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Journal of Plant Breeding and Crop Science

Review

Resistance Mechanisms of Tomato (*Solanum lycopersicum)* to Root-Knot Nematodes (*Meloidogyne* species)

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Root-knot nematodes (Meloidogyne species) are the most devastating and as such they cause significant yield loss in tomato production. They are widely distributed in major tomato producing areas especially in warm climatic conditions. Because of the environmental impact of the application of pesticides, the use of nematode resistant varieties is becoming the most effective alternative to control root-knot nematodes. Several resistance genes are identified from wild tomato and other species. However, Mi-1 resistance gene is the only well characterized and used in many commercial tomato cultivars. A single dominant gene (Mi-1) with a hypersensitive response (HR), which is characterized by a local cell death at the site of nematode penetration and necrotic lesions of the surrounding tissue controls the resistance. Thus, Mi-1 gene either inhibits the penetration of second juvenile stage (J2), reduces number of gall formation, or reduces further development and reproduction rate of the nematode. However, the gene is a temperature dependent and broken by the virulent pathotypes. Plant growth hormones such as salicylic acid (SA) and jasmonic acid (JA) are involved in induced resistance, which is activated after infection. Secondary plant metabolites including amino acids, phenols, and lipophilic molecules were increased in resistant varieties as defense mechanism. The durability of the Mi-gene is a major concern since the resistance lost at high temperature. Heat stable resistance gene (Mi-9) is identified from Solannum arcanum. Hence, pyramiding of the resistance genes in commercial cultivars and genetic modification of plant metabolites might improve the durability of the gene.

Key words: Solanum lycopersicum, Meloidogyne species, Mi-gene, hypersensitive response (HR), induced resistance.

INTRODUCTION

Tomato (Solanum lycopersicum) belongs to Solonacea family and is one of the major vegetable crops in the world. Tomato is produced for the fresh market and processing. It is a major source of minerals, vitamins, and provides health benefits in human consumption (Robertson and Labate, 2007). Tomato ranks fourth among the leading vegetable crops in the world. According to FAO (2013), China, United States, Turkey,

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India, and Egypt are the top five leading tomato producing countries.

Despite its economic value, various pests and diseases influence the productivity of tomato. Plant-parasitic nematodes are soil living microscopic roundworms that affect root system of host plants. Among plant parasitic nematodes, root-knot nematodes (RKN) *Meloidogyne* species: *Meloidogyne incognita, Meloidogyne javanica* and *Meloidogyne arenaria* cause severe damage in vegetable crops including tomato (Sikora and Fernandez, 2005). They are widely distributed in many areas of tomato production field. In a field survey conducted on 25 tomato growing areas of Pakistan, 88% of the sites were infested with root-knot nematodes (Kamran et al., 2010). Of which 52.8% of the infestation was mainly *M. incognita* alone or in combination with *M. javanica*. Another survey conducted in 8 different localities tomato field in India showed that the crop is infected with root-knot nematodes in all 8 localities (Esfahani, 2012). In these areas, *M. javanica* and *M. incognita* were identified occurring either singly or in mixed population. They are potential risk in sandy soils and warm climatic conditions (Greco and Di Vito, 2011). RNK have wide host range and can survive in the soil as eggs or infective second juvenile stage for a long period of time in the absence of suitable host. During infection, the root-knot nematodes form typical symptom of gall, which varies from 1mm up to 2 cm in diameter. Thus, the gall disrupts the normal functioning of xylem tissue of the host plant. The size of the gall depends on age of infection, number of nematodes inside the gall, species of nematodes and host plants (Greco and Di Vito, 2010). The level of crop losses highly depends on the initial population of the nematodes, susceptibility of the crop, age of the plant, cropping sequence, and involvement of other pathogens (Anamika et al., 2011). In susceptible tomato varieties, root-knot nematodes cause delay in flowering, reduction in fresh and dry weight of roots, stems, and leaves; and reduction in fresh fruit yield (Udo et al., 2008). Moreover, seed production per fruit is dramatically reduced. No seed per fruit might occur if the infection is very high (Corbett et al., 2011).

The use of resistant tomato varieties is the most effective, economically feasible, and environmentally method to manage RKN (Sorribas et al., 2005). Due to environmental impact of application of chemical, resistant cultivars are currently the best alternatives for controlling RKN. It is also recommended that resistant cultivars to be used as an integrated management of RKN. In a study conducted for four cropping seasons, crop rotation with resistant tomato reduced the nematode population by 90% and on average high yield has obtained when the resistant tomato cultivated for two consecutive years (Talavera et al., 2009). Resistance genes to RKN are identified from wild species of tomato and other plant species. However, in tomato, a single dominant (*Mi-1*) resistance gene is well known resistance gene to RKN and major insect pests such as white flies and aphids (Casteel et al., 2006). The *Mi-1* gene is used in many breeding programs to develop high yielding hybrid and root-knot nematode resistant tomato varieties (Shrestha et al., 2012).

Several researches have been conducted to investigate how RNK react with tomato and the mechanism by which resistant tomato respond to RKN. Reviewing and discussing scientific papers is indispensable to identify research gaps and propose research areas. The objective of this review paper is, therefore, to briefly review and summarize research progresses on resistance mechanisms of tomato to root-knot nematodes. For this review, different research papers (not older than 2005) are searched from Google scholar and online literature database using the key words ′root-knot nematodes′, ′tomato and root-knot nematodes′, and ‘root-knot nematodes and resistance gene′. In the first aspect of this review, the major resistance gene (*Mi-1*), its resistance mechanisms against RNK and inhibitory effect on nematode penetration are described. In the second aspect, induced resistance, the role of plant hormones in induced resistance to RKN, and the involvement of plant secondary metabolites in defense response of tomato to RKN are discussed. Lastly, the main points of the review and future remarks are pointed out

TOMATO RESISTANCE TO ROOT-KNOT NEMATODES (RKN)

Major RKN resistance gene

Though several genes are identified from wild tomato relative (*Lycopersicon peruvianum*) and other *Solenaceaous* species such as pepper, a single dominant gene (*Mi-1*) has been used for a long period as the only source of resistance to RKN in commercial tomato cultivars. Moreover, Mi-1 is the best-characterized RKN resistance gene. The resistance is mediated by the presence of single dominant Mi-gene with a hypersensitive response (HR). The *Mi-1* gene confers resistance to the three major *Meloidogyne* spp.: *M. incognita, M. arenaria,* and *M. javanica* (Branch et al., 2004). However, the effectiveness of the *Mi-1* gene is temperature dependent. The gene loses its expression at soil temperature of 32°C, which results in high infection by RKN (Zinovieva et al., 2013b). Heat stable resistance gene (*Mi-9*) which is a homolog of *Mi-1* has been identified from *Solanum arcanum* (Jablonska et al., 2007). Besides to temperature sensitivity, virulent populations of *Meloidogyne* spp. have also broken the *Mi-1* gene. In a study conducted to compare the response of susceptible and resistant tomato cultivars to three populations of Tunisian *M. incognita*, the resistant cultivar carrying the *Mi-1* gene did not show

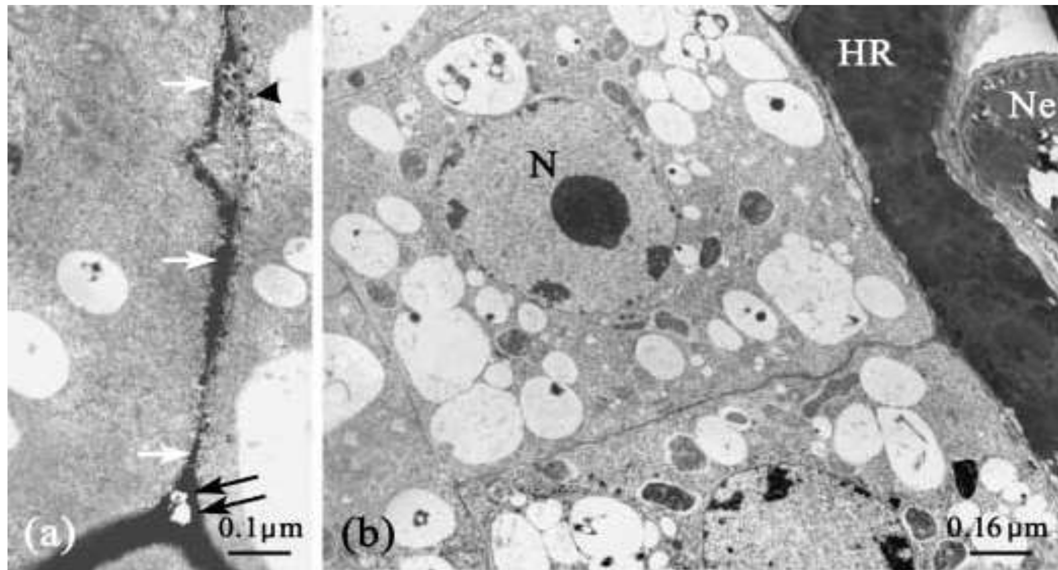

Figure 1. Subcellular localization of H_2O_2 production in resistant tomato roots infected with avirulent nematodes, as shown by electron microscopy. (a) Meristematic cells injured by the invading nematode (12 h after inoculation) show high accumulation of H_2O_2 on their plasma membrane (arrowhead), cell walls (white arrow), and intercellular spaces (double arrows). (b) Hypersensitive reaction of cells selected as feeding site by the nematode is represented by dark, dying cells (HR) at 24 h. Ne, Nematodes; N, nucleus; HR, hypersensitive reaction. Source: Melillo et al. (2006).

hypersensitive response (Regaieg and Horrigue-Raouani, 2012). Hence, J2 penetration and establishment feeding site with high giant cells was observed similar to the susceptible cultivar. The resistance is characterized by local cell death at the site of nematode penetration and necrotic lesions due to the accumulation of toxic substances (Melillo et al., 2006). The incompatible interaction between avirulent pathotype and resistant tomato varieties showed hypersensitive reaction at the site of nematode penetration (Figure 1b). Furthermore, significantly high level of reactive oxygen species (ROS) has been observed at a penetration site and adjacent cells when resistant tomato is infected with avirulent *M. incognita* pathotype (Figure 1a). High accumulation of hydrogen peroxide (H_2O_2) observed on plasma membrane and cell wall with different concentration after inoculation. The effect of H_2O_2 on root-knot nematode has also been confirmed by applying different concentration of exogenous H_2O_2, which results in reduction in reproduction rate (eggs/g fresh root) of *M. Javanica* (Karajeh, 2008). Hence, the possible reasons for the reduction of nematode reproduction have suggested that H_2O_2 has direct killing effect on eggs or juveniles and indirect effect on endogenous H_2O_2 of treated plants.

Mi-1 gene inhibits nematode penetration

Second juvenile stage (J2) of the RKN enters the roots and induces formation of feeding site 'giant cells'. After formation of feeding sites, J2 become sedentary and develop to adult female. Then, the adult female lay eggs in external gelatinous matrix, which is visible as egg mass outside the roots. Infective J2 hatch from the eggs and start infecting other root parts of the same plant or migrate to the neighboring plant. However, in resistant tomato the processes are blocked either by inhibiting J2 penetrating, die after penetration or reducing the reproduction (Gharabadiyan et al., 2012). Hence, one of the resistance mechanisms of tomato to root-knot nematode is inhibiting the penetration of juvenile (J2) during invasion. However, there is variation in numbers of penetrating J2 depending on the nematode population, tomato genotype, and post-inoculation time (Melillo et al., 2006; Verdejo-Lucas et al., 2012). High number of eggs and egg masses per plant, eggs g-1 root, higher infection frequencies and multiplication rate has observed in a virulent population. In a study conducted to compare the resistance response to *M. incognita* of wild tomato species (*L. peruvianum* and *Lycopersicon pimpinellifolium*), local cultivars (CO3, PKM 1), and hybrid Ruchi significant difference was shown between susceptible and resistant wild tomato species with regard to the number of penetrated nematodes after inoculation (Hemaprabha and Balasaraswathi, 2008). In a resistant wild tomato (*L. peruvianum*), the epidermis, endodermis, and vascular tissues of the root cells were intact after inoculation with infective juveniles. Lignin deposition has also been observed on epidermal layer of root section to

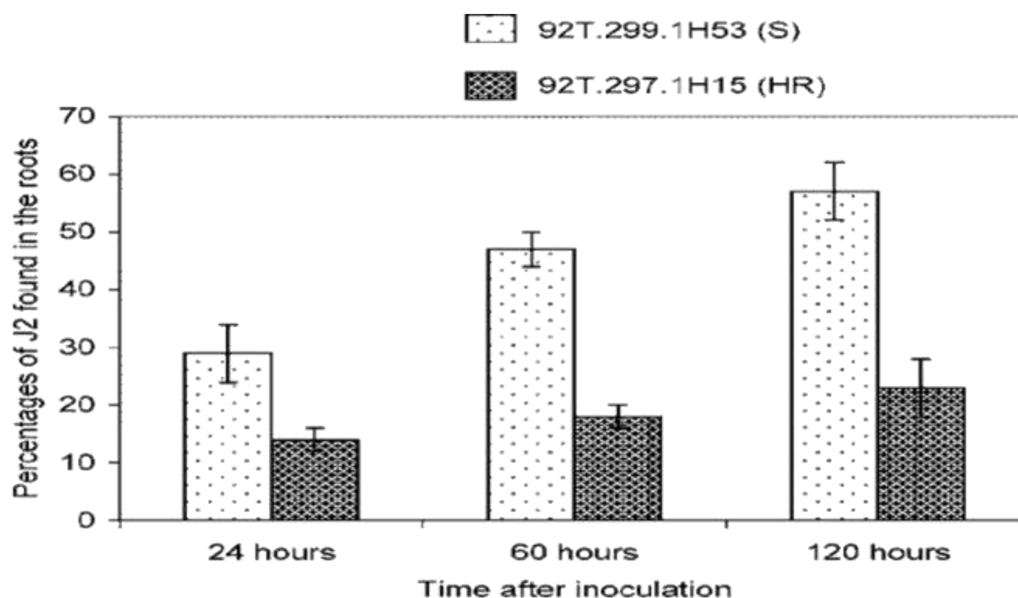

Figure 2. Dynamics of root invasion by second-stage juveniles (J2) of *Meloidogync incognita* in susceptible (S) and resistant (HR) genotypes. Source: Kouassi et al. (2005).

hinder the penetration of nematodes. The level of penetration depends on the period after inoculation. The rate of penetration after 24 h inoculation was double (29%) in susceptible genotype as compared to the resistant one (14%) (Kouassi et al., 2005). In susceptible genotypes, infectivity increases as the time after inoculation increases while there was no significant change in resistant genotype (Figure 2).

Mi-mediated resistance reduces gall formation

After penetration of the root, nematodes induce the formation of a gall in root tissue, which serves as criteria to assess susceptibility of tomato to root-knot nematodes. The resistance response of different genotypes of tomato were evaluated and screened with artificially inoculating J2 of *M. incognita*. The result showed that resistant wild tomato species showed very low number of gall (only 1) and 0.3 gall index (1-5 scale) while the susceptible cultivar showed a mean value of 89.66 galls and gall index of 4 (Hemaprabha and Balasaraswathi, 2008). Disease index (grade scale), calculated based on gall index was high on susceptible varieties and hybrids while very mild on resistant tomato, *L. peruvianum*. In addition, the reason for low number of galls and disease index in resistant tomato (*L. peruvianum*) suggested that low number of nematodes had penetrated the root. However, as shown in Figure 3, the number of gall was significantly increased under high level of inoculum (Gharabadiyan et al., 2012). In another study conducted to evaluate the response of local and commercial tomato cultivars to *M. javanica,* root gall formation was significantly lower on commercial resistant cultivars (Rumbos et al., 2011). Similarly, significantly lower number of galls was observed on tomato cultivar grafted onto root-stock carrying *Mi* resistance gene as compared to the control or non-grafted.

Reducing nematode reproduction

The extent of growth reduction in tomato is directly proportional to the reproduction rate of the nematodes (Kamran et al., 2012). The *Mi-1* mediated resistance significantly reduces the reproductive potential of root-knot nematodes (Corbett et al., 2011). The number of eggs g^{-1} root was very low on resistant cultivar and intermediate on grafted rootstock. Hence, the final population density of *M. javanica* was significantly reduced in resistant tomato having Mi-resistant gene as compared to the susceptible one (Verdejo-Lucas and Sorribas, 2008).

The reproduction of the nematodes was significantly affected by the genetic background of tomato (Cortada et al., 2009; Jacquet et al., 2005). The heterozygous and homozygous allelic condition of *Mi* gene in tomato influenced the reproduction potential of *M. incognita*. According to Jacquete et al. (2005), significant interaction has been observed between the plant genotypes (allelic variants of *Mi* gene) and nematode isolates on the reproduction of RKN. The result showed that the reproduction of a virulent *M. incognita* isolate was significantly higher in heterozygous (Mi/mi) allelic condition than homogenous (*Mi/Mi*) one. The reason for low reproduction potential of *Salvia hisponica* on

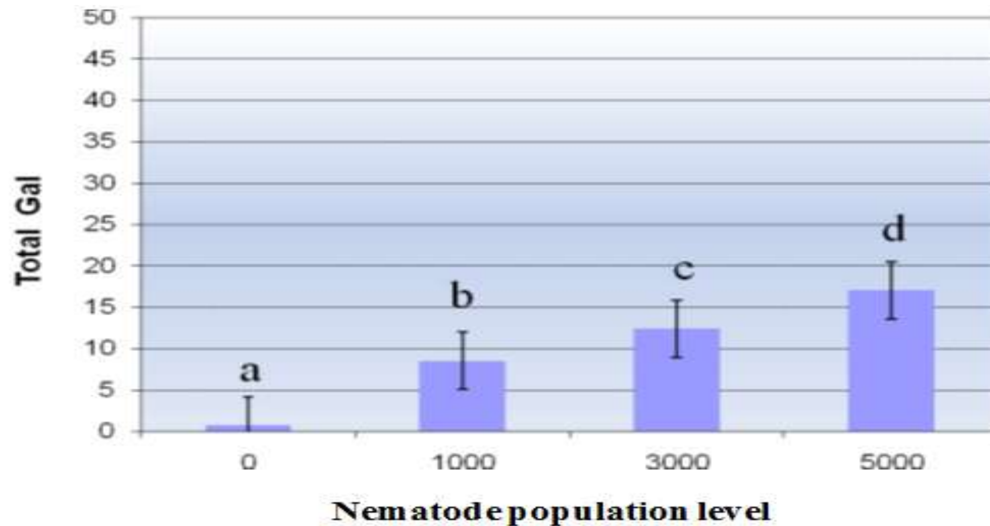

Figure 3. Effect of inoculums levels on gall number.
Source: Gharabadiyan et al. (2012).

homozygous *Mi/Mi* tomato genotype was suggested that the *Mi* gene has dosage effect on nematode reproduction (Maleita et al., 2011). Contrasting reports exist on the effect of tomato genotype (*Mi-1* homozygous or heterozygous locus) on reproduction of RKN. For instance, the heterozygous genotype showed significantly lower reproduction of *M. javanica* while higher nematode reproduction (eggs g^{-1} root) observed on homozygous resistant tomato cultivar (Cortada et al., 2009).

Induced resistance

Induced resistance is a plant defense response, which is activated by feeding damage by herbivorous and other biotic and abiotic factors. The defense mechanism is similar to that of major gene resistance, but unlike the major gene resistance, induced resistance confers partial resistance. For defense response to be activated, certain molecules are required for signal perception and transduction. It has been shown that plant hormones, jasmonic acid (JA) and salicylic acid (SA) have a role in signal transduction of tomato resistance to root-knot nematodes (Cooper et al., 2005; Zhang et al., 2011; Zinovieva et al., 2013b). The involvement of SA and JA in the process of induced resistance has been investigated by exogenous application of SA and JA on resistant and susceptible tomato cultivars infected with *M. incognita* (Zinovieva et al., 2013b). The result showed that SA and JA treatment reduced size of galls, number of galls, number of eggs, and female size on both the susceptible (*Mi*-) and resistance (*M*+) genotypes (Table 1). Moreover, enzyme activity of lypoxygenase (LOX), JA-biosynthesis enzyme, has increased when susceptible (*Mi*-) and resistant (*Mi*+) tomato genotypes are treated with JA (Table 2). SA and JA treatment of resistant and susceptible at high temperature (32°C) significantly reduced the infestation of RKN, number of galls, and fertility of the females. Hence, unlike *Mi*-mediated resistance, JA and SA induced resistance is a stable resistance at high temperature. The number of root-knot nematodes was very low on JA over-expressed tomato mutants as compared to the control one.

In another study, the effect of JA on induced resistance to RKN was investigated by foliar application of JA to tomato cultivars with and without *Mi-1.2* (Cooper et al., 2005). The result showed that JA induces systemic defense response, which results in reduction of nematode reproduction on susceptible tomato varieties. Moreover, application of JA did not show any inhibitory effect on *Mi*-mediated resistance. This shows that there is no signaling conflict between JA-induced resistance and *Mi*-mediated resistance. Comparison between JA-induced resistance and *Mi*-mediated resistance and their combined effect was also studied. Almost complete suppression of avirulent nematode reproduction has observed in *Mi-1.2* mediated resistance while the JA treated susceptible cultivars showed only partial resistance. At high soil temperature (32°C), the effect of *Mi-1.2* significantly reduced while JA induced resistance was temperature independent.

It is also reported that SA has anti-inflammatory role to limit the spread of toxic peroxidative reactions from the nematode infected sites though its involvement on limiting nematode development and reproduction is not well known (Molinari and Loffredo, 2006). In another study, application of SA is found to be effective elicitor of resistance and reduce the reproduction and infestation of root-knot nematodes though the effectiveness depends on the concentration and methods of application

Table 1. Effect of JA and SA on the resistance parameters of tomatoes to M. incognita at different temperatures

Temperature (°C)	Genotype	Treatment	Number of galls/1 g of roots)	Size of galls (mm^2)	Size of female (mm^2)	Number of eggs in ootheca
25	(*Mi*–)	Control	246	1.52	0.33	158
		JA	175	1.43	0.29	118
		SA	202	1.86	0.34	145
	(*Mi*+)	Control	0	No	No	No
		JA	0	-	-	-
		SA	0	-	-	-
32	(*Mi*–)	Control	264	1.84	0.31	162
		JA	205	1.68	0.32	121
		SA	218	1.93	0.34	164
	(*Mi*+)	Control	147	1.32	0.33	63
		JA	73	1.28	0.31	36
		SA	62	1.16	0.24	23
	LSD*	-	40	0.35	0.03	15

*Represents least significant difference (LSD) at P = 0.95.
Source: Zinovieva et al. (2013a).

Table 2. JA effect on LOX activity in leaves of tomatoes infested by the root-knot nematode at different temperatures.

Genotype treatment	LOX activity in plant leaves, E_{234}/(mg/min)			
	25°C		32°C	
(*Mi*–)	Healthy	Infested	Healthy	Infested
Control (water)	0.2	0.34	0.22	0.3
JA	0.24	0.52	0.26	0.44
(*Mi*+)	Healthy	Infested	Healthy	Infested
Control (water)	0.22	0.31	0.24	0.35
JA	0.28	0.3	0.33	0.57
LSD*	0.06	0.06	0.06	0.06

*Represents least significant difference (LSD) at P = 0.95. Source: (Zinovieva et al., 2013a).

(Molinari and Baser, 2010).

Plant secondary metabolites influence the behaviour of root-knot nematodes (Campos et al., 2012; Dutta et al., 2012). A study was conducted to investigate the effect of small lipophilic molecules (SLM), root exudates of *S. lycopersicum*, on RKN. The root exudate was collected from tomato that grown in hydroponic culture in glasshouse chamber. SLMs were extracted through solid phase extraction and bioasssays were developed to test nematode stylet thrusting, motilty, imobility and mortality. The result showed that a significant decrease in stylet thrusting, nematode head movement and reduction in salivary secretion of *Meloidogyne graminicola* and *M. incognita* J2 (Dutta et al., 2012). It has also been observed that SLM inhibits the motility of J2. Moreover, high rate of mortality has been observed with undiluted solution of SLM. Thus, SLM causes nematostatic and nematotoxic effect when applied with diluted and undiluted solution, respectively. This property of SLM has been reported to be similar with volatile organic compounds (VOCs), root exudates of tomato and rice, which have a namatostatic effect, inhibitory effect on the mobility and secretion of salivary secretions of J2 of RKN (Ranganathan and Borges, 2009). It has been reported that many phytochemicals which are exudated from the root can act as repellents, attractants or inhibitors, and toxic effect on plant parasitic nematodes (Curtis et al., 2009).

A study was conducted to evaluate the metabolic responses of susceptible and tolerant tomato to *M. incognita*. The resistant and susceptible tomato cultivars were inoculated with J2 of *M. incogniata*. At time interval

of 0, 24, 48, and 96 h after inoculation, the roots were removed, washed, frozen and freeze-dried for subsequent quantification of aminoacids, phenols, alkalods, and soluble carbohydrates. The result showed that the concentration of amino acids and phenols has increased after inoculation with *M. incognita* in resistant tomato (Campos et al., 2012). The highest concentration of phenols was recorded at 96 h after inoculation. Thus, increasing of phenols concentration after inoculation in resistant tomato suggests that they are involved in preventing the formation of nematode feeding sites. Similarly, the concentration of carbohydrates in resistant tomato varied though its correlation with resistance to nematodes is not well understood.

CONCLUSION AND FUTURE REMARKS

Since application of chemical pesticides has a negative impact on environment, priority has been given to resistant tomato varieties to control root-knot nematode (RKN). The *Mi*-gene of tomato has provided effective control against the three major *Meloidogyne* spp. for many years. The resistance gene (*Mi*-gene) is characterized by hypersensitive response (HR) associated with local cell death at the site of penetration and necrotic lesions on nematode feeding sites. However, the gene loses its effectiveness at high temperature. Moreover, the *Mi* gene is broken by virulent RKN pathotypes. Nowadays, because of climate change the temperature is increasing from time to time in most parts of the world. Thus, the durability and effectiveness of the resistance gene to RKN is uncertain since *Mi*-gene is broken at high temperature. Heat-stable resistance genes to RKN have been identified in different tomato accessions. Furthermore, several resistance genes to RKN have been reported within Solanaceae family. Therefore, strategies need to be developed to improve the durability of the resistance. Pyramiding of these resistance genes in commercial tomato cultivars could be one possible approach to improve the durability of the resistance. Transgenic expression of some resistance genes has shown successful result. Plant secondary metabolites are reported to be involved directly or indirectly in signal transduction of *Mi*- mediated resistance genes. Thus, modification of the metabolic pathways (RKN damage induced expression) of these molecules by using modern biotechnology (genetic engineering) could be an alternative approach for developing resistant tomato varieties to RKN.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic variability studies on bread wheat (*Triticum aestivum* L.) genotypes

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Thirty bread wheat genotypes were tested to assess the genetic variability, among studied genotypes using alpha-lattice design at Tongo sub-center of Assosa Agricultural Research Center and Kulumsa Agricultural Research Center in 2015. Analysis of variance revealed that there were statistically significant differences among the genotypes for most of the traits at individual and across locations. From the combined analysis of variance, significant ($p \leq 0.05$) effect due to location, varieties and G×E was observed for most of the traits. The varieties showed wider variability in mean grain yield of 1284.4-3788.7 kg ha^{-1}, 2588.3-4683.3 kg ha^{-1} and 1936.4 - 4095.6 at Tongo, Kulumsa and across location, respectively. Moderate PCV values (>10%) were obtained for grain yield, 1000 kernel weight, harvest index, tillers per plant and spikes per plant at individual location and across location including days to heading, above ground biomass yield, spike length and kernels per spike at Tongo and above ground biomass yield at Kulumsa. Similarly, moderate GCV values (>10%) were obtained for grain yield, 1000 kernel weight, tillers per plant and spikes per plant at individual location including days to heading, harvest index and kernels per spike at Tongo and above ground biomass yield at Kulumsa. Lower (<10%) was obtained for all traits across location. High heritability estimates (>80%) were obtained for days to heading (86.0%) and days to maturity (85.1%) at Tongo and days to heading (86.2 and 82.69%) and spikes length (80.1 and 82.85%) at Kulumsa and across location. But relatively high genetic advance (>20%) was obtained for grain yield (28.5%) and harvest index (24.3%) at Tongo. Moderate genetic advance (10-20%) was observed for 1000 kernel weight, spikes length and days to heading at individual location and across location including spikelets per spike, tillers per plant, above ground biomass, spikes per plant and plant height at individual location. Generally, it has been observed the presence of variability among the genotypes, heritability in the tested traits of the genotypes studied. Hence, Selection and hybridization on those genotypes based on the trait with high GCV, heritability and genetic advance can be recommended for farther yield improvement of bread wheat at respective location.

Key words: Heritability, genetic advance, traits, phenotypic, genotypic coefficient of variation.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the world's major cereal crops and stable food of many regions grown under both irrigated and rain fed conditions. Unlike rice and maize, which prefer tropical environment, wheat is extensively grown in temperate regions occupying 17% of all crop acreage worldwide. It is the staple food for 40% of the world's population (Goyal and Prasad, 2010; Peng et al., 2011). Currently it is also becoming most important cereals grown on a large scale (Fassil et al., 2000), because of its significance as cash crop, high level of

production per unit area, its major role in supplying the dietary requirements of the society. Wheat is the second only to rice which provides 21% of the total food calories and 20% of the protein for more than 4.5 billion people in 94 developing countries (Braun et al., 2010). Food consumption of wheat is projected at 488 million tonnes, 1.3% higher than in the 2014 season, keeping the average per capital level steady at 67.6 kg (FAO, 2015). Global wheat grain production must increase 2% annually to meet the requirement of consistently increasing world population (around 9 billion) till 2050 (Rosegrant and Agcaoili, 2010).

The leading wheat producing countries are China, India, United States, France, and Russia Federation (FAO, 2015). The Wheat Yield Consortiums an integral part of the wheat strategy to break the genetic yield barrier. In March 2012, 34 research organizations finalized a 10-year integrated research plan. The organizations agreed for sharing advanced scientific expertise, facilities and germplasm, to improve the wheat plant's photosynthesis, ear size and stalk strength working together to succeed in raising the genetic yield potential by up to 50% in the next 20 years. The wheat yield consortium findings will be incorporated into the wheat breeding platform, to deliver high-yielding varieties to farmers' fields in wheat target regions (CGIAR, 2013)

In Ethiopia, bread wheat is an introduced crop, although its time of introduction is immemorial (Hailu, 1991). Wheat can grow in the Ethiopian highlands, which are situated between 6° and 16°N and 35°and 42°E, at altitude ranging from 1500 to 3000 m. However, the most suitable altitude zones of wheat fall between 1900 and 2700 m.a.s.l (Bekele et al., 2000).

Wheat is an important staple food crop and the third highest source of grain-based calories behind corn and sorghum in Ethiopia. It accounts for a little more than 20% of the total calorie supply. 60% of production is used for household consumption, 20% is sold to the market, while the balance is used for seed, in-kind wages, animal feed and other uses. Wheat bran from commercial wheat millers is used as one of the ingredients in commercially-produced, compound animal feed (GAIN, 2015). It grows on 1.6 million hectares of production area with a total production of 3.8 million metric tons and ranks fourth in both area and production among cereal crops in different regions of Ethiopia (CSA, 2015). Ethiopian wheat production self-sufficiency is only 75% and the remaining 25% of wheat imported commercially and through food aid and shares of total cereal consumption is increased by 20% in resent year, making it the second most consumed cereal in Ethiopia after corn (USDA, 2016). Therefore, to meet the self-sufficiency, growing demand of manufacturing industries and reduce the importing, increasing the yield potential would be the solution in the long-run. Farther more increasing wheat production is important to the economic stability and food security of Ethiopia.

Although the productivity of wheat has increased in the last few years in Ethiopia, it is still very low as compared to other wheat producing countries. The national average productivity is estimated to 2.4 tons/ha (CSA, 2015) which is by far below the world's average of 3.27 tons/ha (USDA, 2016).The low productivity is attributed to a number of factors including: Biotic (Diseases, insect pests, and weeds), abiotic (moisture, soil fertility, etc.,) (Zegeye et al., 2001). Among biotic factors, rusts are the most important diseases of wheat, which cause up to 60% loss of wheat yield for leaf or stripe (yellow) rust and 100% loss for stem rust (Park et al., 2007).Wheat and rusts have coevolved for thousand years and resulted in the accumulation of wide spectrum of the pathogens in Ethiopia (Mengistu et al., 1991). Therefore, developments of new varieties which are resistance to different diseases and adaptable to environments with abiotic stress could be a solution for farther grain yield improvement in wheat.

Grain yield and its quality are the principal characters of a cereal crop (Ullah et al., 2010). They are complex quantitative characters, which are influenced by a number of yield contributing characters. Hence, the selection for desirable genotypes should not only be based on yield alone, and the other yield components should also be considered. Direct selection for yield is often misleading in wheat because wheat yield is polygenically controlled. For effective utilization of the genetic stock in crop improvement, information of mutual association between yield and yield components is necessary. It is therefore, necessary to know the correlation of various component characters with yield and among themselves. The correlation coefficients between yield and yield components usually show a complex chain of interacting relationship. Path coefficient analysis partitions the components of correlation coefficient into direct and indirect effects and illuminates the relationship in a more meaningful way. The success of a breeding program depends largely upon the amount of genetic variability present in the population and the extent to which the desired traits are heritable (Majumder et al., 2008).

Several genetic variability studies have been conducted on different crop species based on quantitative and qualitative traits in order to select genetically distant parents for hybridization (Daniel et al., 2011). Genetic improvement to develop varieties with high yield

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potential and resistance/tolerance to a biotic and biotic stresses, with acceptable end-use quality, is the most viable and environment-friendly option to sustainably increase wheat yield. Such improvement of crops requires creation and introduction of genetic variation, inbreeding coupled with selection, and extensive evaluation of breeding materials at multiple locations to identify adapted and stable genotypes with desirable agronomic traits. Several genetic variability studies have been conducted on bread wheat at the different regions of Ethiopia (Adhiena, 2015; Awale et al., 2013; Gezahegn et al., 2015; Mitsiwa, 2013; Obsa, 2014). However, no variability studies have been conducted at Benishangul Gumze Regional State. Therefore, such information is essential for creation of genetic variation and further bread wheat improvement particularly, in the region and generally in the country. Therefore, the current study was carried out to estimate the genetic variability of bread wheat genotypes for yield and yield related traits.

MATERIALS AND METHODS

Experimental sites

The experiments were conducted at two locations, Kulumsa Agricultural Research Center (KARC) and Tongo, under Assosa Agricultural Research Center (AsARC) (Table 1).

The bread wheat genotypes to be studied were given in Table 2.

Experimental design, data collected and field management

The trials were planted in July 04, 2015 at Kulumsa and August 18, 2015 at Tongo. Masood et al. (2008) reported Alpha lattice design provided smaller standard errors of differences, coefficients of variation and error mean squares as compared to randomized complete block design providing efficiency in comparing different entries/lines. Therefore in the current study, thirty genotypes were grown in alpha-lattice design with three replications. Each experimental unit consisted six rows of 2.5 m length with 20 cm spacing between rows. Data were collected from the central four rows for the parameters days to heading, days to maturity, grain filling period, grain yield, 1000 kernel weight, above ground biomass yield, harvest index, hectoliter weight and from randomly sampled plants for the characters; tillers per pant, plant height, kernel per spike, spikelet per spike, spike length and spikes per plant. 1.5 m alleys were left between reps. Non-experimental variables such as seed and fertilizer rates were used as recommended for the specific testing sites. Hence, 73/69 kgha^{-1} N/P_2O_5 were used for Kulumsa and 60/69 kg ha^{-1} N/P_2O_5 for Tongo. A seed rate of 125 kg ha^{-1} was used at both locations.

Data analysis

Analysis of variance (ANOVA)

The analysis of variance (ANOVA) was performed using the SAS version 9.1.3 software for Alpha-Lattice Design. For each location and combined data over locations, analyses of variances, were done using the mean of ten sample plants for the characters like plant height, tillers per plant, spikelets per spike, spike length, kernels per spike and spikes per plant. However, plot values were used for the characters such as days to heading and maturity, grain yield per hectare, harvest index, grain filling period, hectoliter weight, thousand kernels weight, and above ground biomass yield for analysis of variance. The Least Significant Difference (LSD) was used to compare two means at the 5 and 1% level of significance.

Individual locations ANOVA were computed using the following mathematical model:

$$Yijl = \mu + rj + gi + P\,l(j) + \varepsilon ijl$$

Where: Yij =the observed value of the trait Y for the ith genotype in $j^{\,th}$replication; μ= the general mean of trait Y; rj = the effect of $j^{\,th}$ replication; gi= the effect of ith genotypes and $pl(j)$ =block within replicate effect; εijl= the experimental error associated with the trait y for the ith genotype in l^{th} block with in replication and $j^{\,th}$ replication.

Combined ANOVA model:

$$Yijk = \mu + gi + Ej + GEij + Bk(j) + \varepsilon ijk$$

Where, $Yijk$ = observed value of genotype i in block $_k$ of location j; μ = grand mean; Gi = effect of genotype I; Ej = environment or location effect; $GEij$ = the interaction effect of genotype $_i$ with location/environment j; $Bk(j)$ = effect of block k in location/environment j; εijk = random error or residual effect of genotype i in block k of location j.

Estimation of phenotypic and genotypic parameters

Genotypic variance (σ^2_g) = $\frac{MS_g - MS_e}{r}$ (Burton and De vane, 1953)

Where: MS_g = mean square due to genotypes, MS_e = error mean square, r = the number of replication, Environmental variance (σ^2_e) = error mean square=MS_e, and Phenotypic variance ($\sigma^2 p$) = $\sigma^2_g + \sigma^2_e$

Variance components for the data combined over locations were computed in a similar fashion as for individual locations by using the following formulae (Johnson et al., 1955)

$$\sigma^2_e = MS_e$$

$$\sigma^2_{gl} = \frac{MS_{gl} - MS_e}{r}$$

$$\sigma^2_g = \frac{MS_g - MS_{gl}}{rl}$$

$$\sigma^2 p = \sigma^2_g + \frac{\sigma^2}{l} gl + \frac{\sigma^2_e}{lr}$$

Where: $\sigma 2_{\text{gl}}$ = Genetic by location interaction; MS_{e} = error mean square; MS_{gl} = genotype by location interaction mean square; MS_{g} = genotype mean square; r = replication and l = location

Coefficient of variation at phenotypic, genotypic and environmental levels was estimated as:

Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$

Genotypic Coefficient of variation (GCV) = $\frac{\sqrt{\sigma^2 g}}{\bar{x}}$ x100

Where: $\bar{x}$ = grand mean of character.

Table 1. Experimental site analysis.

Site	Altitude	Latitude	Longitude	Annual rain fall (mm)	Average annual temperature (°C)
Tongo	1820	90 23.165' °N	340 24 38°E	1316	24.3 - 26.6
Kulumsa	2200	8.08'N	39.08'E	832	9.78 - 23.12

Table 2. The listed of bread wheat genotypes to be studied.

Entry	Name	Pedigree/genotypes
1	Hidasse	ETBW5795(check 1)
2	ETBW 6861	WAXWING*2/HEILO
3	ETBW 8506	AGUILAL/FLAG-3
4	ETBW 8507	DURRA-4
5	ETBW 7120	QAFZAH-23/SOMAMA-3
6	ETBW 8508	REYNA-8
7	ETBW 7213	CHAM4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB
8	ETBW 8509	REYNA-29
9	ETBW 7038	ATTILA/3*BCN//BAV92/3/TILHI/5/BAV92/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
10	ETBW 8510	HIJLEEJ-1
11	ETBW 7058	ROLF07//TAM200/TUI/6/WBLL1/4/HD2281/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/TACUPETO F2001
12	ETBW 8511	BOW #1/FENGKANG 15/3/HYS//DRC*2/7C
13	ETBW 7147	CROC-1/AE.SQUARROSA(224)// OPATA/3/QAFZAH-21/4/SOMAMA-3
14	ETBW 8512	BABAX/LR42//BABAX*2/3/KURUKU/4/KINGBIRD #1
15	ETBW 7871	PAURAQ/4/PFAU/SERI.1B//AMAD/3/WAXWING
16	ETBW 8513	MUTUS//WBLL1*2/BRAMBLING/3/WBLL1*2/BRAMBLING
17	ETBW 6940	UTIQUE 96/FLAG-1
18	ETBW 8514	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WBLL1*2/BRAMBLING
19	ETBW 7368	D. 56455
20	ETBW 8515	BECARD/3/PASTOR//MUNIA/ALTAR 84
21	ETBW 7364	ACSAD1115
22	ETBW 8516	KACHU/KIRITATI
23	ETBW 7194	VAN'S'/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/4/TEVEE-5
24	ETBW 8517	FRNCLN*2/TECUE #1
25	ETBW 7101	KAMB2/PANDION
26	ETBW 8518	SUP152/AKURI//SUP152
27	ETBW 7872	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
28	ETBW 8519	ATTILA/3*BCN*2//BAV92/3/KIRITATI/WBLL1/4/DANPHE
29	ETBW 6937	AGUILAL/FLAG-3
30	Danda'a	DANPHE#1(check 2)

Estimation of heritability in broad sense

Heritability (H): in broad sense for all characters was computed using the formula given by Falconer (1989). Broad sense heritability (H) expressed as a percentage of the ratio of the genotypic variance **(**σ^2_g**)** to the phenotypic variance **(**σ^2_p) was estimated on genotype mean base (Allard, 1960) as:

$$\text{Heritability } (H^2) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

Where: H^2 = heritability in broad sense; σ^2_p = phenotypic variance; σ^2_g = genotypic variance

Estimation of genetic advance

Genetic advance in absolute unit (GA) and percent of the mean (GAM), were estimated in accordance with the methods illustrated by Johnson et al. (1955) as:

$GA = K\sigma_P H$

Table 3. Mean squares of the 14 traits of bread wheat genotypes tested at Kulumsa and Tongo in 2015/16.

Characters	Tongo						kulumsa					
	source				CV (%)	Efficiency	source				CV (%)	Efficiency
	Rep (DF=2)	Block(Rep) (DF=15)	Genotypes (DF=29)	Error (DF=43)			Rep (DF=2)	Block(Rep) (DF=15)	Genotypes (DF=29)	Error (DF=43)		
DH	17.54*	4.6	104.76**	5.37	4.2	0.96	5.73	2.6	43.21**	2.19	2.63	1.05
DM	3.1	6.86*	63.75**	3.52	1.8	1.24	4.43	9.11	30.34**	4.83	2.16	1.23
GFP	6.41	8.59	18.28**	4.52	4.31	1.23	7.23	4.78*	22.66**	6.2	5.47	0.94
PH	6.35478	17.41336	66.35**	14.32	5	1.06	90.65**	18.74**	69.68**	6.47	3.23	1.49
GY	160354	494552.51**	917407.72**	152281.2	13.62	1.58	687393.61*	231423.8	850298.88**	143794.7	10.39	1.16
TKW	33.6**	9.94	42.29**	5.63	7.09	1.2	3.33	13.29*	67.53**	5.68	5.5	1.35
HW	20.57*	6.01	16.34**	4.63	2.73	1.08	3.05	6.88*	11.90**	2.91	2.33	0.93
AGB	486111	2508648.6*	3800067.3**	1148792	9.22	1.31	975000	1905385	5689567**	1076804	9.59	1.2
HI	0.002*	0.001*	0.004**	5.83x10-4	9.84	1.24	0.01**	1.4X10-2**	5.6X10-2**	1.2X10-2	9.99	1.05
TPP	2.75**	0.58**	0.52**	0.2	15.59	1.49	2.67**	0.49**	0.60**	0.19	16.05	1.42
SPP	3.47**	0.51**	0.57**	0.2	16.45	1.42	2.91**	0.49**	0.47**	0.15	15.15	1.59
SL	1.20**	0.36*	1.66**	0.17	5.11	1.3	6.11**	0.99**	1.34**	0.1	3.79	3.24
SPS	1.6	0.85*	5.25**	0.85	5.16	1	0.89	0.68	3.65**	0.55	6.51	0.07
KPS	9.7	33.3	74.82**	14.28	9.29	1.34	16.02	28.68**	28.53**	8.89	4.06	1.58

DH, Days to heading; DM, Days to maturity; GFP, Grain filling period; GY, Grain yield; TKW, 1000 kernel weight; AGB, Above ground biomass ($kgha^{-1}$); HI, Harvest index; HW, Hectoliter weight; TPP, Tillers per plant; PH, Plant height (cm); SPS, Spikelets per spike; KPS, Kernels per spike; SL, Spike length; SPP; Spikes per plant. LSD= List significant difference; CV= Coefficient of variations.

Where, K=the standardized selection differential at 5% selection intensity (k=2.063); σ_p =phenotypic standard deviation on mean basis; H=heritability in broad sense.

$$GAM = \frac{GA}{\bar{X}} \times 100$$

Where: GAM= genetic advance as percent mean; GA=genetic advance under selection, and $\bar{X}$ = Mean of the population in which selection employed.

RESULTS AND DISCUSSION

Analysis of variance of studied traits

Individual location (Table 3) and across locations (Table 4) ANOVA was carried out for 14 characters recorded at Tongo and Kulumsa. There was a highly significant difference among the genotypes for all traits including days to heading, days to maturity, grain filling period, plant height, grain yield, 1000 kernel weight, hectoliter weight, biological yield, harvest index, tillers per plant, spikes per plant, spikes length, spikelets per spike and kernels per spike studied at individual locations confirming the genetic variability for yield and its components. Obsa (2014) and Awale et al. (2013) also reported considerable genetic variability for grain yield and its component characters in studied bread wheat genotypes in Ethiopia. Other authors also reported considerable genetic variability for grain yield and its component characters in durum wheat (Khan et al., 2013; Mohammed et al., 2011). Gezahegn et al. (2015) reported highly significant and significant differences among genotypes (P<0.01) for days to heading, days to maturity, grain filling period, 1000 kernel weight, plant height, spike length, number of productive tillers per plant, number of spikelet’s per spike and number of grains per plant, grain yield per plot, harvest index and hectoliter weight. However, Mitsiwa (2013) reported non-significant differences among bread wheat genotypes for plant height and spike length and Adhiena (2015) for plant height and number of tillers per plant.

Twelve quantitative characters which had homogeneous error variances were subjected for combined ANOVA over locations (Table 4).

Significant location effects were observed for all the traits except number of spike per plant indicating the differences in growth conditions exhibited at the two locations.

Mean squares of genotypes were significant (P≤0.01) for all characters including days to heading, days to maturity, plant height, grain yield, 1000 kernel weight, hectoliter weight, harvest index, spikes per plant, spikes length, spikelets per spike and kernels per spike except for number of tillers per plant indicating variability in studied genotypes. Hence, selection could be effective for different quantitative characters or for inclusion in crossing program for creating variability. Such variability with in studied genotypes was also reported by Navin et al. (2014).

The location × genotype interaction was significant for days to heading, days to maturity, plant height, grain yield, 1000 kernel weight, hectoliter weight, harvest index, spikes length, spikelets per spike and kernels per spike except number tiller per plant and spike per plant indicating different performance of bread wheat genotype across the two locations or genotypes responded differently to the different environmental conditions suggesting the importance of the assessment of genotypes under different environments in order to identify better performing genotypes for a particular environment. In accordance with Tesfaye et al. (2014) who reported significant differences among genotypes for most of the traits including day to heading, days to maturity, plant height, Septoria disease, thousand seed weight and hector liter weight across environments

Mean, range and estimates of genetic parameters

Mean and range of grain yield and yield components

Range and mean values for the 14 characters are shown in Tables 5 and 6 for Tongo and Kulumsa, respectively. The mean performance of the 30 genotypes for 14 traits is presented in Appendix Tables 5 and 6. Coefficients of variation (CV %) were used to compare the precision of the experimentation, that is, means with lower CV% for most of the characters revealed existence of reliability of the data (Gomez and Gomez, 1984). A range for days to heading at Tongo was 46 to 70 days with minimum values in genotypes ETBW 8518 and the maximum in ETBW 6940 with an average value of 55 days. 46.6% of the genotypes need above the grand mean (55 days) days to heading. The range for days to heading at Kulumsa was 48 to 66 days relatively narrow than days to heading at Tongo with minimum values in genotypes ETBW 8518 and the maximum in ETBW 7213 with an average value of 56 days. 30.0% of the genotypes need above the grand mean (56 days) days to heading. Days to maturity at Tongo and Kulumsa also ranged from 97 (ETBW 7101) to 117 (ETBW 6940) and 97 (ETBW 7101 and ETBW 8517) to 108 (ETBW 6940, ETBW 7147 and ETBW 7213) days, respectively, with an average value of 105 and 102 days, respectively, indicating that the tested genotypes were early to medium maturing category. Grain felling period ranged from 42 to 54.7 and 35 to 49 at Tongo and Kulumsa, respectively, indicating long grain filling period is required at Tongo relative to Kulumsa.

Plant height varied from 63.3 to 83.5 cm at Tongo and 67.2 to 88.7 cm at Kulumsa with a mean height of 75.7 and 78.8 cm, respectively. Number of tillers per plant and spikes per plant were ranged from 2 to 4 and 2 to 4, respectively, at Tongo with a mean of 3 for number of tillers per plant and 3 for number of spike per plant. Similarly, these traits ranged from 2 to 4 and 2 to 4, respectively, at Kulumsa with a mean of 3 for number of tillers per plant and 3 for number of spike per plant. Both number of tillers per plant and number of spike per plant showed similarity in values at both locations indicating most of the tillers were fertile. Spike length ranged from 6.6 to 9.7cm at Tongo and 6.7 to 10.0 cm at Kulumsa with a mean length of 7.9 and 8.5 cm, respectively. The mean number of spikelets per spike and number of kernel per spike were ranged 15 to 22 and 27 to 54, respectively, at Tongo with a mean of 18 for spikelet per spike and 41 for kernels per spike. Similarly, these traits ranged from 15 to 21 and 40 to 54, respectively, at Kulumsa with a mean of 18 for spikelet per spike and 46 for kernels per spike.

The mean 1000 kernel weight ranged from 24.7 g (ETBW 7194) to 38.7 g (ETBW 7364) with an average value of 33.5 g at Tongo and ranged from 27.6 g (ETBW 7058) to 51.3 g (ETBW 8518) with an average value of 43.3 g at Kulumsa. Hectoliter weight provides a rough estimate of flour yield potential in wheat and is important to millers just as grain yield is important to wheat producer. This variable ranged from 71.3 kg/hl (ETBW 8516) to 81.9 kg/hl (ETBW 8510) with an average value of 78.7 kg/hl at Tongo and ranged from 65.4 kg/hl (ETBW 8511) to 75.9 kg/hl (ETBW 8506) with an average value of 73.2 kg/hl at Kulumsa.

Above ground biomass showed a wide range of variation 9000 to 14166.7 kg ha^{-1} with the mean value 11627.8 and 6000 kg ha^{-1} to 15000 kg ha^{-1} with the mean value 10816.7 kg ha^{-1} at Tongo and Kulumsa, respectively. Harvest index (HI) has been used to describe the proportion of harvestable biomass. Current modern wheat varieties have HI of c. 0.45 to 0.50 (spring type) and 0.50 to 0.55 (winter type), approaching its theoretical maximum value (c. 0.64 in winter wheat) (Foulkes et al., 2011; Reynolds et al., 2012). In this study, harvest index ranged from 0.1 to 0.3 with an average value of 0.2 at Tongo and ranged from 0.2 to 0.4 with an average value of 0.3 at Kulumsa. The score of the variable was lower than its theoretical maximum value (0.64) at both locations.

Grain yield is the final result that can be studied through its yield components. Grain yield varied from

Table 4. Mean squares of the 12 traits of bread wheat genotypes tested across location in 2015/16.

Characters	Sources						Mean	CV
	Loc. (DF=1)	Rep(Loc.) (DF=4)	Block(Loc*Rep) (DF=30)	Genotype (DF=29)	Genotype *Loc (DF=29)	Error (DF=86)		
Days to heading	49.09**	20.44*	3.22ns	131.47**	16.496**	3.78	55.78	3.49
Days to maturity	328.05**	3.77ns	7.99*	80.75**	13.34**	4.18	103.18	1.98
Plant height	449.98**	48.50**	18.08ns	107.90**	28.12**	10.39	77.24	4.17
Grain yield	29555458.2**	423874.04*	362988.16**	1086233.14**	681473.46**	148038	3271	11.76
1000 kernel weight	4398.48**	18.47*	11.62**	90.54**	19.29**	5.65	38.41	6.19
Hectoliter weight	1363.89**	11.81*	4.08ns	21.298**	6.94*	3.77	75.97	2.55
Harvest index	0.44**	0.007**	0.0013	0.006**	0.0038**	0.0009	0.29	10.1
Tillers per plant	2.54**	6.29**	1.03**	0.43ns	0.34ns	0.28	2.77	19.42
Spikes per plant	0.91ns	6.33**	0.99**	0.51**	0.3ns	0.25	2.63	19.17
Spikes length	11.12**	3.65**	0.67**	2.75**	0.25*	0.13	8.2	4.46
Spikelets per spike	6.54**	1.25ns	0.76ns	7.03**	1.87**	0.7	18.08	4.63
Kernels per spike	1159.76**	12.86	30.99**	74.996**	28.36**	11.58	43.24	7.87

ns, ** and * indicates non-significant, highly significant at 1% and significant at 5% probability levels, respectively. Rep = Replication; Loc = Location; CV = Coefficient of variations; DF= degree of freedom.

Table 5. Range, mean, variance, broad sense heritability, genotypic and phenotypic coefficient of variability, genetic advance as of mean for the 14 characters of bread wheat genotypes tested at Tongo in 2015/16.

Characters	Range	Mean ± S.E. mean	$\sigma^2 g$	$\sigma^2 p$	H^2	GCV (%)	PCV (%)	GA	GA (%)
Days to heading	46.00-70.33	55.25 ± 0.73	33.13	38.50	86.04	10.42	11.23	10.89	19.71
Days to maturity	97.00-117.00	104.53 ± 0.595	20.08	23.60	85.07	4.29	4.65	8.43	8.06
Grain filling period	42.33-54.67	49.28 ± 0.35	4.59	9.11	50.36	4.35	6.12	3.10	6.29
Plant height	63.26-83.50	75.66 ± 0.60	17.34	31.66	54.77	5.50	7.44	6.29	8.31
Grain yield	1284.40-3788.70	2865.80 ± 74.4	255042.19	407323.35	62.61	17.62	22.27	815.22	28.45
1000 kernel weight	24.67-38.67	33.47 ± 0.47	12.22	17.85	68.44	10.45	12.63	5.90	17.63
Hectoliter weight	71.33-81.87	78.73 ± 0.33	3.90	8.53	45.76	2.51	3.71	2.73	3.46
Above ground biomass	9000.00-14166.70	11627.78 ± 164.5	883758.50	2032550.30	43.48	8.08	12.26	1264.57	10.88
Harvest index	0.13-0.30	0.25 ± 0.004	0.00125	0.00183	68.19	14.41	17.45	0.06	24.28
Tillers per plant	2.20-4.30	2.88 ± 0.0735	0.11	0.31	34.28	11.26	19.23	0.39	13.44
Spikes per plant	2.00-4.20	2.70 ± 0.0771	0.12	0.32	38.44	13.00	20.97	0.44	16.45
Spikes length	6.60-9.67	7.95 ± 0.0963	0.50	0.67	75.17	8.89	10.26	1.25	15.73
Spikelets per spike	15.16-21.53	17.89 ± 0.1812	1.47	2.32	63.22	6.77	8.51	1.96	10.98
Kernels per spike	27.47-54.07	40.7 ± 0.7144	20.18	34.46	58.56	11.04	14.42	7.01	17.23

$\sigma^2 g$ = genotypic variance; $\sigma^2 p$ = phenotypic variance H^2 = Broad sense heritability; GCV = Coefficient of genotypic variance; PCV = coefficient of phenotypic variance; GA = genetic advance.

1284.4 kg ha^{-1} to 3788.7 kg ha^{-1} (mean of 2865.8 kg ha^{-1}) and 2588.3 kg ha^{-1} to 4683.3 kg ha^{-1} (mean of 3676.2 kg ha^{-1}) at Tongo and Kulumsa, respectively. The grain yield performance was better at Kulumsa indicating its potential for wheat production. Gezahegn et al. (2015) reported a wide variation of grain yield per hactar which ranged from 2115 kg ha^{-1} (Menze) to 5955 kg ha^{-1} (Alidoro) in bread wheat. In present study, genotypes ETBW 8514 (3788.7 kg ha^{-1}), Hidasse (3654.4 kg ha^{-1}) and ETBW 8513 (3615.2 kg ha^{-1}) at Tongo and ETBW 7871 (4683.3 kg ha^{-1}), Hidasse (4536.7 kg ha^{-1}) and ETBW 7872 (4495 kg ha^{-1}) at Kulumsa were found to be top yielders (Appendix Tables 1 and 2). The standard check Hidasse was best performed at both location.

Estimates of genetic parameters

The amount of genotypic and phenotypic variability that exist in a species is of utmost importance in breeding to select better varieties and initiating a breeding program. Genotypic and phenotypic coefficients of variation are used to measure the variability that exists in a given genotypes. Estimated genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV), broad sense heritability as well as genetic advance for selection of the traits studied are presented in Tables 5 to 7.

Phenotypic and genotypic coefficients of variation: In general, estimates of phenotypic coefficient of variation in this study were higher than their corresponding genotypic coefficient of variation indicating the influence of environment on the expression of these characters although the differences were small at both locations. Narrower difference between the values of GCV and PCV indicated that the environmental effect was small for the expression of these characters. According to Deshmukh et al. (1986) PCV and GCV values greater than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% to be moderate.

At Tongo the GCV ranged from 2.51% (Hectoliter weight) to 17.62% (Grain yield), whereas PCV ranged from 3.71% (Hectoliter weight) to 22.27% (Grain yield). Among all characters, moderate GCV and PCV values (>10%) were observed for days to heading (10.42 and 11.23%), grain yield (17.62 and 22.27%), 1000 kernel weight (10.45 and 12.63%), harvest index (14.41 and 17.45%), tillers per plant (11.26 and 19.23%), spikes per plant (13.00 and 20.97%), kernels per spike (11.04 and 14.42%), respectively, suggesting sufficient variability and thus scope for genetic improvement through selection for these traits. Navin et al. (2014) reported higher magnitude of GCV and PCV for grain yield per plant, harvest index, tillers per plant, spike length and test weight which support this finding. The rest of the characters grouped under low phenotypic and genotypic coefficients of variation, indicating less scope of selection as they were under the influence of environment.

At Kulumsa the GCV ranged from 0.11% (harvest index) to 13.57% (tillers per plant), whereas PCV ranged from 0.15% (harvest index) to 20.89% (tillers per plant). Moderate GCV and PCV values were observed for grain yield (10.32 and 14.59%), thousand-kernel weight (10.47 and 11.83%), above ground biomass yield (11.46 and 14.95%), tillers per plant (13.57 and 20.89%) and spikes per plant (12.65 and 19.50%), respectively. This indicated that selection will be effective based on these characters and their phenotypic expression would be good indication of the genotypic potential. Similar results of moderate PCV and GCV has been reported for 1000 kernel weight and grain yield in wheat (Gezahegn et al., 2015). The characters days to maturity, grain filling period, plant height, hectoliter weight and harvest index were grouped under low phenotypic and genotypic coefficients of variation. The result is in line with the finding of Mohammed et al. (2011) and Gezahegn et al. (2015) for characters days to maturity, number of spikelets per spike and test weight showed low PCV and GCV (<5%) values. Mitsiwa (2013) also reported low PCV and GCV for grain filling period (1.82 and 1.59%) and days to maturity (3.63 and 3.50%), respectively.

The combined ANOVA results are presented in Table 7. Phenotypic coefficient of variability ranged from 2.89% (hectoliter weight) to 15.68% (spikes per plant). Genotypic coefficient of variability ranged from 2.89% (hectoliter weight) to 8.97% (1000 kernel weight). Generally, the PCV values were higher than GCV values for all the traits studied that reflect the influence of environment on the expression of all the traits. Gezahegn et al. (2015), Gergana and Bozhidar (2015) and Navin et al. (2014) were reported similar result for all studied character. Low GCV (<10%) and moderate PCV (>10%) values were observed for grain yield (7.94 and 14.67%), 1000 kernel weight (8.97 and 10.73%), harvest index (6.60 and 12.43%), tillers per plant (4.32 and 14.81%) and spikes per plant (7.13 and 15.68%), respectively. Adhiena (2015) reported moderate PCV and GCV for spike length, number of grains per spike and harvest index. Similarly, Gezahegn et al. (2015) noted moderate phenotypic and genotypic coefficients of variation for 1000 kernel weight, grain yield and harvest index in sixty four bread wheat genotypes in Ethiopia which are in line with this finding for PCV. The lowest GCV and PCV values were observed for days to heading (7.85 and 8.63%), days to maturity (3.25 and 3.73%), plant height (4.72 and 6.00%), hectoliter weight (2.04 and 2.89%), spikelets per spike (5.13 and 6.56%), spikes length (7.87 and 8.65%) and kernels per spike (6.45 and 9.35%), respectively, indicating less scope of selection as they were under the influence of environment. The result is in line with the finding of Gezahegn et al. (2015) and Arati et al. (2015). Navin et al. (2014) reported higher PCV for grain yield

Table 6. Range, mean, variance, broad sense heritability, genotypic and phenotypic coefficient of variability, genetic advance as of mean for the 14 characters of bread wheat genotypes tested at Kulumsa in 2015/16.

Characters	Range	Mean ± S.E. mean	σ^2g	σ^2p	H^2	GCV (%)	PCV (%)	GA	GA (%)
Days to heading	47.67 - 66.33	56.3 ± 0.45	13.67	15.86	86.20	6.57	7.07	7.08	12.58
Days to maturity	96.67 - 108.00	101.83 ± 0.41	8.50	13.33	63.76	2.86	3.59	4.80	4.72
Grain filling period	34.67 - 49.33	45.53 ± 0.38	5.49	11.68	46.97	5.15	7.51	3.31	7.28
Plant height	67.17 - 88.67	78.82 ± 0.7	21.07	27.54	76.51	5.82	6.66	8.28	10.51
Grain yield	2588.30 - 4683.30	3676.22 ± 68.8	143794.71	287589.42	50.00	10.32	14.59	553.17	15.05
1000 kernel weight	27.61 - 51.26	43.35 ± 0.59	20.62	26.29	78.41	10.47	11.83	8.30	19.14
Hectoliter weight	65.41 - 75.90	73.22 ± 0.27	3.00	5.90	50.73	2.36	3.32	2.54	3.47
Above ground biomass	6000 - 15000	10816.67 ± 183.84	1537587.8	2614391.40	58.81	11.46	14.95	1961.8	18.14
Harvest index	0.24 - 0.44	34.4 ± 0.006	0.00149	0.00267	55.80	0.11	0.15	0.06	0.17
Tillers per plant	1.93 - 4.33	2.73 ± 0.08	0.14	0.33	42.20	13.57	20.89	0.50	18.19
Spikes per plant	1.83 - 4.20	2.59 ± 0.08	0.11	0.26	42.10	12.65	19.50	0.44	16.94
Spikes length	6.67 - 10.00	8.45 ± 0.11	0.41	0.51	80.11	7.60	8.49	1.19	14.04
Spikelets per spike	14.73 - 20.53	18.27 ± 0.14	1.03	1.58	65.26	5.56	6.89	1.69	9.27
Kernels per spike	39.53 - 54.47	45.78 ± 0.49	6.55	15.44	42.41	5.59	8.58	3.44	7.51

σ^2g = genotypic variance; σ^2p = phenotypic variance H^2 = Broad sense heritability; GCV= Coefficient of genotypic variance; PCV= coefficient of phenotypic variance; GA= genetic advance.

Table 7. Range, mean, variance, broad sense heritability, genotypic and phenotypic coefficient of variability, genetic advance as of mean for the 12 characters of bread wheat genotypes tested at across location in 2015/16.

Characters	Range	Mean ± S.E. mean	σ^2gl	σ^2g	σ^2p	H2	GCV (%)	PCV (%)	GA	GA (%)
Days to heading	46.83 - 66.83	55.78 ± 0.43	4.24	19.16	23.17	82.69	7.85	8.63	8.12	14.56
Days to maturity	96.83 - 112.50	103.18 ± 0.38	3.05	11.24	14.85	75.65	3.25	3.73	5.95	5.76
Plant height	66.93 - 85.47	77.24 ± 0.48	5.91	13.30	21.45	61.99	4.72	6.00	5.86	7.58
Grain yield	1936.40 - 4095.60	3271.01 ± 58.92	177811.85	67459.95	230384.82	29.28	7.94	14.67	286.71	8.77
1000 kernel weight	26.14 - 43.41	38.41 ± 0.53	4.54	11.88	16.98	69.96	8.97	10.73	5.88	15.31
Hectoliter weight	69.39 - 78.74	75.97 ± 0.30	1.06	2.39	4.81	49.79	2.04	2.89	2.23	2.93
Harvest index	0.19 - 0.36	0.29 ± 0.005	9.6x 10-4	3.6 x10-4	1.3x10-4	28.21	6.60	12.43	0.02	7.15
Tillers per plant	2.23 - 3.82	2.77 ± 0.05	0.02	0.01	0.17	8.51	4.32	14.81	0.07	2.57
Spikes per plant	2.00 - 3.77	2.63 ± 0.05	0.02	0.04	0.17	20.66	7.13	15.68	0.17	6.61
Spikes length	7.10 - 9.70	8.20 ± 0.07	0.04	0.42	0.50	82.85	7.87	8.65	1.20	14.62
Spikelets per spike	15.20 - 20.55	18.08 ± 0.11	0.39	0.86	1.41	61.19	5.13	6.56	1.48	8.19
Kernels per spike	34.22 - 53.13	43.24 ± 0.47	5.59	7.77	16.36	47.51	6.45	9.35	3.92	9.07

σ^2gl= genotype by environment interaction variance; σ^2g= genotypic variance, σ^2p = phenotypic variance; H^2 = broad sense heritability; GCV = Coefficient of genotypic variance; PCV = coefficient of phenotypic variance; GA = genetic advance.

per plant (28.43), tillers per plant (25.027), above ground biomass yield per plant (23.038), harvest index (23.03) and test weight (18.64) which contradicted this finding.

Estimates of heritability: Broad sense heritability (H^2) which was estimated for the 14 character, ranged from 30.62 to 89.44% at Tongo and 35.82 to 87.81 at Kulumsa (Tables 5 and 6). Pramoda and Gangaprasad, (2007) categorized heritability estimates as low (<40%), medium (40-59%), moderately high (60-79%), and very high (≥80). Accordingly, high heritability estimates were recorded (>80%) at Tongo for the characters; days to heading (86.04%) and days to maturity (85.07%) whereas medium to moderately high heritability were recorded for characters, grain filling period (50.36%), plant height (54.77%), grain yield (62.61%), 1000 kernel weight (68.44%), harvest index (68.19%), spikes length (75.17%), spikelets per spike (63.22%), kernels per spike (58.56%), hectoliter weight (45.76%) and above ground biomass yield (43.48%). The use of breeding will likely be successful in improving these traits or wheat genotype selections based on phenotype are effective. Low heritability estimates were recorded for tillers per plant (34.28%) and spikes per plant (38.44%) (Table 5).

At Kulumsa high heritability estimates were recorded (>80%) for the characters; days to heading (86.20%) and spikes length (80.11%) indicating that the variation observed were mainly under genetic control and were less influenced by the environment and the possibility of progress from selection. Moderate heritability were recorded for days to maturity (63.76%), plant height (76.51%), grain yield (57.99%), 1000 kernel weight (78.41%), hectoliter weight (50.73%), above ground biomass yield (58.81%), harvest index (55.80%), kernels per spike (65.25%). The result of harvest index, grain yield and hectoliter weight were in line with Gezahegn et al. (2015). Medium heritability estimates were recorded (≤50%) for the characters; grain filling period (46.97%), spikelets per spike (42.41%) tillers per plant (42.20%), spikes per plant (42.10%) indicating that the variation observed were mainly due to influence of the environment.

For combined analysis the estimated heritability for the studied traits is presented in Table 7. The heritability values ranged from 8.51 to 82.85 %. High heritability (>80%) was computed for days to heading and spike length indicating selection could be fairly easy and improvement is possible using these traits in breeding. Adhiena (2015) reported high heritability for days to heading which support this finding. Similarly, Gergana and Bozhidar (2015) and Desheva and Cholakov (2014) reported high heritability value for spike length. In the same year Gergana and Bozhidar (2015) reported high estimates of heritability (above 60%) for five characters spike length with awns (74.93%), spike length without awns (80.48%), spikelets per spike (63.96%), grain weight per spike (67.47)% and thousand grain weight (73.51%) in their study on variability, heritability, genetic advance and associations among characters in emmer wheat genotypes. Medium to moderate heritability was recorded for days to maturity (75.65%), plant height (61.99%), 1000 kernel weight (69.96%), hectoliter weight (49.79%), spikelets per spike (61.19%) and kernels per spike (47.51%). Arati et al. (2015), Navin et al. (2014) and Ali et al. (2008) also reported high heritability estimates for grain yield per plant, number of seeds per spike, plant height and 1000 seed weight which support the present findings. Low heritability was recorded for the characters grain yield (29.28%), harvest index (28.21%), tiller per plant (8.51%) and spikes per plant (20.66%). This result is contradicted with the finding of Gergana and Bozhidar (2015) who reported high heritability for tillers per plant and spikes per plant. Selection may be considerably difficult or virtually impractical for less heritable due to the masking effect of the environment.

Estimates of expected genetic advance: Genetic advance as percent mean was categorized as low (0-10%), moderate (10-20%) and high 20% and above (Johnson et al., 1955). Accordingly, the expected genetic advance as the percent of means expressed as a percentage of the mean ranged from 3.46% for hectoliter weight to 28.45% for gain yield at Tongo (Table 5). High GAM was observed in grain yield (28.45%) and harvest index (24.28%). In accordance with finding of Arati et al. (2015) and Navin et al. (2014) who reported similar result with this study. GAM was moderate for days to heading (19.71%), 1000 kernel weight (17.63%), above ground biomass yield (10.88%), tillers per plant (13.44%), spikes per plant (16.45%), spikelets per spike (15.73%) and kernels per spike (10.98%). GAM was low for days to maturity, grain filling period, plant height and hectoliter weight.

At Kulumsa the expected genetic advance expressed as a percentage of the mean ranged from 0.17% for harvest index to 19.14% for 1000 kernel weight (Table 6), indicating that selecting the top 5% of the base population could result in an advance of 0.17 to 19.14% over the respective population mean. GAM was moderate for 1000 kernel weight plot (19.14%) followed by spikelets per spike, tillers per plant, above ground biomass yield, spikes per plant, spikes length, days to heading, plant height in conformity with the findings by Gezahegn et al. (2015) and Awale.et al. (2013) for the traits, 1000 kernel weight per plot (20.13%), grain yield (14.85%), days to 50% heading (14.70%) and number of grains per plant(14.65%) except for harvest index (15.68%).

Genetic advance expressed as percentage of mean from the combined analysis (Table 7) was moderate for days to heading (14.56%), 1000 kernel weight (15.31%) and spikes length (14.62). Gergana and Bozhidar (2015) reported moderate for spikes length (31.83%) and thousand grains weight (33.76%). Mohammed et al. (2011) and Navin et al. (2014) also reported high genetic advance (as percentage of mean) for grain yield and yield related traits like thousand kernel weight and harvest

index which are similar with the present finding. Awale et al. (2013) reported high genetic advance for days to heading, grain filling period, number of tillers, 1000 seed weight, plant height, peduncle length and spike length which are similar with this study except for number of tillers and grain filling period. This suggested selection could be effective in genotypes for these traits and the possibility of improving bread wheat grain yield through direct selection for grain yield related traits. Low genetic advance as percent of the means were recorded for the characters grain yield, harvest index, plant height, spikes per plant, spikelets per spike and kernels per spike, days to maturity, hectoliter weight and tillers per plant. The result is not in line with finding of Gergana and Bozhidar (2015) who reported high genetic advance as a percent of the mean for the characters, number of productive tillers per plant and plant height which are low in this study. Characters like days to heading, 1000 seed weight and spike length showed high heritability coupled with moderate genetic advance. Therefore, these characters should be given top priority during selection breeding in wheat. The results are in accordance with reports of Navin et al. (2014) for the character 1000 kernels weight and Desheva and Cholakov (2014) for spike length indicated that heritability was due to additive gene effects and selection may be effective in early generations for these traits. Gezahegn et al. (2015) reported that high heritability couple with moderate genetic advance as percent of mean for days to 50% heading (82.06 and 14.70%), 1000 kernel weight (74.28 and 20.13%), plant height (69.43 and 10.27%) and spike length (63.66 and 10.34%), respectively, which support the present study. High heritability associated with low genetic advance was exhibited by days to maturity (85.93 and 9.26). This may be because of predominance of non-additive gene action in the expression of this character. The high heritability of these traits was due to favorable influence of environment rather than genotypic and selection for these traits may not be rewarding.

Conclusion

The study revealed the existence of significant genetic variability among the tested genotypes and heritability for different traits confirmed possibility to increase wheat productivity in target area. Attention should be given for traits which has moderate to high heritability and genetic advance in order to bring an effective response of grain yield improvement. Hence, selection and hybridization on those genotypes based on the trait with high GCV, heritability and genetic advance can be recommended for farther yield improvement of bread wheat at respective location.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix

Table 1. Mean performance of 30 bread wheat genotypes tested at Kulumsa.

ENT	Genotypes	DTH	DTM	GFP	PTH	GY	TKW	HLW	BY	HI	TPP	SPP	SL	SPS	KPS
1	Hidasse	56.67	103	46.33	76.67	4536.7	48.81	73.01	11333	0.40	4.33	3.93	7.2	14.73	43.27
2	ETBW 6861	59	104	45	78.33	3845	40.54	73.44	10333	0.37	2.20	2.17	9.43	18.47	49.53
3	ETBW 8506	52.67	97.33	44.67	69.83	2640	44.39	75.90	6000	0.44	2.60	2.43	8.13	16.87	40.8
4	ETBW 8507	55	101.33	46.33	72.5	3151.7	48.28	68.97	10667	0.30	3.00	2.13	8.43	17	41.47
5	ETBW 7120	55.67	105	49.33	82.17	4001.7	46.55	72.66	11333	0.35	2.30	2.27	8.7	19.13	42.47
6	ETBW 8508	56.33	103.67	47.33	67.17	2876.7	41.66	74.53	10000	0.29	2.20	1.83	6.67	17.87	39.63
7	ETBW 7213	66.33	108	41.67	83.67	3538.3	35.65	72.58	11333	0.31	1.93	1.83	9.03	20.53	48.73
8	ETBW 8509	58	106	48	79	3753.3	34.05	73.05	11000	0.34	2.60	2.40	8.17	19.57	52.2
9	ETBW 7038	49.33	97.67	48.33	72.83	3625	45.98	75.16	10500	0.34	2.27	2.23	7.5	18.13	42.83
10	ETBW 8510	52.33	99	46.67	69.67	4126.7	42	75.61	10833	0.38	2.70	2.40	9.2	18.77	44.6
11	ETBW 7058	56.33	100.33	44	88.67	3563.3	46.42	72.93	10833	0.33	2.13	2.10	8.27	17.73	39.53
12	ETBW 8511	63	97.67	34.67	83.83	2588.3	27.61	65.41	10833	0.24	3.13	2.33	9.1	20.27	47.63
13	ETBW 7147	65.67	108	42.33	87	4016.7	34.58	68.47	15000	0.27	3.50	3.50	9.5	18.6	45.2
14	ETBW 8512	51.67	97.33	45.67	74	2935	44.04	73.52	10167	0.29	2.23	2.23	7.47	18.23	47.27
15	ETBW 7871	55.67	99.67	44	81.67	4683.3	44.42	74.48	11500	0.41	2.70	2.47	8.17	19.2	41.5
16	ETBW 8513	56.67	104.33	47.67	83	3215	44.27	74.71	11000	0.29	2.93	2.73	8.9	18.8	48.27
17	ETBW 6940	60	108	48	87.83	4011.7	39.39	73.11	11167	0.36	2.73	2.67	9.2	18.4	46.17
18	ETBW 8514	55.67	101.67	46	76.17	3998.3	45.37	74.35	11000	0.37	2.47	2.40	7.77	17.07	46.93
19	ETBW 7368	56	99.67	43.67	78.5	3790	46.69	75.47	10667	0.36	2.47	2.37	10	18.13	49.1
20	ETBW 8515	54.33	100.33	46	79.17	3730	44.65	73.97	12333	0.31	4.23	4.20	8.5	18.8	54.47
21	ETBW 7364	56.33	102.33	46	76	3308.3	46.21	72.93	9667	0.35	3.00	2.90	8.77	17.8	45.97
22	ETBW 8516	54.67	101	46.33	72.5	3935	49.45	73.91	10500	0.37	3.07	2.87	8.4	17.87	50.73
23	ETBW 7194	62.33	102.33	40	79.83	4180	36.74	72.93	11333	0.37	1.97	1.97	7.93	19.07	45.4
24	ETBW 8517	55.67	100.33	44.67	76.83	3958.3	45.65	73.63	11167	0.36	2.9	2.9	8.23	16.1	43.9
25	ETBW 7101	52.67	96.67	44	79	2753.3	45.6	73.75	9000	0.31	3.27	3.23	8.87	19.47	44.63
26	ETBW 8518	47.67	97	49.33	71	4168.3	51.26	74.89	9667	0.44	2.37	2.17	8	18.47	46.33
27	ETBW 7872	58	104	46	88.67	4495	43.8	74.37	14000	0.32	2.43	2.27	9.17	18.13	47.13
28	ETBW 8519	53.67	100	46.33	77.33	4018.3	46.51	73.27	10500	0.39	2.47	2.57	7.53	17.97	46.23
29	ETBW 6937	56.67	106	49.33	87.5	3350	43.23	73.7	9500	0.36	2.53	2.4	9.87	18.6	45.97
30	Danda'a	55	103.33	48.33	84.37	3493.3	46.81	71.95	11333	0.31	2.37	2.23	7.37	18.27	45.4
Mean		56.3	101.83	45.53	78.82	3676.2	43.35	73.22	10817	0.34	2.7	2.54	8.45	18.27	45.78
CV		2.63	2.16	11.42	3.23	10.39	5.5	2.33	9.59	9.99	16.05	15.15	3.79	6.51	4.06
LSD at 5%		2.44	3.62	4.1	4.19	624.4	3.92	2.81	1708.7	0.06	0.7141	0.6329	0.53	4.91	1.22
LSD at 1%		3.26	4.84	5.48	5.6	834.45	5.24	3.75	2283.5	0.08	0.95	0.85	0.7	6.56	1.63

Table 2. Mean performance of 30 bread wheat genotypes tested at Tongo.

ENT	Genotypes	DH	DM	GFP	PTH	GY	TKW	HW	BY	HI	TPP	SPP	SL	SPS	KPS
1	Hidasse	50	101	51	75.27	3654.4	38	79.23	13000	0.28	4.3	4.2	7.03	15.67	37.27
2	ETBW 6861	59	106.67	47.67	76.1	3293.7	30.67	78.97	12666.7	0.26	2.77	2.5	7.83	17.73	38.67
3	ETBW 8506	47.33	97	49.67	70.1	3287.7	35.33	81.17	11500	0.28	3.37	3.03	6.69	15.77	35.33
4	ETBW 8507	47.67	97.67	50	73.27	2828.3	37.33	79.93	10833.3	0.26	2.97	2.97	7.2	15.17	37
5	ETBW 7120	53.67	106	52.33	72.63	2441.2	38	78.3	11000	0.22	3.37	3.17	8.27	18.63	27.47
6	ETBW 8508	50	102.67	52.67	66.7	2019.9	36	81.17	10333.3	0.2	2.53	2.43	7.53	17.43	28.8
7	ETBW 7213	67.33	114.33	47	79.17	3410	30	78.2	14166.7	0.24	2.2	2.03	9.3	20.13	47.9
8	ETBW 8509	63	111	48	79.37	3019.8	29.33	79.37	13333.3	0.23	2.93	2.77	8.4	21.53	54.07
9	ETBW 7038	47.67	99	51.33	69.5	2650.6	33.33	80.77	10000	0.27	3.63	3.6	7.13	16.67	43.27
10	ETBW 8510	50.67	99	48.33	73.67	3003.5	32	81.87	11666.7	0.26	2.3	2.07	8	18.1	45.37
11	ETBW 7058	55	104.67	49.67	75.57	2815.8	34.67	78.83	11000	0.25	3.6	3.47	7.4	16.9	32.3
12	ETBW 8511	63.33	109	45.67	73.3	1284.4	24.67	73.37	10000	0.13	2.83	2.73	8.8	19.7	43.93
13	ETBW 7147	65	112.33	47.33	75.87	2867.5	28.67	75.23	12000	0.24	2.8	2.57	8.23	18.13	38.17
14	ETBW 8512	48.67	99	50.33	78.33	3180.4	36	80.03	11000	0.29	2.47	2.37	7.2	16.53	43.77
15	ETBW 7871	61.67	104	42.33	77.13	3420.4	31.33	81.03	12333.3	0.28	2.5	2.43	8.3	18.07	41.73
16	ETBW 8513	60	110.67	50.67	78.63	3615.2	37.33	80.9	12333.3	0.29	2.77	2.57	8.37	20	46.33
17	ETBW 6940	70.33	117	46.67	78.57	2614.5	30.67	75.9	13500	0.19	2.93	2.47	9.2	20.3	45.13
18	ETBW 8514	55.67	104.33	48.67	79.37	3788.7	36.67	80.3	12666.7	0.3	2.43	2.27	8.13	19.03	41.57
19	ETBW 7368	55.67	103	47.33	83.5	3242.5	34.67	81.7	12000	0.27	2.4	2.2	9.4	18.5	43.23
20	ETBW 8515	50.33	100	49.67	73.23	2428.2	33.33	77.77	10833.3	0.22	2.63	2.37	7.17	15.9	44.23
21	ETBW 7364	54.33	106	51.67	74.03	2896.3	38.67	78.43	11833.3	0.25	2.7	2.37	7.5	18.27	33.07
22	ETBW 8516	49	102	53	70.37	1923.8	28	71.33	9000	0.21	2.77	2.6	7.97	17.93	42.2
23	ETBW 7194	64.33	108.33	44	73.2	1625.9	24.67	75.5	10833.3	0.15	2.57	2.03	7.5	17.33	36.93
24	ETBW 8517	58	106.67	48.67	80.87	3265	35.33	78.57	12666.7	0.26	3.17	3.5	8.2	17.27	43.37
25	ETBW 7101	48	97	49	78.33	2873	32.67	79.37	10666.7	0.27	2.87	2.53	7.83	17.57	39.5
26	ETBW 8518	46	97.33	51.33	63.27	2594.6	35.33	78.97	9833.3	0.27	2.43	2.4	6.6	15.93	35.63
27	ETBW 7872	56.33	104	47.67	82.27	3342.2	37.33	81.47	12500	0.26	2.83	2.4	9.67	18.87	47.67
28	ETBW 8519	48	100	52	73.3	3157.6	36	77.77	11166.7	0.28	3	2.87	7.3	16.7	43.5
29	ETBW 6937	57	111.67	54.67	81.53	2817.2	30.67	79.27	12833.3	0.21	2.63	2.4	9.13	19.6	44.47
30	Danda'a	54.67	104.67	50	83.4	2611.9	37.33	77.1	11333.3	0.23	3.83	3.6	7.27	17.23	39.13
Mean		55.26	104.53	49.28	75.66	2865.8	33.47	78.73	11627.8	0.25	2.88	2.7	7.95	17.89	40.7
CV		4.2	1.8	4.31	5	13.62	7.09	2.73	9.22	9.84	15.59	16.45	5.11	5.16	9.29
LSD at 5%		3.82	3.09	3.5	6.23	642.57	3.91	3.54	1764.9	0.04	0.74	0.73	0.67	6.22	1.52
LSD at 1%		5.1	4.13	4.68	8.33	858.72	5.22	4.73	2358.6	0.05	0.99	0.98	0.89	8.32	2.03

DH, Days to heading; DM, Days to maturity; GFP, Grain filling period; GY, Grain yield; TKW, 1000 kernel weight; AGB, Above ground biomass ($kgha^{-1)}$; HI, Harvest index; HW, Hectoliter weight; TPP, Tillers per plant; PH, Plant height (cm); SPS, Spikelets per spike; KPS, Kernels per spike; SL, Spike length; SPP; Spikes per plant. LSD= List significant difference; CV= Coefficient of variations.

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Phenotype characterization and diversity assessment of mango (*Mangifera indica* L.) cultivars in Ethiopia

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Little efforts have been made on mango genetic resource assessment in Ethiopia though it is one of the major fruit crops. This study was conducted to assess the diversity of 69 mango cultivars of different growing regions of the country based on 44 phenotypic descriptors. The results of both univariate and multivariate analysis of variance computed for quantitative data, and results from descriptive statistics for qualitative characters indicated the presence of phenotypic variation among the cultivars. Further analysis of Principal Component Analysis (PCA) indicated the first four components explained more than 75% of the total variation in which most fruit, seed and leaf characters contributed much to the observed variation. The cultivars were grouped into 13 clusters by Unweighted Pair Group Method with Arithmetic Means clustering method from the Euclidean distances estimated from phenotypic characters. The three clusters (II, X, and XIII) constructed each by one cultivar while others encompass more than one irrespective of their geographic regions. This indicated the presence of diversity among cultivars in Ethiopia which can be exploited for further improvement, use, and conservation of mango genetic resources.

Key words: Cluster, Euclidean distances, genetic resources, principal component.

INTRODUCTION

Mango (Mangifera indica L.) is amongst the most widely grown tropical and subtropical fruits of the world (Rajwana et al., 2011). The origin of cultivated mango is believed to be eastern India, Assam-Burma region; and South East Asia is believed to be the center of diversity for Mangifera genus (Begum et al., 2014; Kaur et al., 2014). More than 1000 varieties of M. indica L. have been identified all over the world (Rymbai et al., 2014). It is thought to have been introduced to East Africa by the Persians in the 10th century A.D. and the crop started growing in West Africa in the 16th century A.D. (Janick, 2005; Rey et al., 2006).

World mango production is spread over 100 countries that produce over 38.67 million tons of fruit annually (Mitra, 2016). India is the largest producer in the world (18.0 million tons per year), while the leading producer in Africa is Kenya (582,907 ton per year) (FAO, 2015). Mango is the second most important fruit crop in Ethiopia,

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after banana. It constituted 16.01% of 92,362.36 ha of land under fruit crops and 14.76% of 6,797,42.83 tons of produced fruit (CSA, 2015). Moreover, both its area of coverage and production increased by 208.4 and 247%, respectively from 2003 to 2013 (Dessaleg et al., 2014). Western and eastern Ethiopia are among the major growing regions that account for 28 and 23% of the wholesale market share of Addis Ababa (capital city of Ethiopia), respectively (Ssemwanga et al., 2008).

Despite the crop's potential and increasing production trend in Ethiopia, it is hampered by various biotic and abiotic factors (Dessalegn et al., 2014). The majority of farmer cultivars are raised from seedlings arising from a natural cross population and consequently, the trees are of mixed origin and difficult to identify (Bezu et al., 2014). Moreover, growers are replacing the existing locally adapted landraces with recently introduced commercial varieties. Although, the landraces could be having desirable traits such as high and stable yield, low management requirements, low susceptibility to pests and high drought tolerance (Govindaraj et al., 2015; Sennhenn et al., 2013). Very little information has been documented on Ethiopian local mango varieties. Such information is important and could be utilized in the conservation of the valuable genetic resource. There is also much confusion and uncertainty concerning the identity of local mango cultivars due to the usage of different local names for the same varieties.

Characterization of varieties is a necessary requirement for crop improvement, use, and conservation of plant genetic resources (Khan et al., 2015; Krishna and Singh, 2007; Rajwana et al., 2011). Phenotype characterization is the first step before biochemical and molecular markers due to its simplicity, low-cost requirement, standardized, repeatable method and availability of published descriptors for most major crops (Khan et al., 2015; Mohamed and Ahmed, 2015; Ravani and Joshi, 2013). In the last decade, various phenotype markers have been successfully applied in determining the intra cultivar diversity of mango in different parts of the world (Ahmed and Mohamed, 2015; Mohamed and Ahmed, 2015; Preisigke et al., 2013; Rajwana et al., 2011; Rymbai et al., 2014). However, such vital studies have not been conducted on mango in Ethiopia that is on the verge of extinction. Therefore, the objective of this research was to assess the phenotype variation and thereby to estimate the diversity of cultivars across major growing regions of Ethiopia.

MATERIALS AND METHODS

Description of experimental sites

Four major mango growing districts were selected: *Babile* (eastern Hararghe Zone, Oromia Regional State); *Erer-Woldia* and *Sofi* (Harari People's National Regional State) from east and *Asosa* (Benishangul Gumuz Regional State) from western Ethiopia. In addition, mango collections conserved at Melkasa Agricultural Research Center located in central Ethiopia were also included in the study (Figure 1).

Sampling of experimental materials

Survey of mango cultivars was carried out on 113 farmers purposively selected from the four growing districts and Mango orchards of Melkassa Agricultural Research center in 2016. The study sites were selected based on their extensive mango production, as was inferred through consultation with the districts agricultural officers from the Ministry of Agriculture and key informants from the Ethiopian Agricultural Research Institute. Of the germplasm surveyed, 69 cultivars (53 from farmer's field and 16 from Melkassa Agricultural research center) of unknown genetic origin were selected considering their local naming, geographic locations, accessibility, age and distinct features of the trees. The geographic location of each of the sampled trees was recorded using a global positioning system (GPS) along with location information and local names (Table 1).

Phenotype characterization

A total of 44 characters, 15 quantitative and 29 qualitative traits were evaluated according to IPGRI (2006). The qualitative data were collected on the farm while the quantitative data was recorded from randomly collected healthy and undamaged ten leaves and 20 ripe fruits of each sampled tree in Horticulture Laboratory of the Haramaya University, Ethiopia. The collected sample leaves and fruits of each tree were randomly assigned into three replications to conduct an analysis of variance for completely randomized design for a better estimate of error variances (Gomez and Gomez, 1984).

Data analysis

Descriptive statistics (mean, percentage, standard deviation) and Chi-square test of qualitative characters were performed using the Statistical Package for Social Scientists (SPSS) Version 21.0. Variations among the cultivars for all quantitative traits except trunk circumference and crown diameter that were recorded from the single tree were computed using the analysis of variance (ANOVA) for completely randomized design. Moreover, multivariate analysis of variance (MANOVA) was done to test if the combined dependent variables (quantitative traits) were significantly affected by cultivars using SAS software version 9.1. Standardization of data was conducted following the z-score transformation method (Ramette, 2007). Principal Component Analysis (PCA) was done to identify traits that explain the phenotype variability best. Then, clustering of cultivars following the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) (Sneath and Sokal, 1973), where the distance between two clusters is the average distance between all inter-cluster pairs, was made using GENES version 2015.05 program (Cruz, 2013).

RESULTS AND DISCUSSION

Variation of mango cultivars in quantitative traits

The mango cultivars had a wide range of circumference of tree ranging from 59 to 370 cm with a coefficient of variation (CV) of 44.7%. The crown diameter of the tree also ranged from 3.5 to 20 m with a CV of 39.7%. The univariate analysis of variance computed for 13

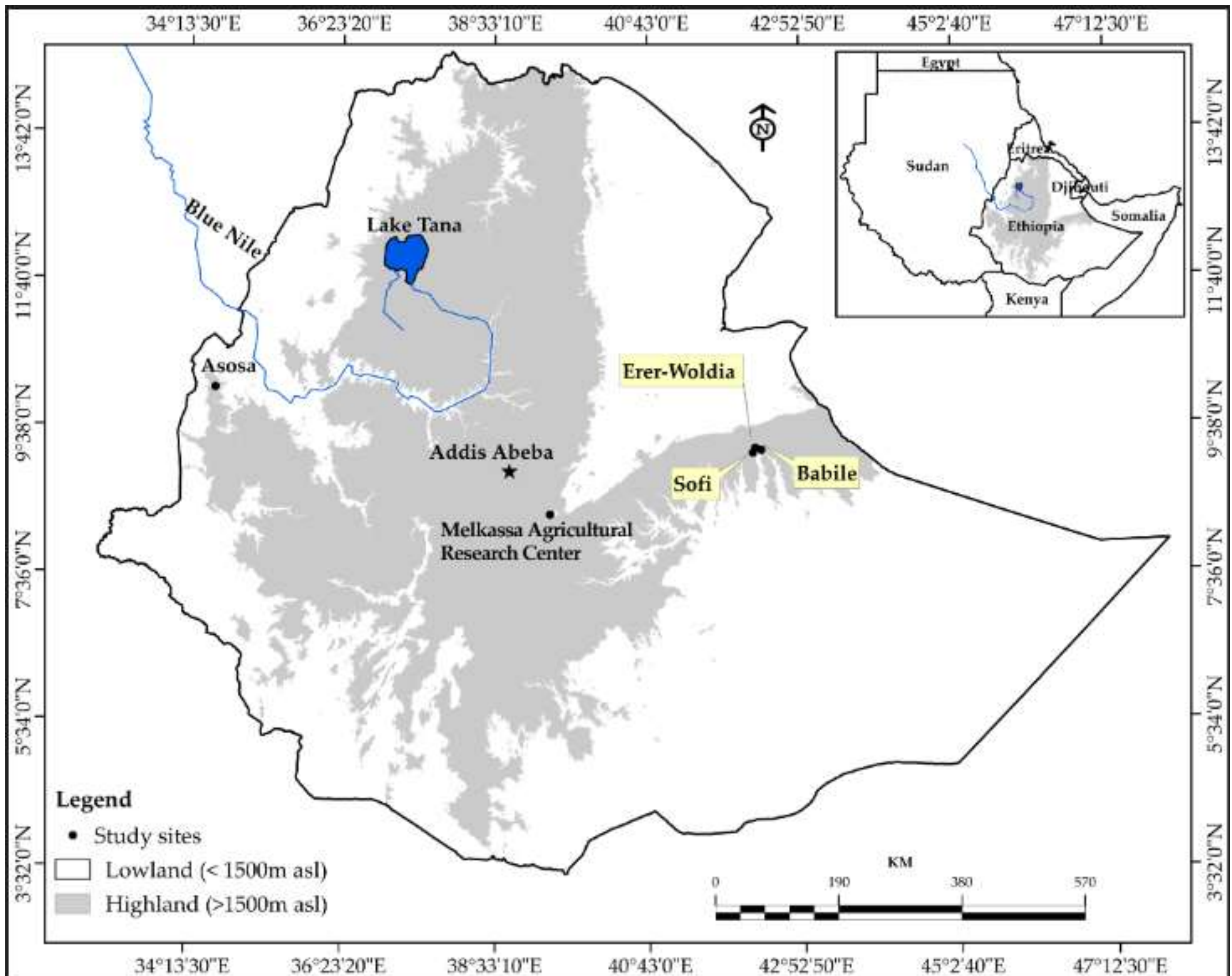

Figure 1. Geographic locations of districts in east, central and western Ethiopia where mango cultivars were sampled.

quantitative traits revealed a highly significant difference ($p < 0.01$) among cultivars (Table 2). In addition, the multivariate analysis of variance as predicted, based on the Wilk's lambda criterion, showed the combined quantitative traits were also significantly ($p < 0.001$) affected by the cultivars (Table 3). This suggested the cultivars varied for all the quantitative traits that could be used for further breeding work. Similarly, authors from India (Singh et al., 2012; Bajpai et al., 2016) and Kenya (Toili et al., 2016; Gitahi et al., 2016) also reported the presence of significant differences among mango cultivars they studied considering similar quantitative traits.

The leaf length and width of the cultivars ranged from 14.3 to 29.9 cm and 1.3 to 6.7 cm, respectively. Fruit weight ranged between 81.3 and 1094 g while petiole length, fruit length and fruit diameter ranged from 2.3 to 6.5 cm, 5.8 to 13.6 cm and 4.3 to 9.8 cm, respectively. The cultivars also showed significant variation for pulp content, stone weight and seed weight that ranged between 59.4 and 1015.6 g, 15.9 and 243.8 g, and 3.5 and 100.6 g, respectively. Similarly, the stone and seed characters exhibited a significant variation (Table 2). Although there is a lack of information about the aforementioned attribute in Ethiopia, the range of the leaf and fruit characters are comparable with Ahmed and Mohamed (2015), Rajwana et al. (2011), Krishnapillai and Wijeratnam (2016), and Gálvez-López et al. (2010) findings on mango cultivars in Sudan, Pakistan, Srilanka and Mexico, respectively. Likewise, the fruit stone and seed characters of studied cultivars were comparable with cultivars studied by Gitahi et al. (2016), Ahmed and Mohamed (2015), Al-Yahyai et al. (2013) and Alcasid et al. (2015). These findings, therefore, indicate the

Table 1. The lists of 69 mango cultivars collected from three geographical regions of Ethiopia.

Cultivars name and codes	No. cultivars	Region		Location		
		Geographic	District	Latitude (N)	Longitude (E)	Altitude (m)
Local -1 (AS01), Local -2, (AS03), Local -3, (AS05), Molala 02 (AS06), Molala 01 (AS07), Debulbul 01 (AS08), Asosa mango (AS09)	7	Western Ethiopia	Assosa	10°08'05" to 10°10'39"	034°37'10" to 034°39'17"	1488 to 1517
Amba Lafe (BA01), Amba Shuto (BA02), Amba Guracha (BA03), Amba Dula (BA04), Amba Adi (BA05), Amba Sabune (BA06), Amba Harewe (BA07), Amba Hudha (BA08), Amba Sukara (BA10), Amba Ako (BA11), Amba Fulla (BA12)	11	Eastern Ethiopia	Babile	09°17'58" to 09°17'59"	042°17'24" to 042°17'27"	1759 to 1768
Amba Teyara (ER01), Amba Ako (ER02), Amba Mucho (ER03), Amba Hula (ER04), Amba Guracha (ER05), Amba Harewe (ER06), Amba Neguse (ER07), Amba Shimbro (ER08), Amba Ahmed Abdulahi (Big) (ER09), Amba Adi (Harewe) (ER10), Amba Umar Alisho Guracha (ER11), Amba Ahmed Lilo (ER12), Amba Sadik (ER13), Amba Sibake (ER14), Amba Amin Abdela Yusuf (ER15), Amba Arejata (ER16), Amba Gerjewi (ER17), Amba Sabid (ER18), Amba UmarAlisho Adi (ER19, Amba Bere (ER20)	20	Eastern Ethiopia	Erer	09°19'58" to 09°21'25"	042°12'20" to 042°13'20"	1370 to 1446
Amba Dada (HA01), Amba Guracha (HA02), Amba Maity (HA03), Amba Seburu jena (HA04), Amba Errero (HA05), Amba Hula-01 (HA06), Amba Hula-02 (HA07), Amba Kukurfa (HA08), Amba Neguse (HA09), Amba Lawe (HA10), Amba Demma (HA11) , Amba Bishaano (HA13), Amba Libanato (HA14), Amba Neguse-01 (HA15), Amba Neguse-02 (HA16)	15	Eastern Ethiopia	Sofi	09°15'40" to 09°16'22.7"	042°10'22.7" to 042°11'29.6"	1493 to 1631
NE1.5 (ML01), NE6.3 (ML02), NE7.6 (ML03), W3.4 (ML04), NE2.4 (ML05), NE3.6 (ML06), ME1.2 (ML07), NE4.1 (ML08), ME2.4 (ML09), ME3.2 (ML10), ME7.5 (ML11), Sodere1.3 (ML12), Sodere2.8 (ML13), Sodere 3.7 (ML14), W1.9 (ML15), Sodere 11.3 (ML16)	16	Central Ethiopia	Melkassa Agricultural Research center	08°24'42" to 08°24'44"	039°19'28" to 039°°19'32"	1539 to 1554

presence of potential cultivars that could be used for breeding as well as commercial purpose.

Variation of mango cultivars for qualitative traits

Tree and leaf characters

The cultivars were largely non-grafted seedlings, irregular (alternate) bearing behavior, tree height range from medium to very tall group, broadly pyramidal to semi-circular crown shape, and spreading growth habit (Table 4). The alternate bearing which is dependent on agronomic practices (Saxena et al., 2014), environmental conditions and genetic makeup (Kaur et al., 2014), is a common phenomenon of mango. Most cultivated mango trees are between 3 and 10 m in height when fully matured depending on the way of pruning (Balley, 2006). However, mango trees can reach a height of 40 m or more (Mukherjee and Litz, 2009) while grafted ones are usually shorter (Khan et al., 2015). Tree canopies vary in genotypes, propagation method, the density of plantation, and prevailing agro-climatic conditions (Khan et al., 2015).

Intermediate foliage density, oblong leaf blade shape, semi-erect to horizontal leaf attitude in relation to branch, a medium category in the angle of secondary veins to the midrib, acuminate apex, acute base shape, and mild leaf fragrance were observed in the majority of the cultivars (Table 4). These characters are among the important attributes that could be utilized for classification of the cultivars (Sharma et al., 2016). Inline to this, Toili et al. (2016), Krishnapillai and Wijeratnam (2016), Joshi et al. (2013), Ribeiro et al. (2013) and Raza et al. (2017) report on mango cultivars in Kenya, Sri Lanka, Indian, Brazil and Pakistan, respectively showed significant variability with the aforementioned characters and suggested for

Table 2. Descriptive statistics and univariate analysis of variance of 13 quantitative traits of mango cultivars from Ethiopia.

Trait	Range	Mean±SE	Mean square		CV (%)
			Cultivars (68)	Error (138)	
Leaf length (cm)	14.3-29.9	20.7±0.3	35.2**	4.4	10.2
Leaf width (cm)	1.3-6.7	5.1±0.1	2.7**	0.3	10.0
Petiole length (cm)	2.3-6.5	3.9±0.18	2.6**	0.5	17.5
Fruit weight (g)	81.3-1094.0	324.0±13.9	112114**	4805.0	21.4
Fruit length (cm)	5.8-13.6	9.0±0.1	8.8**	0.3	6.1
Fruit diameter (cm)	4.3-9.8	7.1±0.1	4.5**	0.2	6.1
Pulp content (g)	59.4-1015.6	254.1±12.1	86320.5**	3028.1	21.7
Stone weight (g)	15.9-243.8	53.6±3.0	4228.2**	268.1	30.6
Stone length (cm)	4.7-11.3	7.1±0.1	7.9**	0.2	6.6
Stone width (cm)	2.1-6.8	3.8±0.1	3.3**	0.1	6.2
Seed weight (g)	3.5-100.6	25.1±1.1	705.5**	49.0	27.9
Seed width (cm)	3.9-10.4	5.9±0.1	3.3**	0.03	6.2
Seed length (cm)	1.4-5.9	2.9±0.1	6.3**	0.1	6.3
Trunk circumference (cm)[a]	59.0-370.0	145.2±7.8	-	-	44.7
Crown diameter (m)[a]	3.5-20.0	8.3±0.4	-	-	39.7

**Significant at $p<0.01$, SE=standard error and CV (%) = coefficient of variation in percent. [a] Analysis of variance was not computed since the data were collected from a single tree.

Table 3. Multivariate analysis of variance (MANOVA) test criteria and F approximations for the hypothesis of no overall cultivars effect on the overall quantitative traits.

Statistic	Value	F Value	Num DF[a]	Den DF[b]	Pr > F
Wilks' Lambda	0.0000000	76.91	884	1641.4	<0.0001
Pillai's Trace	11.7240904	18.38	884	1768	<0.0001
Hotelling-Lawley Trace	5087.0935063	703.23	884	1204.9	<0.0001
Roy's Greatest Root	4140.8113008	8281.62	68	136	<0.0001

[a]The number of degrees of freedom in the model. [b]The number of degrees of freedom associated with the model errors.

characterization of the cultivars they studied.

Fruit, stone and seed characters

The predominant fruit shape of the cultivars was oblong followed by roundish, obtuse fruit apex, absent fruit stalk cavity, absent to slightly neck prominence and perceptible beak type. The majority of the cultivars had orange, greenish yellow to yellow skin color and orange to yellow pulp color when ripe. The cultivars fruit attractiveness was from average to good though there were excellent attractive cultivars (26.1%). Most had low to intermediate fiber in fruit pulp, very juicy, intermediate aroma, and very good to excellent eating quality (Table 5). This indicated the potential of cultivars for the table as well as processing purpose if further studied (Jha et al., 2010; Vijayanand et al., 2015). Most cultivars in Shendi, Sudan also reported oblong fruit shape followed by round and obtuse fruit apex (Ahmed and Mohamed, 2015). A study by Kheshin et al. (2016) on some 'Sukkary' mango genotypes in Egypt also revealed a roundish fruit shape, smooth and waxy yellow skin, and obtuse shape of fruit apex. The predominant fruit shape of mangos in eastern Kenya was roundish and yellow-orange, orange-red and red colors of the fruit skin (Gitahi et al., 2016).

Elevated vein level with the surface of stone (85.5%) and parallel stone venation (85.5%) was recorded from the majority (89.9%) of cultivars. Moreover, 88.4and 62.3% of cultivars seed was reniform shape and monoembryony, respectively (Table 5). The difference in the cultivars embryony type is the most important trait that affects propagation methods (Kuhn et al., 2017). It could be associated with their origin where, most Indian cultivars are mono-embryonic, while generally cultivars from Indonesia, Thailand and the Philippines are reported polyembryonic (Damodaran et al., 2012; Griesbach, 2003).

Principal component analysis

Principal component analysis (PCA) for quantitative traits

Table 4. Summary of tree and leaf qualitative phenotype characters of the 69 mango cultivars from Ethiopia.

Characters[a]	Phenotypic classes[b]	χ^{2c}
Tree type	Seedling (76.8), Grafted (23.2)	19.8***
Regular bearer	Yes (13), No (87)	37.7***
Height of matured tree	Short (34.8), Medium (31.9), Tall (4.3), Very tall (29)	16.2***
Crown shape	Oblong (5.8), Broadly pyramidal (55.1), Semi-circular (30.4), Spherical (8.7)	43.3***
Tree growth habit	Erect (21.7), Spreading (71), Dropping (7.2)	46.3***
Foliage density	Sparse (5.8), Intermediate (91.3), Dense (2.9)	104.4***
Leaf blade shape	Oblong (81.2), Lanceolate (13.1), Elliptic (1.4), Obovate (2.9), Oblanceolate (1.4)	164.6***
Leaf attitude in relation to branch	Semi erect (59.4), Horizontal (37.7), Semi dropping (2.9)	33.7***
Angle of secondary veins to the midrib	Narrow (15.9), Medium (79.7), Wide (4.3)	68.2***
Curvature of secondary veins	Absent (14.5), Present (85.5)	34.8***
Leaf apex shape	Acute (14.5), Acuminate (85.5)	34.8***
Leaf base shape	Acute (78.3), Obtuse (21.7)	22.0***
Leaf fragrance	Absent (30.4), Mild (56.5), Strong (13)	19.8***

[a]Characters according to IPGRI (2006); [b]Numbers in brackets indicate the percentage of cultivars per class of trait. [c]Chi-squared test to indicate significant differences between phenotypic classes; *** significant at 0.1%.

Table 5. Summary of fruit, stone and seed qualitative phenotype characters of the 69 mango cultivars from Ethiopia.

Characters[a]	Phenotypic classes[b]	χ^{2c}
Fruit shape	Oblong (44.9), Elliptic (20.3), Roundish (31.9), Obovoid (2.9)	26.4***
Shape of fruit apex	Acute (5.8), Obtuse (60.9), Round (33.3)	31.4***
Fruit attractiveness	Poor (5.8), Average (33.3), Good (34.8), Excellent (26.1)	14.8***
Depth of fruit stalk cavity	Absent (59.4), Shallow (31.9), Medium (8.7)	26.7***
Fruit neck prominence	Absent (37.7), Slightly prominent (42), Prominent (15.9), Very prominent (4.3)	26.5***
Fruit beak type	Perceptible (72.5), Pointed (15.9), Prominent (7.2), Mammiform (4.3)	84.9***
Skin color of ripe fruit	Green (7.2), Greenish yellow (26.1), Orange (20.3), Yellow (37.7), Green with red blush (5.8), Green with purple patches (1.4)	56.7***
Pulp color of ripe fruit	Light yellow (5.8), Golden yellow (17.4), Yellow-orange (14.5), Orange (31.9), Greenish yellow (2.9), Yellow (23.2), Dark orange (4.3)	33.8***
Quantity of fiber in pulp	Absent (15.9), Low (43.5), Intermediate (33.3), High (7.2)	22.3***
Pulp juiciness	Slightly juicy (18.8), Juicy (33.3), Very juicy (47.8)	8.7**
Pulp aroma	Mild (14.5), Intermediate (60.9), Strong (24.6)	24.6***
Veins level with surface	Depressed (10.1), Elevated (89.9)	43.8***
Pattern of stone venation	Parallel (85.5), Forked (14.5)	34.8***
Seed shape	Ellipsoid (7.2), Oblong (4.3), Reniform (88.4)	94.3***
Type of embryony	Monoembryony (62.3), Polyembryony (37.7)	4.2*
Eating quality	Poor (2.9), Good (24.6), Very good (34.8), Excellent (37.7)	20.6***

[a]Characters according to IPGRI (2006); [b]Numbers in brackets indicate the percentage of cultivars per class of trait. [c]Chi-squared test to indicate significance differences of phenotypic classes; *, ** and *** significant at 0.05, 0.01 and 0.001%, respectively.

Table 6. Principal component loadings of 15 quantitative traits in 69 cultivars of mango in Ethiopia.

Traits	PC-1	PC-2	PC-3	PC-4
Fruit length (cm)	**0.88**	0.10	-0.07	-0.33
Fruit diameter (cm)	**0.70**	-0.06	-0.33	-0.18
Fruit weight (g)	0.43	**0.80**	-0.23	0.08
Pulp content (g)	0.43	**0.71**	-0.29	0.02
Stone length (cm)	**0.85**	0.01	-0.06	-0.33
Stone width (cm)	**0.67**	-0.34	-0.27	-0.05
Stone weight (g)	0.21	**0.80**	0.15	0.30
Seed length (cm)	**0.83**	-0.20	0.26	-0.15
Seed width (cm)	**0.68**	-0.45	0.02	0.17
Seed weight (g)	0.07	**0.81**	0.24	0.13
Trunk circumference (cm)	0.60	-0.26	0.12	**0.66**
Crown diameter (m)	0.55	-0.20	0.19	**0.69**
Leaf blade length (cm)	0.26	0.13	**0.75**	-0.37
Leaf blade width (cm)	0.17	0.03	**0.82**	-0.26
Petiole length (cm)	-0.05	-0.03	**0.56**	0.25
Eigenvalue	4.78	2.95	2.07	1.58
Variability (%)	31.87	19.65	13.81	10.57
Cumulative %	31.87	51.52	65.33	75.89

Values in bold indicate the variables that contributed most to the specific principal component and the squared cosine is the largest.

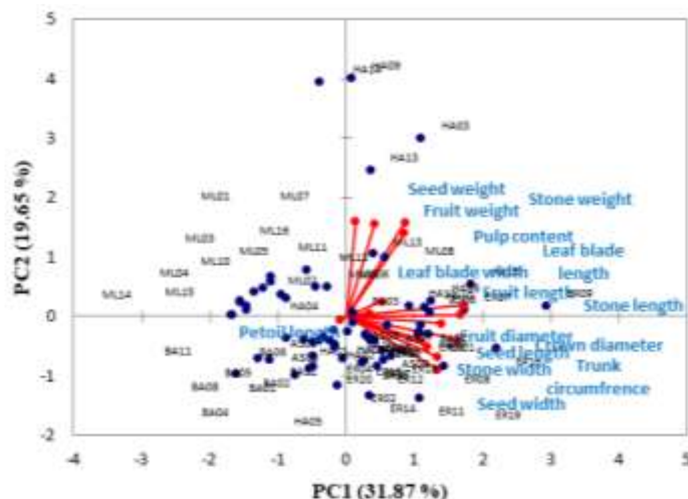

Figure 2. Biplot graphic with two principal components (PC1 and PC2:51.52%) for 15 quantitative traits of 69 mango cultivars in Ethiopia.

showed the first four components with Eigenvalues greater than one explained 75.89% of the total variation (Table 6). The first principal component (PC-1) accounted for 31.87% of the total variation, included fruit length, fruit diameter, stone length, stone width, seed length, and seed width. The second component (PC-2) explained 19.65% of the total variation and was associated with fruit weight, pulp content, stone, and seed weight. The third component (PC-3) that explained 13.80% of the total variation was mainly associated with leaf length, leaf width, and petiole length; and the fourth component (PC-4) accounted for 10.57% of the total variation correlated with trunk circumference and crown diameter. Moreover, the distribution of the cultivars based on the first two components (Figure 2) also showed the phenotypic variation among the cultivars and how widely dispersed they are along the axis. The aforementioned characters which contributed most to the observed variations were also reported by Krishnapillai and Wijeratnam (2016) and Majumder et al. (2013). Hence, it indicated to give greater

Table 7. Range and mean Euclidean distances of 69 mango cultivars in Ethiopia.

Cultivar	Range	Mean	SD	CV (%)	Cluster	Cultivar	Range	Mean	SD	CV (%)	Cluster
AS01	5.1-8.6	6.9	0.8	10.8	II	ER18	3.4-8.0	5.6	0.9	16.3	IX
AS03	4.5-9.7	7.1	1.1	14.8	X	ER19	3.5-8.2	5.7	1.0	17.4	IV
AS05	4.3-7.8	5.9	0.8	13.6	XII	ER20	3.6-8.5	5.7	1.2	21.4	IV
AS06	4.6-8.7	6.4	1.0	15.1	IV	HA01	4.2-8.1	5.9	1.0	16.1	IX
AS07	4.1-9.4	6.3	1.2	19.4	VI	HA02	3.2-7.3	5.1	0.9	17.4	IX
AS08	3.3-7.8	5.8	0.8	14.0	XII	HA03	4.9-9.1	6.8	1.0	14.5	I
AS09	3.9-8.8	6.2	0.9	15.1	IV	HA04	4.7-9.7	6.7	0.9	13.2	I
BA01	3.2-8.4	5.6	1.1	19.2	III	HA05	3.9-8.8	5.6	1.1	19.1	III
BA02	3.3-7.8	5.7	0.9	15.8	XII	HA06	3.5-7.6	5.6	0.9	16.1	IX
BA03	3.3-7.0	5.1	0.8	16.0	XII	HA07	3.7-8.0	5.4	0.9	15.6	VII
BA04	2.7-7.6	5.1	1.0	19.6	III	HA08	3.2-7.0	5.2	0.8	16.3	IX
BA05	3.3-7.9	5.6	0.9	16.5	XII	HA09	3.6-8.2	5.6	1.0	17.9	VI
BA06	3.4-7.3	5.4	0.9	16.7	III	HA10	4.7-9.6	7.0	1.1	16.1	XI
BA07	2.7-8.4	5.5	1.1	20.1	VI	HA11	3.2-7.0	5.0	0.9	18.9	IX
BA08	3.2-9.7	6.2	1.2	19.8	III	HA13	4.2-8.4	6.3	1.0	15.7	I
BA10	3.6-7.9	5.6	0.9	16.6	III	HA14	5.2-9.7	7.4	1.0	14.1	XIII
BA11	3.4-7.2	5.3	0.9	16.3	IX	HA15	3.2-7.8	5.5	1.0	17.9	VI
BA12	4.1-8.8	7.0	1.1	16.1	III	HA16	2.7-8.2	5.7	1.1	19.4	VI
ER01	3.6-8.0	6.0	0.8	13.1	VII	ML01	4.3-9.6	6.3	1.2	19.0	V
ER02	3.5-8.8	6.3	1.2	18.4	IV	ML02	3.0-8.0	5.6	1.0	17.7	V
ER03	4.1-8.8	6.1	1.0	16.0	V	ML03	2.8-8.0	5.9	1.0	16.5	I
ER04	3.6-8.1	5.5	0.9	16.9	III	ML04	3.0-7.3	5.3	0.9	17.0	V
ER05	3.5-7.5	5.5	1.0	17.5	IV	ML05	3.8-8.9	6.0	1.0	16.5	VII
ER06	3.7-8.4	5.4	1.2	21.2	VII	ML06	4.3-9.2	6.9	1.0	14.1	VIII
ER07	3.2-7.9	5.5	1.0	18.0	VI	ML07	2.8-7.8	5.7	1.0	16.9	I
ER08	4.0-8.0	6.1	0.8	13.7	XII	ML08	4.6-8.5	6.2	0.9	14.5	IX
ER09	3.9-9.4	6.5	1.2	18.9	VII	ML09	3.7-8.1	5.5	0.9	16.7	IX
ER10	3.6-8.5	5.6	1.1	19.3	VI	ML10	4.0-9.3	6.5	1.2	18.5	VII
ER11	3.5-8.2	5.7	1.2	21.0	IV	ML11	3.6-8.4	5.5	1.1	19.8	VII
ER12	3.3-7.6	5.5	1.0	17.7	XII	ML12	4.3-9.2	6.9	1.0	14.9	VIII
ER13	3.6-8.8	6.1	1.2	18.9	IV	ML13	3.5-8.1	5.9	1.0	17.1	I
ER14	3.5-8.1	5.8	0.9	15.5	XII	ML14	2.3-7.7	5.7	1.0	17.4	I
ER15	2.7-7.2	5.0	0.9	18.3	III	ML15	2.3-7.3	5.6	1.0	17.2	I
ER16	4.7-9.5	7.0	1.1	15.7	XI	ML16	3.9-8.5	6.3	1.0	15.5	VIII
ER17	3.3-7.7	5.4	1.1	20.0	III						
Overall	2.3-9.7	5.9	1.1	19.4							

Cultivars with the initial letter M (Melkassa district) and A (Assosa district) were from central and western Ethiopia, respectively, while cultivars with initial letter H, E, and B were from eastern Ethiopia Sofi, Erer and Babile districts, respectively. SD: = Standard deviation; CV (%): coefficient of variation.

emphasis on those traits that had a significant contribution to the observed variation for the future breeding program.

Genetic divergence of mango cultivars

Genetic distances of cultivars

The genetic distance of cultivars estimated by Euclidean distance (ED) varied from 2.3 to 9.7 with a mean and a standard deviation (SD) of 5.9 and 1.1, respectively (Table 7). Majority of pairs of cultivars (53.1%) had Euclidean distances less than the overall mean Euclidean distance. Whereas 42.6 and 4.2% of pairs of mango cultivars had Euclidean distances of 5.9 to 7.0 and >7.0 (mean +SD), respectively (Figure 3). The result indicated that considerable mango cultivars of different geographic regions were genetically diverse. The higher the ED of a pair of cultivars indicated the differences of genotypes with more number of genes (alleles) while the lower ED of a pair of cultivars suggested the differences of

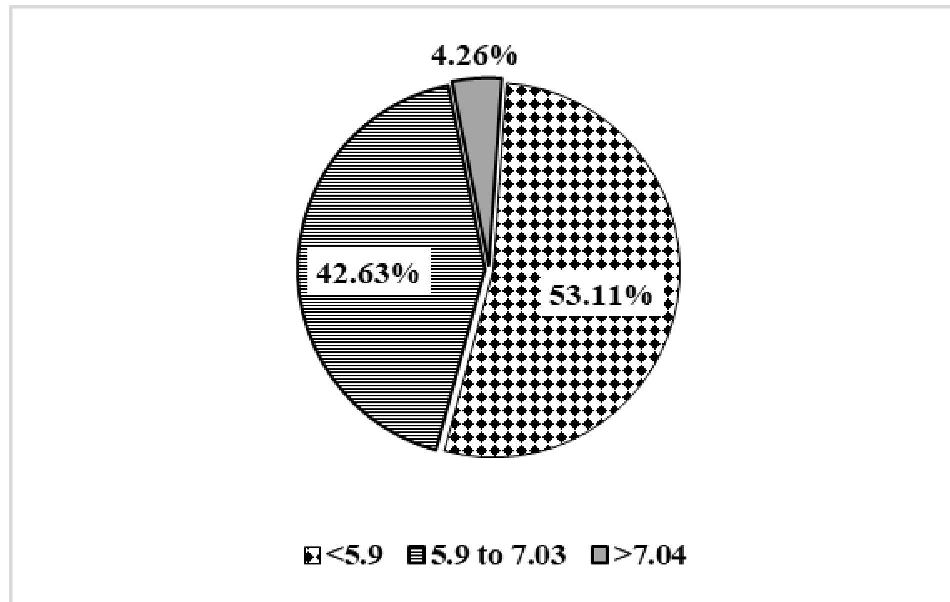

Figure 3. Distribution of 2346 pairs of mango cultivars in respect to Euclidean distance (ED).

genotypes with few genes (alleles) (Bhandari et al., 2017).

The mean Euclidean distance result revealed, the most distant cultivar was HA14 (7.4) followed by AS03 (7.1), HA10 (7.0), BA12 (7.0), and ER16 (7.0). The closest cultivars to others were ML13 (5.9), ML03 (5.9), HA01 (5.9), and AS05 (5.9) (Table 7). The growing regions of the most distant cultivars were from eastern (Harewe, Babile, and Erer Districts) and western (Assosa district) Ethiopia. Whereas, the closest cultivars were from central (Melkassa Research center), eastern (Harewe District), and western (Asosa District) Ethiopia. This suggested the existence of genetic diversity among the cultivars based on their geographic regions. Though, there were cultivars that closely related irrespective of their geographic regions. It is evident that the geographic distance has a contribution to the genetic distances of genotypes (Rao and Hodgkin, 2002). However, the influence of geographical distance on genetic divergence could be suppressed by another factor (s) like genetic drift and natural selection, that could result in the difference in genetic diversity of genotypes of the same location or vice versa (Bhandari et al., 2017; Majumder et al., 2013).

Clustering of mango cultivars

The 69 mango cultivars were grouped into 13 clusters (Table 8 and Figure 4), with mean Euclidean distances of 5.9 with 1.14 and 19.38% standard deviation and coefficient of variation, respectively. Three clusters (II, X, and XIII) constructed each by one cultivar, while others encompass more than one cultivars. Clusters X and XIII had significantly higher mean Euclidean distances than other clusters. The six clusters (I, II, IV, VII, VIII and XI) consisted of 29 cultivars had mean Euclidean distances greater than the overall mean distance of cultivars. Cultivars in Cluster X and XIII were the most divergent of all. While cultivars in the remaining clusters could have the closely related attribute. The formation of the solitary cluster might be due to intensive natural or human selection for diverse adaptive complexes and specific fruit quality in the growing region. The cross-pollination of mango cultivars could also result in specific gene recombination and selection made by growers for propagation might also lead to phenotypic diversity amongst the studied cultivars like most of the existing cultivars in different parts of the world originated (Singh et al., 2016). Although information is lacking on genetic divergence of mango cultivars in Ethiopia, similar study in neighbouring countries such as Kenya (Gitahi et al., 2016; Sennhenn et al., 2013; Toili et al., 2016), Sudan (Ahmed and Mohamed, 2015; Mohamed and Ahmed, 2015) and Egypt (Kheshin et al., 2016) confirmed the existence of phenotype diversity. Hence, the investigated result in Ethiopia is useful for efficient utilization and conservation of the cultivars (Majumder et al., 2013).

Distinguishing characters of clusters

The three solitary clusters (II, X and XIII) were distinguished from others by more than one characters. Cluster II had oblanceolate leaf blade shape, green with red blush ripe fruit skin color, orange ripe fruit pulp color and high fiber in fruit pulp while, Cluster X had dropping

Table 8. Range and mean Euclidean distances of 13 clusters of mango cultivars in Ethiopia.

Cluster	No cultivar	Euclidian distance			
		Range	Mean	SD	CV (%)
I	8	2.25-9.69	6.08	1.06	17.44
II	1	5.15-8.62	6.93	0.75	10.85
III	10	2.74-9.72	5.75	1.18	20.58
IV	8	3.48-8.82	5.95	1.12	18.82
V	4	3.03-9.6	5.82	1.09	18.69
VI	7	2.71-9.37	5.66	1.1	19.44
VII	7	3.61-9.37	5.92	1.14	19.3
VIII	3	3.94-9.23	6.68	1.03	15.44
IX	9	3.19-8.49	5.5	0.97	17.73
X	1	4.47-9.6	7.13	1.06	14.81
XI	2	3.31-9.72	6.97	1.11	15.88
XII	8	5.25-9.72	5.86	1.07	18.25
XIII	1	5.25-9.72	7.39	1.04	14.09
Overall	69	2.25-9.72	5.9	1.14	19.38

SD: = Standard deviation; CV (%): coefficient of variation.

tree growth habit, medium height of matured tree, obovoid fruit shape, green skin colour of ripe fruit, absence of fiber in fruit pulp and intermediate pulp aroma. Cluster XIII had acute leaf base shape, the yellow skin color of ripe fruit and low fiber in fruit pulp.

Clusters VI, VIII and XI each had also distinguishing qualitative traits from others. Cluster VI distinguished by obtuse leaf apex shape of cultivars, while Cluster VIII established from grafted seedlings, short height of matured trees, very good eating quality and parallel pattern of fruit stone venation, mean values for seed length and trunk circumference were lower than the minimum values of cultivars. Cluster XI had the medium depth of fruit stalk cavity, had mean values greater than the maximum mean values of cultivars for fruit length, stone width, and length as well as for seed width and length. Cultivars in cluster I for stone and seed width; cluster V for trunk circumference, and cluster X for petiole length, leaf blade length and width with mean values lower than the minimum values of cultivars. Whereas cultivars in cluster XIII had mean values for fruit diameter, stone width, seed length, and width was greater than the minimum values of cultivars. Mango cultivars grouped in the rest of clusters had mean values of the characters within the ranges of minimum and maximum overall mean values of cultivars and had similarity for two or more qualitative traits.

Grouping of crop genotypes in different clusters is helpful to identify parental lines for breeding or further development of varieties through selection (Karanjalker and Begane, 2016; Majumder et al., 2013). Hence, the resulted cultivar clusters with distinguished characters can be used in mango improvement programs of the country. Likewise, several findings reported on clustering of mango cultivars with their distinguishing traits (Ahmed and Mohamed, 2015; Gitahi et al., 2016; Kheshin et al., 2016; Mohamed and Ahmed, 2015; Sennhenn et al., 2013).

Conclusion

The assessment of phenotypic characters of the studied mango cultivars in Ethiopia revealed the existence of significant phenotype variations. The quantitative characters significantly contributed to the total variation of the cultivars but with varying degree of contribution. The observed range of genetic distances and clustering of cultivars indicated the presence of considerable diversity among cultivars and the existence of cultivars with distinguished characters that can be used for the mango improvement program of the country. However, the results of the study need to be supported by further diversity assessment using molecular markers data, since phenotypic characters are less reliable due to the high influence of environmental factors.

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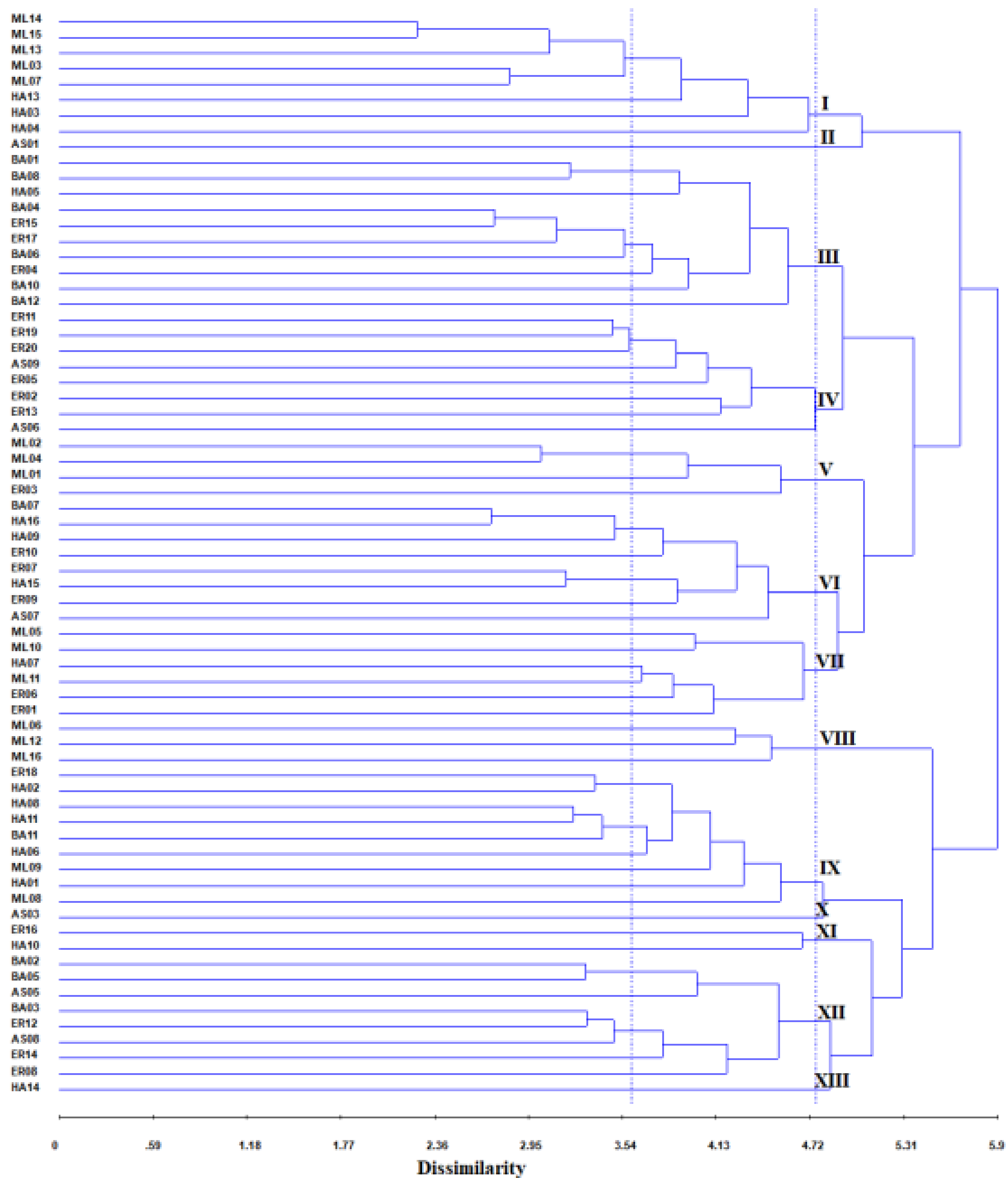

Figure 4. Dendrogram depicting dissimilarity of 69 mango cultivars from the east, central and western Ethiopia obtained by Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) clustering method, based on the Euclidean distances from 44 characters.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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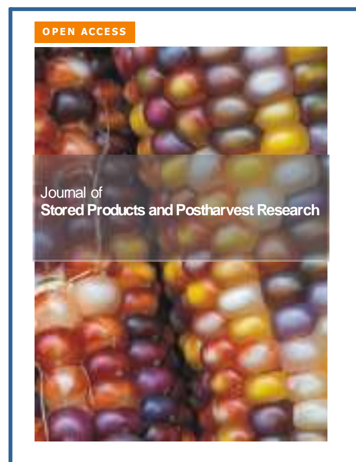

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