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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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African Journal of Plant Science

Table of Content: Volume 9 Number 4, April 2015

ARTICLES

Yield Parameter Response Of Maize (*Oba Super* 2) To Earthworm Cast And Anthill Soil Under Greenhouse Condition
Ezeaku, P. I., Amanambu, C. N., Ede, I. G., Ene, J. and Okebalama, C. B.

Morphological And Molecular Characterization Of *Lepidium Sativum* Population Collected From Ethiopia
Said Mohammed and Kassahun Tesfaye

Participatory Varietal Selection Of Maize (*Zea Mays* L.) In Pawe And Guangua Districts, North Western Ethiopia
Ziggiju Mesenbet Birhanie

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Full Length Research Paper

Yield parameter response of maize (*Oba Super 2*) to earthworm cast and anthill soil under greenhouse condition

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The study of earthworm cast and anthill as plant-soil systems and the surrounding soil is helpful in the appraisal of soil and crop productivity. Therefore, this study was carried out in a tropical and subtropical area to determine the effect of plant-soil systems and the surrounding soil on the yield parameters (plant height, leave surface and dry matter weight) of maize (*Oba Super 2*) under greenhouse condition. Analyses of soil samples of earthworm cast, anthill (termite mound) collected by hand sampling and the surrounding soil collected with core samplers at 10 cm depth and auger samplers at 2 depths (surface, 0 to 15 cm; subsurface, 15 to 30 cm) were examined for chemical and physical properties. Three kilograms each of the soils from three locations (Nsukka, Ede Oballa and Orba) was used for testing maize. Earthworm species (*Eudrilus eugeriae* and *Agrotoreutus nyongii*) and termite species (*Macrotermes* and *Odontotermes* species) that produced the mounds and termite mounds were identified, respectively. The study results found that soil physical and chemical properties were significantly (P<0.05) affected by the plant-soil systems relative to control. Interaction of the plant-soil systems by location and by soil depth was significant (P<0.05) for the measured soil parameters such as soil pH, organic carbon, nitrogen, exchangeable cations (Ca^{+2}, Mg^{+2}, K^{+} except Na^{+}), CEC and available P. Plant height, leaf surface and dry matter yield of maize were found significantly positively (P<0.05) increased. For better and sustainable improvement of soil productivity and yield of maize crop, combined use of earthworm cast and termite mound as plant-soil systems is recommended.

Key words: Maize performance, mounds, termite, cast, earthworm, greenhouse, Nigeria.

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop in the world after wheat and rice with respect to area coverage and productivity (Unagwu et al., 2012). Generally, maize has been in the diet of Nigerians for centuries. In the tropical and subtropical areas, its production derives mainly from three factors: firstly,

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maize can easily be prepared into a variety of meals and this accounts for about 65% of the total daily caloric intake of rural people; secondly, the rising income realizable from the production of maize, and thirdly, that maize not only thrives in intercropping and relay cropping of farmers' cropping system but has quicker biomass recovery with a low economy of production (Ezeaku et al., 2002). In addition, maize provides good sources of raw materials for industries. However, uncertainties and risks associated with agricultural production such as scarcity of inputs, poor morpho-agronomic characteristics of varieties grown, inadequate agro-techniques, high production costs and poor mineral nutrition pose serious problem in meeting maize output levels in tropical and subtropical areas (Ezeaku et al., 2002). It is reported that the high production potential of maize largely depends on availability of essential nutrient elements particularly that of nitrogen, phosphorus and potassium, its nutrient requirement and management (Kang, 1978).

Report had shown that low soil fertility associated mainly with soil acidity is the most important cause of low yield for many crops (Lucia et al., 2010). Hoekenga and Pineros (2004) have reported that about 30% of the world's soils are acidic, and 60% of them are in tropical and subtropical areas associated with long periods of hot and moist weather. Increasing problem of acidification is due to acid rain, removal of natural plant coverage from large production areas and the use of ammonium-based fertilizers (Johnson et al., 1997). Sommers and Lindsa (1979) noted that soil acidity threat to crop production is common in all regions where precipitation is high enough to leach appreciable amounts of exchangeable bases from the soil.

Adequate nutrient, especially N, P and K, supply is essential for optimum production of maize. Most farmers in rural Nigeria hardly afford the use of chemical fertilizers due to scarcity at time of need and high cost when available. Of concern is the acidifying effect of nitrogen component of chemical fertilizer when used without liming. This necessitates research on organic wastes that are cheap, readily available and environmentally friendly, which can be used as fertilizer. Current trend in agriculture supposed to tend towards making the best use of the resources locally available to raise soil fertility and increase food production. Termite mounds and earthworm cast would properly fit into this category.

Cast of earthworm is a digested material that is excreted back into the soil by different species of earthworm. Termite mound is a mixture of soil, organic debris or living plant tissues collected, often over extensive foraging areas, transported to their domain and subjected to intense degradation when it is digested by the termites (Ekundaye and Orhue, 2011). Orhue et al. (2007) noted that plant nutrients and organic material are withheld from circulation in the plant-soil system until they finally decay. Detailed studies of termite mound have been reported in Western (Kang, 1978; Lal, 1988) and the Niger Delta region of Nigeria (Ekundayo and Orhue, 2011). Study on wormcast in relation to soil fertility is limited in eastern region of Nigeria (Mba, 1978). However, study on the effects of combined use of wormcast and termite mound on soil and crop productivity in tropical and subtropical area is lacking.

Reports have shown the benefits of using earthworm cast and termite mound as soil plant-soil system amendments. These include: contribution of nutrients to improve soil fertility and crop productivity (Debruyn and Conacher, 1997); improvement of soil porosity, soil nutrient availability and uptake by plants, minimizing production costs and maximizing yield and profit (Aziz et al., 2010). Increased levels of organic carbon, nitrogen and the formation of water-stable aggregates had been reported (Lal, 1988). Studies have shown significant higher amount of exchangeable cations, micronutrients, organic matter and pH in termite mound soils (Ariha, 1979; Frageria and Baligar, 2004; Ekundayo and Orhue, 2011). Semhi et al. (2008) identified termites as common biological agents that produce significant physical and chemical modifications to tropical and subtropical soils. It is also reported that termite activity increases the content of organic matter in the soils they use for the construction of their nest and also modifies the clay mineral composition of these minerals (Jouquet et al., 2002).

Despite the benefits derivable from the use of plant-soil systems in tropical and subtropical areas, they have their limitations to use by farmers. It is reported that the problem associated with the use of earthworm casts and termite mounds is how to get the huge quantities required to satisfy the nutritional needs of crops. The transportation and handling costs are often beyond the farmer's economy (Lal, 1988). Report by Lee and Wood (1971) showed that the rates of production of the resources materials (earthworm casts and termite mounds) are too small to be used for yearly seasonal crop production and by large commercial farmers.

In spite of the limitations, the long-term benefits in terms of improving soil fertility and enhancing food production cannot be over-emphasized (Fragoso and Lavelle, 1992; Kang, 1978). Studies ascertaining the yield of maize affected by combined use of earthworm cast and termite mound as plant-soil systems are scanty in Nsukka, eastern region of Nigeria. The study aims to provide information on the effects of earthworm cast and termite mound (plant-soil systems) properties on the growth parameters (plant height and leaf surface) and dry matter weight of maize (*Oba Supper 2*).

MATERIALS AND METHODS

Description of study area

The study was conducted in the green house of the University of Nigeria, Nsukka using earthworm cast and termite mound soils collected from three locations: Nsukka, Ede Oballa and Orba. Nsukka and Ede Oballa are situated in Nsukka Local Government

Area (LGA), while Orba is in Udenu LGA. Nsukka LGA lies by latitude 6°51'24" and longitude 7°23'45"E with land area of 45.38 km^2. Udenu LGA lies between coordinates 6°55'N and 7°31'E with a total land area of 897 km^2 (http://www.nipost.gov.ng/postcode.aspx).

Rainfall is bi-modal, the rainy (April - October) and the dry season (November - March). There is usually a short break in August. Average annual rainfall is about 1600 mm. Average minimum and maximum temperature is 22 and 30°C, respectively. The relative humidity is rarely below 60% (Unagwu et al., 2012).

The geomorphology of the study areas is of the highlands stretching through the undulating hills to plain lands. The vegetation belongs to the semitropical rainforest type and complemented by typical grassy vegetation (Ezeaku and Egbemba, 2014).

The soils are very deep, dark reddish brown at the top layer and reddish in the subsoil. They have coarse to medium texture, granular in structure at the top soil, acid in reaction and low in nutrient status. The top soil is characterized by rapid to very rapid permeability (Obi and Asiegbu, 1980). The predominant clay mineralogy is composed mainly of kaolinite and quartz (Akamigbo and Igwe, 1990). The soils are classified in the order of ferrallitic ultisol and entisol which belong to Nkpologwu and Uvuru series, respectively (Mbagwu, 1995; Nwadialo, 1989).

Field work

The study was conducted in 3 ha (300 x 100 m) blocks in each location (Nsuuka, Ede Oballa and Orba). Each block of 1 ha consisted of four land use types: Cassava/yam cultivated field, fallow land, oil palm land, and residential area with land area of 1250, 1250, 2500 and 5000 m^2, respectively. This delineation was done for the 3 ha blocks to avoid the overlap of land use systems.

Prior to sampling in each land use type, the location of earthworm casts and anthills (termite mounds) in each 1 ha block was determined by mapping on a 3 x 3 m grid. Earthworm casts and termite mound soils were collected through hand sampling in the grids replicated trice to cover the 3 ha blocks. Earthworm species predominantly identified were *Eudrilus eugeriae* and *Agrotoreutus nyongii* (Mba, 1978; Fragoso and Lavelle, 1992), while the genera *Macrotermes* and *Odontotermes* species were identified in the termite mounds (Arihad, 1979). The number of earthworm casts and termite mounds in each land use type was recorded. The population count was done within the grid size (3 x 3 m) for the 3 ha block. The collected earthworm casts and termite mounds were carefully put into plastic polyethylene bags, properly labelled and taken to the laboratory for air-drying.

Soil auger samples were randomly taken from the surrounding soils at the depth of 0 to 15 and 15 to 30 cm within an area measuring 50 × 50 m. The surrounding soil is at least 4 m from the grid perimeter. All the soil sampling was done within the field where the earthworm and termite mound sampling were carried out. The depths (0 to 15; 15 to 30 cm) represented the depth of tillage where most nutrients and organic matter are found and usually related to cereal crop yield (Ezeaku et al., 2002). Soil samples from the surrounding field were composited and bulked. Core ring (inner volume of 96.6 cm^3) samples were taken at soil depth of 0 to 10 cm. All soil samples were used for soil property determinations and maize planting in the green house.

Greenhouse studies

The air-dried earthworm casts from each of the location land use types were bulked to form a composite sample. Similar repeated measure was done for termite mound soils. The dried earthworm and termite mound soils were separately crushed to disintegrate the lump structures into smaller aggregate particles and thereafter variously combined to serve as treatments. Composite soil samples from the surrounding field served as control for standardization.

The experimental design was complete randomized design (CRD). The treatments were arranged in CRD and replicated three times. Treatment details were as follows:

T1. Nsukka earthworm cast; T2. Ede Oballa earthworm cast; T3. Orba earthworm cast; T4. Nsukka anthill (Termite mound); T5. Ede Oballa anthill (Termite mound); T6. Orba anthill (Termite mound); T7. Mixture of all earthworm casts; T8. Mixture of all termite mounds; T9. Control soil for Nsukka; T10. Control soil for Ede Oballa; T11. Control soil for Orba.

Phyto assessment

Three (3) kilogram of each site soil was measured into perforated plastic buckets measuring 3.299 cm^3 and allowed for 2 weeks period of incubation. Thereafter, soil in each bucket was turned and properly mixed and watered (200 ml) prior to maize sowing. *Oba Super 2* spp of improved maize seeds were sown into each bucket at the rate of three seeds to a depth of 3 cm, and thinned down to 2 seedlings per bucket after seedling had attained 2-leaf stage. Management that was employed included water application to the soil buckets at intervals and constant weeding during the experiment. Measurements were made on the growth parameters (height and leaf surface) at 2, 4 and 6 weeks after planting. Dry matter yield was determined at the end of the 6 weeks.

Laboratory analysis

Bulk density (Bd) and saturated hydraulic conductivity (Ksat) were determined using Grossman and Keinch (2002) method. The auger soil samples were air-dried in the laboratory ground and passed through a 2 mm sieve. Sieved samples < 2 mm soil fraction was bagged for routine analysis. The fraction of sand, silt and clay was determined using hydrometer method (Gee and Or, 2002) with NaOH as dispersant. Soil pH was determined by McLean (1982) method. Total nitrogen was determined using micro- Kjeldahl (Bremner and Mulvaney, 1982) method. Soil organic carbon was measured by combustion at 840°C (wet-oxidation method) (Wang and Anderson, 1998). Exchangeable bases, Ca^{2+} and Mg^{2+} were obtained by ammonium acetate (NH_4 OAC) method, and Na^+ and K^+ by flame photometer. Cation exchange capacity (CEC) was obtained using Blakemore et al. (1987) method. Exchangeable acidity was determined titrimetrically using 0.05 N NaOH. Available phosphorus was obtained using Bray 11 bicarbonate extraction method as described by Olsen and Sommers (1982).

Data analysis

Data on maize yield variables, soil physical and chemical properties were subjected to analysis of variance (ANOVA) using Genstat Discovery Edition 3, while significant variations in the means were determined using least significance difference ($LSD_{0.05}$) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Distribution of earthworm casts and termite mounds

The population of wormcast and termite mounds (determined from population counts) in four land use

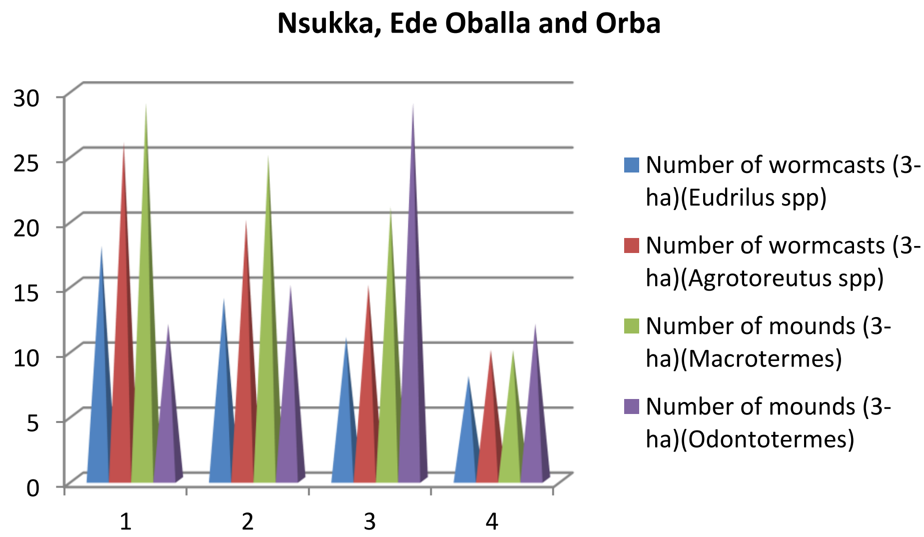

Figure 1. Number of wormcasts and termite mounds across the study location LUTs.

types (LUT 1= cassava/yam cultivated fields, LUT2 = fallow land, LUT 3 = oil palm land and LUT 4 = residential) across the three sites are presented in Figure 1. Generally, wormcast population was higher on fallow and oil palm lands than in other land use types across the locations. Figure 1 shows that total number of casts produced by *Agrotoreutus* spp. (244) was significantly higher ($p<0.05$) than that of *Eudrilus* spp. (194) by 34.7%. This suggests that the benefits derivable from the activities of *Agrotoreutus* spp. could be more useful in improving soil and crop productivity in tropical and subtropical area. This finding accords earlier report that had shown that the number of earthworms can indicate the extent of macropores (earthworm burrows) that are able to quickly drain surface water (Karlen et al., 1997). The report further indicated that earthworm number is 'Level 1' indicator of a soil's ability to accommodate water entry for prolonged periods during high intensity rainfall and frequent irrigation events.

In terms of termite mounds, results in Figure 1 show that the total numbers of *Macrotermes* spp. mounds in LUT 1 and LUT 2 across the study locations (total no. 216) were significantly higher ($p<0.05$) than that of *Odontotermes* (141) by 53.2%. However, total number of mounds produced by *Odontotermes* spp. under LUT 3 (Oil palm land) was higher ($p<0.05$) than in LUT 4.

Effects of worm cast, termite mound, location and soil depth on soil physicochemical properties

The percentage of finer particles in wormcasts and termite mounds could probably be the main effect on physico-chemical properties of soils. The statistical mean values of the physical and chemical properties of wormcasts and termite mounds are presented in Table 1. The textural analysis showed that significantly higher clay contents were obtained in the earthworm cast ($p<0.01$) and termite mound soils ($p<0.05$) than the surrounding soils. The higher clay fractions of wormcast and termite mound soils could be associated with the preferential incorporation of clay by the species of earthworms and termites identified. This confirms earlier report by Kang (1978) and Debruyn and Conacher (1987) who reported significantly higher clay content in termite mounds than the surrounding soil.

In contrast, higher silt content was recorded in the surrounding soil than in the plant-soil systems. This divergent result could be due to variation in site characteristics, soil types, land use and physiographic positions. The physiography of the 3 ha land in the study locations ranged from undulating hills to undulating plains.

The physical properties by location (Table 1) showed that clay, silt and sand fractions were variable but significant ($p<0.05$). Fraction of sand decreased with soil depth, while clay and silt contents increased with depth and could be attributed to illuviation/eluviation phenomena caused by percolating water through the soil column. This is synonymous with report of Obi and Asiegbu (1980). Variation of soil texture from sandy loam to sandy clay loam could be attributed to nature of parent materials and high rainfall that could favor washing away and leaching of silt-sized and clay-sized fractions (Mbagwu, 1995; Lal, 1988).

Soil bulk density (Bd) value recorded in Table 1 was significantly higher ($p<0.05$) in the surrounding soil (1.52 gcm^{-3}) as compared to the earthworm and termite mound soils. Soil Bd decreased by 13.2% in wormcast soil and 5.3% in termite mound soil relative to surrounding soil.

Table 1. Effects of earthworm cast, anthill, location and soil depth on the soil physicochemical properties.

Soil sample	Texture	Clay (%)	Silt (%)	Sand (%)	Tp (%)	Ks cms^{-1}	Bulk density (gcm^{-3})
Plant-soil system							
Earthworm cast	SL	19.5	7.6	72.9	42.6	2.66	1.52
Anthill	SL	22.0	7.5	70.5	45.7	2.82	1.44
Control	SCL	9.7	8.8	81.5	50.2	4.06	1.32
LSD (P<0.05)		13.20**	8.68*	4.64*	-	3.24*	o.14*
Location							
Nsukka	SL	16.0	8.0	76.0	44.2	2.50	1.48
Ede Oballa	SCL	22.0	7.5	70.5	47.5	4.26	1.39
Orba	SCL	21.0	8.0	71.0	46.0	2.43	1.43
LSD (P<0.05)		4.64*	10.6*	15.31*	-	2.80*	0.15*
Soil depth							
0-15 cm	SL	16.0	9.0	75.0	Nd	Nd	Nd
15-30 cm	SCL	20.0	11.0	69.0	Nd	Nd	Nd
LSD (P<0.05)		3.80*	1.38*	Ns	Nd	Nd	Nd

Ks, soil saturated hydraulic conductivity; Bd, soil bulk density; TP, total porosity; NS, not significant; Nd, not determined; SL, sandy loam; SCL, sandy clay loam; LSD (P<0.05), least significant difference at 5% level of probability, *p<0.05, **p<0.01.

Though value of Bd in the termite mound soil (1.44 gcm^{-3}) was higher than in the wormcast soil (1.32 gcm^{-3}), both values are statistically similar.

The higher Bd in the surrounding soil may be associated with seasonal flooding of soils; resulting to continued wetting and drying of soils. There is also the possibility of soil surface crusting and crusting by compaction through raindrop impact and surface erosion. It is quite possible that the higher content of sand in the control soil (Table 1) was due to washing away of clay and silt sized-fractions by rainfall and runoff water and may have contributed to the higher bulk density obtained. The soil bulk density values by location (Table 1) varied from 1.39 to 1.49 gcm^{-3}. Interestingly, all the mean values of soil Bd obtained in the plant-soil systems are within the allowable limit (1.50 gcm^{-3}) for crop production in the subtropics (Lal, 1990).

Soil saturated hydraulic conductivity (Ks) is one of the most important hydrologic properties that could be used to estimate the internal drainage of soils. Results in Table 1 show that Ks was significantly higher (P<0.05) in earthworm cast soil by 34.5% relative to the surrounding soil. The higher Ks (4.06 cms^{-1}) and total porosity (50.2 %) values obtained in the wormcast soils could be attributed to the low soil Bd (1.32 gcm^{-3}) (Table 1). This has implication of lowering run-off and erosion, while increasing aeration and internal drainage. It also suggests that the soil-plant systems present non-limiting conditions in terms of structure as reflected by low Bd, adequate airspaces and higher water transmission. These important soil parameters promote easy crop root growth and proliferation for enhanced crop production. This accords Ezeaku and Egbemba (2014) report that low Bd is a positive productivity indicator as it helps in easing root penetration, and encourages downward movement of water through old root channels. Similarly, the burrow channels created by the activities of earthworms and termites increase soil depth and regulate water flow into the soil. This implies more soil water retention and availability for greater water use efficiency by crops (van Schaik et al., 2014)

The results of measured soil physical properties (Table 1) are corroborated by several study reports. It had been shown that in the presence of earthworms, the hydraulic conductivity increases and soil bulk density decreases due to formed earthworm channels (Lavelle, 1997; Francis and Fraser, 1998; Chan, 2004). Thus, macropores formed by earthworms probably become more important for buffering strong precipitation events in future in humid areas. Next is the formation of macropores earthworm and termite activity creating and stabilizing of soil structure (Six et al. 2004). However, earthworms can also have a negative effect on soil structure and other soil parameters (Laossi et al., 2010).

Effects of earthworm cast, termite mound, location and soil depth on soil chemical properties

The results in Table 2 show that soil pH was slightly acidic in the plant-soil systems (higher soil pH range: 6.2 to 6.3) but more acidic in the surrounding soil (low soil pH of 5.2). Soil pH appreciated by 17.5% in wormcast soil and 16.6% in termite mound soil due to significant (P <

Table 2. Effects of earthworm cast, anthill, location and soil depth on the soil chemical properties.

Soil sample	pH (H_2O)	Kcl	EA	OC	TN	Ca	Mg	K	Na	CEC	Av P
			Cmolkg^{-1}	%		←		Cmolkg^{-1}		→	Mgkg^{-1}
Plant-soil system											
Earthworm cast	6.3	5.7	1.26	4.26	1.128	4.2	1.40	0.42	0.38	20.40	22.6
Anthill	6.2	5.4	1.30	3.30	1.113	4.6	1.20	0.36	0.29	18.00	18.4
Control	5.2	4.8	1.80	2.47	0.166	3.5	0.61	0.12	0.20	12.20	12.1
LSD (P<0.05)	0.29	0.38	Ns	1.46	0.03	0.24	0.01	0.01	0.06	1.68	2.30
Location											
Nsukka	6.3	5.1	1.51	4.08	0.136	3.7	1.20	0.29	0.19	9.20	18.5
Ede Oballa	6.0	5.0	1.40	3.47	0.122	4.2	1.00	0.42	0.34	13.1	16.4
Orba	6.1	5.4	1.22	2.09	0.09	1.2	1.60	0.16	0.18	17.0	13.1
LSD (P<0.05)	Ns	Ns	Ns	0.22	1.37	2.16	0.05	0.01	0.03		2.67
Soil depth											
0-15 cm	6.6	5.0	1.35	4.43	o.12	4.5	1.34	0.33	0.33	20.8	20.0
15-30 cm	6.4	5.5	1.40	4.88	0.32	4.2	1.38	0.38	0.31	22.1	20.20
LSD (P<0.05)	ns	Ns	Ns	0.31	0.01	2.11	0.02	0.06	Ns	1.06	2.54

EA, exchangeable acidity; OC, organic carbon; TN, total nitrogen; Ca^{2+} = calcium; Mg^{2+} = magnesium; Na^{+} = sodium; CEC, cation exchange capacity; Av. P, available phosphorus; Ns, not significant; LSD (P<0.05), least significant difference at 5% level of probability.

0.05) increase in exchangeable cation (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) contents in the soil colloidal complex (Table 2). Low pH value of 5.2 obtained in the control soils might be associated with loss of exchangeable bases resulting from displacement reactions in the soil colloidal complex. Soil acidity has been partly associated with excessive rainfall that necessitates eluviations and leaching loses of cations (Akamigbo and Igwe, 1990) but under field condition.

Organic carbon contents in wormcast soil and termite mound soil were significantly higher (P<0.05) than in the surrounding soil (Table 2). Soil organic carbon (SOC) of wormcast soil increased by 42.0%, while SOC in the termite mound soil increased significantly (P<0.05) by 25.2% relative to control. This increase could be linked to high content of carbon in the plant-soil system (earthworm cast and anthill). Ekundayo and Orhue (2011) and Jouquet et al. (2002) have shown that higher SOC found in termite mound soils could be due to the fact that during the periods of mound construction, organic debris or living plant tissue were collected over extensive foraging area, transported to mounds and subjected to intensive degradation.

In terms of earthworms, they contribute to build-up of organic matter in the soil through a sequence of actions. Earthworms feed on organic materials found on the soil surface and subsurface. At the soil surface, they ingest vegetation, organic materials (fresh and dried) and some fine earth fractions. Earthworms also burrow into the soil subsurface to nibble partially decayed organic materials such as plant roots, twigs and leaves and soil.

The biodegradation of the ingested materials is facilitated by enzymatic reactions from the salivary secretions (Mba, 1978). The waste digests are excreted as casts, which are composited of fine earth materials (clay) and high nutrient elements particularly organic matter and cations that buffer the soil systems. This may have been the reason for the increased clay fractions obtained in both worm cast and termite mound soils (Table 1). Again, earthworm burrowing activity could have advantages of creating more airspace and channels for water transmission, possibly disintegrate hard soil structure for ease of root growth and better crop performance.

The report by Akinrinade et al. (2000) as cited by Ezeaku (2011) had shown that soil organic carbon content of 1.74% or 17.4 gkg^{-1} was suggested as critical level for crop production in the tropic and subtropical soils. The results in this study (Table 2) showed that SOC contents obtained in wormcast (4.26% or 42.6 gkg^{-1}), termite mound (33.0 gkg^{-1}) and surrounding soil (24.7 gkg^{-1}) were above the critical limit and may be related to higher amount of organic residue taken and digested by earthworms and termites. It is also probable that higher quantities of residue are produced in the surrounding soil.

Soil organic carbon mediates nitrogen in the soil. Soil nitrogen increased significantly (P<0.05) by about 85.0% in the two plant-soil systems relative to control (Table 2). The values of total N in the wormcast soil (1.12% or 11.2 gkg^{-1}) and termite mound soil (11.1 gkg^{-1}) were more than 0.15 or 1.5 gkg^{-1} total N at which response to N fertilization is not expected in subtropical soils (Ezeaku, 2011). The value of total N obtained from the surrounding soil was very close to the critical value, suggesting negative implication for crop production. Response to N fertilization is required.

All exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ except Na^+) had significant (P<0.05) increases in the soil (Table 2), an insinuation that amendment soils had effect on the measured parameters. Exchangeable Ca^{2+} increased by 16.7% in wormcast soil and 85.1% in termite mound. Mg^{2+} appreciated by 57.1 (earthworm cast soil) and 50.0% (termite mound). Exchangeable K^+ values are higher in wormcast soil (0.42 cmolkg^{-1}) and termite mound soil (0.36 cmolkg^{-1}) relative to the control. Exchangeable K^+ increased by 71.4% in wormcast soil and 66.6% in termite mound soil. The values of K^+ in the wormcast soil and termite mound soil are higher than the critical values of 0.16 to 0.20 cmolkg^{-1} for crop production (Isirima et al., 2003; Ezeaku, 2011). Johnson et al. (1997) show that the increases in exchangeable cations suggest displacement of active exchangeable Al^{+3} in the cation exchange site by the cations, and could have contributed to the increased soil pH obtained in this study. The increase in exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ except Na^+) is synonymous with Ekundayo and Orhue (2011) report that showed significantly higher (p<0.05) exchangeable cations on termite mound surfaces and mound perimeters than the surrounding soils under some land use types. Similar report in terms of earthworm cast soils had been made (Mba, 1978).

Available P increased significantly (P<0.05) by 46.5% in wormcast soil and 34.4% in termite mound soil (Table 2) relative to control. This could be attributed to high P content in the decayed organic debris or living plant tissues digested (plant-soil system) by the earthworm and termite species. Similar observation has been reported (Kang, 1978; Lal, 1988; Ekundayo and Orhue, 2011).

For the soils of the tropics, 8-12 mgkg^{-1} of available P was considered critical limit for crop production (Ezeaku, 2011). The values of available P obtained in wormcast soil (22.6 mgkg^{-1}) and termite mound (18.4 mgkg^{-1}) (Table 2) were higher than the critical limit. This suggests that available P (12.1 mgkg^{-1}) obtained in the surrounding soil is marginal. Such soil needs phosphorus fertilization.

Enwezor et al. (1990) report as cited in Ezeaku (2011) showed that values of CEC below 6-8 cmolkg^{-1} is low, 6-11 cmolkg^{-1} (medium) and >12 cmolkg^{-1} is considered high. Based on these limits, the amounts of CEC obtained in the plant-soil systems and the surrounding soil (range: 12.2 to 20.4 Cmolkg^{-1}) are generally high (Table 2). The high levels signify no response to N, P and K fertilization for the crop in the tropical and subtropical area.

From Table 2, it can be observed that interaction of wormcast soil and termite mound soil (plant-soil systems) by location and by soil depth was significant (P<0.05) for soil organic carbon, nitrogen, all exchangeable cations (except Na^+) including CEC and available P. The significance of the interaction is a positive soil productivity indicator, suggesting that the nutrients inherent in wormcast and termite mound soils are quite high and could be used as fertilizer to improve crop productivity. These findings are corroborated by Kang (1978), Ariha (1979), Lal (1988), Fragoso and Lavelle (1992), Frageria and Baligar (2004) and Ekundayo and Orhue (2011).

Effects of earthworm cast and termite mound on plant height, leaf area and dry matter yield of maize

The effects of earth worm cast and termite mound (anthill) soils on yield indicators (plant height and leaf area) and maize dry matter yield are shown in Figure 2. The plant-soil systems showed no significant (P<0.05) effect on plant height and leaf area index (LAI) except at 4th and 6th week of sowing (WAS). Soil at T7 (mixture of all earthworm casts) gave the highest plant heights and LAI followed by T8 (mixture of all termite mounds) soil across the measurement periods (2, 4, 6 WAS) (Figure 2). Taking parameter measurements at 6th week for discussion, control soil (T10) had the least plant height value (38.2 cm) and LAI (1.028). In subsequent discussions, T10 would be used as reference standard (control). T7 and T8 increased plant height significantly (P<0.05) by 72.4 and 67.1%, respectively, relative to T10. Also, LAI of soils in T7 and T8 was higher significantly (P<0.05) by 51.6 and 47.1%, respectively as compared to control (T10). The significant (P<0.05) increases in plant height and LAI could be associated with increases in the contents of organic carbon, nitrogen, and exchangeable cations released into the soil exchange site by plant-soil systems (earthworm cast and termite mound soils).

Earthworm cast and termite mound soils had significant (P<0.05) effect on dry matter weight of maize. Though dry matter yield obtained in T7 and T8 soils were statistically similar, T7 soil gave the highest dry matter yield (22.81 g) relative to T10 (9.11 g) (Figure 2). Dry matter yield in T7 and T8 soil increased significantly (P<0.05) by 60.1 and 50.4%, respectively than yield obtained in T10 soil. The significant increases in dry matter yield of the two plant-soil systems (T7 and T8) could be due to high contents of nutrients, their availability and higher assimilation by the roots of the maize plants. This finding agrees with other reports (Aziz, 2010; Ekundayo and Orhue, 2011; Debruyn and Conacher, 1987) which showed that addition of soil-feeding termite nest structures amendments improves soil nutrient availability and uptake by plants.

However, low dry matter yield (9.11 g) obtained in control soil (T10) (Figure 2) could be attributed to inherent low nutrient contents of the surrounding soils. Low nutrient values in soils have been associated with high temperature, high rainfall and leaching loses which characterize tropical soils (Lal, 1988). Most tropical soils have been identified to have coarse to medium texture,

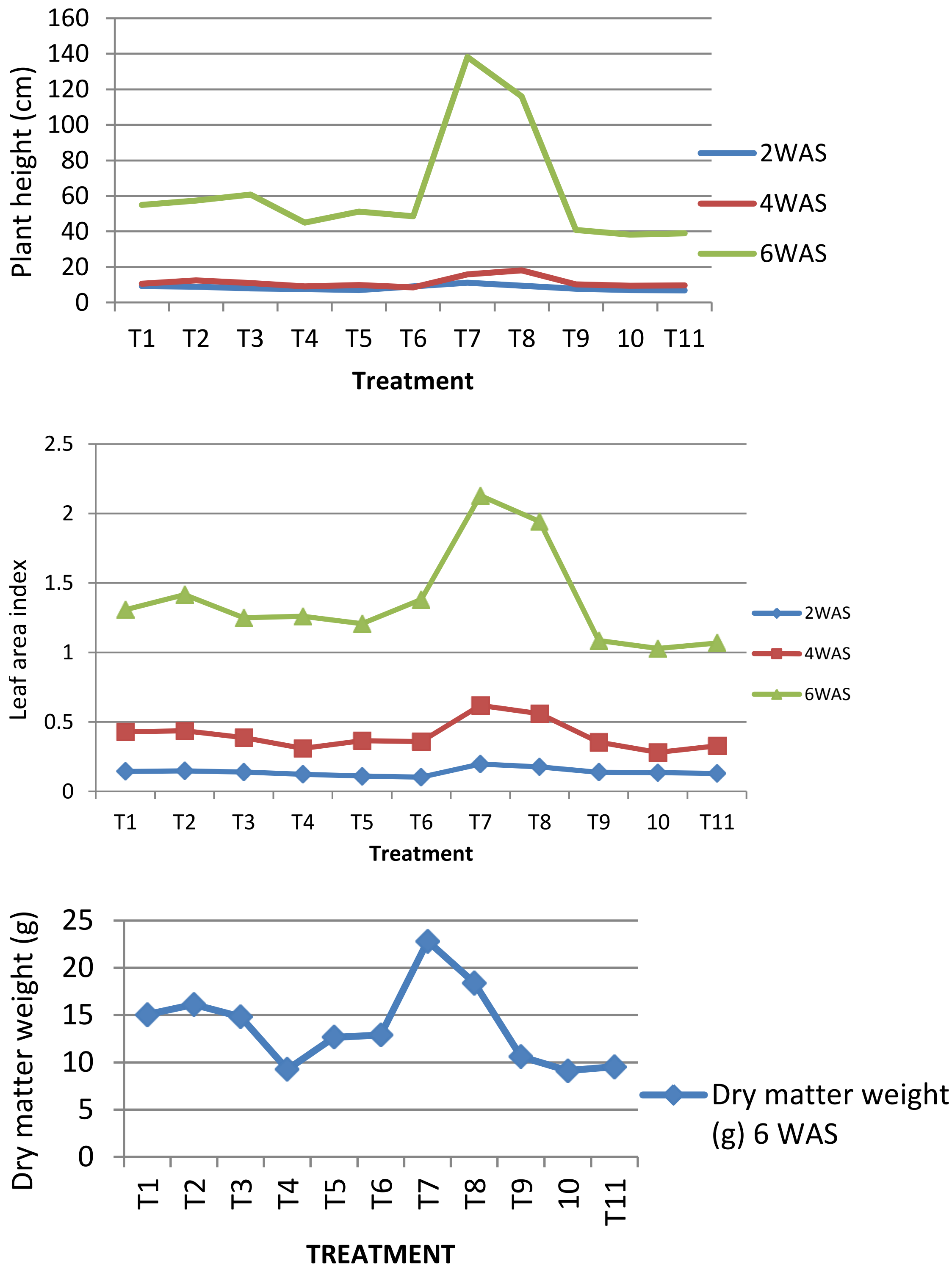

Figure 2. Effects of earthworm cast and anthill on plant height, leaf area index and dry matter yield of maize.

granular in structure, acidic in reaction and low in nutrient status (Akamigbo and Igwe, 1990), hence resulting to low soil and crop productivity. Acid soil as was seen in the control (pH of 5.2) (Table 3) could have contributed to the low dry matter yield of maize. Acid soils have been identified as the main cause of low yield in many tropical crops (Obi and Asiegbu, 1980; Hoekenga and Pineros, 2004).

Table 3. Effects of earthworm cast and anthill on plant height, leaf area index and dry matter yield of maize across the locations.

Treatment	Plant height (WAS) (cm)			Leaf area index (WAS)			Dry matter weight (g)
	2	4	6	2	4	6	6 WAS
T1	9.2	10.5	55.0	0.144	0.429	1.308	15.04
T2	8.8	12.5	57.3	0.148	0.436	1.416	16.12
T3	8.0	11.0	60.8	0.139	0.388	1.249	14.81
T4	7.6	9.0	44.9	0.124	0.310	1.260	9.26
T5	6.9	9.8	51.1	0.110	0.365	1.206	12.65
T6	9.0	8.4	48.5	0.103	0.359	1.381	12.89
T7	11.2	15.8	138.2	0.198	0.620	2.128	22.81
T8	9.5	18.0	116.0	0.177	0.560	1.942	18.39
T9	7.7	10.2	40.8	0.138	0.355	1.086	10.61
10	7.0	9.4	38.2	0.135	0.281	1.028	9.11
T11	6.8	9.6	38.9	0.130	0.329	1.068	9.53
LSD (P<0.05)	ns	1.428*	2.921*	ns	0.0712*	0.1928*	2.071*

WAS, weeks after sowing; T1, Nsukka earthworm cast; T2, Ede Oballa earthworm cast; T3, Orba earthworm cast; T4, Nsukka anthill; T5, Ede Oballa anthill; T6, Orba anthill; T7, Mixture of all earthworm casts; T8, Mixture of all anthills; T9, Control soils (Nsukka); T10, Control soils (ede Oballa); T11, Control soils (Orba); LSD (P<0.05), least significant difference at 5% level of probability, 2, 4 and 6 means the week of measurement.

Conclusion

The results of this study show that clay contents in worm cast and termite mounds soils, regarded as plant-soil system, were higher than the surrounding soils. Silt content, however, was found higher in control soils. Application of the plant-soil systems decreased soil bulk density and increased porosity and saturated hydraulic conductivity.

The study also found that the applied plant-soil systems had significant positive effect on measured soil chemical parameters such as soil pH, OC, total N, exchangeable cations (except Na), CEC and available P. Plant-soil systems (earthworm cast and anthill) by location and by soil depth interaction was significant (P<0.05) for the aforementioned measured soil parameters.

It was also shown that a mixture of all earthworm cast soils (T7) gave higher value in the crop parameters measured than the value obtained in the mixture of all anthill soil (T8), although the difference is not significant. Maize growth performance parameters (plant height and leaf surface) and dry matter yield were significantly increased (p<0.05) in the plot with a mixture of T7 and (T8) than the soils of the surrounding (T10). Thus, the combined use of T7 (mix of all earthworm cast soils) and T8 (mix of all anthill soils) plant-soil systems is recommended for their great potentials to increase soil and crop productivity.

Any soil management practices for optimizing nutrient balance in soil should aim at maintaining vegetation and soil organic materials on the soil surface (mulch). The practices will increase the activities of earthworms and termites, resulting to increases and improvements on soil fertility to maintain the soil nutrients above the threshold values as obtained in the current study. There is need for further research on the effects of the plant-soil systems (earthworm cast and termite mound soils) on maize or any other related crop at field scale in tropical, subtropical and temperate regions so as to explore further their productivity potentials.

Conflict of interest

The authors have not declared any conflict of interest.

REFERENCES

Akamigbo FOR, Igwe CA (1990). Physical and chemical characteristics of four gullied soil location in Anambra State, Nigeria. Nig. Agric. J. 25:29-48.

Arihad MA (1979). The role of *Macrotermes* in soils. Annual Report, Int Centre of Insect. Phyiol. Ecol. 6:20-22.

Aziz TS, Ullah S, Satter A, Nasim M, Farooq, M, Khan MM (2010). Nutrient availability and maize (Zea mays L.) growth in soil amended with organic manures. Int. J. Agric. Biol. 12:621-624.

Blakemore LC, Daly BK, Searle PL (1987). Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report 80. Department of Scientific and Industrial Research, Wellington, New Zealand.

Bremner JM, Mulvaney GS (1982). Nitrogen total. In: Methods of soil analysis, part 2 Vol. 9 (Eds Page A. L. R. H Miller and DR Keeney), Am. Soc. Agron. Madison, W1, pp. 595-624.

Chan KY (2004). Impact of tillage practices and burrows of a native Australian anecic earthworm on soil hydrology. Appl Soil Ecol. 27:89-

96. doi:10.1016/j.apsoil.2004.02.001

Debruyn LAL, Conacher AL (1997). Soil modification by termites in the cereal wheat belt of western Australia. Aust. J. Soil Res. 33:179-193.

Ekundayo EO, Orhue ER (2011). Physical and chemical properties of termite mound and surrounding soil as influenced by land use in the Niger Delta region of Nigeria. Nig. J. Soil Environ. Res. 9:53-58.

Ezeaku PI, Egbemba BO (2014). Yield of maize (Manoma spp) affected by automobile waste and compost manure. Afr. J. Biotechnol. 13(11):1250-1256.

Ezeaku PI, Akamigbo FOR, Asadu CLA (2002). Maize yield predictions based on soil fertility parameters in southeastern Nigeria. J. Agrotech. Ext. 2(2):30-38.

Ezeaku PI (2011). Soil quality as influenced by land use and management in the southern Guinea savanna of Nigeria. Nig. J. Soil Environ. Res. 9:1-11.

Fragoso C, Lavelle P (1992). Earthworm communities of tropical rainforest. Soil Biol. Biochem. 24:1397-1408.

Frageria NK, Baligar VC (2004). Properties of termite mound soils and responses of rice and bean to N, P and K fertilization on such soil. Commun. Soil Sci. Plant Anal. 35:15-16.

Francis GS, Fraser PM (1998). The effects of three earthworm species on soil macroporosity and hydraulic conductivity. Appl. Soil Ecol. 10:11-19. doi:http://dx.doi.org/10.1016/S0929-1393(98)00045-6

Gee GW, Or P (2002). Particle size analysis. In. Dane D.H., Topp G.C. (Eds). Methods of soil Analysis. Part 4: physical methods. Soil Sci. Am Book Series No5, ASA and SSSA, Madison, W1. pp. 255-293.

Grossman RB, Keinch TG (2002). Bulk density and linear Extensibility. In: Dane, J.A., Topp. G.C (Eds). Methods of Soil Analysis Part 4: Physical Methods. Soil Sci. Am. Book Series No.5, ASA and SSSA, Madison, W1 pp. 202-228.

Hoekenga OA, Pineros MA (2004). How do crop plants tolerate acid soils? Mechanisms of Al^{3+} tolerance and phosphorus efficiency. In: Lucia et al (2010). Transcriptional profile of maize roots under acid soil growth. BMC Plant Biol. 10:196.

Johnson JP, Carver BF, Baligar VC (1997). Productivity in Great Plains acid soils of wheat genotypes selected for aluminium tolerance. Bot. Rev. 68:235-269.

Jouquet P, Mamou L, Lepage M, Velde B (2002) Effect of termites on clay minerals in tropical soils; fungus growing termites as weathering agents. Eur. J. Soil Sci. 53(4):521-527.

Laossi KR, Decaens T, Jouquet P, Barot S (2010). Can we predict how earthworm effects on plant growth vary with soil properties? Appl. Environ. Soil Sci. 6pp. doi:10.1155/2010/784342

Lavelle P (1997). Faunal activities and soil processes: Adaptive strategies that determine ecosystem function. Adv. Ecol. Res. 27:93-132. doi:10.1016/s0065-2504(08)60007-0

Kang BT (1978). Effect of some biological factors on soil variability in the tropics III. Effect of macrotermes mounds. Plant Soil 50:241-251.

Lal R (1988). Effect of macrofauna on soil properties in tropical ecosystem. Agric. Ecosys and Environ. 24:101-106.

Lal R (1990). Tropical soils: distribution, properties and management. Res. Manag. Optimization 7:39-52.

Lee KE, Wood TG (1971). Termites and Soils. Academic Press, New York, pp. 251.

Lucia M, Matias K, Felipe R, Renato AJ, Marcelo M (2010). Transcriptional profile of maize roots under acid soil growth. BMC plant Biol. 10:196 http://www.biomedcentral.com/1471-2229/10/196

Mba CC (1978). Fertility status of wormcasts of *Agrotoreutus nyongii* (Shegun) and *Eudrilus eugeniae* (Kinberg). Der Tropenlandwirt, 79:46-53.

Mbagwu JSC (1995). Saturated hydraulic conductivity in relation to physical properties of soils in Nsukka plains, southeastern Nigeria. Geoderma. 68:51-66.

McLean EO (1982). Soil pH and lime. Requirements. In Taga A. L. (Ed). Method of Soil Analysis. Part 2. Chemical and Microbial Properties (2nd Edition). Agronomy Series No. 9. ASA, SSSA. Madison, W.I

Nwadialo BE (1989). Soil-landscape relationship in the Udi-Nsukka plateau. Catena, 16:111-120.

Obi ME, Asiegbu BO (1980). The physical properties of some eroded soils of southeastern Nigeria. Soil Sci. 130:39-48.

Olsen SR, Sommers LE (1982). Phosphorus.. In: Page *et al* (eds) Methods of Soil Analysis. Part 2. Agron 9. ASA, SSSA. Madison, W.I. pp. 403-434.

Orhue ER, Uzu FO, Osaigbovo AU (2007). Influence of activities of termites on some physical and chemical properties of soil under different land use pattern. Int. J. Soil Sci. 2(1):1-14.

Semhi K, Chaudhuri S, Clauer N, Boeglin JL (2008). Impact of termite activity on soil environment: A perspective from their soluble chemical components. Int. J. Environ. Sci. Tec. 5(4):432-444.

Six J, Bossuyt H, Degryze S, Denef K (2004). A history of research on the link between micro) aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79:7-31. doi:10.1016/j.still.2004.03.008

Sommers LE, Lindsay WL (1979). Effect of pH and redox on predicted heavy metal-chelate equilibria in soils. Soil Sci. Soc. Am. J. 43(1):39-47.

Steel RGD, Torrie JH (1980). Principles and procedures of statistics. A Biometrical Approach. 2nd ed. McGraw Hill Book. Coy, Inc, N.Y. USA. pp. 663.

Unagwu BO, Asadu CLA, Ezeaku PI (2012). Maize response to organic (poultry manure) and inorganic fertilizer (NPK 15-15-15) at different soil pH levels. Int J. Environ. Sci. 1(2):126-134.

van Schaik L, Palm J, Klaus J, Zehe E, Schröder B (2014). Linking spatial earthworm distribution to macropore numbers and hydrological effectiveness. Ecohydrol. 7:401-408. doi:10.1002/eco.1358

Wang D, Anderson DW (1998). Direct measurement of organic carbon content in soil by the Leco CR-12 Carbon Analyzer. Commun. Soil Sci. Plant Anal. 29:15-21.

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Full Length Research Paper

Morphological and Molecular Characterization of *Lepidium sativum* population collected from Ethiopia

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***Lepidium sativum* L. (family Brassicaceae), is underutilized medicinal plant with worldwide distribution. In Ethiopia, *L. sativum* occurs in all regions and agro-ecologies at different altitudinal ranges. The study was conducted to assess the genetic diversity of *L. sativum* populations from Ethiopia using molecular marker and agronomic traits. Molecular data generated from inter simple sequence repeat bands recorded was used for computing gene diversity, percent polymorphism, Shannon diversity index and analysis of molecular variance. Moreover, the inter simple sequence repeat data was used to construct unweighted pair group method with arithmetic mean, neighbor joining trees and principal co-ordinate plot using Jaccard's coefficient. Tigray and Amhara *L. sativum* populations showed higher gene diversity (0.24) and Shannon information index (0.35). Both unweighted pair group method with arithmetic mean and principal co-ordinate analysis showed very weak grouping among individuals collected from the same regions. Generally, Tigray and Amhara regions showed moderate to high diversity in inter simple sequence repeat analysis. Different geographical regions of Ethiopia, showed different levels of variation; thus, conservation priority should be given to those regions that have genetic diversity. This result also indicates the presence of genetic diversity that can be exploited to improve the productivity of *L. sativum* in Ethiopia.**

Key words: Genetic diversity, inter simple sequence repeat (ISSR), *Lepidium sativum*, morphology.

INTRODUCTION

The genus *Lepidium* L. comprises about 150 species distributed worldwide. In tropical Africa, only nine species are found. The genus Lepidium belongs to the family Brassicaceae. The garden cress, *Lepidium sativum* L., a fast growing annual herb is native to Egypt and West Asia (Zhan et al., 2009). Medicinal plants are excellent sources of unknown chemical substances with therapeutic effects (Rao, 2004). *L. sativum* seeds contain flavonoids, coumarins, sulphur, glycosides, triterpenes, sterols and various imidazole alkaloids (Radwan et al., 2007; Agarwal and Verma, 2011; Datta et al., 2011). Ethno-medicinal uses of *L. sativum* are: leaves are used as salad, cooked with vegetables, curries and also used as fodder for cattle (Moser et al., 2009; Patel et al., 2009;

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Table 1. List of primers, annealing temperature, primer sequence, amplification quality and repeat motives used for optimization

Primer	Annealing temperature (°C)	Primer sequence	Amplification quality	Repeat motives
810	45	GAGAGAGAGAGAGAGAT	Monomorphic	Dinucleotide
812	45	GAGAGAGAGAGAGAGAA	Polymorphic, reproducible	Dinucleotide
818	48	CACACACACACACACAAG	Monomorphic	Dinucleotide
824	48	TCTCTCTCTCTCTCTCG	Monomorphic	Dinucleotide
834	45	AGAGAGAGAGAGAGAGYT	Polymorphic, reproducible	Dinucleotide
844	45	GAGAGAGAGAGAGAGAYT	No Amplification	Dinucleotide
872	38	GATAGATAGATAGATA	No Amplification	Tetra- nucleotide
873	45	GACAGACAGACAGACA	Polymorphic, reproducible	Tetra- nucleotide
878	45	GGATGGATGGATGGAT	No Amplification	Tetra- nucleotide
880	45	GGAGAGGAGAGGAGA	Polymorphic, reproducible	Penta- nucleotide

Source: Primer kit 900 (UBC 900); Single-letter abbreviations for mixed base positions: R = (A, G) Y = (C, T).

Rehman et al., 2010). The leaves are stimulant, diuretic, used in scorbutic disease and hepatic complaints (Raval and Pandya, 2009).

In Ethiopia, *Lepidium sativum* occurs in all regions and agroecology at different altitudinal range. It is not cultivated widely; instead it is cultivated with teff field and available in all local markets. It is not cultivated in large amount as other crops. The main purpose of its cultivation in Ethiopia is for use as a medicinal plant. It is used for human abdominal ache and diarrhea. Moreover, *L. sativum* is also used to treat skin diseases and other internal problems in livestock.

Despite its medicinal use, there was no genetic diversity study on Ethiopian *L. sativum,* using morphological and molecular markers. Very few studies have been carried out using morphological markers outside Ethiopia. Hence, this study is proposed to investigate the genetic diversity and population structure of *L. sativum* populations collected from Ethiopia. Variation was studied using morphological and molecular markers. This will give the overall genetic variability, patterns of distribution and population structure which will be very critical to design sustainable conservation and use strategy.

MATERIALS AND METHODS

Tissue harvest and DNA extraction

The experiment was designed to characterize these accessions using inter simple sequence repeat (ISSR) markers. Borsch et al. (2003) procedures were used.

Primer selection and optimization

The ISSR marker assay was conducted at Genetics Laboratory of the Microbial, Cellular and Molecular Biology Program Unit, College of Natural Sciences, Addis Ababa University, Addis Ababa. A total of 10 primers, obtained from the Genetic Research Laboratory (Primer kit UBC 900) and primers used by Kim et al. (2002) were used for the initial testing of primers variability and reproducibility.

PCR and gel electrophoresis

The polymerase chain reaction was conducted in Biometra 2003 T3 Thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 1 µl template DNA, 13.45 µl H_20, 5.60 µl dNTP (1.25 mM), 2.6 µl Taq buffer (10XH buffer S), 1.25 µl $MgCl_2$ (50 mM), 0.6 µl primer (20 pmol/µl) and 0.5 µl Taq Polymerase (3 u/µl). The amplification program was 4 min preheating and initial denaturation at 94°C, then 40 x 15 s at 94°C, 1 min primer annealing at (45/48°C) based on primers used, 1.30 min extension at 72°C and the final extension for 7 min at 72°C. The PCR reactions were stored at 4°C until loading on gel for electrophoresis. The amplification products were differentiated by electrophoresis using an agarose gel (1.67% agarose with 100 ml 1xTBE) and 8 µl amplification product of each sample with 2 µl loading dye (6 times concentrated) was loaded on gel. DNA marker 100 bp was used to estimate molecular weight and size of the fragments. The electrophoreses were done for 3 h at constant voltage of 100 V. The DNA was stained with 10 mg/ml ethidium bromide which were mixed with 250 ml distilled water for 30 min and washed with distilled water for 30 min (Table 1).

Statistical analysis

The bands were recorded as discrete characters, presence '1' or absence '0' and '?' for missing data. Based on recorded bands, different softwares were used for analysis. POPGENE version1.32 software (Yeh et al., 1999) was used to calculate genetic diversity for each population as number of polymorphic loci, percent polymorphism, gene diversity (H) and Shannon diversity index (I). Analysis of molecular variance (AMOVA) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier et al., 2006). NTSYS-pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek et al., 1999) softwares were used to calculate Jaccard's similarity coefficient (Table 2).

The unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and compare the population and generate phenogram using NTSYS- pc version 2.02 (Rohlf, 2000).

To further examine the patterns of variation among individual samples on 3D, a principal coordinated analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The

Table 2. Banding patterns generated using the four selected primers, their repeat motifs, amplification patterns and number of scored bands**.**

Primer	Repeat motif	Amplification quality	Number of scored bands
812	(GA)8A	Excellent	14
834	(AG)8YT	Excellent	11
873	(GACA)4	Excellent	16
880	(GGAGA)3	Excellent	12
Total			53

Single-letter abbreviations for mixed base positions: R = (A, G) Y = (C, T).

Table 3. Number of scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H) and Shanon index information (I) of 85 *L. sativum* accessions based on all primers used.

Primer	NSB	NPL	PP	H±SD	I±SD
812	14	9	64.29	0.20±0.20	0.31±0.28
834	11	9	81.82	0.24±0.15	0.38±0.22
873	16	15	93.75	0.36±0.13	0.53±0.18
880	12	10	83.33	0.25±0.15	0.39±0.22
Over all	53	43	81.13	0.27±0.17	0.41±0.24

Table 4. The number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H) and Shannon information index (I) among the five regions of Ethiopia.

Population	NPL	PP	H±SD	I±SD
Amhara	35	66.04	0.24±0.19	0.35±0.28
Oromia	27	50.94	0.17±0.19	0.26±0.28
Tigray	35	66.04	0.24±0.19	0.35±0.27
SNNPR	25	47.17	0.18±0.21	0.27±0.30
Somali	24	45.28	0.18±0.21	0.26±0.30
Total	182	343.39	1.27±1.2	1.87±1.72

calculation of Jaccard's coefficient was made with PAST software version 1.18 (Hammer et al., 2001). The first three axes were used to plot the three dimensional PCO with STATISTICA version 6.0 software (Hammer et al., 2001; Statistica soft, Inc.2001).

RESULTS

Genetic diversity analysis

Of the total 53 loci scored, 81.13% (43) were observed to be polymorphic. From all the populations studied, Amhara and Tigray were 66.04%, Oromia 50.94%, SNNPR 47.17% and Somali 45.28% percent polymorphic. Amhara and Tigray showed more percent polymorphism; while the least polymorphism was detected in population from Somali region. No unique bands were observed for either the accessions or the populations (Table 3).

Among the *L. sativum* accessions evaluated using ISSR markers, samples from Tigray and Amhara exhibited the highest gene diversity (H = 0.24), whereas samples from Oromia had H = 0.17) from SNNPR H = 0.18 and Somali H= 0.18 gene diversity values. The average gene diversity for the total population (H_T) was 0.27 (Table 4).

Primer 873 showed highest gene and Shannon diversity (0.36 and 0.53, respectively) and primer 812 was the least (0.20 and 0.31, gene and Shannon diversity, respectively) (Table 3).

Analysis of molecular variance (AMOVA)

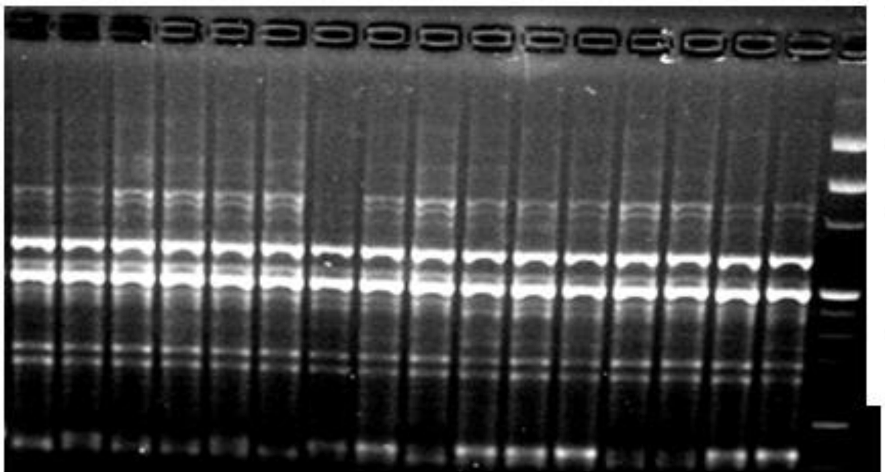
Analysis of molecular variance was carried out on the overall ISSR data score of *L. sativum* accessions without grouping by region or geographic location. AMOVA revealed high percentage of variation (94%) that is attributed to within population variation while the remaining variation is due to among population variation (6%). The variation was found to be highly significant at (P = 0.00). The result shows that there is high gene flow or seed flow among population in different region; this resulted in low genetic variation and differentiation among population (Table 5).

Clustering analysis

UPGMA and Neighbor Joining tree construction methods was used to construct dendrogram for six populations and 85 individuals based on 53 PCR bands amplified by two di-nucleotides (812 and 834), one penta nucleotides (880) and one tetra nucleotide (873). The dendrogram derived from neighbor-joining analysis of the whole ISSR data with 85 *L. sativum* accessions showed four distinct clusters and two sub-clusters within each major cluster. Most of the individual accessions collected from the same region tend to spread all over the tree without forming their own grouping. The wider distribution of *L. sativum*

Table 5. Analysis of molecular variance (AMOVA) of *L. sativum* accessions in Ethiopia without grouping.

Source of variation	Sum of squares	Variance components	Percentage of variation	Fixation	P
Among populations	4.122	0.02834	6.00	0.06	0.00
Within population	34.765	0.44387	94.00		
Total	38.888	0.47221	100		



Amhara	90018-1	208030-2	90004-3	205162-4
Oromia	208769-1	208667-2	212852-3	90002-4
Tigray	219961-1	219960-2	233984-3	
SNNPR	240396-1	242916-2		
Unknown	90009-1	90014-2		
Somali	216815-1			

Figure 1. ISSR fingerprint generated from 16 individual accessions using primer 873.

accession all over the tree shows the low divergence among populations from different localities. UPGMA analysis based on regions of collection of *L. sativum* revealed three major groups. The first cluster contains Oromia, Amhara and Tigray; while the second cluster contains SNNPR and individual from unknown origins. The final major cluster contains the Somali group. However, UPGMA with individual accessions showed intermixing of individuals to different groups, except in two groups where individuals from Oromia clustered together (Figure 2).

Principal co-ordinate (PCO) analysis

All the data obtained using the four ISSR primers were used in PCO analysis using Jaccard's coefficients of similarity. The first three coordinates of the PCO having Eigen values of 4.83, 4.55 and 1.63 with variance of 18.28, 17.26 and 6.20%, respectively were used to show the grouping of individuals using two and three coordinates. In 3D, most of the individual accessions that represent different populations spread all over the plot. Using two coordinates (Figure 3 and 4) almost similar result was observed like that of three coordinates. Overall, no clear grouping was observed among individuals collected from different locality.

DISCUSSION

Molecular diversity and its implications for improvement and conservation

In the present study, ISSR was used for the first time to assess genetic variation of *L. sativum* populations from Ethiopia. This method provides an alternative choice to other system for obtaining highly reproducible markers without any necessity for prior sequence information for various genetic analyses. Because of the abundant and rapidly evolving SSR regions, ISSR amplification has the potential of illuminating much larger number of polymorphic fragments per primer than any other marker system used such as RFLP or microsatellites. ISSRs are regions that recline within the microsatellite repeats and offer great potential to determine intra-genomic and inter-genomic diversity as compared to other arbitrary primers, since they reveal variation within unique regions of the genome at several loci simultaneously. Several property of microsatellite such as high variability among taxa, ubiquitous occurrence and high copy number in eukaryotic genome make ISSRs extremely useful marker for variability analysis (Morgante et al., 2002) (Figure 1).

In this study, bulk sampling approach was chosen, since it permits representation of the vast accession by optimum number of plants. Yang and Quiros (1993)

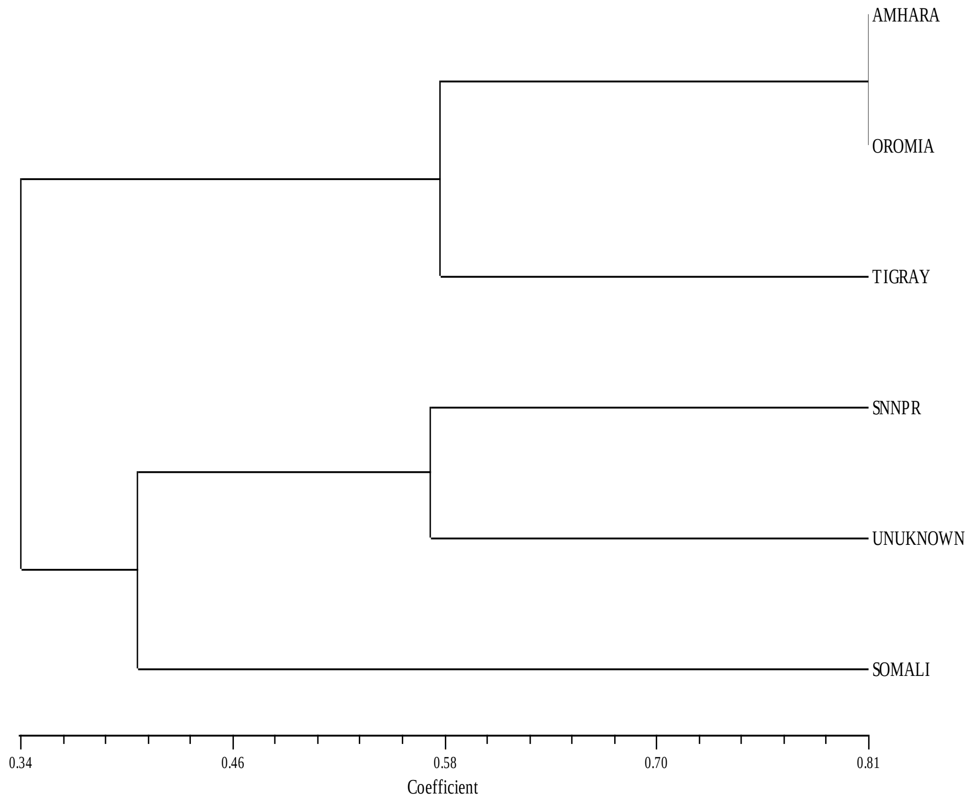


Figure 2. UPGMA based dendrogram for 6 *L. sativum* populations using 4 ISSR (2 di, 1 penta and 1 tetra nucleotide) primers.

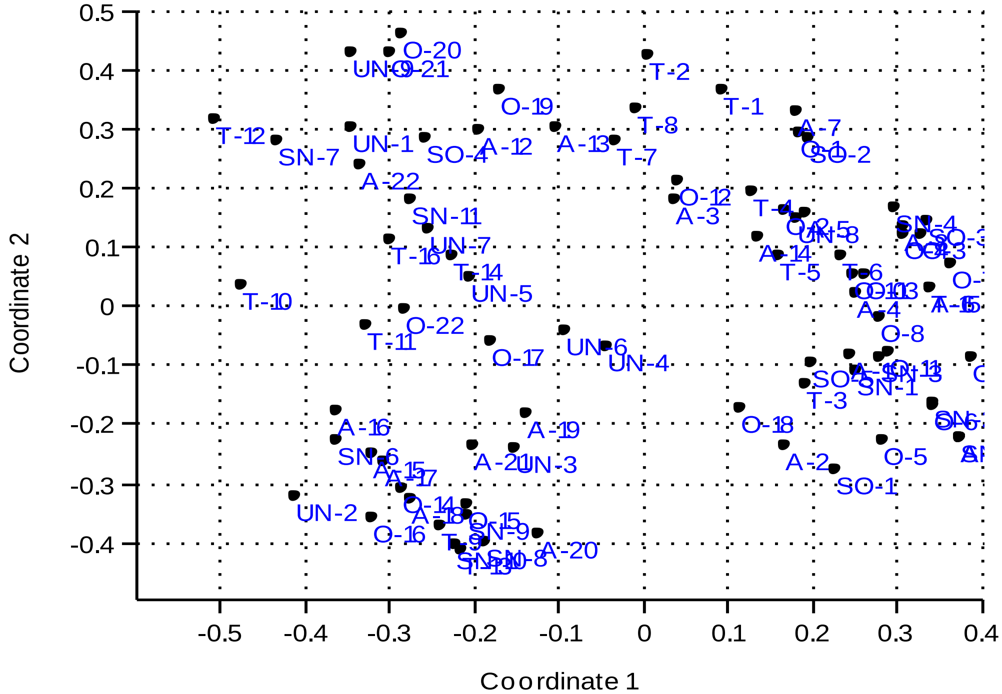


Figure 3. Two dimensional representation of principal coordinate analysis of genetic relationships among 85 accessions of *L. sativum* accessions using ISSR data.

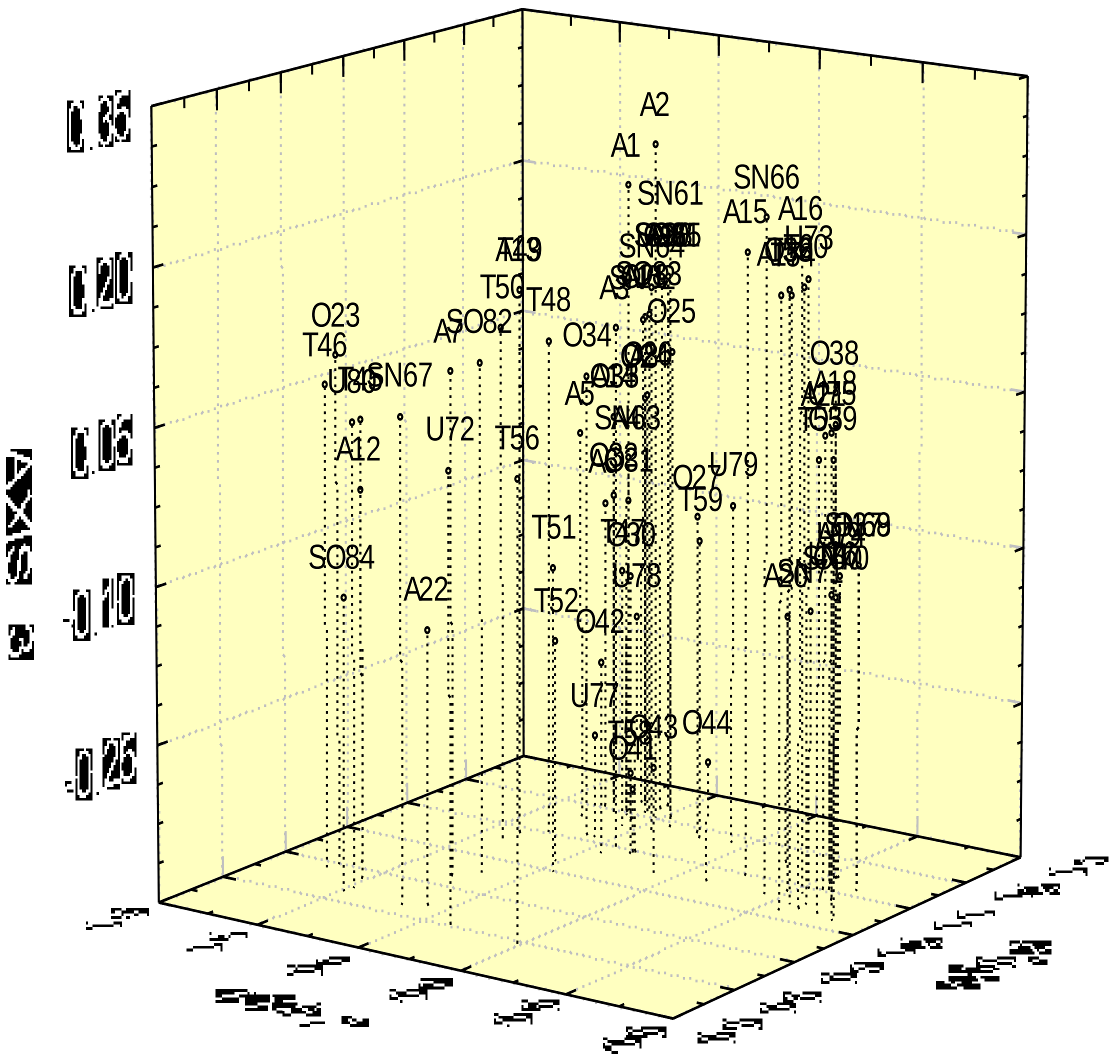


Figure 4. Three dimensional representation of principal coordinate analysis of genetic relationships among 85 accessions of *L. sativum* accessions.

reported that bulked samples with 10, 20, 30, 40 and 50 individuals had resulted in the same RAPD profiles as that of the individual plant constituting the bulk sample. Gilbert et al. (1999) also reported that pooling of DNA from individuals within accessions is the most appropriate strategy for assessing large quantities of plant material and concluded that 2-3 pools of five genotypes is sufficient to represent the genetic variability within and between accessions in the lupin and similar collections. Edossa et al. (2010) used bulked samples for diversity assessment in lentil collected from Ethiopia. The technique revealed higher genetic diversity, and, therefore, validated the usefulness of bulk sample analyses. Dagmawi (2011) also used bulked sample in germplasm diversity study of sesame populations, and found moderate genetic diversity of both Ethiopian and exotic populations.

The present study shows that out of 53 loci generated by four primers, two di, one penta and one tetra; 43 of them were polymorphic with 81.13% polymorphism. In regions based analysis, Amhara and Tigray showed higher percent polymorphism (66.04%); while, SNNPR and Somali showed least polymorphism with 47.17 and 45.28%, respectively. The same patterns of diversity were observed with gene diversity and Shannon index. Generally, *L. sativum* populations from Amhara and Tigray showed higher diversity than the other regions.

Edossa et al. (2010) studied the morphological and molecular diversity of Ethiopian lentil (*Lens culinaris* Medikus) using four ISSR primers and found 59.57% polymorphism with higher percent variation attributed within populations (56.28%). Gezahegne et al. (2009) studied wild and cultivated rice species of Ethiopia using six ISSR primers and reported 38.3 and 28.3% polymorphism of wild and cultivar rice species, respectively. Moreover, higher proportion of genetic diversity was observed within populations of rice (Gezahegne et al., 2009). Hence, the present study showed higher percent polymorphism and higher proportion of diversity within population of *L. sativum* comparable with that of Edossa et al. (2010) and Gezahegne et al. (2009).

In general, Amhara and Tigray had good genetic diversity than Oromia, SNNPR and Somali. But this has to be further studied using proper sampling strategy and multilocation comparison.

AMOVA analysis resulted in high genetic diversity within population (94%) and very low genetic diversity among population (6%). This could be due to high seed exchange among different regions and markets which could lead to intermix of populations between regions. Unlike other landraces of cultivated plants, *L. sativum* in Ethiopia is not restricted to a given area rather it is wildly exchanged among local community and markets. This shows that there is very high gene flow between populations and regions. Jiang et al. (2012) who studied the genetic diversity of *Chimonanthus grammatus* populations by using ISSR marker showed that there was 73.6% within population variation, whereas the rest 26.4% was due to among population variation. As compared to the present study, there was less gene flow. Jiang et al. (2012) recommended that gene flow, genetic drift and evolutionary history might have important influence on genetic structure and diversity of a given population.

L. sativum is both self and cross pollinated plant (Quirós and Cárdenas, 1998). Hence, the proportion of genetic variation is dependent on the type of pollination that the species undergoes. If the species has large proportion of cross pollination, then we expect high genetic variation within population and less divergence among populations. In addition to pollination, behavior of insects; market exchange could facilitate gene flow among regions which could result in higher percent variation within population and less genetic structure. This is also supported by the spread of individual accessions on UPGMA, NJ and PCO graphs.

Dendrogram of the present study by using UPGMA of Jaccard's coefficient of similarity showed Amhara and Oromia populations of *L. sativum* were closely related. Based on this study, the samples with unknown origins could have been probably collected from southern part of Ethiopia since they closely clustered with the SNNPR population.

The Somali population had its own lineage far from the other populations and diverted as anoutlies. Genetic distance is a measure of the allelic substitutions per locus that have occurred during the separate evolution of two populations or species. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. Crosses between distantly related individuals are expected to give better offspring than those between closely related genotypes. Therefore, prior knowledge of the genetic distance between genotypes or accessions is important in designing breeding program.

Genetic diversity of plant populations is largely influenced by factors such as reproduction system, genetic drift, evolutionary history and life history (Loveless and Hamrick, 1984). In broad-spectrum, outcrossing species have higher levels of genetic diversity than selfing and clonal plants (Rossetto et al., 1995).

Conclusions

Analysis of molecular variance for the accessions studied showed that the highest proportion of genetic variation was attributed to within population than among population. It is also highly significant. This confirms that there was a high level of gene flow and low level of genetic differentiation. Based on the UPGMA data, the Amhara, Tigray and Oromia accessions were clustered into one group, whereas the SNNPR and the unknowns in the other cluster. Samples from Somali formed a distinct cluster showing that it is distantly related to accessions from the entire regions.

Conflict of interest

The authors declare that they have no competing interests.

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REFERENCES

Agarwal J, Verma DL (2011). Antioxidative activity and flavonoid composition from Lepidium Sativum. Nat. Sci. 9:21-25.

Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W (2003). Non-coding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. J. Evol. Biol. 16:558-576.

Dagmawi T (2011). Germplasm Study of Ethiopian and Exotic Sesame Populations. M. Sc. Thesis. Addis Ababa University, Addis Ababa.

Datta PK, Diwakar BT, Viswanatha S, Murthy KN, Naidu KA (2011). Safety evaluation studies on garden cress (Lepidium sativum L.) seeds in Wistar rats. Int. J. Appl. Res. Nat. Prod. 4:37-43.

Edossa F, Kassahun T, Endashaw B (2010). A comparative study of morphological and molecular diversity in Ethiopian lentil (Lens culinaris Medikus) landraces. Afr. J. Plant Sci. 4:242-254.

Excoffier L, Laval G, Schneider S (2006). An integrated software package for population genetics data analysis.

Gezahegn G, Kassahun T, Endashaw B (2010). Inter simple sequence repeat (ISSR) analysis of wild and cultivated rice species from Ethiopia. Afr. J. Biotechnol. 9:5048-5059.

Gilbert JE, Lewis RV, Wilkinson MJ, Caligari PDS (1999). Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theor. Appl. Genet. 98:1125-1131.

Hammer O, Harper D, Ryan P (2001) PAST: Paleontological statistics software for education and data analysis. Paleontología Electrónica 4:1-9.

Jiang Y, Li Y, Lu S, Liu Y, Peng J, Zhu D (2012). Genetic diversity of Chimonanthus grammatus populations determined with inter-simple sequence repeats (ISSR) analysis: Implications for conservation. J. Med. Plants Res. 6:1272-1278.

Kim DH, Zur G, Danin-Poleg Y, Lee SW, Shim KB, Kang CW, Kashi Y (2002). Genetic relationships of sesame germplasm collection as revealed by inter-simple sequence repeats. Plant Breed. 121:259-262.

Loveless MD, Hamrick JL (1984). Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. 15:65-69.

Morgante M, Hanafey H, Powell W (2002). Microsatellites are preferentially associated with non-repetitive DNA in plant genome. Nat. Genet. 30:194- 200.

Moser BR, Shah SN, Winkler-Moser JK, Vaughn SF, Evangelisa RL (2009). Composition and Physical Properties of Cress (Lepidium sativum L.) and Field Pennycress (Thlaspi arvense L.) oils. University St., Peoria, USA.

Patel U, Kulkarni M, Undda U, Bhosale A (2009). Evaluation of diuretic activity of aqueous and methanol extracts of Lepidium sativum, garden cress (Cruciferaceae) in rats. Trop. J. Pharm. Res. 8:215-219.

Pavlicek A, Hrda S, Flegr J (1999). Free-tree-software program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia Biol. (Praha) 45:97-99.

Quirós CF, Cárdenas RA (1998). MACA (Lepidium meyenii Walp.). University of California, USA.

Radwan HM, El-Missiry MM, Al-Said WM, Ismail AS, Abdel SH, Seif-Elnasr MM (2007). Investigation of the glucosinolate of Lepidium sativum growing in Egypt and their biological activity. Res. J. Med. Med. Sci. 2:127-132.

Rao NK (2004). Plant genetic resources. Advancing conservation and use through biotechnology. Afr. J. Biotechnol. 3:136-145.

Raval ND, Pandya J N (2009). Clinical trait of Lepidium sativum in management of sandhivata. AYU 30:153-157.

Rehman N, Khan AU, Alkharfy KA, Gilani AH (2010). Pharmacological Basis for the Medical use of Lepidium sativum in Airways Disorders. Department of Biological and Biomedical Sciences, the Aga Khan University.

Rohlf FJ (2000). NTSYS-pc ver 2.11T. Exter Software, Setauket, New York.

Rossetto M, Weaver PK, Dixon KW (1995). Use of RAPD analysis in devising conservation strategies for the rare and endangered Grevillea scapigera (Proteaceae). Mol. Ecol. 4:321-329.

Sneath PHA, Sokal RR (1973). Numerical taxonomy. Freeman, Sanfrancisco.

Stat soft, Inc. (2001). Statistica data analysis system, Statistica software.

Yang X, Quiros C (1993). Identification and classification of celery cultivars with RAPD markers. Theor. Appl. Genet. 83:2-3.

Yeh FC, Yang RC, Boyle TJB, Ye Z, Mao JX (1999). POPGENE, the user Friendly Shareware for Population Genetics Analysis, Version 1.3.1. Molecular Biotechnology center, University of Aleberta, Canada.

Zhan LJ, Fontane E, Tibaldi G, Nicola S (2009). Qualitative and physiological response of minimally processed garden cress (Lepidium sativum L.) to harvest handling and storage conditions. J. Food Agric. Environ. 7:43-50.

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Participatory varietal selection of maize (*Zea Mays* L.) in Pawe and Guangua districts, North Western Ethiopia

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Maize (*Zea mays* L.) plays a critical role in smallholder farmers for food security in Ethiopia. So far, maize variety selection was done without much consideration of farmers' interest. However, farmers have indigenous knowledge to select best performing varieties which suit their environments. This study was aimed to identify more number of preferred maize varieties by farmers in a shorter time (than the conventional system), in accelerating their dissemination and increasing cultivar diversity in Pawe and Guangua district. Ten materials including standard check were evaluated using randomized complete block design (RCBD) design with two replication of two row plot on station and non-replicated three row plots on two farmers' field at Pawe and Guangua district in 2013 cropping season. Both men and women participated in the selection process. At silking, farmers put termite resistance, striga resistance, disease resistance, uniformity, vigorisity, lodging and earliness as criteria during evaluation. In the overall, the top three genotypes were entry 7 (CML395/CML202//CML464), entry 10 (BH547) and entry 4 (DE-78-Z-126-3-2-2-11(g)/CML312//ILOOE-1-9-1-1-1-1-1). The evaluations mean score value for each genotype ranged from 3.6 to 4.9. Entry 7 (4.9) scored the highest value and the lowest was scored by entry 1 (3.6) and 5 (3.6). The genotypes did not show any significant varied stand count at harvest. On the other hand, significant difference was observed among genotypes for plant height, plant aspect, ear aspect, number of cobs and yield. The results revealed that farmers' preferences in some cases coincide with the breeders' selection. However, farmers have shown their own skill in selecting a variety for their localities. Hence, it is a paramount importance to include farmers in a variety of selection process.

Key words: Maize, participatory, varietal selection.

INTRODUCTION

Maize is one of the world's three primary cereal crops. It occupies an important position in world economy and trade as a food, feed and industrial grain crop. Maize consumption is projected to increase by 50% globally and by 93% in sub Saharan Africa from 1995 to 2020 (IFPRI). Though much of the global increase in use of maize is for animal feed, human consumption is increasing and accounts for about 70% of all maize consumption in sub Saharan Africa (Aquino et al., 2001). It is also one of the major crops grown by smallholder farmers in the semi-

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Table 1. List of maize verities tested.

Entry	Variety name
1	X1264DW-1-2-1-1-1-1/F7215//CML312
2	SC22/124-b(109)//Gibe-1-91-1-1-1-1
3	kuleni-320-2-3-1-1-1DE-78-Z-126-3-2-2-1-1(g)//CML312
4	DE-78-Z-126-3-2-2-1-1(g)/CML312//ILOOE-1-9-1-1-1-1-1
5	DE-78-Z-126-3-2-2-1-1purple/Gibe-1-91-1-1-1//CML395
6	CML395/CML202//DE-78-Z-126-3-2-2-1-1 green
7	CML395/CML202//CML464
8 (Check 1)	BH543
9 (Check 2)	BH546
10 (Check 3)	BH547

arid low rainfall areas of Ethiopia. Some reports of diagnostic surveys indicated that 93% of the farmers in the lowlands of Ethiopia are maize growers. Maize grain is used for food, for sale and for marketing local brewery, and the Stover is used for construction, animal feed and domestic fuel (De Groote et al., 2002). Varietal selections of maize in Ethiopia have usually been dominantly based on grain yield. Large numbers of breeding lines have been developed at various research stations and their performance evaluated across multi-location tests over several years and only a few varieties are so far identified. Varietal evaluation and decisions were made only by researchers but, this did not speed up the variety releasing process as expected, or their dissemination afterwards.

Participatory plant breeding/selection has shown success in identifying more number of preferred varieties by farmers in shorter time (than the conventional system), in accelerating their dissemination and increasing cultivar diversity (Joshi et al., 1997). By adding information on farmers' perspectives of the plant and grain trait preferences, it is possible to maximize the variety selection process. Research demands a great deal of money and resources. In PVS, we can overcome this problem and adoption rates increased if the farmers are allowed to participate in variety selection process (Tilahun and Teshom, 1987). Therefore, this experiment was aimed to select promising maize varieties using farmers' input and feedback on the selection of varieties.

MATERIALS AND METHODS

The experiment was conducted at Pawe and Guangua district in 2013/2014 cropping season using 10 maize materials including pipe lines and standard check (Table 1). Randomized complete block design was employed with two replication of two row plot on station and unreplicated three row plots on two farmers' field. Planting was done with 75 cm between rows and 30 cm between plants and all recommended fertilizer rate and cultural practices were applied.

A field day was organized at two stages of the plant that is, silking and maturity and farmers were invited to evaluate the new maize pipeline genotypes with standard checks assisted by district agricultural experts from the Pawe and Guangua district agricultural office and researchers from Pawe Agricultural Research Center. Farmers were grouped and set different criteria for evaluation of maize varieties such as lodging resistance, number of ear/plant, ear size, disease resistance, termite resistance, husk cover, vigorisity, plant height, earliness and seed color. Farmers were discussed and these criteria were put in the order of its importance for selecting a given variety at a particular development stage. These criteria were ranked and top ones were used. Each criterion was scored from 1 to 5 rating scale (1 = very good, 2 = good, 3 = average, 4 = poor and 5 = very poor) for each variety.

At silking, farmers were scored each variety for earliness, vigorisity, striga resistance, termite resistance, disease resistance whereas at maturity, farmers were scored for cob size, plant height, husk cover and seed size. In both silking and maturity, farmers were asked to give an overall assessment of each variety, using the same scale. The methodology used in this study to identify farmers' criteria and to facilitate farmer evaluation of the varieties was very convenient for data collection but not for data analysis (Weltzien et al., 2003).

In addition to farmers' evaluation, all agronomic data were collected by the breeder and subjected for analysis of variance using SAS software.

RESULT AND DISCUSSION

Farmers' evaluation of the varieties: At silking

At silking, farmers were evaluated based on the maize varieties by considering termite resistance, striga resistance, disease resistance, uniformity, vigorisity, lodging, and earliness as criteria. Farmers were also evaluated based on the overall assessment of each genotype independently and score accordingly (Table 2).

As indicated in Table 2, the top three genotypes, entries 7, 10 and 4 were scored better. The evaluations mean score value for genotypes ranged from 3.6 to 4.9 (Table 2). Entry 7 (4.9) scored the highest value and the lowest was scored by entry 1 (3.6) and entry 5 (3.6). Entries 10 (4.7) and 4 (4.1) were ranked second and third best performing varieties by farmers view, respectively. In the evaluation process, both men and woman participated equally. One of the objectives of the partici-

Table 2. Overall mean value of each selection and ranking of genotypes at silking stage.

Entry	Termite resistance	Striga resistance	Disease resistance	Uniformity	Vigorisity	Lodging	Earliness	Total mean score	Rank
1	3	5	2	4	3	3	5	3.6	10
2	5	5	3	3	4	5	3	4.0	4
3	2	5	3	4	4	3	5	3.7	7
4	4	5	3	4	4	4	5	4.1	3
5	5	4	3	2	3	5	3	3.6	9
6	3	5	3	4	4	4	4	3.9	5
7	5	5	4	5	5	5	5	4.9	1
8	5	5	3	3	3	4	4	3.9	5
9	5	4	3	3	2	5	4	3.7	7
10	5	5	4	5	4	5	5	4.7	2

Table 3. Overall mean value of each selection and ranking of genotypes at maturity stage.

Entry	Number of ears	Cob size	Husk cover	Seed size	Plant height	Total mean score	Rank
1	3	5	3	3	4	3.6	5
2	4	3	4	4	4	3.8	4
3	3	4	4	5	5	4.2	2
4	3	3	4	3	3	3.2	7
5	2	4	3	2	5	3.2	7
6	2	3	4	3	3	3.0	8
7	5	4	2	4	5	4.0	3
8	2	3	4	3	4	3.2	7
9	3	4	3	4	3	3.4	6
10	5	4	4	5	4	4.4	1

patory breeding approach is to see how well farmer evaluations of the varieties relate to the selection procedure of the conventional breeding approaches. Therefore, a comparison was made in statistically analyzed scores of the farmer's evaluation and the breeder's analyzed data.

Farmers and researchers used different parameters and methods to evaluate the tested genotypes. Farmers have showed their ability to select well- adapted and preferred varieties under their circumstances using their own criteria. Breeder's must take into account farmers selection traits in their varietal development such as earliness, uniformity and overall field performance.

Evaluation at maturity

From the group discussions, farmers developed the following criteria for evaluating the varieties at all the sites: number of ears, ear size, husk cover, seed size and plant height. Similarly, farmers were also made an overall assessment of the variety independently and scored accordingly.

As shown in Table 3, number of ears for entry 7 was considered better than the two check varieties but equal mean score with entry 10. In cob size, entry 1 (5) scored the highest and followed by entries 3 (4), 5 (4) and 7 (4). The lowest score were recorded by entries 2 (3), 4 (3), 6 (3) and 8 (3).

In the overall assessment, entries 10 (stand. check), 3 and 7 were the top three maize varieties based on the overall mean performance illustrated in Table 3. However, farmers can have the access to select varieties in their trait of interest such as for earliness, yield, biotic resistance etc.

In agreement with De Groote et al. (2002) there was a growing interest among farmers in the use of early maize varieties in short rainfall season and farmers have shown strong interest. Some farmers were willing in adopting the varieties since they were confident of their high yield and earliness. For future trials, sufficient resources need to be made available to assure enough high quality data for statistical analysis.

These data have played a role to adjust the breeders' index in order to make it more responsive to farmers' preferences. In some cases, farmers' preference

Table 4. Mean value of different agronomic traits on station (PARC)

Entry	SH	Ph	PASP	EASP	CH	YLD
1	24.5ab	227.0ab	2.0cb	2.0a	34.0a	51.3abc
2	22.5ab	218.0abc	2.0cb	1.7ab	33.0a	56.5ab
3	28.5a	216.5abc	2.2abc	1.5ab	26.5a	47.7bc
4	20.5ab	186.5cd	2.25abc	1.5ab	28.0a	44.4bc
5	24.5ab	195.7bc	3.0a	1.5ab	30.0a	52.2abc
6	28.0ab	215.2abc	2.2abc	1.25b	34.5a	54.7ab
7	27.5ab	197.3abc	2.5ab	1.5ab	31.5a	53.7ab
8	25.5ab	198.2abc	2.2abc	2.0a	25.0a	37.6c
9	18.0b	227.5a	2.0cb	1.3b	31.0a	53.3abc
10	27.0ab	159.7d	1.5c	1.3b	32.0a	64.1a
CV (%)	18.82	6.8	18.4	14.9	14.36	13.68
Mean	24.6	204.19	2.2	1.55	30.55	51.57
LSD (5%)	10.47	31.55	0.9	0.5	9.9	15.9

SH = stand count at harvest (no.), PH = plant height (cm), PASP = plant aspect (no.), EASP = ear aspect (no.), CH = cob harvested (no.), YLD = yield (Qt/ha), CV = coefficient of variation, LSD = least significance difference.

Table 5. Analysis of variance for agronomic traits on station (PA RC).

Sources of variation	Mean squares						
	d.f.	SH	PH	PASP	EASP	CH	YLD
Replication	1	12.67	7330..47	2.70	0.002	15.40	627.73
Entry	9	22.79^{ns}	1006.05^{*}	0.39^{*}	0.16^{*}	20.45^{*}	109.44^{*}
Error	9	21.44	194.53	0.16	0.05	19.27	49.82

*Indicate significance at P< 0.05, and 'ns' indicate non-significant.

coincides with breeder's selection criteria. In general, farmers have their own indigenous knowledge and skill in selecting a variety for their localities. Hence, it is a paramount importance to include formers in a variety section process.

Agronomic traits of genotype

Agronomic traits that is, stand count at harvest, plant height, plant aspect, ear aspect, number of cobs harvested, and grain yield were collected and analyzed (Table 4). The tested genotypes did not show significant variation for stand count at harvest. Stand count ranged from 18.0 to 28.5 (Table 4). Entry 3 was the highest genotype in stand count at harvest (28.5) followed by entry 6 (28.0) and 7 (27.5). The lowest genotype in stand count was entry 9 (18.0).

A significant difference (p<0.05) was observed among genotypes for plant height, plant aspect, ear aspect, number of cobs and yield. Plant height ranged from 159.7 to 227.5 cm and the tallest was entry 9 (227.5 cm) followed by entry 2 (218.0 cm). The shortest variety was entry 10 (159.7 cm). Plant aspect is an important trait for maize and genotypes had shown significant variation. Entry 10 was a better genotype (1.5) followed by entry 1, entry 2 and entry 9. Significantly, variation was observed among genotype in ear aspect. Best ear aspect was recorded for entry 6 (1.25) followed by entry 9 and entry 10. The lowest was entries 1 and 8.

Number of cobs harvested ranged from 25.0 to 34.5 (Table 4). A significant difference was also observed among genotypes in grain yield performance (Table 5). Grain yield ranged from 37.6 to 64.1 q/ha with the grand mean 51.57 q/ha. The highest grain yield was recorded by entry 10 (64.1 q/ha) followed by entry 2 (56.5 q/ha); while the lowest grain yield was observed by entries 8 (37.6 q/ha) and 4 (44.4 q/ha). Hence, the result clearly showed that high yielding genotypes such as entry 10, entry 2 and entry 6 can be released.

De Groote et al. (2002) stated that, scientists like to control many factors so that they can accurately state that, under their very controlled circumstances, a limited number of traits have improved. A problem here is that these highly controlled circumstances are not often representative of farmers' conditions and the limited number of traits might not represent farmers' preferences.

Conclusion

It has clearly shown that a fair number of the newly pipe line genotypes were better than the two checks except entry 10 both for yield and other characteristics that farmers considered to be important. Farmers' and breeders' evaluation overlaps verities selected by farmers as the best were also the best when actual yield was considered. Still the variety they selected for best yield was the same one breeder's selected when yield was measured. It is also interesting to note that the variety that farmers considered as overall best at silking was not selected at maturity stage because it ranked differently. Entry 7 was the first ranking at silking stage while third at maturity. The methodology has clearly shown that farmers, if included in early evaluation of germplasm, can make a valuable contribution to the breeding effort. Generally, participatory varietal selection was effective and reliable for identifying appropriate cultivars through partnership with resource – poor farmers.

Conflict of interest

The authors have not declared any conflict of interest.

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REFERENCES

Aquino PF, Carrion RC, Flores D (2001). Selected maize statistics. In: Pingali, P.L. (Ed.). 2001. CIMMYT 1999-2000 World Maize Facts and Trends. Meeting World Maize Needs: Technological Opportunities and Priorities for the Public Sector. Mexico. D-F. CIMMYT.

De Groote H, Siambi M, Friesen D, Diallo A (2002). Identifying Farmers' Preferences for new Maize Varieties in Eastern Africa. In Bellon, M.R. and J. Reeves (Eds.) 2002. Quantitative Analysis of Data from Participatory Methods in Plant Breeding. Mexico, DF: CIMMYT.

Joshi KD, Subedi M, Rana RB, Kadayat KB, Sthapit BR (1997). Enhancing on-farm varietal diversity through participatory varietal selection: A case study for chaite rice in Nepal. Exp. Agric. 33:335-344.

Tilahun M, Teshome R (1987). Nazret mixed farming diagnostic survey report: Shewa Region. Research Report (2), IAR, Addis Ababa.

Weltzien E, Smith ME, Meitzner LS, Sperling L (2003). Technical and institutional issues in participatory plant breeding-From the perspective of formal plant breeding. A global analysis of issues, results, and current experience. PPB Monograph No. 1. Cali: PRGA.

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