from Meisso Meteorological Station and presented in Table 1. The rain fall received during cropping period ranged from 150.3 mm (August) to 4.1 mm (November), while the maximum and minimum temperature were 35.5 ^{0}C (June) and 10.4 ^{0}C (November).The minimum and maximum relative humidity were 15% and 96% in September and May respectively.

Table 1.Monthly meteorological data during crop growth period (2018/19) at Meisso.

			Temperature (^{0}C)		Relative humidity (%)	
		Rain fall (mm)	Min.	Max.	Min.	Max.
1	May	56	15	35	22	96
2	June	63	16.3	35.5	24	63
3	July	141.2	15.8	33	35	63
4	August	150.3	15.5	33	36	76
5	September	44.6	10.6	33.5	15	63
6	October	43.1	11.4	34	23	63
7	November	4.1	10.4	33	28	76
	Total/Ave.	502.3	13.57	33.86	26.14	71.43

3.2. Plant materials

Nine hundred forty five sorghum genotypes (940 landraces, 4 released varieties and 1 local check) obtained from Institute of Biodiversity Conservation (EBI) and MARC were used for the study. The landracesweresystematically selected from the EBI collections, representing all sorghum growing regions (Woredas) and altitudes. These genotypes were phenotypedforroot angle in greenhouse experiment, as part of asorghum improvement project at Jimma University (Menamo, 2018 unpublished).

3.3. Experimental design and trial management

The experiment was conducted using-an alpha latticedesign with two replications, having 63 blocks per replication and 15 plots per block. Each plot consisted of a single 3 meter length row and the spacing between rows and plants was 0.75 and 0.2meter, respectively. During planting, the seeds were manually drilled and about 20 days after emergence, thinning was done. Fertilizer was applied at the rates 100kg/ha Urea and 100kg/ha DAP (diammonium

phosphate). DAP was applied during planting. Split application was used for Urea, half of it at planting time and the remaining half at knee stage period(Ayana, 2001). All other field management practices were carried out as per the recommendations.

3.4. Datacollected

Data were recorded on 17quantitative traits. The standard sorghum descriptor (IBPGR/ICRISAT, 1993) was used to score the traits. Table 2 provides the list and description of the scored quantitative traits. All of the traits were measured on individual plant bases where five randomly selected plants were used to represent the genotype in every plot.

Table 2. Full names, units, codes and descriptions of the traits recorded in this study

No	Traits	Units	Codes	Description of the traits
1	Days to emergence	days	DE	The numbers of days from sowing to the date when 50% of the plants in a plot are emerged from the soil
2.	Flag leaf appearance date	days	FLAG	From emergence to when the appearance of flag leaf is clearly visible above the ligule of the previous leave
3	Days to flowering	days	FLOW	From emergence to when 50% of plants flowered half way down the head
4	Days to maturity	days	MATU	from emergence to physiological maturity (black layer formed)
5	Plant height	cm	PHT	The length of the plant from the ground to the panicle tip at physiological maturity
6	Panicle width	cm	PAW	Width of panicle in natural position at the widest part.
7	Panicle length	cm	PAL	Length of panicle from its base to tip.
8	Stem diameter	cm	SD	Stalk diameter was measured 20 cm above ground using digital caliper (World Precision Instruments, Shanghai Trading Co., Ltd).
9	Total number of leaf	count	LFN	Count of total number of leaves per plant (main stalk).

Table2.(*Continued*)

No	Traits	Units	Codes	Description of the traits
10	Leaf area	cm^2	LA	Area of the flag leaf,computed as (Leaf length x Leaf width x 0.69) suggested by (Krishnamurthy *et al*., 1974).
11	Total number of tillers per plant	count	TILLER	The total number of tillers per plant in five randomly selected plants per accession per replication was recorded at harvest.
12	Number of productive tiller	count	ETN	The total number of reproductive tillers from each selected samples in five randomly selected plants per accession per replication was recorded at harvest.
13	Leaf angle	angle	LA	Measured by using Samsung tab field scorer softawe
14	100 seed weight	gram	HSW	Weight of 100 random seed counts
15	Grain yield per unit area	g/m^2	GYPUA	Calculated by dividing adjusted grain yield per plot to the net plot area.
16	Chlorophyll content	number	SPAD	Chlorophyll content was measured from flag leaf at flowering (SPADB) and maturity (SPADM) using Chlorophyll meter, SPAD-502 (Minolta Co. Ltd, Japan).
17	Total number of leaf vein	Count	TLV	Total vein number was measured By using imagej software

3.5. DNA extraction and genotyping

DNA was extracted from 14 days old seedlings. The leaf samples were dried for 18 hours using a freeze drier (Christ, Alpha 1 – 2 LD plus). Grinding was done using geno-grinder (QIAGEN, Tissue Lyser II). Both genomic DNA extraction and genotyping were done at DArT P/L by outsourcing. The samples were genotyped using an integrated DArT and genotyping-by-sequencing methodology involving complexity reduction of the genomic DNA to remove repetitive sequences using methylation sensitive restrictive enzymes prior to sequencingon Next Generation sequencing platforms (DArT,http://www.diversityarrays.com). The detail of methodology for DArT is described by Jaccoud et al. (2001) and Semagn et al. (2006).

3.6. Data analysis

3.6.1. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) for alpha lattice design was performed with R software (R Core Team, 2018)using the following model.

$$Y_{ijl} = \mu + \tau_i + \gamma_j + \rho_{l(j)} + \varepsilon_{ijl}$$

Where, Y_{ijl} =the observed value of the trait Y for the ith genotype under j^{th} replication and l^{th} level of incomplete blocks within replications, μ = the grand mean of trait Y in the experiment, τ_i = effects of ith level of genotype, γ_j = (fixed) effects of jth level of replication,$\rho_{l(j)}$= (random) effects of lth level of incomplete blocks within replications, ε_{ijl}= experimental error. Mean separation was done using the tapply function in R software.

Table 3. Skeleton of Analysis of variance for Alpha lattice design

Source of variation	Degrees offreedom	Sums ofsquares	Mean squares	F
Replicates	r-1	SSr	MSr	
Blocks (replicates)	rs-r	SSb	MSb	
Treatments	t-1	SSt	MSt	F_t
Error	rt-rs-t+1	SSe	MSe	
Total	n-1	SSc		

Abbreviation: SS=sum of squares, MS=mean of squares, r=replication, t=treatments, s=is the number of blocks per replicate

Estimation of Best linear unbiased prediction (BLUP)

Best linear unbiased prediction (BLUP) was calculated by fitting the following linear model in the R package lme4 (Bates *et al*., 2014) for the estimation of breeding values:

$$Y= (1|Genotype) + Rep + (1|Block/Rep)$$

Where, Y is trait data, 1| indicates random effects, Genotype refers to the 940 sorghum genotype and Block/Rep is block nested within replication.

Correlation and principal component analysis

The correlation coefficients were calculated using BLUPs and trait correlation matrix was generated by the Pearson method with the cor function in R software (R core team, 2018). The cor.mtest function in R was used to determine significance for each correlation. The chart.Correlationfunction within the "PerformanceAnalytics"package was used to generate scatter plots and histograms (Peterson *et al*., 2014).In addition, the function prcomp, included in the core package "factoextra" of R software environment (R core team, 2018), wasused to generate PCA (Kassambara and Mundt, 2017).

Estimation of genetic parameters

Genotypic and phenotypic coefficient of variances, heritability in broad sense, genetic advance and genetic advance as a percent of mean were computed as follows:

Genotypic and phenotypic variances were computed using formulas:

$$\sigma^2 g = \frac{MSg - MSe}{r}$$

$$\sigma^2 e = Mse$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e$$

Where:

$\sigma^2 g$ = Genotypic Variance

$\sigma^2 p$ = Phenotypic Variance

$\sigma^2 e$ = Environmental Variance

MSg= mean square of genotype

Mse = Error Mean Squares.

r=replications

Genotypic and phenotypic coefficient of variation

Genotypic and phenotypic coefficientof variation was estimated as suggested by Burton and De vane(1953).

Genotypic coefficient of variability (GCV) = $\frac{\sqrt{\sigma^2 g}}{\bar{x}}$

Phenotypic coefficient of variability (PCV) = $\frac{\sqrt{\sigma^2 p}}{\bar{x}}$

Where:

$\bar{x}$ = Grand mean of trait

The GCV and PCV values are ranked as low, medium and high as suggested byDeshmukhet al.(1986) as follows:-

1-10 – Low

10-20 – Medium

More than 20% - High

Heritability (H^2_B)

Broad sense heritability was computed for each characters based on the formula developed by Allard(1960).

$H^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$

Heritability percentage was categorized as low, medium and high as suggested by Robinsonetal.(1949) as follows:-

0-30%: Low

30-60%: Moderate

60% and above: High.

Genetic advance (GA)

Genetic advance for each trait was calculated by using the formula Allard (1960).

$GA = K * \sigma p * h^2 = k * \sqrt{\sigma^2 p} * \frac{\sigma^2 g}{\sigma^2 p}$

Where:

k = Selection differential which has value of 2.06 at 5% selection intensity.

σp= Phenotypic standard deviation

Genetic advance as a percent of mean (GAM)

$$GAM = \frac{GA}{GM} * 100$$

Where:

GA = Genetic advance

GM = General mean of trait

Genetic advance as percent of mean was classified as low, moderate and high (Johnsonet al., 1955) as follows:-

0-10% - Low

10-20% - Moderate

20% and above – High

3.6.2. Molecular data analysis

Population structure analysis

Population structure of the genotypeswas analyzed using R package's Landscape and Ecological Association (LEA) (Oliver Francios *et al*., 2015). The number of sub-populations was determined using a cross-entropy criterion. The cross-entropycriterion is a value based on the prediction of a fraction of masked genotypes (matrix completion), and on the cross-validationapproach.

Linkage disequilibrium (LD) analysis

LD (in terms of r^2) was calculated for each chromosome separately using window size 50 with TASSEL v5.2.53. As rare alleles induce large variances, only markers with a minor allele frequency of >0.01 were included in the analysis.Statistical tests for eachr^2 were provided by the p value calculated. The critical r^2 for LD decay was determined by values of 0.1, which is considered the minimum threshold for significant association between pairs of loci and to describe the maximum physical distance at which LD is significant (Zhu *et al*., 2008). The LD decay graph was drawn by using ggplot function within the "ggplot2" package of R software environment (Wickham, 2016).

Genome wide association analysis (GWAS)

The initial, 54,080 SNP markers generated for 940 landraces were reduced to 50,367 due to removal of some markers with unknown and *super*-contigs positions. These 50,367 SNP markers were imputed for removal of missing values. Based on the allele frequency distribution in the Ethiopia sorghum collection, 65.5% of the SNPs were rare (MAF < 0.05) due to this the markers filtered MAF >0.01 produced 25,634 robust SNP markers.

Best linear unbiased prediction (BLUP) obtained from 940 sorghum genotypes for all the traits were used as phenotypic values and a total of 25,634 SNP markers were used for GWAS analysis. GWAS was carried out using mixed-linear models (MLM) using the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) package (Zhang *et al*., 2018) in R software (R Core Team, 2018). Log Q–Q plots of p-values were examined to determine how well the models accounted for population structure and familial relatedness. Manhattan plots were visualized using the R package's ShinyAIM (Hussain *et al*., 2018).

Following GWAS, highly significant (based on P-value) SNP markers for selected traits (days to flowering and grain yield per unit area) were scannedfor the nearby genes onto Sorghum bicolor v3.1.1 (McCormick *et al*., 2018) reference genome in Phytozome v12.1. Using JBrowse (Skinner *etal*., 2009). The 50 kb window was used based on average linkage disequilibrium (LD) previously identified in sorghum (Bouchet *et al*., 2012; Mace *et al*., 2013).

4. RESULT AND DISCUSSION

4.1. Variations among genotypes

Analysis of variance (ANOVA) showed highly significant difference (P<0.001) among the 945 sorghum genotypesfor all of the quantitative traits recorded (Table 4), which could be exploited through selection, as variability with in populations is a basic prerequisite for plant breeding program. Endre and Bantte(2016) reported significant differences among 160 sorghum accessions for days to 50 % flowering, plant height and hundred seed weight. Mulualem et al.(2018) similar to the present study,observed highly significant variation for the agronomic traits (days to flowering, days to maturity, grain yield, hundred seed weight, panicle length, panicle weight and plant height) tested at two environments.

Table 4. Analysis of variance for 17 traits of sorghum genotypes evaluated at Miesso in growing season 2018/19

Trait	Mean square				CV%
	Rep	Block(Rep)	Genotypes	Error	
SD	0.15NS	0.17NS	0.45**	0.18**	16.47
PAL	36.56NS	15.61NS	102.12**	18.03**	14.88
PAW	40.35**	3.54NS	14.71**	2.96**	16.27
SPADB	1215.6**	55.27NS	106.89**	47.42**	14.68
SPADM	9.29NS	20.64**	76.35**	14.09**	17.65
LFN	3.34NS	3.83**	19.74**	2.65**	8.37
LA	788.76**	46.29**	54.19**	24.52**	13.99
PHT	158588.89**	1296.29*	4758.71**	993.7**	12.78
LAF	17770.17**	1716.36NS	7878.48**	1718.83**	15.13
TILLER	39.31**	2.44**	15.4**	1.7**	26.46
ETN	4.1*	1.1NS	5.96**	0.99**	29.47
TLV	40.63**	4.42NS	11.81**	4.06**	7.37
FLAG	1894.44**	26.52NS	188.05**	24.5**	5.35
FLOW	8645.29**	112.64**	180.69**	70.55**	7.93
MATU	5769.77**	77.74**	123.82**	50.18**	4.98
HSW	1.63**	0.19NS	0.44**	0.15**	16.57
GYPUA	33239.22**	4058.57NS	21466.18**	3680.97**	22

**= highly significant at P <0.01, * = significant at P <0.05 and NS= non significant, respectively, CV (%) = coefficient of variation.Notes: [@]Quantitative traits abbreviations as indicated in Table 2

4.2. Means and range of traits

The result of descriptive statistics indicated that there was a wide range of variations among the genotypesstudied(Table 6).The genotypes showed considerable variation in days to flowering and maturity.Time to flowering of the genotypesranged from 71.5 to 127.67 days while maturity it ranged from 111 to 160 days. The mean for days to flowering and days to maturity were 105.81 and 142.16 days, respectively.SimilarlyGedifew and Tsige (2019) reported high range of variability among sorghum genotypesfor days to flowering (41-218 days) and days to maturity (130- 170 days). Tirfessa(2009)also reported similar results for days to flowering (48- 160 days) and days to maturity (131 – 211 days).

Among the tested genotypes, the most early flowering were JUS171565 (71.50) followed by JUS171565 (73) and JUS163342 (73). However, JUS173026 (127.67 days) followed by JUS161329 (127 days) had the most late flowering period (Table 5).Identifying early and late flowering is important in choosing genotypes to suit different growing conditions especially for drought prone areas.

The studied genotypes also showed range of variability in their stem diameter (1.03 – 4.08 cm), total leaf number (8.75 – 29.5), number of productive tiller (1 - 11), number of total tillers per plant (1 - 25), leaf angle (19.13 - 47.25), leaf area (18.11 – 604.41), total leaf vein number (18 – 36), flag leaf appearance date (57 - 115.5) with mean values of 2.59, 19.45, 3.39, 4.94, 35.38, 273.9, 27.33 and 92.49, respectively. Plant height ranged from 84.5 – 428 cm (Table 6) with the grand mean of 246.51cm. The maximum plant height was observed for genotype JUS171765 while the minimum number was recorded for genotype JUS163338 (Table 5).The shortest sorghum genotypes are tolerant to drought because genotypes with taller plant height may utilize the available soil water for vegetative development, leaving no moisture for the grain filling stage concomitant with lower current photosynthesis during post flowering stages and decreased grain yield(Kapanigowda *etal*., 2013). Similarly, Murray *et al*. (2008) reported that taller sorghums have the advantage of accumulating more biomass due to greater translocation of photosynthesis from the vegetative tissues resulting in late maturity and low grain yield.

Variation in panicle length, panicle width, hundred seed weight, chlorophyll content measured at flowering and maturity time was also observed in this study with a range of variability

from 10 - 51.5 cm, 4 - 19.88 cm, 0.65 – 4.97 g, 19.3- 68.4 and 3.8 – 41.7, respectively(Table 6). For grain yield per unit area, which is one of the most important traits in most breeding programs the genotypes showed wide range of variability i.e 117.42 – 628.99 g/m^2 with a mean of 275.66 g/m^2(Table 6).The highest grain yield per unit area was recorded for genotype (JUS171325) and the lowest was recorded for genotype (JUS173471) (Table 5).

Sorghum genotypes characterized by early flowering and early maturity, small number of leaves per plant, small leaf area, erect leaf type (small leaf angle), larger stem diameter, small number of productive tiller, small leaf area, high grain yield per unit areaand short plant heightare most suitable for lowland areas with a limited rain fall and short growing season(Gebrekidan, 1981; Adugna and Tirfessa, 2014; Farshadfar *etal*., 2013; Edmeades *et al*., 1999)(Table 5).Generally the result showed the presence of genetic variability among the genotypes for the important phenological and yield related traits. This provides an opportunity for sorghumimprovement through selection for the target area.

Table 5. Mean performance genotypes for selected traits among sorghum genotypes evaluated at Miesso 2018/19 season

No.	Genotype	Trait SD	No.	Genotype	Trait LFN	No.	Genotype	Trait LA	No.	Genotype	Trait PHT
Bottom five genotypes											
1	JUS163342	1.03	1	JUS163342	8.75	1	JUS171132	19.13	1	JUS163338	84.50
2	JUS173348	1.16	2	JUS163338	9.50	2	JUS171224	20.75	2	JUS163342	102.50
3	JUS163348	1.23	3	JUS163434	9.63	3	JUS173826	20.88	3	JUS173111	107.17
4	JUS163434	1.27	4	JUS163348	10.25	4	JUS171717	21.63	4	JUS173146	112.34
5	JUS163341	1.31	5	JUS163341	10.38	5	JUS161338	21.63	5	JUS163434	116.00
Top five genotypes											
1	JUS173644	4.08	1	JUS171765	29.50	1	JUS171549	47.25	1	JUS171765	428.00
2	JUS173768	4.04	2	JUS173768	27.75	2	JUS173462	47.13	2	JUS173175	382.34
3	JUS173661	4.01	3	JUS173452	27.50	3	JUS171017	47.00	3	JUS163363	375.50
4	JUS173807	3.95	4	JUS171029	26.84	4	JUS171509	46.88	4	JUS173703	370.34
5	JUS173317	3.78	5	JUS173790	26.84	5	JUS173348	46.88	5	JUS171238	351.50
	Mean	2.59			19.45			35.38			246.51
	LSD	**			**			**			**

Notes: @ Quantitative traits abbreviations as indicated in Table 2

Table 5. (*Continued*)

Bottom five genotypes											
		Trait			Trait			Trait			Trait
No.	Genotype	FLOW	No.	Genotype	MATU	No.	Genotype	GYPUA	No.	Genotype	ETN
1	JUS163341	71.50	1	JUS163341	111.00	1	JUS173471	117.42	1	JUS171444	1.00
2	JUS171565	73.00	2	JUS163342	112.50	2	JUS173212	121.69	2	JUS171366	1.00
3	JUS163342	73.00	3	JUS171565	113.00	3	JUS173026	127.86	3	JUS171784	1.00
4	JUS163434	75.75	4	JUS173611	115.50	4	JUS173373	131.57	4	JUS171280	1.00
5	JUS173611	76.50	5	JUS163434	115.75	5	JUS173118	133.30	5	JUS171686	1.00
Top five genotypes											
1	JUS173026	127.67	1	JUS173708	160.00	1	JUS171325	628.99	1	JUS173377	11.00
2	JUS161329	127.00	2	JUS173026	159.75	2	JUS171349	621.89	2	JUS173463	11.00
3	JUS173764	126.13	3	JUS161329	159.50	3	JUS171784	603.74	3	JUS171547	10.00
4	JUS171229	125.88	4	JUS173790	158.67	4	JUS171672	601.80	4	JUS173483	10.00
5	JUS173756	125.50	5	JUS173317	158.00	5	JUS171265	576.92	5	JUS173160	10.00
	Mean	105.81			142.16			275.66			3.39
	LSD	**			**			**			**

Notes: @ Quantitative traits abbreviations as indicated in Table 2

4.3. Phenotypic and genotypic coefficients of variation

The amounts of genotypic and phenotypic variations that exist in a crop species areessential in initiating a breeding program. The genotypic and phenotypic coefficient of variation wereestimatedtoobserve the extent of variability between the genotypes.Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were categorized as high (>20%), moderate (10-20%) and low(<10%) (Deshmukh*et al*., 1986).

Phenotypic coefficient of variation (PCV) ranged from 5.53 % for days to maturity to 56.17 % for the total number oftillers per plant, while genotypic coefficient of variation (GCV) ranged from 4.27 % for days to maturity to 52.96 % for the total number of tillers per plant. With these ranges, high value for PCV was obtained for the total number of tillers per plant (56.17%) followed by the number of productive tiller(50.92 %), grain yield per unit area (37.58%), panicle width (25.66%), panicle length (25.49%), chlorophyll content at maturity (29.06%) and leaf area (22.92%). Similarly the maximum value of GCV also obtained fromnumber of total tiller per plant followed by number of productive tiller, grain yield per unit area, panicle length, panicle width, chlorophyll content at maturity and leaf area with a value of 52.96, 46.5, 34.21, 23.13, 22.93, 26.24 and 20.26% respectively (Table6). These high results of PCV and GCV revealed that the genotypes have a broad base genetic background and existence of substantial variability to facilitate improvement through selection.Similarly,Abraha et al.(2015) reported that high level of genotypic variance for grain yield, productive tiller, panicle width and panicle length. Tirfessa(2009) also reported high GCV value for panicle length.

Low PCV and GCV values were computed fortotal leaf vein number, flag leaf appearance date, days to flowering and days to maturity. This showed that these traits were more influenced by the environment for their phenotypic expression and relatively smaller variability.Mulualem et al.(2018) reported lower values of GCV and PCV fordays to flowering and days to maturity. Further, Low GCV and PCV value was also reported for days to maturity by Abraha et al.(2015).

4.4. Estimate of heritability and expected genetic advance

Assessment of heritable and non-heritable components in the total variability is vital in adopting suitable breeding method. Heritability is the proportion of the observed variation in a

progeny that is inherited. Heritability estimate indicates the possibility and extent to which improvement is possible through selection (Robinson *et al*., 1956). Genotypic coefficient of variation (GVC) and heritability estimate would give better information about the efficiency of selection (Burton, 1952). In addition, it measures the genetic relationship between parents and their progeny.

Broad sense heritability which is the ratio of the genotypic variance to the total phenotypic variance ranged from 54.75 for leaf angle to 88.9 for the total number oftillers per plant (Table6). Robinson et al.(1949) classified the ranges of heritability into low(0-30%) where selection may be considerably difficult or impractical due to the masking effect of environment, moderate (30-60%) and high (60% and above). Accordingly, stem diameter (60%), panicle length (82.34%), panicle width (79.88%), total leaf number (86.57%), leaf area (78.18%), plant height (79.12%), number of total tiller per plant (88.90%), number of productive tiller (83.39%), total leaf vein density (65.62%), flag leaf appearance date (86.97), days to flowering (60.96%), hundred seed weight (65.91%) and grain yield per unit area (82.86%) had high heritability (Table 6).High heritability of the traits suggests that they are less influenced by environment and selection for such traits could be easier. This result is in agreement with Abraha et al. (2015) who reported high broad sense heritability estimates for days to flowering, plant height, number of leaves per plant, panicle length and productive number of tillers per plant. In addition, Gedifew and Tsige (2019) reported high heritability value for days to flowering, leaf area, number of leaves per plant and plant height.

Medium heritability was recorded for chlorophyll content measured at flowering (55.64%), leaf angle (54.75%) and days to maturity (59.47%).Similar result was previously reported in sorghum byTirfessa(2009) for days to maturity (49%).

High heritability estimate doesnot necessarily mean high genetic gain (genetic advance)(Mulualem *etal*., 2018; Johonson *et al*., 1955). The utility of heritability increases when it is used to estimate genetic advance (Johonson *et al*., 1955). Thus, genetic advance has an added edge over heritability as a guidingfactor to plant breeders. The genetic advance (GA) and genetic advance as the percentage of the mean (GAM) at 5% selection intensity is presented in (Table6).

Genetic advance as percent of mean ranged from 6.78% for days to maturity to 102.87 % for number oftotal tiller per plant. Johnson et al.(1955) classified genetic advance as percentage of mean (GAM); values from 0%-10% are low, from 10%-20% moderate and 20% and above are high. Based on this, traits like stem diameter, panicle length, panicle width, total leaf number, leaf area, plant height, number of productive tiller, chlorophyll content measured at maturity, number oftotal tiller per plant, hundred seed weight and grain yield per unit area showed high genetic advance as percent of mean. Similar results of high genetic advance as a percent of mean was found for plant height, panicle width and hundred seed weight (Mulualemet al.,2019), number of leaves per plant and panicle length by (Tirfessa,2009).

Among the traits with high genotypic coeffiecient of variation (GCV)and heritability estimate, panicle length, panicle width, leaf area, number of total tiller per plant, number of productive tiller and grain yield per unit area were in conjunction with higher values of genetic advance as percentage of mean (Table6), reflecting thatthese traits are controlled by additive genetic (Panse, 1957) factors and less environmental influence in the phenotypic expression. Breeding methods based on progeny testing and mass selection could be useful in improving these traits (Nyadanu*et al*., 2014).

High heritability might not necessarily lead to increased genetic advance. In this study, total leaf vein number (65.62%) and days to flowering (60.96%) possessing high estimate of heritability, but they fail to show high estimate of genetic advance as percentage of mean.This is the indication of non-additive (dominance and epistasis) gene actionswhich could be exploited through heterosis breeding.If a trait is governed by non-additive gene action, it may give high heritability but low genetic gain, whereas, if it is governed by additive gene action heritability and genetic gainwould be high(Panse, 1957).

Table 6. Range, mean and genetic parameters for 17 quantitative traits of sorghum genotypes evaluated at Miesso, 2018/19

No.	Trait	Mean±SD	Range		σ^2g	σ^2e	σ^2p	GCV	PCV	H^2	GA	GAM
			Min	Max				%	%	%		%
1	SD	2.59±0.61	1.03	4.08	0.14	0.09	0.23	14.24	18.39	60.00	0.59	22.72
2	PAL	28.53±8.72	10.00	51.50	42.05	9.02	51.06	23.13	25.49	82.34	12.12	43.24
3	PAW	10.58±3.37	4.00	19.88	5.88	1.48	7.36	22.93	25.66	79.88	4.46	42.22
4	SPADB	48.29±9.30	19.30	68.40	29.74	23.71	53.45	11.29	15.14	55.64	8.38	17.35
5	SPADM	21.26±7.78	3.80	41.70	31.13	7.05	38.18	26.24	29.06	81.55	10.38	48.82
6	LFN	19.45±3.74	8.75	29.5	8.54	1.33	9.87	15.03	16.16	86.57	5.60	28.81
7	LA	35.38±6.86	19.13	47.25	14.84	12.26	27.10	10.89	14.71	54.75	5.87	16.59
8	PHT	246.51±62.58	84.5	428	1882.51	496.85	2379.36	17.60	19.79	79.12	79.50	32.25
9	LAF	273.90±77.29	18.11	604.41	3079.83	859.42	3939.24	20.26	22.92	78.18	101.09	36.91
10	TILLER	4.94±3.39	1.00	25.00	6.85	0.86	7.70	52.96	56.17	88.90	5.08	102.87
11	ETN	3.39±2.27	1.00	11.00	2.49	0.50	2.98	46.50	50.92	83.39	2.97	87.48
12	TLV	27.33±3.05	18.00	36.00	3.88	2.03	5.91	7.20	8.89	65.62	3.28	12.02
13	FLAG	92.49±11.82	57.00	115.50	81.78	12.25	94.03	9.78	10.48	86.97	17.37	18.78
14	FLOW	105.81±12.85	71.5	127.67	55.07	35.28	90.35	7.01	8.98	60.96	11.94	11.28
15	MATU	142.16±10.83	111	160	36.82	25.09	61.91	4.27	5.53	59.47	9.64	6.78
16	HSW	2.38±0.61	0.65	4.97	0.15	0.08	0.22	16.07	19.79	65.91	0.64	26.87
17	GYPUA	275.66±136.83	117.42	628.99	8892.61	1840.49	10733.10	34.21	37.58	82.85	176.82	64.14

SD= standard deviation, Notes: @ Quantitative traits abbreviations as indicated in Table 2.

4.5. Correlation among traits

The correlation coefficients of seventeen quantitative traits were used in characterizing the 945 sorghum genotypes. The correlation coefficients of seventeen quantitative traits showed that there was a significant positive and negative correlation among traits (Figure2 and AppendixTable 2). Grain yield per unit area was positively correlated with panicle width (0.32), chlorophyll content measured at flowering (0.36), number of productive tiller (0.29) and hundred seed weight (0.31).But it was significantly and negatively correlated with total leaf number, stem diameter, chlorophyll content measured at maturity, flag leaf appearance date, days to 50 % flowering and days tomaturity. Similarly, Abraha et al. (2015) reported significant negative correlation of grain yield with days to flowering and days to maturity under drought stress condition. Bekele (2008) also reported significant positive correlation of grain yield with plant height, panicle width, hundred seed weight and productive tiller of Ethiopian sorghum landraces tested under moisture stressed area. The negative correlation with days to 50% flowering was desirable which indicated that selection for earliness might lead to improvement in grain yield for drought tolerance due to short life cycle of the crop.

The interrelationship among yield component; total leaf number, flag leaf appearance date, days to flowering and days to maturity had negative correlation with panicle width and length. These correlations were desirable and indicated that improvement in panicle width or length might bring improvement in small number of leaves per plant and earliness. Stem diameter was highly correlated with all traits under studied except for leaf area even though the correlation coefficient was low (Figure2and Appendix 2).

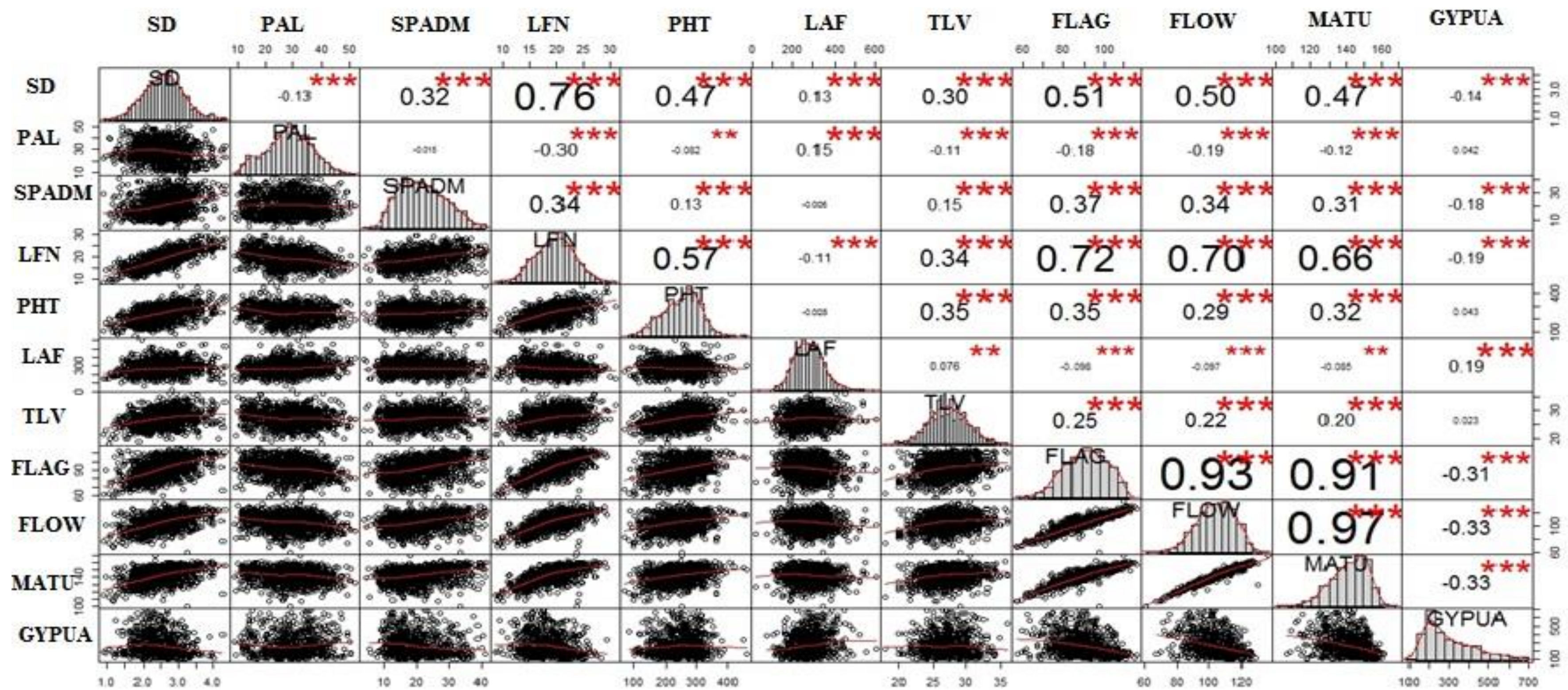

Figure 2.Variation and coefficients of correlations among 11 traits. Histograms for the 11 traits (SD, PAL, SPADM, LFN, PHT, LAF, TLV, FLAG, FLOW, MATU and GYPUA) are displayed along the diagonal. To the left and below the diagonal are scatter plots containing measured individuals from the 945 sorghum genotypes. The red line through the scatter plot represents the line of best fit. Pearson correlation coefficients are shown above and to the right of the diagonal. The correlation significance levels are: *p = 0.05, **p = 0.01, and ***p = 0.001

4.6. Principalcomponent analysis

In order to assess the pattern of genetic diversity, principal component analysis was done by considering all the 17 quantitative traits. The first five principal components with eigenvalues greater than one accounted for 68 %of the total genotypic variation (Table 7).

Table 7. Principal Component analysis of 17 quantitative traits in 945 sorghum genotypes

Trait	PC1	PC2	PC3	PC4	PC5
SD	**0.31**	0.07	-0.26	0.03	-0.02
PAL	-0.11	**0.40**	-0.11	**-0.44**	0.12
PAW	-0.19	**0.31**	-0.25	**-0.42**	0.15
SPADB	-0.28	0.01	-0.21	-0.06	-0.03
SPADM	0.18	0.18	0.07	-0.07	0.16
LFN	**0.37**	0.01	-0.16	0.16	0.06
LA	-0.09	0.07	-0.24	0.04	**0.59**
PHT	0.21	0.20	**-0.39**	0.18	0.28
LAF	-0.07	0.04	**0.34**	-0.27	**-0.61**
TILLER	-0.10	**0.55**	0.20	**0.35**	-0.13
ETN	-0.18	**0.48**	0.13	**0.39**	-0.10
TLV	0.14	0.01	**-0.37**	0.08	-0.15
FLAG	**0.39**	0.09	0.01	-0.04	-0.08
FLOW	**0.38**	0.10	0.04	-0.05	-0.08
MATU	**0.37**	0.12	0.03	-0.07	-0.08
HSW	-0.16	**-0.31**	-0.29	0.32	0.10
GYPUA	-0.16	0.08	-0.42	0.32	-0.23
Eigenvalues	5.60	1.96	1.57	1.28	1.14
Standard deviation	2.37	1.40	1.25	1.13	1.07
% Variance	0.33	0.12	0.09	0.08	0.07
% Cumulative	0.33	0.45	0.54	0.61	0.68

Notes: [@]Quantitative traits abbreviations as indicated in Table 2

The PCA bi-plot analysis showed that most of the traits that accounted for 33 % of the phenotypic variation were laid in the first PC (Figure 3) and 11.5 % variation was contributed to the second PC. Numberof total tiller per plant with high loading value (Figure 3) was found to be the top trait to discriminate the genotypes. The second highest contributor to the phenotypic variation was number of productive tiller followed by the panicle length. The remaining traits such as stem diameter, hundred seed weight and panicle width contributed similar amount of phenotypic variation. In addition, total leaf number and days to

maturity contributed similar amount of phenotypic variation.This implies that these traits are vital for the variation in sorghum genotypes. Hence due consideration should be given to the high contributor traits while planning a breeding program for improving drought related traits. Comparative results were reported by Abraha etal. (2015) and Bekele (2008) who worked on different agro-morphological traits in sorghum genotypes.

The seventeen quantitative traits were dispersed in the three quadrants with the majority of the traits were found on the two positive quadrants of the second PC. The first PC classified plant height, SPAD at maturity, total leaf vein number, stem diameter, days to 50 % flowering, days to maturity, flag leaf appearance date and total leaf numberin one group or inthe first quadrant with positive principal scores and the rest of the traits with negative scores in the second and third quadrant. The second PC also classified all the traits in the first and secondquadrant with positive principal scores except hundred seed weight, which was found in the third quadrant with negative principal score. Traits in the same quadrant are positively correlated with each other.

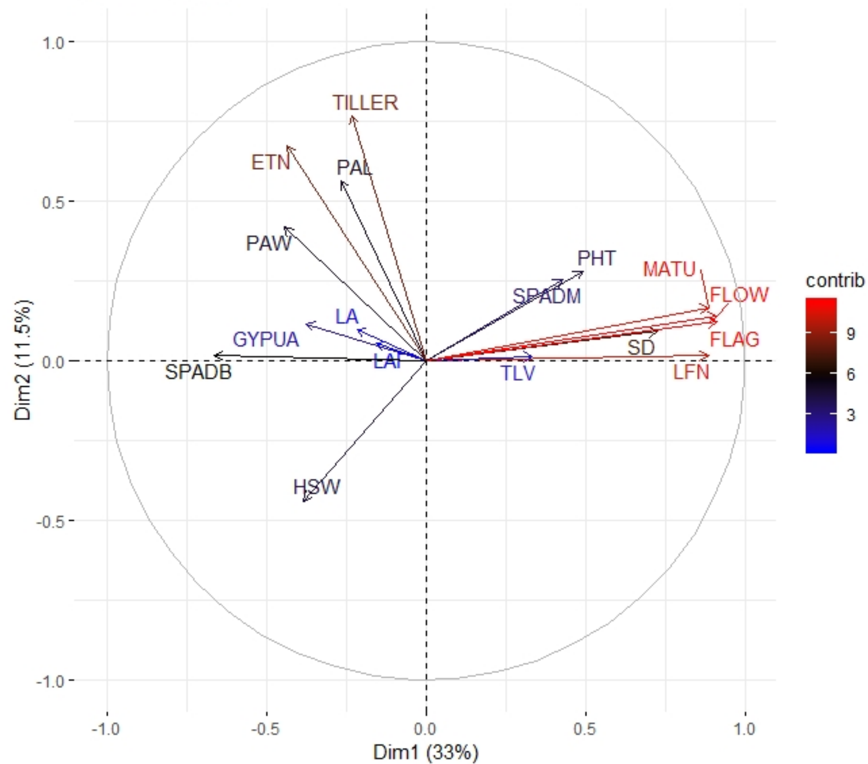

Figure 3.Biplot of the first and second principal components (Dim1 and Dim2) among various quantitative traits in the study. The right side color legend (contrib) indicates the contribution of each trait to the first two PCs. Notes: @ Quantitative traits abbreviations as indicated in Table 2

4.7. Populationstructure

Structure-like population genetic analysis was used to analyze the structure of the landrace population. A range of sub-populations (K=1:10) were tested and a K-value of 5 was determined to best capture the structure of the population structure based on minimal cross-entropy (Figure 4). As indicated in Figure 5, 940 sorghum genotypes were distributed across five subpopulations, which were denoted S1, S2, S3, S4 and S5 respectively. A total of 692 genotypes (73.62%) assigned to either one of the 5 sub-populations with the admixture coefficient value >60%, while the remaining 248 genotypes (26.38%) were categorized as admixtures. 15, 59, 76, 67 and 31 genotypes were categorized as admixtures (Figure 5)from subpopulation (1, 2, 3, 4 and 5), respectively.

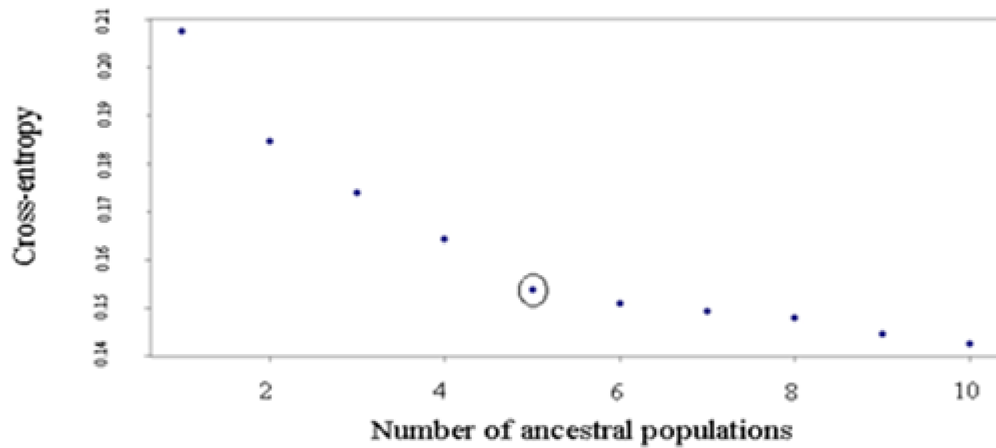

Figure 4. Values of k (x-axis) with cross-entropy values used to detect the true k (y-axis) of five groups (k=5). Circle indicates the “elbow” point

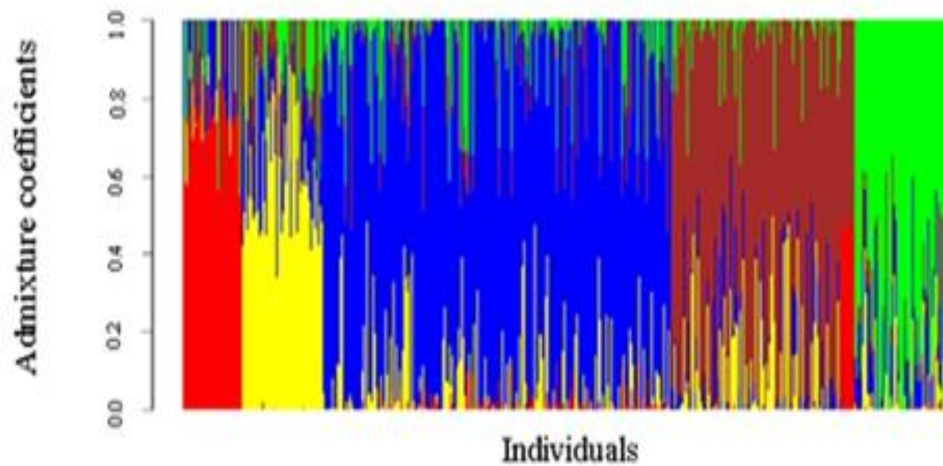

Figure 5. Population structure of 940 sorghum genotypes at K = 5. Each vertical bar represents a single genotype; the length of each bar represents the proportion contributed by each sub-population. Sub-population 1 (color-coded red), sub-population 2 (color-coded yellow), sub-population 3 (color-coded blue), sub-population 4 (color-coded brown) and sub-population 5 (color-coded green).

The Sub-population 1 contained the lowest number of genotypes (73) of which 30 from Oromia, 15 from Amhara, 14 from Tigray, 4 from Benishangul Gumuz, 3 from Somali and 3 from Gambella, 2 from Dire, 1 from Afar and 1 from SNNP. The sub-population 2 was made up of 99genotypes: 32 were from Gambella, 23 from Oromia, 16 from Tigray, 14 from SNNP, 9 from Amhara, 2 from unknown, 1 from Somali, 1 from Afar and1 from Dire.Sub-population

3 contained the largest number of genotypes (425) of which 127 from Amhara, 105 from Oromia, 73 from Tigray, 30 from SNNP, 20 from Somali, 25 from Dire, 23 from Gambella, 22 from Afar, 6 from Benishangul Gumuz and 3 from unknown region.The Sub-population 4 comprised of225 genotypes of which 105 from Oromia, 55 from Amhara, 24 from SNNP, 22 from Tigray, 9 from Gambella, 3 from Somali, 3 from Afar and 3 from Dire.

The Sub-population 5 consisted of 118 genotypes of which 40 form Gambella, 26 from SNNP, 18 from Amhara, 14 from Oromia, 13 from Tigray, 3 from unknown region, 2 from Afar and from Benishangul Gumuz.The distribution of genotypes into the five groups without reflecting their region of origin might indicate the existence of wide variations among genotypes within the regions as well as lack of strong regional differentiation which might be due to gene flow between the regions. Girma et al. (2019) and (Endre and Bantte *et al*., 2016) studied on sorghum genotypes and showed the existence of different groups of population structure.

4.8. Linkage disequilibrium

The LD level of the whole genome of the sorghum genotypes was estimated using 25,634 SNPs. Based on the threshold r^2 value 0.1, the average r^2 value started to decay between 50 to 100kbp (Figure 6).As LD is broken down by recombination, and recombination is not distributed uniformly across the genome(Phillips *et al*., 2003), blocks of LD are expected. The present LD decay result is similar to previously published values in sorghum of 50–150 kb (Bouchet *et al*., 2012) and 75–150 kb (Morris *et al*., 2013). In contrast, Hamblin et al. (2005) who found that LD in sorghum largely decays by 10–15 kb. Differences among studies might be due to low genome coverage of markers and use of few genotypes.

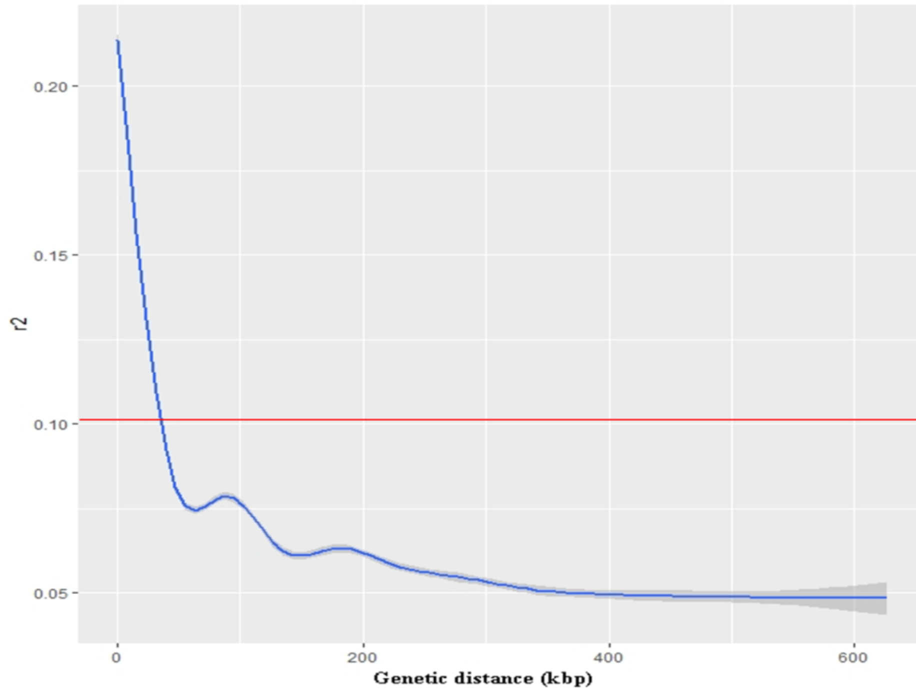

Figure 6. Decay of LD (r^2) as a function of genetic distance (kbp) between pairs of loci on all chromosomes

4.9. Genome wide association study by trait

In total, genome wide association mapping identified 91 different SNPs with significant association ($p \leq 5.21E\text{-}5$) to stem diameter, panicle length and width, chlorophyll content measured at flowering and maturity, total leaf number, leaf angle, plant height, leaf area, number of total tiller per plant, number of productive tiller, total leaf vein number, flag leaf appearance date, days to 50% flowering, days to maturity, hundred seed weight and grain yield per unit area (Figure9 and Table 8).

Table 8.Summary of significant single nucleotide polymorphisms (SNPs) representing different regions across sorghum chromosomes for the 17 quantitative traits

No.	Trait	QTL name	Chromosome	Position	P-value	R^2
1	ETN	qETN4.1	4	67645303	1.5E-05	0.0844
2	ETN	qETN6.1	6	54709080	2.8E-05	0.0804
3	ETN	qETN9.1	9	59291283	2.1E-08	0.0907
4	FLAG	qFLAG2.1	2	43489886	1.6E-07	0.1604
5	FLAG	qFLAG6.1	6	197792	5.8E-10	0.1756
6	FLAG	qFLAG6.2	6	53110589	2.1E-06	0.1610
7	FLAG	qFLAG10.1	10	2411603	1.3E-08	0.1694
8	FLOW	qFLOW2.1	2	68567714	8.7E-06	0.1269
9	FLOW	qFLOW4.1	4	16133049	1.5E-05	0.1320
10	FLOW	qFLOW6.1	6	197792	1.2E-08	0.1313
11	FLOW	qFLOW6.2	6	53110589	9.1E-06	0.1202
12	FLOW	qFLOW10.1	10	2411592	8.8E-06	0.1284
13	GYPUA	qGYPUA3.1	3	62340261	6.6E-06	0.0690
14	GYPUA	qGYPUA5.1	5	5358597	2.1E-09	0.0826
15	HSW	qHSW5.1	5	8329053	2.5E-10	0.2159
16	HSW	qHSW6.1	6	50377580	3.3E-07	0.2267
17	HSW	qHSW6.2	6	56994982	6.9E-07	0.2068
18	HSW	qHSW7.1	7	6366453	3.1E-07	0.2153
19	LA	qLA1.1	1	21735985	2.1E-05	0.1271
20	LA	qLA1.2	1	78024416	9.5E-06	0.1177
21	LA	qLA6.1	6	390629	2.9E-05	0.1182
22	LAF	qLAF1.1	1	1702319	5.8E-06	0.0437
23	LAF	qLAF3.1	3	10499490	3.2E-12	0.0632
24	LAF	qLAF8.1	8	55927895	2.7E-09	0.0381

Notes: [@] Quantitative traits abbreviations as indicated in Table 2

Table 8. (*Continued*)

No.	Trait	QTL name	Chromosome	Position	P-value	R^2
25	LAF	qLAF8.2	8	58849962	2.1E-07	0.0412
26	LFN	qLFN1.1	1	27579022	2.6E-09	0.311
27	LFN	qLFN2.1	2	7835726	7.9E-06	0.3057
28	LFN	qLFN2.2	2	68577889	1.1E-06	0.3036
29	LFN	qLFN3.1	3	73820556	1.14E-07	0.3116
30	LFN	qLFN6.1	6	464463	2.24E-08	0.3075
31	LFN	qLFN6.2	6	197792	5.17E-12	0.3213
32	LFN	qLFN8.1	8	59293437	2.48E-06	0.3088
33	LFN	qLFN8.2	8	59746724	1.58E-07	0.3161
34	LFN	qLFN10.1	10	9880771	4.15E-07	0.3129
35	MATU	qMATU1.1	1	1702319	1.91E-12	0.0763
36	MATU	qMATU1.2	1	75637943	2.38E-06	0.0689
37	MATU	qMATU2.1	2	70400579	1.1E-07	0.0682
38	MATU	qMATU3.1	3	10499490	7.11E-08	0.0693
39	MATU	qMATU3.2	3	62627564	6.46E-08	0.07
40	MATU	qMATU4.1	4	65106947	6.41E-08	0.0682
41	PAL	qPAL1.1	1	4452552	2.91E-05	0.3542
42	PAL	qPAL1.2	1	66717712	3.07E-06	0.359
43	PAL	qPAL3.1	3	60215678	1.64E-07	0.3542
44	PAL	qPAL3.2	3	65495629	6.2E-07	0.3494
45	PAL	qPAL4.1	4	53962403	8.45E-07	0.3516
46	PAL	qPAL5.1	5	61974965	3.81E-07	0.3497
47	PAL	qPAL6.1	6	46495183	5E-09	0.3682
48	PAL	qPAL6.2	6	46717993	2.77E-05	0.3609
49	PAL	qPAL6.3	6	53235367	7.77E-07	0.3624
50	PAL	qPAL9.1	9	10474739	2.06E-06	0.353

Notes: [@] Quantitative traits abbreviations as indicated in Table 2

Table 8. (*Continued*)

No.	Trait	QTL name	Chromosome	Position	P-value	R^2
51	PAL	qPAL9.2	9	48713159	1.57E-08	0.3562
52	PAL	qPAL10.1	10	6431459	2.7E-06	0.3493
53	PAW	qPAW1.1	1	20750932	2.64E-07	0.0989
54	PAW	qPAW1.2	1	61354196	7.94E-06	0.0938
55	PAW	qPAW3.1	3	4692320	1.64E-05	0.0897
56	PAW	qPAW6.1	6	49485063	3.51E-08	0.1065
57	PAW	qPAW10.1	10	3472549	8.82E-08	0.094
58	PHT	qPHT1.1	1	10969119	7.61E-06	0.2279
59	PHT	qPHT3.1	3	73820556	1.55E-10	0.2305
60	PHT	qPHT4.1	4	3672315	1.59E-06	0.2383
61	PHT	qPHT4.2	4	49526648	1.31E-07	0.2326
62	PHT	qPHT5.1	5	61974965	0.000026	0.2283
63	PHT	qPHT5.2	5	62014861	6.77E-12	0.2476
64	PHT	qPHT6.1	6	2933576	2.33E-06	0.2314
65	PHT	qPHT7.1	7	1521349	4.34E-06	0.2298
66	PHT	qPHT7.2	7	60263939	2.55E-05	0.2313
67	PHT	qPHT8.1	8	1443545	1.05E-07	0.2399
68	PHT	qPHT10.1	10	15431893	1.85E-06	0.2318
69	PHT	qPHT10.2	10	9880771	2.51E-07	0.2398
70	SD	qSD1.1	1	58767784	3.83E-07	0.1495
71	SD	qSD3.1	3	4061345	1.34E-05	0.153
72	SD	qSD6.1	6	464463	4.81E-09	0.1567
73	SD	qSD8.1	8	48257103	2.18E-05	0.1578
74	SD	qSD8.2	8	59746724	8.97E-06	0.1518
75	SD	qSD8.3	8	55691107	1.87E-07	0.1574
76	SD	qSD9.1	9	42951252	8.09E-08	0.1586

Notes: [@] Quantitative traits abbreviations as indicated in Table 2

Table 8. (*Continued*)

No.	Trait	QTL name	Chromosome	Position	P-value	R^2
77	SPADB	qSPADB1.1	1	61257247	1.64E-05	0.2161
78	SPADB	qSPADB1.2	1	74818662	5.07E-07	0.2143
79	SPADB	qSPADB4.1	4	19733505	1.11E-07	0.2181
80	SPADM	qSPADM6.1	6	197792	1.05E-05	0.0863
81	SPADM	qSPADM10.1	10	7480369	1.28E-07	0.0926
82	TILLER	qTILLER3.1	3	56093897	1.77E-05	0.1323
83	TILLER	qTILLER5.1	5	4217940	3.28E-07	0.1364
84	TILLER	qTILLER5.2	5	6782254	5.63E-06	0.1379
85	TLV	qTLV1.1	1	39008622	7.12E-07	0.1094
86	TLV	qTLV3.1	3	63955223	2.78E-05	0.1052
87	TLV	qTLV3.2	3	73831651	1.74E-05	0.1074
88	TLV	qTLV4.1	4	62049328	3.51E-10	0.1113
89	TLV	qTLV6.1	6	6775273	1.62E-06	0.1137
90	TLV	qTLV9.1	9	10474739	5E-07	0.1087
91	TLV	qTLV10.1	10	15431893	2.22E-06	0.1094

Notes: [@] Quantitative traits abbreviations as indicated in Table 2

Stem diameter (SD): Manhattan plots (Figure 9) showed that a total of seven SNP markers (Table 8) that were above the threshold ($p \leq 5.21E-5$) for the GWAS results were associated with stem diameter and were distributed on chromosome 1, 3, 6, 8 and 9. R^2 explaining the total phenotypic variation in stem diameter with these SNPs ranged from 14.95– 15.86 %. SPNs markers that control stem diameter in sorghum were previously reported on chromosome 1, 3 and 8 by Zhao et al. (2016) using genome wide association study and on chromosome 6 by Zou etal. (2012) using bi-parental QTL mapping.

Panicle width (PAW): A total of five SNP marker-trait associations withpanicle width were detected on chromosome 1, 3, 6 and 10, with the total phenotypic variance of 8.97 % - 10.65 % (Table 8). Two QTLs linked to panicle width was previously reported on chromosome on 1 and 3 by Hmon et al. (2014).Six QTLs on chromosome 6, two on chromosome 10, eight on

chromosome 1 and seven on chromosome 3 that are linked to panicle width was also reported by Mace et al.(2018) or (https://aussorgm.org.au/sorghum-qtl-atlas/).

Plant height (PHT): A total of 12 SNPs on chromosome 1, 3, 4, 5, 6, 7, 8 and 10 were significantly ($p \leq 5.21E-5$) associated with plant height. These SNPs accounted for up to 24.76 % of the total phenotypic variance for the trait. SPN markers that control plant height in sorghum using genome wide association study were previously reported on chromosome 5, 7 and 8 by Girma et al. (2019) and on chromosome 6 by Zhao et al. (2016). A single QTL linked to plant height was also reported on chromosome 10 by Fakrudin et al. (2013).Nine QTLson chromosome 5, twenty one on chromosome 3, four on chromosome 8, twenty on chromosome 4, fourteen on chromosome 10, and twenty eight on chromosome 1 that are linked to plant height were also reported by Mace et al. (2018) or (https://aussorgm.org.au/sorghum-qtl-atlas/).

Total leaf number (LFN): GWAS for leave number per plant identified a total of 9 SNPs/genomic regions across six chromosomes (1, 2, 3, 6, 8 and 10) significantly associated with leave number per plant. The effect of these SNPs on the phenotypic variation ranged from 30.36 -32.13%. A QTL controlling number of leaves per plant were previously reported on chromosome six by Lopez et al. (2017) and on chromosome 1 and 3 by Reddy et al. (2013). Four QTLs were dissected out for number of leaves per plant, of which three QTLs located on chromosome 10 and another one on chromosome 1 (Fakrudin et al., 2013).Three QTLs on chromosome 1, six on chromosome 3 and one on chromosome 10 that are linked to leave number per plant was also reported by Mace etal. (2018) or (https://aussorgm.org.au/sorghum-qtl-atlas/).

Panicle length (PAL): The 12 SNPs associated with the panicle length were identified on chromosome 1, 3, 4, 5, 6, 9 and 10. The R^2-value of all the SNPs reported here are the highest among the studied traits, explaining 36.82 % of the total phenotypic variance for panicle length (Figure 9 and Table 8). Similarly, Zhao et al. (2016), using genome wide association mapping confirmed the presence of SNPs markers for panicle length on chromosome 3, 5 and 10 in sorghum. Fakrudin et al. (2013) also identified three QTLs for panicle length located on chromosome 3 and 10 in RILs derived from the cross E36-1 × SPV70.

Leaf area (LAF): Four SNPs or genomic regions associated with flag leaf area were recorded on chromosome 1, 3 and 8, and explained 17.56 % phenotypic variation. QTL linked to green leaf area at flowering was previously reported on chromosome 1 by Reddy et al. (2014). A QTL linked to flag leaf area was also identified on chromosome 8 by Mace et al. (2012).

Number of productive tiller (ETN): Three SNPs associated with the number of productive tiller per plant were located on chromosome 4, 6 and 9. The R^2 explaining the total variance in number of productive tiller per plantfor all the SNPs is about 9.07 %. A region significant for number of productive tiller per plant on chromosome 4 and 9 was previously reported by Zhao et al. (2016) using GWAS.Nine QTLson chromosome 9, fifteen on chromosome 4 and twenty four on chromosome 6 that are linked to productive tiller number was also reported by Mace et al. (2018) or (https://aussorgm.org.au/sorghum-qtl-atlas/).

Number of total tiller per plant (Tiller): Three significant marker trait associations were detected on chromosomes 3 and 5, explaining 13.23% to 13.79% of the variation of the total tiller number per plant.

Hundred seed weight (HSW): A total of four loci exhibited significant associations ($p \leq$ 5.21E-5) with hundered seed weight, which was distributed on chromosome 5, 6 and 7. The percentage of the variation explained by the markers ranged from 20.68 % to 22.67%. QTL linked to hundred grain weight was previously reported on chromosome 5 and 7 by Han et al. (2015).

Flag leaf appearance date (FLAG): In total, four SNPs were identified as being significantly associated with the flag leaf appearance date at a threshold of ($P \leq$ 5.21E-50), and they explained 16.04–17.56 % of the phenotypic variance. These SNPs were located on chromosome 2, 6 and 10.

Days to 50% flowering (FLOW): A total of 5 SNPs/genomic regions were associated with days to 50% flowering (Figure 9 and Table 8) were detected on chromosome 2, 4, 6 and 10. The percentage of the phenotypic variance explained by each marker (R^2) ranged from 5.3% to 14%. A region significant associated with days to flowering on chromosome 10 was previously reported by Zhao et al. (2016). Two significant QTLs controlling days to 50% flowering was also identified on chromosome 6 by Zou et al. (2012).Eighty four QTLs on chromosome 6, forty two on chromosome 2, forty on chromosome 10 and twenty nine on

chromosome 4 that are linked to days to flowering were also reported by Mace etal. (2018).Genes identified(SORBI_3006G001100 and SORBI_3006G001200)around qFLOW6.1 SNP marker on chromosome 6 for days to flowering (Figure 7). The identified genes were hypothetical proteins (something that has not been experimentally shown).

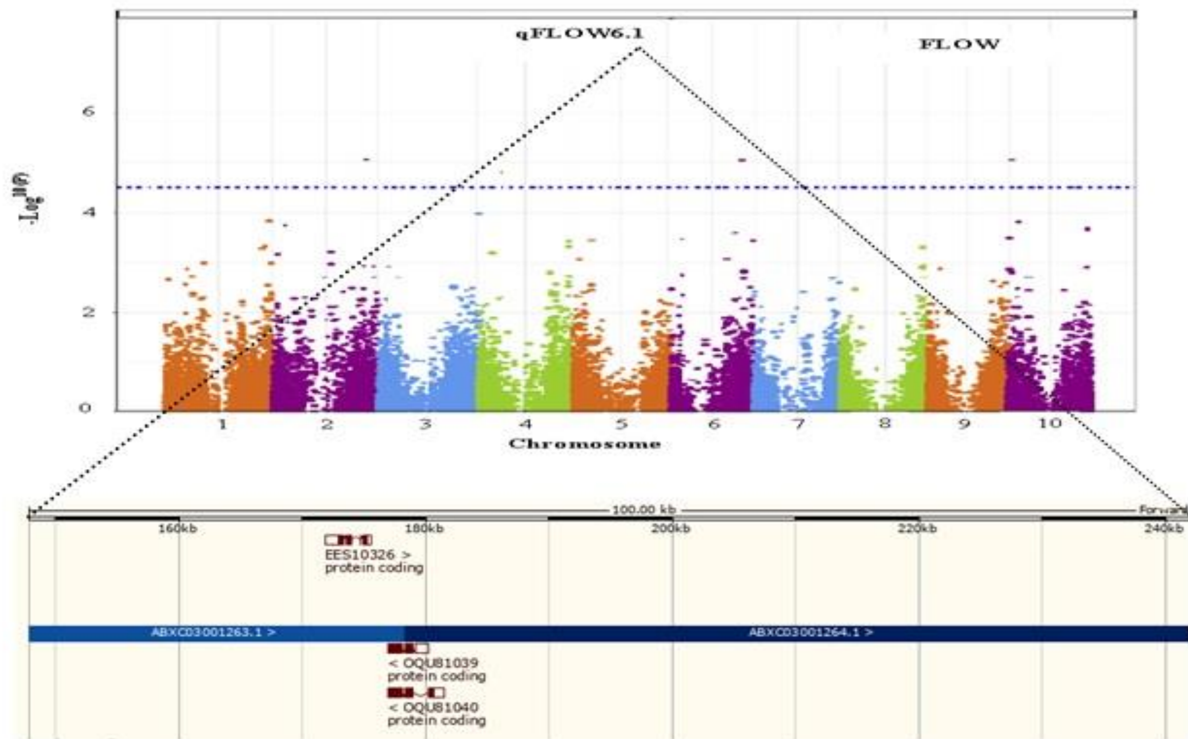

Figure 7. Days to flowering candidate genes in linkage disequilibrium with significant SNP (qFLOW6.1) identified from association mapping

Days to maturity (MATU): Marker-trait associations for the days to maturity were detected on chromosomes 1, 2, 3 and 4. Associations for days to maturity explained 6.82 –7.63 % variation in days to maturity. In total, 6 regions across the genome were identified as having significant association with days to maturity. Among the eight QTLs identified for days to maturity in the study of Reddy et al. (2013), two QTLs werelocated eachof chromosome 1 and 2 and one QTL on chromosome 3.Two QTLs each on chromosome (1, 4 and 3) and three on chromosome 2 that are linked to days to maturity was also reported by Mace etal. (2018).

Grain yield per unit area (GYPUA): Two SNP markers explaining 8.26 % phenotypic variation were identified as loci significantly associated with grain yield per unit area on chromosome 3 and 5, and (Figure9 and Table 8).A QTL controlling grain yield was

previously reported on chromosome three by Reddy et al. (2013). A QTL linked to seed yield per plant was also reported by Fakrudin et al. (2013). Similarly, QTL identified for grain yield per panicle in the present study was also reported on chromosome 3 by Reddy et al. (2014).Seven QTLs on chromosome 5 and twenty four on chromosome 3 that are linked to grain yield were also reported by Mace etal. (2018).Genes identified(SORBI_3005G052700,SORBI_3005G053100,SORBI_3005G053200,SORBI_3005G053800,SORBI_3005G053501andSORBI_3005G053700) around qGYPUA5.1 SNP marker on chromosome 5 for grain yield per unit area (Figure 8). The identified genes were hypothetical proteins (something that has not been experimentally shown).

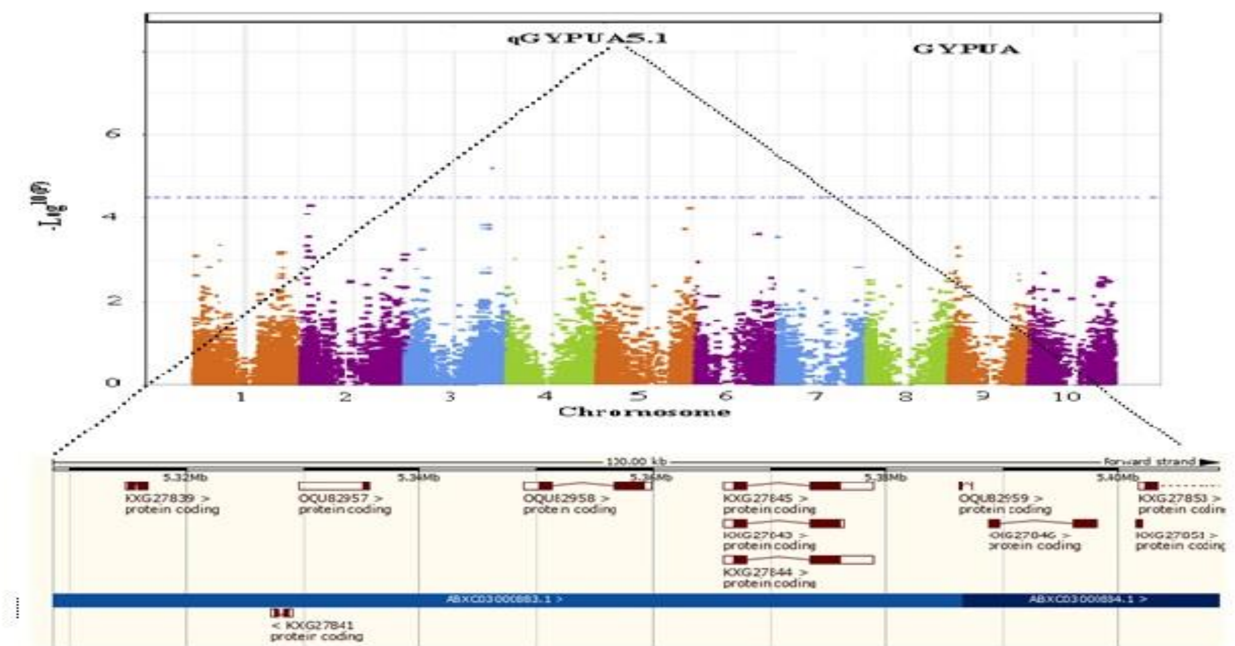

Figure 8. Grain yield per unit areacandidate genes in linkage disequilibrium with significant SNP (qGYPUA5.1) identified from association mapping

Chlorophyll content measured at flowering (SPADB): A total of three SNP markers were associated with Chlorophyll content measured at flowering (Figure 9), and two association signals were found on chromosome 1, which were distributed on two regions of chromosome 1 and a single association signal was found on chromosome 4.The R^2 explaining the total phenotypic variation of the trait ranges between 21.43 and 21.81%. One significant QTL controlling total chlorophyll content was previously identified on chromosome 4 by Chen et

al. (2017). QTL identified for chlorophyll content measured at flowering in the present study was also reported on chromosome 1 by Reddy et al. (2014).

Chlorophyll content measured at maturity (SPADM): Two marker trait associations were detected for chlorophyll content measured at maturity on chromosomes 6 and 10. These markers explained 8.63% to 9.26 % of the variation. QTL identified for chlorophyll content measured at maturity in the present study was also reported on chromosome 10 by Reddy et al. (2014). Similarly, Gelli et al. (2017) also identified two QTLs for chlorophyll content measured at maturity located on chromosome 10 in RILs derived from cross CK60 × San Chi San.

Total leaf vein number (TLV): Significant marker trait associations for total vein number were detected on chromosomes 1, 3, 4, 6, 9, and 10, explaining 10.52 % to 11.37 % of the total phenotypic variation in total vein number**.** In total, 7 regions across the genome were identified as having significant association with total vein number.

Leaf angle (LA): Manhattan plots (Figure 9) and genome wide association analysis showed that two SNPs on chromosome 1 and a single SNP on chromosome 6 were significantly associated with Leaf angle. These markers explained 11.77 % to 12.71 % of the variation in leaf angle. SNP markers that control leaf angle in sorghum were previously reported on chromosome 1 and 6 by Zhao et al. (2016) using genome wide association study.Nine QTLs on chromosome 1 and four on chromosome 6 that are linked to leaf angle were also reported by Mace etal. (2018) or (https://aussorgm.org.au/sorghum-qtl-atlas/).

4.10. SNPs associated with more than one traits

Loci, qSPADM6.1, qLFN6.1, qFLAG6.1 and qFLOW6.1 located on chromosome 6 (pos: 197,792 bp)showed significant association with chlorophyll content measured at maturity, total leaf number, days to 50 % flowering and flag leaf appearance date.This observation was supported by the highly significant positive correlation amongthese traits (Table 9).The co-localization of the SNP identified for these traits can help in improvement of these traits at a time using the same linked markers.qPHT10.2 and qTLV10.1 on chromosome 10 (pos: 15,431,893 bp)were found to be significantly associated with totalleaf vein number and plant height. qPHT10.1, qLFN10, qPHT3.1 and qLFN3.1were significantly associated with plant

height and total leaf number.qMATU1.1, qLAF1.1, qMATU3.1 and qLAF3.1were detected to be significantly associated with days to maturity and leaf area.

Table 9. SNPs detected in more than one trait

QTL name	Traits	Chromosome	Position
qSD8.3& qLFN8.2	LFN& SD	8	59746724
qLFN6.2 &qSD6.1	LFN & SD	6	464463
qPAL9.1 &qTLV9.1	TLV & PAL	9	10474739
qPHT5.1& qPAL5.1	PHT & PAL	5	61974965
qMATU1.1 & qLAF1.1	MATU & LAF	1	1702319
qMATU3.1 & qLAF3.1	MATU & LAF	3	10499490
qFLOW6.2 & qFLAG6.2	FLOW & FLAG	6	53110589
qPHT10.1& qLFN10.1	PHT & LFN	10	9880771
qPHT3.1& qLFN3.1	PHT & LFN	3	73820556
qPHT10.2&qTLV10.1	TLV & PHT	10	15431893
qSPADM6.1, qLFN6.1, qFLAG6.1 & qFLOW6.1	SPADM, LFN, FLAG & FLOW	6	197792

Notes: [@]Quantitative traits abbreviations as indicated in Table 2

qSD8.3 and qLFN8.2on chromosome 8 (pos: 59,746,724 bp) and qLFN6.2 and qSD6.1on chromosome 6 (pos: 464,463 bp) were significantly associated with total leaf number and stem diameter.This is supported by the highly significant positive correlation between stem diameter and total leaf number (r=0.79), suggesting that plant with more number ofleaves per plant can increase the stem diameter, by means of transportof increased photosynthate from leaf to stem diameter (Fakrudin *etal*., 2013).

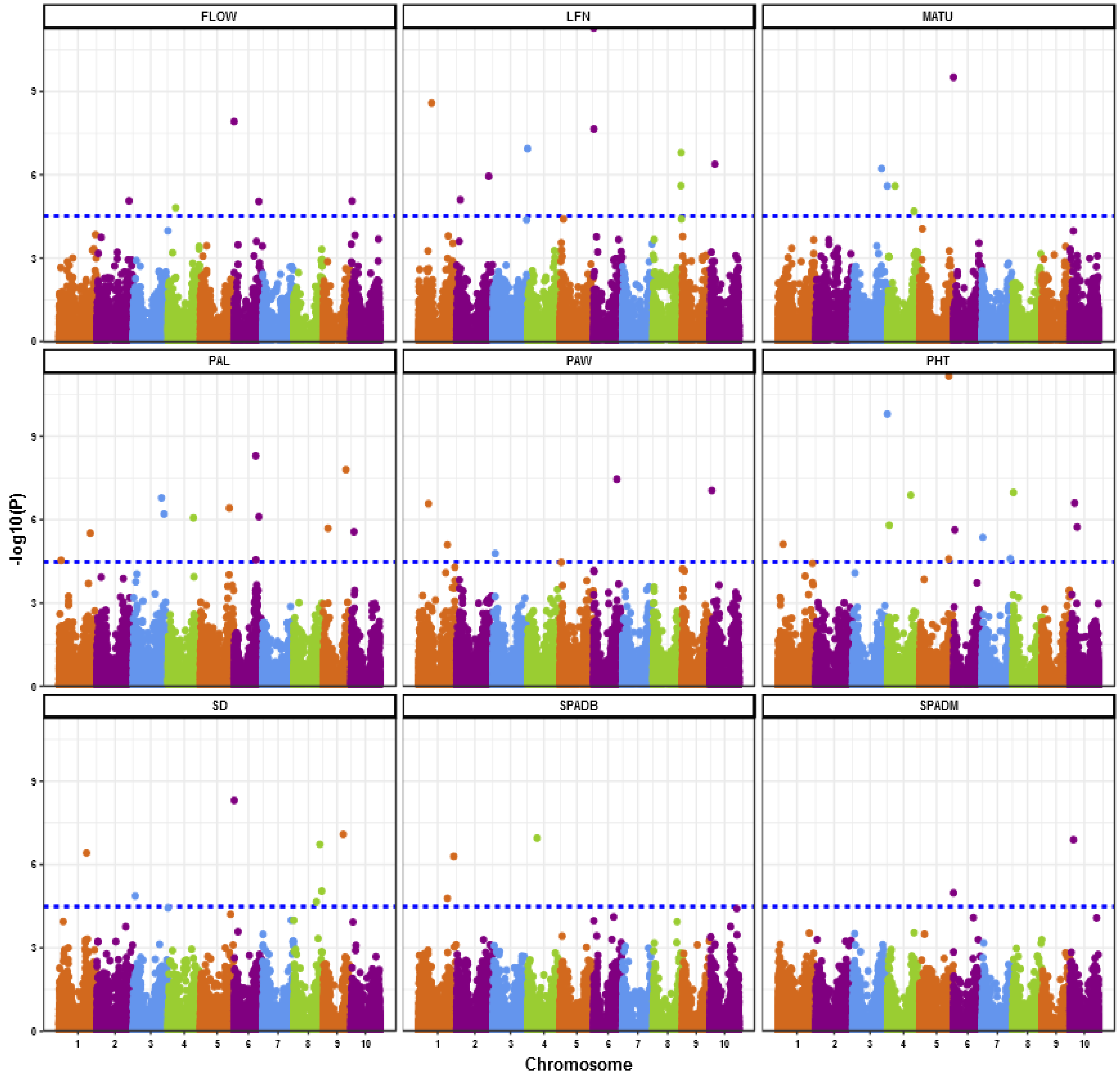

Figure 9. GWAS across 940 Ethiopian sorghum genotypes collection using 25,634 SNPs. Chromosome coordinates are displayed along the X-axis with the –log 10 of the association P value for each single nucleotide polymorphism displayed on the Y-axis. A greater –log 10 indicates stronger association with the trait. The blue line denotes the significance threshold.Notes: [@]Quantitative traits abbreviations as indicated in Table 2

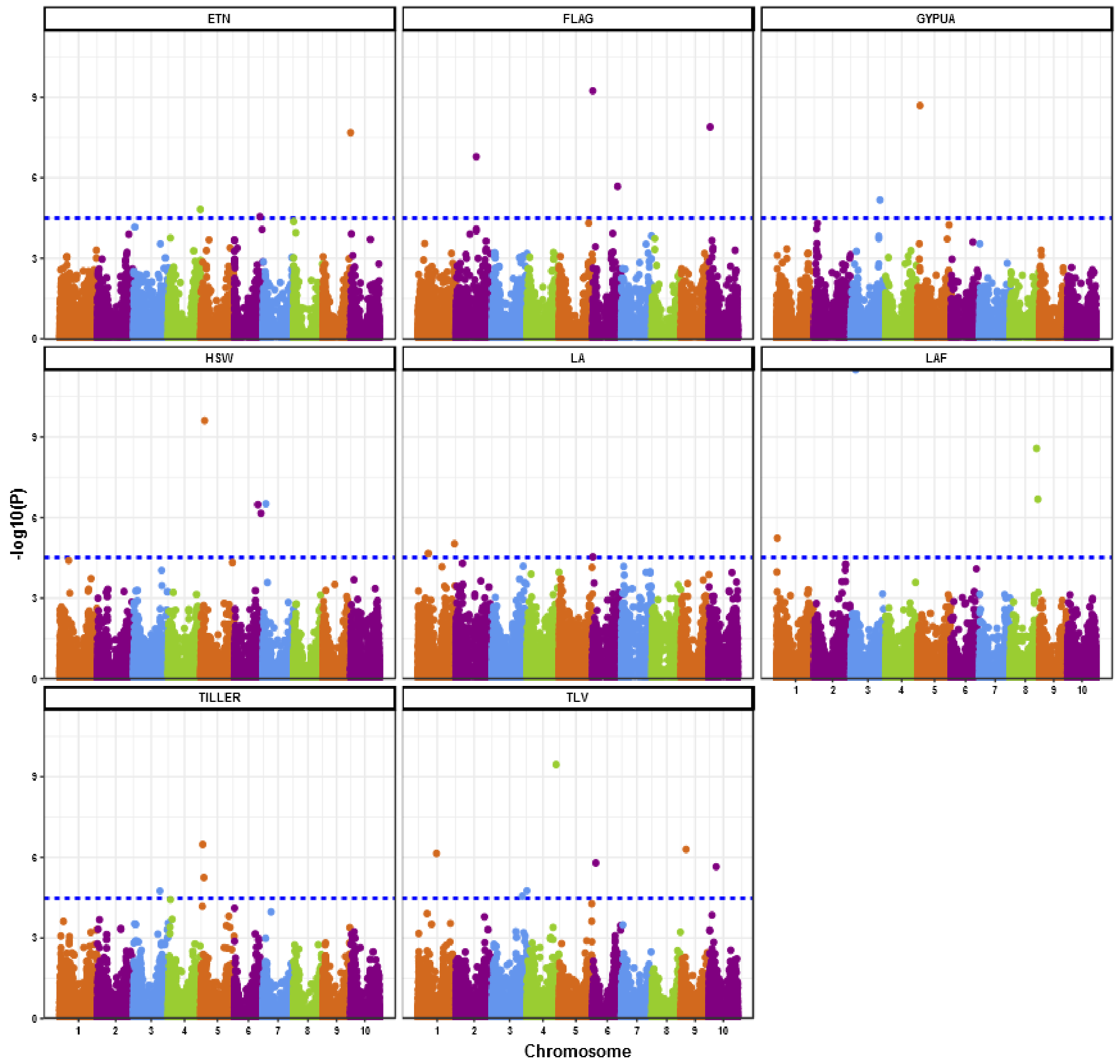

Figure 9.(*Continued*)

For all seventeen quantitative traits, 91 associated SNPs markers and out of these eleven markers were detected in more than one trait (Table 8).The highest number of marker trait associationswasrecorded for panicle length (12) and plant height (12), while the lowest number of marker trait associationswas recorded for chlorophyll content measured at maturity (2) and grain yield per unit area (2) (Table 8). The largest fraction of significant SNPs (19.78%) was detected on chromosome 6, followed by chromosomes 1 and 3with 16.48% and 14.29%, respectively (Table 8).The smallest concentration of significant SNP markerswas observed on chromosome 7 for hundred seed weight and plant height.

4. SUMMARY AND CONCLUSION

As a C4 species, sorghum has greater transpiration efficiency and hence survives and grows better than most other cereal crops under water-stress conditions. However, drought is still the major constraint for its production and productivity.Therefore, understanding the extent and pattern of genetic variability for such key traits have paramount importance for sorghum breeding under water limited condition.

A total of 945sorghum genotypes from different geographical locations were evaluated for variation in drought tolerance under drought stress condition during the main cropping season of 2018/19 at Miessosubstation, MARCusing alpha latticedesign with two replications.

The result revealed the presence of significant genetic variability among the tested genotypes for the different traits,which could be exploited through selection, as variability within populations is the basic prerequisite for crop improvement. The coefficient of correlation among the traits (Figure 2, AppendixTable 2) showed that most of the trait revealed a significant positive and negative association with each other. For the principal analysis of traits, the first five principal components (PCs) with eigenvalues greater than one accounted for 68 % of the total genotypic variation, the remaining 32 % accounted from the left twelve principal components.

For all of the traits measured, higher phenotypic over genotypic coefficient of variation were observed with range of GCV 4.27% for days to maturity to 52.96 % for number of total tillers per plant, PCV 5.53 % for days to maturity to 56.17 % for number of total tillers per plant.H^2 54.75% for leaf angle to 88.9 % for number of total tillers per plant and GAM 9.64 % for days to maturity to 102.87 % for number of total tillers per plant. Among the traits with high genotypic coeffiecient of variationand heritabilityestimate, panicle length and width, leaf area, number of total tiller per plant and number of productive tiller were in conjunction with higher values of genetic advance as percentage of mean, reflecting the variability of these traits is controlled by additive genetic factors and less environmental influence in the phenotypic expression.

A total of 91 different SNPs with significant association to different traits were detected.The highest number of marker trait associations was recorded for panicle length (12) and plant

height (12), while the lowest number of marker trait associations was recorded for chlorophyll content measured at maturity (2) and grain yield per unit area (2).

In general , this study showed the existence of genetic variability in sorghum genotypes for different traits grown under moisture stress condition, providing opportunity to select a number of promising parents with key traits related to drought tolerance. Therefore, it can be concluded that number leaves per plant, flag leaf appearance date, days to flowering, days to maturity and hundred seed weight could be considered as important selection criteria for sorghum yield improvement.

Based on theextent of genetic variability revealed by both morphological and molecular genetic markersamong thegenotypes,the following recommendations were suggested.

1. The sorghum genotypes showed highly significant variation for all traits under drought stress condition and could be utilized by sorghum breeders to develop new and economically important sorghum varieties.
2. The identified SNP markers linked with different traits cloud be used for marker assisted selection following proper validation.
3. The studied sorghum genotypes were grouped into five subgroups with regional independency. Therefore, further studies should be done based onpartitioning of the genetic diversity into within and between regions of origins and also based on theiraltitude variation.

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6. APPENDIX

Table 10Appendix 1. List of sorghum genotypes used in the study arranged according to their collection regions and zone

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
1	JUS171645	Amara	Misrak Gojam	25	JUS171369	SNNP	Bench Maji
2	JUS171639	Amara	Semen Gondar	26	JUS171746	Amara	Semen Gondar
3	JUS171480	Oromiya	Mirab Shewa	27	JUS171223	Amara	Debub Wello
4	JUS171325	Amara	Semen Shewa	28	JUS171435	Oromiya	Misrak Shewa
5	JUS171380	Oromiya	Semen Shewa	29	JUS171241	Affar	Zone 1
6	JUS171741	Amara	Semen Gondar	30	JUS171538	Amara	Semen Gondar
7	JUS171403	Oromiya	Arsi	31	JUS171321	Tigray	Debubawi
8	JUS171757	Amara	Debub Wello	32	JUS171779	Amara	Semen Gondar
9	JUS171422	SNNP	Hadiya	33	JUS171599	Amara	Semen Gondar
10	JUS171509	Gambella	Zone 1	34	JUS171597	SNNP	Bench Maji
11	JUS171289	Tigray	Mirabawi	35	JUS171463	Oromiya	Semen Shewa
12	JUS171358	Amara	Semen Shewa	36	JUS171506	Gambella	Zone 1
13	JUS171475	Oromiya	Semen Shewa	37	JUS171784	Amara	Debub Wello
14	JUS171259	Amara	Semen Wello	38	JUS171472	Oromiya	Semen Shewa
15	JUS171700	Oromiya	Mirab Shewa	39	JUS171316	Tigray	Debubawi
16	JUS171622	Oromiya	Misrak Wellega	40	JUS171378	SNNP	Bench Maji
17	JUS171560	Amara	Semen Wello	41	JUS171627	Oromiya	Misrak Shewa
18	JUS171534	Amara	Semen Gondar	42	JUS171792	Tigray	Mehakelegnaw
19	JUS171521	Amara	Debub Wello	43	JUS171666	Tigray	Mirabawi
20	JUS171290	Tigray	Mirabawi	44	JUS171660	Tigray	Mehakelegnaw
21	JUS171615	Tigray	Mehakelegnaw	45	JUS171520	Oromiya	Illubabor
22	JUS171503	SNNP	Bench Maji	46	JUS171677	Tigray	Debubawi
23	JUS171711	Tigray	Mehakelegnaw	47	JUS171547	Tigray	Mehakelegnaw
24	JUS171782	Amara	Semen Gondar	48	JUS171344	Amara	Semen Shewa

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
49	JUS171508	Oromiya	Misrak Shewa	73	JUS171291	Tigray	Mirabawi
50	JUS171638	Amara	Debub Gondar	74	JUS171766	Oromiya	Misrak Harerge
51	JUS171460	Amara	Misrak Gojam	75	JUS171640	Amara	Semen Gondar
52	JUS171544	Amara	Debub Gondar	76	JUS171352	Amara	Semen Shewa
53	JUS171637	Amara	Semen Gondar	77	JUS171252	Amara	Semen Wello
54	JUS171537	Amara	Debub Gondar	78	JUS171387	Oromiya	Mirab Shewa
55	JUS171649	Amara	Agew Awi	79	JUS171505	Gambella	Zone 1
56	JUS171754	Amara	Oromiya	80	JUS171464	Oromiya	Semen Shewa
57	JUS171519	Oromiya	Mirab Harerge	81	JUS171515	Amara	Semen Wello
58	JUS171542	Amara	Debub Gondar	82	JUS171349	Amara	Semen Shewa
59	JUS171385	Oromiya	Mirab Shewa	83	JUS171786	Amara	Debub Wello
60	JUS171625	SNNP	Sidama	84	JUS171562	Amara	Semen Wello
61	JUS171500	Oromiya	Misrak Wellega	85	JUS171574	Amara	Debub Wello
62	JUS171478	Oromiya	Semen Shewa	86	JUS171623	Oromiya	Illubabor
63	JUS171556	Oromiya	Mirab Harerge	87	JUS171345	Amara	Semen Shewa
64	JUS171652	Oromiya	Bale	88	JUS171641	Tigray	Debubawi
65	JUS171414	Oromiya	Misrak Shewa	89	JUS171512	SNNP	Bench Maji
66	JUS171163	Tigray	Debubawi	90	JUS171686	Oromiya	Borena
67	JUS171441	SNNP	Sidama	91	JUS171280	Tigray	Mirabawi
68	JUS171248	Affar	Zone 1	92	JUS171368	SNNP	Bench Maji
69	JUS171496	Amara	Debub Wello	93	JUS171807	Amara	Semen Gondar
70	JUS171329	Amara	Semen Shewa	94	JUS171447	Oromiya	Semen Shewa
71	JUS171665	Tigray	Mirabawi	95	JUS171459	Amara	Misrak Gojam
72	JUS171808	Somali	Jigjiga	96	JUS173351	Oromiya	Mirab Harerge

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
97	JUS173360	SNNP	Bench Maji	121	JUS173343	Tigray	Debubawi
98	JUS173389	Tigray	Debubawi	122	JUS173391	Tigray	Mirabawi
99	JUS171251	Amara	Semen Wello	123	JUS171395	Amara	Semen Gondar
100	JUS161002	Oromiya	Mirab Shewa	124	JUS161004	Oromiya	Mirab Harerge
101	JUS161329	SNNP	Bench Maji	125	JUS161331	Tigray	Misrakawi
102	JUS173221	Gambella	Zone 1	126	JUS173219	Affar	Zone 1
103	JUS173421	SNNP	Bench Maji	127	JUS173214	Affar	Zone 1
104	JUS173361	Tigray	Debubawi	128	JUS173358	Tigray	Mirabawi
105	JUS173367	Tigray	Mirabawi	129	JUS173368	Tigray	Mirabawi
106	JUS173373	SNNP	Bench Maji	130	JUS173376	Tigray	Mirabawi
107	JUS171590	Tigray	Misrakawi	131	JUS171334	Amara	Semen Shewa
108	JUS173350	Tigray	Debubawi	132	JUS173356	Tigray	Mirabawi
109	JUS173344	Tigray	Debubawi	133	JUS173372	Tigray	Debubawi
110	JUS173390	SNNP	Bench Maji	134	JUS171168	Oromiya	Mirab Harerge
111	JUS171593	Amara	Semen Gondar	135	JUS171298	Tigray	Mirabawi
112	JUS161003	Oromiya	Misrak Wellega	136	JUS161325	Oromiya	Illubabor
113	JUS161330	Oromiya	Arssi	137	JUS161332	DDP	Dire Dawa
114	JUS173241	Affar	Zone 1	138	JUS173312	Tigray	Debubawi
115	JUS173423	SNNP	Bench Maji	139	JUS173420	SNNP	Bench Maji
116	JUS173363	SNNP	Bench Maji	140	JUS173355	SNNP	Bench Maji
117	JUS173369	Tigray	Mirabawi	141	JUS173216	Gambella	Zone 1
118	JUS173375	Tigray	Mirabawi	142	JUS173374	Tigray	Debubawi
119	JUS171497	Tigray	Debubawi	143	JUS171336	Amara	Semen Shewa
120	JUS173354	Tigray	Debubawi	144	JUS173347	SNNP	Bench Maji

Appendix 1. (*Continued*)

	Place of Collection		Place of Collection

List	Genotypes	Region	Zone	List	Genotypes	Region	Zone
145	JUS173342	Tigray	Debubawi	169	JUS173340	SNNP	Bench Maji
146	JUS173236	Affar	Zone 1	170	JUS153001	Unknown	Unknown
147	JUS171256	Amara	Semen Wello	171	JUS161008	Somali	Jigjiga
148	JUS161326	Affar	Zone 1	172	JUS173222	Gambella	Zone 1
149	JUS161333	Oromiya	Illubabor	173	JUS173310	Oromiya	Mirab Harerge
150	JUS173311	Oromiya	Mirab Wellega	174	JUS173425	SNNP	Bench Maji
151	JUS173419	SNNP	Bench Maji	175	JUS173365	SNNP	Bench Maji
152	JUS173357	SNNP	Bench Maji	176	JUS173381	SNNP	Bench Maji
153	JUS173379	SNNP	Bench Maji	177	JUS173348	Tigray	Debubawi
154	JUS173377	Tigray	Mirabawi	178	JUS171619	Tigray	Mirabawi
155	JUS171601	SNNP	Semen Omo	179	JUS173345	Tigray	Mirabawi
156	JUS173346	SNNP	Bench Maji	180	JUS173215	Affar	Zone 1
157	JUS173341	Tigray	Debubawi	181	JUS161001	Amara	Semen Wello
158	JUS173225	Affar	Zone 1	182	JUS161328	Oromiya	Mirab Shewa
159	JUS171526	Amara	Debub Gondar	183	JUS173226	Affar	Zone 1
160	JUS161327	Amara	Misrak Gojam	184	JUS173309	Gambella	Zone 1
161	JUS173227	Gambella	Zone 1	185	JUS173362	SNNP	Bench Maji
162	JUS173305	Gambella	Zone 1	186	JUS173366	SNNP	Bench Maji
163	JUS173424	Oromiya	Mirab Harerge	187	JUS173382	Somali	Jigjiga
164	JUS173364	Tigray	Debubawi	188	JUS173349	Tigray	Debubawi
165	JUS173380	Tigray	Mirabawi	189	JUS173217	Affar	Zone 1
166	JUS173352	Tigray	Debubawi	190	JUS173515	Tigray	Mirabawi
167	JUS171365	Amara	Semen Shewa	191	JUS173258	Gambella	Zone 1
168	JUS173359	SNNP	Bench Maji	192	JUS173244	Affar	Zone 1

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone

193	JUS173207	Affar	Zone 1	217	JUS173205	Gambella	Zone 1
194	JUS173235	Gambella	Zone 1	218	JUS173238	Gambella	Zone 1
195	JUS173307	Gambella	Zone 1	219	JUS173303	Gambella	Zone 1
196	JUS173297	Gambella	Zone 1	220	JUS173294	Gambella	Zone 1
197	JUS173315	Oromiya	Mirab Harerge	221	JUS173317	Oromiya	Illubabor
198	JUS173330	Amara	Semen Wello	222	JUS173328	Gambella	Zone 1
199	JUS173525	Amara	Semen Wello	223	JUS173527	Amara	Semen Wello
200	JUS173506	Tigray	Debubawi	224	JUS173508	Affar	Zone 1
201	JUS173213	Amara	Debub Wello	225	JUS173211	Gambella	Zone 1
202	JUS173516	Amara	Semen Wello	226	JUS173518	Affar	Zone 1
203	JUS173254	Amara	Debub Wello	227	JUS173252	Affar	Zone 1
204	JUS173458	Gambella	Zone 1	228	JUS173460	Oromiya	Mirab Wellega
205	JUS173206	Affar	Zone 1	229	JUS173204	Amara	Debub Wello
206	JUS173237	Affar	Zone 1	230	JUS173231	Amara	Debub Wello
207	JUS173304	Gambella	Zone 1	231	JUS173302	Oromiya	Misrak Harerge
208	JUS173296	Oromiya	Misrak Harerge	232	JUS173295	Tigray	Debubawi
209	JUS173318	Oromiya	Misrak Harerge	233	JUS173333	Gambella	Zone 1
210	JUS173329	Oromiya	Jimma	234	JUS173313	Gambella	Zone 1
211	JUS173526	Tigray	Mirabawi	235	JUS173513	Amara	Semen Wello
212	JUS173507	Amara	Debub Wello	236	JUS173509	Amara	Semen Wello
213	JUS173212	Affar	Zone 1	237	JUS173210	Amara	Debub Wello
214	JUS173517	Amara	Semen Wello	238	JUS173519	Amara	Semen Wello
215	JUS173250	Amara	Semen Gondar	239	JUS173257	Gambella	Zone 1
216	JUS173459	Oromiya	Misrak Harerge	240	JUS173461	SNNP	Bench Maji

Appendix 1. (*Continued*)

		Place of Collection				Place of Collection	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
241	JUS173220	Amara	Debub Wello	265	JUS173240	Amara	Debub Wello

242	JUS173242	Affar	Zone 1	266	JUS173299	Gambella	Zone 1
243	JUS173301	Oromiya	Mirab Harerge	267	JUS173320	Tigray	Mirabawi
244	JUS173293	Oromiya	Misrak Harerge	268	JUS173316	Gambella	Zone 1
245	JUS173332	Unknown	Unknown	269	JUS173522	Tigray	Debubawi
246	JUS173521	Tigray	Debubawi	270	JUS173504	Amara	Semen Wello
247	JUS173514	Tigray	Mirabawi	271	JUS173512	Amara	Debub Wello
248	JUS173510	Amara	Semen Wello	272	JUS173208	Affar	Zone 1
249	JUS173230	Gambella	Zone 1	273	JUS161343	Amara	Mirab Gojam
250	JUS173306	Oromiya	Illubabor	274	JUS173229	Affar	Zone 1
251	JUS173251	Gambella	Zone 1	275	JUS173234	Gambella	Zone 1
252	JUS173462	Oromiya	Mirab Shewa	276	JUS173232	Affar	Zone 1
253	JUS173233	Affar	Zone 1	277	JUS173298	Gambella	Zone 1
254	JUS173239	Gambella	Zone 1	278	JUS173319	Gambella	Zone 1
255	JUS173300	Oromiya	Mirab Harerge	279	JUS173314	Gambella	Zone 1
256	JUS173334	DDP	Dire Dawa	280	JUS173524	Tigray	Debubawi
257	JUS173331	Tigray	Debubawi	281	JUS173505	Affar	Zone 1
258	JUS173523	Amara	Semen Wello	282	JUS173520	Tigray	Debubawi
259	JUS173503	Tigray	Debubawi	283	JUS173463	Oromiya	Misrak Harerge
260	JUS173511	Amara	Semen Wello	284	JUS173439	Gambella	Zone 1
261	JUS173209	Amara	Debub Wello	285	JUS173466	Gambella	Zone 1
262	JUS173308	Oromiya	Illubabor	286	JUS173622	Gambella	Zone 1
263	JUS173223	Affar	Zone 1	287	JUS173445	Oromiya	Misrak Harerge
264	JUS173228	Affar	Zone 1	288	JUS173449	Gambella	Zone 1

Appendix 1. (*Continued*)

		Place of Collection				Place of Collection	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
289	JUS173479	SNNP	Bench Maji	313	JUS173624	Amara	Debub Wello
290	JUS173588	Gambella	Zone 1	314	JUS173626	Gambella	Zone 1

291	JUS173575	Gambella	Zone 2	315	JUS173573	Gambella	Zone 1
292	JUS173596	Gambella	Zone 1	316	JUS173623	Gambella	Zone 1
293	JUS173572	Gambella	Zone 1	317	JUS173601	Amara	Semen Wello
294	JUS173594	Gambella	Zone 1	318	JUS173615	Oromiya	Mirab Harerge
295	JUS173464	SNNP	Bench Maji	319	JUS173442	Oromiya	Misrak Harerge
296	JUS173453	SNNP	Bench Maji	320	JUS173450	Somali	Shinile
297	JUS173485	Oromiya	Misrak Harerge	321	JUS173621	SNNP	Bench Maji
298	JUS173743	Amara	Debub Wello	322	JUS173727	Oromiya	Misrak Harerge
299	JUS173446	SNNP	Bench Maji	323	JUS173457	SNNP	Bench Maji
300	JUS173448	Oromiya	Misrak Harerge	324	JUS173469	Oromiya	Mirab Wellega
301	JUS161345	Oromiya	Arssi	325	JUS173616	SNNP	Bench Maji
302	JUS173589	Gambella	Zone 1	326	JUS173630	Gambella	Zone 2
303	JUS173574	Gambella	Zone 1	327	JUS173609	Gambella	Zone 1
304	JUS173597	Gambella	Zone 1	328	JUS173598	Gambella	Zone 1
305	JUS173604	Gambella	Zone 1	329	JUS173625	Gambella	Zone 1
306	JUS173610	Gambella	Zone 1	330	JUS173611	Gambella	Zone 1
307	JUS173465	Gambella	Zone 1	331	JUS173443	Oromiya	Misrak Harerge
308	JUS173444	Oromiya	Mirab Harerge	332	JUS173440	Oromiya	Misrak Harerge
309	JUS173578	Gambella	Zone 1	333	JUS173576	Oromiya	Mirab Shewa
310	JUS173728	Somali	Jigjiga	334	JUS173725	Oromiya	Misrak Harerge
311	JUS173456	Oromiya	Misrak Harerge	335	JUS173482	Oromiya	Mirab Wellega
312	JUS173468	SNNP	Bench Maji	336	JUS173470	Oromiya	Jimma

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
337	JUS173618	SNNP	Bench Maji	361	JUS173581	Gambella	Zone 1
338	JUS173590	Gambella	Zone 2	362	JUS173585	Gambella	Zone 1
339	JUS173587	SNNP	Bench Maji	363	JUS173600	Gambella	Zone 1

340	JUS173614	Gambella	Zone 2	364	JUS173591	Gambella	Zone 1
341	JUS173579	Gambella	Zone 1	365	JUS173617	Gambella	Zone 1
342	JUS173613	SNNP	Bench Maji	366	JUS173452	SNNP	Bench Maji
343	JUS173447	SNNP	Bench Maji	367	JUS173455	Oromiya	Illubabor
344	JUS173441	Oromiya	Mirab Wellega	368	JUS173620	Gambella	Zone 1
345	JUS173607	Gambella	Zone 1	369	JUS173481	Somali	Shinile
346	JUS173722	DDP	Dire Dawa	370	JUS173478	SNNP	Bench Maji
347	JUS173474	Oromiya	Mirab Shewa	371	JUS173584	Gambella	Zone 1
348	JUS173471	Gambella	Zone 1	372	JUS173580	Gambella	Zone 1
349	JUS173606	SNNP	Bench Maji	373	JUS173595	Gambella	Zone 2
350	JUS173582	Gambella	Zone 1	374	JUS173577	Gambella	Zone 1
351	JUS173608	SNNP	Bench Maji	375	JUS171191	Gambella	Zone 1
352	JUS173603	Gambella	Zone 1	376	JUS173454	Gambella	Zone 1
353	JUS173619	SNNP	Bench Maji	377	JUS173720	Amara	Debub Wello
354	JUS173612	Gambella	Zone 2	378	JUS173731	Somali	Jigjiga
355	JUS173451	Gambella	Zone 1	379	JUS173707	Amara	Oromiya
356	JUS173467	Amara	Semen Wello	380	JUS173695	Somali	Jigjiga
357	JUS173586	SNNP	Bench Maji	381	JUS173708	Somali	Jigjiga
358	JUS173480	Oromiya	Mirab Wellega	382	JUS173783	Tigray	Mehakelegnaw
359	JUS173472	SNNP	Bench Maji	383	JUS173762	Somali	Jigjiga
360	JUS173605	Gambella	Zone 1	384	JUS173663	Amara	Semen Wello

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
385	JUS173672	Benishangul Gumuz	Metekel	409	JUS173679	Oromiya	Mirab Wellega
386	JUS173666	Oromiya	Mirab Harerge	410	JUS173645	Oromiya	Jimma
387	JUS173735	DDP	Dire Dawa	411	JUS173632	Gambella	Zone 1
388	JUS173657	Oromiya	Mirab Wellega	412	JUS173659	SNNP	Bench Maji

389	JUS173702	Oromiya	Misrak Harerge	413	JUS173704	Amara	Debub Wello
390	JUS173734	Oromiya	Misrak Harerge	414	JUS173729	Oromiya	Misrak Harerge
391	JUS173705	Amara	Debub Wello	415	JUS173696	Amara	Debub Wello
392	JUS173701	DDP	Dire Dawa	416	JUS171171	Oromiya	Mirab Harerge
393	JUS173712	Oromiya	Mirab Harerge	417	JUS173714	Amara	Debub Wello
394	JUS173759	Somali	Jigjiga	418	JUS173755	Somali	Jigjiga
395	JUS173764	Somali	Jigjiga	419	JUS173782	Amara	Debub Wello
396	JUS173677	Oromiya	Mirab Harerge	420	JUS173648	Amara	Semen Wello
397	JUS173671	Amara	Semen Wello	421	JUS173639	Oromiya	Mirab Harerge
398	JUS173646	SNNP	Bench Maji	422	JUS173644	Amara	Debub Wello
399	JUS173633	Oromiya	Mirab Harerge	423	JUS173652	Gambella	Zone 2
400	JUS173658	Oromiya	Mirab Shewa	424	JUS173660	Oromiya	Mirab Harerge
401	JUS173726	Oromiya	Misrak Harerge	425	JUS173706	Somali	Jigjiga
402	JUS173733	Amara	Debub Wello	426	JUS173721	Amara	Debub Wello
403	JUS173703	Oromiya	Misrak Harerge	427	JUS173697	Oromiya	Misrak Harerge
404	JUS173694	SNNP	Bench Maji	428	JUS173692	Dire Dawa provisiona	Dire Dawa
405	JUS173713	Amara	Oromiya	429	JUS173715	Amara	Semen Wello
406	JUS173754	Benishangul Gumuz	Metekel	430	JUS173756	Tigray	Mirabawi
407	JUS173765	Benishangul Gumuz	Metekel	431	JUS173792	Oromiya	Jimma
408	JUS173664	Amara	Debub Wello	432	JUS173650	Tigray	Mehakelegnaw

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
433	JUS173638	Oromiya	Jimma	457	JUS173641	Tigray	Debubawi
434	JUS173643	Unknown	Unknown	458	JUS173655	Somali	Jigjiga
435	JUS173653	Amara	Debub Wello	459	JUS173719	DDP	Dire Dawa
436	JUS173661	SNNP	Bench Maji	460	JUS173711	Oromiya	Mirab Harerge
437	JUS173724	DDP	Dire Dawa	461	JUS173709	Amara	Debub Wello

438	JUS173744	Amara	Debub Wello	462	JUS173699	DDP	Dire Dawa
439	JUS173687	Amara	Semen Wello	463	JUS173718	Somali	Jigjiga
440	JUS173700	Oromiya	Misrak Harerge	464	JUS173758	Somali	Jigjiga
441	JUS173716	Oromiya	Misrak Harerge	465	JUS161337	SNNP	Hadiya
442	JUS173757	DDP	Dire Dawa	466	JUS173670	Gambella	Zone 1
443	JUS173767	Amara	Semen Wello	467	JUS173634	Oromiya	Jimma
444	JUS173649	SNNP	Bench Maji	468	JUS173640	Amara	Debub Wello
445	JUS173637	Amara	Oromiya	469	JUS173656	Amara	Oromiya
446	JUS173642	Gambella	Zone 1	470	JUS173732	Amara	Debub Wello
447	JUS173654	Amara	Semen Wello	471	JUS173688	Somali	Jigjiga
448	JUS173662	Amara	Semen Wello	472	JUS173153	Oromiya	Illubabor
449	JUS173723	Amara	Semen Wello	473	JUS173823	Gambella	Zone 1
450	JUS173710	SNNP	Bench Maji	474	JUS173817	Oromiya	Misrak Harerge
451	JUS173698	Oromiya	Mirab Harerge	475	JUS173802	Somali	Jigjiga
452	JUS173717	Amara	Semen Wello	476	JUS173807	Amara	Debub Wello
453	JUS173760	Benishangul Gumuz	Metekel	477	JUS173789	Tigray	Mehakelegnaw
454	JUS173768	Somali	Jigjiga	478	JUS173780	Oromiya	Illubabor
455	JUS173668	Oromiya	Illubabor	479	JUS173777	Oromiya	Misrak Harerge
456	JUS173636	Oromiya	Mirab Harerge	480	JUS173174	Tigray	Mehakelegnaw

Appendix 1. (*Continued*)

		Place of Collection				**Place of Collection**	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
481	JUS173856	Oromiya	Mirab Shewa	505	JUS173169	Oromiya	Mirab Shewa
482	JUS173149	Oromiya	Mirab Shewa	506	JUS173161	Tigray	Mirabawi
483	JUS173691	Amara	Semen Wello	507	JUS173689	Oromiya	Misrak Harerge
484	JUS173152	Oromiya	Mirab Shewa	508	JUS173158	Oromiya	Jimma
485	JUS173881	Oromiya	Jimma	509	JUS173829	Amara	Semen Wello
486	JUS173819	Amara	Debub Wello	510	JUS173831	Tigray	Mehakelegnaw

487	JUS173799	Amara	Debub Wello	511	JUS173801	Somali	Jigjiga
488	JUS173809	Somali	Jigjiga	512	JUS173811	Amara	Debub Wello
489	JUS173790	Oromiya	Misrak Harerge	513	JUS173885	Unknown	Unknown
490	JUS173770	Amara	Debub Wello	514	JUS173772	Oromiya	Illubabor
491	JUS173778	Tigray	Mehakelegnaw	515	JUS173781	Somali	Jigjiga
492	JUS173175	SNNP	Bench Maji	516	JUS173147	Oromiya	Mirab Shewa
493	JUS173155	Oromiya	Mirab Shewa	517	JUS173170	DDP	Dire Dawa
494	JUS173159	Oromiya	Mirab Shewa	518	JUS173160	Tigray	Mirabawi
495	JUS173690	DDP	Dire Dawa	519	JUS173797	Amara	Semen Shewa
496	JUS173151	Oromiya	Illubabor	520	JUS173150	DDP	Dire Dawa
497	JUS173826	Oromiya	Mirab Harerge	521	JUS173830	Tigray	Debubawi
498	JUS173820	Amara	Semen Wello	522	JUS173832	Oromiya	Jimma
499	JUS173800	DDP	Dire Dawa	523	JUS173803	Benishangul Gumuz	Metekel
500	JUS173810	Oromiya	Mirab Harerge	524	JUS173784	Oromiya	Jimma
501	JUS173791	DDP	Dire Dawa	525	JUS173793	Benishangul Gumuz	Metekel
502	JUS173771	Oromiya	Illubabor	526	JUS173773	Somali	Jigjiga
503	JUS173779	Somali	Jigjiga	527	JUS173191	Oromiya	Mirab Shewa
504	JUS173146	Tigray	Mehakelegnaw	528	JUS173148	DDP	Dire Dawa

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
529	JUS173171	Tigray	Debubawi	553	JUS173165	SNNP	Bench Maji
530	JUS173162	Tigray	Mehakelegnaw	554	JUS173763	Somali	Jigjiga
531	JUS173798	Amara	Semen Wello	555	JUS173824	Amara	Semen Wello
532	JUS161340	SNNP	Semen Omo	556	JUS173816	Tigray	Debubawi
533	JUS173814	Amara	Semen Wello	557	JUS173806	Benishangul Gumuz	Metekel
534	JUS173833	Gambella	Zone 1	558	JUS173788	DDP	Dire Dawa
535	JUS173804	Amara	Debub Wello	559	JUS173796	Oromiya	Illubabor

536	JUS173786	Oromiya	Illubabor	560	JUS173776	Somali	Jigjiga
537	JUS173794	Oromiya	Jimma	561	JUS173183	DDP	Dire Dawa
538	JUS173774	Benishangul Gumuz	Metekel	562	JUS173185	Tigray	Mehakelegnaw
539	JUS173190	Oromiya	Misrak Shewa	563	JUS173163	DDP	Dire Dawa
540	JUS173144	Tigray	Mirabawi	564	JUS173157	Oromiya	Mirab Shewa
541	JUS173172	Tigray	Mehakelegnaw	565	JUS173834	Oromiya	Jimma
542	JUS173164	Oromiya	Illubabor	566	JUS173143	Oromiya	Mirab Shewa
543	JUS173785	Amara	Oromiya	567	JUS173142	Tigray	Mirabawi
544	JUS173156	DDP	Dire Dawa	568	JUS171565	SNNP	Debub Omo
545	JUS173815	Oromiya	Jimma	569	JUS173129	Amara	Debub Wello
546	JUS173805	Somali	Jigjiga	570	JUS173098	Amara	Debub Wello
547	JUS173787	DDP	Dire Dawa	571	JUS161338	SNNP	Semen Omo
548	JUS173795	Oromiya	Mirab Harerge	572	JUS173113	Unknown	Unknown
549	JUS173775	Oromiya	Illubabor	573	JUS173840	Oromiya	Mirab Shewa
550	JUS173177	Oromiya	Misrak Shewa	574	JUS173186	DDP	Dire Dawa
551	JUS173145	DDP	Dire Dawa	575	JUS173184	DDP	Dire Dawa
552	JUS173166	DDP	Dire Dawa	576	JUS173167	Amara	Oromiya

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
577	JUS173835	Oromiya	Jimma	601	JUS173124	SNNP	Bench Maji
578	JUS173675	Amara	Oromiya	602	JUS173647	Gambella	Zone 1
579	JUS171425	SNNP	Hadiya	603	JUS171577	SNNP	Semen Omo
580	JUS171576	SNNP	Kembata Alabana Temb	604	JUS171116	Amara	Mirab Gojam
581	JUS173126	SNNP	Bench Maji	605	JUS173105	SNNP	Bench Maji
582	JUS173106	SNNP	Bench Maji	606	JUS171569	Oromiya	Arssi
583	JUS161335	SNNP	Debub Omo	607	JUS173121	Amara	Semen Wello
584	JUS173114	Oromiya	Misrak Harerge	608	JUS173111	Unknown	Unknown

585	JUS173841	Oromiya	Mirab Shewa	609	JUS173822	Oromiya	Jimma
586	JUS173187	DDP	Dire Dawa	610	JUS173179	Oromiya	Mirab Shewa
587	JUS173154	DDP	Dire Dawa	611	JUS173173	Tigray	Mirabawi
588	JUS173218	Amara	Debub Wello	611	JUS173173	Tigray	Mirabawi
589	JUS173116	Oromiya	Illubabor	612	JUS173095	Oromiya	Mirab Harerge
590	JUS173651	Tigray	Mehakelegnaw	613	JUS173117	Tigray	Debubawi
591	JUS171595	SNNP	Keficho Shekicho	614	JUS173676	Amara	Debub Wello
592	JUS171029	SNNP	Debub Omo	615	JUS171600	SNNP	Semen Omo
593	JUS173118	Oromiya	Illubabor	616	JUS171602	SNNP	Semen Omo
594	JUS173099	SNNP	Bench Maji	617	JUS173103	SNNP	Bench Maji
595	JUS173122	Amara	Semen Wello	618	JUS173808	Amara	Oromiya
596	JUS173112	SNNP	Bench Maji	619	JUS173110	SNNP	Bench Maji
597	JUS173821	Oromiya	Mirab Shewa	620	JUS173836	Tigray	Mehakelegnaw
598	JUS173188	Tigray	Mehakelegnaw	621	JUS173828	Gambella	Zone 1
599	JUS173180	Oromiya	Misrak Shewa	622	JUS173189	Tigray	Mirabawi
600	JUS173483	SNNP	Bench Maji	623	JUS173182	Unknown	Unknown

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
624	JUS173285	Oromiya	Illubabor	648	JUS173127	Oromiya	Illubabor
625	JUS173096	Amara	Debub Wello	649	JUS173203	Amara	Debub Wello
626	JUS173279	Gambella	Zone 1	650	JUS171424	SNNP	Hadiya
627	JUS171433	Amara	Bahir Dar Special	651	JUS173102	Tigray	Mehakelegnaw
628	JUS171089	SNNP	Semen Omo	652	JUS173100	Tigray	Mehakelegnaw
629	JUS173104	SNNP	Bench Maji	653	JUS173115	Oromiya	Illubabor
630	JUS173108	SNNP	Bench Maji	654	JUS173839	Oromiya	Mirab Shewa
631	JUS173120	Amara	Semen Wello	655	JUS173818	Amara	Semen Wello
632	JUS173837	Gambella	Zone 1	656	JUS173193	Tigray	Mehakelegnaw

633	JUS173813	Oromiya	Jimma	657	JUS173178	DDP	Dire Dawa
634	JUS173194	DDP	Dire Dawa	658	JUS171669	Tigray	Mehakelegnaw
635	JUS173181	Tigray	Mirabawi	659	JUS171016	Amara	Debub Wello
636	JUS173278	Tigray	Debubawi	660	JUS171017	Amara	Debub Wello
637	JUS173125	Tigray	Debubawi	661	JUS171323	Amara	Semen Shewa
638	JUS173761	Tigray	Mehakelegnaw	662	JUS171143	Oromiya	Mirab Harerge
639	JUS171511	SNNP	Gurage	663	JUS171077	Tigray	Mehakelegnaw
640	JUS173097	Oromiya	Misrak Harerge	664	JUS171106	Somali	Jigjiga
641	JUS173109	Amara	Semen Wello	665	JUS171429	Amara	Semen Shewa
642	JUS173119	Oromiya	Illubabor	666	JUS171250	Amara	Semen Wello
643	JUS173838	Oromiya	Misrak Harerge	667	JUS171319	Tigray	Debubawi
644	JUS173827	Oromiya	Mirab Shewa	668	JUS171462	Oromiya	Semen Shewa
645	JUS173195	Oromiya	Mirab Shewa	669	JUS171216	Oromiya	Mirab Harerge
646	JUS173192	Amara	Oromiya	670	JUS171176	Oromiya	Illubabor
647	JUS173593	Gambella	Zone 2	671	JUS171034	Gambella	Zone 1

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
672	JUS171510	Oromiya	Misrak Harerge	695	JUS171072	Oromiya	Misrak Harerge
673	JUS171613	Tigray	Mehakelegnaw	696	JUS171635	Tigray	Mirabawi
674	JUS171728	Tigray	Debubawi	697	JUS171580	SNNP	Bench Maji
675	JUS171651	Oromiya	Misrak Wellega	698	JUS171267	Somali	Jigjiga
676	JUS171030	Amara	Misrak Gojam	699	JUS171084	Oromiya	Misrak Harerge
677	JUS171351	Amara	Semen Shewa	700	JUS171126	Oromiya	Misrak Harerge
678	JUS171061	Gambella	Zone 1	701	JUS171421	SNNP	Hadiya
679	JUS171240	Affar	Zone 1	702	JUS171076	Oromiya	Mirab Harerge
680	JUS171183	Tigray	Mehakelegnaw	703	JUS171080	Oromiya	Misrak Shewa
681	JUS171528	Tigray	Misrakawi	704	JUS171181	Tigray	Mehakelegnaw

682	JUS171606	Amara	Misrak Gojam	705	JUS171440	SNNP	Hadiya
682	JUS171606	Amara	Misrak Gojam	706	JUS171454	Amara	Misrak Gojam
683	JUS171192	Gambella	Zone 1	707	JUS171019	Oromiya	Mirab Harerge
684	JUS171684	Unknown	Unknown	708	JUS171753	Amara	Semen Shewa
685	JUS171626	Oromiya	Misrak Shewa	709	JUS171653	Oromiya	Bale
686	JUS171740	Amara	Semen Gondar	710	JUS171135	Oromiya	Misrak Harerge
687	JUS171673	Amara	Semen Gondar	711	JUS171117	Oromiya	Misrak Harerge
688	JUS171161	Tigray	Debubawi	712	JUS171794	Oromiya	Misrak Harerge
689	JUS171056	SNNP	Bench Maji	713	JUS171049	Gambella	Zone 2
690	JUS161339	SNNP	Semen Omo	714	JUS171548	Tigray	Debubawi
691	JUS171107	Oromiya	Misrak Harerge	715	JUS171707	Tigray	Debubawi
692	JUS171121	Oromiya	Misrak Harerge	716	JUS171672	Tigray	Debubawi
693	JUS171297	Tigray	Mirabawi	717	JUS171386	Oromiya	Mirab Shewa
694	JUS171549	Tigray	Mehakelegnaw	718	JUS171791	SNNP	Bench Maji

Appendix 1. (*Continued*)

		Place of Collection				Place of Collection	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
719	JUS171810	Tigray	Mehakelegnaw	743	JUS171736	Tigray	Mirabawi
720	JUS171494	Gambella	Zone 1	744	JUS171088	Tigray	Misrakawi
721	JUS171219	Gambella	Zone 1	745	JUS171608	Amara	Bahir Dar Special
722	JUS171146	Oromiya	Misrak Harerge	746	JUS171170	Oromiya	Mirab Harerge
723	JUS171040	Gambella	Zone 1	747	JUS171361	Amara	Semen Shewa
724	JUS171265	Tigray	Mehakelegnaw	748	JUS171094	Oromiya	Misrak Harerge
725	JUS171432	Amara	Bahir Dar Special	749	JUS171575	Amara	Semen Gondar
726	JUS171773	Amara	Semen Gondar	750	JUS171139	Oromiya	Misrak Harerge
727	JUS171218	Oromiya	Jimma	751	JUS171705	Amara	Semen Shewa
728	JUS171332	Amara	Semen Shewa	752	JUS171366	Amara	Semen Shewa
729	JUS171003	Amara	Misrak Gojam	753	JUS173879	Oromiya	Jimma

730	JUS171458	Amara	Misrak Gojam	754	JUS171260	Amara	Debub Wello
731	JUS171588	Amara	Debub Gondar	755	JUS171199	Amara	Semen Gondar
732	JUS171255	Amara	Semen Wello	756	JUS171628	Oromiya	Misrak Shewa
733	JUS171809	Amara	Semen Gondar	757	JUS171207	Amara	Semen Gondar
734	JUS171008	Oromiya	Mirab Harerge	758	JUS171360	Amara	Semen Shewa
735	JUS171127	Oromiya	Misrak Harerge	759	JUS173394	Tigray	Debubawi
736	JUS171484	Oromiya	Misrak Shewa	760	JUS171018	Oromiya	Mirab Harerge
737	JUS171238	Amara	Debub Wello	761	JUS171763	Oromiya	Mirab Harerge
738	JUS171101	Oromiya	Misrak Harerge	762	JUS171379	Oromiya	Semen Shewa
739	JUS171737	Tigray	Mirabawi	763	JUS171717	SNNP	Gurage
740	JUS171559	Amara	Oromiya	764	JUS171747	Tigray	Debubawi
741	JUS171203	Amara	Semen Gondar	765	JUS173553	Amara	Semen Wello
742	JUS171167	Oromiya	Mirab Harerge	766	JUS171347	Amara	Semen Shewa

Appendix 1. (*Continued*)

		Place of Collection				Place of Collection	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
767	JUS171778	Amara	Semen Gondar	791	JUS171057	Amara	Semen Gondar
768	JUS171195	Gambella	Zone 1	792	JUS171322	Tigray	Mirabawi
769	JUS171776	Amara	Semen Gondar	793	JUS171706	Amara	Semen Shewa
770	JUS171453	Amara	Misrak Gojam	794	JUS171603	Amara	Debub Wello
771	JUS171035	Gambella	Zone 1	795	JUS171611	Oromiya	Mirab Harerge
772	JUS173861	Oromiya	Jimma	796	JUS173627	Oromiya	Mirab Harerge
773	JUS171450	Oromiya	Semen Shewa	797	JUS171132	Oromiya	Misrak Harerge
774	JUS171388	Oromiya	Mirab Shewa	798	JUS171487	Unknown	Unknown
775	JUS173392	Tigray	Mirabawi	799	JUS171295	Tigray	Mirabawi
776	JUS171296	Tigray	Mirabawi	800	JUS171664	Tigray	Mehakelegnaw
777	JUS171331	Amara	Semen Shewa	801	JUS171231	Amara	Debub Wello
778	JUS171335	Amara	Semen Shewa	802	JUS171530	Amara	Semen Gondar

List	Genotypes	Region	Zone	List	Genotypes	Region	Zone
779	JUS171594	Amara	Debub Gondar	803	JUS171050	Gambella	Zone 2
780	JUS171293	Tigray	Mirabawi	804	JUS171642	Oromiya	Borena
781	JUS173138	Amara	Debub Wello	805	JUS171208	Amara	Misrak Gojam
782	JUS171566	Amara	Misrak Gojam	806	JUS171632	Amara	Semen Gondar
783	JUS171169	Oromiya	Mirab Harerge	807	JUS171489	Oromiya	Mirab Harerge
784	JUS171724	Oromiya	Misrak Wellega	808	JUS171217	Oromiya	Mirab Harerge
785	JUS171096	Oromiya	Misrak Harerge	809	JUS171397	Oromiya	Arssi
786	JUS171443	Oromiya	Misrak Shewa	810	JUS171504	Oromiya	Misrak Shewa
787	JUS173261	Amara	Debub Wello	811	JUS171284	Tigray	Mirabawi
788	JUS171266	Tigray	Mehakelegnaw	812	JUS171716	Oromiya	Mirab Shewa
789	JUS171229	Amara	Debub Wello	813	JUS171631	Amara	Debub Wello
790	JUS171772	Amara	Semen Gondar	814	JUS171224	Amara	Debub Wello

Appendix 1. (*Continued*)

		Place of Collection				Place of Collection	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
815	JUS171190	Gambella	Zone 1	839	JUS171350	Amara	Semen Shewa
816	JUS173490	Oromiya	Mirab Harerge	840	JUS171755	Amara	Debub Wello
817	JUS171226	Amara	Debub Wello	841	JUS171444	Oromiya	Misrak Shewa
818	JUS171633	Amara	Bahir Dar Special	842	JUS171557	Oromiya	Mirab Harerge
819	JUS171436	Oromiya	Misrak Shewa	843	JUS171221	Oromiya	Misrak Harerge
820	JUS171095	Oromiya	Misrak Harerge	844	JUS171420	SNNP	Hadiya
821	JUS171093	Oromiya	Misrak Harerge	845	JUS171162	Tigray	Debubawi
822	JUS171555	Oromiya	Mirab Harerge	846	JUS171340	Amara	Semen Shewa
823	JUS171585	Tigray	Debubawi	847	JUS173090	Amara	Semen Wello
824	JUS171202	Amara	Semen Gondar	848	JUS173091	Tigray	Debubawi
825	JUS171644	Amara	Semen Gondar	849	JUS173086	Oromiya	Mirab Shewa
826	JUS171330	Amara	Semen Shewa	850	JUS173043	SNNP	Bench Maji
827	JUS171038	Gambella	Zone 1	851	JUS173044	Benishangul Gumuz	Metekel

List	Genotypes	Region	Zone	List	Genotypes	Region	Zone
828	JUS171341	Amara	Semen Shewa	852	JUS173042	Benishangul Gumuz	Metekel
829	JUS171696	Amara	Debub Wello	853	JUS173045	Amara	Oromiya
830	JUS171173	Oromiya	Mirab Harerge	854	JUS173046	Amara	Semen Wello
831	JUS171726	Oromiya	Misrak Wellega	855	JUS173047	Somali	Shinile
832	JUS171134	Oromiya	Semen Shewa	856	JUS173048	Amara	Debub Wello
833	JUS171765	Oromiya	Mirab Harerge	857	JUS173033	Amara	Semen Wello
834	JUS173682	Oromiya	Mirab Harerge	858	JUS173034	Amara	Debub Wello
835	JUS171346	Amara	Semen Shewa	859	JUS173035	Oromiya	Mirab Harerge
836	JUS171026	Amara	Misrak Gojam	860	JUS173085	Oromiya	Misrak Harerge
837	JUS171774	Amara	Semen Gondar	861	JUS173036	Oromiya	Mirab Wellega
838	JUS171193	Gambella	Zone 1	862	JUS173037	Gambella	Zone 1

Appendix 1. (*Continued*)

		Place of Collection				Place of Collection	
List	**Genotypes**	**Region**	**Zone**	**List**	**Genotypes**	**Region**	**Zone**
863	JUS173087	Gambella	Zone 2	887	JUS173006	Affar	Zone 1
864	JUS173038	SNNP	Bench Maji	888	JUS173001	Amara	Semen Wello
865	JUS173039	Oromiya	Mirab Shewa	889	JUS173002	Tigray	Mirabawi
866	JUS173040	Oromiya	Mirab Wellega	890	JUS173076	Amara	Semen Wello
867	JUS173012	Oromiya	Illubabor	891	JUS173075	Tigray	Debubawi
868	JUS173084	Oromiya	Illubabor	892	JUS173077	Oromiya	Mirab Shewa
869	JUS173013	Oromiya	Jimma	893	JUS173088	Oromiya	Misrak Harerge
870	JUS173014	Tigray	Mehakelegnaw	894	JUS173089	Somali	Jigjiga
871	JUS173016	Oromiya	Mirab Wellega	895	JUS173056	Oromiya	Illubabor
872	JUS173017	Oromiya	Jimma	896	JUS173054	Tigray	Debubawi
873	JUS173015	Oromiya	Mirab Harerge	897	JUS173021	Oromiya	Mirab Harerge
874	JUS173018	Oromiya	Misrak Harerge	898	JUS173057	Amara	Debub Wello
875	JUS173019	Somali	Shinile	899	JUS173022	Oromiya	Jimma
876	JUS173020	Oromiya	Mirab Wellega	900	JUS173055	Amara	Debub Wello

877	JUS173007	DDP	Dire Dawa	901	JUS173053	Gambella	Zone 2
878	JUS173008	Oromiya	Mirab Wellega	902	JUS173031	Oromiya	Jimma
879	JUS173092	Oromiya	Mirab Wellega	903	JUS173071	Oromiya	Jimma
880	JUS173009	Tigray	Mirabawi	904	JUS173049	Tigray	Debubawi
881	JUS173010	Tigray	Debubawi	905	JUS173032	Amara	Debub Wello
882	JUS173011	Oromiya	Illubabor	906	JUS173070	Oromiya	Jimma
883	JUS173083	Gambella	Zone 1	907	JUS173072	Oromiya	Jimma
884	JUS173003	Amara	Semen Gondar	908	JUS173051	Benishangul Gumuz	Metekel
885	JUS173004	Amara	Debub Wello	909	JUS173074	Oromiya	Misrak Harerge
886	JUS173005	Amara	Debub Wello	910	JUS173068	Oromiya	Illubabor

Appendix 1. (*Continued*)

List	Genotypes	Place of Collection		List	Genotypes	Place of Collection	
		Region	Zone			Region	Zone
911	JUS173073	Benishangul Gumuz	Metekel	935	JUS173094	Oromiya	Misrak Harerge
912	JUS173052	Oromiya	Misrak Harerge	936	JUS173080	Amara	Semen Wello
913	JUS173050	Tigray	Mehakelegnaw	937	JUS173079	Amara	Debub Wello
914	JUS173041	Tigray	Mehakelegnaw	938	JUS173082	Oromiya	Misrak Harerge
915	JUS173027	Tigray	Debubawi	939	JUS173081	Amara	Debub Wello
916	JUS173059	Tigray	Debubawi	940	JUS173078	Oromiya	Illubabor
917	JUS173024	Oromiya	Mirab Shewa	941	ICSR50	Realesed variety	
918	JUS173025	Amara	Semen Wello	942	S35	Realesed variety	
919	JUS173023	Tigray	Mirabawi	943	Gobiye	Realesed variety	
920	JUS173093	Oromiya	Misrak Shewa	944	ETS_2752	Realesed variety	
921	JUS173061	Oromiya	Mirab Shewa	945	Dagnechew	Local check	
922	JUS173060	Oromiya	Mirab Shewa				
923	JUS173065	Oromiya	Mirab Shewa				
924	JUS173062	Tigray	Mehakelegnaw				
925	JUS173026	Tigray	Mehakelegnaw				
926	JUS173058	DDP	Dire Dawa				

927	JUS173063	Amara	Debub Wello
928	JUS173028	Amara	Semen Wello
929	JUS173067	Oromiya	Mirab Harerge
930	JUS173066	Oromiya	Mirab Harerge
931	JUS173030	Oromiya	Mirab Harerge
932	JUS173069	Oromiya	Mirab Harerge
933	JUS173064	Oromiya	Misrak Harerge
934	JUS173029	Oromiya	Misrak Harerge

Table 11Appendix Table 2.Correlation among traits of sorghum landraces. The correlation coefficient was computed using the mean values of the traits for each of 945 landraces

	SD	PAL	PAW	SPADB	SPADM	LFN	LA	PHT	LAF	TILLER	ETN	TLV	FLAG	FLOW	MATU	HSW	GYPUA
SD	1																
PAL	-0.13*	1															
PAW	-0.23**	0.5**	1														
SPADB	-0.32**	0.18*	0.34**	1													
SPADM	0.32**	0.01	-0.1*	-0.22**	1												
LFN	0.76**	-0.26**	-0.38**	-0.52**	0.34**	1											
LA	-0.22*	0.11	0.18	0.198*	-0.06	-0.14**	1										
PHT	0.47**	0.01	0.22*	-0.25**	0.18*	0.57**	0.08	1									
LAF	0.13**	0.12	0.18	0.21	-0.13	-0.16	-0.03	-0.11*	1								
TILLER	-0.25*	0.22	0.15	0.07	0.03	-0.19*	0.01	0.02	-0.01	1							
ETN	-0.27**	0.29*	0.19*	0.26**	-0.07	-0.33**	0.12	-0.1*	0.01	0.76**	1						
TLV	0.30*	-0.11*	-0.07*	-0.13*	0.15	0.34**	-0.04	0.35*	0.1	-0.1	-0.15*	1					
FLAG	0.51**	-0.16*	-0.33**	-0.57**	0.37**	0.72**	-0.16*	0.35**	-0.08	-0.13	-0.31**	0.26*	1				
FLOW	0.50**	-0.14*	-0.34**	-0.55**	0.34**	0.70**	-0.15*	0.29**	-0.08	-0.11	-0.28**	0.25*	0.93**	1			
MATU	0.47**	-0.12*	-0.3**	-0.51**	0.31**	0.66**	-0.14*	0.32**	-0.06	-0.09	-0.27**	0.31*	0.91**	0.97**	1		
HSW	-0.18*	-0.11	-0.03	0.29**	-0.27**	-0.21*	0.16	-0.09*	0.05	-0.17	-0.01	-0.06	-0.37**	-0.37**	-0.38**	1	
GYPUA	-0.14**	0.07	0.32*	0.36**	-0.18**	-0.19**	0.09	0.04	0.19**	0.13	0.29*	0.03	-0.31**	-0.33**	-0.33**	0.31**	1

Notes: @ Quantitative traits abbreviations as indicated in Table 2

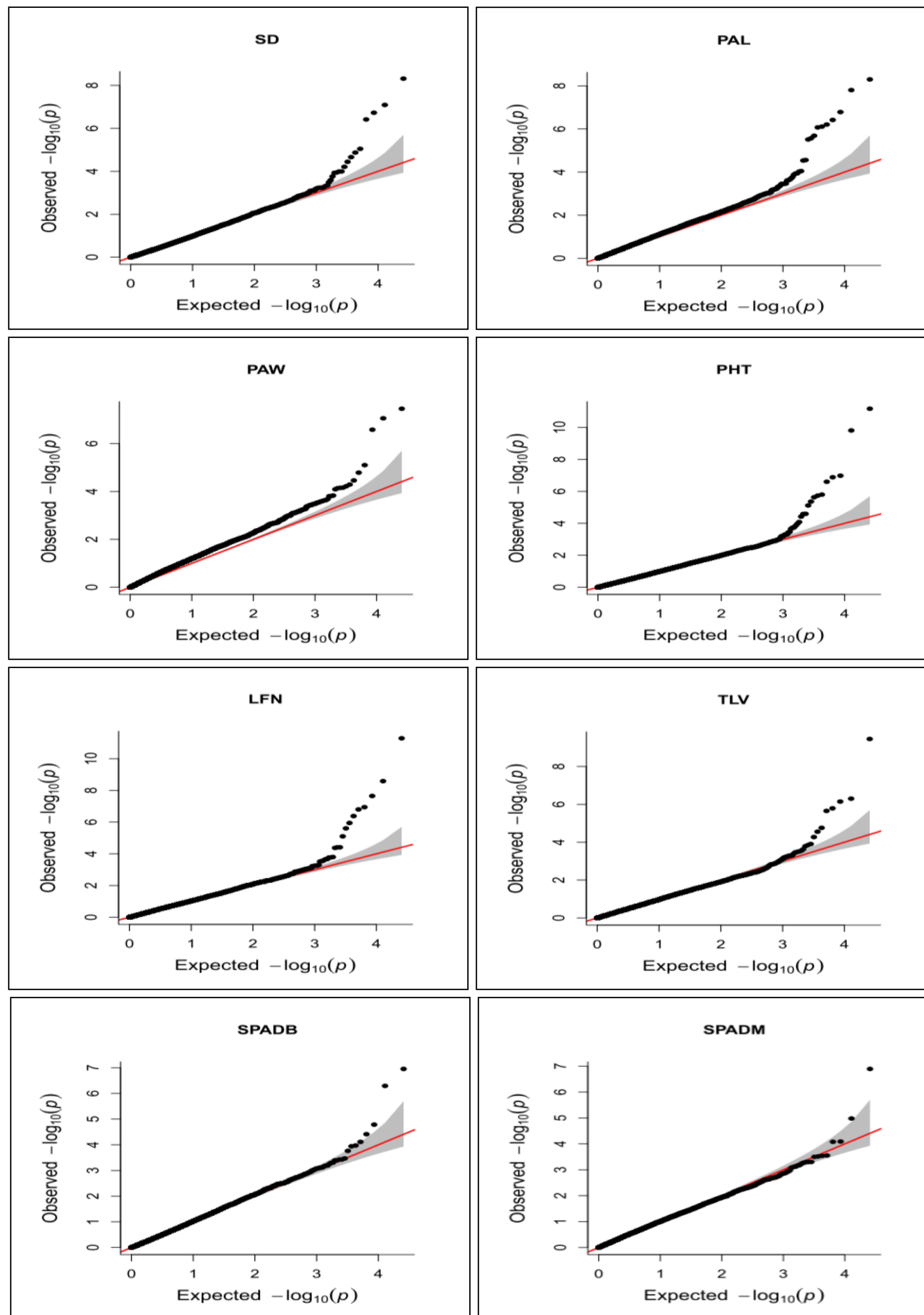
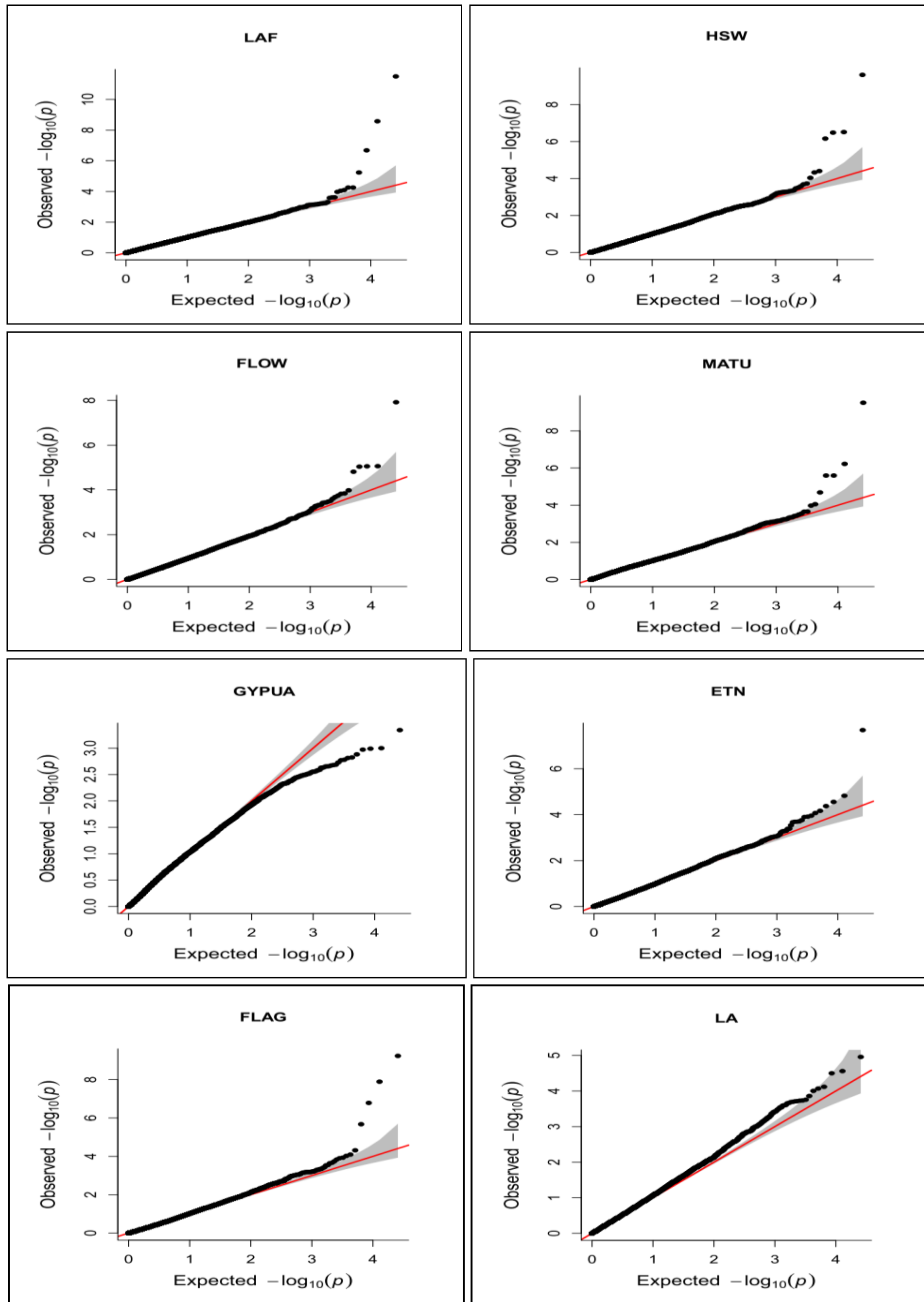


Figure 10Appendix Figure1. Quantile-Quantile (QQ) plot used to evaluate the performance of the mixed linear model used for of GWAS for the 17 quantitative traits using mixed linear model (MLM). Notes: @ Quantitative traits abbreviations as indicated in Table 2



Appendix 1.(*Continued*)

Appendix B. Plant DNA extraction protocol for Diversity Arrays Technology (DArT)

Plant DNA Extraction Protocol for DArT

BUFFER STOCK SOLUTIONS

EXTRACTION BUFFER STOCK

To make 500 ml:

0.35 M sorbitol	31.9 g sorbitol
0.1 M TrisHCl pH 8.0	50 ml 1M TrisHCl pH 8.0
5 mM EDTA pH 8.0	5 ml 0.5 M EDTA pH 8.0 fill up to 500 ml MiliQ H_2O

LYSIS BUFFER

STOCK To make 500 ml:

0.2 M Tris HCl pH 8.0	100 ml 1M Tri HCl pH 8.0
0.05 M EDTA pH 8.0	50 ml 0.5 M EDTA pH 8.0
2M NaCl	200 ml 5 M NaCl
2% CTAB	10 g CTAB
	fill up to 500 ml with MilliQ H_20

SARCOSYL STOCK 5% (w/v)

FRESH BUFFER WORKING SOLUTION*:

0.5 % (w/v) sodiumdisulfite (= sodium metabisulfite)

2 % (w/v) PVP-40 (K29-32) Sigmadissolve in required volume of extraction buffer stock; add same volume of lysis buffer stock and 0.4 volume of extraction (=lysis) buffer stock of sarcosyl stock.

For example to make 120 ml:

Add 0.6 g sodiumdisulfite (= sodium metabisulfite) and 2.4 g PVP-40 (K29-32) to 50 ml extraction buffer stock and dissolve; add 50 ml lysis buffer stock and 20 ml sarcosyl stock

For example to make 30 ml:

Add 0.15 g sodiumdisulfite (= sodium metabisulfite) and 0.6 g PVP-40 (K29-32) to 12.5 ml extraction buffer stock and dissolve; add 12.5 ml lysis buffer stock and 5 ml sarcosyl stock

*This buffer may settle into two layers on standing. Heat to 65ºC and shake immediately before adding to extraction tubes.

PROTOCOL

For 15 ml Sarsted tubes:

- aliquot 6 ml of freshly prepared preheated to 65ºC well mixed “fresh buffer solution” and place tubes to the 65ºC incubator or water bath, (3, 4 days old “fresh buffer solution” works fine),
- grind required amount (same across all samples) of plant material in mortar and pestle under liquid nitrogen to fine powder,

- suspend powder in 6 ml “fresh buffer solution” kept at 65ºC (make sure there are no clumps, vortex if necessary),
- incubate at 65ºC for 1 h (can extend for another 30 min), invert tubes in every 20 minutes or incubate with gentle shaking,
- cool down for 5 min and add 6 ml of chloroform : isoamyl alcohol (24 : 1) mixture,
- mix well for 30 min,
- spin 20 min, 3000 x g, RT,
- transfer water phase to fresh tube, add same volume of ice cold isopropanol and invert tube ~ 10 times, nucleic acids should become visible,
- spin 30 min, 3000 x g, RT,
- discard supernatant, wash pellet with 2 ml 70 % EtOH,
- discard EtOH, dry pellet and dissolve in 200 µl – 500 µl 1 x TE (10 mM TrisHCl pH 8.0, 1 mM EDTA pH 8.0),
- check DNA quality and quantity on 0.8 % agarose gel. (If RNA quantity is several fold less thanDNA, RNase treatment is not necessary for DArT applications).

For 2 ml Eppendorf tubes:

- aliquot 1 ml of freshly prepared preheated to 65ºC, well mixed “fresh buffer solution” and place tubes to the 65ºC incubator or water bath, (3, 4 days old “fresh buffer solution” works fine),
- grind required amount (same across all samples) of plant material in mortar and pestle under liquid nitrogen to fine powder,
- suspend powder in 1 ml “fresh buffer solution” kept at 65ºC (make sure there are no clumps, vortex if necessary),
- incubate at 65ºC for 1 h (can extend for another 30 min), invert tubes in every 20 minutes or incubate with gentle shaking,
- cool down for 5 min and add 1 ml of chloroform : isoamyl alcohol (24 : 1) mixture,
- mix well for 30 min,
- spin 20 min, 10000 x g, RT,
- transfer water phase to fresh tube, add same volume of ice cold isopropanol and invert tube ~ 10 times, nucleic acids should become visible,
- spin 30 min, 10000 x g, RT,
- discard supernatant, wash pellet with 2 ml 70 % EtOH,
- discard EtOH, dry pellet and dissolve in 250 µl of 1 x TE (10 mM TrisHCl pH 8.0, 1 mM EDTA pH8.0),
- check DNA quality and quantity on 0.8 % agarose gel. (If RNA quantity is several fold less thanDNA, RNase treatment is not necessary for DArT applications).