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African Journal of Plant Science

Full Length Research Paper

Sorption characteristics, growth and yield response of wheat *(Triticum aestivum* L.) to application of essential nutrients on nitisol and vertisol of Central Highland of Ethiopia

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Wheat growth and yield response were evaluated in a greenhouse experiment using two major soils, Nitosols and Vertisols. Sorption capacities of the soils and crop response were employed to determine the availability of nutrients in the two soils. Eight fertilizer treatments (Optimum (Opt.),Optimum-N, Optimum-P,Optimum-K,Optimum-S,Optimum-B,Optimum-Zn and control) in Nitosols and six fertilizer treatments (Optimum, Optimum-N, Optimum-P, Optimum-S, Optimum-B and control) in Vertisols were arranged in completely randomized design (CRD) with five replications using wheat variety (Digalu) as a test crop. Deficiency in total N, available P, S and B was observed in the two soils. Besides, K and Z in Nitisols were less than three times the critical values. The result indicated that applications of optimum fertilizer significantly ($P<0.05$) increased plant height, spike length, number of seeds per spike, straw yield, grain yield and total biomass yield. Similarly, it resulted in an increase in grain yield of 75 and 68% over the controls in Nitosols and vertisols respectively. Omission of N, P, S, and B were resulted in grain yield reduction by 65.6, 23.4, 4.7, and 3.1% in Nitosols and by 69.4, 22.4, 14.1, and 15.3% in vertisols. Omission of K and Zn in Nitisols also causes up to 9.4 and 4.7% grain yield reduction. Thus, external supplies of these nutrients could be recommended for optimum production of wheat.

Key words: Grain yield, Nitisols, nutrient concentrations in plants, soil nutrient contents, Vertisols.

INTRODUCTION

Cereal crops are the largest group in terms of their share in area cultivated, production, productivity and consumption in Ethiopia (CSA, 2018). Wheat is one of the major cereals widely grown in the highlands of Ethiopia. The country is the second largest wheat producer in sub-Saharan Africa, next to South Africa (ECEA, 2008). Wheat ranks fourth after teff (*Eragrostis tef*), maize (*Zea mays*) and sorghum (*Sorghum bicolor*) in area coverage and total production (CSA, 2018). Wheat production has grown significantly following several

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government programs and initiatives implemented to drive agricultural growth and food security in the country. Ethiopian wheat production is estimated at 4.5 million tonnes in 2018/19 and almost 1.65 million hectares were dedicated to wheat cultivation (GAIN, 2019). According to Global Agricultural Information Network (GAIN, 2019) report yields are close to 2.7 tons per hectare. However production still falls short of meeting domestic needs and the country remains a net importer of wheat.

Soil fertility depletion is a major constraint to agricultural production and food security worldwide, particularly in wheat and rice production areas of the developing world (Tan et al., 2005). Similarly, one of the basic limiting factors for cereal crop yield including that of wheat in Ethiopia is poor soil fertility (Louis, 2010). The problem is more serious in the highlands where most of the human and livestock population is inhabited (Hailu, 2010). Nitisols and Vertisols are among the most extensive agricultural soils in the Ethiopian highlands but soil degradation threatens their productive capacity (Hillette et al., 2015; Eyasu, 2017). The most recent survey indicates the extent of Nitisols coverage is about one million hectares accounting for 31% of the agricultural lands in the Ethiopian highlands (Elias, 2016). Nitisols are among the most productive agricultural soils along with Vertisols, Luvisols, and Planosols (Stocking, 1988). Vertisols also cover 13 million hectares of land mass, while more than half (8.6 million ha) of the Vertisols are found in the central highlands of the country (Debele, 1985; Jutzi et al., 1987). Ethiopia ranks third in Vertisols abundance in Africa after Sudan and Chad (Jutzi et al., 1987). In addition to the high P fixing characteristics of Vertisols (Abunyewa et al., 2004), lack of response to P application on central highland Vertisols of Ethiopia may be due to deficiency of nutrients other than P.

Previously, only nitrogen (N) and phosphorus (P) were considered to be the limiting nutrients in Vertisols of Ethiopia (Mamo et al., 1988). However, many soils in the highlands of Ethiopia are poor in available plant nutrients and organic matter content (Mamo et al., 2002). Hence, the national gross nutrient depletion rate was estimated to be -122 kg N ha^{-1}, −13 kg P ha^{-1} and −82 kg K ha^{-1} (Haileslassie et al., 2005). The field level nutrient balances on Nitisols from southern Ethiopia (−102, −45 and −67 kg ha^{-1} for N, P and K respectively) are even more threatening (Elias, 2002). Soil erosion also contributes significantly to soil fertility depletion, as the rates of losses are estimated to be 130 tons ha^{-1} for cultivated fields, which is one of the highest in Africa (FAO, 1986; Elias, 2016). Nitrogen and phosphorus are not the only yield constraining factors, but others such as S, Zn, B, Fe, Cu and K-deficiencies are also common soil fertility problems due to the low inherent soil fertility status and/or poor management (Tegbaru. 2015). Mining of nutrients due to low and unbalanced fertilizer application favored the emergence of multi nutrient deficiencies in Ethiopian soils (Desta, 1984, Abiye et al., 2004). The recent national soil fertility survey conducted by Ethiopian Agricultural Transformation Agency (ATA) revealed that in addition to nitrogen and phosphorus, potassium, sulfur, and zinc deficiencies are widespread in Ethiopian soils, while some soils are also deficient in boron and copper (ATA, 2013). These all potentially limit crop productivity despite continued use of nitrogen and phosphorus fertilizers as blanket recommendation over decades.

The nutrients usually applied as a fertilizer for crop production in Ethiopia are nitrogen and phosphorus in the form of Urea and DAP (Hillette et al., 2015). However, if the level of any one of the other essential nutrients falls below the critical level, the yield response to nitrogen and phosphorus would be seriously affected. Therefore, in order to set priorities among the different plant nutrients, it is important to identify the status of the limiting nutrients in various soils. Thus, this research was carried out to evaluate wheat growth and yield response to most essential nutrients under Nitisols and Vertisols from central highlands of Ethiopia.

MATERIALS AND METHODS

Description of the study area

Pot experiment was conducted under greenhouse conditions at National Soil Testing Center in Addis Ababa, Ethiopia using soil samples collected from Wolmera district of Oromia Regional State, central Ethiopia. The soils used for the study were classified as Nitisol and Vertisol, covering large production areas of central highlands of Ethiopia (Debele, 1985; Jutzi et al., 1987; Elias, 2016). Wolmera is one of the districts in West Shewa Zone of Oromia Regional State, Ethiopia (Figure 1). It is located at about 30 km west of Addis Ababa on the main road to Ambo city. It is situated at an altitude of 2000 to 3380 m above sea level. The area receives an average annual rainfall of 1067 mm and average temperature of 18°C (BoA, 2013).

Soil sampling, preparation and analysis

Soil samples (0-20 cm depth) were randomly taken from 40 sampling points, 20 each for Nitisols and Vertisols using an auger. The soil samples were bulked into two composite samples, one each for Nitisols and Vertisols. The composite samples were then homogenized and crushed for a pot trial in the greenhouse experiment. Sub-samples were taken from the composites, air-dried and ground with mortar and pestle to pass through a 2 mm sieve and subjected to physicochemical analyses and sorption study. For determinations of organic carbon (OC) and total nitrogen (TN), however, a 0.5 mm sieve was used.

Soil particle size was done by using the modified sedimentation hydrometer procedure (Bouyoucos, 1951), bulk density (BD) was determined according to BSI,(1975) and soil water holding at filed capacity and permanent wilting point was determined according to the procedure outlined by Van Reeuwijk (1993). Soil pH and electrical conductivity (EC) were measured in the supernatant

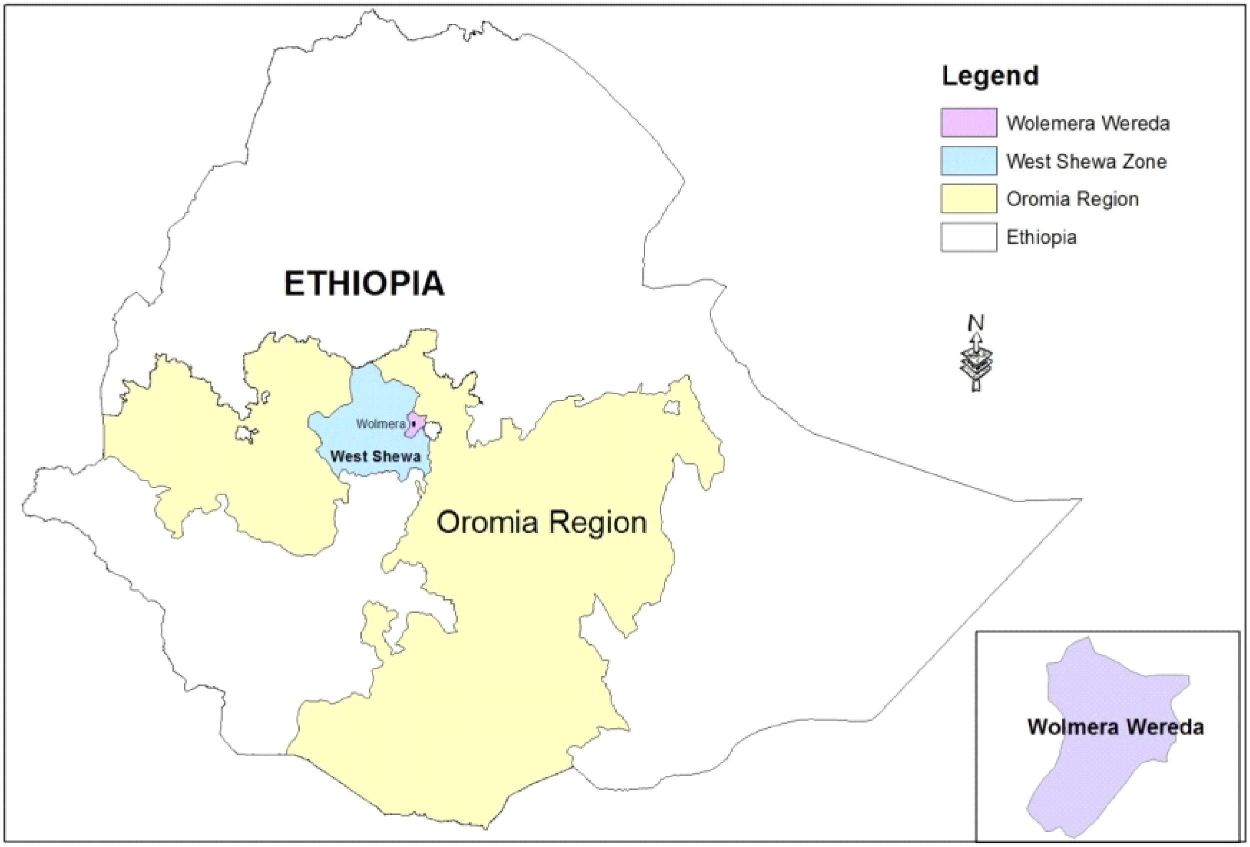

Figure 1. Map of the soils sampling area.

suspension of a 1:2.5 soil:water mixture by using a pH meter and EC meter, respectively (Van Reeuwijk, 1993). Soil organic carbon was determined by using wet oxidation method of Walkley and Black (Walkley and Black, 1934), while total nitrogen was analyzed by wet-oxidation procedure of the Kjeldahal method (Bremner and Mulvaney, 1982). Available phosphorus was determined by Olsen method (Olsen and Sommer, 1982). Exchangeable basic cations and cation exchange capacity (CEC) of the soils were determined by leaching the soils with neutral 1M ammonium acetate (Van Reeuwijk, 1993). The exchangeable cations, calcium (Ca) and magnesium (Mg), in the leachate were determined by Atomic Absorption Spectrophotometer (AAS), whereas potassium (K) and sodium (Na) were determined by flame photometer. Sulfate was determined turbid-metrically using barium sulfate precipitation method (Motsara and Roy, 2008). Available micronutrients iron (Fe), manganese (Mn), zinc (Zn) and copper(Cu) contents of the soils were extracted by diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell, 1978) and the contents of each in the extract were determined by atomic absorption spectrophotometer. The concentration of water-soluble boron was determined by hot water extraction (Watson, 2011).

Preparation of sorption solutions

The laboratory analysis result of the soil samples showed that total N, available P, S, and B are deficient in both soil types. In addition, K and Zn in Nitisols were below three times the critical levels of the respective elements, while the other nutrients were found to be sufficient for crop production. Based on the analysis result sorption solutions for phosphorus (P), sulfur (S), boron (B), potassium (K) and zinc (Zn) were prepared and the amount of a particular element necessary to bring the level to three times its critical level was determined from the sorption solution curves. A series of five sorption solutions were prepared in polyethylene bottles with a control in replications. The actual amounts were varying according to the concentration of a particular element in the soil (Table 1).

Ten gram of soil sample along with 10 ml of sorption solution was added to each bottle, while 10 ml of distilled water was added for the control. Then, the bottles were gently shake to ensure complete mixing of the solution with the soil and allowed to air dry. The air dried samples were extracted and analyzed for the elements. A sorption curve was constructed for each element by plotting the amount of element extracted against the added amount. These sorption curves were used to determine the optimum amount of element to be added in the treatments of the greenhouse experiment except for N. N was added based on the recommendations given by Holeta Agricultural Research Center (150 DAP and 100 Urea per hectare for Nitisols and 150 DAP and 200 Urea per hectare for Vertisols) (personal communication). The critical levels used for the nutrients were phosphorus, 12 mg; potassium, 121 mg; sulfur 10 mg, zinc, 0.5 mg and boron 0.5 mg per kg of soil (Havlin et al. 2010; Landon, 2014).

Experimental design and treatments

The treatments were determined by using soil analysis and sorption

Table 1. Concentration of nutrients in sorption solutions.

Sorption solution	Concentration (mgL^{-1})				
	P	K	S	Zn	B
Control	0	0	0	0	0
1	20	25	10	1	0.25
2	40	50	20	2	0.5
3	80	100	30	4	1.0
4	160	200	40	8	2.0
5	320	400	50	16	4.0

Table 2. Treatments and amount of elements added (mg kg^{-1} soil) to the two experimental soils.

Nitisols	Vertisols
Opt. (N: 39.5, P: 114, K:29.3, S:36, B:2.4 and Zn:1.4)	Opt. (N: 62.5, P: 110, S:44 and B: 2.8)
Opt. – N	Opt. – N
Opt. – P	Opt. – P
Opt. – K	Opt. – S
Opt. – S	Opt. – B
Opt. – B	Control
Opt. – Zn	
Control	

Opt. = Optimum treatment, Opt. - = Optimum treatment without the indicated element, Control = without any element.

results, except for N, for both soils. Three kilogram of the composite soil sample was placed on the plastic sheet and measured amounts of nutrients as per the treatments (Table 2) were applied and mixed thoroughly before filling the plastic pots (20 cm × 14.5 cm × 16 cm). Plastic pots filled with soils were watered to the field capacity three days before seed sowing. Wheat variety Digalu, obtained from Holeta Agricultural Research Center, and that is commonly used by the farmers in the study area, was used as a test crop. Six seeds of wheat were sown in each pot and thinned to four plants at two weeks after germination. The pots were kept in a greenhouse and watered using deionized water regularly to maintain moisture level at about field capacity. Under each pot, a saucer was placed to collect drainage losses of the nutrients. The treatments were arranged in a completely randomized design (CRD) with five replications.

Plant data collection and sample analysis

Plant data collection

Eight weeks after germination, two replications were randomly selected and the plants in each pot were sampled for determination of nutrient contents in the shoot. Nutrient uptake by the shoot for each treatment was determined quantitatively by multiplying shoot dry weight of each treatment by the respective nutrient content of the shoot. At maturity growth parameters including plant height, spike length, spike number, total biomass and grain yield were measured from the remaining three replications. Plant height was measured from the ground level to the tip of the spike using a ruler. Spike length was measured from its base to the tip. Spike number was determined by counting the number of fertile spikes per plant using the four plant samples and number of seeds per spike was counted. Total biomass yield was determined by weighing the total above ground plant biomass before threshing to separate the grain. Grain yield was measured by taking the weight of the grains threshed from each plant after adjusting the grain moisture content to 12.5%. Straw yield was calculated as the difference between the total above ground plant biomass and grain yield. Plant tissue samples (grain and straw) from each pot were put in envelopes and oven dried at 70°C to constant weight and finely ground using a stainless steel grinder to pass through 0.5 mm mesh sieve and analyzed for nutrient concentrations.

Plant sample analysis

Plant samples were analyzed following dry ashing method, whereby the plant material is calcinated in a muffle furnace, dissolved in nitric acid, and filtered for the determination of nutrient elements. The concentration of P in the filtrate was determined by spectrophotometer using the vanado-molybdate method, and K was determined by a flame photometer, whereas Ca, Mg, and micronutrients were determined by atomic absorption spectrophotometer (Wolf, 1982). B was measured colorimetrically using Azomethine-H (Sippola and Ervio, 1977). N in the plant material was analyzed by wet-oxidation of the modified Kjeldahl procedure (Nelson and Sommers, 1973). S was determined by di-acid digestion method as described by Motsara and Roy (2008).

Statistical analysis

The data collected from greenhouse experiment and laboratory analysis were subjected to analysis of variance using SAS statistical software version 9.2 (SAS, 2008). Duncan's multiple

Table 3. Physical characteristics of the surface soils of Wolmera district.

Soil characteristics	Nitisols	Vertisols
Sand (%)	18	16
Silt (%)	28	26
Clay (%)	54	58
Textural Class	Clay	Clay
Bulk density (g cm^{-3})	1.22	1.21
Field Capacity (%)	28.16	39.69
Permanent Wilting Point (%)	18.36	27.90

Table 4. Chemical characteristics of the surface soils of Wolmera district.

Soil characteristics	Nitisols	Vertisols
pH in water (1:2.5)	5.6	6.1
EC (1:2.5) (dsm^{-1})	0.080	0.094
OC (%)	1.55	2.45
TN (%)	0.19	0.24
Av. P ($mgkg^{-1}$ soil)	9.62	10.19
Av. K ($mgkg^{-1}$ soil)	343	438
Av. S ($mgkg^{-1}$ soil)	8.24	6.93
Na (cmol(+)kg^{-1} soil)	0.07	0.23
K (cmol(+)kg^{-1} soil)	0.92	1.13
Ca (cmol(+)kg^{-1} soil)	7.48	21.93
Mg (cmol(+)kg^{-1} soil)	2.50	5.93
CEC (cmol(+)kg^{-1} soil)	34.62	53.57
Base Saturation (%)	34.82	54.26
Fe (mg kg^{-1} soil)	44.10	51.91
Mn (mg kg^{-1} soil)	57.26	36.29
Zn (mg kg^{-1} soil)	0.94	2.29
Cu (mg kg^{-1} soil)	4.27	5.04
B (mg kg^{-1} soil)	0.41	0.33

range tests was used to separate significantly differing treatment means at $P < 0.05$.

RESULTS AND DISCUSSION

Selected physical and chemical properties of the experimental soils

Soil physical properties

The surface soils (0-20 cm depth) of the experimental sites were dominated by clay fraction, which is 54% for Nitisols and 58% for Vertisols (Table 3). The relatively high clay content observed in this study agrees with the findings of (Abebe et al*.,* 2013; and Hillette et al., 2015*)*, which showed high clay contents for Nitisols and Vertisols. The high clay content indicates better water and nutrient holding capacity of the soils. The bulk density of the two soils were very similar and within the optimum range for mineral soils (1.21 - 1.22 g cm^{-3}). According to the rate established by Handreck and Black (1984), the bulk density values of both soils do not restrict root penetration and are suitable for plant growth. The soil moisture contents at field capacity and permanent wilting point were 28.16 and 18.36% for Nitisols and 39.69 and 27.90% for Vertisols, respectively. These moisture contents are considered suitable for plant growth and soil microbial activity. However, the soil moisture content of Nitisol was lower than that of Vertisol by 40.9% at field capacity and by 51.9% at wilting point. Thus, this requires further study to elucidate weather the wheat crop response could be similar with such moisture content differenec between the two soils.

Soil chemical properties

As per the ratings established by Tekalign (1991) for Ethiopian soils, the soil pH is moderately acidic for Nitisols and slightly acidic for Vertisols (Table 4), which is

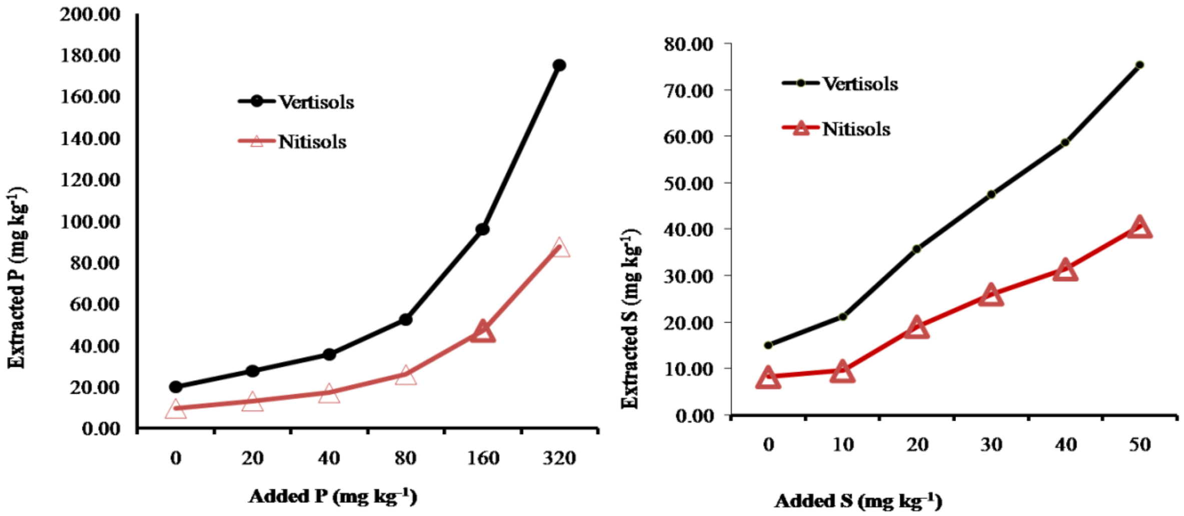

Figure 2. Phosphorus and Sulfur sorpotin characteristics of Nitisols and Vertisols.

favorable for most crops. The electrical conductivity of both study soils were low (Table 4), indicating that these soils contain low levels of soluble salts and thus, the problem of salinity is not expected. The organic carbon and total N contents of both soils (Table 4) could be grouped under moderate range, based on the ratings of soil test values established by Tekalign (1991). However, the total organic carbon and total N contents of Vertisols were higher than that of Nitisols by 58.1 and 26.3%, respectively. Available P was low for both soils (<12 mg kg^{-1}) according to the rating of Havlin et al. (2010), while low for Nitisols and medium for Vertisols according to Cottenie (1980), who classified <10 mg kg^{-1} of soil available P as low and between 10 and 17 mg kg^{-1} of soil as medium. The present results are in agreement with the findings of Getachew et al. (2015) and Hillette et al. (2015). Available S contents for both soil types were found to be low according to Havlin et al. (2010) but in medium range according to Horneck et al. (2011). The results are in agreement with the findings of Assefa et al. (2015a) who reported S deficiency in central highlands of Ethiopia.

The exchangeable Ca followed by Mg was the dominant cations in both soil types. Relatively higher values of exchangeable Ca and Mg were recorded for Vertisols as compared to Nitisols (Table 4). Similarly, higher value (27.7%) of exchangeable K was recorded for Vertisols as compared to Nitisols. The concentrations of basic cations (Ca, Mg and K) in the two soil types were in adequate ranges for crop production and responses of crops to applications of fertilizers containing these elements may not be expected, except for K in Nitisols (Landon, 2014). According to the rating of Landon (2014), cation exchange capacities (CEC) of the studied soils were high for Nitisols and very high for Vertisols. The very high value of CEC in Vertisols is mainly due to both high clay and organic matter content of the soil. The status of micronutrients was found to be sufficient in both soil types, except boron in both soil types and zinc in Nitisols (Table 4).

Soprtion characteristics of Nitisols and Vertisols

The results showed that both Nitisols and Vertisols have a relatively strong sorption capacity for P, S and B, while Nitisols also had a strong capacity for retention of Zn (Figures 2, 3, 4). Retention of K was relatively low when compared to the other plant nutrients tested in Nitisols. Considering the laboratory analyses and sorption studies P, S, B and Zn had high potential to limit yield, while there is also a probability for K to limit yield in Nitisols.

The soluble P added to the soils was strongly fixed by both soil types although the fixation is relatively greater in Vertisols as compared to Nitisols. The high clay content of the soils in this study could increase P fixation due to its high surface area. Havlin et al. (1999) reported that P fixation tends to be more pronounced and ease of P release tends to be lowest in soils with higher clay content. At low initial P addition, P retention was maximum, while at high P addition, P retention was minimum. As increment of P addition increased, P retention decreased. It can be concluded that soil P saturation can decrease adsorption, that is when the soil is saturated with P, rate of adsorption decreased. This reduction in percent of P adsorption could be due to increasing concentration of applied P causing excess P on soil adsorption sites. This results in P release into solution. Sulfur was also fixed by both soil types. However the fixation was relatively lower as compared to P fixation. The sorption might be due to low soil pH, S adsorbed in oxides and hydroxides of iron and clay

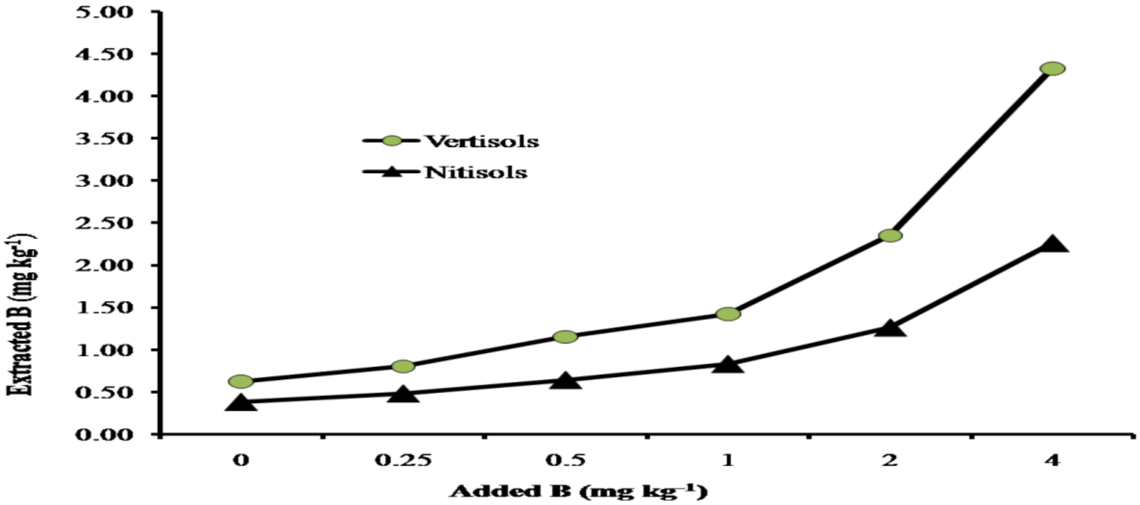

Figure 3. Boron sorption characteristics of Nitisols and Vertisols.

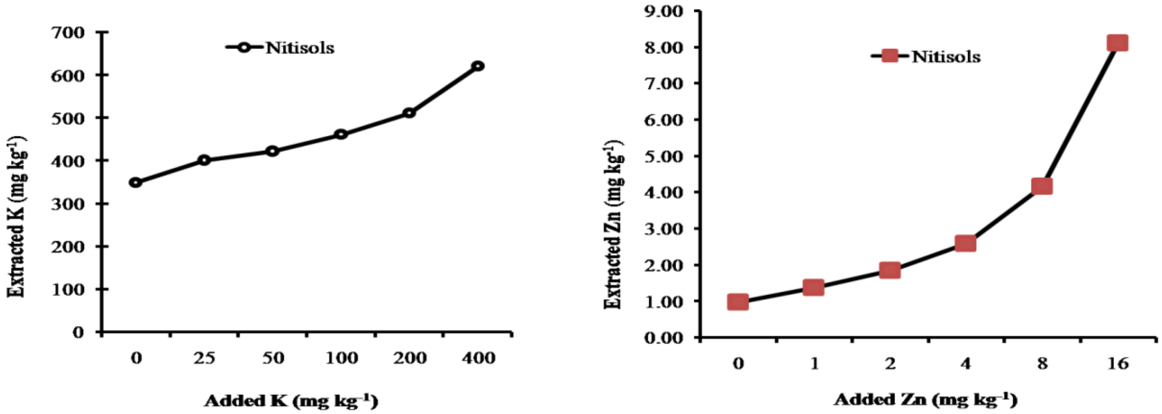

Figure 4. Potassium and Zinc sorption characteristics of Nitisols and Vertisols.

minerals. Similar to phosphate, sulphate is adsorbed to clay minerals and sesquioxides, thus presence of $H_2PO_4^-$ may affect SO_4^{2-} adsorption, because the binding strength for sulphate is not as strong as that for phosphate. The relative strength of anion retention by soil colloids varies in order: phosphate > sulphate > nitrates = chlorides (Blair, 1988).

Although the sorption results indicated that boron was highly fixed by the two soils, the fixation is greater in Vertisols (Figure 3). This could be due to relatively high pH in Vertisols than Nitisols and the types of clay mineral, which might have been dominated by kaolinite. According to Havlin et al. (1999), increasing pH, clay content, organic matter and presence of Al compounds favor $H_4BO_4^-$ adsorption, and B-adsorption capacity generally follows the order mica> montmorillonite > kaolinite.

The results of K sorption indicated that this nutrient was also fixed by the soil in small amount. This may be due to presence of kaolinite clay mineral in the soil. According to Havlin et al. (2010), K fixation represents the re-entrapment of K^+ between the layers of the 2:1 clays, predominantly hydrous mica, but 1:1 minerals such as kaolinite do not fix K. The sorption results indicated that Zn was also highly fixed by Nitisols. This could be due to relatively low pH values and high clay minerals in this soil.

Effects of nutrient omissions on shoot dry weight

Comparison of the mean values of shoot dry weight showed that the lowest values were obtained from the control, followed by Opt.-N and Opt.-P in both Nitisols and Vertisols (Tables 5 and 6). In Nitisols shoot dry weight was reduced by 93, 70, and 50%, for the control and treatments with omission of N (Opt.-N) and P (Opt.-

Table 5. Shoot dry weight, nutrients concentration and uptake in the shoot as influenced by treatments in Nitisols.

Treatment	(g plant^{-1})	Shoot nutrient concentration						Shoot nutrient uptake					
		(%)				(mg kg^{-1})		(mg plant^{-1})					
	SDW	N	P	K	S	Zn	B	N	K	P	S	Zn	B
Opt.	2.49^{a}	1.70^{c}	0.15^{e}	2.51^{b}	0.53^{a}	29.80^{bc}	69.51^{d}	42.26^{a}	3.71^{ab}	62.17^{a}	13.11^{a}	0.074^{a}	0.173^{b}
Opt. -N	0.74^{e}	1.17^{d}	0.23^{a}	1.70^{f}	0.43^{c}	21.57^{e}	107.61^{ab}	8.56^{b}	1.68^{c}	12.43^{d}	3.13^{e}	0.016^{d}	0.079^{de}
Opt. -P	1.25^{d}	2.91^{a}	0.19^{bc}	3.00^{a}	0.51^{ab}	45.94^{a}	91.92^{c}	35.86^{a}	2.36^{c}	37.22^{c}	6.24^{d}	0.057^{bc}	0.114^{cd}
Opt. -K	2.00^{bc}	1.82^{c}	0.18^{d}	2.16^{d}	0.47^{bc}	30.63^{b}	98.65^{bc}	36.25^{a}	3.52^{b}	42.91^{bc}	9.37^{c}	0.061^{abc}	0.197^{b}
Opt. -S	2.40^{a}	1.89^{bc}	0.19^{bc}	2.09^{e}	0.46^{c}	27.34^{d}	114.34^{a}	45.63^{a}	4.49^{a}	50.01^{b}	10.88^{b}	0.066^{ab}	0.275^{a}
Opt. -B	1.63^{cd}	2.25^{b}	0.20^{b}	2.44^{c}	0.45^{c}	29.01^{c}	31.40^{e}	36.38^{a}	3.28^{b}	39.51^{c}	7.31^{d}	0.048^{c}	0.051^{e}
Opt. –Zn	2.12^{ab}	1.95^{bc}	0.19^{cd}	2.12^{de}	0.45^{c}	28.99^{c}	60.54^{d}	41.22^{a}	3.91^{ab}	44.75^{bc}	9.52^{c}	0.062^{abc}	0.128^{c}
Control	0.17^{f}	1.31^{d}	0.09^{f}	0.76^{g}	0.32^{d}	13.05^{f}	15.71^{f}	2.20^{b}	0.14^{d}	1.27^{e}	0.54^{f}	0.002^{d}	0.003^{f}
LSD (0.05)	0.383	0.354	0.012	0.053	0.041	1.30	9.32	11.06	0.77	9.69	1.31	0.152	0.409
CV (%)	10.41	8.19	2.86	1.11	3.92	2.00	5.48	15.45	11.6	11.58	7.57	13.71	13.91

Opt. = Optimum treatment, Opt.- = Optimum treatment without the indicated element, Control = without any element, SDW = Shoot dry weight, `LSD = List significant difference, CV (%) = Coefficient of variation. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$.

Table 6. Shoot dry weight, nutrients concentration and uptake in the shoot as influenced by treatments in Vertisols.

Treatment	(g plant^{-1})	Shoot nutrient concentration						Shoot nutrient uptake					
		(%)				(mg kg^{-1})		(mg plant^{-1})					
	SDW	N	P	K	S	Zn	B	N	P	K	S	Zn	B
Opt.	3.14^{a}	1.61^{a}	0.17^{cd}	1.91^{c}	0.55^{a}	14.70^{e}	76.23^{b}	50.44^{a}	5.16^{a}	59.87^{a}	17.05^{a}	0.046^{b}	0.239^{a}
Opt. –N	0.70^{c}	1.58^{a}	0.27^{a}	1.63^{d}	0.40^{b}	16.10^{d}	82.95^{ab}	10.96^{c}	1.83^{d}	11.35^{c}	2.82^{d}	0.011^{d}	0.058^{d}
Opt. –P	1.83^{b}	1.73^{a}	0.16^{d}	2.28^{a}	0.29^{c}	27.91^{b}	87.44^{a}	31.53^{b}	2.88^{c}	41.43^{b}	5.26^{c}	0.051^{b}	0.160^{c}
Opt. –S	2.01^{b}	1.78^{a}	0.22^{b}	2.32^{a}	0.31^{c}	16.61^{d}	73.99^{b}	35.51^{b}	4.30^{b}	46.30^{b}	6.24^{c}	0.034^{c}	0.149^{c}
Opt. –B	3.10^{a}	1.62^{a}	0.18^{c}	1.98^{b}	0.39^{b}	22.48^{c}	65.02^{c}	49.83^{a}	5.54^{a}	61.23^{a}	11.90^{b}	0.070^{a}	0.202^{b}
Control	0.46^{c}	1.19^{b}	0.23^{b}	1.37^{e}	0.24^{d}	39.21^{a}	58.30^{c}	5.42^{d}	1.03^{d}	6.20^{c}	1.07^{e}	0.018^{d}	0.027^{d}
LSD (0.05)	0.347	0.287	0.017	0.07	0.054	1.40	8.96	4.97	0.82	6.71	1.12	0.009	0.036
CV (%)	7.58	7.41	3.54	1.49	6.15	2.50	4.95	6.63	9.71	7.27	6.2	9.14	10.58

Opt. = Optimum treatment, Opt. - = Optimum treatment without the indicated element, Control = without any element, SDW= Shoot dry weight, LSD = List significant difference, CV (%) = Coefficient of variation. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$.

P), respectively, whereas the corresponding reductions were 85, 78 and 42%, respectively, in Vertisols, as compared to the optimum treatment. This indicates that N and P in both soil types were the most limiting nutrients to support good wheat growth, perhaps due to inherent poor N and P status of the experimental soils. Hence, external supply of these nutrients is required to enhance wheat growth and development. These results are in line with Hillette et al. (2015) who reported deficiency of N and P nutrients for wheat on Vertisols of central Ethiopia, and P deficiency on Nitisols of central Ethiopian highlands (Getachew et al., 2015). In addition, omission of B, K and Zn resulted in 35, 20 and 15% shoot yield reduction of wheat, respectively in Nitisols. On the other hand, S showed 36% yield reduction in Vertisols, indicating that S, K, Zn and B are also limiting nutrients to support good wheat growth in the soils and the need for external supply of these nutrients (Tables 5 and 6). These responses to the nutrients are in line with the soil analysis results (Table 4).

Effects of nutrient omissions on nutrient concentrations and uptakes

Nitrogen omission showed a significant ($P<0.05$) effect on nutrient concentrations in shoots and uptake of the nutrients by wheat in Nitisols (Table 5), while it only showed a significant effect on shoot nutrient uptakes in Vertisols (Table 6). The N contents varied from 1.17 to 2.91% for Nitisols and from 1.19 to 1.78% for Vertisols (Tables 5and 6), which were below the critical value of 3.6% for wheat (Engel and Zubriski, 1982). Thus, the low concentrations of N in the wheat tissue could be due to inadequate rate of N used in the present pot experiments and low soil total nitrogen content. The other most probable explanation is that the critical values of the nutrient in the plant could be soil and crop variety specific. Perhaps, growing conditions may also influence the growth performance and nutrient uptake of crops.

Although the N concentrations in all treatments for both soils were generally below the critical range, the values of N concentration and uptake in plant material of N-omitted treatments were even very low as compared to the other treatments, except the control. This signifies that N was one of the limiting nutrients in these soils.

Nitrogen concentrations in P-omitted pots were very high for both Nitisols (2.91%) (Tables 5) and Vertisols (1.73%) (Table 6). These high concentrations of N in the P-omitted pots might have resulted from the dry matter reduction that occurs when plants are under nutritional stress. But the N uptake in P-omitted treatments was relatively low, since the dry matter yield in these treatments were also very low. Differences in nutrient uptakes in both soil types were better explained by differences in dry matter production rather than by nutrient concentration in the shoot.

Phosphorus omission showed highly significant ($P < 0.05$) differences in shoot nutrient concentrations and uptakes of nutrients by wheat in both Nitisols and Vertisols (Tables 5 and 6). Phosphorus concentration in the plant ranged from 0.09 to 0.23% and from 0.16 to 0.27% for Nitisols and Vertisols, respectively. According to Plank and Donohue (2000) these values are below the critical range, but were close to the lower limit of sufficiency range. According to the authors' ratings, the sufficiency range for P in wheat is between 0.2 and 0.5%. These low concentrations of P may be due to a dilution effect by high biomass production, particularly when at optimum N supply, low soil available P content and high P fixation. The phosphorus concentrations in N-omitted treatments were high (0.23%) for Nitisols (Table 5) and (0.27%) for Vertisols (Table 6). This high concentration of P in the N-omitted treatments might have resulted from the combined effects of element accumulation and dry matter reduction that occurred when plants are under nutritional stress. The P uptake in N-omitted treatments was very low, since nitrogen deficiency causes a marked reduction in uptake of P (Mengel and Kirby 2001). Additinally, the low P uptake is also due to low biomass production.

Potassium concentration and uptake in wheat shoot showed significant difference ($P < 0.05$) among treatments for both soils (Tables 5 and 6). The concentration of potassium in the plants ranged from 0.76 to 3.00% for Nitisols and from 1.37 to 2.32 % for Vertisols. The concentrations of potassium were above the critical range for both soils, except for the controls. According to Jones et al. (1991) the critical range of K in plant material of wheat is 1.5 to 3.0%. The low concentration of K in the controls may be due to inadequate soil available K. The uptake of K in N-omitted treatments was very low for both soils. This low uptake may be due to nitrogen deficiency, which causes a great reduction in uptake of K (Mengel and Kirby, 2001). Sulfur concentration and uptake in wheat plants showed significant differences ($P < 0.05$) among the treatments in both soil types (Tables 5 and 6). The concentrations of sulfur in the plant material range from 0.32 to 0.53 % for Nitisols and from 0.24 to 0.55 % for Vertisols. According to Jones et al. (1991) this is within the critical range. Low uptake of S in the control, N-omitted and P-omitted treatments may be due to low concentration of N and P, because N, P and S are component of protein molecule and omission of these nutrients reduce the uptake of S.

Zinc concentration and uptake in wheat plants were significantly ($P < 0.05$) different among the treatments in both soil type. Zinc concentration in wheat plants ranged from 13.05 to 45.94 mg kg^{-1} for Nitisols, which is below the critical for the control and within the critical range for other treatments. According to Plank and Donohue (2000), the critical range of Zn in plant material of wheat is 18 to 70 mg kg^{-1}. Low uptake of Zn in control and N-omitted treatments may be due to low concentration of Zn in control and N in both treatments. According to Mengel and Kirby (2001), nitrogen deficiency causes a marked reduction in uptake of Zn. The result is also in agreement with the low Zn and N content of the initial soil (Table 4).

Boron concentration and uptake in the plants were

Table 7. Treatment effects on Yield and Yield components of Wheat in Nitisols.

Treatment	PH (cm)	SL (cm)	NSPS	SY (g pot^{-1})	GY (g pot^{-1})	BY(g pot^{-1})	HI
Optimum	88.5^{a}	8.5^{a}	35.7^{a}	6.8^{ab}	6.4^{a}	13.1^{a}	48.5^{a}
Opt. -N	66.2^{d}	5.6^{c}	23.2^{c}	2.3^{d}	2.2^{c}	4.4^{c}	49.0^{a}
Opt. -P	79.3^{bc}	7.8^{b}	28.6^{b}	5.5^{c}	4.9^{b}	10.4^{b}	47.4^{a}
Opt. -K	72.9^{cd}	7.6^{b}	30.4^{b}	6.8^{ab}	5.8^{a}	12.5^{a}	45.9^{a}
Opt. -S	82.6^{ab}	7.4^{b}	31.6a^{b}	6.5^{b}	6.1^{a}	12.7^{a}	48.1^{a}
Opt. -B	80.8^{b}	7.8^{b}	32.2^{ab}	6.7^{ab}	6.2^{a}	12.8^{a}	48.0^{a}
Opt. -Zn	90.0^{a}	7.7^{b}	29.3^{b}	7.3^{a}	6.1^{a}	13.4^{a}	45.6^{a}
Control	49.4^{e}	5.4^{c}	18.5^{c}	1.7^{d}	1.6^{c}	3.3^{d}	49.5^{a}
LSD (0.05)	7.4	0.4	4.7	0.7	0.6	0.8	3.7
CV (%)	4.2	2.3	7.0	5.3	5.2	3.5	3.4

Opt. = Optimum treatment, Opt. - = Optimum treatment without the indicated element, Control= without any element, LSD= List significant difference, CV (%) = Coefficient of variation, PH= plant height (cm), SL= spike length (cm), NSPS= number of seeds per spike, SY= straw yield (g pot^{-1}), GY= grain yield (g pot^{-1}), BY= total biomass yield (g pot^{-1}) and HI= harvest index. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$.

Table 8. Treatment effects on Yield and Yield components of Wheat in Vertisols.

Treatment	PH (cm)	SL (cm)	NSPS	SY (g pot^{-1})	GY (g pot^{-1})	BY(g pot^{-1})	HI
Optimum	81.0^{a}	8.0^{a}	38.1^{a}	10.2^{a}	8.5^{a}	18.7^{a}	45.4^{bc}
Opt. -N	62.4^{c}	5.5^{b}	23.2^{c}	2.8^{d}	2.6^{d}	5.4^{d}	48.3^{a}
Opt. -P	81.8^{a}	7.5^{a}	30.2^{b}	7.4^{c}	6.6^{c}	14.1^{c}	47.4^{ab}
Opt. -S	74.5^{ab}	7.8^{a}	33.5^{ab}	8.5^{b}	7.3^{b}	15.8^{b}	46.5^{abc}
Opt. –B	81.8^{a}	8.0^{a}	31.7^{b}	9.0^{b}	7.2^{b}	16.2^{b}	44.7^{c}
Control	70.4^{b}	5.2^{b}	22.8^{c}	3.2^{d}	2.7^{d}	5.8^{d}	45.7^{bc}
LSD (0.05)	7.3	0.6	5.0	0.7	0.3	0.8	2.4
CV (%)	4.0	3.5	6.8	4.1	2.3	2.5	2.1

Opt. = Optimum treatment, Opt. - = Optimum treatment without the indicated element, Control= without any element, LSD= List significant difference, CV (%) = Coefficient of variation, PH= plant height (cm), SL= spike length (cm), NSPS= number of seeds per spike, SY= straw yield (g pot^{-1}), GY= grain yield (g pot^{-1}), BY= total biomass yield (g pot^{-1}) and HI = harvest index. Means followed by the same letter(s) within a column are not significantly different at $P< 0.05$.

significantly ($P < 0.05$) affected by treatments in both soils (Tables 5 and 6). Boron concentration in plants ranged from 15.71 to 114.34 mg kg^{-1} and from 58.30 to 87.44 mg kg^{-1} for Nitisols and Vertisols, respectively, which were within and above the critical range.

Effects of omissions of nutrients on plant height, spike length and number of seeds per spike

Analysis of variance revealed that plant height, spike length and number of seed per spike were significantly ($P < 0.05$) affected by omission of nutrients (Table 7). The higher values were measured for optimum and Zn-omitted treatments for Nitisols and for optimum, P-omitted and B-omitted treatments for Vertisols. As expected, the lowest plant heights were recorded for the controls and N-omitted treatments for both soils, indicating that vegetative growth is highly affected by omission of N. This might be attributed to the role and presence of N in many essential compounds. The most important function of N in wheat is promotion of rapid growth through increases in height, tiller number, size of leaves and length of roots (Chatterjee and Maiti, 1985). The highest spike length and number of seeds per spike were obtained from optimum treatments, whereas the least values were recorded for the controls and N-omitted treatments in both soils. The spike length for the P, S and B omission treatments were not significantly different from the optimum treatment in Vertisols (Table 8).

Effects of omissions of nutrients on straw and grain yields

The results showed that omission of some nutrients significantly ($P <0.05$) influenced straw yield (SY), grain yield (GY) and total biomass yields (BY) of wheat in both

Nitisols and Vertisols (Tables 7 and 8). The highest grain and total biomass yields were recorded for the optimum nutrients application in both soils, although the values were statistically at par with those obtained from K, S, B and Zn-omitted treatments for Nitisols. On contrary, the highest straw yield (7.3 g pot^{-1}) was recorded for Zn-omitted treatment for Nitisols. For Vertisols, the highest straw yield (10.2 g pot^{-1}) was obtained from the optimum treatment, whereas the lowest values were recorded for the control and N-omitted treatments in both soils.

The total biomass yield results in Nitisols showed N and P to be the main yield limiting nutrients (Table 7). Omission of these nutrients reduced total biomass yield by 66.4 and 20.6%, respectively, as compared to the optimum treatment. Omission of N, P, S, and B from the Vertisols markedly reduced the total biomass yield (Table 8). The yield reductions due to omission of these nutrients were 71.1, 24.6, 15.5 and 13.4%, respectively, as compared to the optimum treatment indicating that nutrients, such as N and P in Nitisols and N, P, S and B in Vertisols were limiting to support good crop growth. These findings are in line with the soil analysis results (Table 4).

The highest grain yields were recorded for the optimum treatments, while the lowest grain yields were obtained from the controls and N-omitted treatments in both soils (Table 7 and 8). Grain yield increased by 75 and 68.2% due to optimum treatments over the controls in Nitisols and Vertisols, respectively. The grain yield reduction due to omission of N, P, S, and B were 65.6, 23.4, 4.7, and 3.1%, respectively, for Nitisols and 69.4, 22.4, 14.1 and 15.3%, respectively, for Vertisols. The results are in line with the findings of Assefa et al*.* (2015a), who reported that wheat responded well to applied N, S and P fertilizers in central highlands of Ethiopia. Eyasu (2013) also found strong wheat grain yield response to nitrogen, phosphorus and potassium (NPK) fertilizers under field condition on Rhodic Nitisols in south western Ethiopia. Similar study also showed nitrogen and phosphorus fertilizers significantly increased grain yield, biomass yield, seeds per spike, effective tiller number and plant height of bread wheat in southern Tigray (Assefa et al*.,* 2015b). Likewise, it was observed that grain yields of different genotypes of wheat significantly increased by application of boron as compared to the control (Soylu et al*.,* 2004; Jana et al*.,* 2005).

Conclusion

The surface soils of both Nitisols and Vertisols were dominated by clay fraction and the pH was in favorable range for most crops in both soils. The nutrient elements P, K, S, B and Zn in Nitisols and P, S and B in Vertisols were below three times the critical levels of the elements. Total biomass yield in Nitisols showed N and P to be the main yield limiting nutrients. Omission of these nutrients reduced total biomass yield by 66.4 and 20.6%, respectively, as compared to the optimum treatment in Nitisols. Omission of N, P, S, and B markedly reduced the total biomass yield in Vertisols. The yield reductions due to omission of these nutrients were 71.1, 24.6, 15.5 and 13.4%, respectively as compared to the optimum treatment. Thus, omission of nutrients, such as N and P in Nitisols and N, P, S and B in Vertisols, was limiting crop growth. Grain yield increased by 75 and 68.2% due to optimum treatment over the controls in Nitisols and Vertisols, respectively. The reduction in grain yield due to omission of N, P, S, and B was 65.6, 23.4, 4.7 and 3.1% for Nitisols and 69.4, 22.4, 14.1 and 15.3% for Vertisols, respectively. Omission of K and Zn in Nitisols also causes up to 9.4% and 4.7% grain yield reduction, respectively. This indicates that order of requirement for Nitisols were N > P > K > S=Zn≈ B, whereas N > P > B ≈ S for Vertisols.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Heterosis and combining ability of highland adapted maize (*Zea mays. L)* DH lines for desirable agronomic traits

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Maize (*Zea mays* L.) is one of the cereals that provide calorie requirements in the majority of Ethiopians diet. The national average maize yield in Ethiopia is low and thus knowledge of combining ability and heterosis is a prerequisite to develop high yielding maize varieties. The objective of the present study is to estimate combining abilities of double haploid (DH) maize inbred lines for grain yield and related agronomic traits, and to identify crosses with higher standard heterosis. A total of 36 diallel crosses generated by crossing nine maize DH lines using half diallel mating scheme and four standard checks were studied for different desirable agronomic traits during 2017 cropping season at Ambo and Kulumsa Agricultural Research Centers. The genotypes were evaluated in alpha lattice design replicated twice in both locations. Analyses of variances showed significant mean squares due to crosses for most traits studied. The highest grain yields were obtained from crosses L1 x L3, L3 x L8, L4 x L8 and L8 x L9. GCA mean squares were significant for all studied traits, while SCA mean squares were significant only for grain yield, days to anthesis, ear per plant and ear diameter. Relatively larger GCA over SCA variances were observed in the current study for most studied traits revealing the predominance of additive gene action in controlling these traits. Of the DH inbred lines, L3 and L8 were the best general combiners for grain yield, and hence are promising parents for hybrid development. Inbred lines L2, L4, L6, L7 and L8 were good combiners for earliness whereas, L1, L2 and L6 showed negative and significant GCA effects for plant and ear height. In this study, none of the crosses showed positive and significant standard heterosis for grain yield.

Key words: Combining ability, general combining ability, highland maize, standard heterosis, specific combining ability.

INTRODUCTION

Currently, maize is one of the most important field crops to fulfill food security in Ethiopia. It contributes the greatest share of production and consumption along with other major cereal crops, such as tef, wheat and sorghum

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Table 1. Description of testing sites.

Research center	Altitude (masl)	RF (mm)	Temp (°C)		Latitude	Longitude	Soil type
			Min	Max			
Ambo	2225	1050	10.4	26.3	8°57'N	38°7'E	Black vertisol
Kulumsa	2180	830	10	23.2	8°5'N	39°10'E	Luvisol/eutric nitosols

(CSA, 2017). It has a significant importance in the diets of rural Ethiopia and has gradually penetrated into urban centers. This is particularly evidenced by green maize cobs being sold at road sides throughout the country as a hunger-breaking food available during the months of May to August annually (Twumasi et al*.,* 2012).

The high altitude sub-humid areas including the highland transition and true highland of Ethiopia is next to mid-altitude in maize production. It is estimated that the highland sub-humid agro-ecology covers 20% of the land devoted annually to maize cultivation and 30% of small-scale farmers in the area depend on maize production for their livelihood (Twumasi et al., 2001). In this agro-ecology, maize production is characterized by low yields owing to unimproved varieties coupled with biotic constraints such as turcicum leaf blight, common leaf rust, stalk lodging, stalk borers, and storage pests and abiotic stresses such as frost, hailstorm and low soil fertility (Twumasi et al., 2001). Because of these constraints, the highland areas have been facing great challenges in maize production which occasionally lead to food insecurity, malnutrition, reduced income and widespread poverty (Demissew et al., 2014). Therefore, it remains important to develop high yielding, nutritionally enhanced and stress tolerant maize varieties which fit the diverse highland agro-ecology of the country.

Combining ability studies are of primary importance in maize hybrid development since it provides information for the selection of parents, identification of promising hybrids and on the nature and magnitude of gene actions. On the other hand, heterosis occurs when two inbred lines of out bred species are crossed, as much as when crosses are made between pure lines. It is practically exploited to develop hybrid varieties (George, 2007).

Several studies on combining ability and heterosis of maize inbred lines for grain yield and yield related traits were conducted for different sets of locally developed/introduced inbred lines in Ethiopia (Hadji, 2004; Dagne et al*.,* 2010; Demissew et al*.,* 2011; Yoseph et al., 2011; Shushay et al., 2013; Umar et al., 2014; Girma et al., 2015; Beyene, 2016; Tolera et al*.,* 2017; Dufera et al*.,* 2018). However, it is always mandatory for any breeding program to generate such information for any new batch of inbred lines generated locally or received outside of the program. Currently, at Ambo highland maize research program there are a number of new batches of inbred lines generated through different methods of inbred line development. Little or no information is available on the particular sets of new inbred lines used for this study regarding the combing ability effects of the parental lines to be used for future hybrid development.

The focus of the current study was, therefore to generate information on nine elite maize inbred lines crossed using half diallel mating scheme following Griffing (1956) with the objectives of identifying best inbred lines having good general and specific combining ability effects, and determine the magnitude of standard heterosis for yield and yield related traits for further breeding and/or cultivar development.

MATERIALS AND METHODS

Descriptions of experimental sites

The experimental sites used for this experiment were two representative sites of highland sub-humid agro-ecology in Ethiopia, *viz*., Ambo Agricultural Research Centre (AARC) and Kulumsa Agricultural Research Centers (KARC) (Table 1).

Experimental materials

Nine inbred lines obtained from Ambo highland maize breeding program were crossed using diallel mating design during the main cropping season of 2016 and thirty-six single cross hybrids were generated. The list of inbred lines and their origin is presented in Table 2. The DH lines used in the crosses were originally obtained from CIMMYT-Zimbabwe and were locally selected based on previous field performances in test-cross evaluations for adaptation, disease reaction and general combining ability by the highland maize breeding program at AARC. The thirty-six F_1 crosses together with four commercial hybrid checks: Arganne, Kolba, Jibat and Wenchi were used in the hybrid trial evaluations in 2017.

Experimental design trial management and data collection

The 36 F_1 crosses plus the four hybrid commercial checks adapted to the highland agro-ecology of Ethiopia were planted using alpha lattice design (Patterson and Williams, 1976) with two replications each of which have eight blocks with five entries in each of the blocks. Design and randomization of the trials were generated using CIMMYT's Field book software (Bindiganavile et al., 2007).

The trials were hand planted with two seeds per hill, which later thinned to one plant per hill at the 2-4 leaf stage to get a total plant population of 53,333 per hectare. Reliable moisture level of the soil was assured before planting so as to insure good germination and seedling development. Pre-emergence herbicide, Premagram Gold660 at the rate of 5 lt ha^{-1}, was applied three days after

Table 2. The list of inbred lines used to make the diallel crosses for the study.

Entry	Pedigree	Seed Source
1	(INTA-F2-192-2-1-1-1-B*9/CML505-B)DH-3060-B-B-#	AHMBP*
2	(LPSC7-C7-F64-2-6-2-1-B/CML488)DH-3033-B-B-#	AHMBP*
3	(CML444/CML539)DH-3091-B-B-#	AHMBP*
4	(CML144/CML159)DH-3049-B-B-#	AHMBP*
5	([LZ956441/LZ966205]-B-3-4-4-B-5-B*7-B/DTPWC9-F109-2-6-1-1-B)DH-3001-B-B-#	AHMBP*
6	(CML545/CML505)DH-10-B-#	AHMBP*
7	(CML545/CML505)DH-44-B-#	AHMBP*
8	([CML312/[TUXPSEQ]C1F2/P49-SR]F2-45-3-2-1-BB//INTA-F2-192-2-1-1-1-B*4]-1-5-1-2-1-B*6/CML505)DH-11-B-#	AHMBP*
9	(CML312/CML442)DH-3002-B-B-#	AHMBP*

*AHMBP = Ambo Highland Maize Breeding Program.

planting of the seeds to control weeds followed by hand weeding at a later stage of crop emergence. Each entry was placed in a one-row plot of 5.25 m long and 0.75 m x 0.25 m apart between and within rows spacing, respectively. The recommended rate of inorganic fertilizers, that is, 150 and 200 kg ha^{-1} of DAP and urea, respectively, were used. Urea was applied in two splits, *viz*., half of it was applied when plants had six to eight leaves, and the remaining half was applied at flag leaf emergence before flowering at both sites. Other standard cultural and agronomic practices were followed in trial management as per recommendations for the areas.

The procedure of data collection followed CIMMYT's manual for managing trials and reporting data (CIMMYT, 1985). Data on grain yield and other important agronomic traits were collected on a plot and sampled plants base. Data collected on a plot basis include: days to 50% anthesis (DA), days to 50% silking (DS), anthesis-silking interval (ASI), grain yield (GY) (t -ha^{-1}), thousand kernel weight (TKW) (g). Data collected on plant base include: ear height (EH) (cm), plant height (PH) (cm), ear length (EL) (cm), ear diameter (ED) (cm), number of ears per plant (EPP), number of rows per ear (RPE), number of kernels per row (KPR).

Statistical analyses

Before data analyses, anthesis-silking interval (ASI) was normalized using $\ln\sqrt{ASI + 10}$ as suggested by Bolanos and Edmeades (1996). Analysis of variance (ANOVA) per individual and across locations was carried out using PROC MIXED method = type3 procedure in SAS (2003) by considering genotypes as fixed effects and replications and blocks within replications as random effects for individual site analyses. In the combined analyses, environments, replications within environments and blocks within replications and environments were considered as random while genotypes remained as fixed effects following same procedure of Moore and Dixon (2015). Combined analyses were performed for traits that showed significant genotypic differences in the individual location analyses, and after testing homogeneity of error variance using Bartlett's test (Gomez and Gomez, 1984). In the combined analyses, entry and location main effects were tested using entry x location interaction mean squares as error term, while entry x location interaction mean squares were tested against pooled error.

Combining ability analyses

Combining ability analyses were done for traits that showed significant differences among genotypes and thus Griffing's Method IV (crosses only) and Model I (fixed) of diallel analyses (Griffing, 1956) was used to estimate combining ability effects and associated standard errors using a modification of the DIALLEL-SAS program (Zhang et al., 2005). The significance of GCA and SCA effects were tested against the respective standard errors of GCA and SCA effects, respectively, using t-test (Griffing, 1956; Singh and Chaudhary, 1985). In the across locations combining ability analyses, the significance of GCA and SCA mean squares were tested using the corresponding interactions with location as error term. The mean squares attributable to all the interactions with locations were tested against pooled error.

The linear mathematical model developed by Griffing (1956) for an observation made on the genotype for Method IV and model I was used as follows:

$$X_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{bc}\sum_k \sum_l e_{ijkl}$$

$$\begin{cases} i,\ j = 1,\dots,\ p, \\ k = 1,\dots, b, \\ l = 1,\dots, c. \end{cases}$$

Where, X_{ij} = the value of a character measured on cross of ith and jth parents; μ = Population mean; g_i (g_j) = the general combing ability effects of the ith and jth parents, s_{ij} = the specific combing ability effects of the crosses, e_{ijkl} = is the error effect, p, b and c = number of parents, blocks and sampled plants, respectively.

Estimation of standard heterosis

Standard heterosis or economic heterosis was calculated for the characters that showed significant differences for genotypes following the method suggested by Falconer and Mackay (1996). This was computed as percentage increase or decrease of the cross performances over the best standard check. Kolba was used as the best standard check.

$$SH\ (\%) = \frac{(F1 - SV)}{SV} \times 100$$

Where, F1 = Mean value of a cross, SV = Mean value of standard

Table 3. Combined analyses of variance for grain yield and yield related traits of 36 diallel crosses and four hybrid checks evaluated at Ambo and Kulumsa.

Trait	Sources of variation								
	Loc (DF = 1)	**Rep(Loc) (DF = 2)**	**Blk(Loc,rep) (DF = 28)**	**Genotype (DF = 39)**	**Genotype*Loc (DF = 39)**	**Error (DF =50)**	**Grand mean**	**SE(m)**	**CV(%)**
GY	228.90**	0.57^{ns}	1.20*	4.81**	1.89**	0.62	8.34	±0.56	9.48
DA	739.60**	3.70^{ns}	3.13^{ns}	41.56**	3.40^{ns}	2.62	90.32	±1.14	1.79
DS	288.90**	6.23^{ns}	4.59*	47.64**	3.79^{ns}	2.71	92.25	±1.16	1.78
ASI	0.20**	0.0005^{ns}	0.001^{ns}	0.004**	0.003**	0.0015	1.23	±0.03	3.15
PH	48546.05**	694.08*	226.27^{ns}	951.19**	196.36^{ns}	183.59	214.36	±9.58	6.32
EPP	0.48**	0.008^{ns}	0.03^{ns}	0.08**	0.05 ^{ns}	0.02	1.42	±0.1	10.85
ED	1.64**	0.17 ^{ns}	0.03^{ns}	0.11**	0.03**	0.03	4.53	±0.12	3.54
TKW	217378.16 ^{ns}	828.46^{ns}	1143.64^{ns}	4907.65**	1207.44^{ns}	1436.51	343.41	±26.8	11.04

**Significant at 0.01 level of probability; * = significant at 0.05 level of probability; ns = non-significant; Loc= location; Rep= replication; Blk= block; DF= degrees of freedom; SE(m)= standard error of mean; GY= grain yield; DA= number of days to anthesis; DS= number of days to silking; ASI= anthesis silking interval; PH= plant height; EPP= number of ears per plant; ED= ear diameter and TKWT =1000-kernel weight.

check, SH= Standard heterosis expressed as percentage. Variety test of significance for percent heterosis was made using the t-test. The standard errors of the difference for heterosis and t-value were computed as follows (Singh, 1985).

$$\text{t (standard cross)} = \frac{F1-SV}{SE(d)}$$

$$\text{SE (d) for SH} = \sqrt{2MSe/r}$$

Where, SE (d) = standard error of the difference, SH= standard heterosis, Me = error mean square, r = number of replications. The computed t value was tested against the t tabular-value at error degree of freedom .

RESULTS AND DISCUSSION

Analyses of variance (ANOVA)

Combined analyses of variances revealed highly significant (P<0.01) differences among the 40 genotypes including checks for all traits studied under combined analyses (Table 3). This indicates the presence of inherent variation among the materials, which makes selection possible. Desirable genes from these genotypes can effectively be utilized to develop high performing hybrids. Similarly, several previous studies reported significant differences among genotypes for grain yield and yield related traits in different sets of maize genotypes (Dagne et al., 2007; Demissew, 2014; Habtamu et al., 2015; Amare et al., 2016; Tolera et al*.,* and Dufera et al*.,* 2018).

The interaction between genotypes and locations (G x LOC) was significant for grain yield, Anthesis-silking interval and ear diameter, indicating that genotypes performed differently across locations, which means that the relative performances of the genotypes were influenced by the varying environmental conditions for these traits. On the other hand, days to anthesis, days to silking, plant height, number of ears per plant and thousand kernel weight showed non-significant difference for genotype by location interaction (Table 2), indicating that the relative performance of the genotypes for these traits was not influenced by the varying environmental conditions. Consistent with the present finding, Gudeta (2007) reported significant G x LOC interaction for grain yield, number of rows per ear and ear diameter and non-significant G x LOC interaction for number of ears per plant.

Genotypes performances

The combined means from across locations' analyses are given in Table 3. Overall mean grain yield of the genotypes was 8.34 t/ha with a range of 6.16 t/ha to 11.07 t/ha. Kolba (11.07 t/ha) followed by Jibat (10.91 t/ha), Wenchi (10.43 t/ha) and Argane (10.15t/ha) had higher grain yield, while crosses L5 x L9 (6.16 t/ha) and L2 x L9 (6.74 t/ha) showed lower grain yield. The high heritability value (0.64) for this trait indicated more contributions of genetic factors rather than environmental effects on this trait, implying selection for this character could be more effective. In line with this, Dagne et al*.* (2010), Amare et al. (2016); Beyene (2016), Dufera et al*.* (2018) also identified genotypes that performed better than the checks used in their studies for grain yield.

Days to anthesis ranged from 84.25 days (L4 x L6) to 102 days (L5 x L9) with overall mean of 90.33 days. Mean number of days to silking was 92.26 with a range of 85.5 (L4 x L6) to 103.5 (L5 x L9). Most of the crosses showed longest number of days to anthesis and silking. This shows that those crosses could be grouped as late maturing types. Late maturing crosses are important in the breeding programs for development of high yielding hybrids in areas that receive sufficient rain fall (Girma et al*.,* 2015). The heritability values for both days to anthesis and silking were very high (0.92 and 0.93 respectively) indicating the traits were not greatly influenced by environment. Thus, it shows selection for these traits could be more effective and easy since the genetic variability was detected clearly because of low environmental influence (Table 4). Anthesis-silking interval ranged from 1.14 days (L2 x L8) to 1.29 days (L4 x L5) with a mean of 1.23 days (Table 4). In general, all crosses exhibited short ASI or short gaps between anthesis and silking days which is a desired character for good seed setting. The positive ASI observed for all of the genotypes studied is an expected result as maize is a protoandrous plant in which anthesis normally begins 1-3 days before silk emergence (Rahman et al., 2013).

Plant height ranged from 185.25 cm (L2 x L6) to 251.25 cm (Kolba) with a mean of 214.37 cm. Genotypes with shorter plant height could be used as sources of genes for the development of shorter statured varieties for highland agro-ecology of Ethiopia. In agreement with this result, Beyene (2016), Abiy (2017) and Tolera et al*.* (2017) also identified genotypes with short and long plant and ear heights. Mean number of ears per plant of genotypes was 1.42 ranged from 1.18 (L1 x L7) to 1.74 (L1 x L3). Seven crosses exhibited higher number of ears per plant than the best check, Kolba (Table 4). Desirability of higher number of ears for grain yield improvement was suggested by various authors such as Dagne et al*.* (2010), Demissew et al*.* (2011), Girma et al*.* (2015), Ram et al*.* (2015), Amare et al*.* (2016).

The mean for ear diameter ranged from 4.05 to 5.05 cm with overall mean of 4.53 cm. The cross L3 x L9 (4.05 cm) had the smallest diameter as compared to other hybrids, while cross L4 x L8 (5.05 cm) displayed the largest ear diameter. The crosses with wider ear diameter could be used for grain yield improvement since increasing ear diameter could lead to increase in number of rows per ear. Thousand kernel weight ranged from 214.18 g for (L3 x L9) to 410.9 g for (Jibat) with overall mean of 343.41 g.

Standard heterosis

The estimate of standard heterosis over the best standard check (Kolba) was computed for grain yield and yield related traits that showed significant differences among genotypes and the result is presented in Table 5. Standard heterosis for grain yield over the best check Kolba ranged from -44.35% (L5 x L9) to -8.31% (L1 x L3). Out of the 36 hybrids studied, none of the hybrids had positive and significant as well as negative and significant heterosis over the standard check Kolba (Table 5). All hybrids exhibited non-significant and negative standard heterosis over the best standard check Kolba. This indicates that the check hybrid Kolba was more prolific than all the F1 hybrids and indicating lack of significant heterosis among the crosses used in the current study. The highest negative standard heterosis was manifested by L5 x L9 (-44.35 %) followed by L2 x L9 (-39.11 %) and L1 x L7 (-36.49 %) over Kolba for grain yield. Positive standard heterosis was considered to be desirable for grain yield as it indicates increased yield over the existing standard check. In contrast to this finding, several other authors reported positive and significant heterosis for grain yield over best standard check indicating the possibility of increasing yield by exploiting heterotic potential of maize genotypes (Tiwari, 2003; Twumasi et al., 2003; Amiruzzaman et al., 2010; Wali et al., 2010; Habtamu et al., 2015; Ziggiju and Legesse, 2016; Dufera et al*.,* 2018).

Negative standard heterosis was considered as desirable for days to anthesis and silking as it indicates earliness of a genotype and the reverse is true for the crosses with positive and significant standard heterosis. Standard heterosis over best check Kolba ranged from -3.71 to 16.57% and -5.00 to 15.00%, respectively, for days to anthesis and silking which was revealed by crosses (L4 x L6) and (L5 x L9), respectively, for both traits. Out of the 36 hybrids studied, ten crosses exhibited negative and non-significant standard heterosis for days to anthesis, while twenty of the hybrids showed significant heterosis and the rest six hybrids exhibited positive and non-significant heterosis for days to anthesis in undesired direction. For days to silking, out of 36 hybrids, twelve crosses revealed negative heterosis, while only two crosses (L4 x L6) and (L6 x L8) revealed significant heterosis in desired direction over best standard check. Twenty four crosses showed positive heterosis over best standard check. Among them, seventeen of the crosses revealed significant heterosis in undesired direction. Negative heterosis for these traits indicated earliness as compared to the standard check (Kolba). Similar to this study, Natol et al. (2017) also reported negative and non-significant, and positive and significant heterosis for days to anthesis and silking in their study on standard heterosis of maize inbred lines for grain yield and yield related traits at southern Ethiopia. In addition, previous investigators reported significant negative and positive standard heterosis for days to anthesis and silking over standard check (Bayisa, 2004; Mahantesh, 2006; Shushay, 2014; Ziggiju and Legesse, 2016; Abiy, 2017).

For anthesis silking interval, standard heterosis ranged from -9.52 % (L2 x L8) to 2.38 % (L4 x L5) over Kolba. Almost all crosses showed negative standard heterosis over the best check for anthesis silking interval, indicating the tendency of the crosses to have short interval between anthesis and silking dates than Kolba, which is

Table 4. Mean values of yield and yield related traits of 36 diallel crosses and four commercial checks evaluated at Ambo and Kulumsa in 2017.

Genotype	Traits							
	GY	**DA**	**DS**	**ASI**	**PH**	**EPP**	**ED**	**TKW**
L1*L2	8.80	87.25	88.50	1.21	199.75	1.37	4.53	379.10
L1*L3	10.05	91.75	94.50	1.27	210.75	1.74	4.50	306.48
L1*L4	7.87	88.00	90.25	1.25	198.00	1.22	4.88	363.33
L1*L5	9.03	92.75	95.25	1.26	228.75	1.38	4.58	335.78
L1*L6	7.09	87.00	89.50	1.26	187.50	1.20	4.53	377.33
L1*L7	7.03	88.50	90.50	1.24	192.00	1.18	4.43	358.48
L1*L8	8.25	88.00	89.50	1.21	207.75	1.29	4.83	370.28
L1*L9	7.49	92.75	95.50	1.27	218.75	1.31	4.70	326.03
L2*L3	8.96	91.00	93.25	1.25	219.25	1.62	4.35	329.10
L2*L4	8.85	86.75	88.50	1.23	205.50	1.44	4.60	343.85
L2*L5	7.48	92.75	94.75	1.24	216.75	1.23	4.45	357.15
L2*L6	7.44	86.25	87.25	1.19	185.25	1.48	4.35	350.35
L2*L7	9.06	88.50	90.00	1.22	209.00	1.54	4.40	378.85
L2*L8	8.07	88.50	88.50	1.14	202.25	1.27	4.65	381.90
L2*L9	6.74	94.75	96.00	1.21	212.75	1.41	4.40	276.68
L3*L4	8.55	91.75	94.25	1.26	232.50	1.39	4.48	313.25
L3*L5	8.00	96.00	98.25	1.25	237.50	1.44	4.40	290.83
L3*L6	8.75	91.25	92.75	1.22	199.00	1.66	4.33	330.23
L3*L7	7.41	92.00	94.75	1.27	219.50	1.47	4.25	306.90
L3*L8	9.68	92.50	95.50	1.28	224.25	1.71	4.43	328.65
L3*L9	7.34	98.75	100.50	1.23	226.00	1.63	4.05	214.18
L4*L5	8.03	90.50	93.75	1.29	204.50	1.37	4.43	316.20
L4*L6	7.54	84.25	85.50	1.21	194.50	1.23	4.58	312.90
L4*L7	8.78	86.75	89.50	1.27	207.00	1.36	4.75	384.00
L4*L8	9.41	86.50	87.00	1.17	228.50	1.30	5.05	378.73
L4*L9	7.59	91.25	93.50	1.25	213.25	1.41	4.58	312.10
L5*L6	7.29	91.00	92.25	1.21	208.75	1.36	4.48	359.75
L5*L7	7.54	93.50	96.00	1.26	232.25	1.29	4.63	334.38
L5*L8	8.13	93.00	95.00	1.24	240.25	1.49	4.73	308.53
L5*L9	6.16	102.00	103.50	1.22	224.00	1.30	4.58	274.20
L6*L7	7.18	86.25	87.50	1.21	188.25	1.25	4.45	405.68
L6*L8	8.01	85.75	86.25	1.17	191.75	1.42	4.63	399.95
L6*L9	7.62	92.50	94.75	1.25	200.75	1.59	4.43	327.28
L7*L8	8.23	86.50	89.00	1.26	198.25	1.26	4.65	389.18
L7*L9	8.42	92.25	94.75	1.26	226.00	1.55	4.40	327.98
L8*L9	9.26	91.75	93.25	1.22	235.50	1.63	4.70	301.28
Argane	10.15	87.75	89.50	1.23	222.50	1.48	4.50	383.55
Kolba	11.07	87.50	90.00	1.26	251.25	1.57	4.55	408.03
Jibat	10.91	88.75	90.00	1.19	239.75	1.55	4.55	410.90
Wenchi	10.43	88.50	91.50	1.28	235.00	1.54	4.50	383.18
Mean	8.34	90.33	92.26	1.23	214.37	1.42	4.53	343.41
LSD $_{(0.05)}$	1.12	2.30	2.34	0.055	19.24	0.22	0.23	53.83
CV (%)	9.48	1.79	1.78	3.15	6.32	10.9	3.54	11.04
R^2	0.95	0.96	0.96	0.88	0.92	0.86	0.88	0.89
H^2	0.64	0.92	0.93	0.12	0.81	0.37	0.76	0.81
Min	6.16	84.25	85.5	1.14	185.25	1.18	4.05	214.18
Max	11.07	102	103.5	1.29	251.25	1.74	5.05	410.9

GY= grain yield; DA= number of days to anthesis; DS= number of days to silking; ASI= anthesis silking interval; PH= plant height; EH= ear height; EPP= number of ears per plant; EL= ear length; ED= ear diameter; RPE= number of kernel rows per ear; KPR= number of kernels per row; and TKWT =1000-kernel weight; R^2 **=** Coefficient of determination; H^2= heritability in broad sense; Min= minimum; Max= maximum.

desirable for synchronization of anthesis and silking, and for seed setting. In line with this study, Dufera et al*.* (2018) reported negative standard heterosis over best checks in their study on combining ability, heterosis and heterotic grouping of quality protein maize inbred lines at bako, western Ethiopia. The magnitude of standard heterosis for plant height ranged from -26.27 % (L2 x L6) to -4.38 (L5 x L8) (Table 4). For this trait, all of the crosses showed negative and non-significant heterosis over the best check. This implies that all crosses were shorter in plant height than kolba, which is favorable trait for lodging resistance. This result is in agreement with the findings of Shushay (2014).

For number of ears per plant, standard heterosis among hybrids varied from -24.84 (L1 x L7) to 10.83 % (L1 x L3). Seven hybrids showed positive standard heterosis over the check kolba. This result indicated the prolificacy of the new hybrids over the standard check, Kolba. The rest 29 crosses showed negative standard heterosis over best check and are undesirable for high number of ear per plant. Similarly significant positive and negative standard heterosis was observed by Koppad (2007), Shushay (2014) and Ziggiju and Legesse, 2016 for number of ears per plant.

Standard heterosis for ear diameter varied between -10.99 (L3 x L9) and 10.99 % (L4 x L8) over kolba (Table 5). Sixteen crosses showed positive heterosis over best standard check. Among them only one cross (L4 x L8) showed significant and positive standard heterosis over kolba. Among twenty crosses those showed negative standard heterosis, only one hybrid (L3 x L9) had negative and significant standard heterosis over Kolba for ear diameter. Positive standard heterosis shows that the F1 crosses had larger ear diameter than the standard check which is important to increase number of kernel rows per ear and thus important to increase grain yield while negative heterosis depicts that the check hybrids had larger ear diameter than the F1 hybrids. Similar result was previously reported by Beyene (2016). Standard heterosis for thousand kernel weight varied from -47.51 (L3 x L9) to -0.58 % (L6 x L7). All of the crosses showed negative and non-significant standard heterosis over the standard check Kolba (Table 5). Similar to the current study, both desirable and undesirable heterosis for thousand kernel weight in maize has been reported by previous investigators (Amiruzzaman et al*.,* 2010; Shushay, 2014).

Combining ability analyses

Combining ability analysis across the two locations is presented in Table 6. The results showed that mean squares due to GCA and SCA were significant for grain yield, days to anthesis, number of ears per plant and ear diameter. This indicates that both additive and non-additive gene actions are important in the inheritance of these traits. Reports on similar studies by Dagne et al. (2007) showed that both GCA and SCA mean squares were significant for ear height, plant height and days to maturity. Similarly, Yoseph et al. (2011) observed significant GCA and SCA for anthesis date, anthesis silking interval, ear height and plant height in elite maize inbred lines developed by CIMMYT for insect resistance.

The contribution of GCA variances was much greater than that of SCA variances for most of the traits except for grain yield at Kulumsa and across locations, number of ears per plant at Ambo and anthesis silking interval at both Ambo and Kulumsa, which showed higher contribution of SCA variance for these traits at these particular locations. The higher percentage relative contribution of GCA sum of squares over SCA sum of squares showed the predominant role of additive gene action over non-additive gene action in the inheritance of the traits studied. The breeding implication of this predominance of additive gene action is that the genotypes having this character can be used to develop hybrid and/or synthetic varieties. Similar results were reported by other authors in their study on combining ability for yield and yield related traits in maize (Chandel and Mankotia, 2014; Amare et al., 2016; Beyene, 2016; Bitew et al*.,* 2017 and Tolera et al*.,* 2017). They reported predominance of additive gene action over non-additive for most of the traits they studied.

GCA and SCA mean squares were significant for grain yield across the two locations. This significant GCA and SCA mean squares indicated the importance of both additive and non-additive gene actions in governing grain yield. This has breeding implications, since hybridization methods such as reciprocal recurrent selection which utilizes both additive and non-additive gene effects simultaneously, could be useful in genetic improvement of the population characters under consideration. Similar to the present study Hadji (2004) found highly significant mean squares due to GCA and SCA for grain yield in diallel study of quality protein maize inbred lines. In addition, Dagne et al*.*, 2011; Demissew et al*.,* 2011; Shushay et al*.*, 2013 and Bitew et al*.,* 2017 also reported the importance of both additive and non-additive gene actions in governing grain yield in maize.

For number of days to anthesis and silking, mean squares due to GCA were significant at across the two locations. Mean square due to SCA was significant for days to anthesis but for days to silking, mean square due to SCA was non-significant. In agreement with this study, Tolera et al*.* (2017) found the importance of both additive and non-additive gene effects for days to anthesis. GCA sum of squares were larger than SCA sum of squares for anthesis and silking dates. In line with this study, Ahmad and Saleem (2003) reported the preponderance of additive gene action in the inheritance of days to anthesis and silking.

For plant height, mean squares due to GCA were highly significant ($p<0.01$). While it showed non-significant

Table 5. Standard heterosis of F1 hybrids over Kolba for grain yield and related traits evaluated at Kulumsa and Ambo in 2017.

Crosses	GY	DA	DS	ASI	PH	EPP	ED	TKW
L1*L2	-20.51^{ns}	-0.29^{ns}	-1.67^{ns}	-3.97^{ns}	-20.5^{ns}	-12.74^{ns}	-0.44^{ns}	-7.09^{ns}
L1*L3	-8.31^{ns}	4.86*	5.00**	0.79^{ns}	-16.12^{ns}	10.83^{ns}	-1.10^{ns}	-24.89^{ns}
L1*L4	-28.91^{ns}	0.57^{ns}	0.28^{ns}	-0.79^{ns}	-21.19^{ns}	-22.29^{ns}	7.25^{ns}	-10.96^{ns}
L1*L5	-18.43^{ns}	6.00**	5.83**	0.00^{ns}	-8.96^{ns}	-12.10^{ns}	0.66^{ns}	-17.71^{ns}
L1*L6	-35.95^{ns}	-0.57^{ns}	-0.56^{ns}	0.00^{ns}	-25.37^{ns}	-23.57^{ns}	-0.44^{ns}	-7.52^{ns}
L1*L7	-36.49^{ns}	1.14^{ns}	0.56^{ns}	-1.59^{ns}	-23.58^{ns}	-24.84^{ns}	-2.64^{ns}	-12.14^{ns}
L1*L8	-25.47^{ns}	0.57^{ns}	-0.56^{ns}	-3.97^{ns}	-17.31^{ns}	-17.83^{ns}	6.15^{ns}	-9.25^{ns}
L1*L9	-32.34^{ns}	6.00**	6.11**	0.79^{ns}	-12.94^{ns}	-16.56^{ns}	3.30^{ns}	-20.10^{ns}
L2*L3	-19.06^{ns}	4.00*	3.61^{ns}	-0.79^{ns}	-12.74^{ns}	3.18^{ns}	-4.40^{ns}	-19.34^{ns}
L2*L4	-20.05^{ns}	-0.86^{ns}	-1.67^{ns}	-2.38^{ns}	-18.21^{ns}	-8.28^{ns}	1.10^{ns}	-15.73^{ns}
L2*L5	-32.43^{ns}	6.00**	5.28**	-1.59^{ns}	-13.73^{ns}	-21.66^{ns}	-2.20^{ns}	-12.47^{ns}
L2*L6	-32.79^{ns}	-1.43^{ns}	-3.06^{ns}	-5.56^{ns}	-26.27^{ns}	-5.73^{ns}	-4.40^{ns}	-14.14^{ns}
L2*L7	-18.16^{ns}	1.14^{ns}	0.00^{ns}	-3.17^{ns}	-16.82^{ns}	-1.91^{ns}	-3.30^{ns}	-7.15^{ns}
L2*L8	-27.10^{ns}	1.14^{ns}	-1.67^{ns}	-9.52^{ns}	-19.50^{ns}	-19.11^{ns}	2.20^{ns}	-6.40^{ns}
L2*L9	-39.11^{ns}	8.29**	6.67**	-3.97^{ns}	-15.32^{ns}	-10.19^{ns}	-3.30^{ns}	-32.19^{ns}
L3*L4	-22.76^{ns}	4.86*	4.72*	0.00^{ns}	-7.46^{ns}	-11.46^{ns}	-1.54^{ns}	-23.23^{ns}
L3*L5	-27.73^{ns}	9.71**	9.17**	-0.79^{ns}	-5.47^{ns}	-8.28^{ns}	-3.30^{ns}	-28.72^{ns}
L3*L6	-20.96^{ns}	4.29*	3.06^{ns}	-3.17^{ns}	-20.80^{ns}	5.73^{ns}	-4.84^{ns}	-19.07^{ns}
L3*L7	-33.06^{ns}	5.14**	5.28**	0.79^{ns}	-12.64^{ns}	-6.37^{ns}	-6.59^{ns}	-24.78^{ns}
L3*L8	-12.56^{ns}	5.71**	6.11**	1.59^{ns}	-10.75^{ns}	8.92^{ns}	-2.64^{ns}	-19.45^{ns}
L3*L9	-33.69^{ns}	12.86**	11.67**	-2.38^{ns}	-10.05^{ns}	3.82^{ns}	-10.99**	-47.51^{ns}
L4*L5	-27.46^{ns}	3.43^{ns}	4.17*	2.38^{ns}	-18.61^{ns}	-12.74^{ns}	-2.64^{ns}	-22.51^{ns}
L4*L6	-31.89^{ns}	-3.71^{ns}	-5.00**	-3.97^{ns}	-22.59^{ns}	-21.66^{ns}	0.66^{ns}	-23.31^{ns}
L4*L7	-20.69^{ns}	-0.86^{ns}	-0.56^{ns}	0.79^{ns}	-17.61^{ns}	-13.38^{ns}	4.40^{ns}	-5.89^{ns}
L4*L8	-15.00^{ns}	-1.14^{ns}	-3.33^{ns}	-7.14^{ns}	-9.05^{ns}	-17.20^{ns}	10.99**	-7.18^{ns}
L4*L9	-31.44^{ns}	4.29*	3.89*	-0.79^{ns}	-15.12^{ns}	-10.19^{ns}	0.66^{ns}	-23.51^{ns}
L5*L6	-34.15^{ns}	4.00*	2.50^{ns}	-3.97^{ns}	-16.92^{ns}	-13.38^{ns}	-1.54^{ns}	-11.83^{ns}
L5*L7	-31.89^{ns}	6.86**	6.67**	0.00^{ns}	-7.56^{ns}	-17.83^{ns}	1.76^{ns}	-18.05^{ns}
L5*L8	-26.56^{ns}	6.29**	5.56**	-1.59^{ns}	-4.38^{ns}	-5.10^{ns}	3.96^{ns}	-24.39^{ns}
L5*L9	-44.35^{ns}	16.57**	15.00**	-3.17^{ns}	-10.85^{ns}	-17.20^{ns}	0.66^{ns}	-32.80^{ns}
L6*L7	-35.14^{ns}	-1.43^{ns}	-2.78^{ns}	-3.97^{ns}	-25.07^{ns}	-20.38^{ns}	-2.20^{ns}	-0.58^{ns}
L6*L8	-27.64^{ns}	-2.00^{ns}	-4.17*	-7.14^{ns}	-23.68^{ns}	-9.55^{ns}	1.76^{ns}	-1.98^{ns}
L6*L9	-31.17^{ns}	5.71**	5.28**	-0.79^{ns}	-20.10^{ns}	1.27^{ns}	-2.64^{ns}	-19.79^{ns}
L7*L8	-25.65^{ns}	-1.14^{ns}	-1.11^{ns}	0.00^{ns}	-21.09^{ns}	-19.75^{ns}	2.20^{ns}	-4.62^{ns}
L7*L9	-23.94^{ns}	5.43**	5.28**	0.00^{ns}	-10.05^{ns}	-1.27^{ns}	-3.30^{ns}	-19.62^{ns}
L8*L9	-16.35^{ns}	4.86*	3.61^{ns}	-3.17^{ns}	-6.27^{ns}	3.82^{ns}	3.30^{ns}	-26.16^{ns}
Kolba (mean)	11.07	87.50	90.00	2.50	251.25	1.57	4.55	408.03
SE(d)	0.79	1.62	1.65	0.04	13.55	0.14	0.17	37.90

**Significant at 0.01 level of probability; * = significant at 0.05 level of probability; ns = non-significant; SE(d)= standard error of difference; GY= grain yield; DA= number of days to anthesis; DS= number of days to silking; ASI= anthesis silking interval; PH= plant height; EPP= number of ears per plant; ED= ear diameter and TKWT =1000-kernel weight.

SCA mean square across locations (Table 6). In this study, additive gene action than non-additive gene action was important for plant height. In consistent with this finding, Dagne (2002), Hadji (2004) and Demissew et al*.* (2011) reported the importance of additive and non-additive gene action in the inheritance of plant height. Combining ability analyses revealed highly significant GCA and SCA effects for ear per plant. Similar to the present study, Malik et al. (2004) reported significant GCA and SCA mean squares for number of ears per plant in a diallel study of nine quality protein maize (QPM) inbred lines.

Both GCA and SCA mean squares for ear diameter were significantly different ($p<0.05$) across the two

Table 6. Across locations combing ability analyses of variance for grain yield and other agronomic traits of 36 diallel crosses evaluated at Ambo and Kulumsa (2017).

Source of variation	Mean squares							
	DF	GY	DA	DS	PH	EPP	ED	TKW
Location (LOC)	1	201.17**	720.03**	315.06**	43646.17**	0.29**	1.65**	189667.5**
Replication (LOC)	2	0.71 ^{ns}	5.14 ^{ns}	8.26 ^{ns}	730.03*	0.0042 ^{ns}	0.17**	1393.92 ^{ns}
Crosses	35	3.12**	57.57**	66.16**	952.29**	0.09**	0.14**	6551.29**
GCA	8	5.89*	235.78**	274.24**	3189.48**	0.23**	0.47**	22818.03**
SCA	27	2.30*	4.76*	4.51 ^{ns}	289.42 ^{ns}	0.05**	0.04*	1731.52 ^{ns}
GCA*LOC	8	3.22**	7.40*	3.87 ^{ns}	263.14 ^{ns}	0.09**	0.03 ^{ns}	953.31 ^{ns}
SCA*LOC	27	1.50*	4.45 ^{ns}	3.14 ^{ns}	239.98 ^{ns}	0.04*	0.03 ^{ns}	1725.43 ^{ns}
Error	70	0.81	2.87	3.33	189.83	0.02	0.03	1331.38
% GCA		43.18	93.62	94.74	76.55	55.91	76.54	79.61
% SCA		56.82	6.38	5.26	23.45	44.09	23.46	20.39

**Significant at 0.01 level of probability, * = significant at 0.05 level of probability, ns = non-significant, GY= grain yield, DA= number of days to anthesis, DS= number of days to silking, PH= plant height, EPP= number of ears per plant, ED= ear diameter and TKW=1000-kernel weight.

locations indicating that both additive and non-additive gene effects were important in agreement with the study of Dagne (2002), Hadji (2004) and Gudeta (2007). Mean squares due to GCA for thousand kernel weight were highly significant ($p<0.01$) across locations (Table 6) but mean squares due to SCA were not significant. This study showed additive than non-additive gene actions were important in governing this trait. In contrast to this finding, Dagne (2002), Dagne et al. (2007), Gudeta (2007) and Beyene (2016) reported the importance of both additive and non-additive gene actions for this trait.

GCA × Loc mean squares were significant for grain yield, days to anthesis and ears per plant indicating that GCA effects associated with parents were not consistent for these traits over the two environments (Table 6). But the interaction was not significant for days to silking, plant height, ear diameter and thousand kernel weight, indicating that GCA effects associated with parents were consistent over the two environments. SCA × Loc mean squares were significant for grain yield and ear per plant showing that SCA effects of these traits associated with crosses were not consistent over the two environments, while, SCA × Loc showed non-significant mean squares for the rest of traits, indicating that SCA effects associated with crosses were consistent over the two environments. Similar findings were reported by Dagne et al. (2007) in their study on heterosis and combining ability for grain yield and its component in selected maize inbred lines.

General combining ability effects

The general combining ability effects of parental inbred lines were computed for the traits exhibited significant general combining ability (GCA) mean squares in combining ability analyses of variance (Table 6). The Estimates of GCA effects for parental lines showed significant differences for various traits. General combining ability effects of grain yield and related agronomic traits for across locations analyses are presented in Table 7.

GCA effects of lines for grain yield ranged between -0.59 t/ha (L9) to 0.61 t/ha (L3) (Table 7). Five inbred lines showed positive GCA effects for grain yield. Two inbred lines L3 (0.61 t/ha) and L8 (0.62 t/ha) showed positive and significant GCA effects. This indicates the potential advantage of these inbred lines for the development of high-yielding hybrids and/or synthetic varieties, as the lines can contribute desirable alleles in the synthesis of new varieties. Four inbred lines (L5, L6, L7 and L9) showed negative and non-significant GCA (Table 7), indicating these lines were poor combiners for grain yield. Results of the current study are similar to the findings of several authors (Kanagarasu et al., 2010; Yoseph et al., 2011; Girma et al., 2015; Amare et al., 2016; Beyene, 2016; Dufera et al., 2018) who reported significant positive and negative GCA effects for grain yield in maize germplasm.

GCA effects of lines for days to anthesis ranged between -2.90 (L6) to 4.49 (L9), while for days to silking it ranged from -3.44 (6) to 4.56 (L9) (Table 7). Six inbred lines (L1, L2, L4, L6, L7 and L8) showed negative and significant GCA effects for days to anthesis. This indicates that these lines were good general combiners for early maturity while three inbred lines (L3, L5 and L9) exhibited significant and positive GCA effects for days to anthesis and that these lines have tendency to increase late maturity. L9 had higher and positive GCA effect for days to silking (4.45) whereas L6 had lower and negative GCA effect (-3.44). All the three inbred lines which showed positive GCA effects had significant GCA effects

Table 7. Estimates of general combining ability effects (GCA) of nine inbred lines across the two locations (2017).

Line	GY	DA	DS	PH	EPP	ED	TKW
L1	0.14^{ns}	-1.22*	-0.90*	-7.35^{ns}	-0.09**	0.10**	16.66^{ns}
L2	0.09^{ns}	-1.26*	-1.87**	-6.32^{ns}	0.01^{ns}	-0.07*	13.82^{ns}
L3	0.61*	2.92**	3.42**	10.58**	0.19**	-0.21**	-40.08**
L4	0.26^{ns}	-2.69**	-2.51**	-1.57^{ns}	-0.08*	0.15**	3.45^{ns}
L5	-0.44^{ns}	3.85**	4.14**	14.00**	-0.06^{ns}	0.0008^{ns}	-17.62^{ns}
L6	-0.54^{ns}	-2.90**	-3.44**	-19.85**	-0.02^{ns}	-0.07*	23.33*
L7	-0.15^{ns}	-1.47**	-1.12**	-3.21^{ns}	-0.06^{ns}	-0.04^{ns}	26.46**
L8	0.62*	-1.72**	-2.26**	4.83^{ns}	0.01^{ns}	0.20**	22.61*
L9	-0.59^{ns}	4.49**	4.56**	8.89*	0.08*	-0.06^{ns}	-48.64**
SE(g_i)	0.31	0.57	0.44	4.45	0.034	0.037	10.01

**Significant at 0.01 level of probability, * = significant at 0.05 level of probability, ns = non-significant, GY= grain yield, DA= number of days to anthesis, DS= number of days to silking, PH= plant height, EPP= number of ears per plant, ED= ear diameter and TKW=1000-kernel weight.

for days to silking while six inbred lines exhibited significant and negative GCA effects for this trait. L1 (-0.90), L2 (-1.87), L4 (-2.51), L6 (-3.44), L7 (-1.12) and L8 (-2.26) were the best general combiners for early maturity (Table 7). Lines with negative and significant GCA effects for days to anthesis and silking are desirable when the objective is to develop early maturing hybrids, as hybrids generated using these lines tend to flower earlier. Similarly, lines with positive and significant GCA effects for days to flowering are desirable when the objective is to develop late maturing hybrids. Thus, there is possibility of making effective selection for these traits, which could lead to considerable genetic improvement for earliness and lateness. Desirability of negative GCA for days to anthesis and silking for earliness and desirability of positive GCA for these traits for lateness was suggested by various authors such as Shushay et al. (2013), Umar et al. (2014), Girma et al. (2015), Beyene, (2016) and Abiy (2017).

Even though five inbred lines showed negative GCA effects for plant height in combined analyses across locations (Table 7), only one inbred line L6 (-19.85) showed significant GCA effect, implying the tendency of this line to reduce plant height, which is very important for development of genotypes resistant to lodging. All the four inbred lines that showed positive GCA (L3, L5, L8 and L19) were poor general combiners for short plant height as they showed positive and significant GCA effects. In line with the present study, Dagne et al. (2010), Demissew et al. (2011) and Dufera et al. (2018) found significant positive and negative GCA effects for plant height.

For number of ears per plant, four inbred lines showed positive GCA effects among them two inbred lines L3 (0.19) and L9 (0.08) had significant GCA effects. L3 had positive and highly significant GCA effect for number of ears per plant, hence, it was the best general combiner for prolificacy. Two inbred lines L1 (-0.09) and L4 (-0.08) showed significantly negative GCA effects for ears per plant, hence are considered as poor combiners for number of ears per plant. L1 had the smallest GCA effect of -0.09 for ears per plant. Similar to the present findings, Dagne et al. (2007) reported significant positive and negative GCA effects for number of ears per plant.

In combined analyses across the two locations, four inbred lines showed positive GCA effects for ear diameter among them three inbred lines had significant GCA effects. L1 (0.1), L4 (0.15) and L8 (0.20) were the best general combiners for ear diameter, that is these lines have the tendency to increase ear diameter as they had highly significant and positive GCA effect (Table 7). On the other hand, three inbred lines had significantly negative GCA effects. The present study is in agreement with Melkamu (2013), Rahman et al. (2013) and Habtamu (2015) who reported significant positive and negative GCA effects for ear diameter.

Significantly positive and negative GCA effects were obtained for thousand kernel weight across the two locations. From a total of six inbred lines which showed positive GCA effects for thousand-kernel weight, three of the inbred lines L6 (23.33), L7 (26.46) and L8 (22.61) showed significant and positive GCA effects, indicating that the inbred lines were the best general combiners for thousand-kernel weight. On the other hand, L3 (-40.08) and L9 (-48.64) showed negative and significant GCA effects, which are undesirable. In support of this findings, Amiruzzaman et al. (2010) and Demissew et al. (2011) recorded significant positive and negative GCA effects for thousand kernel weights.

Specific combining ability effects

Specific combining ability effects for grain yield and

Table 8. Estimates of specific combining ability effects (SCA) of 36 diallel crosses evaluated at Ambo and Kulumsa in 2017.

Crosses	GY	DA	EPP	ED
L1*L2	0.47 ns	-0.84 ns	0.04 ns	-0.032 ns
L1*L3	1.47*	-0.52 ns	0.22**	0.08 ns
L1*L4	-0.64 ns	1.34 ns	-0.03 ns	0.09 ns
L1*L5	1.23 ns	-0.45 ns	0.12 ns	-0.06 ns
L1*L6	-0.59 ns	0.55 ns	-0.11 ns	-0.036 ns
L1*L7	-1.27 ns	0.63 ns	-0.09 ns	-0.16 ns
L1*L8	-0.60 ns	0.38 ns	-0.05 ns	-0.0071 ns
L1*L9	-0.15 ns	-1.09 ns	-0.09 ns	0.13 ns
L2*L3	0.18 ns	-1.23 ns	0.0012 ns	0.10 ns
L2*L4	0.39 ns	0.13 ns	0.101 ns	-0.01 ns
L2*L5	-0.27 ns	-0.41 ns	-0.13 ns	-0.007 ns
L2*L6	-0.21 ns	-0.16 ns	0.07 ns	-0.035 ns
L2*L7	1.02 ns	0.66 ns	0.17*	-0.014 ns
L2*L8	-0.73 ns	0.91 ns	-0.16*	-0.007 ns
L2*L9	-0.86 ns	0.95 ns	-0.09 ns	0.0036 ns
L3*L4	-0.39 ns	0.95 ns	-0.13 ns	0.00 ns
L3*L5	-0.24 ns	-1.34 ns	-0.11 ns	0.08 ns
L3*L6	0.61 ns	0.66 ns	0.07 ns	0.08 ns
L3*L7	-1.33 ns	-0.02 ns	-0.09 ns	-0.03 ns
L3*L8	0.38 ns	0.73 ns	0.09 ns	-0.096 ns
L3*L9	-0.76 ns	0.77 ns	-0.05 ns	-0.21*
L4*L5	0.11 ns	-1.23 ns	0.102 ns	-0.26**
L4*L6	-0.28 ns	-0.73 ns	-0.09 ns	-0.04 ns
L4*L7	0.57 ns	0.34 ns	0.08 ns	0.11 ns
L4*L8	0.43 ns	0.34 ns	-0.04 ns	0.16 ns
L4*L9	-0.19 ns	-1.13 ns	0.00053 ns	-0.05 ns
L5*L6	0.18 ns	-0.52 ns	0.03 ns	0.014 ns
L5*L7	0.04 ns	0.55 ns	-0.0063 ns	0.14 ns
L5*L8	-0.14 ns	0.30 ns	0.125 ns	-0.007 ns
L5*L9	-0.91 ns	3.09*	-0.128 ns	0.104 ns
L6*L7	-0.21 ns	0.05 ns	-0.09 ns	0.032 ns
L6*L8	0.15 ns	-0.19 ns	0.0087 ns	-0.04 ns
L6*L9	0.66 ns	0.34 ns	0.12 ns	0.025 ns
L7*L8	-0.33 ns	-0.88 ns	-0.10 ns	-0.04 ns
L7*L9	1.07 ns	-1.34 ns	0.12 ns	-0.03 ns
L8*L9	1.14 ns	-1.58 ns	0.13 ns	0.03 ns
SE(s_{ij})	0.75	1.39	0.083	0.09

**Significant at 0.01 level of probability, * = significant at 0.05 level of probability, ns = non-significant, GY= grain yield, DA= number of days to anthesis, DS= number of days to silking, PH= plant height, EPP= number of ears per plant, ED= ear diameter and TKW=1000-kernel weight.

related agronomic traits for across location is presented in Table 8. The crosses showed considerable variation in their SCA effects for the different traits.

In combined analyses across the two locations, positive SCA effects were found in seventeen of the crosses for grain yield. The cross L1 x L3 was the only best positive and significant ($p<0.05$) cross combination with SCA value of 1.47. Thus, this cross could be selected for its specific combining ability to improve grain yield. Crosses with higher value of SCA effects also showed higher values of mean grain yield, indicating good correspondence between SCA effects and mean grain yield. Hence such cross combinations could effectively be exploited in hybrid breeding program in maize research. Nineteen crosses showed negative SCA effects for grain yield (Table 8) which are undesirable as these crosses showed a tendency to reduce grain yield performance. In line with the current finding, Kamara et al*.* (2014), Girma al*.* (2015), Ram et al*.* (2015), Bullo and Dagne (2016) reported significant positive and negative SCA for grain yield. They suggested that, when high yielding specific combinations are desired, especially in hybrid maize development, SCA effects could help in the selection of parental material for hybridization.

For days to anthesis, only one cross L5 x L9 (3.09) showed positive and significant SCA effect (Table 8). Thus, this cross could be used for late maturity for the locations with sufficient rainfall. In agreement with this finding several researchers reported significant positive and negative SCA effects for days to anthesis (Kanagarasu et al*.,* 2010, Dagne et al*.,* 2011, Aminu and Izge, 2013; Aminu et al*.,* 2014).

Positive SCA effects were found in eighteen of the crosses for ear per plant. The crosses L1 x L3 and L2 x L7 were the two best positive and significant cross combinations with SCA values of 0.22 and 0.17, respectively. Thus, these crosses could be selected for their specific combining ability to improve number of ears per plant. Eighteen crosses showed negative SCA effects in undesired direction for ear per plant with only one significant and negative SCA, L2 x L8 (-0.16) (Table 8). This indicates that this hybrid combination is poor for number of ears per plant. Similar results were reported by Berhanu (2009) and Bello and Olawuyi (2015). They indicated the capacity of the crosses to produce hybrids having increased number of ears per plant.

Sixteen of the crosses showed positive SCA effects for ear diameter but none of them were significant (Table 8). On other hand, twenty of the crosses showed negative SCA effects, but only two of the crosses L3 x L9 (-0.21) and L4 x L5 (-0.26) showed significant and negative SCA effects for this trait. This indicates that none of these crosses were significantly good specific combinations for ear diameter. Amiruzzaman et al. (2010) found significant positive and negative SCA effects for ear diameter.

Conclusion

From the study, it can be concluded that better performing inbred lines with desirable GCA, cross combinations with desirable SCA effects and crosses with noticeable level of heterosis above the standard check for grain yield and other grain yield related traits were successfully identified. These genotypes constitute a source of valuable genetic materials that could be successively used for future breeding work in the development of maize cultivars with desirable traits' composition for highland sub-humid agro-ecology of Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The effects of gypsum on pod-yield and pre-harvest aflatoxin contamination in selected peanut cultivars of Zambia

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Good agricultural practices are an effective means of minimizing pre-harvest aflatoxin contamination in peanuts. A field experiment was conducted to evaluate the effect of gypsum on pod yield and aflatoxin contamination in three peanut cultivars (Kadononga, MGV 4 and MGV 5) in Zambia. The experiment was conducted in Chongwe and Lusaka districts. Gypsum (15.6 % calcium) was applied at rates of 0 and 400 kg/ha at flowering stage. Although gypsum had no significant effect on aflatoxin contamination, there were significant differences (p = 0.009) in cultivar susceptibility to aflatoxin contamination. The cultivar with the smallest kernels had 18.8% lower aflatoxin content than the large-kernelled cultivar. Additionally, gypsum did not have a clear effect on pod yield. For instance, gypsum was associated with 44.8% more grain-filled pods in Kadononga (p = 0.005) at the site in Lusaka, but this result did not apply to the other two cultivars. At the site in Chongwe, gypsum was associated with 34.6% higher pod yield of MGV 5 only (p = 0.006). These results further suggest that plant factors such as kernel size may have an influence on natural resistance to aflatoxin contamination in peanuts.

Key words: Aflatoxin, gypsum, peanut cultivar, pod-yield, Zambia.

INTRODUCTION

The prevalence of high aflatoxin contamination in peanuts is a recurrent problem in most tropical climates including Zambia (Njoroge et al., 2017). This has prompted concerted efforts to combat aflatoxin contamination at various stages of the peanut value chain. In the pre-harvest stages, there is need to implement good agricultural practices to minimize contamination during crop growth. Measures that minimize plant stress especially during the pod development stage are recommended so as to minimize

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colonization of pods by toxigenic *Aspergillus* fungi and subsequent aflatoxin contamination in kernels (Waliyar et al., 2013; Torres et al., 2014).

One of the critical elements in the development of sound groundnut pods and kernels is calcium (Cox et al., 1976; Jain et al., 2011). Well-developed mature pods are not easily perforated by insects and this minimizes the entry of fungi into the seed tissue. It is on this principle that calcium-containing soil amendments such as gypsum are used to minimize pre-harvest aflatoxin contamination in peanuts (Reding et al., 1993; Gebreselassie et al., 2014). Additionally, amending soils with gypsum at the rate of 250 kg/ha was associated with higher grain yields compared with the control (Bairagi et al., 2017). According to Kabir et al. (2013) amending the soil with gypsum as a source of calcium resulted in higher number of pods per plant and 100 pod weights. Therefore, sufficient calcium fertilization in peanuts can both minimize aflatoxin contamination and increase kernel yield.

However, cultivar response to calcium inputs partly depends on kernel size. Large seeded cultivars require higher inputs of calcium than small-seeded kernels (Jordan et al., 2014). The objective of this study was to assess the effect of gypsum amendment on kernel yield and pre-harvest aflatoxin contamination on aflatoxin-susceptible cultivars. Pod-yield and aflatoxin contamination in three peanut cultivars of Zambia were evaluated following a gypsum amendment on two soils with contrasting exchangeable calcium content.

MATERIALS AND METHODS

Location and soil properties

A field experiment was conducted at the University of Zambia (UNZA), Field Research Station (15° 28.646' S, 28° 20. 278' E) in Lusaka district and at Kasisi Agricultural Training Centre (KATC15°14.989' S, 28° 29.013' E) in Chongwe district. The experiment was done under rain-fed conditions from mid-December 2016 to April 2017. Both research sites are situated in the agro-ecological region II of Zambia with mean annual rainfall ranging from 800 to 1000 mm (Soil Survey Branch, 2002). The two research sites were characterised by soil with contrasting chemical properties in terms of soil pH and exchangeable calcium content. The soil at KATC was characterised by strong acidity (pH = 4.22) and very low exchangeable calcium (0.06 cmol/kg) while the soil at UNZA had near neutral pH of 6.98 and high exchangeable calcium content of 5.14 cmol/kg. It should be noted that most acidic soils are associated with calcium deficiency (Brady and Weil, 2010).

Treatments and experimental design

The treatments in the experiment were two rates of gypsum and three cultivars. The cultivars were: MGV 5, MGV 4 and Kadononga. The chosen cultivars were among the most popular ones among small-holder farmers and seed companies. MGV 5 and MGV 4 were among the most common commercial cultivars while Kadononga was among the most common local landrace mostly preferred for its early maturity and 'tasty' kernels. All the three cultivars have a bunch type growth habit. MGV 5 and MGV 4 are Virginia market types taking between 120 and 130 days to physiological maturity with potential yields of 2.5 to 3.0 metric ton/ha and 1.5 to 2.5 metric ton/ha, respectively. Kadononga is a Spanish type that takes between 90 and 100 days to maturity with a yield potential of 1.0 to 1.5 metric ton/ha. In terms of seed size, MGV 5 is large-seeded; MGV 4 is medium-seeded while Kadononga is a small-seeded cultivar. The treatments were laid out in split-plot design with gypsum as the main plot factor and cultivar as the sub-plot factor, respectively. The experimental plots measured 4 m by 4 m with a 1 m isle between plots. Each factor was replicated thrice resulting in 18 experimental plots. Gypsum was applied on the soil surface covering the entire plant row span at flowering stage rates of 0 and 400 kg/ha. The time of application and the rate of 400 kg/ha of gypsum were adopted from the literature (Waliyar et al., 2013).

Seeding and field management

Seedbed preparation was done by ploughing with a tractor-mounted disc plough followed by levelling using a disc harrow. Seeding was done on a flat seedbed in 5 cm-deep planting holes made using a hand-hoe. The recommended seeding rate for each cultivar was followed. The fields were kept weed, pest and disease-free throughout the growing season. Weeds were uprooted by hand or dug-out using a hoe just as soon as they appeared. Appropriate pesticides and fungicides were sprayed regularly to control pests and fungal infections, respectively.

Harvesting and determination of pod-yield

Harvesting of peanut pods was done at physiological maturity. Plants were dug out using a hand hoe. Pod yield was determined by counting grain-filled pods from each of the 6 randomly selected plants from the middle plant rows of each experimental plot. The pods were then dried in an electric vacuum oven (Hereanus, Germany) set at 45°C to a gravimetric moisture content of 10%. The dried pods were then shelled by hand and prepared for aflatoxin testing.

Sampling and aflatoxin analysis

One third of the total kernel yield per treatment constituted the laboratory sample. The sample was constituted by aggregating several 100 g scoops from a single bulk sample. The bulk sample was shaken after each 100 g sub-sample was taken. Duly constituted laboratory samples were then ground into fine flour using an ordinary kitchen grinder (LM2211BM, Moulinex, China). Ground samples were homogenized by thorough shaking. Total aflatoxin content in the flour was extracted using 65% ethanol reconstituted from an original product (UN1170, Xilong Scientific Co., Shantou City, China) with a concentration of 95%. For each treatment, three sub-samples each weighing 10.0 g were mixed with 30 ml of ethanol and shaken on a rotary shaker (ISO-9001-2000, Navyug, India) at 120 rpm for 3 min. After shaking, the mixture was filtered through Whitman 42 filter paper. Total aflatoxin concentration in each sample was determined using Neogen Afla Reveal® Q+ aflatoxin kit (Neogen Corporation, USA). The lower and upper limits of detection of aflatoxin concentration were 1 and 50 µg/kg, respectively. All the tested samples had total aflatoxin concentrations within detection limits.

Statistical analysis

Data on pod yield and aflatoxin content were subjected to the

Table 1. Effects of gypsum on the number of grain-filled pods per plant for each variety.

Site	Cultivar	Gypsum level (kg/ha)	Number of pods per plant ± standard error mean	P-value
UNZA	Kadononga	0 400	29 ± 3.1 42 ± 3.2	0.005[a]
	MGV 4	0 400	51 ± 5.9 44 ± 4.3	0.410
	MGV 5	0 400	57 ± 3.8 65 ± 6.1	0.284
KATC	Kadononga	0 400	23 ± 2.2 20 ± 1.4	0.199
	MGV 4	0 400	30 ± 2.2 28 ± 2.6	0.509
	MGV 5	0 400	26 ± 1.5 35 ± 2.7	0.006[a]

[a]Gypsum was associated with higher number of grain-filled pods per plant in selected cases.

analysis of variance test at 95% confidence interval. The separation of statistically significant treatment means was done using Fisher's protected Least Significant Difference.

RESULTS AND DISCUSSION

Effects of gypsum on pod-yield in selected peanut cultivars

Gypsum applied at flowering stage of peanuts resulted in higher number of grain-filled pods only in selected cultivars (Table 1). Although gypsum was associated with higher number of pods for Kadononga at UNZA, no similar result was observed at KATC. Similarly, gypsum was associated with a significantly higher number of grain-filled pods for MGV 5 at KATC and not at UNZA. MGV 4 did not show any response to gypsum at both sites. In general, these results are contrary to literature (Cox et al., 1976; Jain et al., 2011; Jordan et al., 2014; Bairagi et al., 2017) that report higher yields due to calcium-containing inputs such as gypsum. According to Kabir et al. (2013) peanut plants fertilized with calcium inputs recorded higher 100 pod weights compared with plants that did not receive a calcium input. As earlier noted by Smith et al. (1993) calcium is an essential element for pod filling in peanuts and a lack of it is reported to cause fruit abortions, resulting in fewer grain-filled pods.

According to Cox et al. (1976), peanut response to calcium-containing inputs is dependent on soil characteristics such as the exchangeable calcium content. Recommendations must therefore consider soil type and in particular the native calcium content at which additional amounts would trigger a response. The choice of 400 kg/ha in the current study was based on the recommendation by Waliyar et al. (2013). The poor response to gypsum observed in the study suggests that the chosen rate of gypsum application may not be adequate for the soil types at the two sites.

The observed result in this study could be attributed to the native exchangeable calcium content of the soil at the two experimental sites. Although the soil at Kasisi had low exchangeable calcium, it was still able to meet the calcium requirements for Kadononga and MGV 4. As for MGV 5 at the same site, the positive response to gypsum input could signify the need for extra calcium for optimal growth. In the case of the soil at UNZA that had high native exchangeable calcium content, the logical expectation would be that the small-kernelled Kadononga would not respond to additional calcium, unlike the larger-kernelled MGV 4 and MGV 5. On the contrary, only the small-seeded Kadononga responded, making it difficult to attribute the response to gypsum amendment.

Additionally, differences in plant nutrient and moisture requirements between cultivars have an effect on crop performance. For instance, small-kernelled peanut cultivars have a lower nutrient and soil moisture requirement than larger ones (Jordan et al., 2014). Thus, if there is a limited supply of nutrients, especially exchangeable calcium and plant-available-water in the soil, the small-kernelled cultivars would grow normally

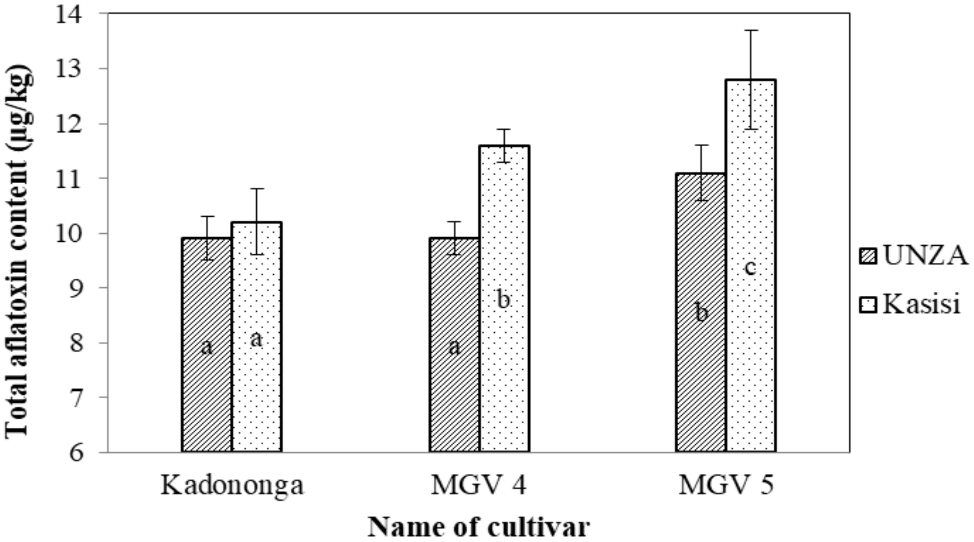

Figure 1. Total aflatoxin content of peanuts kernels from respective cultivar. Error bars represent standard error of the mean. Letters within each data bar indicate statistical significance between treatments for each site.

under given soil conditions while the larger-kernelled cultivars would need external inputs.

Effect of gypsum and cultivar on total aflatoxin content in kernels at harvest

Results from the study showed that the gypsum amendment had no effect on total aflatoxin content in kernels ($p > 0.05$). In contrast, other authors such as Reding et al. (1993) and Gebreselassie et al. (2014) reported decreased aflatoxin content in peanut kernels due to gypsum amendment. Nonetheless, there were significant differences ($p < 0.01$) in mean total aflatoxin content in the three cultivars (Figure 1). The aflatoxin content varied according to kernel size. The cultivar with smallest kernel size was the least contaminated. This pattern was observed at both experimental sites. The mean total aflatoxin concentrations across the two sites were 10.1, 10.8 and 12 ppb for Kadononga, MGV 4 and MGV 5, respectively, in the same order as their kernel size starting with the smallest to the largest. Although the aflatoxin contamination in all the cultivars was within the permissible limits of less than 15 ppb according to the Zambia Bureau of Standards ZS 723 safety standard, the result in this study suggests that more effort is needed to manage aflatoxin contamination in larger-kernelled cultivars than in smaller ones.

Soil moisture status assessed in terms of mean daily rainfall received during the pod-development phase of the crop did not show significant differences ($p > 0.05$) across cultivars at each of the two sites. Therefore, the observed differences in mean total aflatoxin levels cannot be explained by the moisture status. Reding et al. (1993) attributed the reduction in aflatoxin contamination following the application of gypsum amendment to inherent plant factors. Similarly, results from the current study suggest that there could be plant factors such as pod strength that may influence aflatoxin contamination. According to Waliyar et al. (2013), well-developed mature pods tend to be less susceptible to fungal infection and subsequent aflatoxin contamination than immature and weak pods. In a study involving ten peanut genotypes, it was reported that although all the studied cultivars supported the growth of *Aspergillus flavus*, one cultivar recorded significantly less aflatoxin contamination (< 20 ppb) regardless of the amount of fungal accumulation (Korani et al., 2017). This result may suggest that some peanut genotypes are naturally more resistant to aflatoxin contamination than others.

Conclusions

Gypsum at the rate of 400 kg/ha did not have a clear influence on pod yield and no significant effect on total aflatoxin contamination in harvested kernels. However, this study showed significant differences in cultivar susceptibility to aflatoxin contamination. For the three cultivars in the study, results showed a negative relationship between pre-harvest aflatoxin content and kernel size indicating that inherent factors such as kernel size may have a role in determining aflatoxin resistance. Further, it can be concluded that management of aflatoxin contamination in larger-kernelled cultivars requires more effort than in smaller ones.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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